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Morphological differentiation between diploid and polyploid species of green toads (Anura: Bufonidae: *Bufotes*) in Central Asia

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Abstract. We examined morphometric variation in green toads of the genus *Bufotes* using univariate and multivariate statistics in order to identify diagnostic characters. This study describes patterns of variation in two diploid (*B. sitibundus* and *B. perrini*) and a tetraploid (*B. pewzowi*) species and triploid hybrids of *B. perrini* and *B. pewzowi* distributed in Kazakhstan and Kyrgyzstan (14 localities). Since body proportion characters were highly correlated, we divided them into each other and obtained 190 indices. Using multivariate analyses we selected among them five most valuable. Discriminant analysis showed 95% of specimens correctly classified. The greatest importance for discrimination of diploid and tetraploid species had two indices related to the parotoid gland size, distance between nostrils and length of foot. Between diploid species the biggest differences were observed by two indices related to length of foot, radius bone size, and diameter of the eye. Based on these features we obtained two multiplicative indices that allow us to reliably identify 85–94% of specimens of each species. Hybrids usually had intermediate values of the indices compared to parental species (*B. perrini* and *B. pewzowi*), mostly closer to the latter. The application of these diagnostic indices will allow non-invasive identification of individuals of these diploid and tetraploid species during field research and study of museum specimens.

Key words. Amphibia, polyploidy, morphological variation, external characters, Kazakhstan, Kyrgyzstan.

INTRODUCTION

Polyploidy is an important evolutionary phenomenon. The process of increase in the number of chromosome sets and its consequences concerning morphological and ecological differentiation of polyploids and their ancestors, phylogenetic origin and genome evolution of polyploids, role of polyploidy in diversification have attracted much attention (Soltis & Soltis 2012).

Eurasian green toads of the genus *Bufotes* Rafinesque, 1815 are one of the most famous examples of polyploid speciation among amphibians (Litvinchuk et al. 2016). The genus includes ten diploid and five polyploid species (Dufresnes et al. 2019). Based on phylogenetic reconstructions, diploid species of the genus can be divided into two main groups. The *B. latastii* group consists of three species restricted to Iran, Iraq, Pakistan and the Indian Himalayas. The *B. viridis* group includes seven species distributed in North Africa, Europe, and the western part of Asia. Some species from these two groups interbred and gave origin to three triploid and two tetraploid species which inhabit Central Asia and the Himalaya (Stöck et al. 2001, 2006; Litvinchuk et al. 2012; Betto-Colliard et al. 2018; Dufresnes et al. 2019). In the *B. viridis* group, species are morphologically quite similar and usually can only be identified using genetic characteristics (Stöck et al. 2006; Dufresnes et al. 2019). Variation of their morphological characters is poorly studied.

Two diploid and one tetraploid species of green toads are distributed in the northern part of Central Asia (Kazakhstan and Kyrgyzstan). The variable toad, *B. sitibundus* (Pallas, 1771), represents a diploid species that inhabits Western Asia, the Arabian Peninsula, the Caucasus, the left bank of Volga River, and the southern Ural in Russia, as well as western and northern Kazakhstan (Fig. 1). The Perrin's toad (*B. perrini* Mazepa, Litvinchuk, Jablonski, and Dufresnes, 2019) is a recently described diploid species that is distributed in northeastern Iran, northern Afghanistan, Turkmenistan, Uzbekistan, northern Kyrgyzstan, and southern Kazakhstan (Fig. 1). The Pewzow's toad, *B. pewzowi* (Bedriaga, 1898) is a tetraploid species of hybrid origin (Dufresnes et al. 2019) whose maternal species is *B. perrini* and whose paternal species is another diploid species *B. latastii* (Boulenger, 1882). The spe-

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cies inhabits mostly mountainous regions in the east of Central Asia and the Altai Mountains in Siberian Russia (Fig. 1). In Kazakhstan and Kyrgyzstan ranges of *B. perrini* and *B. pewzowi* are parapatric and in some places are slightly overlapping. Here the species can hybridize with formation of a triploid offspring (Borkin et al. 1986a, 2001, 2007; Castellano et al. 1998, 2003; Odierna et al. 2004; Litvinchuk et al. 2006).

Analyses of morphological features in various green toads were carried out by many researchers (Peters 1971; Pisanets 1977; Hemmer et al. 1978; Pisanets & Szczerbak 1979; Borkin et al. 1986b, 2007; Ataev 1987; Stugren & Tassoula 1987; Castellano et al. 1998, 2000; Tok 1999; Tosonoğlu 1999; Sartaeva & Vashetko 2005; Kurtup et al. 2006; Lada 2012). However, diagnostic morphological characters which allow determining most of these species were not revealed (Dufresnes et al. 2019). This prevents the successful identification of species during field research and study of museum specimens. Therefore, the aim of the present paper was to find morphological differences which allow distinguishing species of the genus *Bufotes*, which inhabit Kazakhstan and Kyrgyzstan.

MATERIAL AND METHODS

During the period from 1994 to 2001, a total of 209 adult males of *B. sitibundis* (n=13), *B. perrini* (n=64), *B. pewzowi* (n=113) and hybrids of *B. perrini* and *B. pewzowi* (n=19) from 14 localities were sampled in Kazakhstan and Kyrgyzstan (Table 1; Fig. 1). Among them, 54 specimens of all three species were studied for the first time. Other 155 specimens of *B. perrini*, *B. pewzowi* and their hybrids were previously analyzed by Castellano et al. (1998, 2000). In the study, only adult males were used, since they are in the spawning water bodies for a long time, and therefore they are much easier to catch than

Table 1. Localities, coordinates, altitude, ploidy level, year of collection and sample size (N) of various species of green toads of the genus *Bufotes*.

	Locality	Country	Coordinates	Altitude	Ploidy	Year	N
			B. sitibundus				
1	Atyrau	Kazakhstan	47.233° N, 51.933° E	-20	2n	2001	13
			B. perrini				
2	Tulek	Kyrgyzstan	43.109° N, 74.092° E	758	2n	1994–1995	16
3	Kok-jar	Kyrgyzstan	42.810° N, 74.641° E	935	2n	1995, 1997	20
4	Koi-Tash	Kyrgyzstan	42.684° N, 74.670° E	1350	2n	1994, 1998	2
5	Kopa	Kyrgyzstan	43.417° N, 75.783° E	750	2n	1994, 1995, 1998	23
6	Kapchagai	Kazakhstan	43.966° N, 77.362° E	554	2n	1997	3
			B. perrini* × *B. pewzowi				
3	Kok-jar	Kyrgyzstan	42.810° N, 74.641° E	935	3n	1995, 1997	5
4	Koi-Tash	Kyrgyzstan	42.684° N, 74.670° E	1350	3n	1994	11
6	Kapchagai	Kazakhstan	43.966° N, 77.362° E	554	3n	1997	3
			B. pewzowi				
3	Kok-jar	Kyrgyzstan	42.810° N, 74.641° E	935	4n	1995	1
4	Koi-Tash	Kyrgyzstan	42.684° N, 74.670° E	1350	4n	1994, 1998	10
6	Kapchagai	Kazakhstan	43.966° N, 77.362° E	554	4n	1997	2
7	Kizilkum	Kazakhstan	42.065° N, 67.703° E	210	4n	1995	7
8	Jabagly	Kyrgyzstan	42.433° N, 70.467° E	1100	4n	1995	11
9	Karaoi	Kazakhstan	45.842° N, 74.802° E	344	4n	1995	11
10	Zhidely	Kazakhstan	45.300° N, 75.200° E	370	4n	1995	3
11	Ayakoz	Kazakhstan	48.000° N, 80.417° E	700	4n	1997	10
12	Alma-Ata	Kazakhstan	43.250° N, 76.956° E	846	4n	1994–1995	28
13	Big Lake	Kazakhstan	43.052° N, 76.988° E	2509	4n	1994	12
14	Issyk-Kul	Kyrgyzstan	42.200° N, 76.669° E	1750	4n	1994	18

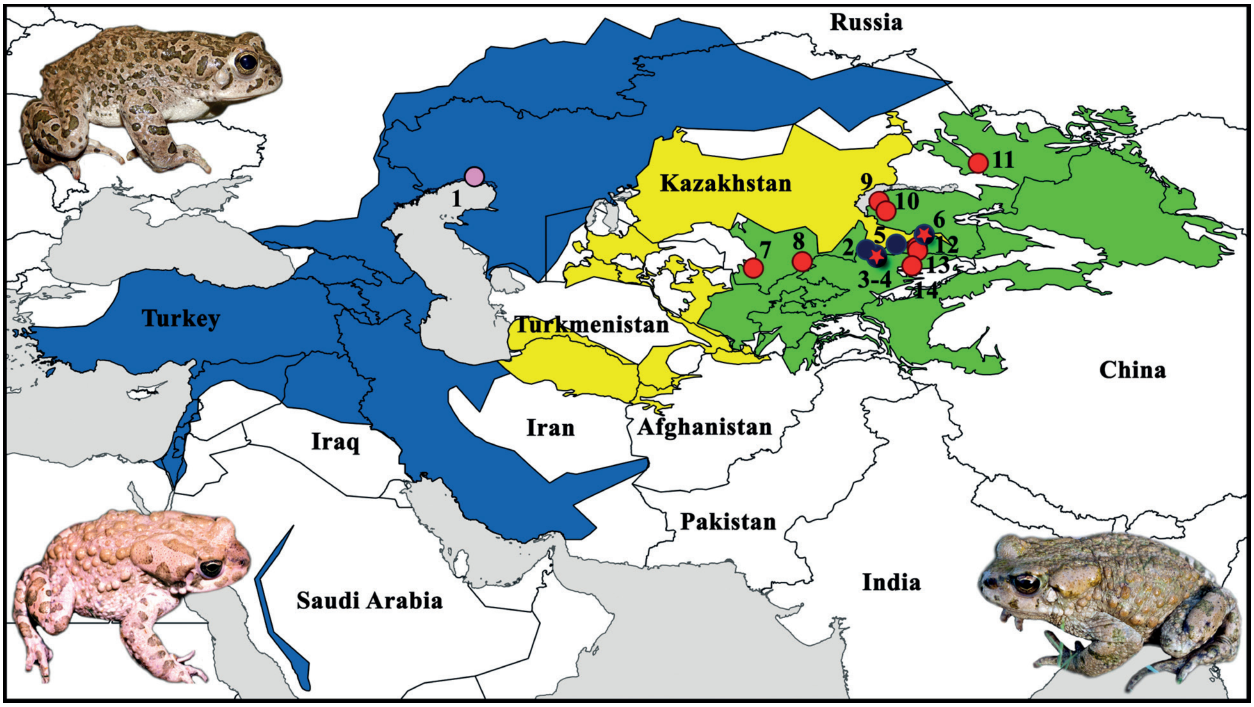

Fig. 1. Distribution and localities of *Bufotes sitibundus* (blue range and rose circle), *B. perrini* (yellow range and dark blue circles), and *B. pewzowi* (green range and red circles). Localities, where triploid hybrids of *B. perrini* and *B. pewzowi* were found together with parental species, are designated by red stars. Numbers for localities are given in Table 1. Photo credit: LSN and DTN (male of *B. pewzowi* from Kazakhstan in the right corner; male of *B. perrini* from Kazakhstan in the lower left corner; and male of *B. sitibundus* from Kazakhstan in the upper left corner).

females, which are breeding in water bodies for a very short time.

Animals were anaesthetized in 1% solution of tricaine methane-sulfonate (MS-222) and measured to the nearest 0.01 mm with a digital caliper. Voucher specimens are deposited in herpetological collections of the Department of Life Sciences and Systems Biology of University of Turin (Turin, Italy) and the Institute of Zoology of the Republic of Kazakhstan (Almaty, Kazakhstan). We measured twenty body proportion characters following Castellano & Giacoma (1998): snout-vent length (SVL); length of the head which was measured from the tip of snout until the posterior edge of the mandible (LHEAD); width of the head (distance between the posterior edges of mandibles; WHEAD); minimum distance between the nostrils (INTNOS); distance between nostrils and the tip of snout (NOSTIP); minimum distance from the nostril opening to the anterior corner of the eye (NOSEYE); minimum distance from the eye to the tympanum (EYETYM); horizontal diameter of the eye (DEYE); vertical diameter of the tympanum (DTYM); length of parotoid glands (LPAR); distance between the elbows when animal is kept with humerus at 90° degree angle with respect to the body axis (WGRASP); length of the radio-ulna (RADUL); length of the hand (LHAND); length of the first finger of the hand (LIFING); length of the femur (LFEM); length of the tibia (LTIB); length of the tarsus (LTARS); length of the foot (LFOOT); minimum distance from the distal extremity of the inner metatarsal tubercle to the web between the third and fourth finger (WEB); and length of the metatarsal tubercle (LMET).

Since all characters were highly correlated with each other (n=209; r=0.55–0.98; P<0.05), we divided them into each other and obtained 190 ratios (indices). Then, for all indices the natural logarithm conversion was made. Using the method of principal components, we selected the 118 most variable indices (factor loadings more than |0.035|) on the first two axes (30.6% and 15.5% of variance). This reduction in the number of indices allowed us to use the discriminant analysis (species as a grouping variable), which was applied in order to find most valuable indices for differentiation of the three studied species. Based on the results, five most distinguishing indices (NOSTIP/INTNOS, LFOOT/RADUL, LFOOT/LPAR, LPAR/INTNOS, and WEB/DEYE) were selected and used for all subsequent calculations. Additionally, to estimate position of hybrid samples we performed the second discriminant analysis with populations (n≥3) as a grouping variable. All these analyses were performed using Past ver. 4.03 (https://past.en.lo4d.com/). To perform univariate analyses of variance, we applied the one-way ANOVA for comparison of means and the Sheffe test for post-hoc comparisons (P<0.001) which were calculated in Statistica ver. 8.0.

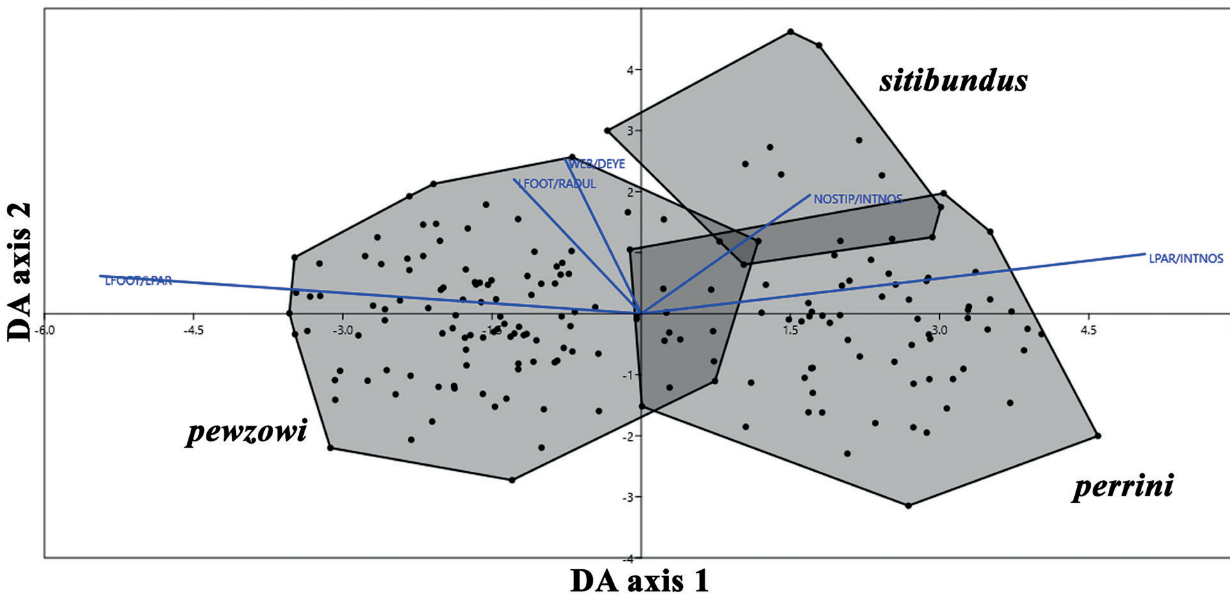

Fig. 2. Discriminant analysis biplot (species as a grouping variable) of morphometric characters and indices for males of *Bufotes sitibundus* (2n), *B. perrini* (2n) and *B. pewzowi* (4n).

To obtain karyotypes for each specimen, venous blood was incubated 100–200 µl for 4 days at 25°C in MEM (Minimal Eagle Medium, GIBCO) with 20% calf serum and 3% Phytohaemogglutinin M. Chromosome preparations were produced by conventional air-drying method, using KCl 0.075M as a hypotonic solution. Standard staining method was performed using Giemsa 5% in phosphate buffer pH 7 (Castellano et al. 1998).

RESULTS

The one-way ANOVA analysis showed significant differences among species for all five selected indices (F=57.6, df=10, $p < 0.001$). The post-hoc comparisons revealed that *B. pewzowi* significantly differed from both diploid species by relatively higher values of the index LFOOT/LPAR and relatively lower values of indices NOSTIP/INTNOS and LPAR/INTNOS (Table 2). Additionally,

Table 2. Variability (X±SD; range) of snout-vent length and morphometric indices in three species of green toads and triploid hybrids between *Bufotes perrini* and *B. pewzowi*. N = sample size.

Character	*sitibundus*	*perrini*	*perrini* × *pewzowi*	*pewzowi*
N	13	64	19	113
SVL	67.5±8.1 (57.0–80.1)	87.0±7.1 (70.1–100.0)	75.3±5.2 (64.8–87.3)	71.5±6.4 (49.4–84.8)
NOSTIP/INTNOS	1.34±0.11 (1.14–1.53)	1.27±0.08 (1.09–1.51)	1.20±0.09 (1.04–1.36)	1.17±0.12 (0.76–1.43)
LFOOT/RADUL	1.86±0.08 (1.74–1.97)	1.68±0.06 (1.52–1.83)	1.80±0.05 (1.71–1.89)	1.82±0.07 (1.65–2.00)
LFOOT/LPAR	1.75±0.13 (1.50–2.00)	1.62±0.13 (1.36–2.04)	2.08±0.15 (1.81–2.41)	2.19±0.21 (1.51–2.65)
LPAR/INTNOS	4.36±0.37 (3.77–4.91)	4.44±0.44 (3.51–5.62)	3.53±0.43 (2.82–4.55)	3.40±0.53 (2.11–5.20)
WEB/DEYE	3.01±0.23 (2.79–3.45)	2.78±0.18 (2.48–3.23)	2.96±0.20 (2.58–3.30)	2.94±0.23 (2.42–3.53)
MI1	2.51±0.39 (1.93–3.28)	2.78±0.43 (1.72–3.82)	1.72±0.33 (1.17–2.48)	1.59±0.41 (0.83–3.44)
MI2	5.74±0.59 (4.87–6.75)	4.68±0.40 (3.95–5.82)	5.32±0.43 (4.65–5.95)	5.35±0.48 (4.34–6.55)

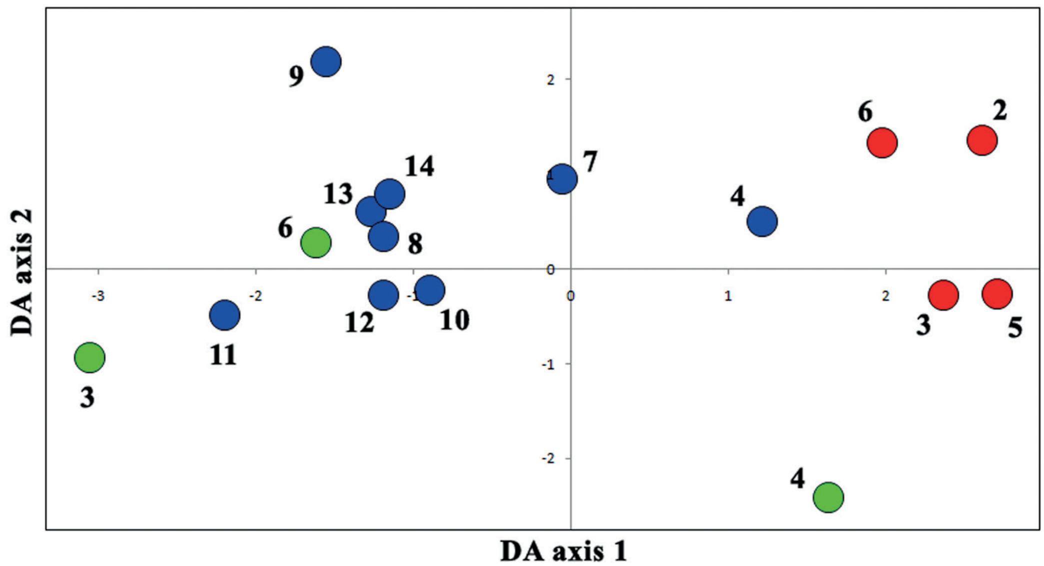

Fig. 3. Plot of centroids for males of *Bufotes perrini* (red circles), *B. pewzowi* (blue) and triploid hybrids between *B. perrini* and *B. pewzowi* (green) in the space of the first and second canonical discriminant axes. Numbers for localities see in Table 1.

B. pewzowi differed from *B. perrini* by having a smaller body length and higher values of indices LFOOT/RADUL and WEB/DEYE. Among diploids, *B. perrini* differed from *B. sitibundus* by having a larger body length and lower values of indices WEB/DEYE and LFOOT/RADUL. Hybrids had usually intermediate values of the indices as compared to their parental species (*B. perrini* and *B. pewzowi*). Among *B. pewzowi* populations, toads from Kizilkum obviously differed from other populations by indices LFOOT/LPAR and LPAR/INTNOS that related to the parotoid gland size (Table 3).

In the discriminant analysis, the first axis included 89% of variation and the second 11%. All three species formed distinct clusters with some overlap (Fig. 2). In general, the overall correct classification rate was 94.7%, where 93.8% were for *B. perrini* (two individuals were confused with *B. sitibundus* and two with *B. pewzowi*), 94.7% for *B. pewzowi* (two individuals were confused with *B. perrini* and four with *B. sitibundus*), and 100% for *B. sitibundus*. In the discriminant analysis with populations as a grouping variable (Fig. 3), centroids of samples of *B. perrini* and *B. pewzowi* formed separate clusters. Two hybrid triploid populations were close to *B. pewzowi*, but a triploid population from Koi-Tash (locality 4) was in intermediate position between *B. perrini* and *B. pewzowi*.

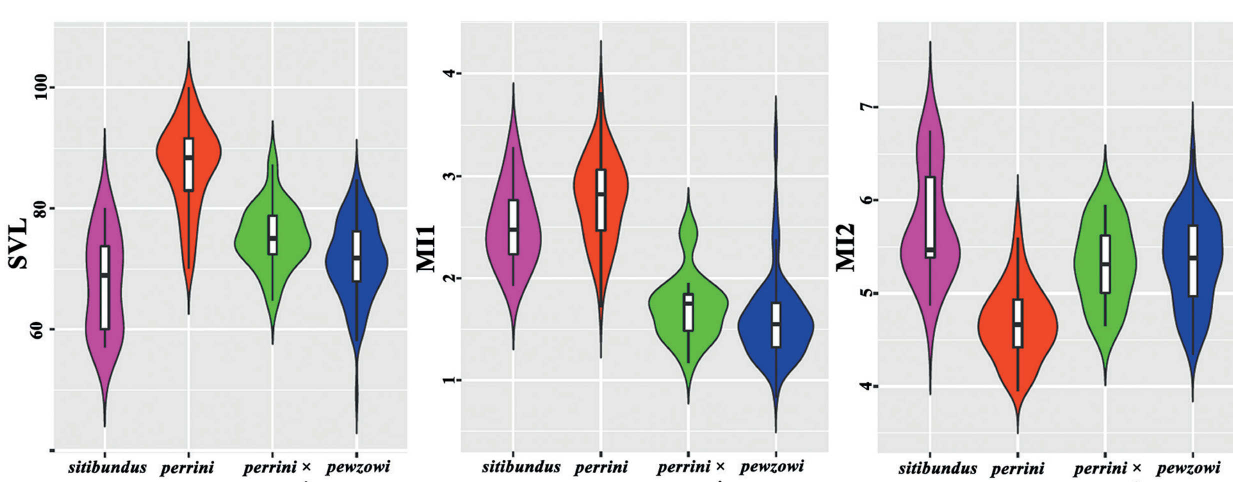

Fig. 4. Variability (violin plots) of SVL, MI1 and MI2 indices in males of *Bufotes sitibundus* (2n), *B. perrini* (2n), *B. pewzowi* (4n), and triploid hybrids between *B. perrini* and *B. pewzowi*.

Table 3. Variation (X±SD) of snout-vent length and morphometric indices in populations of green toads of the genus *Bufotes*. N = size. Numbers for localities see in Table 1 and Fig. 1.

	Locality	N	SVL	NOSTIP/INTNOS	LFOOT/RADUL	LFOOT/LPAR	LPAR/INTNOS	WEB/DEYE	MI1	MI2
					B. sitibundus					
1	Atyrau	13	67.5±8.1	1.34±0.11	1.86±0.08	1.75±0.13	4.36±0.37	3.09±0.23	2.51±0.39	5.74±0.59
					B. perrini					
2	Tulek	16	87.4±8.2	1.25±0.08	1.64±0.06	1.60±0.09	4.45±0.32	2.66±0.14	2.81±0.33	4.37±0.29
3	Kok-jar	20	84.2±7.3	1.28±0.09	1.72±0.05	1.64±0.13	4.50±0.48	2.87±0.20	2.78±0.46	4.95±0.42
4	Koj-Tash	2	89.8±1.5	1.31±0.10	1.67±0.03	1.53±0.09	5.08±0.76	2.90±0.13	3.34±0.69	4.84±0.13
5	Kopa	23	88.6±7.3	1.25±0.06	1.68±0.06	1.60±0.13	4.31±0.43	2.78±0.14	2.73±0.43	4.66±0.31
6	Kapchagai	3	88.9±2.0	1.35±0.04	1.67±0.02	1.73±0.19	4.52±0.39	2.70±0.13	2.65±0.50	4.53±0.17
					B. perrini* × *B. pewzowi					
3	Kok-jar	5	74.9±6.2	1.18±0.06	1.84±0.05	2.09±0.11	3.44±0.29	2.96±0.24	1.65±0.21	5.44±0.48
4	Koi-Tash	11	73.6±3.5	1.20±0.10	1.77±0.05	2.11±0.14	3.43±0.33	2.99±0.15	1.64±0.25	5.30±0.38
6	Kapchagai	3	82.1±4.5	1.21±0.10	1.83±0.05	2.04±0.16	3.68±0.53	2.92±0.26	1.83±0.41	5.34±0.51
					B. pewzowi					
3	Kok-jar	1	73.9	1.14	1.89	2.51	3.10	3.04	1.23	5.75
4	Koi-Tash	10	73.1±5.7	1.18±0.07	1.82±0.09	2.11±0.20	3.63±0.35	2.98±0.21	1.74±0.29	5.42±0.54
6	Kapchagai	2	78.7±7.6	1.22±0.13	1.77±0.03	2.13±0.26	3.61±0.09	2.73±0.02	1.71±0.25	4.84±0.10
7	Kizilkum	7	71.9±3.7	1.16±0.08	1.86±0.08	1.69±0.12	4.47±0.62	2.70±0.15	2.68±0.54	5.04±0.36
8	Jabagly	11	73.3±3.8	1.28±0.08	1.79±0.08	2.22±0.18	3.59±0.34	2.92±0.13	1.63±0.23	5.23±0.37
9	Karaoi	11	62.7±3.8	1.18±0.10	1.82±0.08	2.12±0.10	3.34±0.30	2.61±0.12	1.58±0.15	4.75±0.38
10	Zhidely	3	59.7±11.3	1.21±0.07	1.77±0.08	2.21±0.14	3.54±0.02	2.74±0.29	1.61±0.11	4.84±0.59
11	Ayakoz	10	68.3±2.1	0.98±0.15	1.86±0.06	2.36±0.17	2.64±0.38	3.00±0.24	1.12±0.18	5.61±0.53
12	Alma-Ata	28	74.7±4.7	1.22±0.11	1.80±0.07	2.24±0.18	3.51±0.40	3.09±0.18	1.58±0.28	5.57±0.42
13	Big Lake	12	77.0±5.8	1.16±0.09	1.83±0.06	2.12±0.08	3.42±0.31	2.96±0.11	1.62±0.19	5.42±0.28
14	Issyk-Kul	18	68.9±4.9	1.11±0.1	1.84±0.06	2.29±0.09	3.00±0.16	2.95±0.18	1.31±0.11	5.42±0.36

The greatest impact to discrimination of diploid and tetraploid species had the indices LPAR/INTNOS and LFOOT/LPAR (Table 2, Fig. 2). We divided the first index by the second and obtained the first multiplicative index $MI1 = LPAR^2/(INTNOS \times LFOOT)$. A comparison of diploids and polyploids with use of this index showed 93% of correct identifications in total. Most males of *B. perrini* (94%) and *B. sitibundus* (85%) have values of more than 1.90, and vice versa, values of *B. pewzowi* (94%) and triploid hybrids (100%) were lower than that (Figs 4–5).

The indices LFOOT/RADUL and WEB/DEYE demonstrated the biggest differences between diploid species (Table 2; Fig. 2). We multiplied these indices by each other and obtained the second multiplicative index $MI2 = (LFOOT \times WEB)/(RADUL \times DEYE)$. In total, 86% of identifications using the index were correct. Most individuals of *B. perrini* (88%) have values of the index below 1.27 and most *B. sitibundus* (77%) have values above that number (Figs 4–5).

DISCUSSION

Previous studies of the morphometric variation in amphibians suggest that climatic and ecological conditions are correlated with each other and thus may drive differences in overall body shape (e.g., Castellano & Giacoma 1998; Amor et al. 2011). Based on previous studies of morphological variation in green toads, several general trends can be identified. For example, diploid species can be divided by body length (SVL) into two main groups which were also revealed in phylogenetic reconstructions (Dufresnes et al. 2019). Representatives of the *B. viridis* group are characterized by relatively large body length, while members of the *B. latastii* group are usually small.

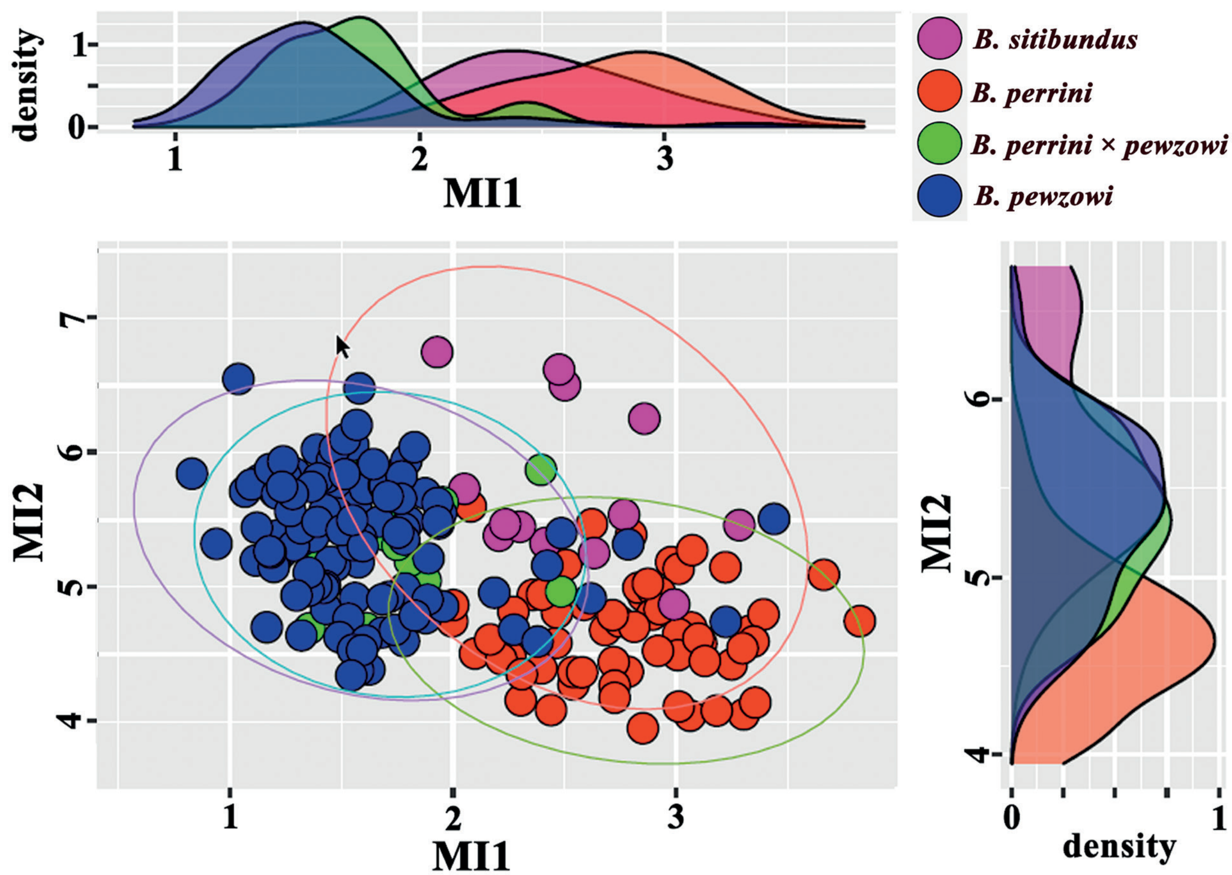

Fig. 5. Distribution of indices MI1 and MI2 in males of *Bufotes sitibundus* (2n), *B. perrini* (2n), *B. pewzowi* (4n), and triploid hybrids between *B. perrini* and *B. pewzowi*.

Polyploid species of hybrid origin occupy an intermediate position. Our findings support this regularity. In Kazakhstan and Kyrgyzstan, adult males of diploid species of the *B. viridis* group have usually larger average SVL (80–87 mm in *B. perrini* and 68–80 mm in *B. sitibundus*) as compared to polyploid *B. pewzowi* (62–73 mm). In other regions, maximum values of average body length of males of diploid toads were 83 mm for *B. sitibundus* and 82 mm for *B. perrini*, whereas the maximum value of average body length of tetraploid *B. pewzowi* was 71 mm (Table 4).

The study on Israeli green toads has demonstrated that variation of body size was partly a function of climate humidity (Nevo & Schneider 1976). In amphibians, volume to surface ratios in larger species would help conserve water in unpredictable environments, and therefore larger species will have greater tolerance to desiccation (Bidau et al. 2011). For the Central Asian green toads this suggestion is only partially true. As a rule, *B. perrini* with its largest body size inhabits very dry deserts. Intermediate *B. sitibundus* live in more moist steppes. However, *B. pewzowi* with smallest SVL as a rule inhabits both relatively moist mountain regions as well as dry mountains (Eastern Tien-Shan) and deserts of premontane and intermontane depressions (Stöck et al. 2001, 2006) with extreme continental climate and maximal humidity deficiency (Murzayev 1966; Vilesov et al. 1986).

Several mechanisms ensure the resistance of amphibians to land conditions among which the skin plays an important role. Unlike diploid green toad species, the skin of tetraploid *B. pewzowi* contains unusual mucous glands ("mosaic glands"), which along with typical mucopolysaccharide components, produce a high amount of proteins (Fedotovskikh et al. 2020). The predominance of protein secretion is considered a characteristic of the terrestrial amphibians (Fujikura et al. 1988; Duellman & Trueb 1994) and we cannot exclude that the "proteinization" of mucous glands enhances the protective properties of the toad's skin and provides additional resistance to desiccation without increasing the overall size of the animals.

The size of the parotoid glands is probably also associated with the described phenomenon, since the mosaic glands were found directly in the parotoid and the dorsal skin. In our study, *B. perrini* had the longest (and largest) parotoids, *B. sitibundus* had intermediate ones, while *B. pewzowi* had the shortest. Similar regularities in the gland size variability among green toad species were previously observed by other authors (Eiselt & Schmidtler 1973; Pisanets 1977; Andrén & Nilson 1979; Borkin et al.

Table 4. Variation (X±SD) of snout-vent length in adult males of three species of green toads of the genus *Bufotes* (N≥3). N = sample size.

Country	N	X±SD (range)	References
		B. sitibundus	
Kazakhstan	13	67.5±8.1 (57.0–80.1)	Present paper
Kazakhstan	3	80.1±0.5 (79.7–80.6)	Borkin et al. (2007)
Russia (Ural)	29	61.5	Toporkova (1981)
Russia (Dagestan)	26	67.2±1.1 (54.7–75.6)	Pysanets (2014)
Russia (Dagestan)	90	70.6	Khonyakina (1980)
Russia (Chechnya)	12	53.0±6.5	Pisanets (1977)
Russia and Georgia	?	65.0 (55.0–75.0)	Hemmer et al. (1978)
Georgia	9	(52.0–86.6)	Tarkhnishvili & Gokhelashvili (1999)
Armenia	181	70.2	Melkumyan & Pisanets (1987)
Azerbaijan	12	56.3±3.9	Melkumyan & Pisanets (1987)
Turkey	7	67.9±5.7 (60.0–76.0)	Flindt & Hemmer (1968)
Turkey	5	75.4	Altunişik et al. (2015)
Turkey	57	62.6 (47.9–82.8)	Altunişik & Özdemir (2015)
Turkey	50	70.0	Kurtup et al. (2006)
Turkey	54	69.2	Kurtup et al. (2011)
Turkey	90	64.1	Altunişik et al. (2021)
Israel	85	75.2 (60.0–88.0)	Nevo & Schneider (1976)
Egypt (Sinai)	16	83.3 (71.0–93.0)	Nevo & Schneider (1976)
Iran (Cheshmeh-ye Sefied)	5	65.6±2.7 (62.0–69.0)	Andren & Nilson (1979)
Iran (Central Zagros)	26	72.7±3.8	Ashkavandi et al. (2012)
Iran, Iraq and Israel	25	67.1 (57.0–78.0)	Eiselt & Schmidtler (1973)
Iran (Kerman Province)	?	70.0 (60.0–80.0)	Hemmer et al. (1978)
Iran	7	75.9±8.6 (63.1–88.8)	Dufresnes et al. (2019)
		B. perrini	
Kazakhstan	64	87.0±7.1 (70.1–100.0)	Present paper
Kazakhstan	?	80.0 (55.3–95.5)	Sartaeva & Vashetko (2005)
Uzbekistan, Turkmenistan, Afghanistan and Iran	?	(45.0–80.0)	Hemmer et al. (1978)
Uzbekistan and Turkmenistan	3	81.9±5.3 (76.1–86.6)	Dufresnes et al. (2019)
Turkmenistan	7	71.1±7.0	Pisanets (1977)
Turkmenistan	121	77.2 (56.7–91.3)	Pisanets & Mezhzherin (1996)
		B. pewzowi	
Kazakhstan	113	71.5±6.4 (49.4–84.8)	Present paper
Kazakhstan	12	(68.0–77.8)	Bassalaeva et al. (1998)
Kazakhstan	?	64.2 (48.3–77.0)	Sartaeva & Vashetko (2005)
Kazakhstan	11	62.1 (55.1–67.5)	Borkin et al. (2007)
Kyzgyzstan	?	(45.0–60.0)	Andrushko (1951)

Table 4. Continued.

Country	N	X±SD (range)	References
		B. pewzowi	
Kyrgyzstan	5	72.8 (56.4–82.7)	Pisanets & Szczerbak (1979)
Mongolia, China, Kazakhstan, Kyrgyzstan, Uzbekistan, and Tajikistan	?	(50.0–80.0)	Hemmer et al. (1978)
Mongolia	?	(?–78.0)	Peters (1971)
Mongolia	?	(?–71.9)	Borkin et al. (1986b)
Mongolia	5	67.5±2.8 (65.3–72.3)	Dufresnes et al. (2019)
China	42	66.1 (51.6–76.5)	Fei et al. (1999)
China	7	57.7±5.0 (50.1–63.0)	Stöck et al. (2001)
China	3	65.4±6.0 (59.4–71.5)	Dufresnes et al. (2019)
Uzbekistan	77	64.2 (48.3–77.0)	Sartaeva & Vashetko (2005)
Uzbekistan	13	69.1±4.8	Pisanets (1977)
Tajikistan	7	71.1±2.9	Pisanets (1977)
Tajikistan	8	65.7±6.1	Pisanets (1977)
Tajikistan	45	68.1 (57.1–79.2)	Pisanets & Szczerbak (1979)
Tajikistan and Uzbekistan	20	68.4±8.1 (47.6–80.7)	Kondratova et al. (2020)
Uzbekistan	12	64.6 (57.1–60.0)	Pisanets & Szczerbak (1979)

1986b; Ataev 1987; Pisanets & Mezhzherin 1996; Castellano et al. 1998; Fei et al. 1999; Dufresnes et al. 2019). In our study, this trend is reflected by the indices associated with the length of the parotoids (LPAR/INTNOS, LFOOT/LPAR and MI1), which were the most useful indices for discriminating diploid and polyploid species. However, the length of the parotoids can be affected by other environmental factors. For example, among diploid green toads lotic breeders, such as *B. surdus* (Boulenger, 1983) and *B. luristanicus* (Schmidt, 1952), have relatively smaller parotoids, as compared to lentic species (other eight species). Probably, small parotoids help to improve the streamlining of these toads. However, all green toad species in Central Asia are lentic breeders and therefore, this regularity should not affect the size of their parotoids here.

It should be noted, that previously Castellano et al. (1998) concluded that the best criteria for discriminating the Central Asian green toad species are their body size and the relative dimensions of their head and limbs. According to our data, the multiplicative indices MI1 and MI2 were most informative for the distinction between three local green toad species. Use of these indices allows to identify 85–94% of specimens of each species. However, our findings need to be confirmed in a broader study covering a larger number of samples (especially *B. sitibundus*). It is possible that an addition of such characters as the form of parotoid glands to the analysis will increase success in the species identification.

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