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# Sexual Size Dimorphism and Pattern Polymorphism of the *Bufotes sitibundus* (Syn. *Bufotes variabilis*) Pallas, 1771 in Azerbaijan

Gulbaniz Gasimova[1,2*]

[1]Institute Zoology of Azerbaijan National Academy of Sciences, AZ1073, passage 1128, block 504, Baku, Azerbaijan
[2]Khazar University, Life Sciences department, AZ1096, 41 Mehseti Street, Baku, Azerbaijan
* gqasimova@mail.ru
* Orcid No: 0000-0003-4916-0112

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**Abstract**

The paper contains data on sexual size dimorphism of *Bufotes sitibundus* Pallas, 1771 with samples from the "Greater Caucasus", "Lankaran" and "Absheron" territories of Azerbaijan. The 139 adult specimens (62♂ and 77♀) from 3 populations were collected. They were released after morphometric measurements and pattern morphs analysis. Reliability of differences was estimated using Independent Sample t-test. Statistical analysis of morphological features showed that there are differences in different features between males and females in different populations. In each of the populations studied, the femur (FmL) and tibia (TbL) were longer in males than in females. Investigation of the pattern polymorphism in 126 specimens has shown that dorsal pattern with spots separated by short distance between them is dominant in 2 studied populations ("Absheron" and "Lankaran"). There were not found any differences between males and females according to pattern characteristics.

**Keywords:** Amphibia, Azerbaijan, *Bufotes sitibundus*, pattern polymorphism, sexual size, dimorphism.

## 1. Introduction

The vital activity of animal organisms mainly reflects the characteristics of their growth and development, life expectancy, the sexual maturity, reproduction and the degree of endurance. These characteristics are to some extent hereditary and form the basis of natural selection. However, abiotic factors such as temperature, quantity and quality of food, and humidity affect the life of each individual to varying degrees, which causes variability among populations. In addition to these factors, the duration of the period of activity can also affect body size. For example, it has been found that the growth rate in individuals of the same age with a long annual period of activity is higher than in individuals with a low period of activity. Therefore, changing environmental factors can affect body size, which is a genetic feature. Ectotherms, such as amphibians and reptiles are more sensitive to such factors. Changes that an organism undergoes under the influence of environmental factors are studied at the level of individuals, populations and species [1,2].

The taxonomy of the genus *Bufo* has changed several times in recent years. First, Frost et al. (2006) merged the former "*Bufo*" *viridis* group with a new genus, described as *Pseudepidalea*, and proposed to divide *Bufo* into several genera [3]. Then Dubois and Boer (2010) showed that *Pseudepidalea* is a junior synonym for *Bufotes* (Rafinesque, 1815) [4]. They also recommended 3 different subgenus of the same genus *Bufo* (*Bufo*, *Bufotes*, *Epidalea*); therefore, *Pseudepidalea variabilis* was changed to *Bufo* (*Bufotes*) *variabilis* (Pallas, 1769) [5].

A molecular study showed that green toads of Asia Minor, the Middle East, and northern Eurasia form a separate clade [6]. Since the range of this clade includes the type locality, they referred to these populations as *B. variabilis* (Pallas, 1769) [7]. Recently, Dufresnes et al. (2019) stated that the Middle Eastern green toads might instead be considered *Bufotes sitibundus* (Pallas, 1771), which is the oldest name for this species [8].

Therefore, we use *B. sitibundus* as the scientific name in this study. *B. sitibundus* spreads from Greece eastwards through Türkiye to Syria, Jordan, and Lebanon.

It is also reported from Iraq, Iran and is distributed through the Caucasus and Russia to Kazakhstan [9].
In Azerbaijan territory *B. sitibundus* was first registered by Menetrie near the city of Baku in 1830 [10]. In our republic the species is distributed in all regions having favourable biotops including territories at the altitudes up to 2100 m a.s.l., Guba dstrict, Khinalig village. The aim of this study was a comparative study of morphometric measurements, sexual size dimorphism and pattern polymorphism of specimens taken from 3 populations of the widely distributed *B. sitibundus.*

## 2. Materials and Methods

Material collection covered the years of 2006-2016 in seasons when the amphibians are active. Totally 139 specimen of *B. sitibundus* were analyzed. From them 82 specimen (40♂ and 42♀) were from the "Greater Caucasus" (northern Azerbaijan) population; 31 specimens (7♂ and 24♀) from the "Absheron" (Absheron Peninsula, eastern Azerbaijan) population; 26 specimens (15♂ and 11♀) from the "Lankaran" (south-eastern Azerbaijan) population (Table 1). The coordinates of the areas where the amphibians were found were recorded using the Garmin eTrex GPS device. ArcGIS 10.3 the electronic mapping software have been used for preparing the map based on the collected materials (Figure1).

**Table 1.** Coordinates, sample sizes and altitude for each population.

| Populations | N | Locality | Coordinates | | Altitude (m) | Capture date |
|---|---|---|---|---|---|---|
| | | | N | E | | |
| "Greater Caucasus" | 82 | Qakh | 41.375556 | 46.801111 | 249 | 20.04.2011 |
| | | Qakh/Ilisu | 41.460556 | 47.048333 | 1372 | 15.05.2013 |
| | | Oghuz/Deymedere | 40.943056 | 47.559722 | 392 | 03.05.2012 |
| | | Zagatala SNR | 41.750278 | 46.500556 | 893 | 04.06.2006 |
| | | Zagatala/Qebizdere | 41.703889 | 46.593333 | 543 | 07.06.2006 |
| | | İsmayilli/Buynuz | 40.917778 | 48.060833 | 757 | 11.08.2006 |
| | | Balakan/Katex | 41.687222 | 46.527222 | 1089 | 21.07.2010 |
| | | Balakan/Mazymchay | 41.794444 | 46.323611 | 438 | 05.05.2012 |
| | | Quba Khinalig | 41.181667 | 48.118889 | 2063 | 07.05.2016 |
| | | Quba Khinalig | 41.176944 | 48.127222 | 2131 | 07.05.2016 |
| "Absheron" | 31 | Gobustan Boyukdash | 40.112222 | 49.375833 | 171 | 18.04.2010 |
| | | Gobustan Gizil Gaya | 40.113889 | 49.377222 | 171 | 11.05.2011 |
| | | Baku/Nardaran | 40.573611 | 49.988056 | 11 | 05.04.2012 |
| | | Baku/8th kilometer | 40.41 | 49.938056 | 46 | 25.06.2014 |
| | | Baku/Saray | 40.532222 | 49.710278 | 35 | 17.05.2007 |
| | | Baku/Incirlik | 40.527222 | 49.846111 | 65 | 12.04.2007 |
| | | Baku/Zykh | 40.345278 | 49.977778 | -21 | 19.05.2008 |
| | | Baku/Ahmedly | 40.384167 | 49.959722 | 88 | 24.03.2009 |
| "Lankaran" | 26 | Lerik, Cangamiran | 38.7575 | 48.4375 | 1126 | 18.04.2010 |
| | | Lankaran, Ashagy Nuvadi | 38.710833 | 48.8575 | -15 | 12.04.2006 |
| | | Astara, Mashkhan | 38.5475 | 48.815833 | 69 | 23.05.2008 |
| | | Astara, Chayoba | 38.633056 | 48.806111 | -4 | 14.04.2006 |

The specimens were collected using a handmade butterfly net and by hand in streams, brooks and cultivation waterways. Snout-urostyle (SUL), femur length (FmL), tibia length (TbL), first toe length (T1L), inner metatarsal tubercle length (IMTL) of collected specimens were measured and the ratios SUL/TbL, FmL/TbL, TbL/IMTL, T1L/IMTL were computed. The morphometric measurements were taken with the calipers in each adult specimen to the nearest 0.1 mm in the standardized manner. After morphometric measuring specimens were released to nature.Data obtained were processed in the STATISTICA StatSoft 10 program. To test significance of sexually dimorphic characters, Independent Sample t-test at the significance level of 0.01 were employed. Color photographs were made and the pattern characteristics of the live specimens only were taken.

The pattern polymorphism in populations of amphibian species was identified based on variations in the pattern of the dorsal and ventral sides of the body [11, 12]. For the *B. sitibundus* in Azerbaijan 7 pattern morphs were established: a-with small spots with shorter distance between them, b-with large spots with shorter distance between them, c-with small spots with greater distance between them, d-with large spots with greater distance between them (Figure 2), e-dense ventral spots, f-sparse ventral spots, g-without ventral patterns.

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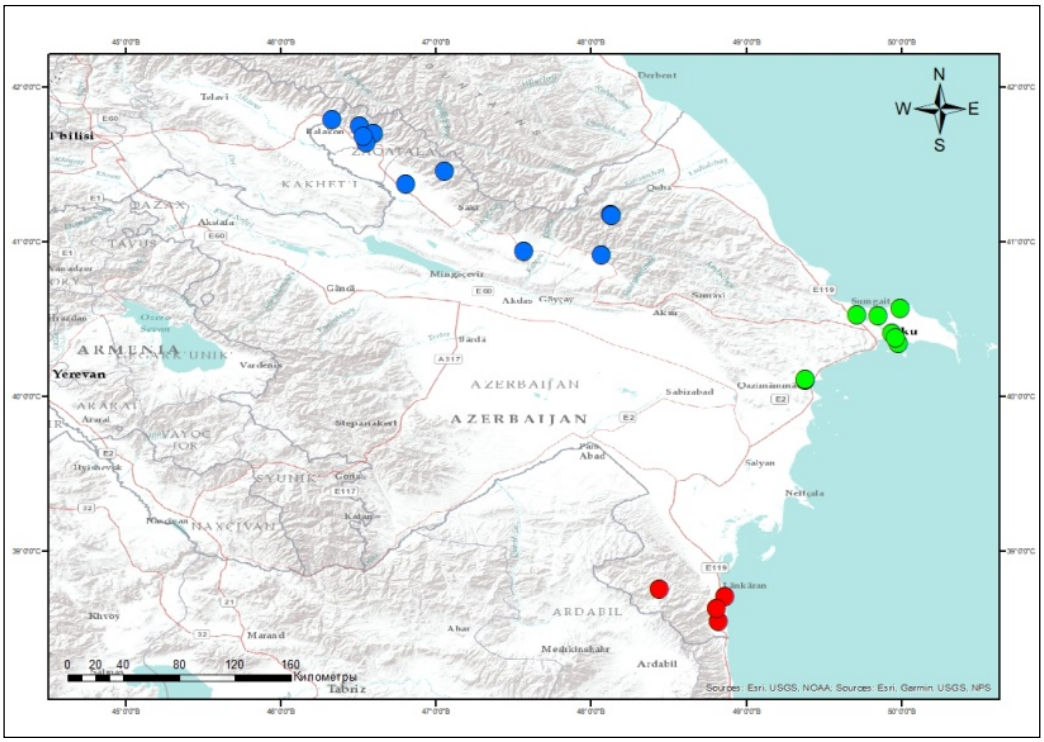


**Figure 1.** Map showing the study area in Azerbaijan (green circle - "Absheron", blue circle - "Greater Caucasus" and red circle - "Lankaran").

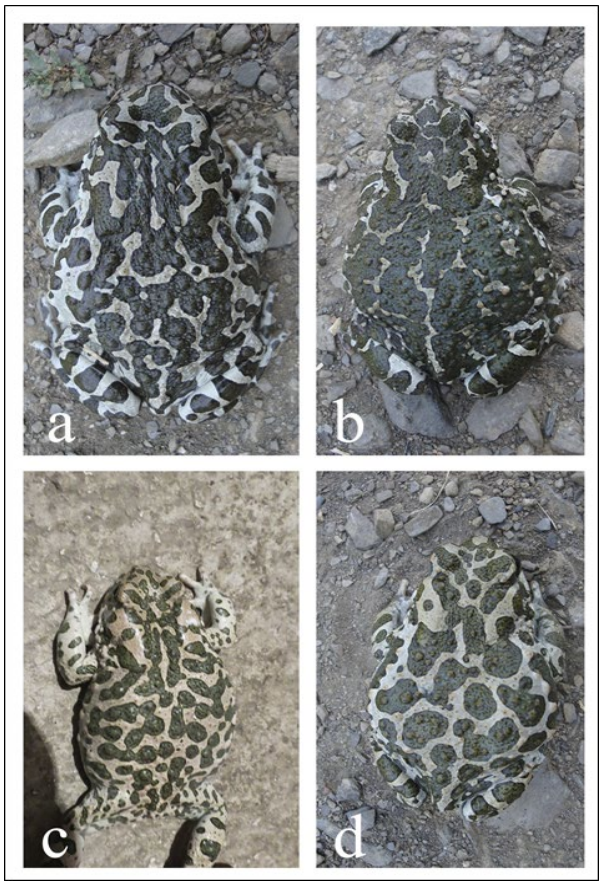


**Figure 2.** Four types of dorsal pattern as observed in the specimens of *B. sitibundus* Pallas, 1771 from the Azerbaijan: a-with small spots with shorter distance between them, b-with large spots with shorter distance between them, c-with small spots with greater distance between them, d-with large spots with greater distance between them.

## 2. Results and Discussion

Anuran amphibians grow throughout life [13]. Therefore, it is recommended that individuals compared in size be from the same age group. Taking this condition into account, the study compared only adults of the species *B. sitibundus* taken from 3 populations of Azerbaijan. Statistical analysis of the size and proportions of the body of individuals from the populations of the Greater Caucasus, Absheron, and Lankaran is shown in Table 2.

From the morphometric analysis of samples taken from all three populations, it can be seen that specimens of the Greater Caucasus population are larger in size (T1L), and specimens of the Absheron population in some sizes and ratios (FmL, IMTL, TbL / IMTL) than individuals of the Lankaran population. In terms of body length (SUL), specimens from the Greater Caucasus population differ slightly from those from the other two populations.

**Table 2.** Comparison of morphometric characteristics of *B. sitibundus* from "Absheron", "Lankaran" and "Greater Caucasus" populations.

| Character | Greater Caucasus (n=82♀♂) | | Lankaran (n=26♀♂) | | Absheron (n=31♀♂) | |
|---|---|---|---|---|---|---|
| | M±SD | Range | M±SD | Range | M±SD | Range |
| SUL | 68.43±13.01 | 33.50-91.10 | 70.74±49.90 | 43.40-80.80 | 70.15±9.43 | 46.40-87.50 |
| FmL | 27.58±5.76 | 1.90-39.50 | 28.70±14.15 | 17.80-33.90 | 27.54±3.43 | 18.00-35.20 |
| TbL | 25.47±5.12 | 11.10-38.10 | 26.81±11.34 | 16.90-31.60 | 24.56±3.29 | 16.60-29.80 |
| T1L | 5.95±1.64 | 3.40-11.20 | 5.57±5.90 | 3.40-11.40 | 6.18±1.89 | 3.50-11.70 |
| IMTL | 3.85±0.78 | 2.10-6.50 | 4.85±1.67 | 2.20-7.40 | 3.88±0.77 | 2.60-5.20 |
| SUL/TbL | 2.71±0.23 | 1.88-3.37 | 2.66±0.05 | 2.22-2.98 | 2.87±0.35 | 2.39-4.43 |
| FmL/TbL | 1.09±0.14 | 0.09-1.45 | 1.07±0.00 | 0.87-1.17 | 1.13±0.13 | 1.01-1.73 |
| TbL/IMTL | 6.66±1.27 | 4.00-12.07 | 5.78±1.10 | 3.81-7.68 | 6.51±1.18 | 3.61-8.62 |
| T1L/IMTL | 1.59±0.49 | 0.97-3.37 | 1.23±0.35 | 0.57-2.40 | 1.59±0.35 | 0.81-2.54 |

The sizes of males and females were compared separately for each population (Table 3). Results of Independent Sample t-test (2-tailed) presented in Table 3 detect significant sexual size dimorphism ($p \leq 0.01$) in some morphometric parameters (FmL and TbL) of the females and males of *B. sitibundus* taken from the "Absheron", "Lankaran" and "Greater Caucasus" populations. There are also differences in some ratios (SUL/TbL and TbL/IMTL) of specimens taken from the "Greater Caucasus" population and "Lankaran". However the results of inner metatarsal tubercle length (IMTL) measures were different in "Absheron" and "Lankaran" populations. The snout-urostyle length (SUL) measures of specimens taken from "Greater Caucasus" and "Absheron" population differed from "Lankaran" populations. Comparison of morphometric characters in each population has shown that males are larger than females.

**Table 3.** Comparison of morphometric characters (mm) in males and females of *B. sitibundus*. n: number; M:arithmetic mean; SE: standard error of mean; significant at level*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Morphometric abbreviations: SUL (snout–urostylelength), FmL (femur length), TbL (tibia length), T1L (first toe length), IMTL (inner metatarsal tubercle length), SUL/TbL; FmL/TbL; TbL/IMTL; T1L/IMTL.

| | SEX | | SUL | FmL | TbL | T1L | IMTL | SUL/TbL | FmL/TbL | TbL/IMTL | T1L/IMTL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| "Greater Caucasus" | ♂ | M | 71.63 | 29.61 | 27.51 | 5.70 | 3.88 | 2.65 | 1.09 | 7.14 | 1,49 |
| | (n=40) | SE | 0.74 | 0.39 | 0.52 | 0.26 | 0.10 | 0.03 | 0.01 | 0.19 | 0,08 |
| | ♀ | M | 65.38 | 25.65 | 23.54 | 6.20 | 3.82 | 2.76 | 1.09 | 6.20 | 1,68 |
| | (n=42) | SE | 2.65 | 1.11 | 0.90 | 0.25 | 0.14 | 0.04 | 0.03 | 0.18 | 0,07 |
| | p | | 0,021 * | 0.002 ** | 0.001 *** | 0.139 | 0.612 | 0.034 * | 0.923 | 0.002 ** | 0.052 |
| "Absheron" | ♂ | M | 76.13 | 30.21 | 27.61 | 7.30 | 4.57 | 2.75 | 1.09 | 6.06 | 1,59 |
| | (n=7) | SE | 3.34 | 1.05 | 0.62 | 0.87 | 0.13 | 0.08 | 0.02 | 0.13 | 0,17 |
| | ♀ | M | 68.41 | 26.76 | 23.67 | 5.85 | 3.68 | 2.91 | 1.14 | 6.64 | 1,60 |
| | (n=24) | SE | 1.85 | 0.66 | 0.64 | 0.34 | 0.15 | 0.08 | 0.03 | 0.27 | 0,07 |
| | p | | 0,040 * | 0.046 * | 0.007 ** | 0.183 | 0.045 * | 0.289 | 0.377 | 0.947 | 0.865 |
| "Lankaran" | ♂ | M | 71.91 | 29.81 | 27.97 | 6.38 | 5.18 | 2.58 | 1.07 | 5.57 | 1,32 |
| | (n=15) | SE | 1.12 | 0.67 | 0.60 | 0.75 | 0.28 | 0.06 | 0.02 | 0.25 | 0,18 |
| | ♀ | M | 69.14 | 27.19 | 25.24 | 4.45 | 4.40 | 2.75 | 1.07 | 6.07 | 1,12 |
| | (n=11) | SE | 2.92 | 1.40 | 1.20 | 0.21 | 0.45 | 0.07 | 0.01 | 0.34 | 0,12 |
| | p | | 0,385 | 0.046 * | 0.034 * | 0.159 | 0.021 * | 0.002 ** | 0.738 | 0.030 * | 0.831 |

The dorsal part of *B. sitibundus* is light olive-gray, surrounded by a narrow black border with large and small dark green spots. Specimens of this species have different patterns, and it is difficult to find two identical individuals in the number, location and color of spots. The study of 126 specimens of *B. sitibundus* has revealed 4 dorsal (a-with small spots with shorter distance between them, b-with large spots with shorter distance between them, c-with small spots with greater distance between them, d-with large spots with greater distance between them), and 3 ventral (with dense spots, with sparse spots, without patterns) pattern forms.

Pattern consisted of small spots with shorter distance between them accounted for 28.57%in the "Greater Caucasus" population, 30% in the "Absheron" population, and 84%in the "Lankaran" population; pattern consisted of large spots with shorter distance between them accounted for 22.22% in the "Greater Caucasus" population, 50% in the "Absheron" and 4% in the "Lankaran" population; pattern consisted of small spots with greater distance between them accounted for 12.69 % in the "Greater Caucasus" population, 13.33% in "Absheron" population, 0% in the "Lankaran" population; pattern consisted of large spots with greater distance between them 36.50% in the "Greater Caucasus" population, 6.67% in the "Absheron" population and 12% in "Lankaran" population. Pattern with small spots with shorter distance between them predominated in two populations ("Absheron" and "Lankaran"). The dense ventral spots of individual from "Greater Caucasus" population are observed. We have not recorded specimens without ventral patterns in "Lankaran" population (Table 4). According to the theory of evolution, sexual selection is a special form of natural selection that has led to sexual dimorphism.

Sexual dimorphism consists of phenotypic differences between males and females of the same species. Kuo et al. [14] point to morphological differences between males and females in shape and size, while Selander [15] points to differences in behavior [14, 15]. Sexual dimorphism in terms of body size is a common feature in the animal kingdom (*Regnum Animale*), differing in size and direction in different classes [16,17].

The reasons for sexual size dimorphism in all animals are explained by 3 accepted hypotheses: 1) Large body size in males has an influence on superiority in the process of sexual selection. During the breeding season, males with larger body sizes are more likely to mate, competing with other males [18]; 2) The large body size of females has a positive effect on their reproductive performance during reproduction [19]; 3) The large body size of individuals of both sexes has an effect on intraspecific competition from an ecological point of view [20, 21].

**Table 4.** Occurrence of pattern morphs in populations of *B. sitibundus* in the studied biotopes in Azerbaijan. (*- 6 specimens without patterns in Greater Caucasus populations).

| Morph | | Populations | | | | | |
|---|---|---|---|---|---|---|---|
| | | "Greater Caucasus" | | "Absheron" | | "Lankaran" | |
| | | n=69* (♂♀) | % | n=30 (♂♀) | % | n=25 (♂♀) | % |
| Dorsal | A | 18 | 28.57 | 9 | 30 | 21 | 84 |
| | B | 14 | 22.22 | 15 | 50 | 1 | 4 |
| | C | 8 | 12.69 | 4 | 13.33 | - | - |
| | D | 23 | 36.50 | 2 | 6.67 | 3 | 12 |
| Ventral | e | 37 | 53.62 | 13 | 43.33 | 17 | 68 |
| | F | 25 | 36.23 | 10 | 33.33 | 8 | 32 |
| | G | 7 | 10.14 | 7 | 23.33 | - | - |

In 90% of anuran amphibians, females are larger than males [22]. However it is known that in some species of frogs, males are larger than females. The morphometric analysis of the *B. sitibundus* species was carried out to resolve the issue of the size of the sexes of this species in the territory of Azerbaijan. Statistical analysis of morphological characters by sex showed that males and females of different populations differ in different characters. However, as can be seen from Table 3, in each of the three studied populations, the length of the femur and tibia in males was greater than that of females. It is known that in the amplexus position during reproduction, the male squeezes the female's abdomen with his hind limbs and helps her to lay eggs. In this regard, we believe that the length of the femur and tibia in males plays an important role during mating.

The sexual size dimorphism that we observe in *B. sitibundus*, that is, the fact that males are larger than females in some morphological characteristics, can be caused by several reasons. One of these reasons can be

explained by the first of the above considerations. However, more research is needed to determine other causes of sexual size dimorphism.

It is believed that the degree of polymorphism of a species is directly proportional to the diversity of its habitat. Thus, the degree of polymorphism is high in species living in different landscapes and having a wide range.

According to the results of the analysis of to the shape of the waist and abdomen of the specimens of the genus *B. sitibundus* from 3 populations, it can be said that individuals of the "Greater Caucasus" population show a higher degree of polymorphism than individuals of other populations. The reason for this is the capture of individuals of the "Greater Caucasus" population from different landscapes. Such differences in the degree of polymorphism of individuals within a population can also be associated with intraspecific variability and genetic factors. Therefore, more research is needed in this direction.

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## Author's Contributions

**Gulbaniz Gasimova:** Literature research, data collection, field survey and measurement, article writing.

## Ethics

There are no ethical issues after the publication of this manuscript.

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