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# Body size and color polymorphism in *Bufotes viridis* complex (Anura: Bufonidae) inhabiting two semi-natural areas in Plovdiv City, Bulgaria

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**Abstract.** The current study presents data about the variations in body size and color polymorphism of toads from the *Bufotes viridis* complex inhabiting two semi-natural habitats – "Hulm Bunardzhik" Hill (Site 1) and "Mladezhki hulm" Hill (Site 2) located in a highly urbanized area of Plovdiv City, Bulgaria. The purpose of the analyses was to obtain data allowing a better understanding of the adaptive capabilities of this anuran complex, allowing it to survive in diverse, incl. anthropogenically transformed habitats. The results of our study showed that for the parameter snout-vent length (SVL) the main effect of the habitat (site) was significant, while the effects of sex and the combination sex and site were insignificant. We found significant interactions between all three tested variables (site, sex and combination sex and site) for the parameter body weight (BW). The population from "Mladezhki hulm" Hill was strongly dominated by individuals from the two dark-background morphs (C and D), while the population from "Hulm Bunardzhik" Hill was dominated by the light-colored (A and B morphs) individuals. Our results support the hypothesis that color polymorphism in anurans is also influenced by environmental factors and habitat characteristics.

**Key words**: green toad, body sizes, color polymorphism, urban areas, south Bulgaria.

## Introduction

The European green toad *Bufotes viridis* (Laurenti, 1768) (Frost 2013), synonym *Pseudepidalea viridis* (Frost et al. 2006), is a species from the European green toads *Bufotes viridis* subgroup (Dubois & Bour 2010). *B. viridis* diploid complex is one of the most polytopic amphibian groups of the Palearctic (Stöck et al. 2006, Özdemir & Kultrup 2007, Fayzulin et al. 2018). It includes at least 12 major evolutionary lineages and 14 morphologically similar species distributed across Europe, Asia and Northern Africa and overall the taxonomy is still controversial and unspecified (Batista et al. 2006, Litvinchuk et al. 2007, Stöck et al. 2008, Özdemir et al. 2014, Vences et al. 2019). In Bulgaria *B. viridis* complex occurs across the territory of the whole country (Beschkov & Nanev 2002). This terrestrial and nocturnal toad inhabits a wide range of habitats and is commonly found in less disrupted areas (Stojanov et al. 2011), as well as in human-modified habitats: urbanized (Mollov 2019) and industrial areas (Zhelev et al. 2014).

The ecology, biology and biometrical characteristics of *B. viridis* complex populations in Europe are fairly well known (Biserkov et al. 2007, Stojanov et al. 2011, Zhelev et al. 2017, Mollov 2019). In Bulgaria *Bufotes viridis* complex is relatively well-studied, but some aspects of its ecology, such as variations in color polymorphism and their possible role, are still poorly understood.

In animal biology, coloration plays a vital role in various aspects. In many amphibians, cryptic or aposematic coloration enhances their protection against predators (Hoffman & Blouin 2000, Kang et al. 2016). Coloration can also provide valuable information to co-specific about sex, vitality, maturity or breeding availability (Vitt & Caldwell 2009, Jablonski et al. 2014). Anurans are ectothermic animals and some color variations (including color anomalies) may influence their survival and behavior (Clusella Trullas et al. 2007, Silva & Mahaulpatha 2019). Coloration and patterns of green toads from the *B. viridis* complex varies across their distributional range, but in most cases the background coloration is light to dark brownish, covered with randomly dispersed darker green patches with various sizes (Stojanov et al. 2011, Arnold & Ovenden 2002). Despite the differences in tint or intensity of both background color and pattern, color aberrations within this species complex are rarely observed. However, a number of authors report some color deviations from the typical color pattern for this toad complex: axanthism (Jablonski et al. 2014), erythrism (Lanza & Canestrelli 2002, Kolenda et al. 2017) and albinism (Andrä 2011).

Peskova (2003, 2006) describes four color morphs in *B. viridis* for Southern Russia: with an even background: 1) light or 2) dark; green spots against this background: a) individual, small or b) merging. Accordingly, the following 4 morphs can be distinguished: A – light back background, individual spots; B – light back background, merging spots; C – dark back background, individual spots; D – dark back background, merging spots (4). Based on this methodology, we registered these four color morphs in green toad populations from South Bulgaria (Zhelev et al. 2014). In another recent study, conducted in Southeastern Russia (Samara region) the four morphs were also confirmed (Fayzulin et al. 2019).

The aim of the study is to present the results from a parallel evaluation of body size and color polymorphism of a representative, random sample from the *B. viridis* complex that inhabit two semi-natural habitats – "Hulm Bunardzhik" Hill and "Mladezhki hulm" Hill (protected areas) located in a highly urbanized area of Plovdiv City, Bulgaria. The purpose of the analyses is to obtain data allowing a better understanding of the adaptive capabilities of this anuran complex, allowing it to survive in diverse habitats (incl. anthropogenically transformed). In this study, we examined the relationship between sex and dorsal coloration (color by sex), as well as the influence of the habitats in the sites on the coloration of individuals of the *B. viridis* complex. We also examined the relationship between sex and the type of spots - individual or merging (spots by sex), as well as between the type of spots and the habitat (spots by sites).

**Material and Methods**

Sampling area

NL "Halm Bunardzhik" Hill (Site 1) is situated on 22.0 hectares, with peak altitude at 265 m a.s.l., and NL "Mladezki halm" Hill (Site 2) - on 36.2 hectares with highest point at 285.5 m a.s.l. Both sites are located in the center of the city of Plovdiv (Figure 1) and by origin are syenite hills formed during the Paleogene. Both hills were declared as protected natural monuments by the Bulgarian Ministry of Environment and Waters (MOEW), aiming to conserve the natural landscape and the unique geomorphologic formations. However, despite the prohibitions on public and business activities, these sites are subject to tourism and recreation, and - especially during the weekends - a significant flow of people visit them. In addition, the hills are basically built up, in the time before they were declared as protected (Mollov 2019).

Capturing toads and morphological analyses

The current study is based on the results from the analyzed samples of 30 female and 30 male toads from each site captured in April 2017. All toads were captured by hand using flashlight at night. Exact age could not be determined and the toads were considered adults, based on the snout–vent length (SVL; Bannikov et al. 1977). The toads collected in the present study were considered adults and sexually mature at SVL > 60.0 mm (Bannikov et al., 1977; Sinsch, et al., 2007; Altunişik & Özdemir, 2015). The toads were grouped according to their sex, on the basis of the secondary sexual characteristics such as the presence of vocal sac and "marital corns" on the first finger in male individuals. The analyses were done with live animals, on the next day after their collection. For each individual, we measured SVL using a digital caliper with an accuracy of 0.1 mm and total weight to the nearest 0.1 g. Body weights (BW) were estimated using a digital weighing balance (KERN EMB 600-2, Germany). After the analyses the toads were brought back to their habitats.

Identification of color polymorphism

The color polymorphism in *B. viridis* complex individuals was determined based on variations in the coloration of the dorsal side of the body, according to the methodology proposed by Peskova (2003, 2006). The morph is not a taxonomical category and especially in *B. viridis* complex the mechanisms of genetic control on the coloration of the dorsal side are poorly studied (Stöck et al. 2008). Some authors suspect a connection between the color of the background (dark or light) on the dorsal side of this toad with the environmental factors (Zhelev et al. 2014, Fayzulin et al. 2019). The number of spots is not under strict genetic control and is also influenced by environmental factors (Arnold & Ovenden 2002, Peskova 2006).

Statistical analyses

Mathematical processing of the data was carried out with standard statistical procedures, using the statistical package R-3.1.2 (R Development Core Team 2015). Measured morphological data (SVL and BW) were analyzed by a two-way analysis of variance (ANOVA), which included factorial analyses of group (sites, habitats), sex and group-sex interactions, using the values from individual toads. The data distribution of both variables SVL and BW was visually inspected, using histogram graphs. Due to the heavier females the BW data had two modes and was not normally distributed. As the assumption of ANOVA is a normality of the residuals from the model and the diagnostics of the models showed normally distributed residuals we proceeded with reporting the results from the parametric tests.

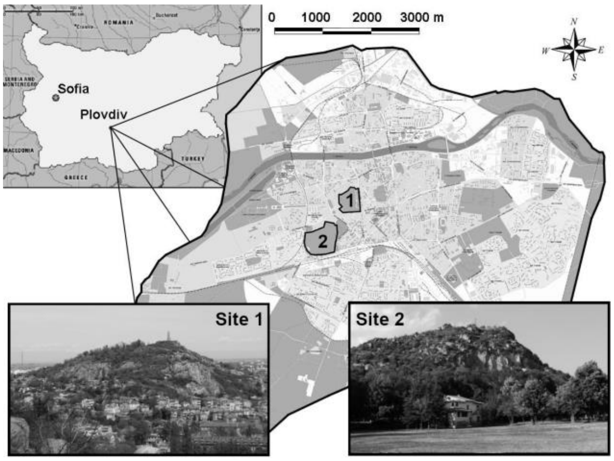


Figure 1. Indicative map of the sites of the Plovdiv city where individuals of the *B. viridis* complex were captured in 2017. Legend: Site 1 – NL "Halm Bunardzhik" Hill, Site 2 – NL "Mladezki halm" Hill.

The statistical significance of the differences between the groups was evaluated using Tukey HSD post hoc test. Results with $p < 0.05$ [$\alpha = 5\%$] were considered significant. Data were given as mean values ±SEM, a Minimum–Maximum. The significance of relationship of the skin color and type of the morph with the habitat was tested using chi-squared test of independence or Fisher's exact test (in the case where the numbers of animals in a category were below five). The data analyzed with chi-squared or Fisher's exact test were not tested for normality as no normality is expected.

**Results**

Morphological parameters (SVL and BW): descriptive statistics and ANOVA.

Results from two-way ANOVA analysis are presented in Table 1.

For the parameter SVL the main effect of habitat (site) was significant ($p < 0.05$). The effects of sex and the combination sex and site were insignificant ($p > 0.05$). We found significant interaction between all three tested variables (site, sex and combination sex and site) for the parameter BW ($p < 0.001$). The significance of the differences between the means of the four groups for two studied parameters was tested using Tukey HSD (Table 1). Comparisons between individuals of both sexes from sites 1 and 2 did not show any statistically significant differences for the SVL parameter. For the BW parameter, all pair-wise comparisons showed significant differences, except for the comparison between the males from the two biotopes. The females from both sites were significantly heavier than the males, which was the reason for the reliable interaction effect of sites and sex for this parameter. The effect of the habitat varied by sex.

Color polymorphism

At site 1, all four morphs: A, B, C and D (Figure 2), were registered for the individuals of both sexes, while for those inhabiting site 2, morph D was absent for the females. For both sexes, individuals in site 1 were dominated by two dark-background morphs (C and D), while those from site 2, the two morphs with light-background were dominant - A and B (see Table 2). The analysis of the interaction between the

Table 1. Morphological parameters in individuals of the *B. viridis* complex from two investigated sites. Legend: $A_{df}$ - sites, $B_{df}$ - sex, $C_{df}$ - sites × sex, $D_{df}$ - Residuals; MS - mean square, SVL - snout–vent length, BW - body weight, *n* - number of individuals. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns $p > 0.05$.

| Two-way ANOVA: tests of between-subjects effects | | | | | |
|---|---|---|---|---|---|
| SVL | | | | BW | |
| $A_1$: MS = 68.056, F = 6.631* | | | | $A_1$: MS = 193.70, $F_1$ = 14.811*** | |
| $B_1$: MS = 12.956, F = 1.262 ns | | | | $B_1$: MS = 1912.97, $F_1$ = 146.268*** | |
| $C_1$: MS = 9.753, F = 0.951 ns | | | | $C_1$: MS = 632.31, $F_1$ = 48.348*** | |
| $D_{116}$: MS = 10.264 | | | | $D_{116}$: MS = 13.08 | |
| Descriptive statistics (Mean±SEM; Min–Max). The sings < and > compare mean values of the parameters | | | | | |
| Parameters | Site 1 | | Site 2 | | Comparisons (Tukey HSD) |
| | Female (1) n = 30 | Male (2) n = 30 | Female (3) n = 30 | Male (4) n = 30 | |
| SVL (mm) | 66.63±3.72 (60.19–74.27) | 65.41±3.17 (61.17–72.31) | 67.56±2.99 (61.04–74.15) | 67.47±2.87 (62.47–74.52) | 1/2ns, 3/4ns, 1/3ns, 1/4ns, 2/3ns, 2/4ns |
| BW (g) | 39.61±3.87 (35.11–47.61) | 27.02±3.27 (21.31–34.69) | 32.47±3.46 (27.03–38.84) | 29.07±3.83 (23.62–36.51) | 1>2***, 3>4**, 1>3***, 1>4*, 2<3***, 2/4ns |

Table 2. Frequency of occurrence of color morphs in *B. viridis* complex in the studied sites. Legend: n – number of individuals, % - morphs share, A and C - individual spots of the dorsal side of the body, B and D - merging spots of the dorsal side of the body, LB - light background, DB - dark background.

| Sites | Sex | Morphs (n / %) | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | LB | C | D | DB |
| Site 1 | Female | 3 / 10.0% | 6 / 20.0% | 9 / 30.0% | 11 / 36.67% | 10 / 33.33% | 21 / 70.0% |
| | Male | 2 / 6.67% | 2 / 6.67% | 4 / 13.34% | 14 / 46.66% | 12 / 40.0% | 26 / 86.66% |
| Site 2 | Female | 13 / 43.33% | 15 / 50.0% | 28 / 93.33% | 2 / 6.67% | 0 / 0 | 2 / 6.67% |
| | Male | 14 / 46.67% | 13 / 43.33% | 27 / 90.0% | 1 / 3.33% | 2 / 6.67% | 3 / 10.0% |

sex and the dorsal coloration (color by sites) did not show statistically significant association for the individuals from site 1, as well as those from site 2 (Figure 3b). The assessment of the habitat influence on the dorsal coloration (color by sites) showed strong statistically significant association - Fisher's exact test F = 37.934, $p < 0.0001$ (Figure 3c). The analysis on the connection between the sex and the type of spots (spots by sex) did not show any statistically significant results for toads from both site 1 (Figure 4a) and site 2 (Figure 4b). We did not record statistically significant association between habitat and type of spots as well (spots by sites, Figure 4c).

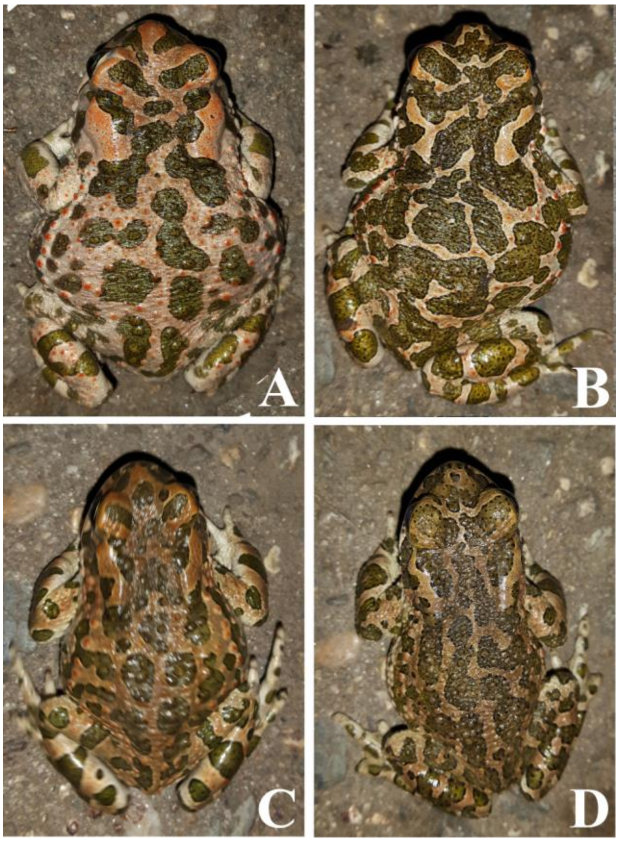

Figure 2. Photos of the four identified color morphs in *B. viridis* complex from the two investigated sites. Legend: A – light back background, individual spots of the dorsal side of the body; B – light back background, merging spots; C – dark back background, individual spots); D – dark back background, merging spots.

**Discussion**

Body size in *B. viridis* complex individuals from the study sites in Plovdiv City

In the present study, all studied individuals were adults, but not divided into age groups based on accurate skeletochronology, as our work was not strictly oriented to demographic parameters. The measured SVL and BW parameters give an overall picture of body size for the four samples of 30 adult green toads inhabiting the two study sites in Plovdiv City.

It is well known that, of the two parameters of body size in our study, the BW is the more variable. The BW in anurans can be influenced by various factors, such as competition, food availability, unfavorable climatic conditions or a poor environmental quality (Reading & Clarke 1995, Nabil et al. 2011). Anurans are ectothermic animals and usually the

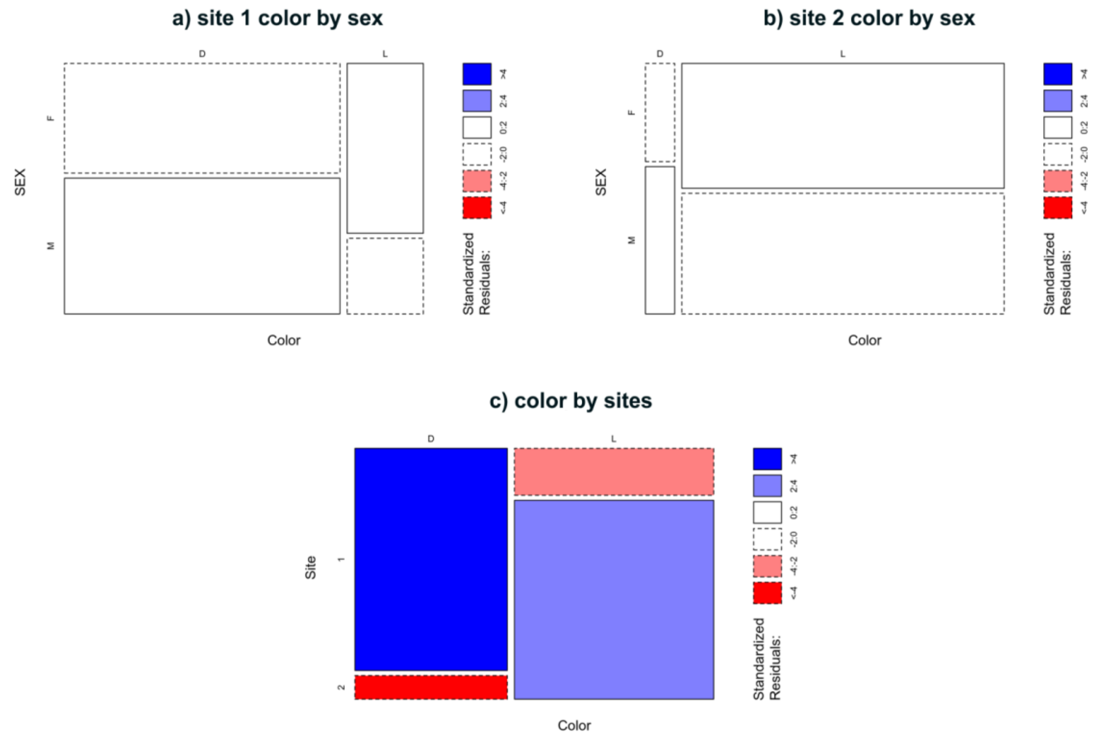


Figure 3. Grouping by sex of individuals of *B. viridis* complex from each site by color (a, b) and coloring toads from two sites, without regard for sex (c). Legend: F – females, M – males, L – light background of the dorsal side of the body, D – dark background of the dorsal side of the body. *Statistics: F: Fisher's exact test - 4a) F = 0.365, p = 0.209, 4b) F = 0.047, p = 1.0, 4c) F = 37.934, p = $1.729^{e-15}$.

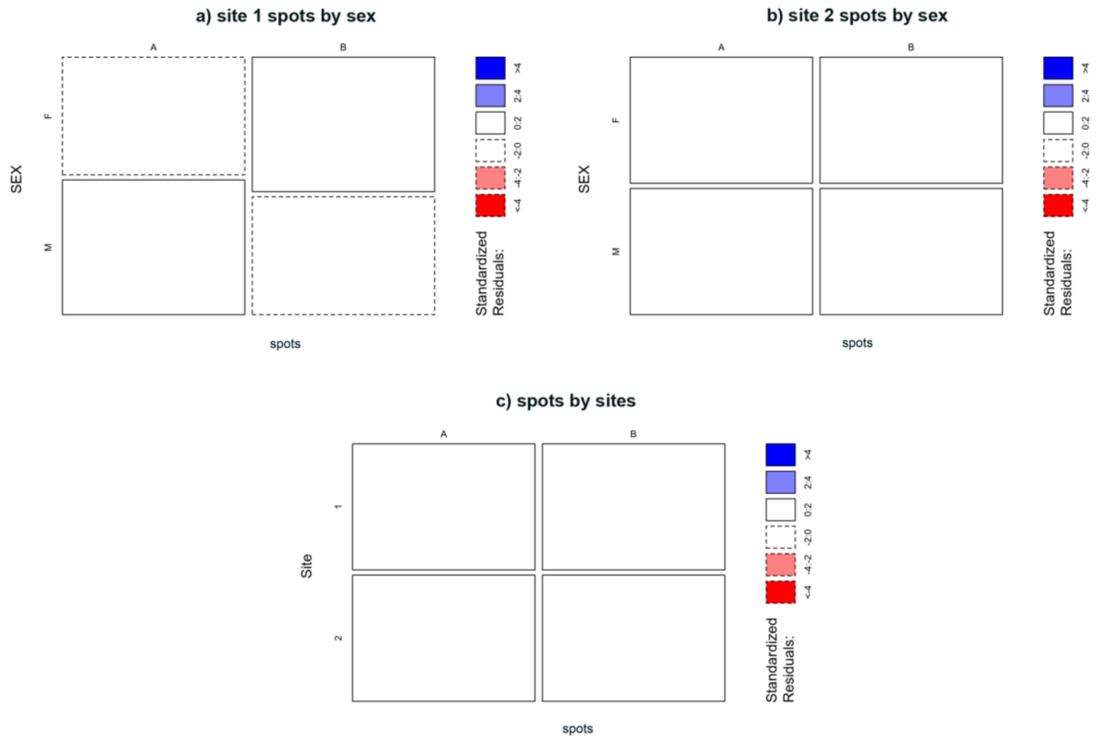


Figure 4. Distribution of spots by sex in *B. viridis* complex from each site (a, b) and the type of spots at toads from two sites, without regard to sex (c). Legend: F – females, M – males, A – individual spots of the dorsal side of the body, B - merging spots of the dorsal side of the body. *Statistics: $\chi^2$: Pearson's Chi-squared test with Yates' continuity correction, F: Fisher's exact test.5a) *$\chi 2$ = 0.067, p = 0.796, 5b) $\chi 2$ = 0, p = 1.0, 5c) F = 1.0, p = 1.0.

Bergmann's rule does not apply for them (Berrigan & Charnov 1994, Ashton 2002, Adams & Church 2008), however individuals from other wide-ranging endothermic animal species, living in a cold climate tend to be larger than individuals living in a warm climate. There are even records that show some anuran species, contrary to the Bergmann's rule, shrink their body size at colder climate (Ma et al. 2009). Another important abiotic factor connected with the variations of body size is altitude (Altunişik & Özdemir 2015). In amphibians commonly the combination of high-altitude and cooler temperatures, leads to a larger body size (Üzüm & Olgun 2009). Parameters such as food availability, habitat quality, and predation pressure on age of maturity and limited altitudinal gradients have effects on the body size (Morrison & Hero 2003). Specifically for *B. viridis* complex data suggest that climatic factors, ecological and trophic influence can lead to differences in body size of the green toads (Giacoma et al. 1997, Castellano & Giacoma 1998).

According to our results, no statistically significant differences in body length were recorded between individuals of both sexes inhabiting site 1 and site 2 (see Table 1). The SVL parameter varies in individuals from site 1 in the range of 60.19–74.27 mm and in those in site 2 in the range of 61.04–74.52 mm. For the other basic body size parameter, BW, our results revealed clear sexual dimorphism – the females from both sites were heavier than the males. For Bulgaria, according to Beshkov & Nanev (2002), Biserkov et al. (2007) and Stojanov et al. (2011) the linear body size length of the male green toads vary from 48 to 100 mm and from 50 to 120 mm for the females, accordingly. Although both sites are located in the center of a large city, they are unique "green islands" that have preserved a unique flora and fauna not influenced as strongly by anthropogenic pressure. Their relative altitude in respect to the city of Plovdiv (164 m) is 143 m for "Mladezhki hulm" Hill and 108 m for "Hulm Bunardzhik" Hill, and climatic conditions are identical to those of the surrounding city. Therefore, factors such as altitude, temperature, competition, food shortage or anthropogenic pressure are unlikely to affect significantly the body size of green toads inhabiting the two sites. In our opinion, the most probable reason for the bigger BW of the female individuals from the two sites could be associated with the reproductive period, when the female shows a physiological increase in body weight, related to the production of the eggs. Similar differences in BW between female and males are reported for various species from the *Bufotes viridis* complex from different parts of the world (Sinsch et al. 2007, Lo Valvo & Giacalone 2013, Trochet et al. 2014, Henle et al. 2014).

Color polymorphism in *B. viridis* complex individuals from investigated sites in Plovdiv City

In the two studied sites, four color morphs were identified among individuals of the *B. viridis* complex. Statistical analyses have showed a strong statistically significant association between habitat and dorsal coloration (color by sites). Site 2 was strongly dominated by individuals from the two dark-background morphs (C and D), while site 1 was dominated by the light-colored (A and B morphs) individuals. There is currently no consensus in the scientific community as to what causes variations in color polymorphism in amphibians and specifically in anurans. According to Hartl & Jones (1998), four sources contribute to phenotypic variations in anurans: a) genetic variation – the phenotype is genetically determined; b) environmental variation – the phenotype is set by differences in environments; c) genotype-environment variation – the phenotype is assigned by interaction of genotype and environment and d) genotype-environment association – the phenotype is assigned by association of genotype and environment. Field studies support the hypothesis that natural selection leads to the emergence of polymorphic forms (morphs) among anurans as camouflage protection against predators (Hoffman & Blouin 2000, Kang et al. 2016, Kolenda et al. 2017, Silva & Mahaulpatha 2019). However, there is evidence to support the hypothesis that color polymorphism in anurans is also influenced by environmental factors (Stöck et al. 2008, Summers et al. 2003, Jazayeri & Saberi 2018). In the particular case of *B. viridis* complex, there is even evidence of "selective advantages" for individuals with color morphs A and C when living in an anthropogenically transformed environment (Zhelev et al. 2014, Fayzulin et al. 2019). Of course, more field analyses, accompanied by genetic and molecular biological analyses, are needed to prove such a hypothesis. The results of this study clearly indicate that the reason for the dominance of dark-background individuals in site 1 and light-backgrounds in site 2 must be sought in the environmental characteristics of each of the habitats. As stated in the material and methods section, site 2 is characterized by low-stemmed herbaceous and shrubby vegetation. This is an open area without trees and shady places. The bright coloration on the back of the body enables frogs to blend well with the landscape, reducing the likelihood of being detected by visually oriented predators. The light background may also play a certain thermodynamic role, since outdoors, in direct sunlight especially during the summer season, temperatures are quite high. Quite the opposite argument would explain the dominance of dark-background individuals of C and D inhabiting in site 1, where the vegetation is predominantly of a tree type and has many shady places. However, further field studies are needed to understand the selective advantages of polymorphism in amphibian anurans.

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**References**

Adams, D.C., Church, J.O. (2008): Amphibians do not follow Bergmann's rule. Evolution 62: 413-420.

Altunişik, A., Özdemir, N. (2015): Life history traits in *Bufotes variabilis* (Pallas, 1769) from 2 different altitudes in Turkey. Turkish Journal of Zoology 39: 153-159.

Andrä, E. (2011): Aspekte der Biologie der Wechselkröte. Wechselkröten-Symposium des LBV am 13. Mai 2011, München, Germany, pp. 1-13.

Arnold, E.N., Ovenden, D. (2002): A field guide to the reptiles and amphibians of Britain and Europe. 2nd Edition. Harper Collins Publishers, London.

Ashton, K.G. (2002): Do amphibians follow Bergmann's rule? Canadian Journal of Zoology 80: 708-716.

Bannikov, A.G., Darevskii, I.S., Ishtenko, V.G., Rustamov, A.K., Shterbak, N.N. (1977): *A Guide to the Amphibians and Reptiles of the USSR*. Prosveshtenie, Moscow. [in Russian]

Batista, V., Carranza, S., Carretero, M.A., Harris, D.J. (2006): Genetic variation within *Bufo viridis*: evidence from mitochondrial 12S and 16S rRNA DNA sequences. Bulletin de la Societe Herpetologue de France. 17: 24-33.

Berrigan, D., Charnov, E.L. (1994): Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos 70: 474-478.

Beschkov, V., Nanev, K. (2002): *Amphibians and reptiles in Bulgaria*. Pensoft, Sofia and Moscow. [in Bulgarian, English summary]

Biserkov, V., Naumov, B., Tchankov, N., Stoyanov, A., Petrov, B., Dobrev, D., Stoev, P. (2007): *A field guide to Amphibiansand Reptiles of Bulgaria*. Green Balkans, Sofia. [in Bulgarian, English summary]

Castellano, S., Giacoma, C. (1998): Morphological variation of the green toad *Bufo viridis*, in Italy: a test of causation. Journal of Herpetology 32(4): 540-550.

Clusella Trullas, S., Van Wyk, J.H., Spotila, J.R. (2007) Thermal melanism in ectotherms. Journal of Thermal Biology 32: 235-245.

Dubois A., Bour R. (2010): The nomenclatural status of the nomina of amphibians and reptiles created by Garsault (1764), with a parsimonious solution to an old nomenclatural problem regarding the genus *Bufo* (Amphibia, Anura), comments on the taxonomy of this genus, and comments on some nomina created by Laurenti (1768). Zootaxa 2447: 1-52.

Fayzulin, A.I., Chikhlyaev, I.V., Knyazev, A.E., Kuzovenko, A.E., Mikhailov, R.A. (2019): Characteristics of green toads *Bufotes viridis* (Laurenti, 1768) polymorphism in urbanized territories of the Samara region. Bulletins Samara Scientific Center Russian Academic of Science 21(2): 154-158.

Fayzulin, A.I., Svinin, A.O., Ruchin, A.B., Skorinov, D.V., Borkin, L.J., Rosanov, Yu.M., Kuzovenko, A.E., Litvichuk, S.N. (2018): *Distribution and contact zone of two forms of the green toad from the Bufotes viridis complex (Anura, Amphibia), differing in genome size, in the Volga region*. Current Studies of Herpetology 18(1-2): 35-45. [in Russian, English Summary]

Frost, D.R. (2013): Amphibian Species of the World: An Online Reference. Version 5.6 (9 January 2013). Electronic Database. American Museum of Natural History, New York, USA. <http://research.amnh.org/herpetology/amphibia/index.html>

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., De Sa, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C. (2006): The amphibian tree of life. Bulletin of the American Museum of Natural History 297: 1-370.

Giacoma, C., Zugolaro, C., Beani, L. (1997): The advertisement call of the green toad (*Bufo viridis*): consistency, variability and role in mate choice. Herpetologica 53: 454-64.

Hartl, D.L., Jones, E.W. (1998): Genetics: Principles and Analysis, 4th ed, Jones & Bartlett, Sudbury.

Henle, K., Dubois, A., Rimpp, K., Vershinin, V. (2017): Mass anomalies in green toads *(Bufotes viridis)* at a quarry in Roßwag, Germany: inbred hybrids, radioactivity or an unresolved case? Mertensiella 25: 185-242.

Hoffman, E.A., Blouin, M.S. (2000): A review of colour and pattern polymorphisms in anurans. Biological Journal of the Linnean Society 70(4): 633-665.

Jablonski, D., Alena, A., Vlček, P., Jandzik, D. (2014): Axanthism in amphibians: A review and the first record in the widespread toad of the *Bufotes viridis* complex (Anura: Bufonidae). Belgian Journal of Zoology 144(2): 93-101.

Jazayeri A., Saberi F. (2018): Color polymorphism study on marsh frog populations (*Pelophylax ridibundus*) in the northern and the southern habitats of Khuzestan province. Journal of Animal Environment 10(2): 107-114.

Kang, C., Kim, Y.E., Jang, Y. (2016): Colour and pattern change against visually heterogeneous backgrounds in the tree frog *Hyla japonica*. Scientific Reports 6(3): 1-12.

Kolenda, K., Najbar, B., Najbar, A., Kaczmarek, P., Kaczmarski, M., Skawiński, T. (2017): Rare colour aberrations and anomalies of amphibians and reptiles recorded in Poland. Herpetology Notes 10: 103-109.

Lanza, B., Canestrelli, D. (2002): Atypische Färbung bei *Salamandrina terdigitata* (Lacepede, 1788) und *Bufo viridis viridis* Laurenti, 1768. Salamandra (Rheinbach) 38: 105-108.

Litvinchuk, S.N., Rosanov, J.M., Usmanova, N.M., Borkin, L.J., Mazanaeva, L.F., Kazakov, V.I. (2007): Variability ofmicrosatellites BM224 and Bcal7 in populations of green toads (*Bufo viridis* complex) differing by nuclear DNA content andploidy. Cell and Tissue Biology 1(1): 65-79.

Lo Valvo, M., Giacalone, G. (2013): Biometrical analyses of a sicilian green toad, *Bufo siculus* (Stöck *et al.* 2008), population living in Sicily (Italy). International Journal of Morphology 31(2): 681-686.

Ma, X., Lu, X., Merila, J. (2009): Altitudinal decline of body size in a Tibetan frog. Journal of Zoology 279: 364-371.

Mollov I. (2019): Urban ecology studies of the Amphibians and Reptiles in the city of Plovdiv, Bulgaria, Cambridge Scholars Publishing: Newcastle.

Morrison, C., Hero, J.M. (2003): Geographic variation in life-history characteristics of amphibians: a review. Journal of Animal Ecology 72: 270-279.

Nabil, A., Sarra, F., Slim, B.Y., Merella, P., Khaled, S. (2011): Morphological Variation of the African Green Toad, *Bufo boulengeri* (Amphibia: Anura) in Tunisia. Pakistan Journal of Zoology 43(5): 921-926.

Özdemir, N., Kultrup, B. (2007): Intraspecific variation of Turkish green toads, *Bufo* (*Pseudepidalea*) *viridis* Laurenti, 1768, based on 16S ribosomal RNA sequences (Anura: Bufonidae). Herpetozoa 20(1-2): 3-10.

Özdemir, N., Gül, S., Poyarkov, N.A., Kutrup, B., Tosunoğlu, M., Doglio, S. (2014): Molecular systematics and phylogeography of *Bufotes variabilis* (syn. *Pseudepidalea variabilis* Pallas, 1769) in Turkey. Turkish Journal of Zoology 38(4): 412-420.

Peskova, T.Yu. (2003): *Intrapopulation polymorphism of green toad color*. pp. 90-91. In: Bakiyev, A.G. (ed.), Current Problems of Herpetology and Toxicology. IEVB publishing: Tolyatti. [in Russian, English summary]

Peskova, T.Yu. (2006): *Seasonal dynamics of the polymorphism of the green toad in clean and anthropogenically polluted habitats of the Western Predkavkaz Region*. pp. 130-146. In: Bakiyev, A.G. (ed.), Current Problems of Herpetology and Toxicology. IEVB publishing: Tolyatti. [in Russian, English summary]

R Development Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

Reading, C.J., Clarke, R.T. (1995): The effects of density, rainfall and environmentaltemperature on body condition and fecundity in the common toad *Bufo bufo*. Oecologia 102(4): 453-459.

Silva, G., Mahaulpatha, W. (2019): Distribution of colour and pattern polymorphism in montane frogs of Sri Lanka. Journal of Entomology and Zoology Studies 7(2): 486-491.

Sinsch, U., Leskovar, C., Drobig, A., König, A., Grosse, W-R. (2007): Life-history traits in green toad (*Bufo viridis*) populations: indicators of habitat quality. Canadian Journal of Zoology 85: 665-673.

Stöck, M., Roth, P., Podloucky, R., Grossenbacher, K. (2008): Wechselkröten unter Berücksichtigung von *Bufo viridisvirdis* Laurenti, 1768; *Bufo variabilis* (Pallas, 1769); *Bufoboulengeri* Lataste, 1879; *Bufo balearicus* Böttger, 1880 und *Bufo siculus* Stöck, Sicilia, Belfiore, Lo Brutto, Lo Valvo und Arculeo. Handbuch der Reptilien und AmphibienEuropas. Bd. 5 (Froschlurche II). Wiebelsheim, Aula Verlag, S., 413-498.

Stöck, M., Moritz, C., Hickerson, M., Frynta, D., Dujsebayeva, T., Eremchenko, V., Macey, J.R., Papenfuss, T.J., Wake, D.B. (2006): Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. Molecular Phylogenetics and Evolution 41(2): 663-689. doi:10.1016/j.ympev.2006.05.026

Stojanov, A.J., Tzankov, N., Naumov, B. (2011): Die Amphibien und Reptilien Bulgariens. Chimaira, Frankfurt am Main.

Summers, K., Cronin, T.W., Kennedy, T. (2003) Variation in spectral reflectance among populations of *Dendrobates pumilio*, the strawberry poison frog, in the Bocas Del Toro Archipelago, Panama. Journal of Biogeography 30(1): 35-53.

Trochet, A., Moulherat, S., Calvez, O., Stevens, V., Clobert, J., Schmeller, D. (2014): A database of life-history traits of European amphibians. Biodiversity Data Journal 2: e4123.

Üzüm, N., Olgun, K. (2009): Age, size and growth in two populations of the southern crested newt, *Triturus karelinii* (Strauch 1870) from different altitudes. Herpetologica 65: 373-383.

Vences, M., Bina Perl, R.G., Giesen, K., Schluckebier, R., Simon, K., Schmidt, E., Steinfartz, S., Zieg, T. (2019): Development of new microsatellite markers for the Green Toad, *Bufotes viridis,* to assess population structure at its northwestern range boundary in Germany. Salamandra 55(3): 191-198.

Vitt, L.J., Caldwell, J.P. (2009): Herpetology: An introductory biology of Amphibians and Reptiles; 3rd edition. Academic Press, San Diego.

Zhelev, Zh., Arnaudov, At., Boyadzhiev, P. (2014): Colour polymorphism, sex ratio and age structure in the populations of *Pelophylax ridibundus* and *Pseudepidalea viridis* (Amphibia: Anura) from anthropogenicalli polluted biotopes in Southern Bulgaria and their usage as bioindicators. Trakia Journal of Sciences 12(1): 1-22.

Zhelev, Zh.M., Georgieva, K.N., Todorov, O.B., Peeva, K.G. (2017): Haematological parameters of *Bufotes viridis* (Laurenti, 1768) (Anura: Bufonidae) from southern Bulgaria. Acta Zoologica Bulgarica 69(3): 335-343.