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TETRAPLOID GREEN TOAD SPECIES (*Bufo pewzowi*) FROM THE ALTAY MOUNTAINS: THE FIRST RECORD FOR RUSSIA

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Tetraploid green toad, *Bufo pewzowi* Bedriaga, 1898 was found in Altay Republic. This is the first record of the species for Siberia and Russia in general. The distribution of diploid and tetraploid toads in Siberia and adjacent territories of eastern Kazakhstan and north-western China is outlined. *Bufo pewzowi* is recommended to list in the Red Data Book of Russian Federation and in regional Red Data Book of Altay Republic.

Keywords: polyploidy, green toads, genome size, *Bufo pewzowi*, *B. viridis*, Altay, Siberia, Russia.

INTRODUCTION

Polyploidy is one of the most important processes involved in evolution of the Western Palearctic green toads of the *Bufo viridis* complex comprising diploid, triploid, and tetraploid bisexual reproducing taxa (Mazik et al., 1976; Borkin et al., 1986, 1995, 2001b; Dujsebayeva et al., 1997, 2004; Stöck et al., 2001, 2006). In western Siberia, the distribution of the *B. viridis* complex is poorly studied. Formerly, it has been assumed that the south-western part of the region is inhabited by diploid *B. viridis* Laurenti, 1768 (Malkov and Malkov, 1976; Yakovlev, 1995, 1996, 1999, 2007; Kuzmin, 1999; Vozniyuk and Kuranova, 2008). Indeed, our study of the genome size variation in a sample of green toads collected in the vicinities of Novosibirsk City, south-western Siberia, revealed only diploid specimens (Litvinchuk et al., 2006). However, tetraploid *B. pewzowi* Bedriaga, 1898 is widely distributed in adjacent territories of Kazakhstan, China, and Mongolia (Borkin et al., 1986; Stöck et al., 2006; Zhang et al., 2008).

The aim of our study was to check ploidy level in green toads from localities in Altay Republic (Russia), which are close to the north-western border of currently recognized range of tetraploid *B. pewzowi* in Kazakhstan.

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MATERIAL AND METHODS

Nine specimens of green toads from Altay Republic and one hundred and six specimens (22 localities) from adjacent regions were used in the study of nuclear DNA content (or genome size) variation (Table 1). After anesthesia (using etoxetan), blood was taken by a micro-pipette from the heart or the femoral vein. Peripheral blood cells of the grass frogs, *Rana temporaria* Linnaeus, 1758, collected in St. Petersburg and Pskov provinces (north-western Russia), were used as a reference standard. Flow cytometry was performed using a microscope-based fluorimeter. Details of the technique have been previously published (Borkin et al., 2001a). Specimens studied are deposited at the Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

Two subadult toads from Altay Republic were used for studying karyotypes. Chromosomes were prepared from intestine, as described by Schmid (1978). Giemsa staining followed Schmid (1978). We counted chromosomes in 23 metaphases.

The range maps are based on the coordinates and altitude determined by a GPS receiver, or identified ex post facto from military maps of «General'nyi shtab VS SSSR» [= USSR General Military Staff] at a scale of 1:100,000 (for old literature data). For most references, for which appropriate geographic coordinates were not given in original sources, geographic positions of collecting sites were estimated using the Microsoft Encarta World Atlas (1998 Edition), GoogleEarth, and/or FallingRain Gazetteer (<http://gnswww.nga.mil/geonames/GNS/index.jsp>).



Fig. 1. Churgun' Point in the Kyzyl-Tash River valley (Altay Republic, Russia). *Bufo pewzowi* was collected in the swampy hillock area.

Maximum entropy modeling (Phillips et al., 2006) of the green toads geographic distribution was performed by the Maxent software (www.cs.princeton.edu/~schapire/maxent) using the 20 climatic (Bio1–19) and elevation variables (WorldClim database, 1 km² spatial resolution, available in <http://www.worldclim.org/current>). A model was developed using 75% of the data randomly selected as training data and the remaining 25% as testing ones.

RESULTS AND DISCUSSION

In May 21, 2009, S. N. Litvinchuk and V. I. Kazakov found green toads in a swamped area in Churgun' Point (or "urochishche"), Kosh-Agach District, Altay Republic, Russia (Fig. 1). Nine subadult specimens (Fig. 2) were taken at the distance of ca. 300 m in wet moss near small springs in the valley of Kyzyl-Tash River, Chuya River tributary (50°13'44.7" N 87°53'11.1" E, 1475 m

TABLE 1. Genome Size (pg) Variation in the *Bufo viridis* Complex from the Altay Mountains and Adjacent Regions of Russia, North-Western China, Western Mongolia, and Eastern Kazakhstan

Species	Locality	n	Mean ± S.D.	Range
<i>Bufo pewzowi</i>	Churgun', Altay Republic, Russia	9	20.26 ± 0.06	20.19 – 20.36
	Eastern Kazakhstan (19 samples)	96	20.31 ± 0.16	19.86 – 20.92
	Chovd Aimag, Mongolia	3	20.68 ± 0.03	20.65 – 20.70
	Turfan, China	4	20.11 ± 0.14	20.01 – 20.31
<i>Bufo viridis</i>	Novosibirsk, Russia	3	9.19 ± 0.06	9.15 – 9.25

a.s.l.). At 16:15 of a local time, the temperature of water in a spring was 5.5°C and in the river was 9.4°C. In this place, an adult brown frog *Rana arvalis* Nilsson, 1842 and two subadult lizards *Zootoca vivipara* Jacquin, 1787 were recorded as well.

According to our cytogenetic data, the green toad sample proved to contain tetraploid specimens belonging to *B. pewzowi*. Genome size varied between 20.19 and 20.36 pg (Table 1). The karyotype consisted of 44 chromosomes with six tetrads of large chromosomes and five tetrads of small ones (Fig. 3).

To outline the distribution of green toads in southwestern Siberia and neighboring parts of Kazakhstan and China, we summarized all currently known records (Fig. 4 and Appendix). Unfortunately, data on ploidy level (di- or tetraploidy) are quite limited (Table 2). Only four samples from Siberia and Kazakhstan were examined cytologically (karyology and/or genome



Fig. 2. Subadult *Bufo pewzowi* from Churgun' Point (Altay Republic, Russia).

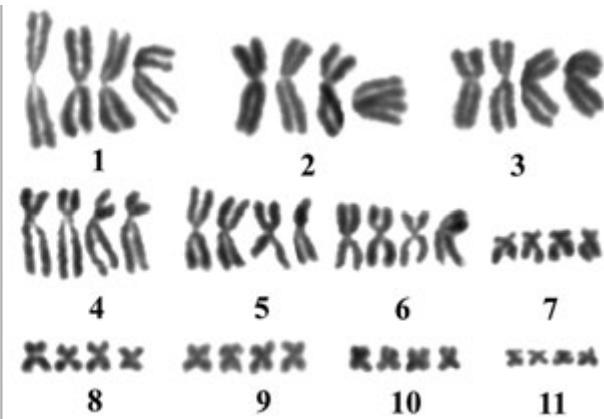
size), and nine samples from China and Kazakhstan by means of mtDNA haplotypes, which were ploidy- and species-specific. However, no ploidy data were available for the majority of samples, known from various publications and/or museum collections (Appendix). In such cases, we suggested that all samples located to the east of 80°E may have $4n$, because neither $2n$ nor $3n$ toads have been detected as yet in that region (Borkin et al., 2001b; Stöck et al., 2001, 2006; Dujsebayeva, 2008). Vice versa, some localities, situated relatively not so far from the regions of Qaraghandy and Novosibirsk cities, were preliminarily attributed by us to diploids, because diploidy was previously confirmed for green toads of these regions.



Fig. 4. Distribution of tetraploid *Bufo pewzowi* (green squares), diploid *B. viridis* (violet squares), and green toads with unknown ploidy (dark blue squares) in the Altay Mountains and adjacent territories of Russia, China, and Kazakhstan. Numbers of localities are the same as in Table 2 and Appendix.



Fig. 3. Tetraploid karyotype of *Bufo pewzowi* from Churgun' Point, Altay Republic, Russia.



According to reliable data and our assumptions (Table 2, Fig. 4, and *Appendix*), tetraploid toads (*B. pewzowi*) inhabit mountain regions in Russia (Altay Mountains: 500 – 1700 m a.s.l.) and adjacent parts of north-

western China and eastern Kazakhstan (Altay and Tarbagatai Mountains: as a rule, 403 – 1220 m a.s.l.). The highest tetraploid locality is associated with thermal springs (Rakhman Qaynary, Kazakhstan Altay, 1815 m

TABLE 2. Methods of Ploidy Identification for Green Toads from the Altay Mountains and Adjacent Territories (for details see Fig. 4, and the *Appendix*)

No.	Locality	Cytometry	Karyology	mtDNA	Geography	Reference
Tetraploids						
1	Churgun'	+	+			Present paper
2	Kuray			+	"	
3	Koksu River			+	"	
4	Karagem River			+	"	
5	Argut River			+	"	
6	Uymon Steppe			+	"	
7	Edigan River			+	"	
8	Aletai		+		Zhang et al., 2008	
9	Mahartai			+	Present paper	
10	Tumandy River			+	Stöck et al., 2001	
11	Rakhman Qaynary			+	Stöck et al., 2006	
12	Terekty		+		"	
13	Prirechnoe		+		"	
14	Bukombay Mts.			+	"	
15	Kukuryum			+	Present paper	
16	Aksiiir		+		Borkin et al., 1986	
17	Mayqapshaghay			+	Present paper	
18	Uydene River			+	Berezovikov, 2008	
19	Zaisan			+	Present paper	
20	Kyzylkaiyn River			+	Berezovikov, 2008	
21	Tasbastau			+	"	
22	Bozsha			+	"	
23	Esne River			+	"	
24	Karatay River		+		Stöck et al., 2006	
25	Akzhar			+	Berezovikov, 2008	
26	Lasty River			+	"	
27	Tebeske River		+		Stöck et al., 2006	
28	Karabuga River			+	Berezovikov, 2008	
29	Bazar River			+	"	
30	Shet-Bugaz River			+	"	
31	Tarbaghatay District			+	Present paper	
32	Akshauli			+	Berezovikov, 2008	
33	Karabulak			+	"	
34	Munbulak			+	"	
35	Ayakoz		+		Bassalaeva et al., 1998	
Diploids						
35	Ayakoz		+		Odierna et al., 2004	
40	Qaraghandy		+		Stöck et al., 2006	
41	Kokpekti			+	Present paper	
42	Temirtau			+	"	
43	Peredovik			+	Stöck et al., 2006	
48	Novosibirsk		+		Litvinchuk et al., 2006	
49	Krivodanovka			+	Present paper	
50	Ob'			+	"	
51	Pristan'-Pochta			+	"	
52	Akademgorodok			+	"	
53	Ordynskoe District			+	"	

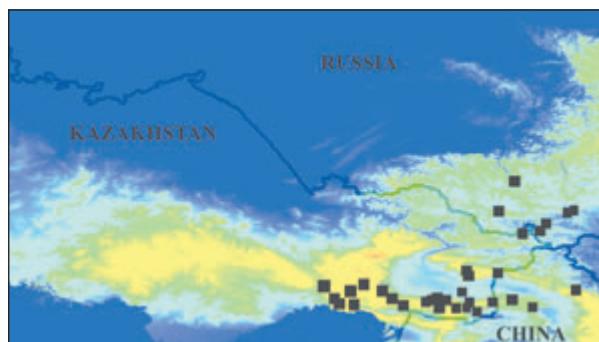


Fig. 5. Predicted potential geographic distribution for tetraploid *Bufo pewzowi* in the Altay Mountains and adjacent territories of Russia, China, and Kazakhstan made using all known records (black squares) and 20 environmental variables. All areas with orange (high), yellow (medium), and green (low suitability) colors likely represent suitable environmental conditions.

a.s.l.). Diploid green toads were found in lowlands and foothills only (95 – 700 m a.s.l.). Green toads with unknown ploidy were recorded between distributional ranges of diploids and tetraploids in Altay Kray of Russia (Fig. 4:46 – 47) and eastern Kazakhstan between 73°6' E and 80°25' E (Fig. 4:36 – 39 and 44 – 45).

Maximum entropy modeling of the distribution of tetraploid *B. pewzowi* demonstrated that the species prefers to inhabit highlands (48.3% of contribution of elevation) and territories with relatively high precipitation (19.7% of contribution of precipitation in driest month). Territories with suitable environmental conditions for tetraploids are revealed westward and north-eastward to known localities of *B. pewzowi* (Fig. 5).

Thus, our cytological data demonstrated that southwestern Siberia harbors two species of green toads: diploid *B. viridis* (Novosibirsk oblast') and tetraploid *B. pewzowi* (the Altay Mountains). The Altay sample is the first record of tetraploid species for the region and for Russia in general.

We would like to recommend to list *B. pewzowi* in the Red Data Book of Russian Federation under the category 3 (a rare species). Formerly, green toads named *B. viridis* (a presumed diploid species) were listed in the Red Data Book of Altay Republic with the category III (Yakovlev, 2007). Based on our data, we recommend to change the name of the toad to *B. pewzowi* (a tetraploid species) with keeping the same protection category.

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APPENDIX⁴**Altay Republic, Russia**

1. Churgun' Point, 50°13'44.7" N 87°53'11.1" E, 1475 m a.s.l., Kyzyl-Tash River, Kosh-Agach District (our data).
2. Kuray village, 50°14' N 87°55' E, about 1500 m a.s.l., Kosh-Agach District, N. P. Malkov, June 1978 (Malkov and Malkov, 1976; Kuchin, 2001; Yakovlev, 1987, 2007); Chuya River, Kuray Steppe, A. M. Kolosov, 7/28/1936, ZMMU 223; N. P. Malkov, July 1978 (Yakovlev, 1987, 1995, 1996; Vozniy-chuk and Kuranova, 2008).
3. Koksu River, 49°39' N 87°2' E, about 1700 m a.s.l., Kosh-Agach District, 7/18/1981, ZMK 1678.
4. Karagem River, lower part, 49°52' N 87°10' E, about 1296 m a.s.l., Kosh-Agach District, N. P. Malkov and Yu. P. Malkov, 8/27/1970 (Malkov and Malkov, 1976; Zolotarenko, 1985; Kuchin, 2001; Yakovlev, 1995, 1996, 1999, 2007; Vozniy-chuk and Kuranova, 2008).
5. Argut River mouth, 50°14' N 86°40' E, about 765 m a.s.l., Onguday District, V. V. Sapozhnikov, 7/12/1911, ZMTU 173 (Vozniy-chuk and Kuranova, 2008).
6. Uymon Steppe, 50°15' N 85°45' E, about 1000 m a.s.l., Ust'-Koksky District, V. Dymov, 1984 (Yakovlev, 1996, 1999, 2007; Kuchin, 2001).
7. Edigan River, 51°7' N 86°13' E, about 500 m a.s.l., 2 – 3 km lower of Edigan village along Edigan River, Chemal District, S. G. Livanov, 7/12/1988 and 8/27/1988 (Yakovlev, 1996, 1999, 2007; Kuchin, 2001); Katun' River valley (Vozniy-chuk, 2007).

Xinjiang Uygur Autonomy, China

8. Aletai village, 47°52' N 88°07' E, 857 m a.s.l., CIB 029820, CIB 029822 and CIB 029832 (Zhang et al., 2008).
9. Mahartai [= Mukurtay] point, 47°20' N 86°48' E, about 550 m a.s.l. (Kaščenko, 1909; Nikolsky, 1918).
10. Tumandy River, 47°33' N 86°11' E, about 750 m a.s.l., G. N. Potanin, 6/28/1876, ZISP 1052 (Potanin, 1883; Bedriaga, 1898; Nikolsky, 1918; Bannikov, 1958; Peters, 1971; Borkin et al., 1986; Borkin and Kuzmin, 1988; Stöck et al., 2001).

Vostochno-Kazakhstanskaya Province, Kazakhstan

11. Rakhman Qaynary [= Rakhmanovskie Klyuchi] kurort, 49°33' N 86°31' E, about 1815 m a.s.l., Arasan Lake, Bereli River, G. K. Tjumenzew, 1878, ZISP 1015 [Thermae Arasanenses pr. fl. Bukhtarma]; A. A. Silantjew, 8/18/1897, ZISP 2114 [precise locality pointed out in Silantiev (1903: XVIII)]; V. V. Sapozhnikov, 7/23 – 24/1905, ZMTU 61; B. K. Polenov, 1882, SPbU. 135; A. N. Sedel'nikov, 7/16/1914, ZISP 2808; B. N. Shvanovich, 1916, ZISP 2768; V. G. Kolbintsev, 7/25/2002 (Nikolsky, 1883, 1918; Kaščenko, 1902; Dorowatowsky, 1913; Heptner, 1936; Kolosov, 1938; Iskakova, 1959, 1962; Kuzmin, 1999; Stöck et al.,

⁴ **Institutional abbreviations:** CIB, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA; ZISP, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; ZMTU, Zoological Museum of Tomsk State University, Tomsk, Russia; ZMK, Zoological Museum, National Scientific Natural History Museum, National Academy of Sciences, Kiev, Ukraine; ZMMU, Zoological Museum of Moscow State University, Russia; SPbU is Department of Vertebrate Zoology, St. Petersburg State University, St. Petersburg, Russia.

2006; Dujsebayeva, 2008; Vozniy-chuk and Kuranova, 2008; Zhang et al., 2008).

12. Terekty [= Terekty, Alekseyevka] village, 48°27' N 85°44' E, 618 m a.s.l., T. N. Dujsebaeva, 8/10/2001 (Stöck et al., 2006; Dujsebayeva, 2008; Zhang et al., 2008).

13. Prirechnoe village, 48°27' N 84°48' E, about 900 m a.s.l., T. N. Dujsebaeva, 8/8/2002 (Stöck et al., 2006; Dujsebayeva, 2008; Zhang et al., 2008).

14. Southern Bukombay Mountains, 48°15' N 84°48' E, about 850 m a.s.l., T. N. Dujsebaeva, 8/6/2001 (Stöck et al., 2006; Dujsebayeva, 2008).

15. "Kus-Murum" [= Kukuryum village], 47°49' N 84°38' E, 403 m a.s.l., E. N. Zorin, 6/19/1911, ZMMU. 202; Zaisan Lake, A. S. Khakhlov, 1894, ZMTU 63 (Kaščenko, 1902; Nikolsky, 1918).

16. Aksiir farm, 47°30' N 85°35' E, about 780 m a.s.l., 12 km to the north-west of Mayqapshaghay [= Maikangal] village, M. L. Golubev, July 1979, ZMK 945 (Shcherbak and Golubev, 1981; Borkin et al., 1986, 2001; Dujsebaeva et al., 1997; Kuzmin, 1999; Stöck et al., 2001; Berezovikov, 2008; Dujsebayeva, 2008); Aksiir River, bridge on the road in Mayqapshaghay village (Berezovikov, 2008).

17. Maygapshaghay [= Maikapchagay] village, 50 km to the south, about 47°10' N 85°08' E, M. L. Golubev, 4/6/1979, ZMK 1143.

18. Uydene River, 47°22.380' N 84°47.900' E, 846 m a.s.l., reservoir, Saur Mountains, N. N. Berezovikov, 5/24/2001 (Berezovikov, 2008).

19. Zaisan Town, 47°28' N 84°52' E, 660 m a.s.l., Yu. N. Isaev and A. V. Medyanovsky, 8/20/1978, ZMK 707.

20. Kyzylkaiyn River, lower part, 47°37'13.5" N 84°8'20.3" E, 678 m a.s.l., 3 – 4 km lower of canyon, Manrak Mountains, N. N. Berezovikov, 5/15/2000 (Berezovikov, 2008).

21. Tasbastau village, 47°19' N 84°30' E, 1220 m a.s.l., Kandysu River, Chilikty Valley, N. N. Berezovikov, 5/30/2001 (Berezovikov, 2008).

22. Bozsha village, 47°19' N 83°59' E, 960 m a.s.l., reservoir, Kandysu River Valley, Manrak Mountains, N. N. Berezovikov, 5/31/2000 (Berezovikov, 2008).

23. Esne River, upper part, 47°35.397' N 83°53.535' E, 715 m a.s.l., Manrak Mountains, N. N. Berezovikov, 5/15/2000 (Berezovikov, 2008).

24. Karatal River, 47°38' N 83°48' E, 621 m a.s.l., between Akzhar and Baytigi villages, M. A. Chirikova, 2002 (Dujsebaeva et al., 2005; Stöck et al., 2006; Dujsebayeva, 2008).

25. Akzhar Settlement, 47°35.340' N 83°42.110' E, 630 m a.s.l., swamps in valleys of Kandysu and Sharga rivers, N. N. Berezovikov, 6/1/2001 (Berezovikov, 2008).

26. Lasty River, 47°30.839' N 83°35.684' E, 630 m a.s.l., lower of Shet-Ulasty and Orta-Lasty rivers confluence, between Kuygan and Zhambyl villages, Tarbagatay Mountains, N. N. Berezovikov, 6/1/2001 (Berezovikov, 2008).

27. Tebeske [= Tebiske] River, 47°25' N 82°52' E, 971 – 984 m a.s.l., N. N. Berezovikov, 5/11/2000 and M. A. Chirikova, 2002 (Stöck et al., 2006; Berezovikov, 2008; Dujsebayeva, 2008; Zhang et al., 2008).

28. Karabuga [= Kargyba] River, 47°37'24" N 82°34'40" E, 780 m a.s.l., Tarbagatay Mountains, N. N. Berezovikov, 6/4/2000 (Berezovikov, 2008).

29. Bazar River, 47°51' N 82°15' E, 787 m a.s.l., Tarbagatay Mountains, N. N. Berezovikov, 6/4/2000 (Berezovikov, 2008).

30. Shet-Bugaz River, 48°02' N 81°52' E, 818 m a.s.l., Kezen'-Chilik point, Northern Tarbagatay Mountains, N. N. Berezovikov, 5/7/2000 (Berezovikov, 2008).

31. Tarbaghatay [= Tarbagatay] District (Berdibaeva, 1984).
32. Akshauli [= Znamenka] village, 2 km to south, 47°26'46.2" N 81°21'30.2" E, 1105 m a.s.l., Tarbagatay Mountains, N. N. Berezovikov, 5/20/2001 (Berezovikov, 2008).
33. Karabulak village, 47°30' N 80°54' E, about 930 m a.s.l., Tarbagatay Mountains (Berezovikov, 2008).
34. Mynbulak village, 47°37'27.6" N 80°47'19.8" E, 945 m a.s.l., Tarbagatay Mountains, N. N. Berezovikov, 5/20/2001 (Berezovikov, 2008).
35. Ayakoz [= Ayaguz, Sergiopol] Town, 48°0' N 80°25' E, 700 m a.s.l., A. A. Kuschakewitsch, 1881, ZISP 1511 (Nikolsky, 1918; Iskakova, 1959; Bassalaeva et al., 1998; Borkin et al., 2001; Odierna et al., 2004; Dujsebayeva, 2008); Chingiztau Mountains (Iskakova, 1959).
36. Degelen Hills, 49°50' N 78°5' E, about 570 m a.s.l., I. N. Magda, 8/26/2005 (T. N. Dujsebaeva, personal communication); Uzun-Bulak Spring, 49°46' N 78°4' E, I. N. Magda, 8/26/2005 (T. N. Dujsebaeva, personal communication); Semipalatinsk Nuclear Testing Site (Khromov, 1998).
- Qaraghandy Province, Kazakhstan**
37. Kent Mountains, 49°3' N 76°11' E (Iskakova, 1959).
38. Kyzylrat Mountains, 48°30' N 75°26' E (Iskakova, 1959; Atakhanova and Bigaliev, 1995).
39. Qarqaly [= Karakalinsk] City, 49°25' N 75°7' E, about 715 m a.s.l., Nura River valley (Iskakova, 1959; Atakhanova and Bigaliev, 1995); Karkarali Mounts, I. A. Slowtzow, 1877, ZISP 997 (Nikolsky, 1918).
40. Qaraghandy [= Karaganda] City, 49°48' N 73°6' E, about 550 m a.s.l., to the north from the railway near the city center, M. Stöck, MVZ.Herp 249173 (Iskakova, 1959; Kapitonov, 1987; Atakhanova and Bigaliev, 1995; Stöck et al., 2006).
41. Kokpekti [= Solonichki] Settlement, 49°56' N 73°16' E, about 516 m a.s.l., Botakara [= Bukhar-Zhyrauzsky] District (Atakhanova and Bigaliev, 1995).
42. Temirtau Town, 50°4' N 72°58' E, about 530 m a.s.l., enterprise "Karbit" (Atakhanova and Bigaliev, 1995).
- Akmola Province, Kazakhstan**
43. Peredovik village, 51°08' N 71°16' E, about 340 m a.s.l., the southwestern vicinities of Astana [= Akmola] City, M. Stöck, MVZ.Herp 249168 – 249169 (Stöck et al., 2006).
- Pavlodar Province, Kazakhstan**
44. Kyzylkak Lake, 53°26' N 73°39' E, about 42 m a.s.l., 6/20 – 7/2/1898 (Berg and Ignatow, 1901).
45. Bayanaul [= Bayan-Aul] National Park, 50°47' N 75°42' E, 468 m a.s.l., Bayanaul District (Atakhanova and Bigaliev, 1995).
- Altaisky Kray, Russia**
46. Kulunda Steppe⁵, A. I. Dzens-Litovsky, 1928, ZISP 3323.
47. Barnaul City, 53°21' N 83°45' E, F. A. Gebler, 1834, ZISP 353⁶ (Boulenger, 1880; Bedriaga, 1891, 1898; Kaščenko, 1902; Nikolsky, 1918; Kuzmin, 1999).

Novosibirsk oblast', Russia

48. Novosibirsk City, 55°02' N 82°50' E, about 95 m a.s.l., sand quarry in 2 – 3 km from Ob' River left bank (Zolotarenko, 1985; Kuzmin, 1999; Chernyshova et al., 2002; Litvinchuk et al., 2006 [south-western Siberia]; Balatsky, 2009); Novosibirsk-Zapadnyi railway station, Ob' River valley (Zolotarenko, 1985); Zael'tsovsky Park, Botanical Forestry, and Dendrarium (Chernyshova, 2009); Chernyshovsky Bor (Chernyshova, 2009).
49. Krivodanovka village, 55°05' N 82°39' E, about 95 m a.s.l., Kolyvansk District (Balatsky, 2009).
50. Ob' Town, 54°59' N 82°43' E, about 105 m a.s.l., Kolyvansk District (Balatsky, 2009; Chernyshova, 2009).
51. Pristan'-Pochta Settlement, 55°27' N 82°59' E, about 125 m a.s.l., Kolyvansk District (Balatsky, 2009).
52. Akademgorodok, 54°51' N 83°06' E, about 120 m a.s.l., Novosibirsk District (Chernyshova, 2009).
53. Ordynskoe District (Balatsky, 2009).

Additional distributional notes. Questionable records of green toads were listed for some other regions of Siberia. For example, *B. viridis* is in the Red Data Books of Krasnoyarsk Kray (Achinsk District and the right bank of Krasnoyarskoe Reservoir) and Khakasiya Republic (<http://res.krasu.ru/nature/a.html>). Additionally, “spotted” (perhaps, green) toads from Znamenka (Aleksandrovsky zavod, Lugavka River; 53°32' N 91°54' E) and Beya (Beyskoe; 53°03' N 90°54' E) villages were mentioned in the catalogue of Minusinsk Museum, Krasnoyarsk Kray (Martyanov, 1881). Later, S. M. Čugunov (1914) examined these collections and found here common toads, *B. bufo* (Linnaeus, 1758) only. According to E. I. Strelkov (1963), two specimens of *B. viridis* were found in the vicinities of Tomsk City in 1956. However, lately, the record was not confirmed (Kuranova, 2000). In herpetological catalogue of St. Petersburg State University (Dorowatosky, 1913), a specimen of *B. viridis* (SPbU. 134, collector A. M. Nikolsky, 1882) was mentioned for Tourak [= Turok] village (51°34' N 85°01' E), Altai Kray, Russia. However, according to A. M. Nikolsky (1883, 1918) himself, he collected there a large specimen of common toad *B. bufo* only. We studied this specimen and confirm A. M. Nikolsky's identification. S. L. Kuzmin (1999) noted a record of *B. viridis* from Markakol' Nature Reserve (Kazakhstan). However, neither *B. viridis*, nor *B. pewzowi* were found in the territory of reserve (Dujsebaeva, 2001; Utyasheva, 2006).

⁶ According to morphological characters (coloration and form of parotid glands), this specimen could be assigned to *B. pewzowi*. We assume that this green toad was collected outside the Barnaul City vicinities. In 1834, the collector visited the Altay Mountains in the border between Russia and Kazakhstan, where he could collect this specimen. However, in description of the travel (Gebler, 1837), he mentioned no green toads.

⁵ Kulunda Steppe is vast lowland (100,000 km²), which is distributed in the south-west of Altaisky Kray (Russia) and the adjacent territory of Pavlodar Province (Kazakhstan).