## Some hematologic parameters of *Elaphe sauromates* (PALLAS, 1811)

Most studies of ophidian hematology refer to counts and sizes of blood cells (RYERSON 1949; HARTMAN & LESSLER 1964; HUTCHISON & SZARSKI 1965; DUGUY 1970; SAINT-GIRONS 1970; ARIKAN et al. 2004; TOK et al. 2006; ARIKAN et al. 2009), and fewer to parameters such as hematocrit, hemoglobin concentration or mean cell hemoglobin concentration (WOJTASZEK 1991, 1992; TROIANO et al. 1997; LAMIRANDE et al. 1999; TROIANO et al. 2000; DUTTON & TAY-LOR 2003).

Hematologic studies on snake species occurring in Turkey are not many and largely restricted to counts and descriptions of size and morphology of the blood cells (DUGUY 1970; SAINT-GIRONS 1970; WOJTA-SZEK 1991, 1992; ARIKAN et al. 2004, 2009; TOK et al. 2006; for the species studied see Table 2). The present paper presents information about the cytomorphometry of the blood and some additional hematologic parameters of *Elaphe sauromates* (PALLAS, 1811). This snake species has a wide distributional range in Turkey and was not yet studied in this respect.

A single apparently healthy female specimen of *Elaphe sauromates* captured in the posthibernation reproductive period was used in the study. It was collected near Edirne, (WGS84: 41.674 N, 26.561 E; UTM: 35 T 0466654, 4524996), Turkish Thrace, in April 2010. The blood sample was taken from the postorbital sinus of the living specimen by means of a heparinized hematocrit capillary (MACLEAN et al. 1973; ARIKAN et al. 2003). Staining of the blood smears was done with Wright's stain, a broadly used substance. The specimen was later released back to where it was collected.

The red blood cell counts (RBC [cells per mm<sup>3</sup>]) and white blood cell counts (WBC [cells per mm<sup>3</sup>]) were carried out using a Neubauer hemocytometer, where standard Hayem's solution for red blood cells and Turk's solution for white blood cells were used as diluting agents.

Hematocrit was determined using the micro-hematocrit method (TANYER 1985). The tubes were spun in a micro-hematocrit

centrifuge for 5 min at 12000 rpm and the hematocrit (HCT [%]) was calculated from the proportion of the blood cell volume in the total blood volume. Hemoglobin concentration (Hb [g/dl]) was measured colorimetrically with a Sahli hemoglobinometer (TANYER 1985). The derived values 'mean cell volume of erythrocytes' (MCV = HCT /  $100 \cdot \text{RBC}$  [fl]), 'mean cell hemoglobin' (MCH = Hb / RBC [pg]) and 'mean cell hemoglobin concentration' (MCHC = Hb·100 / HCT [%]) were mathematically calculated from the results of the abovementioned analyses (TANYER 1985).

Blood cell measurements were done under a microscope (total magnification factor 1500x) with an Olympus 15x ocular micrometer. On each blood smear 40 erytrocytes were randomly chosen. Erythrocyte lengths (L) and widths (W), nucleus lengths (NL) and widths (NW) were measured. Erythrocyte (S) and nucleus size (NS) were computed according to the formulas:  $S = L \cdot W \cdot \pi/4$  and NS = NL  $\cdot NW \cdot \pi/4$ .

The descriptive statistics were performed using Minitab® statistical software package. Cytomorphological data were described by their means, standard deviations (SD) and range values and presented in a table along with the numbers of cells measured.

The results are shown in Tables 1 and 2 (under 'present study'). Values below are presented in the order (minimum - mean  $\pm$  SD - maximum).

Erythrocyte size (S) and nucleus size (NS) were found to measure  $(87.95 - 138.32\pm18.38 - 172.76)$  and  $(11.78 - 25.33\pm6.71 - 38.87) \mu m^2$ , respectively. The ratios erythrocyte length / erythrocyte width (L/W), nucleus length / nucleus width (NL/NW), and nucleus size / erythrocyte size (NS/S) were found to be  $(1.50 - 1.87\pm0.21 - 2.44)$ ,  $(1.20 - 1.60\pm0.26 - 2.17)$  and  $(0.10 - 0.18\pm0.05 - 0.26)$ , respectively.

The erythrocytes (Fig. IA) of *Elaphe* sauromates were about oval, and their nuclei were located centrally. The erythrocyte length, erythrocyte width, nucleus length and nucleus width in  $\mu$ m were found to be (16.00 - 18.02±1.08 - 20.00), (7.00 - 9.76±1.00 - 12.00), (5.00 - 7.10±1.19 - 9.00) and (3.00 - 4.49±0.66 - 5.50), respectively.

Lymphocytes (Fig. 1B) were the most abundant leucocytes in the blood. They were of spherical shape and measured (6.00 -  $6.70\pm0.72 - 8.00$ ) µm in diameter, their nucleus was in a central position. The cytoplasm of the lymphocytes was stained in pale blue and their nuclei were dark purple-blue.

Monocytes (Fig. 1C) were the second most frequently observed leucocytes. Their diameters were (12.00 -  $14.40\pm1.65$  - 17.00)  $\mu$ m, and there was some granulation in their cytoplasm. Their kidney-shaped nuclei were at least as large as half of the cell. The cytoplasm appeared light purple and the nucleus dark blue.

Eosinophils (Fig. 1D) were  $(15.00 - 17.10\pm1.29 - 19.00)$  µm in diameter. Their cytoplasms were stained in light blue and their nuclei in dark blue. Their cytoplasms were characterized by large roundish bright reddish granules.

Basophils (Fig. 1E) were observed to have diameters of  $(6.00 - 8.00 \pm 1.49 - 10.00)$ µm. The plasm was stained in light blue, the dark purplish-bluish granules partially masked the dark blue nucleus.

Neutrophils were not encountered on the blood smear preparations of *E. sauromates* in this study.

The length of the somewhat irregularly shaped thrombocytes (Fig. 1F) was (4.50 -  $5.60\pm0.82$  - 6.50) µm, their width was  $(3.80 - 4.46\pm 0.56 - 5.00)$  µm. The cytoplasm was stained in pale blue and the nucleus in dark purple. The large oval nucleus was in a lateral position within the thin irregular cytoplasmic envelope. Thrombocytes were susceptible to clustering on the blood smear preparations.

The results of additional hematologic analyses and calculations were as follows: red blood cell count: 450000 per mm<sup>3</sup>, white blood cell count: 8800 per mm<sup>3</sup>, hematocrit: 16.5%, hemoglobin concentration: 5.60 g/dl, mean cell volume: 366.66 fl, mean cell hemoglobin: 124.44 pg, and mean cell hemoglobin concentration: 33.93%. Total protein concentration in the blood (plasm plus cells) was determined refractometrically to be 8.50 g/dl.

In *Elaphe sauromates*, the red blood cell count, white blood cell count and hematocrit were found to be clearly lower than those of other colubrid species of the study area, *Coronella austriaca* LAURENTI, 1768, *Natrix natrix* (LINNAEUS, 1758), *Platyceps collaris* MÜLLER, 1878, *Eirenis modestus* (MARTIN, 1838) (Table 2). MCV, MCH and MCHC levels were higher in *E. sauromates* than in *N. natrix* (Table 2) which was the only colubrid species from which comparative data were available (WOJTAZSEK 1991).

Table 1: Established measurements and sizes of the blood cells of a specimen of *Elaphe sauromates* (PALLAS, 1811) from Edirne, Turkey.

n - number of cells measured, SD - standard deviation, L - erythrocyte length  $[\mu m]$ , W - erythrocyte width  $[\mu m]$ , S - erythrocyte size  $[\mu m^2]$ , NL - nucleus length  $[\mu m]$ , NW - nucleus width  $[\mu m]$ , NS - nucleus size  $[\mu m^2]$ , N/C – nucleo-cytoplasmic ratio, MD - maximum diameter  $[\mu m]$ , TL - thrombocyte length  $[\mu m]$ , TW - thrombocyte width  $[\mu m]$ .

Character	n	Mean	SD	Range
Erythrocyte measurements				
L (µm)	40	18.02	1.08	16.00-20.00
W (um)	40	9.76	1.00	7.00-12.00
L/Ŵ	40	1.87	0.21	1.50-2.44
$S(\mu m^2)$	40	138.32	18.38	87.95-172.76
NĽ (um)	40	7.10	1.19	5.00-9.00
NW (µm)	40	4.49	0.66	3.00-5.50
NL/NW	40	1.60	0.26	1.20-2.17
NS $(\mu m^2)$	40	25.33	6.71	11.78-38.87
NS/Š	40	0.18	0.05	0.10-0.26
Leucocyte measurements				
Monocytes MD (µm)	10	14.40	1.65	12.00-17.00
Eosinophils MD (µm)	10	17.10	1.29	15.00-19.00
Lymphocytes MD (µm)	10	6.70	0.72	6.00-8.00
Basophils MD (µm)	10	8.00	1.49	6.00-10.00
TL (um)	5	5.60	0.82	4.50-6.50
TW (μm)	5	4.46	0.56	3.80-5.00

B

D





A





С





Е



Fig. 1: Blood cells of a specimen of *Elaphe sauromates* (PALLAS, 1811) from Edirne, Turkey. A - erythrocytes, B - lymphocyte, C - monocyte, D - eosinophils, E - basophil, F - a cluster of thrombocytes.

Table 2: Some RBC - Red Blo Mean Cell Volume [fl] [µm], S - erythrocyte s	hematologic values in colubrid snake species od Cell Count [per mm <sup>3</sup> ], WBC - White Blc MCH - Mean Cell Hemoglobin [pg], MCH ize [µm <sup>2</sup> ], NL - nucleus length [µm], NW - r	: occurring in Turke ood Cell Count [per C - Mean Cell Hem ucleus width [µm],	y, according to mm <sup>3</sup> ], Hb - He oglobin Concer NS - nucleus si	various autho emoglobin co atration [%], ze [µm <sup>2</sup> ].	ors. Incentration L - erythroc	[g/dl], HCT yte length [µ	- Hematocr m], W - ery	t [%], MCV - hrocyte width
Reference	Species	RBC [mm <sup>-3</sup> ]	WBC [mm <sup>-3</sup> ]	[lb/g] dH	HCT [%]	MCV [f]	MCH [pg]	MCHC [%]
Present Study DESSAUER (1970)	Elaphe sauromates (PALLAS, 1811) Natrix natrix (LINNAEUS, 1758)	450000 -	-	5.6 -	16.5 37	366,66 -	124.44 -	33.93 -
DUGUY (1970)	Natrix tessellata (LAURENTI, 1758) Coronella austriaca LAURENTI, 1768 Natrix natrix (LINNAEUS, 1758)	-580000-1406000 668000-1302000			χ. 			
WOJTAZSEK (1991)	Zamenis longissimus (LAURENTI, 1768) Natrix natrix (LINNAEUS, 1758)	622000-1410000 ずず 1748000 ゃぃ 1545000	- & & 16710 00 16030	- ở ở 5.75 00 5.08	- 0 0 36.3 0 0 1	- م م 191.3 د د 100.7	- م م 53.09 ٥ ٥ ٤٦ ٥٤	ਰਾਰਾ 28.1 000778
Tok et al. (2006)	Platyceps collaris (MULLER, 1878) Natrix natrix (LINNAEUS, 1758) Eirenis modestus (MARTIN, 1838)	1310000 759000 843000	++	9 9 + +	t. 1 + +	+ +	+ + 0	9. 74 +
Reference	Species	L [µm]	[µm]	S [μm <sup>2</sup> ]	NL [µm]	[mμ] WN	NS [μm <sup>2</sup> ]	
Present Study Harrman & LessLer	Elaphe sauromates (PALLAS, 1811) Pantherophis guttatus (LINNAEUS, 1766)	18.02 18.9	9.76 11.8	138.32 -	7.10 6.2	4.49 3.6	25.33 -	
SAINT GIRONS (1970)	Coronella austriaca LAURENTI, 1768 Zamanis longiscimus (1 AUDENTI, 1768)	17.5 18.3	9.7	140.0 152 1	7.3	3.6 4.1	20.6	
Worтazseк (1991) Ток et al. (2006)	Natrix natrix (LINNAEUS, 1758) Platyceps collaris (MULER, 1878) Natrix natrix (LINNAEUS, 1758)	16.31 15.41 17.39	10.02 10.66 9.94	- 129.59 136.29	7.19 7.29 7.39	3.71 4.02	23.25	
ARIKAN et al. (2009)	Eirenis modestus (MARTIN, 1838) Telescopus fallax (FLEISCHMANN, 1831) Dolichophis caspius (GMELIN, 1789)	17.07 18.33 14.91	10.71 10.33 7.64	143.86 148.80 89.88	7.77 7.53 10.01	3.93 5.06 4.84	24.02 29.87 38.08	

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However, the hemoglobin concentration was almost identical in both species (Table 2). In *E. sauromates* the value of the mean erythrocyte volume (MCV) was proportionately higher whereas the value of the red blood cell count was lower as compared with other snakes.

Some authors found that there was both a great intraspecific and interspecific variation in the blood cell counts of snakes, the intraspecific variation being affected by sex, age, season, and health status of the animal (DUGUY 1970; ARIKAN et al. 2004; ToK et al. 2006; ARIKAN et al. 2009). Our study supports the above observation in that the blood cell counts of *E. sauromates* extended the range of interspecific variation of this blood parameter considerably.

No neutrophils were encountered on the blood smear preparations of *E. sauromates* paralleling the finding of ARIKAN et al. (2009) who reported that there was no neutrophil leucocyte in the blood samples of the viperids and colubrids they had examined.

The authors are fully aware that there is intraspecific, age-dependant, seasonal and sexual variation in reptilian blood cell counts and other blood parameters which in addition may be influenced by the health status of the animal. Thus, the results obtained from a single specimen may reveal a rough picture of its hematologic data only and cannot specify the hematologic reference intervals for *E. sauromates*.

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