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Haematological Parameters of *Bufotes viridis* (Laurenti, 1768) (Anura: Bufonidae) from Southern Bulgaria

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Abstract: This study presents the reference ranges of some haematological parameters in adult specimens of *Bufotes viridis* from wild populations living in southern Bulgaria. All manipulations were done in laboratory conditions with live animals, 6-8 hours after their capture. In 1 mm^3 of blood, erythrocyte and leukocyte counts were estimated at 935000 for ♀ and 939210 for ♂, and 8747 for ♀ and 8813 for ♂, respectively. The measured haemoglobin level (Hb) was 13.21 g/dL and 12.33 g/dL, the haematocrit value (PCV) was 48.0% and 46.0%, respectively for females and males. The other values were as follows: mean corpuscular volume (MCV) 561.66 fL and 521.11 fL, mean corpuscular haemoglobin (MCH) 151.37 pg and 137.91 pg, mean corpuscular haemoglobin concentration (MCHC) 27.78% and 26.97%, respectively for females and males. In both sexes, the differential blood count was: lymphocytes (57%), neutrophils (15%) and low number of basophils, monocytes and eosinophils. Differences between males and females were statistically significant only relative to the haemoglobin.

Key words: Haematology, blood cells, *Bufotes viridis*, southern Bulgaria

Introduction

Amphibians are the smallest class of vertebrates, with 7,543 species in the world (AmphibiaWeb 2016). In Bulgaria, there are 24 species of amphibians of two orders: Caudata – eight species in one family, and Anura – 16 species in five families. These numbers, compared to most European countries, make the group relatively well-represented (Stojanov et al. 2011, Tzankov & Popgeorgiev 2014).

Tailless amphibians (Anura) have fully developed blood-circulatory and immune systems (Manning & Horton 1982). Being poikilothermic, they are a heterogeneous group of vertebrates with regard to their blood parameters. Depending on species, season and health conditions, many factors affect the blood parameters in amphibians (Boral & Deb 1970, Cabagna Zenklusen et al. 2011, Gül et al. 2011, Mahapatra et al. 2012, Grace & Akinola 2013, Arikan & Çiçek 2014, Meesewat et al. 2016, Zhelev et al. 2016).

Blood, being highly differentiated and an inner reactive medium, reflects all changes in its functional state, which is associated with changes of its basic parameters depending on environmental factors (Davis et al. 2008). This can explain the growing recent interest towards studies on using changes in haematological parameters in anuran species for bioindication and biomonitoring (Aliko et al. 2012, Priyadarshani et al. 2015, Salinas et al. 2015, Zhelev 2016).The haematology of Bulgarian anurans is poorly studied (Nikolov & Daraktchiev 1988), probably due to the fact that most of them are strongly protected and special permits are required

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for their catch. For several decades, there have been an increasing number of publications on changes in the blood parameters of *Pelophylax ridibundus* (Pallas, 1771), one of the anurans resistant to anthropogenic pollution (Zhelev et al. 2013, 2014a, 2015a). The green toad *Bufotes viridis* (Laurenti, 1768) (species name according to Frost (2014), synonyms *Bufo viridis* and *Pseudepidalea viridis*), together with *P. ridibundus*, inhabits the territory of the whole country (Beschkov & Nanev 2002). This terrestrial and nocturnal toad is not only common in less disrupted areas in southern Bulgaria (Stojanov et al. 2011) but also maintains populations of high numbers in human-modified habitats (Zhelev 2012, Zhelev et al. 2014b, 2014c). Both amphibian species often use the same water bodies for breeding. Usually, the marsh frog remains in the same reservoir where its development has completed, while the green toad leaves it and continues its life on land (Bannikov 1977).

This study aims to explore some haematological parameters of *Bufotes viridis* from populations in southern Bulgaria and to compare them with haematological data of the same species from other areas as well as with those of other terrestrial anurans.

Materials and Methods

The Ethics Board for Experimental Animals, the Faculty of Biology at Plovdiv University, approved the animal catch and laboratory methodology. All experiments were conducted in accordance with national and international guidelines of the European Parliament and the Council on the protection of animals used for scientific purposes (Directive 2010/63/EU). Manipulations were done in laboratory conditions, in line with the ethical standards for research work with live animals (Beaupre et al. 2004), within 6-8 hours after capture. After the analyses, animals were released back to their original environments.

Study area, toads capturing and morphological measurements

The study was conducted in southern Bulgaria, with a total of 76 (38 ♂, 38 ♀) adult (Snout-Vent Length > 60.0 mm) mature individuals of *Bufotes viridis*. The animals were caught at night-time with the help of electric torch. They were separated according to their sex using secondary sexual characteristics, the so-called "marital corns" on the first finger in males. The sample specimens were collected May – June 2015 from four areas in the vicinity of Plovdiv (198 m. a.s.l., 10 ♂, 10 ♀), Kardzhali (280 m. a.s.l., 8 ♂, 9 ♀), Galabovo (200 m. a.s.l., 9 ♂, 9 ♀) and Rakitnitsa (200 m. a.s.l., 11 ♂, 10 ♀) (Fig. 1).

The specimens were measured (Snout-Vent Length, SVL) to the nearest 1 mm and weighed (total weight, BW) to the nearest 0.1 g. Body weights were estimated using a digital weighing balance (KERN EMB 600-2, Germany). The health of the toads in this study was estimated using the formula:

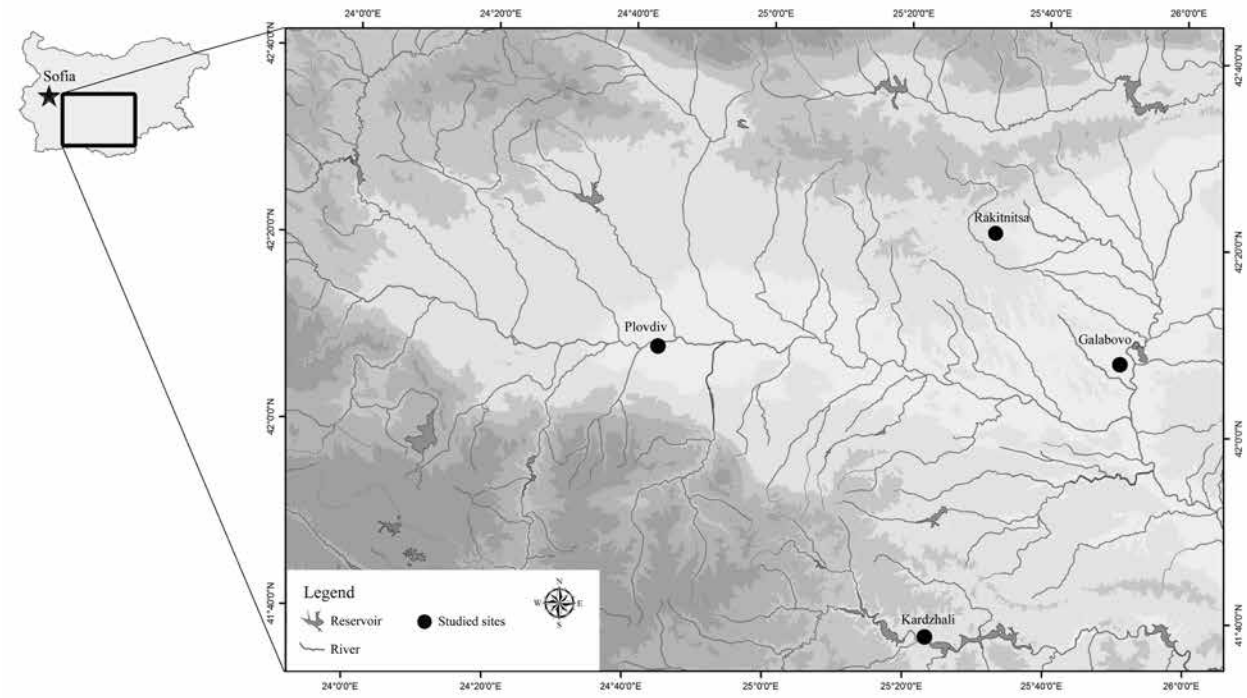

Fig. 1. Geographical location of the studied areas

Table 1. Statistically significant differences in the haematological parameters of *Bufotes viridis* (♂+♀) from the four populations inhabiting southern Bulgaria (n: number of individuals, M: mean value, SD: standard deviation, the signs > and < compare mean values of the parameters. Legend: MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, Ly: lymphocytes

Parameters	Populations								Comparisons
	Rakitnitsa n = 21		Plovdiv n = 20		Kardzhali n = 17		Galabovo n = 18		
	M	SD	M	SD	M	SD	M	SD	
MCH (pg)	161.96	27.91	98.42	15.41	157.43	26.83	163.72	25.17	1 = 3 = 4 > 2 $F_{3, 75}$ = 33.054, P < 0.001
MCHC (%)	28.25	3.91	28.42	4.73	28.40	3.13	24.22	2.36	1 = 2 = 3 > 4 $F_{3, 75}$ = 5.617, P < 0.001
MCV (fl)	578.58	123.44	354.31	64.51	560.65	116.06	687.64	121.69	4 > 1 = 3 > 2 $F_{3, 75}$ = 31.593, P < 0.001
Ly	57.33	4.26	60.91	4.05	56.41	2.65	56.33	2.08	1 = 3 = 4 < 2 $F_{3, 75}$ = 7.479, P < 0.001

BW (g) / SVL^3 (cm) × 10^2 proposed by Pauly (1983) and modified for anurans (Jelodar & Fazli 2012, Zhelev et al. 2015b).

Haematological analysis

The animals were anaesthetized with ether following Stetter (2001). We worked with blood (0.20 ml) drawn by means of cardiac ventricular puncture using small heparinised needle (20 mm length). The erythrocyte (RBCs) and leukocyte (WBCs) count was determined according to the method of Wierord using a Burker chamber (Pavlov et al. 1980). For dilution of erythrocytes, a standardised Hayem solution was used via Thoma pipettes, while for the leucocytes we used Turck's solution. Dilution was carried out 200 times for the erythrocyte count and 20 times for the leukocyte count. The haemoglobin concentration (Hb) was determined with the cyanhaemoglobin method by reading of absorbance at 540 nm with UV/Visible spectrophotometer (Eppendorf BioSpectrometer®, Germany). The packed cell volume PCV (or haematocrit value) was determined with heparinised haematocrit capillaries. Blood was centrifuged for 5 min at 3000 rpm constant-rotation (Eppendorf 5430R, Germany) in thin-walled capillary tubes and the value obtained was read from the scale and recorded in % (Blaxhall & Daisley 1973). The derivative haematological parameters (MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration and MCV: mean corpuscular volume) were calculated according to Brown's (1980) formulae. MCV was calculated by dividing haematocrit per litre of blood by total RBC count. We prepared dry blood smears and coloured them using Romanowsky-Giemsa's method (Pavlov et al. 1980). The differential leukocyte count was determined on the basis of 100 leukocytes per slide with Olympus stereo microscope (SZX16, resolve 900 line pair/mm, Germany) using Schilling's method (Pavlov et al. 1980).

Statistical analysis

We tested the normal distribution of all morphological and haematological data with Shapiro-Wilk test. The fact that the individual count from every population was limited served as a reason to unite all the studied parameters obtained for both sexes from a single population (♀+♂) and to compare them with the parameters obtained from other populations. The statistical analysis was done using one-way ANOVA (with Bonferroni post-hoc test) or non-parametric Kruskal-Wallis test (depending on distributions). The performed analysis demonstrated statistically valuable changes only in some of the examined haematological parameters between the individuals of different population groups. Due to this and the fact that the four studied areas were located in the same region (southern Bulgaria, a region with homogeneous climate conditions without temperature shifts, and with similar altitudes), the data obtained from the four populations were collated and analysed together in order to obtain reference value for every single haematological parameter related to the animals of both sexes. Then, the parametric Student's t-test (with those variables where normality was proved) or non-parametric Mann-Whitney – Wilcoxon Z-test (for variables not normally distributed) were performed to test statistically significant differences between sexes. P-values below 0.05 were considered statistically significant. Results were presented as means and standard deviations.

Logistic regression was used to build the model with cross-sectional odds ratio (OR) for statistically significant morphological and haematological vari-

Table 2. Morphological and haematological values of females and males *Bufotes viridis* from southern Bulgaria (n: number of individuals, Min: minimum value, Max: maximum value, M: mean, SD: standard deviation). Legend: SVL: Snout-vent length, BW: body weight, CF: condition factor, RBCs: erythrocyte count, WBCs: leukocyte count, Hb: haemoglobin concentration, PCV: packed cell volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, St: stab neutrophils, Sg: segmented nuclei neutrophils, Ba: basophils, Eo: eosinophils, Mo: monocytes, Ly: lymphocytes

Parameters	Sex						Comparisons (♀/♂)
	Females (n = 38)			Males (n = 38)			
	Min–Max	M	SD	Min–Max	M	SD	
SVL (cm)	6.6–8.7	7.82	0.51	6.3–8.7	7.47	0.63	t = 2.563, df = 74, p = 0.012
BW (g)	39.64–87.16	66.60	9.31	36.08–86.16	61.57	10.91	t = 2.162, df = 74, p = 0.034
CF	10.44–19.56	13.99	1.72	11.67–16.95	14.64	1.51	t = -1,770, df = 74, p = 0.081
RBCs (/mm^3)	670,000–1,450,000	935,000.1	1,650,675.5	700,000–1,220,000	939,210.5	1,114,615.8	Z = -0.551, df = 74, p = 0.585
WBCs(/mm^3)	4200–16900	8747.37	4554.45	4600–16500	8813.16	4149.58	Z = -0.353, df = 74, p = 0.727
Hb (g/dL)	8.14–16.63	13.21	2.91	7.08–16.21	12.33	2.84	Z = -2,187, df = 74, p = 0.028
PCV (%)	(23.0–65.0)	48.0	1.32	23.0–70.0	46.0	1.22	Z = -0.847, df = 74, p = 0.401
MCH (pg)	83.18–218.67	151.37	39.61	78.41–195.37	137.91	32.57	Z = -1,844, df = 74, p = 0.065
MCHC (%)	20.96–39.29	27.78	3.93	19.27–34.31	26.97	4.19	t = 0.878, df = 74, p = 0.383
MCV (fl)	273.55–939.28	561.66	184.45	258.33–783.44	521.11	135.71	t = 1.092, df = 74, p = 0.279
Differential leukocyte count (N/100 WBC)							
St	3–7	5.50	0.95	4–8	5.79	1.02	Z = -1,109, df = 74, p = 0.268
Sg	3–14	9.24	3.85	3–14	9.29	3.92	Z = -0.068, df = 74, p = 0.948
Ba	4–23	13.0	5.53	4–23	12.18	5.56	Z = -0.893, df = 74, p = 0.376
Eo	1–12	3.74	3.72	1–11	3.92	3.91	Z = -0.173, df = 74, p = 0.866
Mo	5–21	10.76	3.94	5–20	10.92	3.74	Z = -0.360, df = 74, p = 0.722
Ly	50–68	57.76	3.90	50–66	57.89	3.91	t = -0.147, df = 74, p = 0.884

Table 3. Logistic regression model: variables in the equation

Predictor variables	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP (B)	
							Lower	Upper
MCH	-0.018	0.008	4.960	1	0.026	0.983	0.968	0.998
St neutrophils	0.847	0.315	7.247	1	0.007	2.332	1.259	4.319
SVL	-1.456	0.495	8.653	1	0.003	0.233	0.088	0.615
Constant	8.891	3.643	5.957	1	0.015	7269.441	–	–

ables. The equation of the logistic regression for assessing the probability (Sex = 1) has the form:

P (Sex = 1) =1 / (1 + e^{-z}), where *e* is the basis of natural logarithms (e ≈ 2.7183).

$Z = B_0 + B_1*X_1 + B_2*X_2 + \ldots + B_n*X_n$, where X_1, X_2 … Xn are indications of the values of factors in the model, while B_0, B_1, B_2 … B_n are coefficients of the model, whose evaluations are calculated using the data from the sample.

The rule is: if P (Sex = 1) for object N is > 0.5 so, then N refers to the group of sex 1; if N is < = 0.5 so, then N refers to the group of sex 0. All statistical analyses were performed with the IBM SPSS Statistics 19 software.

Results

The comparison of the haematological parameters of *Bufotes viridis* (♀+♂) individuals from the four populations from southern Bulgaria illustrated statistically valuable differences in only three derivative parameters (MCH, MCHC and MCV) and one parameter corresponding with the differential blood formula (Ly; Table 1). These differences raise questions for future studies on the link between the haematological parameters changes in *B. viridis* and the environmental conditions, considering also the anthropogenic pollution in some areas. These opened questions were not investigated in this study.

The female specimens of *B. viridis* are bigger than males: the mean differences are statistically significant for variables SVL and BW. There were no sex differences in the values of CF between males and females (Table 2).

Sex differences were found only in the haemoglobin concentration: the value of Hb in females was 13.21 ± 2.9 and was significantly higher than that in males (12.33 ± 2.84). There were no significant differences for the mean values of other haematological variables (Table 2) between sexes.

The logistic regression model obtained consisted of the morphological parameter (SVL) and two haematology (MCH and St neutrophils) variables as predictor variables. The model correctly classified 71.1% females and 68.4% males, while the overall accuracy was 69.7%.

Here Z = 8.891 - 0.018* MCH +0.847* St neutrophils -1.456* SVL (see Table 3).

The increase of MCH with one unit reduced the "risk" N to be of sex 1 from 1 to 0.983. The increase of St with one unit increased the "risk" N to be of sex 1 from 1 to 2.332. The increase of SVL with one unit reduced the "risk" N to be of sex 1 from 1 to 0.233. Thus the expression of Z, the equation for P and decisive rule can be used to classify each object of interest.

Discussion

The literature data about the haematological parameters in *Bufotes viridis* is insufficient and unsystematic. We found significant differences between males and females of *B. viridis* specimens only for haemoglobin concentration. Gül et al. (2011) reported that in Turkey in the blood of female individuals of *B. viridis*, there was a slight difference in the number of erythrocytes (937.7 × 10^6/mm^3), haemoglobin was higher (15.76 g/dL) and haematocrit lower (43.75%) in comparison with males (975.0 ×10^6/mm^3; 12.95 g/dL; 58.50%, respectively; Table 4). Statistically significant changes in the number of erythrocytes and the amount of haemoglobin in both males and females during the transition from summer to winter (in the pre-hibernation period) were found in populations of *B. viridis* in the Western Caucasus (Zhukova & Kubantcev 1978; Table 4).

Several authors mentioned sex-determined differences concerning haematological parameters in various terrestrial anurans. Dönmez et al. (2009) reported that the RBC count, Hb, PCV and MCV of *Bufo bufo* were higher in males than in females (Table 4). Similar were the observations of Grace & Akinola (2013) in *Bufo regularis* where males during the rainy season showed a higher RBC count (520.0 ×10^6/mm^3), Hb (11.20 g/dL) and PCV (34.30%) than females (RBCs: 420.0 ×10^6/mm^3, Hb: 10.40 g/dL, PCV: 33.20%). Mahapatra et al. (2010) reported a higher RBC count (530.0 ×10^6/mm^3), haemoglobin concentration (7.80 g/dL) and mean corpuscular haemoglobin concentration (33.49%)

Table 4. Haematological parameters (mean values) from previous studies for adult *Bufotes viridis* and other species Anura with terrestrial life (F – females, M – males, T – total)

Literature	Species	Sex	RBCs (× 10^6/mm^3)	WBCs (/mm^3)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (%)	MCV (fl)
Present study	*Bufotes viridis*	F	935000	8747	13.21	48.0	151.37	27.78	561.66
		M	939210	8813	12.33	46.0	137.91	26.97	521.11
Boral & Deb (1970)	*Bufo melanostictus* Schneider, 1799	$T_{January}$	676800	–	14.91	47.68	–	31.22	742.56
		T_{April}	679000	–	12.64	40.95	–	30.82	616.70
		T_{August}	482500	–	10.62	34.55	–	30.97	725.98
Zhukova & Kubantcev (1978)	*Bufo viridis*	F_{summer}	485500	–	9.60	–	–	–	–
		F_{winter}	574400	–	11.50	–	–	–	–
		M_{summer}	523300	–	10.40	–	–	–	–
		M_{winter}	605000	–	11.70	–	–	–	–
Carmena-Suero et al. (1980)	*Hyla septentrionalis* Duméril & Bibron, 1841	T	–	–	6.20	22.40	–	27.70	–
Ostejic et al. (2000)	*Bufo spinosus limensis* Daudin, 1803	T	634000	–	–	39.53	172.63	28.06	621.90
Arikan et al. (2003)	*Pelodytes caucasicus* Boulenger, 1896	T	777600	2560	–	–	–	–	–
Dönmez et al. (2009)	*Bufo bufo* Linnaeus, 1758	F	870000	–	10.10	33.08	115.95	30.81	378.90
		M	900000	–	11.80	42.36	131.02	27.83	470.74
Gül & Tok (2009)	*Bufo viridis*	T	721750	1646	–	–	–	–	–
	Bufo bufo	T	534722	2325	–	–	–	–	–
	Hyla arborea Linnaeus, 1758	T	579583	2980	–	–	–	–	–
	Pelobates syriacus Boettger, 1889	T	657100	1216	–	–	–	–	–
Cabagna Zenklusen et al. (2011)	*Rhinella fernandezae* Gallardo, 1957	T	505000	4500	9.18	27.37	180.15	33.50	535.48
Gül et al. (2011)	*Pseudepidalea viridis*	F	937666	5900	15.76	43.75	133.93	29.67	450.18
		M	975000	6775	12.95	58.50	174.18	27.24	638.63
	Pelobates syriacus Boettger, 1889	F	765909	2600	10.38	38.0	141.37	24.21	577.35
		M	652500	3250	9.30	40.0	138.59	28.86	468.20
	Hyla arborea	F	733636	3602	12.21	45.0	194.08	26.19	878.72
		M	647500	2800	11.82	50.18	186.89	24.68	758.10
Mahapatra et al. (2012)	*Polypedates maculatus* Gray, 1830	F	570000	16642	7.80	23.80	135.82	33.49	419.56
		M	480000	14628	6.56	28.65	137.51	26.05	582.01
Grace & Akinola (2013)	*Bufo regularis* Reuss, 1833	$F_{rainy\ season}$	360000	–	9.0	28.20	–	–	–
		$M_{rainy\ season}$	520000	–	11.20	34.30	–	–	–
		$F_{dry\ season}$	420000	–	10.40	33.20	–	–	–
		$M_{dry\ season}$	390000	–	10.10	31.0	–	–	–
Das & Mahapatra (2014)	*Polypedates teraiensis* Dubois, 1986	F	620000	1215	5.82	51.55	96.71	11.75	822.41
		M	590000	1212	5.95	50.62	100.05	11.74	851.31

Table 5. Differential leukocyte count data (N/100 WBCs – TN: Total neutrophils, Ba: basophils, Eo: eosinophils, Mo: monocytes and Ly: lymphocytes) from previous studies for adults *Bufotes viridis* and other species Anura with terrestrial life (F – females, M – males, T – total)

Literature	Species	Sex	TN	Ba	Eo	Mo	Ly
Present study	*Bufotes viridis*	F	14.74	13.0	3.74	10.76	57.76
		M	15.08	12.18	3.92	10.93	57.89
Nikolov & Daraktchiev (1988)	*Bufo viridis*	T	28.95	21.54	4.98	9.50	42.0
	Bufo bufo	T	29.32	20.84	2.30	4.32	41.80
Friedsohn 1910 (cited in Jordan 1938)	*Bufo vulgaris* Laurenti, 1768	T	18.30	6.70	1.30	0.0	73.0
Cannon & Cannon (1979)	*Bufo alvarius* Girard, 1859	T	48.0	1.0	9.0	5.0	37.0
Cabagna et al. (2005)	*Bufo arenarum* Hensel, 1867	T	20.90	0.0	13.70	1.30	64.0
Chiesa et al. (2006)	*Bufo arenarum*	T	27.30	3.80	3.70	1.70	60.90
Forbes et al. (2006)	*Bufo americanus* Holbrook, 1836	T	68.0	7.40	3.30	1.50	20.0
Ponsen et al. (2008)	*Glyphogloossus molossus* Gunther, 1868	T	26.30	8.30	1.10	22.70	41.60
Davis & Durso (2010)	*Acris crepitans* Baird, 1854	T	22.40	5.0	1.60	2.70	68.30
Mahapatra et al. (2012)	*Polypedates maculatus*	F	22.0	3.72	12.72	7.43	50.29
		M	27.42	2.71	12.59	7.57	49.71
Das & Mahapatra (2014)	*Polypedates teraiensis*	F	26.66	2.80	9.97	5.70	54.92
		M	23.52	3.50	11.02	6.42	55.52

in females than in males (RBCs: 480.0 $\times 10^6$/mm^3, Hb: 6.56 g/dL, MCHC: 26.05%) of *Polypedates maculates*. A similar observation for the RBC count was found by Gül et al. (2011) in *Hyla arborea* and *Pelobates syriacus* and by Das & Mahapatra (2014) in *Polypedates teraiensis* (Table 4).

Gül et al. (2011) reported that, among five species of anurans with different ways of life, the number of erythrocytes is higher in terrestrial (*B. viridis*) and aquatic species (*P. ridibundus*) as compared to that of semi-aquatic species (*Rana dalmatina*). Moreover, haemoglobin concentration, haematocrit value and related derivative indices MCV and MCH are higher in the terrestrial *B. viridis*, *P. syriacus* and *H. arborea*.

Interpreting white blood cell counts and changes in the differential blood count of any animal requires some knowledge about how many cells of each type are normally found in it for many amphibians, this basic information is not available. One amphibian species, for which there are little data on changes of the blood counts, is *B. viridis*. In comparison with other amphibians, proportions of white blood cell of green toad from southern Bulgaria appeared generally typical, with the most abundant cell type being lymphocytes (57% of white blood cells in both sexes), followed by neutrophils (15% in both sexes), then basophil monocytes and eosinophils. Similar type of changes in the differential blood count in *B. viridis* from Bulgaria are reported by Nikolov & Daraktchiev (1988), with the difference that they have found lower number of lymphocytes (42%) and higher number of neutrophils (28%) and basophils (21%). According to the data of Davis et al. (2008), there is a close link between leukocyte profiles and glucocorticoid levels. Specifically, these hormones act to increase the number and percentage of neutrophils, while decreasing the number and percentage of lymphocytes. This phenomenon has been seen in all five vertebrate taxa in response to either natural stressors or exogenous administration of stress hormones. There is evidence that higher than normal proportions of neutrophils in circulation can point to infections, while eosinophil numbers are thought to be associated with parasitism defence (Kieseker 2002). Monocytes are phagocytic cells that engulf foreign material and are commonly found in animals with bacterial infections, while lymphocytes are involved in a variety of immunological functions such as immunoglobulin production and modulation of immune defence (Davis et al. 2008). In this aspect, studying the changes in differential white blood cell counts can provide important information that concerns the health status of the organism.

High levels of neutrophils and lower values of lymphocytes were found in the blood of *Bufo alvarius* (see Cannon & Cannon 1979) and *Bufo americanus* (see Forbes et al. 2006; Table 5). Ponsen et al. (2008) reported high levels of neutrophils (26%) and monocytes (22%) in the blood of *Glyphogloossus molossus* (Table 4). Mahapatra et al. (2012) reported higher values of neutrophils (27%) in males than in females (22%) of the *P. maculatus*, while Das & Mahapatra (2014) found higher values of neutrophils in females (26%) than in males (23%) in *P. teraiensis* (Table 5).

A number of haematological tests were conducted in different terrestrial species of Anura, without taking into consideration the gender of animals. The data (Tables 4 and 5) show quite a wide range of variation in the values of various haematological parameters in representatives of the Anura inhabiting different parts of the world with their specific climatic and geographical characteristics (Boral & Deb 1970, Carmena-Suero et al. 1980, Nikolov & Daraktchiev 1988, Ostejic et al. 2000, Arikan et al. 2003, Cabagna et al. 2005, Chiesa et al. 2006, Forbes et al. 2006, Ponsen et al. 2008, Gül & Tok 2009, Davis & Durso 2010 and Cabagna Zenklusen et al. 2011).

In conclusion, the haematology of *B. viridis* appears to be similar to that of other terrestrial anurans. There were small differences between sexes; however, in terms of values of haematological parameters, they were statistically insignificant. Differences between males and females were statistically significant only regarding Hb concentration. As the study represents the first detailed haematological examination on *Bufotes viridis* in southern Bulgaria, the values can serve as general reference for future research with this species or other anurans.

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