Reducing the threat to environmental security through the conservation of biodiversity of territories

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Abstract. The nature of the variability of the population structure of green toads (Bufo danatensis Pis.) depending on the natural and anthropogenic differentiation of landscapes is a prerequisite for solving the problems of protection and utilization of biodiversity. Today, the problem of disturbing natural ecosystems due to human activity is the most pressing one. The paper presents an analysis of the population of green toads (Bufo danatensis Pis.) living in the Pamir-Alay mountain system, southwestern regions of Tien Shan, eastern part of the Fergana Valley. This population is of value for the ecosystem of the studied territories and its natural population, which proves variability if species depending on the terrain elevation. In order to perform morphometric and phenetic analysis of Bufo danatensis populations, the series of 40-90 species of the same size group in various landscape zones were used, preserved in 4% formalin. Capture and observations were performed in the daytime and at night time, depending on the nature of diurnal activity. We have determined the body weight (M), body length (L), length of thigh (F), length of second thigh (T), length of the first (inner) finger of a hindlimb (D.p.), total length of the inner calcaneal tuber (C.int.l.), and the height of the calcaneal tuber (C.int.h.). We have calculated proportions used in Anura taxons: L/T, F/T, D.p./C.int.l., T/C.int.l., C.int.h./C.Int.l. We have determined the features of tuberosity, as well as the features of the pattern on the limbs (on the forelimbs – antibrachium, on the hindlimbs – second thigh) for a green toad. The maculation on the belly and throat, as well as features of a dorsal stripe were individually assessed. The measurements were carried out with a caliper with an accuracy of 0.1 mm; 219 specimens of a green toad were treated. The paper contains an analysis of morphometric and phenetic structures of green toads in various landscape zones of the Pamir-Alay. Following the results of research, it was found that the difference between populations of green toads in terms of body size and a dorsomedial stripe in various altitudinal belts is highly significant; in addition, a wedge-like variability has been identified.

1. Introduction
Revealing the nature of the variability of the population structure of animals depending on the natural and anthropogenic differentiation of landscapes is a prerequisite for solving the problems of protection and utilization of biodiversity. Amphibians as an integral part of biogeocenoses play an important role in the functioning of the latter; combined with systems, they are a convenient target of population research. A new direction of herpetological research, landscape herpetology, is actively developing at present. Its most important task is to assess consistent patterns and mechanisms that determine the relationship of variability of spatial differentiation of species with natural and anthropogenic...
differentiation of landscapes in various regions [7]. Solving this problem is necessary for understanding the processes of adaptation of populations to changes in environmental conditions resulting from the transformation of landscapes, as well as for organizing efficient protection of lower vertebrate animals [15, 16].

2. Material and Methodology

The paper is based on the results of many years of field studies (1992-2017) that were carried out in the Pamir-Alay mountain system, and, to a certain degree, south-western regions of Tien Shan, as well as the eastern part of the Fergana Valley.

11 characters were used in the determination of the nature of landscape and geographical variability of morphometric and phenetic structures of populations. 9 characters of them have been described in academic literature. Nevertheless, we have used 2 of them for the first time.

In order to perform morphometric and phenetic analysis of green toad populations, the series of 40-90 species of the same size group in various landscape zones were used, preserved in 4% formalin. Capture and observations were performed in the daytime and at night time, depending on the nature of diurnal activity.

We have determined the body weight (M), body length (L), length of thigh (F), length of second thigh (T), length of the first (inner) finger of a hindlimb (D.p.), total length of the inner calcaneal tuber (C int.l.) and the height of the calcaneal tuber (C.int.h.). We have calculated proportions used in Anura taxons: L/T, F/T, D.p./C.int.l., T/C.int.l., C.int.h./C.Int.l. [2, 5, 6, 11, 13, 14].

We have identified the features of tuberosity, the features of the pattern on the limbs (on the forelimbs – antibrachium, on the hindlimbs – second thigh) for a green toad [8, 9]. The maculation on the belly and throat, as well as features of a dorsal stripe were individually assessed.

The measurements were carried out with a caliper with an accuracy of 0.1 mm; 219 specimens of a green toad were treated.

In order to eliminate the influence of the size of impuberal species on average data by population samples, only mature specimens [3, 8, 9], i.e. with body length over 50 mm, were treated.

3. Results and their Discussion

Morphometric structure of populations. We took only mature species for our morphological analysis in accordance with comments of Ishchenko, V.G. [2]. Mean values of characters and indices are presented in Table 1.

According to Kanep, S.V. [3] and Pisanets, E.M. [9], the largest green toads live in the “optimum zone” of their range, which includes Caucasus and plains and foothills of Central Asia. The average body length of toads here reaches 70-83 mm. The body size decreases outside of the “optimum zone”. Thus, in the east of the range in Tien Shan, Pamir-Alay and western Alay, the average body length of a toad is only 51-71 mm [9].

According to Toktosunov [12], the body size of toads living at various altitudes were as follows: the average body length of a green toad in the Chuya valley (the altitude of 650-700 meters) is 75.2±1.7 mm, in Issyk-Kul hollow (the altitude of 1,660 m) – 66.6±0.7 mm, in Tyulek (the altitude of 2,700 m) – 59.8±1.1 mm.

Our research has shown that the body size of a green toad can be described as follows depending on altitudinal belts: in plain and foothill regions, in the neighborhood of Osh (the altitude of no more than 1,150 m), the body length of a green toad is 69.1±0.80 mm; in medium-altitude mountain regions, in the north-western part of Kichi-Alay range of mountains (the altitude of 1,200 - 2,000 m), the body length of a green toad is 73.1±0.69 mm.

In the high mountain regions, on the northeastern and southeastern macroslope of the Alay Range, as well as in the Alay Valley (the altitude of 2,000 - 3,500 m), the average body length of a green toad is 57.3±0.50 mm. Formerly, we have found that only young species of a green toad can be met in the
high-altitude regions. According to Davydov (1971-1972) and Nalivaev (1974-1975), this fact was noted at an altitude of 3,300 and 3,500 and 3,826 m, respectively (Yashchilkul Lake, Tadjikistan) [cited by Said-Aliev, 10].

In addition, we have found young species of a green toad at altitudes from 3,300 to 3,500 m, where they are common. The maximum body length of female species was 44.9 mm, while the body weight was 7.3 g.

Table 1. Morphological characters of a green toad (Bufo danatensis Pis.) in various landscape zones of the Pamir-Alay and plain and foothill regions of Belarus.

| Indicators          | Plains and foothills n=90 | Medium-altitude mountain regions n=87 | High mountain regions n=72 | Plain and foothill regions of Belarus n=35 |
|---------------------|---------------------------|--------------------------------------|-----------------------------|------------------------------------------|
| L                   | 69,13±0,80                | 73,15±0,69                           | 67,30±0,50                  | 61,18±0,87                              |
| L/T                 | 2,62±0,01                 | 2,64±0,01                            | 2,72±0,01                   | 2,69±0,02                               |
| F/T                 | 1,02±0,006                | 1,05±0,007                           | 1,06±0,006                  | 1,02±0,008                              |
| D.p./C.int.l.       | 2,26±0,03                 | 2,35±0,03                            | 2,09±0,03                   | 2,15±0,03                               |
| T/C.int.l.          | 6,49±0,08                 | 6,92±0,10                            | 5,90±0,12                   | 0,14±0,10                               |
| C.int.h./C.Int.l.   | 0,60±0,007                | 0,61±0,01                            | 0,54±0,009                  | 0,54±0,02                               |

According to Pisanets [9] and Toktosunov [12], the body size of a green toad decreases as the altitude decreases; this fact is proved in our materials. However, Melkumian [4] has shown that the body size of toads in mountain regions of Armenia increases as the altitude increases.

As can be seen from the above, the results of our research indicate that the difference between populations of green toads by body size in various altitudinal belts is highly significant (Table 1). We can talk about the presence of the optimum zone, a zone which includes plains and foothills as well as medium-altitude mountain regions for living of a green toad. The size and weight figures decrease starting from medium-altitude mountain regions towards high mountain regions, on the one hand, and towards plain regions, on the other hand. The nature of distribution of size and weight characteristics of a green toad by altitudinal belts in the studied regions of the Pamir-Alay is at variance with data for Armenia.

The ratio of the body length to the length of second thigh (L/T). There is virtually no difference in indicators of L/T ratio of a green toad in samples from various landscape zones (plains and foothills, medium-altitude mountain regions) (2.62±0.01 as against 2.64±0.01), except for the minimum and the maximum values (Table 1). There is somewhat less difference between landscape zones “plains and foothills” and “high mountain regions” (2.62±0.01 as against 2.72±0.01 in adult species, respectively).

As a result, the length of second thigh in various green toad populations decreases with the transition from plains and foothills to high mountain regions (according to mean indicators, see Table 1).

Proportions: the ratio of the length of thigh to the length of second thigh and the height of the calcaneal tuber to its length (F/T and C.int.h./C.int.1.). No difference in the abovementioned proportions of the body was found in a green toad regardless of species living in various landscape zones. There is virtually no difference in the distribution of values of proportions (Table 1).

The ratio of length of the first (inner) finger to the length of the calcaneal tuber and the length of second thigh to the length of calcaneal tuber (D.p./C.int.1. and T/C.int.1.). The variables of proportions D.p./C.int.1. and T/C.int.1. of a green toad from various landscape zones vary greatly. This difference between populations from plains and foothills and medium-altitude mountain zones correlate as 2.26±0.03 as against 2.35±0.03 and 6.49±0.08 as against 6.92±0.10 (respectively), while between plains and foothills and high mountain regions they correlate (2.26±0.03 / 2.09±0.03 / 6.49±0.08 /
5.90±0.12 (Table 1). As a result, green toads living in the plain and foothill zone differ from species living in high-mountain regions in the fact that they have a larger calcaneal tuber.

When studying the phenetic structure of green toad populations in various landscape zones, we have identified various variants of wartiness of the dorsal region of the body and features of the pattern on the limbs (determined using the methodology of Pisanets [9]). Besides, we have identified different variants of maculation on the belly and the frequency of occurrence of species with a dorsal stripe, as well as the presence and the degree of manifestation of a dorsomedial stripe.

Wartiness of the dorsal region of the body. According to Pisanets [8, 9], the features of tuberosity (wartiness) have certain sex specifics. Our data was analyzed without regard to the gender of amphibians in the general group of studied species, since the paper was aimed at identifying the general nature of variability of green toad populations in various landscape zones.

According to Pisanets [8, 9], the degree of expression of this character can be described by the three states of it: pointed tuberosity, rounded tuberosity, and lack of tuberosity. The material of our research has been individually processed for the three landscape zones: plain and foothill zone, medium-altitude mountain zone, and high mountain zone. Obtained results are presented in Table 2.

**Table 2.** The features of tuberosity on the back of a green toad (Bufo danatensis Pis.) in various landscape zones of the Pamir-Alay.

| Landscapes                         | N   | Wartiness of the dorsal region |
|-----------------------------------|-----|--------------------------------|
|                                   |     | a     | b     | c     |
| Plains and foothills              | 74  | 79.72±4.61 | 18.92±4.55 | 1.36±1.34 |
| Medium-altitude mountain regions  | 69  | 76.81±5.08 | 21.74±4.96 | 1.45±1.43 |
| High mountain regions             | 60  | 81.67±4.99 | 15.0±4.60  | 3.33±2.31 |

Note: N – the number of species; a – pointed tuberosity; b – rounded tuberosity; c – no tuberosity.

As can be seen from the Table above, the occurrence of species with pointed tuberosity somewhat decreases with the transition from the plain and foothill zone to medium-altitude mountain regions (76.8±5.08% against 79.7±4.67%), whereas it increases with the transition from medium-altitude mountain regions to high mountain regions (81.6±4.99% against 76.8±5.08%), although this difference is not statistically-valid.

The frequency of occurrence of rounded tuberosity increases with the transition from the plain and foothill zone to the medium-altitude mountain regions (18.92±4.55% / 21.74±4.96% / 15.0±4.60%). Apparently, this is due to environmental conditions. In summer, the climate in plains and foothills and high mountain regions is drier than that in the medium-altitude mountain regions. High mountain regions are represented by the rather arid Alay Valley. At the same time, medium-altitude mountain regions are more favorable for a green toad, since there is damper climate there.

Features of the pattern on the limbs. In order to analyze the phenetic structure of populations by the features of the pattern on the limbs in various landscape zones, we have used the following variants (individually for antibrachium and for second thigh). According to the description presented by Pisanets [8], these characters were divided into 5 states: 1) three or more horizontal spotty stripes; 2) two horizontal spotty stripes; 3) single horizontal spotty stripes; 4) spots and stripes are not prominent; 5) spotty stripes are arranged lengthwise (along the main axis of a limb) (Tables 3-4).

The variability of this character in various landscape zones of a green toad is presented in Tables 3 and 4. As can be seen from Tables, the state of this character in animals from various landscape zones varies greatly. The populations of green toads from high-mountain regions differ the most (41.66±3.36% and 30.0±5.91%). Green toad populations in plains and foothills and in high mountain regions have many spotty stripes on both limbs (antibrachium and second thigh), whereas the number of green toad populations in high mountain regions (Alay Valley) significantly decreases (77.02±4.89% and 68.11±5.61% as against 41.66±3.36% respectively). Green toads in high mountain
regions differ significantly by the frequency of occurrence of species with variants of the character on both limbs (antibrachium and second thigh), except for a single horizontal stripe.

Table 3. Features of the pattern on the hindlimbs (second thigh) of a green toad (Bufo danatensis Pis.) in various landscape zones of the Pamir-Alay.

| Landscapes                | N   | Features of the pattern on the hindlimbs (%) | 1   | 2   | 3   | 4   | 5   |
|---------------------------|-----|---------------------------------------------|-----|-----|-----|-----|-----|
| Plains and foothills      | 74  | 77,0±4,89                                   | 4,0±2,29 | 0,0 | 18,9±4,55 | 0,0 |
| Medium-altitude mountain regions | 69  | 68,1±5,61                                   | 13,0±4,05 | 1,4±1,43 | 17,4±4,56 | 0,0 |
| High mountain regions     | 60  | 41,6±3,36                                   | 30,0±5,91 | 1,6±0,99 | 20,0±5,16 | 6,7±3,22 |

Note: N - the number of species; 1 - three or more horizontal spotty stripes; 2 - two horizontal spotty stripes; 3 - single horizontal spotty stripe; 4 - spots and stripes are not prominent; 5 - the spotty stripe is arranged lengthwise (vertically).

Table 4. Features of the pattern on the forelimbs (antibrachium) of a green toad (Bufo danatensis Pis.) in various landscape zones of the Pamir-Alay.

| Landscapes                | N   | Features of the pattern on the forelimbs (%) | 1   | 2   | 3   | 4   | 5   |
|---------------------------|-----|---------------------------------------------|-----|-----|-----|-----|-----|
| Plains and foothills      | 74  | 77,3±4,86                                   | 18,9±4,55 | 0,0 | 6,7±2,91 | 0,0 |
| Medium-altitude mountain regions | 69  | 73,9±5,28                                   | 13,0±4,05 | 1,4±1,43 | 11,6±3,91 | 0,0 |
| High mountain regions     | 60  | 31,7±6,00                                   | 40,0±6,32 | 3,3±2,31 | 20,0±5,16 | 5,0±2,81 |

Note: N – the number of species; 1 – three or more horizontal spotty stripes; 2 – two horizontal spotty stripes; 3 – single horizontal spotty stripes; 4 – spots and stripes are not prominent; 5 – the spotty stripe is arranged lengthwise (vertically).

Hence, a significant variability of green toad populations from various landscape zones can be observed. That gives rise to a suggestion that conditions of formation of populations in certain landscape zones (particularly in Alay valley) were rather specific. The specificity of populations from high mountain regions is clearly visible by the abovementioned characters, whereas species of populations from medium-altitude mountain regions as well as plains and foothills are almost the same in terms of the degree of manifestation of all variants of the pattern on the limbs of a green toad.

Variations of dorsomedial stripe of a green toad. The most important result of our research consists in the fact that we have found a character that cannot be observed in a green toad in other geographical regions.

There are only individual reports on the presence of a light dorsomedial stripe of a green toad [17]. According to Borkin et al., three specimens of a green toad with thin light stripes were found in Mongolia. Among species from the oasis Uliastain-Gol in Mongolia, 11% of toads (male and female species) had a thin small light stripe in the centre of their back [17].

Based on the processing of representative materials, we were able to describe this character, which is an intermittent clearly visible narrow stripe, lighter that the general background, runs along the middle of the back, starting from the tip of the muzzle. The stripe has several variants according to the degree of its manifestation: 1) from the tip of the muzzle to the gap between the nostrils (short); 2)
from the tip of the muzzle to the gap between the eyes (average); 3) from the tip of the muzzle to the center of the back (long); 4) from the tip of the muzzle to the anus (continuous). The stripe is not expressed in all species. Due to the relatively small size of samples, we could not reliably estimate the frequency of occurrence of species with various variants of the degree of manifestation of the character, and only its presence or absence was analyzed. The variant of manifestation of a very long stripe can be found in very rare cases (in occasional species). The first three variants of the character could mainly be observed.

Obtained results by the frequency of occurrence of species with a character of a dorsal stripe in various landscape zones are presented in Table 5. As can be seen from Tables, the frequency of occurrence of species with this character in green toad populations decreases with the transition from plains and foothills to high mountain regions. Their number in plain and foothill regions is 1.35±1.34%, in the medium-altitude mountain regions – 11.59±3.85%, whereas species with a dorsal stripe are clearly prevalent in high mountain regions – 70.0±5.91%.

**Table 5.** Frequency of occurrence of a green toad with dorsomedial stripe (Bufo danatensis Pis.) in various landscape zones of Pamir-Alay.

| Landscapes                  | N  | Dorsomedial stripe |
|-----------------------------|----|--------------------|
|                             |    | there is no stripe | there is no stripe |
| Plains and foothills        | 74 | 1.4±1.36           | 98.6±1.36          |
| Medium-altitude mountain    | 69 | 11.6±3.85          | 88.4±3.85          |
| High mountain regions       | 80 | 70.0±5.91          | 30.0±5.91          |

As can be seen from the above, the represented data shows the greater degree of specificity of high-altitude populations which differ the most by the frequency of occurrence of the character of the presence of a light dorsomedial stripe from populations of medium-altitude mountain regions as well as populations of plains and foothills.

The features of pigmentation of the belly of a green toad. The pigmentation of the belly of a green toad is expressed in maculation. Various degree of maculation on the belly is shown in Table 6. According to Toktosunov [12], the belly side of the green toad is light gray or yellowish with black speckles. As the terrain elevation increases, the frequency of occurrence of species with leopard-spotted coloration decreases. Whereas large-spotted toads prevail in the high mountain region. According to Toktosunov [12], the belly side of the green toad is light gray or yellowish with black speckles. As the terrain elevation increases, the frequency of occurrence of species with leopard-spotted coloration decreases. Whereas large-spotted toads prevail in the high mountain region.

In the research of specimens of a green toad in various landscape zones, we have observed different degree of variation of the character of belly maculation. Unlike with the data from the abovementioned author, we have identified the pigmentation of the throat and the pigmentation of the belly of a toad as separate characters. The throat and the belly have different pigmentation. If there is no maculation on one of the abovementioned parts of the body, it can be present on some other body part. Sometimes the maculation is expressed on both sides of the abdomen (throat and belly). The size of spots on the belly in various landscape zones slightly differed. Therefore, we have analyzed only the following variants of the character of spotting of a green toad: 1) only the throat is spotted; 2) only the belly is spots; 3) the spotty throat and belly; 4) maculation on the belly is virtually absent (Table 6).

As is shown in Table 6 for a green toad, as the terrain elevation increases, the number of species which have large spot on the belly, only increases (16.2±4.28% against 51.6±6.45%). Toads without maculation (68.9±5.38%) are prevalent in plains and foothills. The medium-altitude mountain regions stand in between. Pigmentation characters (phenes) can be least frequently observed in all landscape zones only on the throat (1.35±1.34% in plains and foothills, 5.79±2.81% in the medium-altitude mountain regions, and 5.00±2.81% in the high mountain regions) [1].
Table 6. The belly pigmentation of a green toad (Bufo danatensis Pis.) in various landscape zones of Southern Kyrgyzstan.

| Landscapes                  | Total number of species | Pigmentation of throat n | Pigmentation of belly n | Pigmentation of throat and belly n | No pigmentation n |
|-----------------------------|-------------------------|--------------------------|-------------------------|-----------------------------------|-------------------|
| Plains and foothills        | 74                      | 1                        | 12                      | 10                                | 51                |
|                             |                         | 1.3±1.3                  | 16.2±4.3                | 13.5±1.4                          | 68.9±5.4          |
| Medium-altitude mountain regions | 69                      | 4                        | 26                      | 20                                | 19                |
|                             |                         | 5.7±2.8                  | 37.6±5.8                | 28.9±5.5                          | 27.5±5.4          |
| High mountain regions       | 60                      | 3                        | 31                      | 11                                | 15                |
|                             |                         | 5.0±2.8                  | 51.6±6.5                | 18.3±5.0                          | 25.0±5.6          |

4. Conclusions
A comparison study of a number of phenetic characters of green toad populations (Bufo danatensis Pis.) from the foothill regions, medium-altitude mountain regions, and high mountain regions of the Pamir-Alay area has shown the specificity of populations from various landscape zones.

1. We have identified a very significant difference of remote populations by certain phenetic characters. First and foremost, we should point out a very interesting fact of rather high occurrence of species having a light dorsomedial stripe. It is most noteworthy that the wedge-like variability of frequency of occurrence of striped species can be observed: 1.4±1.3% – the population of the plains and foothills, 11.6±3.8% in the medium-altitude mountain regions, and 70.0±5.9% in the high mountain regions. Such a high frequency of occurrence of species with a dorsomedial stripe among populations of the complex of a Palearctic green toad has been revealed for the first time.

2. We have found strong specificity of mountain populations of green toads in the Pamir-Alay region; at the same time, species with a dorsomedial stripe in populations from Belarus and other regions of Europe are virtually uncommon.

3. In addition, we have identified the specificity of high-altitude populations by the features of the pattern on the limbs and maculation on ventricular body parts.

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