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MORPHOLOGY OF PERIPHERAL BLOOD CELLS FROM SOME LACERTID LIZARDS FROM TURKEY

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The present study is on the morphologies and sizes of peripheral blood cells (erythrocytes, leukocytes, and thrombocytes) on blood smears, stained with Wright's stain, in some lacertid lizards species [*Apathya cappadocica* (Werner, 1902), *Acanthodactylus boskianus* (Daudin, 1802), *Acanthodactylus harranensis* Baran et al. 2005, *Anatolacerta danfordi* (Günther, 1876), *Darevskia praticola* (Eversmann, 1834), *D. uzzelli* (Darevsky and Danielyan, 1977), *D. valentini* (Boettger, 1892), *Parvilacerta parva* (Boulenger, 1887), *Lacerta pamphylica* Schmidtler, 1975, *L. trilineata* Bedriaga, 1886, *L. viridis* (Laurenti, 1768), *Ophisops elegans* Menetries, 1832, *Mesalina brevirostris* Blanford, 1876, *Podarcis muralis* (Laurenti, 1768), *P. sicula* (Rafinesque-Schmaltz, 1810), *Timon princeps* (Blanford, 1874)] from Turkey. As a result of our survey, it was determined that the blood cells of the investigated species are shows significant variations in sizes and of leukocytes, agranulocytic leukocytes (lymphocytes and monocytes) are present as predominant cells. Moreover, of granulocytes, neutrophils were no observed in A. *danfordi*, *D. praticola*, *D. uzzelli*, and *P. parva*.

Keywords: Lacertidae; blood cell morphology; blood smears; Wright's stain.

INTRODUCTION

The first investigations about hematology of reptiles consist of comparing blood cells of reptiles with other vertebrates' ones (Gulliver, 1840, 1842). The later works include sexual and seasonal variations on cell counts and sizes (Gulliver, 1875; Werzberg, 1910; Leowenthal, 1928, 1930; Wintrobe, 1933; Jordan, 1938; Ryerson, 1949; Altman and Dittmer, 1961; Hartman and Lessler, 1964; Hutchison and Szarski, 1965; Szarski and Czopek, 1966; Saint Girons and Saint Girons, 1969; Saint Girons, 1970; Duguy, 1970; Canfield and Shea, 1988; Sevinç and Uğurtaş, 2001; Atatür et al. , 2001).

In present study, the blood cell morphologies and sizes of 16 lacertid lizard species known from Turkey were investigated comparatively and obtained data discussed in literature.

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MATERIAL AND METHODS

Specimens of 16 species in family Lacertidae were collected different date and various localities from Turkey.

The blood samples were obtained according to MacLean et al. (1973), from postorbital sinuses of the specimens via heparinized glass capillaries (MacLean et al., 1973). Blood smears were prepared and stained with Wright's stain to facilitate the measurements of morphological and size parameters of the blood cells. The 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 measurements of their lengths (L), widths (W), nuclear lengths (NL) and nuclear widths (NW). The sizes of the erythrocytes (ES) and their nuclei (NS) were computed from $ES = LW\pi/4$ and $NS = NLNW\pi/4$. 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 leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) and thrombocytes (TL, TW) were also taken to determine their sizes. One

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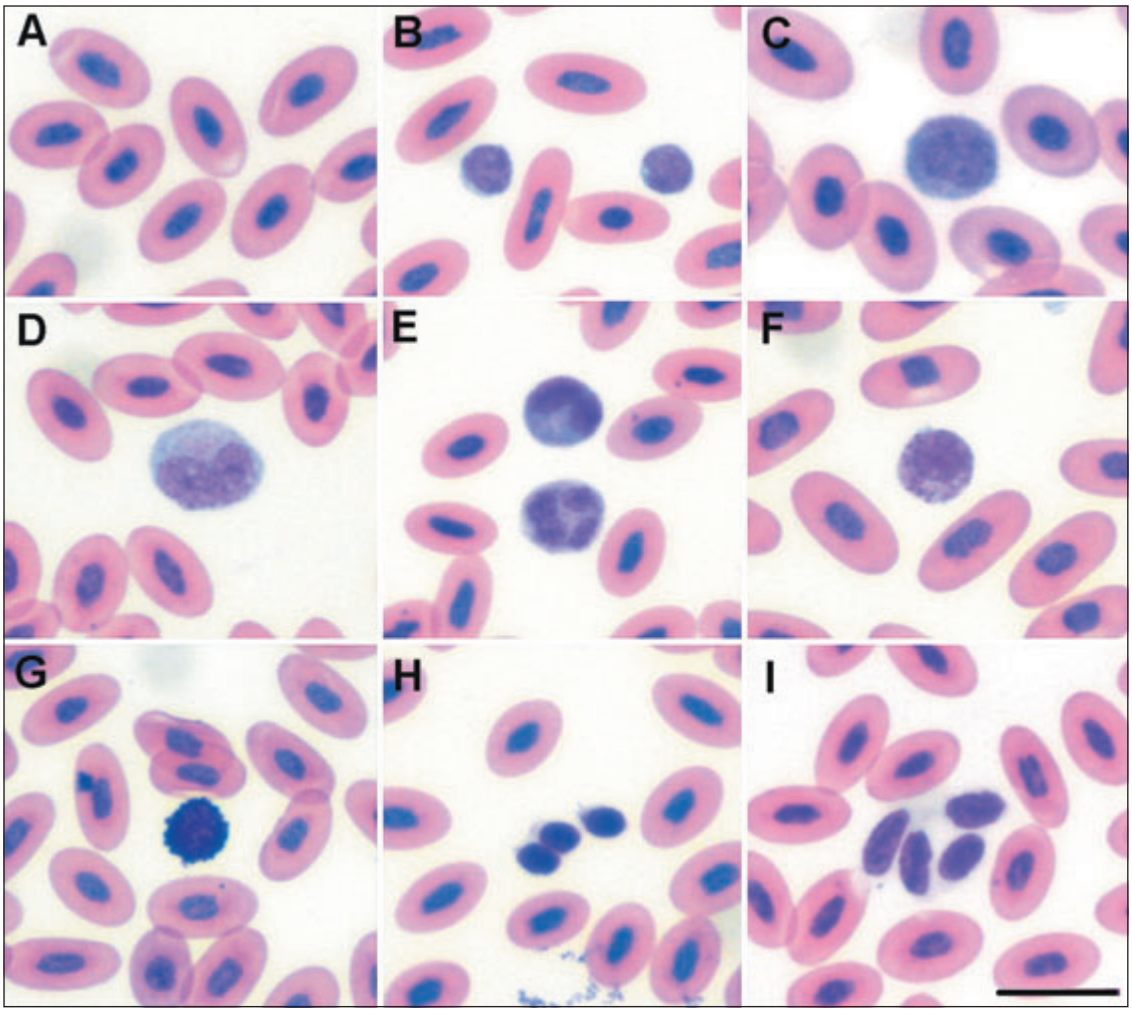

Fig. 1. Photomicrographs of peripheral blood cells of various lizards from Turkey, Wright's stain: **A**, normal erythrocytes (*Apathya cappadocica*); **B**, small lymphocyte (*Lacerta trilineata*); **C**, large lymphocyte (*Anatolacerta dandordi*); **D**, monocyte (*Mesalina brevirostris*); **E**, neutrophil (*Ophisops elegans*); **F**, eosinophile (*Lacerta pamphylica*); **G**, basophil (*A. cappadocica*); **H**, almost nearly spherical shaped thrombocytes (*O. elegans*); **I**, a group of spindle shaped thrombocytes (*M. brevirostris*). Scale bar is 15 μm.

way ANOVA test was utilized in the comparisons of the obtained data, $\alpha = 0.05$ 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 erythrocytes in lacertid lizard is oval and with nuclei just like those of all other vertebrates except of mammals. Nuclei are also ellipsoidal, more or less as regular shape uniformly localized in a central place of the cell (Fig. 1**A**). On smears stained with Wright's stain, the cytoplasms were light yellowish pink, 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.

Regarding the erythrocyte length, width and size, it was detected significant interspecific, in some cases even intraspecific variations. A statistical comparison of the species showed that the longest erythrocytes were found in *L. pamphylica* ($F_{15,624} = 30.463$, $P = 0.000$), the shortest, the narrowest and the smallest in *O. elegans*, the widest *Anatolacerta danfordi* ($F_{15,624} = 27.001$, $P = 0.000$) and the largest in *A. harranensis* ($F_{15,624} = 29.210$, $P = 0.000$). Regarding the L/W ratio, the most strongly ellipsoidal erythrocytes were observed

TABLE 1. The Erythrocyte Measurements in the Peripheral Blood Cells of 16 Lacertid Lizards Species from Turkey

Species	Erythrocytes			
	L, μm	W, μm	L/W	ES, μm^2
A. cappadocica	13.42 ± 0.90 12.00 – 15.75	7.94 ± 0.47 6.50 – 8.75	1.69 ± 0.14 1.45 – 2.12	83.73 ± 8.13 70.16 – 102.00
A. boskianus	14.22 ± 0.98 12.25 – 16.50	7.92 ± 0.41 7.00 – 8.50	1.80 ± 0.14 1.58 – 2.03	88.45 ± 8.37 74.53 – 110.10
A. harranensis	15.46 ± 1.24 12.50 – 18.00	8.56 ± 0.59 7.25 – 10.00	1.81 ± 0.13 1.47 – 2.07	104.22 ± 13.53 73.59 – 137.38
A. danfordi	14.14 ± 1.17 11.50 – 16.50	9.09 ± 0.51 7.75 – 10.00	1.56 ± 0.11 1.35 – 1.85	101.13 ± 12.10 69.96 – 122.46
D. praticola	13.08 ± 0.97 11.50 – 15.25	8.01 ± 0.38 7.25 – 9.00	1.64 ± 0.13 1.36 – 1.97	82.34 ± 7.78 67.71 – 101.76
D. uzzelli	13.65 ± 0.96 11.50 – 15.50	7.84 ± 0.48 7.00 – 8.75	1.74 ± 0.12 1.48 – 2.00	84.22 ± 9.33 64.57 – 100.09
D. valentini	13.32 ± 0.93 12.00 – 16.25	7.73 ± 0.57 6.50 – 9.00	1.73 ± 0.14 1.47 – 2.04	80.97 ± 9.47 63.78 – 102.05
P. parva	13.63 ± 0.86 11.75 – 15.50	8.01 ± 0.44 7.25 – 9.00	1.70 ± 0.12 1.42 – 1.93	85.80 ± 8.39 73.59 – 107.74
L. pamphylica	15.61 ± 1.00 13.25 – 18.00	7.89 ± 0.52 6.75 – 9.00	1.99 ± 0.16 1.64 – 2.30	96.77 ± 9.91 75.51 – 127.17
L. trilineata	14.39 ± 1.01 12.00 – 17.00	7.63 ± 0.49 6.75 – 9.00	1.89 ± 0.12 1.56 – 2.18	86.31 ± 10.22 68.88 – 116.77
L. viridis	14.94 ± 1.04 12.50 – 17.50	8.16 ± 0.56 7.25 – 9.25	1.83 ± 0.1 1.58 – 2.12	96.03 ± 11.78 71.14 – 118.00
O. elegans	12.43 ± 0.65 11.25 – 14.00	7.51 ± 0.25 7.00 – 8.00	1.66 ± 0.09 1.52 – 2.00	73.27 ± 4.88 63.19 – 83.21
M. brevirostris	14.06 ± 0.91 12.50 – 16.50	8.07 ± 0.42 6.75 – 8.75	1.75 ± 0.14 1.52 – 2.10	89.09 ± 7.52 106.86 – 118.00
P. muralis	13.93 ± 0.95 11.75 – 16.25	8.43 ± 0.59 7.00 – 9.50	1.66 ± 0.11 1.47 – 1.96	92.46 ± 11.32 65.94 – 114.81
P. sicula	13.89 ± 0.94 12.25 – 16.00	8.1 ± 0.35 7.25 – 8.75	1.74 ± 0.12 1.50 – 2.00	87.41 ± 7.85 72.12 – 109.90
T. princeps	14.98 ± 1.14 12.00 – 17.75	8.43 ± 0.47 7.50 – 9.25	1.78 ± 0.14 1.45 – 2.15	99.27 ± 10.83 77.72 – 120.20

Note. L, erythrocyte length; W, erythrocyte width; ES, erythrocyte size.

in *L. pamphylica*, the least ellipsoidal in *A. danfordi* (Table 1).

In nuclear measurements, the largest nuclei were found in *L. trilineata* ($F_{15,624} = 11.947$, $P = 0.000$), the shortest in *D. uzzelli*, the widest and the largest in *A. danfordi* ($F_{15,624} = 90.886$, $P = 0.000$; $F_{15,624} = 21.825$, $P = 0.000$), the narrowest in *O. elegans* and *M. brevirostris*, the smallest in *T. princeps*. Regarding NL/NW, the most strongly ellipsoidal nuclei were observed in *L. trilineata*, the least ellipsoidal in *D. uzzelli* ($F_{15,624} = 24.704$; $P = 0.000$). Nucleocytoplasmical ratio (NS/ES) was the highest in *O. elegans*, the lowest in *T. princeps* ($F_{15,624} = 28.336$, $P = 0.000$; Table 2).

On blood smears belonging to investigated species, lymphocytes as predominant cell amongst the different types of leukocytes were observed both small and large types. In small lymphocytes, fairly chromophilic nuclei almost filled the whole cell (Fig. 1**B**). Cytoplasm was pushed to a side as a small peripheral zone. In small lymphocytes diameter was ranged between 7.19 – 7.96 μm (Table 3). Large lymphocytes had a relatively wider zone of cytoplasm. Cytoplasm was stained a pale blue, nuclei purplish blue with Wright's stain (Fig. 1**C**). In large lymphocytes diameter was ranged from 10.07 to 13.04 μm (Table 3).

Of the agranulocytes, monocytes were similar to large lymphocytes in size but could be easily differentiated by their kidney shaped nuclei. Cytoplasm was stained a light gray, the nuclei dark purplish blue with Wright's stain. In monocytes, diameter was ranged from 10.07 to 13.39 μm (Table 3).

Of granulocytes, the neutrophils or heterophils are spheroidal cells; their nuclei were consisted of 2 – 4 lobes (Fig. 1**E**). No neutrophils were observed in *A. danfordi*, *D. praticola*, *D. uzzelli*, *P. parva*, and *P. sicula*. In

TABLE 2. The Erythrocyte Nuclei Measurements in the Peripheral Bloods of 16 Lacertid Lizards Species from Turkey

Species	NL, µm	NW, µm	NL/NW	NS, µm^2	NS/ES
A. cappadocica	6.33 ± 0.46 5.50 – 7.00	4.11 ± 0.13 4.00 – 4.25	1.54 ± 0.13 1.29 – 1.75	20.42 ± 1.58 17.27 – 22.52	0.25 ± 0.02 0.21 – 0.30
A. boskianus	6.24 ± 0.55 5.25 – 7.50	4.02 ± 0.12 3.75 – 4.25	1.56 ± 0.15 1.24 – 1.88	19.69 ± 1.70 16.49 – 23.55	0.23 ± 0.02 0.19 – 0.31
A. harranensis	6.59 ± 0.50 5.50 – 7.75	4.07 ± 0.13 3.75 – 4.25	1.62 ± 0.15 1.29 – 1.94	21.02 ± 1.49 18.06 – 25.02	0.21 ± 0.02 0.16 – 0.26
A. danfordi	6.73 ± 0.61 5.50 – 8.50	4.41 ± 0.13 4.25 – 4.75	1.53 ± 0.14 1.28 – 1.89	23.32 ± 2.30 18.35 – 30.03	0.23 ± 0.03 0.19 – 0.36
D. praticola	6.19 ± 0.43 5.25 – 7.00	4.31 ± 0.12 4.00 – 4.50	1.44 ± 0.02 1.17 – 1.69	20.93 ± 1.46 18.35 – 23.84	0.26 ± 0.02 0.21 – 0.32
D. uzzelli	5.98 ± 0.36 5.50 – 6.75	4.34 ± 0.14 4.00 – 4.50	1.38 ± 0.11 1.22 – 1.69	20.36 ± 1.22 18.35 – 23.84	0.25 ± 0.03 0.20 – 0.33
D. valentini	6.13 ± 0.48 5.25 – 7.25	4.28 ± 0.16 4.00 – 4.50	1.43 ± 0.10 1.24 – 1.61	20.63 ± 2.03 17.27 – 25.61	0.26 ± 0.03 0.20 – 0.33
P. parva	6.12 ± 0.48 5.25 – 7.25	3.98 ± 0.10 3.75 – 4.25	1.54 ± 0.12 1.31 – 1.75	19.15 ± 1.67 16.19 – 24.19	0.22 ± 0.02 0.18 – 0.28
L. pamphylica	6.33 ± 0.41 5.75 – 7.25	4.23 ± 0.11 4.00 – 4.50	1.49 ± 0.09 1.28 – 1.71	21.01 ± 1.61 18.06 – 25.61	0.22 ± 0.02 0.18 – 0.27
L. trilineata	6.93 ± 0.52 6.00 – 8.25	3.93 ± 0.11 3.75 – 4.00	1.77 ± 0.16 1.50 – 2.20	21.38 ± 1.56 17.66 – 25.12	0.25 ± 0.02 0.21 – 0.31
L. viridis	6.64 ± 0.52 5.75 – 7.75	4.36 ± 0.13 4.25 – 4.50	1.53 ± 0.14 1.28 – 1.76	22.68 ± 1.73 19.18 – 27.38	0.24 ± 0.02 0.19 – 0.31
O. elegans	6.51 ± 0.34 5.75 – 750	3.84 ± 0.15 3.50 – 4.25	1.70 ± 0.11 1.41 – 1.93	19.63 ± 1.22 16.93 – 23.55	0.27 ± 0.02 0.23 – 0.30
M. brevirostris	6.46 ± 0.49 5.50 – 7.75	3.84 ± 0.15 3.50 – 4.25	1.69 ± 0.17 1.38 – 2.07	19.48 ± 1.34 16.93 – 22.81	0.22 ± 0.02 0.18 – 0.29
P. muralis	6.36 ± 0.54 5.00 – 7.50	4.35 ± 0.12 4.25 – 4.50	1.46 ± 0.12 1.18 – 1.76	21.74 ± 2.06 16.68 – 25.61	0.24 ± 0.02 0.19 – 0.31
P. sicula	6.59 ± 0.51 5.75 – 7.75	4.19 ± 0.12 4.00 – 4.50	1.57 ± 0.13 1.35 – 1.82	21.69 ± 1.82 18.84 – 25.86	0.25 ± 0.02 0.21 – 0.32
T. princeps	6.04 ± 0.53 5.00 – 7.50	3.99 ± 0.11 3.75 – 4.25	1.52 ± 0.15 1.25 – 2.00	18.89 ± 1.67 15.70 – 22.08	0.19 ± 0.02 0.15 – 0.24

Note. NL, nucleus length; NW, nucleus width; NS, nucleus size; NS/ES, nucleocytoplasmic ratio

TABLE 3. The Mean Measurement Values of Agranulocytic Leukocytes, Granulocytic Leukocytes, and Thrombocytes in the Peripheral Bloods of 16 Lacertid Lizards Species from Turkey, Together with Their Standard Deviation

Species	Lymphocyte (small), µm	Lymphocyte (large), µm	Monocyte, µm	Neutrophil, µm	Eosinophil, µm	Basophil, µm	TL, µm	TW, µm
A. cappadocica	7.73 ± 0.26	10.68 ± 0.72	11.34 ± 0.55	10.40 ± 0.29	10.12 ± 0.30	9.70 ± 0.33	6.35 ± 0.32	5.33 ± 0.21
A. boskianus	7.96 ± 0.34	13.04 ± 1.16	13.39 ± 1.14	13.38 ± 0.97	10.20 ± 0.21	9.95 ± 0.21	7.68 ± 0.53	5.10 ± 0.32
A. harranensis	7.65 ± 0.24	10.86 ± 0.71	11.25 ± 0.57	11.13 ± 0.32	9.95 ± 0.21	9.33 ± 0.13	7.08 ± 0.45	4.96 ± 0.23
A. danfordi	7.90 ± 0.58	10.57 ± 0.82	12.33 ± 1.09	—	10.38 ± 0.14	10.56 ± 0.13	6.80 ± 0.33	5.30 ± 0.20
D. praticola	7.48 ± 0.19	11.22 ± 0.51	11.63 ± 0.55	—	9.80 ± 0.21	9.20 ± 0.21	6.28 ± 0.53	4.69 ± 0.26
D. uzzelli	7.42 ± 0.18	10.34 ± 0.67	11.00 ± 0.45	—	9.54 ± 0.17	8.93 ± 0.31	6.70 ± 0.37	4.89 ± 0.30
D. valentini	7.33 ± 0.29	10.07 ± 0.86	11.28 ± 1.00	9.42 ± 0.14	9.88 ± 0.14	9.40 ± 0.38	6.40 ± 0.39	4.69 ± 0.24
P. parva	7.47 ± 0.40	10.86 ± 0.77	11.59 ± 0.79	—	10.04 ± 0.19	9.46 ± 0.23	8.19 ± 0.47	5.07 ± 0.25
L. pamphylica	7.50 ± 0.19	10.31 ± 0.95	11.20 ± 0.78	10.15 ± 0.14	9.75 ± 0.20	9.95 ± 0.19	6.95 ± 0.44	4.90 ± 0.38
L. trilineata	7.31 ± 0.26	10.18 ± 1.52	10.07 ± 0.47	9.83 ± 0.38	9.95 ± 0.21	9.50 ± 0.20	7.66 ± 0.63	5.00 ± 0.46
L. viridis	7.54 ± 0.23	11.15 ± 0.71	11.68 ± 0.61	10.95 ± 0.41	10.50 ± 0.43	9.25 ± 0.25	7.28 ± 0.42	4.88 ± 0.21
O. elegans	7.19 ± 0.37	10.03 ± 0.67	10.70 ± 0.45	10.05 ± 0.31	9.38 ± 0.75	8.75 ± 0.25	6.30 ± 0.35	4.50 ± 0.20
M. brevirostris	7.50 ± 0.37	11.03 ± 1.26	12.30 ± 0.93	11.55 ± 0.48	10.25 ± 0.39	9.36 ± 0.18	7.29 ± 0.44	5.14 ± 0.33
P. muralis	7.75 ± 0.31	10.78 ± 1.03	12.48 ± 1.13	10.38 ± 0.14	10.15 ± 0.29	10.00 ± 0.40	7.18 ± 0.46	5.20 ± 0.39
P. sicula	7.90 ± 0.36	12.19 ± 1.27	12.27 ± 0.71	—	9.96 ± 0.19	9.25 ± 0.46	8.42 ± 0.25	5.31 ± 0.21
T. princeps	7.42 ± 0.42	10.61 ± 0.41	10.73 ± 0.62	10.37 ± 0.32	9.88 ± 0.14	9.50 ± 0.31	7.46 ± 0.42	5.35 ± 0.24

Note. TL, thrombocyte length; TW, thrombocyte width.

neutrophils, diameter was ranged from 10.05 to 13.38 μm (Table 3).

Eosinophils were easily distinguished with large bright red granules in their cytoplasm. The darkly stained nuclei were masked by the cytoplasmic granules so that their nuclei shape was not readily distinguishable (Fig. 1**F**). In eosinophils, diameter was ranged from 9.38 to 10.50 μm (Table 3).

Basophils were rarely seen on blood smears. Purplish black granules in their cytoplasm were characteristic for these cells. Nuclei were masked by the cytoplasmic granules as being in eosinophils nuclei (Fig. 1**G**). Basophils diameter was ranged from 8.75 to 10.56 μm (Table 3).

In some species, thrombocytes were observed nearly spheroidal while they were spindle shaped in some species. Quite chromophilic nuclei were large as well as almost filling the whole cell (Fig. 1**H**). Nucleoplasmic ratio in spindle shaped thrombocytes was very high. On blood smears, they were observed in groups of two or more cells (Fig. 1**I**). The length (diameter) and width of thrombocytes was ranged from 6.28 to 8.42 μm and from 4.50 to 5.35 μm, respectively (Table 3).

DISCUSSION AND CONCLUSION

Among vertebrates, the largest erythrocytes were found in urodeles (Hartman and Lessler, 1964; Foxon, 1964). Reptiles constitute a heterogeneous group among vertebrates, regarding their blood cell morphologies (Hartman and Lessler, 1964; Szarski and Czopek, 1966; Szarski, 1968; Saint Girons, 1970). According to Saint Girons and Saint Girons (1969), the largest erythrocytes are found in *Sphenodon punctatus*, member of old group, the later in turtles and crocodiles; the smallest in lacertid lizards. The numbers of erythrocytes in reptiles were lower than aves and mammals; lizards have the more high numbers than turtles in reptiles. Consequently, there is a negative correlation between the number of erythrocytes and the body sizes (Duguy, 1970).

According to various researchers (Hartman and Lessler, 1964; Szarski and Czopek, 1966; Saint Girons and Saint Girons, 1970; Sevinç et al., 2000; Atatür et al., 2001), sizes of erythrocytes in lizards varied not only amongst the families, even sometimes amongst different species in same family. In the present study, it is determined that the size of the erythrocytes varied not only amongst species, but also on the different blood smears of the same species in investigated 16 lacertid lizards coinciding the present literature. The largest erythrocytes were observed in *A. harranensis*, the smallest in *O. elegans*. We believe that these differentiations were stemmed from the possible different activation levels or environmental factors. Regarding L/W ratios, the more ellipsoidal erythrocytes were found in *L. pamphylica*, the least ellipsoidal or nearly spheroidal ones in *A. danfordi*. Atatür et al. (2001) reported that the erythrocytic nuclei were showed some interspecific variation in scincid lizards, and except of *Eumeces schneiderii* their nuclei were generally regular and more or less spherical in shape, and also there were a positive correlation between the sizes of the erythrocytes and their nuclei. The present work on blood smears established variations between the sizes of erythrocyte nuclei of 16 species which belong to the family Lacertidae. It was also determined that the nuclei were more ore less regular in shape and regarding NL/NW ratio, except some species (*L. trilineata*, *O. elegans*, and *M. brevirostris*) were established nearly spherical shape. The present study also established that the size of leukocytes varied between the species; in all of the investigated lizards agranulocytes were dominant cells; of granulocytic leukocytes, the nuclei of eosinophils and basophils were not easily seen because of the dense granulations of their cytoplasms. This finding supports Saint Girons (1970), Arıkan et al. (2004) and Arıkan et al. (in press). Of granulocytic leukocytes, no neutrophils were also observed in blood smears of *A. danfordi*, *D. praticola*, *D. uzzelli*, and *P. parva*. These results agree with the observations of Cannon et al. (1996) on some lizards, of Arıkan et al. (2004) on some viperids and of Arıkan et al. (in press) in one elapid species, *Walterinnesia aegyptia*.

Thrombocytes have been described as small, ovoidal cells with centrally localized extremely chromophilic nuclei by various Authors (Taylor and Kaplan, 1961; Saint Girons, 1970; Camfield and Shea, 1988; Sevinç and Uğurtaş, 2001; Arıkan, et al., 2004). The present study established the presence of both small, ovoidal cells and nearly spheroidal thrombocytes.

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