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Morphology of peripheral blood cells from various Turkish snakes

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Abstract. The present study is on the morphologies and sizes of peripheral blood cells (erythrocytes, leucocytes and thrombocytes) of thirty two Turkish snake species from blood smears, stained with Wright's stain. The investigated species and their families are as follows: Leptotyphlopidae [*Leptotyphlops macrorhynchus* (Jan 1861)], Typhlopidae [*Typhlops vermicularis* Merrem 1820], Boidae [*Eryx jaculus* (Linnaeus 1758)], Colubridae [*Dolichophis jugularis* (Linnaeus 1758), *D. schmidti* (Nikolsky 1909), *D. caspius* (Gmelin 1789), *C. ventromaculatus* Gray 1834, *Hemorrhois nummifer* (Reuss 1834), *H. ravergieri* (Ménétriés 1832), *Platyceps collaris* (Müller 1878), *P. najadum* (Eichwald 1831), *Zamenis hohenackeri* (Strauch 1873), *Natrix natrix* (Linnaeus 1758), *N. tessellata* (Laurenti 1768), *Eirenis rothi* Jan 1863, *E. levantinus* Schmidtler 1993, *E. coronella* (Schlegel 1837), *E. barani* Schmidtler 1988, *E. eiselti* Schmidtler & Schmidtler 1978, *E. decemlineatus* (Duméril & Bibron 1854), *E. modestus* (Martin 1838), *Rhynchocalamus melanocephalus* (Jan 1862), *Telescopus fallax* (Fleischmann 1831), *T. nigriceps* (Ahl 1924), *Malpolon monspessulanus* (Hermann 1804), *Spalerosophis diadema* (Schlegel 1837), Viperidae [*Montivipera xanthina* (Gray 1849), *M. wagneri* (Nilson & Andrén 1984), *M. albizona* (Nilson,, Andrén & Flärdh 1990), *Vipera eriwanensis* (Reuss 1933), *Macrovipera lebetina* (Linnaeus 1758)] and Elapidae [*Walterinnesia aegyptia* Lataste 1887].

Keywords. Leptotyphlopidae, Typhlopidae, Boidae, Colubridae, Viperidae, Elapidae, blood cells, hematology

Introduction

The cellular metabolism of ectothermic animals must remain viable over broad temperature ranges, thus, their homeostatic control mechanisms and cellular requirements are some times less restricted than those found in mammals. As a result the blood composition of reptiles varies greatly between major groups (Dessaurer 1970).

In different reptiles, the studies on the comparative morphologies of the peripheral blood cells mainly concentrate on sexual and seasonal variations of cell counts and sizes, and blood parasites (Gulliver 1842, 1875, Wintrobe 1933, Jordan 1938, Ryerson 1949, Pienaar 1962, Saint Girons & Saint Girons 1969, Duguy 1970, Saint Girons 1970, Sypek & Borysenko 1988, Alleman et al. 1999, Knotková, et al. 2002, Uğurtaş et al. 2003, Arıkan et al. 2004). While there are several works on the taxonomy and distribution of Turkish snakes (Baran 1976, Başoğlu & Baran 1980, Baran & Atatür 1998,

Uğurtaş et al. 2001, Budak & Göçmen 2005, Göçmen et al. 2007), there is only one study on their blood cell morphologies and sizes, that of Arıkan et al. (2004), based on some viperid snakes.

Here the blood cell morphologies and sizes of 32 Turkish snake species belonging to six families were investigated comparatively.

Materials and Methods

Specimens of 32 snake species belonging to six families were collected from different parts of Turkey in different dates. Blood samples were obtained according to MacLean et al. (1973), from postorbital sinuses of the specimens via heparinized glass capillaries. Blood smears were prepared and stained with Wright's stain to facilitate the measurements of morphological and size parameters of the blood cells. Cells were measured under a light microscope, using a MOB-1-15x micrometrical ocular. From each blood smear, 40 erythrocytes were randomly chosen for the measurement of their lengths (L), widths (W), nuclear lengths (NL) and nuclear widths (NW). Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed from ES= LWπ/4 and NS= NLNWπ/4. Comparisons of cell and nuclear shapes were done from L/W and NL/NW ratios and that of nucleus/cytoplasm from NS/ES ratio. From the blood smears of each species, measurements of leucocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) and thrombocytes (TL, TW) were also taken to determine their sizes. One way ANOVA test was utilized in the comparisons of the obtained data, α= 0.05 in all of the analyses. The photomicrographs of the blood cells were taken with Olympus BX51-Altra 20 Soft Imaging System.

Results

The characteristic shape of snake erythrocytes is oval, just like those of other ectothermic vertebrates and those of birds. Except those of *Montivipera xanthina*, the erythrocytes of other Turkish snakes have more or less ellipsoidal nuclei uniformly localized in a central place of the cell (Fig. 1A). In *M. xanthina*, the nuclei were irregularly shaped and their placement within the cell was not uniform (Fig. 1B). The erythrocytes of *Eirenis modestus* were almost spheroidal (Fig. 1C), and those of *Typhlops vermicularis* strongly ellipsoidal (Fig. 1D). On smears stained with Wright's stain, the cytoplasms were light yellowish pink and the chromophilic nuclei were dark purplish blue.

The erythrocyte measurements (lengths and widths), sizes and L/W ratios are given in Table 1; nuclear measurements of the erythrocytes and nucleocytoplasmic ratios (NS/ES) are given in Table 2. Because of the irregular nuclear shapes of the erythrocytes in *Montivipera xanthina,* no related measurements were made, thus no values were given in Table 2.

Regarding erythrocyte size, the blood smears demonstrated significant interspecific, in some cases even intraspecific variations. The longest and largest erythrocytes were observed in *Telescopus nigriceps* ($F_{31,\ 1248}$= 44.198, P= 0.000; $F_{31,\ 1248}$= 66.908, P= 0.000), the widest in *Malpolon monspessulanus* ($F_{31,\ 1248}$= 139.863, P= 0.000), the shortest in *Eirenis eiselti,* the narrowest in *Montivipera xanthina* and the smallest in *Eirenis modestus.* Regarding L/W ratio, the most strongly ellipsoidal cells were observed in *M. xanthina,* the least ellipsoidal (nearly spherical) ones in *Malpolon monspessulanus* ($F_{31,\ 1248}$= 153.317, P= 0.000; Table 1).

The longest nuclei were observed in the erythrocytes of *Montivipera wagneri* ($F_{30,\ 1209}$= 182.964, P= 0.000), the widest nuclei in *Telescopus fallax* ($F_{30,\ 1209}$= 55.317, P= 0.000), the largest nuclei in *Dolichophis jugularis* ($F_{30,}$

$_{1209}$= 160.779, *P*= 0.000); the shortest nuclei in *Macrovipera lebetina,* the narrowest nuclei in *Eirenis rothi* and the smallest ones in *Coluber ventromaculatus*. Regarding the NL/NW ratio, the most strongly ellipsoidal nuclei were observed in *Montivipera wagneri,* the least ellipsoidal in *Macrovipera lebetina obtusa* ($F_{30, 1209}$= 123.162, *P*= 0.000; Table 2). Nucleocytoplasmical ratio (NS/ES) was highest in *Eirenis modestus,* lowest in *C. ventromaculatus* ($F_{30, 1209}$= 271.863, *P*= 0.000; Table 2).

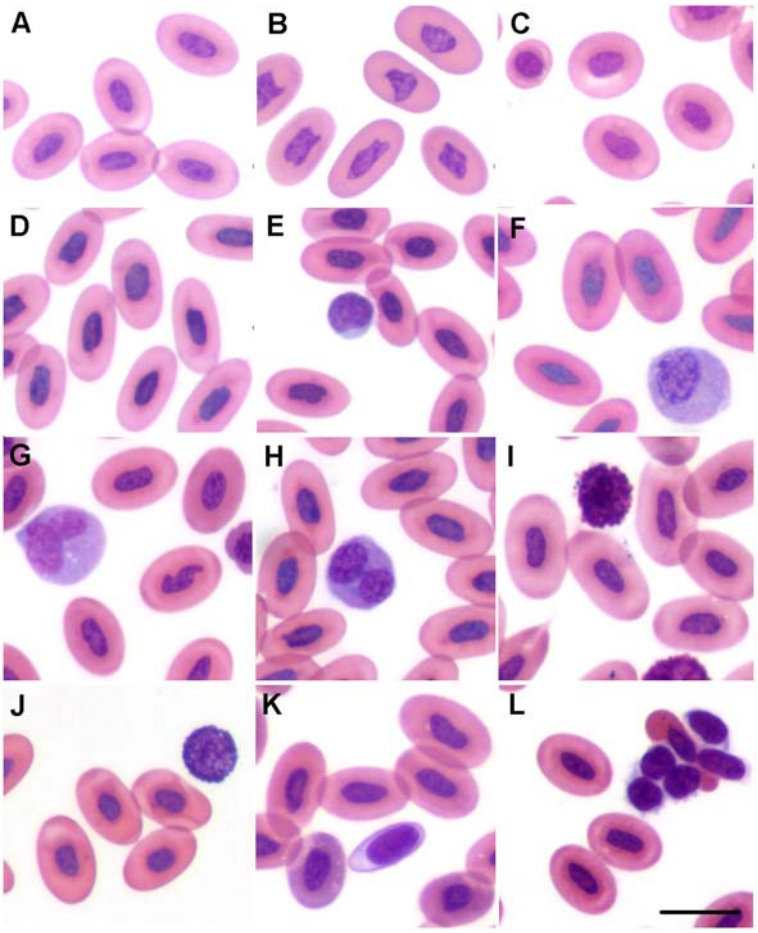

Figure 1. Photomicrographs of peripheral blood cells of various snakes from Turkey, Wright's stain. **A**: Normal erythrocytes (*H. ravergieri*); **B**: Erythrocytes with irregular nuclei (*M. xanthina*); **C**: Nearly spheroidal erithrocytes (*E. modestus*); **D**: Rather ellipsoidal erythrocytes (*T. vermicularis*); **E**: Small lymphocyte (*L. macrorhynchus*); **F**: Large lymphocyte (*Z. hohenackeri*); **G**: Monocyte (*P. najadum*); **H**: Neutrophile (*T. vermicularis*); **I**: Eosinophile (*M. wagneri*); **J**: Basophile (*S. diadema*); **K**: A spindle shaped thrombocyte (*V. eriwanensis*), **L**: A group of thrombocytes (*P. najadum*). Horizontal bar: 15 µm.

Table 1/A. The erythrocyte measurements established in the peripheral bloods of 32 snake species from Turkey belonging to six families [L: Erythrocyte length, W: Erythrocyte width, ES: Erythrocyte size].

Species	Erythrocytes			
	L (µm)	W (µm)	L/W	ES (µm²)
L. macroryhnchus	15.86±0.11 (14.50-17.25)	9.29±0.08 (8.50-10.50)	1.71±0.02 (1.49-1.89)	115.75±1.45 (98.42-136.00)
T. vermicularis	16.57±0.17 (14.50-18.75)	9.13±0.06 (8.25-10.00)	1.82±0.02 (1.54-2.03)	118.76±1.60 (100.38-137.38)
E. jaculus	16.36±0.19 (13.25-18.50)	8.77±0.07 (7.50-9.50)	1.87±0.02 (1.61-2.18)	112.83±2.01 (85.81-134.24)
C. ventromaculatus	15.94±0.17 (14.25-18.00)	10.67±0.11 (7.75-11.50)	1.50±0.02 (1.30-1.94)	133.60±2.14 (91.26-160.24)
D. jugularis	16.29±0.18 (14.00-19.00)	7.48±0.07 (6.50-8.25)	2.18±0.02 (1.87-2.41)	95.81±1.69 (74.18-123.05)
D. schmidti	16.21±0.14 (14.50-18.00)	9.88±0.07 (8.75-10.75)	1.64±0.01 (1.49-1.86)	125.82±1.71 (102.44-151.90)
D. caspius	14.91±0.16 (13.00-17.00)	7.64±0.12 (6.50-9.25)	1.96±0.02 (1.70-2.22)	89.88±2.21 (66.33-120.11)
H. nummifer	15.61±0.10 (14.25-17.00)	9.33±0.04 (9.00-10.00)	1.68±0.01 (1.50-1.84)	114.30±0.90 (105.98-126.29)
H. ravergieri	14.76±0.16 (13.00-18.00)	9.95±0.11 (8.50-12.00)	1.49±0.02 (1.19-1.80)	115.38±1.91 (86.74-141.30)
P. collaris	14.40±0.12 (13.25-15.75)	10.04±0.08 (9.25-11.25)	1.44±0.01 (1.26-1.65)	113.63±1.54 (98.81-136.00)
P. najadum	15.47±0.14 (13.75-17.25)	10.23±0.13 (8.25-12.00)	1.52±0.01 (1.23-1.72)	124.50±2.41 (89.05-160.14)
Z. hohenackeri	17.66±0.24 (15.50-23.50)	9.91±0.09 (8.75-10.75)	1.79±0.03 (1.48-2.29)	137.55±2.51 (106.47-189.09)
N. natrix	16.87±0.18 (14.50-19.00)	10.15±0.08 (9.25-12.00)	1.67±0.02 (1.31-1.87)	134.46±1.66 (111.86-154.01)
N. tessellata	15.98±0.21 (13.50-19.25)	7.92±0.09 (6.75-9.00)	2.02±0.03 (1.76-2.39)	99.61±2.13 (74.18-128.45)
E. rothi	14.77±0.15 (13.25-17.25)	8.73±0.06 (7.75-9.50)	1.69±0.02 (1.50-1.97)	101.24±1.44 (82.13-118.49)
E. levantinus	16.60±0.15 (14.75-18.75)	10.04±0.09 (9.00-11.25)	1.66±0.02 (1.40-1.85)	130.84±1.77 (111.27-158.23)
E. coronella	16.59±0.23 (13.25-19.25)	10.22±0.11 (8.50-11.75)	1.63±0.02 (1.45-1.95)	133.52±2.81 (88.41-170.64)
E. barani	16.18±0.12 (14.75-18.00)	9.68±0.05 (9.00-10.50)	1.67±0.01 (1.51-1.95)	122.98±1.27 (108.92-144.24)

Table 1/B. The erythrocyte measurements established in the peripheral bloods of 32 snake species from Turkey belonging to six families [L: Erythrocyte length, W: Erythrocyte width, ES: Erythrocyte size].

Species	Erythrocytes			
	L (μm)	W (μm)	L/W	ES (μm²)
E. eiselti	14.13±0.13 (12.50-16.50)	9.62±0.07 (8.50-10.75)	1.47±0.01 (1.35-1.61)	106.84±1.65 (83.41-136.00)
E. decemlineatus	14.75±0.14 (13.25-16.50)	10.03±0.07 (8.75-11.00)	1.47±0.01 (1.29-1.68)	116.25±1.55 (97.88-142.48)
E. modestus	14.47±0.15 (12.50-17.00)	7.45±0.08 (6.50-8.50)	1.95±0.02 (1.67-2.36)	84.78±1.53 (66.33-108.43)
R. melanocephalus	17.96±0.20 (15.50-21.00)	9.85±0.07 (8.75-10.50)	1.83±0.02 (1.56-2.21)	138.88±1.90 (115.59-168.97)
T. fallax	18.33±0.23 (15.00-21.25)	10.33±0.10 (9.25-12.00)	1.78±0.02 (1.46-2.10)	148.80±2.57 (111.86-178.83)
T. nigriceps	18.55±0.20 (16.50-22.00)	10.43±0.11 (9.25-13.25)	1.78±0.02 (1.50-2.18)	152.14±2.79 (130.11-228.83)
M. monspessulanus	15.24±0.13 (13.75-16.75)	11.16±0.09 (9.50-12.50)	1.37±0.01 (1.20-1.56)	133.60±1.77 (102.54-161.91)
S. diadema	15.74±0.18 (13.50-19.00)	9.52±0.13 (8.00-10.75)	1.66±0.02 (1.47-1.88)	118.10±2.69 (90.08-156.61)
M. xanthina	17.08±0.16 (15.25-20.00)	7.20±0.10 (6.25-8.75)	2.38±0.03 (1.94-2.80)	96.78±2.08 (77.27-137.38)
M. albizona	17.16±0.26 (14.50-20.25)	9.67±0.13 (8.00-12.00)	1.78±0.03 (1.38-2.13)	130.72±3.31 (99.60-186.05)
M. wagneri	17.63±0.20 (15.25-19.75)	7.62±0.10 (6.00-8.75)	2.32±0.03 (1.97-2.92)	105.71±2.22 (82.43-130.11)
V. eriwanensis	16.98±0.17 (14.00-19.25)	7.58±0.08 (6.75-9.00)	2.25±0.03 (1.89-2.64)	101.16±1.65 (76.93-123.64)
M. lebetina	17.21±0.25 (13.00-21.25)	9.83±0.10 (7.75-10.75)	1.75±0.02 (1.44-2.13)	133.11±2.61 (79.09-166.81)
W. aegyptia	16.20±0.15 (14.00-18.50)	10.14±0.10 (8.75-12.50)	1.60±0.02 (1.38-1.87)	129.12±2.05 (100.68-169.27)

Leucocytes are spheroidal cells. Both small and large lymphocytes were observed in blood smears. In small lymphocytes, fairly chromophilic nuclei almost filled the whole cell. Cytoplasm was pushed to a side as a small peripheral zone (Fig. 1E). Lymphocytes were significantly the most abundant leucocytes in the blood smears. Regarding small lymphocytes, the smallest mean diameter was in *Platyceps najadum* (5.95 μm), the biggest in *E. eiselti* (8.92 μm). Large lymphocytes had a relatively wider

zone of cytoplasm (Fig. 1F). Cytoplasm was stained a pale blue, nuclei purplish blue with Wright's stain. Regarding large lymphocytes, the smallest mean diameter was in *M. monspessulanus* (10.62 µm), the biggest in *Platyceps collaris* (14.45 µm; Table 3).

Table 2/A. The erythrocyte nuclei measurements established in the peripheral bloods of 32 snake species from Turkey belonging to six families [NL: Nucleus length, NW: Nucleus width, NS: Nucleus size; NS/ES: Nucleocytoplasmic ratio].

	Nuclei				
	NL (µm)	**NW (µm)**	**NL/NW**	**NS (µm²)**	**NS/ES**
L. macroryhnchus	7.33±0.08 (6.00-8.25)	4.45±0.02 (4.00-4.75)	1.65±0.02 (1.26-1.88)	25.58±0.31 (20.85-29.14)	0.22±0.01 (0.18-0.26)
T. vermicularis	7.27±0.08 (6.00-8.25)	4.54±0.02 (4.50-4.75)	1.60±0.02 (1.26-1.83)	25.93±0.29 (21.20-29.83)	0.22±0.01 (0.18-0.25)
E. jaculus	7.16±0.09 (6.50-8.25)	4.39±0.02 (4.25-4.75)	1.63±0.02 (1.37-1.94)	24.67±0.33 (21.69-29.14)	0.22±0.02 (0.18-0.29)
C. ventromaculatus	6.91±0.06 (6.00-7.75)	4.49±0.03 (4.00-5.00)	1.54±0.02 (1.32-1.88)	24.33±0.22 (21.20-27.38)	0.18±0.01 (0.15-0.26)
D. jugularis	10.57±0.06 (9.75-11.50)	4.98±0.04 (4.50-5.50)	2.13±0.02 (1.90-2.44)	41.27±0.37 (36.21-46.41)	0.44±0.01 (0.34-0.58)
D. schmidti	7.67±0.08 (6.75-9.00)	4.27±0.02 (4.00-4.50)	1.80±0.02 (1.50-2.12)	25.70±0.31 (22.77-30.03)	0.20±0.01 (0.18-0.24)
D. caspius	10.01±0.08 (8.75-11.00)	4.84±0.04 (4.50-5.50)	2.07±0.02 (1.81-2.39)	38.08±0.52 (30.91-46.41)	0.43±0.01 (0.29-0.56)
H. nummifer	6.92±0.08 (6.00-8.00)	4.52±0.01 (4.25-4.75)	1.53±0.02 (1.26-1.88)	24.53±0.24 (22.8-27.38)	0.21±0.01 (0.18-0.24)
H. ravergieri	7.49±0.09 (6.25-9.50)	4.91±0.04 (4.50-5.75)	1.53±0.02 (1.32-1.79)	28.90±0.50 (23.30-41.02)	0.25±0.01 (0.19-0.37)
P. collaris	7.29±0.07 (6.50-8.50)	4.62±0.02 (4.50-4.75)	1.58±0.02 (1.37-1.79)	26.42±0.26 (23.84-31.69)	0.23±0.02 (0.19-0.29)
P. najadum	8.44±0.23 (7.25-10.75)	5.03±0.05 (4.50-5.75)	1.69±0.05 (1.35-2.15)	33.32±0.97 (26.49-42.19)	0.27±0.01 (0.22-0.48)
Z. hohenackeri	8.49±0.13 (6.75-10.00)	4.44±0.02 (4.25-4.75)	1.92±0.03 (1.47-2.35)	29.53±0.41 (23.84-35.33)	0.22±0.01 (0.19-0.26)
N. natrix	7.95±0.07 (7.25-8.75)	4.56±0.02 (4.25-4.75)	1.74±0.02 (1.58-1.89)	28.49±0.30 (25.61-32.63)	0.21±0.01 (0.17-0.24)
N. tessellata	10.21±0.09 (9.00-11.25)	5.04±0.04 (4.50-5.75)	2.03±0.02 (1.82-2.26)	40.46±0.61 (31.79-49.65)	0.41±0.01 (0.31-0.54)
E. rothi	7.84±0.12 (6.25-9.50)	4.14±0.02 (4.00-4.50)	1.90±0.03 (1.47-2.31)	25.45±0.38 (20.85-31.69)	0.25±0.01 (0.20-0.29)
E. levantinus	8.12±0.08 (7.00-9.00)	4.47±0.02 (4.25-4.75)	1.82±0.02 (1.56-2.12)	28.47±0.29 (24.73-33.56)	0.22±0.01 (0.19-0.26)
E. coronella	7.43±0.09 (6.50-8.75)	4.58±0.02 (4.25-4.75)	1.62±0.02 (1.42-2.06)	26.66±0.34 (22.96-31.69)	0.20±0.01 (0.15-0.28)

Table 2/B. The erythrocyte nuclei measurements established in the peripheral bloods of 32 snake species from Turkey belonging to six families [NL: Nucleus length, NW: Nucleus width, NS: Nucleus size; NS/ES: Nucleocytoplasmic ratio].

	Nuclei				
	NL (µm)	**NW (µm)**	**NL/NW**	**NS (µm²)**	**NS/ES**
E. barani	7.94±0.08 (6.25-8.75)	4.54±0.02 (4.25-4.75)	1.75±0.02 (1.32-2.06)	28.32±0.29 (23.30-31.69)	0.23±0.01 (0.20-0.26)
E. eiselti	7.29±0.05 (6.50-8.00)	4.54±0.02 (4.50-4.75)	1.60±0.01 (1.42-1.78)	26.00±0.22 (22.96-28.90)	0.25±0.01 (0.20-0.30)
E. decemlineatus	7.68±0.10 (6.75-9.00)	4.50±0.03 (4.25-5.00)	1.71±0.03 (1.35-2.00)	27.12±0.37 (23.35-31.79)	0.23±0.01 (0.21-0.27)
E. modestus	10.05±0.06 (9.25-10.75)	4.92±0.07 (4.50-7.25)	2.06±0.02 (1.31-2.28)	38.80±0.55 (32.68-54.07)	0.46±0.01 (0.36-0.62)
R. melanocephalus	7.95±0.08 (7.00-9.25)	4.47±0.02 (4.25-4.75)	1.78±0.02 (1.47-2.18)	27.88±0.28 (24.73-30.91)	0.20±0.01 (0.15-0.24)
T. fallax	7.53±0.06 (6.50-8.25)	5.06±0.04 (4.75-5.75)	1.49±0.02 (1.30-1.65)	29.87±0.34 (25.51-34.98)	0.20±0.02 (0.17-0.29)
T. nigriceps	7.96±0.10 (6.75-9.25)	4.60±0.02 (4.25-4.75)	1.73±0.02 (1.42-2.06)	28.73±0.37 (24.73-33.56)	0.19±0.02 (0.15-0.23)
M. monspessulanus	7.42±0.09 (6.25-8.75)	4.84±0.03 (4.50-5.25)	1.54±0.02 (1.25-1.83)	28.17±0.38 (23.84-34.34)	0.21±0.02 (0.18-0.26)
S. diadema	6.81±0.10 (5.75-8.25)	4.67±0.02 (4.25-5.00)	1.46±0.02 (1.21-1.74)	24.98±0.42 (21.20-30.76)	0.21±0.02 (0.17-0.26)
M. wagneri	10.61±0.07 (9.75-11.75)	4.70±0.05 (400-5.50)	2.27±0.02 (1.95-2.63)	39.19±0.55 (32.97-48.42)	0.38±0.01 (0.29-0.56)
M. albizona	7.39±0.14 (6.00-10.00)	4.36±0.05 (3.50-5.00)	1.70±0.01 (1.26-2.29)	25.32±0.62 (20.41-39.25)	0.20±0.01 (0.15-0.26)
V. eriwanensis	10.58±0.06 (10.00-11.50)	4.91±0.04 (4.50-5.50)	2.16±0.02 (1.95-2.44)	40.77±0.45 (35.33-48.57)	0.41±0.01 (0.31-0.52)
M. lebetina	6.68±0.12 (5.00-8.50)	4.74±0.05 (4.25-5.75)	1.41±0.03 (1.11-1.89)	24.87±0.51 (17.66-31.40)	0.19±0.03 (0.14-0.27)
W. aegyptia	7.53±0.08 (6.25-8.75)	4.82±0.04 (4.25-5.50)	1.57±0.02 (1.25-1.83)	28.52±0.41 (22.52-36.06)	0.22±0.01 (0.18-0.28)

Of the agranulocytes, monocytes were similar to large lymphocytes in size but could be easily differentiated by their kidney shaped nuclei (Fig. 1G). Cytoplasm was stained a light gray, the nuclei dark purplish blue with Wright's stain. They are the most abundant cells after the lymphocytes. Regarding monocytes, the smallest mean diameter was in *Dolichophis jugularis* (11.50 µm), the biggest in *Eirenis decemlineatus* (14.13 µm; Table 3). No monocytes were observed in *Macrovipera lebetina.*

Table 3. The mean measurement values of agranulocytic leucocytes established in the peripheral bloods of 32 snake species from Turkey belonging to six families, together with their standard errors.

Species	Lymphocyte (small) (µm)	Lymphocyte (large) (µm)	Monocyte (µm)
L. macroryhnchus	8.33±0.11	12.71±0.21	12.22±0.17
T. vermicularis	8.33±0.09	13.64±1.19	13.20±0.25
E. jaculus	7.89±0.09	13.71±0.12	12.72±0.30
C. ventromaculatus	8.41±0.13	14.29±0.29	13.45±0.81
D. jugularis	7.19±0.08	10.95±0.12	11.50±0.35
D. schmidti	8.34±0.13	14.00±0.27	12.21±0.65
D. caspius	7.31±0.13	10.84±0.14	13.24±0.22
H. nummifer	8.86±0.12	10.96±0.12	12.00±0.57
H. ravergieri	7.31±0.10	11.33±0.17	12.89±0.19
P. collaris	8.08±0.07	14.45±0.19	13.67±0.31
P. najadum	7.68±0.14	10.71±0.13	11.70±0.45
Z. hohenackeri	8.08±0.12	13.35±0.26	12.80±0.43
N. natrix	7.88±0.07	14.36±0.27	12.69±0.35
N. tessellata	8.59±0.42	11.30±0.27	12.72±0.25
E. rothi	8.14±0.14	12.71±0.31	12.81±0.21
E. levantinus	8.06±0.08	13.25±0.27	12.78±0.31
E. coronella	7.96±0.18	12.50±0.29	12.54±0.24
E. barani	8.11±0.09	11.98±0.17	12.40±0.23
E. eiselti	8.92±0.08	11.66±0.14	11.96±0.36
E. decemlineatus	8.25±0.11	13.30±0.35	14.13±0.30
E. modestus	8.22±0.18	10.50±0.17	12.68±0.20
R. melanocephalus	8.32±0.07	13.33±0.49	12.84±0.25
T. fallax	8.91±0.22	12.11±0.18	13.68±0.24
T. nigriceps	8.40±0.10	13.34±0.29	13.00±0.41
M. monspessulanus	8.25±0.21	11.62±0.26	12.22±0.17
S. diadema	8.44±0.09	12.41±0.21	12.40±0.23
M. xanthina	6.74±0.22	12.65±0.29	13.88±0.41
M. wagneri	6.60±0.12	11.61±0.09	13.83±0.48
M. albizona	6.30±0.27	13.20±0.21	13.17±0.25
V. eriwanensis	7.48±0.18	11.33±0.60	13.06±0.37
M. lebetina	7.75±0.31	12.40±0.58	-
W. aegyptia	8.42±0.21	12.80±0.29	13.84±0.24

Of granulocytes, the neutrophis are spheroidal cells. Their nuclei usually consisted of 2-4 lobes and placed close to the cells periphery (Fig. 1H). No neutrophils were observed in *Dolichophis jugularis*, *D. shmidti*, *D. caspius*, *Platyceps collaris*, *Z. hohenackeri*, *Natrix natrix*, *Eirenis rothi*, *E. levantinus*, *E. coronella*, *Rhyncocalamus melanocephalus*, *Spalerosophis diadema*, *Montivipera xanthina*, *M. wagneri*, *M. albizona*, *Vipera eriwanensis*, *Macrovipera lebetina* and *Walterinnesia aegyptia*. The biggest neutrophils were measured in *C. ventomaculatus* (15.40 µm), the smallest in *E. jaculus*' (10.33 µm; Table 4).

The most abundantly observed granulocytes were eosinophils. Their cytoplasm was stained a light yellowish color with Wright's stain, and was full of large, bright red granules, a characteristic of these cells. The darkly stained nuclei were masked by the cytoplasmic granules (Fig. 1I). The largest eosinophils were observed in *Typhlops vermicularis* (13.00 µm), the smallest in *Malpolon monspessulanus* (10.00 µm; Table 4).

Basophils were rarely seen in blood smears. Their large, purplish black cytoplasmic granules masked the nuclei, so their shapes were not readily distinguishable (Fig. 1J). The largest cells were seen in *Telescopus fallax* (11.92 µm), the smallest in *Eirenis barani* (9.96 µm; Table 4).

In blood smears stained with Wright's stain, nuclei of the thrombocytes were observed as quite chromofilic, filling the whole cell. Thus, the nucleocytoplasmic ratio was very high. While the thrombocytes were usually spindle shaped, some were nearly spheroidal (Fig. 1K). In blood smears, they were observed as groups of two or more cells (Fig. 1L). Measurements on thrombocytes were given in Table 4. Accordingly, the longest thrombocytes belonged to *Walterinesia aegyptia* (17.57 µm), the shortest to *Eryx jaculus* (6.25 µm).

A statistical comparison of the families showed that the longest erythrocytes were found in Viperidae (17.21 µm), the shortest in Leptotyphlopidae (15.86 µm) ($F_{5,\ 234}$= 12.029, P= 0.000). The widest erythrocytes belonged to Elapidae (10.14 µm), the narrowest to Viperidae (8.38 µm) ($F_{5,\ 234}$= 76.174, P= 0.000). A comparison of the L/W ratios showed that the most ellipsoidal cells were seen in Viperidae (2.10 µm), the most spheroidal ones in Elapidae (1.61 µm) ($F_{5,\ 234}$= 125.352, P= 0.000). Sizewise, the largest cells belonged to Elapidae (129.12 µm), the smallest to Boidae (112.83 µm) ($F_{5,\ 234}$= 15.760, P= 0.000).

Regarding nuclear length, the longest ones were seen in Viperidae (9.07 µm), the shortest ones in Boidae (7.16 µm) ($F_{5,\ 234}$= 103.883, P=0.000). Widest nuclei were observed in Elapidae (4.82 µm), the narrowest in Boidae (4.39 µm) ($F_{5,\ 234}$= 33.258, P= 0.000). A comparison of NL/NW ratios showed that the most ellipsoidal nuclei occurred in Viperidae (2.02 µm), the most spheroidal in Elapidae (1.57 µm) ($F_{5,\ 234}$= 88.329, P= 0.000). Regarding nuclear sizes, the largest nuclei were observed in Viperidae (32.36 µm), the smallest in Boidae (24.67 µm) ($F_{5,\ 234}$= 84.232, P= 0.000). Regarding nucleocytoplasmic ratios, the highest ratios belonged to Viperidae (0.30 µm) and Colubridae (0.26 µm), while in the other families the values were close to each other ($F_{5,\ 234}$= 114.23, P= 0.000).

Among Colubridae, the largest erythrocytes belonged to *Telescopus nigriceps*, the smallest to *Eirenis modestus* ($F_{22,\ 897}$= 82.289,

Table 4. The mean measurement values of granulocytic leucocytes and thrombocytes established in the peripheral bloods of 32 snake species from Turkey belonging to six families, together with their standard errors [TL: Thrombocyte length, TW: Thrombocyte width].

	Neutrophil (µm)	Eosinophil (µm)	Basophil (µm)	TL (µm)	TW (µm)
L. macroryhnchus	12.25±0.38	10.88±0.07	10.08±0.08	8.97±0.31	5.75±0.07
T. vermicularis	11.83±0.20	13.00±0.01	10.61±0.16	8.83±0.17	6.33±0.05
E. jaculus	10.33±0.08	10.65±0.06	10.17±0.08	6.25±0.08	4.45±0.05
C. ventromaculatus	15.40±0.44	10.75±0.09	11.25±0.14	14.09±0.22	6.45±0.11
D. jugularis	-	10.14±0.07	10.08±0.05	10.61±0.23	5.80±0.09
D. schmidti	-	11.25±0.18	10.91±0.27	10.15±0.14	5.46±0.08
D. caspius	-	10.13±0.13	10.06±0.16	8.81±0.23	5.19±0.07
H. nummifer	11.50±0.10	10.65±0.06	10.30±0.15	8.21±0.20	5.50±0.11
H. ravergieri	10.75±0.07	11.05±0.16	10.65±0.16	11.21±0.20	5.79±0.08
P. collaris	-	10.92±0.08	10.67±0.8	9.33±0.46	5.58±0.08
P. najadum	11.00±0.01	11.01±0.83	10.73±0.79	9.39±0.73	5.68±0.44
Z. hohenackeri	-	10.81±0.06	10.31±0.12	10.00±0.20	5.72±0.10
N. natrix	-	11.96±0.25	10.25±0.11	9.32±0.31	5.86±0.12
N. tessellata	12.98±0.10	11.50±0.24	10.72±0.06	11.07±0.22	7.10±0.08
E. rothi	-	10.58±0.17	10.00±0.18	10.55±0.37	5.65±0.06
E. levantinus	-	10.75±0.08	10.20±0.09	7.83±0.11	5.63±0.06
E. coronella	-	10.75±0.10	10.25±0.10	8.04±0.10	5.75±0.09
E. barani	11.44±0.12	10.69±0.12	9.96±0.10	7.82±0.11	5.89±0.11
E. eiselti	10.90±0.13	10.70±0.12	10.25±0.10	8.25±0.21	5.60±0.15
E. decemlineatus	12.65±0.13	11.10±0.19	10.30±0.17	14.14±0.29	6.43±0.17
E. modestus	12.47±0.20	11.25±0.22	10.30±0.09	11.08±0.23	6.22±0.11
R. melanocephalus	-	10.75±0.08	10.56±0.06	10.48±0.29	6.10±0.08
T. fallax	10.91±0.16	11.39±0.16	11.92±0.36	11.36±0.19	6.25±0.35
T. nigriceps	11.55±0.09	11.85±0.13	11.50±0.10	8.90±0.06	5.70±0.05
M. monspessulanus	12.13±0.13	10.00±0.01	10.13±0.16	13.85±0.47	6.17±0.21
S. diadema	-	10.81±0.06	11.13±0.16	8.75±0.13	5.83±0.07
M. xanthina	-	10.45±0.12	10.32±0.11	11.25±0.33	6.15±0.06
M. albizona	-	10.44±0.19	10.69±0.06	9.48±0.16	6.45±0.19
M. wagneri	-	12.39±0.21	11.21±0.40	7.58±0.84	5.20±0.50
V. eriwanensis	-	11.95±0.22	10.33±0.22	14.37±0.23	9.07±0.16
M. lebetina	-	10.56±0.21	10.13±0.16	7.92±0.09	5.03±0.08
W. aegyptia	-	-	-	17.57±0.38	6.60±0.33

P= 0.000); the largest nuclei were seen in *Dolichophis jugularis*, the smallest in *Coluber ventromaculatus* ($F_{22, 897}$= 170.139, *P*= 0.000).

Among Viperidae, no significant differences were seen regarding cell lengths ($F_{4, 195}$= 1.377, *P*= 0.243). Regarding cell sizes, the largest erythrocytes belonged to *Macrovipera lebetina*, the smallest to *Montivipera xanthina* ($F_{4, 195}$= 49.339, *P*= 0.000). The largest nuclei were seen in *Vipera eriwanensis*, the smallest in *Macrovipera lebetina* ($F_{3, 156}$= 256.030, *P*= 0.000).

Among the venomous species, the largest erythrocytes were observed in *Telescopus nigriceps*, the smallest in *Montivipera xanthina* ($F_{10, 429}$= 66.254, *P*= 0.000); the largest nuclei were seen in *Vipera eriwanensis*, the smallest in *Macrovipera lebetina* ($F_{9, 390}$= 180.964, *P*= 0.000).

Discussion and Conclusion

Reptiles constitute a heterogeneous group among vertebrates, regarding their blood cell morphologies (Hartman & Lessler 1964, Szarski & Czopek 1966, Szarski 1968, Saint Girons 1970). Wintrobe (1933) opined that the size of the erythrocytes reflected the place of a species within the evolutionary scale, the lower vertebrates and those species which were evolutionarily not successful had large and nucleated erythrocytes, while the higher vertebrates had small and enucleated ones. From this viewpoint, reptiles are regarded intermediate between amphibians and birds (Szarski & Czopek 1966, Szarski 1968). Among reptiles, the largest erythrocytes are found in *Sphenodon punctatus,* turtles and crocodiles (Hartman & Lessler 1964, Saint Girons 1970), the smallest in lacertid lizards (Hartman & Lessler 1964, Saint Girons & Saint Girons 1969, Sevinç et al. 2000).

Saint Girons and Saint Girons (1969) pointed out that the snakes constituted a homogenous group regarding their erythrocyte sizes, except *Typhlops vermicularis*; of *Typhlops, T. vermicularis* and *T. punctatus* differ greatly, erythrocytes were small and elongated with relatively large nuclei in *Typhlops vermicularis,* and thus their nucleocytoplasmic ratio was quite high.

The present work on blood smears established variations between the erythrocyte morphologies of 32 species which belonged to six families, and even some variations within the same species. The largest erythrocytes were found in *Telescopus nigriceps,* the smallest in *Eirenis modestus.* Regarding L/W ratios, the most ellipsoidal erythrocytes were observed in *Montivipera xanthina,* the least ellipsoidal or nearly spheroidal ones in *Malpolon monspessulanus*.

The presence of more or less irregular nuclei in the erythrocytes of viperid and elapid snakes was pointed out (Gulliver 1875, Jordan 1938, Saint Girons & Saint Girons 1969, Arıkan et al. 2004). Saint Girons and Saint Girons (1969) recorded that the erythrocytes and their nuclei were slightly more spherical and had greater surface areas in viperid snakes. Our findings indicate that especially in *M. xanthina* the erythrocytes possessed iregular nuclei and in *Macrovipera lebetina* the erythrocytes and their nuclei were nearly spherical. These results are similar to those of Saint Girons and Saint Girons (1969).

Saint Girons (1970) and Arıkan et al. (2004) reported that the size of the leucocytes varied not only between species, but also within a species; in blood smears of different reptiles the agranulocytes were

dominant; in eosinophils and basophils the nuclei were not easily seen because of the dense granulations of their cytoplasm. The present study also established that the size of the leucocytes varied not only between the species, but also in the blood smears of the same species. In all of the investigated snakes small and large lymphocytes were the dominant cells. Of agranulocytes, no monocytes were observed in *Macrovipera lebetina*. Of granulocytic leucocytes, forms and structures of nuclei were not apparent in eosinophils and basophils because of dense granulations of their cytoplasms. This finding supports Saint Girons (1970) and Arıkan et al. (2004). No neutrophils were observed in the viperids (*Montivipera. Xanthina*, *M. wagneri*, *M. albizona*, *V. eriwanensis*, *Macrovipera lebetina*) and of the colubrids in *Dolichophis jugularis*, *D. shmidti*, *D. caspius*, *Platyceps collaris*, *Zamenis hohenackeri*, *Eirenis rothi*, *E. levantinus*, *E. coronella*, *Natrix natrix*, *Rhynchocalamus melanocephalus* and *Spalerosophis diadema.* These results agree with the observations of Cannon et al. (1996) on some lizards and of Arıkan et al. (2004) on some viperids. Our results indicate that no granulocytic leucocytes were present in *Walterinesia aegyptia*.

Some authors described the thrombocytes in various reptiles as small, ovoidal cells with centrally localized extremely chromophilic nuclei (Taylor & Kaplan 1961, Saint Girons 1970, Canfield & Shea 1988, Arıkan et al. 2004). The present study established the presence of both small, ovoidal cells and nearly spheroidal thrombocytes; the largest of which was observed in *Walterinesia aegyptia,* the smallest in *Eryx jaculus*.

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