# Morphology of peripheral blood cells from various species of Turkish Herpetofauna

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**Abstract.** In this study, measurements of morphological and size parameters of peripheral blood cells (erythrocyte, leucocyte, thrombocyte) on blood smear preparation devices stained with Wright's stain were given for 87 species from Turkish herpetofauna (19 amphibian species including 7 urodeles and 12 anurans as well as 68 reptile species including 4 turtles, 30 lizards and 34 snakes).

It was determined that erythrocyte and nucleus sizes showed great variations among the species of herpetofauna and even among the preparations of the same species; the largest blood cells (erythrocyte, leucocyte, thrombocyte) were found in urodeles; aquatic and semiaquatic species had larger erythrocytes than terrestrials, and the largest erythrocytes were in turtles among the reptile species examined. Lymphocytes were determined as the predominant cells among the blood leucocytes in blood smears of all the examined species.

Keywords. Amphibians, reptiles, blood smears, blood cell morphology.

#### INTRODUCTION

Blood analyses are useful, widely used tools that aid in the diagnosis and monitoring of animal health and disease and in the differentiation of physiologic processes (Christopher et al., 1999). These techniques are used with several wildlife species, especially for threatened or endangered populations, and help to indicate ecosystem health (Deem et al., 2006). However, much of our knowledge regarding vertebrate blood and blood cells is based on mammalian references (Claver and Quaglia, 2009). The studies of nonmammalian vertebrate blood is relatively new (e.g., Canfield, 1998; Mader, 2000; Campbell, 2004; Allander and Fry, 2008), and blood cell morphology of reptiles still completely unknown (e.g., Frye, 1991; Mader, 2000; Campbell, 2004; Strik et al., 2007, Sykes and Klaphake, 2008).

The studies on the comparative morphologies of the peripheral blood cells in different amphibians and reptiles mainly concentrate on seasonal and sexual variations of counts (Vernberg, 1955; Altman and Dittmer, 1961; Foxon, 1964; Hutchison and Szaski, 1965; Dessaurer, 1970; Duguy, 1970; Jerrett and Mays, 1973; Alleman et al., 1999; Wojtaszek and Adamowicz, 2003; Solís et al., 2007) and sizes of blood cells (erythrocyte, leucocyte, thrombocyte) (Gulliver, 1875; Wintrobe, 1933; Hartman and Lessler, 1964; Szarski, 1968; Saint Girons and Saint Girons, 1969; Saint Girons, 1970; Wojtaszek et al., 1997; Harr et al., 2001; Knotková et al., 2002; Salakij et al., 2002), and blood parasites (Espinosa-Avilés et al., 2008; Roca and Galdón, 2010). There are several review on morphology of blood cells in amphibians and reptiles (e.g., Hutchison and Szaski, 1965; Hartman and Lessler, 1964; Szarski and Czopek, 1966; Canfield, 1998; Mader, 2000; Campbell, 2004; Allander and Fry, 2008, Sykes and Klaphake, 2008).

Since 1989, there have been several studies on morphology of peripheral blood cells in Turkish amphibians (e.g., Arıkan, 1989; Atatür et al., 1998, 1999; Arıkan et al., 2001, 2003a, 2003b, 2010; Gül and Tok, 2009) and reptiles (e.g., Arıkan et al., 2004, 2009a, 2009b; Atatür et al., 2001; Sevinç et al., 2000; Uğurtaş et al., 2003). The objective of the present study is to obtain detailed information on morphology and size of peripheral blood cell in 87 amphibian and reptile species in Turkey comparatively, and the results were discussed with literature.

#### MATERIAL AND METHODS

Individuals of 87 species belonging to sexually-mature amphibians and reptiles were collected from Anatolian and Thracian parts of Turkey (Table 1). The field studies were carried out in April-May for amphibians and in April-June for reptiles. The individuals were primarily collected on several herpetofaunal trips of previous studies or projects performed between 1989 and 2009. Blood samples were obtained from heart ventriculus of amphibians via heparinized glass capillaries, from caudal vein of turtles via heparinized injector (Hutchison and Szarski, 1965; Szarski and Czopek, 1966), and from postorbital sinuses of lizard and snake individual via heparinized glass capillaries according to MacLean et al. (1973). After obtaining blood samples in reptiles, they were released to their natural environments.

For each individual, approximately 4-5 blood smears were prepared and stained with Wright's stain for the measurements of morphology and size parameters of blood cells. Blood cells were

| Species                    | n Locality                  | Latitude  | Longitude |
|----------------------------|-----------------------------|-----------|-----------|
| Urodela                    |                             |           |           |
| Lissotriton vulgaris       | 5 Bornova – Izmir           | 38.472031 | 27.262555 |
| Lyciasalamandra atifi      | 4 Alanya – Antalya          | 36.594658 | 32.123258 |
| Mertensiella caucasica     | 3 Akçaabat – Trabzon        | 41.000657 | 39.569261 |
| Neurergus strauchii        | 10 Yam village – Bitlis     | 38.375950 | 42.091056 |
| Ommatotriton vittatus      | 5 Mezitli – Mersin          | 36.857369 | 34.397636 |
| Salamandra infraimmaculata | 4 Harbiye – Antakya (Hatay) | 36.138361 | 36.143428 |
| Triturus karelinii         | 4 Kalecik - Ankara          | 40.097222 | 33.408333 |

Table 1. Collecting localities of 87 species from Turkish Herpetofauna [n: number of individuals].

(continued).

| Table 1. | (continued). |
|----------|--------------|
|----------|--------------|

| Species                     | n  | Locality                     | Latitude  | Longitude |
|-----------------------------|----|------------------------------|-----------|-----------|
| Anura                       |    |                              |           |           |
| Pelophylax bedriagae        | 5  | Bornova-Izmir                | 38.472031 | 27.262555 |
| Pelophylax caralitanus      | 10 | Beyşehir – Konya             | 37.676389 | 31.726111 |
| Rana dalmatina              | 4  | Belgrad forest - Istanbul    | 41.194309 | 28.951383 |
| Rana holtzi                 | 5  | Mountain Bolkar – Niğde      | 37.438813 | 34.604279 |
| Rana macrocnemis            | 6  | Mountain Uludağ – Bursa      | 40.072066 | 29.216721 |
| Bufo bufo                   | 7  | Marmaris – Muğla             | 36.863411 | 28.275040 |
| Pseudepidalea variabilis    | 10 | Sülüklüpınar – Adana         | 37.040136 | 37.040136 |
| Pelobates syriacus          | 8  | Seydişehir – Konya           | 37.423871 | 31.850475 |
| Pelodytes caucasicus        | 10 | Uzungöl – Trabzon            | 40.622109 | 40.285267 |
| Bombina bombina             | 5  | Büyükdöllük – Edirne         | 41.760133 | 26.603753 |
| Hyla arborea                | 5  | Fethiye – Muğla              | 36.651389 | 29.123056 |
| Hyla savignyi               | 6  | Alanyalı village – Mersin    | 37.094734 | 34.501284 |
| Testudines                  |    |                              |           |           |
| Emys orbicularis            | 10 | Lake Yayla – Denizli         | 38.059675 | 28.778826 |
| Mauremys caspica            | 8  | Nusaybin – Mardin            | 37.066667 | 41.216667 |
| Mauremys rivulata           | 3  | Northern Cyprus              | 35.237616 | 33.471477 |
| Testudo graeca              | 8  | Izmir                        | 38.418850 | 27.128720 |
| Lacertilia                  |    |                              |           |           |
| Ablepharus chernovi         | 4  | Çamardı – Niğde              | 37.832029 | 34.986486 |
| Chalcides ocellatus         | 6  | Finike – Antalya             | 36.300827 | 30.144497 |
| Eumeces schneideri          | 5  | Meke saltern – Konya         | 37.682887 | 33.636460 |
| Ophiomorus punctatissimus   | 4  | Kaş – Antalya                | 36.204441 | 29.638982 |
| Trachylepis aurata          | 4  | Karapınar – Konya            | 37.714821 | 33.552237 |
| Trachylepis vittata         | 4  | Kırobası – Mersin            | 36.722014 | 33.909358 |
| Acanthodactylus boskianus   | 4  | Adana                        | 36.999996 | 35.321314 |
| Acanthodactylus harranensis | 7  | Şanlıurfa                    | 37.149994 | 38.799857 |
| Anatololacerta danfordi     | 4  | Çamlıyayla - Mersin          | 37.170139 | 34.608260 |
| Apathya cappadocica         | 8  | Ulukışla – Niğde             | 38.055150 | 34.310216 |
| Darevskia praticola         | 4  | Kırklareli                   | 41.733333 | 27.216667 |
| Darevskia uzzelli           | 5  | Kars                         | 40.592680 | 43.077692 |
| Darevskia valentini         | 12 | Ardahan                      | 41.110477 | 42.702174 |
| Lacerta pamphylica          | 3  | Mut – Mersin                 | 36.644337 | 33.435555 |
| Lacerta trilineata          | 7  | Çamlıyayla – Mersin          | 37.170139 | 34.608260 |
| Lacerta viridis             | 4  | Hendek – Adapazarı (Sakarya) | 40.805100 | 30.749291 |
| Mesalina brevirostris       | 2  | Akçakale – Şanlıurfa         | 36.711006 | 38.947988 |
| Ophisops elegans            | 6  | Mut – Mersin                 | 36.644337 | 33.435555 |
| Parvilacerta parva          | 2  | Çamardı – Niğde              | 37.832029 | 34.986486 |
| Podarcis muralis            | 2  | Kırklareli                   | 41.733333 | 27.216667 |
| Podarcis siculus            | 2  | Istanbul                     | 41.005270 | 28.976960 |
| Timon princes               | 2  | Eruh – Siirt                 | 37.750000 | 42.183333 |
| Eublepharis angramainyu     | 3  | Birecik – Şanlıurfa          | 37.025002 | 37.976955 |

(continued).

| Table 1 | l. (con | tinued). |
|---------|---------|----------|
|---------|---------|----------|

| Species                       | n | Locality                       |   | Latitude  | Longitude |
|-------------------------------|---|--------------------------------|---|-----------|-----------|
| Cyrtopodion heterocercum      | 2 | Mardin                         | 3 | 37.301906 | 40.730414 |
| Cyrtopodion scabrum           | 2 | Şanlıurfa                      | 3 | 37.120305 | 38.784801 |
| Hemidactylus turcicus         | 3 | Northern Cyprus                | 3 | 35.237616 | 33.471477 |
| Laudakia stellio              | 2 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Trapelus lessonae             | 2 | Birecik – Şanlıurfa            | 3 | 37.025002 | 37.976955 |
| Chamaeleo chamaeleon          | 2 | Northern Cyprus                | 3 | 35.237616 | 33.471477 |
| Varanus griseus               | 1 | Viranşehir – Şanlıurfa         | 3 | 37.178374 | 39.761510 |
| Serpentes                     |   |                                |   |           |           |
| Leptotyphlops macrorhynchus   | 2 | Birecik – Şanlıurfa            | 3 | 37.025002 | 37.976955 |
| Typhlops vermicularis         | 5 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Eryx jaculus                  | 2 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Dolichophis caspius           | 2 | Ulukışla – Niğde               | 3 | 38.055150 | 34.310216 |
| Dolichophis jugularis         | 2 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Dolichophis schmidti          | 2 | Suruç – Şanlıurfa              | 3 | 36.974652 | 38.424516 |
| Eirenis barani                | 1 | Kahramanmaraş                  | 3 | 37.583309 | 36.933403 |
| Eirenis coronella             | 2 | Birecik – Şanlıurfa            | 3 | 37.025002 | 37.976955 |
| Eirenis decemlineatus         | 1 | Diyarbakır                     | 3 | 37.914409 | 40.230624 |
| Eirenis eiselti               | 2 | Diyarbakır                     | 3 | 37.914409 | 40.230624 |
| Eirenis levantinus            | 1 | Samandağ - Antakya (Hatay)     | 3 | 36.082392 | 35.999324 |
| Eirenis modestus              | 2 | Çamlıyayla - Mersin            | 3 | 37.170139 | 34.608260 |
| Eirenis punctatolineatus      | 2 | Eruh – Siirt                   | 3 | 37.750000 | 42.183333 |
| Eirenis rothii                | 2 | Küplüce – Kilis                | 3 | 36.757230 | 37.237016 |
| Hemorrhois nummifer           | 2 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Hemorrhois ravergieri         | 1 | Sakçagözü - Gaziantep          | 3 | 36.715370 | 37.117360 |
| Malpolon monspessulanus       | 2 | Çiğli – İzmir                  | 3 | 38.499432 | 27.038216 |
| Natrix natrix                 | 4 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Natrix tessellata             | 4 | Beyşehir – Konya               | 3 | 37.676389 | 31.726111 |
| Platyceps collaris            | 1 | Midyat – Mardin                | 3 | 37.416667 | 41.369719 |
| Platyceps najadum             | 1 | Mut – Mersin                   | 3 | 36.644337 | 33.435555 |
| Platyceps ventromaculatus     | 1 | Harran – Şanlıurfa             | 3 | 36.866667 | 39.033331 |
| Rhynchocalamus melanocephalus | 1 | Antakya (Hatay)                | 3 | 36.401829 | 36.349788 |
| Spalerosophis diadema         | 2 | Birecik – Şanlıurfa            | 3 | 37.025002 | 37.976955 |
| Telescopus fallax             | 2 | Northern Cyprus                | 3 | 35.237616 | 33.471477 |
| Telescopus nigriceps          | 2 | Kilis                          | 3 | 36.718399 | 37.121220 |
| Zamenis hohenackeri           | 2 | Antakya (Hatay)                | 3 | 36.401829 | 36.349788 |
| Zamenis longissimus           | 1 | Zonguldak                      | 4 | 41.456406 | 31.798752 |
| Macrovipera lebetina          | 2 | Dikmen – Northern Cyprus       | 3 | 35.268159 | 33.324760 |
| Montivipera albizona          | 1 | Mountain Balık – Kahramanmaraş | 3 | 37.516405 | 36.449976 |
| Montivipera wagneri           | 2 | Karakurt – Kars                | 4 | 40.169027 | 42.605943 |
| Montivipera xanthina          | 2 | Gümüldür – Izmir               | 3 | 38.076415 | 27.022031 |
| Vipera eriwanensis            | 2 | Ardahan                        | 4 | 41.110477 | 42.702174 |
| Walterinnesia morgani         | 1 | Tek Tek Mountains – Şanlıurfa  | 3 | 36.812953 | 39.252838 |

measured using a MOB-1-15× micrometrical ocular. Lengths (L) and widths (W) of 40 randomly chosen erythrocytes as well as nuclear lengths (NL) and nuclear widths (NW) were measured for each blood smear. Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed from ES=  $LW\pi/4$  and NS= NLNW $\pi/4$ . Cells and nuclear shapes were compared with L/W and NL/NW ratios, and nucleus/cytoplasm with NS/ES ratio. In addition, from the blood smears of each species, measurements of leucocytes (lymphocytes, monocytes, heterophils, eosinophils, basophils) and thrombocytes (TL, TW) were also taken to determine their sizes. The photomicrographs of the blood cells were taken with Olympus BX51-Altra 20 Soft Imaging System. Correlation between body size and erythrocyte size were analyzed by non-parametric kendall  $\tau$  test.

## RESULTS

Characteristic erythrocyte shape of amphibians and reptiles we analyses is oval, similar to that of vertebrate fish and birds. Except for *Montivipera xanthina*, the erythrocytes of the examined species have a somewhat ellipsoidal nucleus, uniformly located in the centre of the cell (Fig. 1A). However, in *M. xanthina*, the irregularly shaped nuclei were determined in erythrocytes (Fig. 1L). On smears stained with Wright's stain, the cytoplasms were light yellowish pink and the chromophilic nuclei were dark purplish blue.

The blood smears of the examined species demonstrated interspecific and even intraspecific variations in terms of the lengths, widths and sizes of the erythrocytes and nuclei. The erythrocyte measurements (lengths and widths), sizes, L/W ratios, nuclear measurements and nucleocytoplasmic ratios are given in Table 2.

Among the amphibian and reptile species of Turkish herpetofauna, the largest erythrocyte was observed in urodele species (Fig. 1A). Mean length, width and size of erythrocytes in urodeles ranged respectively between 28.06  $\mu$ m-33.28  $\mu$ m, 16.63  $\mu$ m-20.13  $\mu$ m and 367.05  $\mu$ m<sup>2</sup>-523.44  $\mu$ m<sup>2</sup>. In addition, L/W ratio, mean lucleus length, mean nucleus width, mean nucleus size, NL/NW ratio and nucleocytopasmic ratio (NS/ES) were found to change between 1.63-1.80, 13.86  $\mu$ m-16.86  $\mu$ m, 8.53  $\mu$ m-10.46  $\mu$ m, 92.85  $\mu$ m<sup>2</sup>-138.51  $\mu$ m<sup>2</sup>,1.56-1.69 and 0.22-0.34, respectively. The biggest erythrocytes and nuclei were observed in *Salamandra infraimmaculata* and the smallest in *Ommatotriton vittatus*. Similarly; the most strongly ellipsoidal erythrocytes and nuclei were observed in *Mertensiella caucasica* and the least ellipsoidal in *Triturus karelinii*. And, the shortest nucleus was observed in *Lissotriton vulgaris*, and the least ellipsoidal nuclei in *Neurergus strauchii* (Table 2).

Anurans were determined to have smaller erythrocytes and nuclei than urodeles (Fig. 1B, C). Mean erythrocyte length, ranged between 15.29  $\mu$ m-24.36  $\mu$ m, erythrocyte width 9.68  $\mu$ m-15.05  $\mu$ m, erythrocyte size 116.42  $\mu$ m<sup>2</sup>-276.62  $\mu$ m<sup>2</sup>, L/W ratio 1.63-2.35, mean nucleus length 6.21  $\mu$ m-9.59  $\mu$ m, nucleus width 3.47  $\mu$ m-5.03  $\mu$ m, nucleus size 18.13  $\mu$ m<sup>2</sup>-36.66  $\mu$ m<sup>2</sup>, NL/NW ratio 1.61-2.35 and nucleocytoplasmic ratio 0.10-0.14. Among the anuran species examined, the largest and the most strongly ellipsoidal erythrocytes were observed in aquatic *Pelophylax caralitanus* (Fig. 1C) and the smallest erythrocytes in terrestrial *Pelodytes caucasicus* (Fig. 1C). The least ellipsoidal cells were found in *Pseudepidalea varibilis* and the largest nuclei in *Bombina bombina;* in addition, the shortest nuclei in *Pelobates syriacus*, the most strongly ellipsoidal nuclei in *Hyla arborea*, and the least ellipsoidal nuclei in *Rana dalmatina* (Table 2).

Of the reptiles, the largest erythrocytes were observed in turtles. And among the turtles, the largest erythrocytes were observed in aquatic species (e.g. 200.67  $\mu$ m<sup>2</sup> in *Emys orbicularis*), and the smallest erythrocytes in a terrestrial species *Testudo greaca* as 163.81  $\mu$ m<sup>2</sup> (Fig. 1D, E). In turtles; mean erythrocyte length ranged between 17.35  $\mu$ m-19.99  $\mu$ m, erythrocyte width 11.90  $\mu$ m-12.76  $\mu$ m, erythrocyte size 163.81  $\mu$ m<sup>2</sup>-200.67  $\mu$ m<sup>2</sup>, L/W ratio 1.47-1.61, mean nucleus length 6.09  $\mu$ m-7.15  $\mu$ m, nucleus width 4.91  $\mu$ m-6.31  $\mu$ m, nucleus size 23.60  $\mu$ m<sup>2</sup>-35.64  $\mu$ m<sup>2</sup>, NL/NW ratio 1.14-1.25 and nucleocytoplasmic ratio 0.15-0.20 (Table 3).

In this regard; the longest, widest and largest erythrocytes were observed in *E. orbicularis*; the most strongly ellipsoidal erythrocytes and the least ellipsoidal nuclei in *Mauremys caspica*. In addition, the smallest and the least ellipsoidal cells were found in *T. graeca* and the longest, widest and largest nuclei in *M. caspica*. However, the shortest, narrowest and the most strongly ellipsoidal nuclei were determined in *T. graeca*; the biggest nuclei



Fig. 1. Photomicrographs of erythrocytes of some species belong to Turkish Herpetofauna. A: O. vittatus, B: P. caralitanus, C: P. caucasicus, D: E. orbicularis, E: T. graeca, F: O. elegans, G: M. brevirostris, H: A. danfordi, I: L. trilineata, J: L. macrorhynchus, K: H. ravergieri, L: M. xanthina. Horizontal bar: 20 µm.

|   |                    |                  |                   |                       | ,                |                   |                 |                       |                  |
|---|--------------------|------------------|-------------------|-----------------------|------------------|-------------------|-----------------|-----------------------|------------------|
| Crossing.                                   |                    | Erythre          | ocytes            |                       |                  |                   | Nuclei          |                       |                  |
| oberres                                     | L (µm)             | W (µm)           | L/W               | ES (μm <sup>2</sup> ) | NL (µm)          | NW (µm)           | NL/NW           | NS (μm <sup>2</sup> ) | NS/ES            |
| Urodela                                     |                    |                  |                   |                       |                  |                   |                 |                       |                  |
|   |                    |                  |                   |                       |                  |                   |                 |                       |                  |
| Lissotriton vulgaris                        | $30.02\pm0.16$     | $17.81\pm0.08$   | $1.69\pm0.01$     | $419.44\pm3.11$       | $13.86\pm0.13$   | $8.53 \pm 0.07$   | $1.62 \pm 0.02$ | 92.85±0.68            | $0.22 \pm 0.002$ |
| Lyciasalamandra atifi                       | $33.28 \pm 0.17$   | $19.44 \pm 0.09$ | $1.73\pm0.02$     | $507.54\pm3.35$       | $14.99\pm0.11$   | $9.44 \pm 0.09$   | $1.59 \pm 0.02$ | $111.14\pm0.64$       | $0.22 \pm 0.002$ |
| Mertesiella caucasica                       | $31.69 \pm 0.29$   | $17.69 \pm 0.29$ | $1.80 \pm 0.02$   | $440.44\pm 5.79$      | $16.64 \pm 0.16$ | $9.84{\pm}0.09$   | $1.69 \pm 0.02$ | $128.60 \pm 0.92$     | $0.29 \pm 0.002$ |
| Neurergus strauchii                         | $31.20 \pm 0.21$   | $18.93 \pm 0.08$ | $1.65 \pm 0.01$   | $463.82 \pm 4.35$     | $15.45\pm0.07$   | $9.88 {\pm} 0.03$ | $1.56\pm0.03$   | $120.10\pm0.77$       | $0.26 \pm 0.002$ |
| <b>Ommatotriton</b> vittatus                | $28.06 \pm 0.16$   | $16.63\pm0.12$   | $1.70 \pm 0.01$   | $367.05\pm3.82$       | $16.03 \pm 0.14$ | $9.86 \pm 0.09$   | $1.63 \pm 0.02$ | $124.14\pm0.70$       | $0.34 \pm 0.002$ |
| Salamandra infraimmaculata                  | $33.10 {\pm} 0.20$ | $20.13\pm0.13$   | $1.65 \pm 0.02$   | 523.44±4.79           | $16.86 \pm 0.19$ | $10.46 \pm 0.11$  | $1.61 \pm 0.02$ | $138.51 \pm 0.84$     | $0.27 \pm 0.002$ |
| Triturus karelinii                          | $29.50 \pm 0.16$   | $18.14 \pm 0.09$ | $1.63 \pm 0.01$   | $420.37\pm 2.96$      | $14.98 \pm 0.11$ | $9.44 \pm 0.07$   | $1.59 \pm 0.03$ | $111.06\pm0.74$       | $0.26 \pm 0.002$ |
| Anura<br>Ranidae                            |                    |                  |                   |                       |                  |                   |                 |                       |                  |
| Pelophylax bedriagae                        | $23.14\pm0.22$     | $13.10 \pm 0.09$ | $1.61 {\pm} 0.03$ | 265.63±2.53           | $8.51 \pm 0.11$  | $5.01 \pm 0.01$   | $1.58 \pm 0.03$ | $38.34\pm0.74$        | $0.14{\pm}0.003$ |
| Pelophylax caralitanus                      | $24.36 \pm 0.23$   | $14.46\pm0.11$   | $1.69 \pm 0.01$   | 276.62±3.86           | $8.13 \pm 0.13$  | $5.03 \pm 0.03$   | $1.61 \pm 0.02$ | $32.15\pm0.62$        | $0.12 \pm 0.001$ |
| Rana dalmatina                              | $19.99 \pm 0.24$   | $12.11\pm0.11$   | $1.65\pm0.02$     | $190.47 \pm 3.54$     | $8.78 \pm 0.09$  | $5.59 \pm 0.02$   | $1.57\pm0.02$   | $38.48 \pm 0.42$      | $0.20 \pm 0.001$ |
| Rana holtzi                                 | $19.10\pm0.12$     | $12.80 \pm 0.06$ | $1.64 \pm 0.10$   | $192.81 \pm 1.83$     | $7.84 \pm 0.04$  | $4.13 \pm 0.04$   | $1.93\pm0.02$   | $25.46 \pm 0.27$      | $0.13 \pm 0.002$ |
| Rana macrocnemis                            | $20.55\pm0.12$     | $13.46\pm0.05$   | $1.54\pm0.01$     | $217.68\pm1.76$       | $8.66 \pm 0.06$  | $4.14 \pm 0.04$   | $2.14\pm0.03$   | $28.03\pm0.28$        | $0.13 \pm 0.002$ |
| Bufonidae                                   |                    |                  |                   |                       |                  |                   |                 |                       |                  |
| Bufo bufo                                   | $20.85 \pm 0.10$   | $13.45\pm0.07$   | $1.55\pm0.01$     | $221.22\pm1.90$       | $7.81 \pm 0.10$  | $4.34{\pm}0.10$   | $1.86 \pm 0.05$ | $26.60 \pm 0.63$      | $0.12 \pm 0.002$ |
| <i>Pseudepidalea viridis</i><br>Pelobatidae | 17.86±0.07         | $12.71 \pm 0.04$ | $1.38 \pm 0.01$   | 179.18±0.96           | 6.25±0.13        | 3.72±0.04         | $1.96 \pm 0.05$ | 18.13±0.56            | $0.11 \pm 0.002$ |
| Pelobates syriacus                          | $17.56 \pm 0.08$   | $11.70 \pm 0.07$ | $1.50 \pm 0.01$   | 161.85±1.31           | 6.63±0.09        | $3.47\pm0.04$     | $1.96 \pm 0.05$ | 18.13±0.56            | $0.11 \pm 0.002$ |
| relouinade                                  |                    |                  |                   |                       |                  |                   |                 |                       |                  |
| Pelodytes caucasicus                        | $17.56\pm0.08$     | 9.68±0.09        | $1.58\pm0.01$     | $116.42\pm 2.07$      | $6.21 \pm 0.07$  | $3.81 \pm 0.05$   | $1.63\pm0.02$   | $18.71 \pm 0.31$      | $0.16\pm0.002$   |
| Bombinatoridae                              |                    |                  |                   |                       |                  |                   |                 |                       |                  |
| Bombina bombina                             | $21.80 \pm 0.12$   | $15.05\pm0.08$   | $1.45\pm0.02$     | $258.14\pm 2.36$      | $9.59 \pm 0.16$  | $4.88 \pm 0.06$   | $1.98 \pm 0.04$ | 36.66±0.82            | $0.14 \pm 0.002$ |
| Hylidae                                     |                    |                  |                   |                       |                  |                   |                 |                       |                  |
| Hyla arborea                                | $19.80 \pm 0.10$   | $12.89 \pm 0.06$ | $1.54\pm0.01$     | $200.33 \pm 1.66$     | $7.94\pm0.10$    | $3.50 \pm 0.08$   | $2.35\pm0.07$   | $21.88 \pm 0.61$      | $0.11 \pm 0.001$ |
| Hyla savignyi                               | $18.63 \pm 0.18$   | $12.41\pm0.08$   | $1.50 \pm 0.02$   | 181.44±1.98           | 7.09±0.09        | 3.97±0.08         | $1.82 \pm 0.04$ | 22.30±0.55            | $0.12 \pm 0.002$ |

|                             |                  | Erythr           | ocytes            |                       |                 |                   | Nuclei          |                       |                  |
|-----------------------------|------------------|------------------|-------------------|-----------------------|-----------------|-------------------|-----------------|-----------------------|------------------|
| Species                     | L (µm)           | W (µm)           | L/W               | ES (μm <sup>2</sup> ) | NL (µm)         | NW (µm)           | NL/NW           | NS (μm <sup>2</sup> ) | NS/ES            |
| Testudines<br>Emydidae      |                  |                  |                   |                       |                 |                   |                 |                       |                  |
| Emys orbicularis            | $19.99 \pm 0.11$ | $12.76\pm0.09$   | $1.58 \pm 0.01$   | 200.67±1.88           | $7.15\pm0.05$   | $6.26 \pm 0.19$   | $1.19 \pm 0.01$ | $35.37\pm1.10$        | $0.18 \pm 0.01$  |
| Geoemydidae                 |                  |                  |                   |                       |                 |                   |                 |                       |                  |
| Mauremys caspica            | $18.99 \pm 0.09$ | $11.90 \pm 0.07$ | $1.61 {\pm} 0.01$ | 177.64±1.42           | $7.15\pm0.05$   | $6.31 {\pm} 0.04$ | $1.14 \pm 0.01$ | $35.64 \pm 0.41$      | $0.20 \pm 0.01$  |
| Mauremys rivulata           | $19.02\pm0.12$   | $12.19\pm0.08$   | $1.57 \pm 0.01$   | $182.74\pm1.97$       | $6.72 \pm 0.04$ | $5.89 \pm 0.04$   | $1.15\pm0.01$   | $31.22\pm0.35$        | $0.18 \pm 0.01$  |
| Testudinidae                |                  |                  |                   |                       |                 |                   |                 |                       |                  |
| Testudo graeca              | $17.35\pm0.14$   | $11.96\pm0.11$   | $1.47 \pm 0.01$   | $163.81\pm 2.34$      | $6.09 \pm 0.05$ | $4.91 \pm 0.04$   | $1.25\pm0.01$   | $23.60{\pm}0.34$      | $0.15\pm0.01$    |
| Squamata, Sauria            |                  |                  |                   |                       |                 |                   |                 |                       |                  |
| Scincidae                   |                  |                  |                   |                       |                 |                   |                 |                       |                  |
| Ablepharus chernovi         | $14.13\pm0.07$   | $7.58 \pm 0.03$  | $1.87 \pm 0.01$   | $84.12 \pm 0.57$      | $6.12 \pm 0.05$ | $2.50 \pm 0.00$   | $2.45\pm0.02$   | $12.01\pm0.10$        | $0.14 \pm 0.001$ |
| Chalcides ocellatus         | $14.68 \pm 0.06$ | $7.92\pm0.04$    | $1.86 \pm 0.01$   | 91.33±0.61            | $5.15\pm0.03$   | $2.64{\pm}0.03$   | $1.98 \pm 0.02$ | $10.70 {\pm} 0.13$    | $0.12 \pm 0.001$ |
| Eumeces schneideri          | $15.17\pm0.06$   | $7.74\pm0.04$    | $1.97 \pm 0.01$   | 92.31±0.56            | $7.11\pm0.04$   | $2.54\pm0.02$     | $2.81 \pm 0.02$ | $14.20\pm0.13$        | $0.15\pm0.001$   |
| Ophiomorus punctatissimus   | $15.14\pm0.06$   | 7.73±0.04        | $1.96 \pm 0.01$   | 92.08±0.64            | $6.05 \pm 0.05$ | $2.68 \pm 0.04$   | $2.30 \pm 0.03$ | $12.70\pm0.21$        | $0.14 \pm 0.002$ |
| Trachylepis aurata          | $14.27\pm0.08$   | 7.56±0.02        | $1.90 \pm 0.01$   | $84.88 \pm 0.54$      | $5.06 \pm 0.02$ | $2.52 \pm 0.01$   | $2.01 \pm 0.01$ | $10.02 \pm 0.07$      | $0.12 \pm 0.001$ |
| Trachylepis vittata         | $14.14\pm0.08$   | $7.55\pm0.02$    | $1.87 \pm 0.01$   | 83.77±0.53            | $6.14 \pm 0.05$ | $2.50 \pm 0.00$   | $2.46\pm0.02$   | $12.06\pm0.10$        | $0.14 \pm 0.001$ |
| Lacertidae                  |                  |                  |                   |                       |                 |                   |                 |                       |                  |
| Acanthodactylus boskianus   | $14.22\pm0.98$   | $7.92 \pm 0.41$  | $1.80 {\pm} 0.14$ | $88.45\pm 8.37$       | $6.24 \pm 0.55$ | $4.02 \pm 0.12$   | $1.56 \pm 0.15$ | $19.69 \pm 1.70$      | $0.23 \pm 0.02$  |
| Acanthodactylus harranensis | $15.46\pm 1.24$  | 8.56±0.59        | $1.81 \pm 0.13$   | $104.22\pm 13.53$     | $6.59 \pm 0.50$ | $4.07 \pm 0.13$   | $1.62 \pm 0.15$ | $21.02 \pm 1.49$      | $0.21 \pm 0.02$  |
| Anatololacerta danfordi     | $14.14\pm 1.17$  | $9.09 \pm 0.51$  | $1.56 \pm 0.11$   | $101.13\pm 12.10$     | $6.73 \pm 0.61$ | $4.41 \pm 0.13$   | $1.53 \pm 0.14$ | $23.32\pm2.30$        | $0.23 \pm 0.03$  |
| Apathya cappadocica         | $13.42\pm0.90$   | $7.94\pm0.47$    | $1.69 \pm 0.14$   | 83.73±8.13            | $6.33 \pm 0.46$ | $4.11\pm0.13$     | $1.54{\pm}0.13$ | $20.42 \pm 1.58$      | $0.25\pm0.02$    |
|                             |                  |                  |                   |                       |                 |                   |                 |                       | (continued).     |

Table 3. The exthrocyte and their nuclei measurements ( $\pm$  with their standard errors) established in the peripheral bloods of 4 turtles species belong to 3

| continued). |
|-------------|
| Table 3. (  |

| Currier                  |                  | Erythr            | ocytes          |                       |                 |                 | Nuclei          |                  |                 |
|--------------------------|------------------|-------------------|-----------------|-----------------------|-----------------|-----------------|-----------------|------------------|-----------------|
| operes                   | L (μm)           | (mu) W            | L/W             | ES (μm <sup>2</sup> ) | NL (µm)         | NW (µm)         | MN/TN           | NS ( $\mu m^2$ ) | NS/ES           |
| Darevskia praticola      | $13.08 \pm 0.97$ | $8.01{\pm}0.38$   | $1.64 \pm 0.13$ | 82.34±7.78            | $6.19 \pm 0.43$ | $4.31 \pm 0.12$ | $1.44{\pm}0.02$ | 20.93±1.46       | $0.26\pm0.02$   |
| Darevskia uzzelli        | $13.65 \pm 0.96$ | $7.84 \pm 0.48$   | $1.74 \pm 0.12$ | 84.22±9.33            | $5.98 \pm 0.36$ | $4.34 \pm 0.14$ | $1.38 \pm 0.11$ | $20.36 \pm 1.22$ | $0.25\pm0.03$   |
| Darevskia valentini      | $13.32 \pm 0.93$ | 7.73±0.57         | $1.73 \pm 0.14$ | 80.97±9.47            | $6.13 \pm 0.48$ | $4.28 \pm 0.16$ | $1.43 \pm 0.10$ | $20.63\pm 2.03$  | $0.26\pm0.03$   |
| Lacerta pamphylica       | $15.61\pm 1.00$  | 7.89±0.52         | $1.99 \pm 0.16$ | 96.77±9.91            | $6.33 \pm 0.41$ | $4.23 \pm 0.11$ | $1.49 \pm 0.09$ | $21.01\pm1.61$   | $0.22 \pm 0.02$ |
| Lacerta trilineata       | $14.39\pm 1.01$  | 7.63±0.49         | $1.89 \pm 0.12$ | $86.31 \pm 10.22$     | $6.93 \pm 0.52$ | $3.93 \pm 0.11$ | $1.77 \pm 0.16$ | $21.38 \pm 1.56$ | $0.25\pm0.02$   |
| Lacerta viridis          | $14.94\pm 1.04$  | $8.16 \pm 0.56$   | $1.83 \pm 0.10$ | 96.03±11.78           | $6.64 \pm 0.52$ | $4.36 \pm 0.13$ | $1.53 \pm 0.14$ | 22.68±1.73       | $0.24 \pm 0.02$ |
| Mesalina brevirostris    | $14.06 \pm 0.91$ | $8.07 \pm 0.42$   | $1.75 \pm 0.14$ | 89.09±7.52            | $6.46 \pm 0.49$ | $3.84{\pm}0.15$ | $1.69 \pm 0.17$ | $19.48 \pm 1.34$ | $0.22 \pm 0.02$ |
| Ophisops elegans         | $12.43\pm0.65$   | $7.51\pm0.25$     | $1.66 \pm 0.09$ | 73.27±4.88            | $6.51 \pm 0.34$ | $3.84 \pm 0.15$ | $1.70 \pm 0.11$ | $19.63\pm1.22$   | $0.27\pm0.02$   |
| Parvilacerta parva       | $13.63 \pm 0.86$ | $8.01 \pm 0.44$   | $1.70 \pm 0.12$ | 85.80±8.39            | $6.12 \pm 0.48$ | $3.98 \pm 0.10$ | $1.54\pm0.12$   | $19.15\pm 1.67$  | $0.22\pm0.02$   |
| Podarcis muralis         | $13.93 \pm 0.95$ | 8.43±0.59         | $1.66 \pm 0.11$ | 92.46±11.32           | $6.36 \pm 0.54$ | $4.35\pm0.12$   | $1.46\pm0.12$   | $21.74\pm 2.06$  | $0.24\pm0.02$   |
| Podarcis siculus         | $13.89 \pm 0.94$ | $8.10 \pm 0.35$   | $1.74 \pm 0.12$ | 87.41±7.85            | $6.59 \pm 0.51$ | $4.19 \pm 0.12$ | $1.57 \pm 0.13$ | $21.69 \pm 1.82$ | $0.25\pm0.02$   |
| Timon princeps           | $14.98\pm 1.14$  | $8.43 \pm 0.47$   | $1.78 \pm 0.14$ | 99.27±10.83           | $6.04{\pm}0.53$ | $3.99 \pm 0.11$ | $1.52\pm0.15$   | $18.89 \pm 1.67$ | $0.19\pm0.02$   |
| Euplebharidae            |                  |                   |                 |                       |                 |                 |                 |                  |                 |
| Eublepharis angramainyu  | $16.57\pm0.17$   | $8.93 \pm 0.08$   | $1.86 \pm 0.02$ | $116.29\pm 1.95$      | 7.38±0.07       | $4.38 \pm 0.02$ | $1.69 \pm 0.02$ | $25.35\pm0.24$   | $0.22 \pm 0.01$ |
| Gekkonidae               |                  |                   |                 |                       |                 |                 |                 |                  |                 |
| Cyrtopodion heterocercum | $16.17\pm0.20$   | $8.81 {\pm} 0.06$ | $1.84 \pm 0.03$ | 111.77±1.57           | 7.57±0.08       | $4.59 \pm 0.02$ | $1.65 \pm 0.02$ | 27.27±0.33       | $0.24 \pm 0.01$ |
| Cyrtopodion scabrum      | $14.83 \pm 0.10$ | $8.34{\pm}0.07$   | $1.78 \pm 0.01$ | 97.13±1.26            | 7.08±0.06       | $4.38 \pm 0.02$ | $1.62 \pm 0.01$ | $24.34\pm0.24$   | $0.25\pm0.01$   |
| Hemidactylus turcicus    | $16.56\pm0.21$   | $8.91 {\pm} 0.06$ | $1.86 \pm 0.02$ | $115.89 \pm 1.93$     | 7.44±0.09       | $4.40 \pm 0.02$ | $1.69 \pm 0.02$ | $25.71 \pm 0.32$ | $0.22 \pm 0.01$ |
| Agamidae                 |                  |                   |                 |                       |                 |                 |                 |                  |                 |
| Laudakia stellio         | $16.85 \pm 0.18$ | $9.12 \pm 0.06$   | $1.85\pm0.02$   | 120.71±1.71           | $7.84 \pm 0.08$ | $4.40 \pm 0.02$ | $1.79 \pm 0.02$ | 27.08±0.29       | $0.23 \pm 0.01$ |
| Trapelus lessonae        | $14.75\pm0.16$   | 8.69±0.08         | $1.70 \pm 0.02$ | $100.78\pm 1.67$      | $6.91 \pm 0.06$ | $4.58 \pm 0.02$ | $1.51 \pm 0.01$ | $24.83\pm0.25$   | $0.25\pm0.01$   |

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| Table 3. (continued).                 |                  |                  |                   |                       |                  |                 |                 |                       |                 |
|---------------------------------------|------------------|------------------|-------------------|-----------------------|------------------|-----------------|-----------------|-----------------------|-----------------|
| Canadian                              |                  | Erythr           | ocytes            |                       |                  |                 | Nuclei          |                       |                 |
| opecies                               | L (µm)           | (mu) W           | L/W               | ES (μm <sup>2</sup> ) | NL (µm)          | NW (µm)         | MN/TN           | NS (μm <sup>2</sup> ) | NS/ES           |
| Chamaeleonidae                        |                  |                  |                   |                       |                  |                 |                 |                       |                 |
| Chamaeleo chamaeleon                  | $15.97\pm0.16$   | 9.75±0.08        | $1.64 \pm 0.02$   | $122.34\pm 1.81$      | 7.72±0.09        | $4.85\pm0.03$   | $1.59 \pm 0.02$ | 29.37±0.35            | $0.24 \pm 0.01$ |
| Varanidae                             |                  |                  |                   |                       |                  |                 |                 |                       |                 |
| Varanus griseus                       | $16.24\pm0.13$   | $10.21 \pm 0.09$ | $1.59 \pm 0.01$   | $130.33\pm1.99$       | $7.09\pm0.07$    | $4.69 \pm 0.03$ | $1.51\pm0.02$   | $26.12\pm0.34$        | $0.20 \pm 0.01$ |
| Squamata, Ophidia<br>Leptotyphlopidae |                  |                  |                   |                       |                  |                 |                 |                       |                 |
| Leptotyphlops macrorhynchus           | $15.86 \pm 0.11$ | 9.29±0.08        | $1.71 \pm 0.02$   | $115.75\pm 1.45$      | 7.33±0.08        | $4.45\pm0.02$   | $1.65 \pm 0.02$ | $25.58 \pm 0.31$      | $0.22 \pm 0.01$ |
| Typhlopidae                           |                  |                  |                   |                       |                  |                 |                 |                       |                 |
| Typhlops vermicularis                 | $16.57\pm0.17$   | $9.13 \pm 0.06$  | $1.82 \pm 0.02$   | $118.76\pm 1.60$      | 7.27±0.08        | $4.54 \pm 0.02$ | $1.60 \pm 0.02$ | $25.93 \pm 0.29$      | $0.22 \pm 0.01$ |
| Boidae                                |                  |                  |                   |                       |                  |                 |                 |                       |                 |
| Eryx jaculus                          | $16.36 \pm 0.19$ | 8.77±0.07        | $1.87 \pm 0.02$   | $112.83\pm 2.01$      | $7.16\pm0.09$    | $4.39 \pm 0.02$ | $1.63 \pm 0.02$ | $24.67 \pm 0.33$      | $0.22 \pm 0.02$ |
| Colubridae                            |                  |                  |                   |                       |                  |                 |                 |                       |                 |
| Dolichophis caspius                   | $14.91\pm0.16$   | 7.64±0.12        | $1.96 \pm 0.02$   | 89.88±2.21            | $10.01 \pm 0.08$ | $4.84 \pm 0.04$ | 2.07±0.02       | $38.08 \pm 0.52$      | $0.43 \pm 0.01$ |
| Dolichophis jugularis                 | $16.29\pm0.18$   | $7.48\pm0.07$    | $2.18 \pm 0.02$   | 95.81±1.69            | $10.57 \pm 0.06$ | $4.98 \pm 0.04$ | $2.13\pm0.02$   | $41.27 \pm 0.37$      | $0.44 \pm 0.01$ |
| Dolichophis schmidti                  | $16.21\pm0.14$   | 9.88±0.07        | $1.64 \pm 0.01$   | 125.82±1.71           | 7.67±0.08        | $4.27 \pm 0.02$ | $1.80 \pm 0.02$ | $25.70\pm0.31$        | $0.20 \pm 0.01$ |
| Eirenis barani                        | $16.18\pm0.12$   | 9.68±0.05        | $1.67 \pm 0.01$   | $122.98\pm 1.27$      | $7.94{\pm}0.08$  | $4.54 \pm 0.02$ | $1.75\pm0.02$   | 28.32±0.29            | $0.23 \pm 0.01$ |
| Eirenis coronella                     | $16.59\pm0.23$   | $10.22 \pm 0.11$ | $1.63 \pm 0.02$   | $133.52\pm 2.81$      | 7.43±0.09        | $4.58 \pm 0.02$ | $1.62 \pm 0.02$ | $26.66 \pm 0.34$      | $0.20 \pm 0.01$ |
| Eirenis decemlineatus                 | $14.75\pm0.14$   | $10.03 \pm 0.07$ | $1.47 \pm 0.01$   | $116.25\pm 1.55$      | $7.68 \pm 0.10$  | $4.50 \pm 0.03$ | $1.71 \pm 0.03$ | $27.12 \pm 0.37$      | $0.23 \pm 0.01$ |
| Eirenis eiselti                       | $14.13\pm0.13$   | 9.62±0.07        | $1.47 \pm 0.01$   | $106.84 \pm 1.65$     | 7.29±0.05        | $4.54 \pm 0.02$ | $1.60 \pm 0.01$ | $26.00\pm0.22$        | $0.25\pm0.01$   |
| Eirenis levantinus                    | $16.60 \pm 0.15$ | $10.04 \pm 0.09$ | $1.66 \pm 0.02$   | $130.84 \pm 1.77$     | $8.12 \pm 0.08$  | $4.47\pm0.02$   | $1.82 \pm 0.02$ | 28.47±0.29            | $0.22 \pm 0.01$ |
| Eirenis modestus                      | $14.47\pm0.15$   | $7.45\pm0.08$    | $1.95 \pm 0.02$   | $84.78 \pm 1.53$      | $10.05\pm0.06$   | $4.92 \pm 0.07$ | $2.06 \pm 0.02$ | $38.80 {\pm} 0.55$    | $0.46 \pm 0.01$ |
| Eirenis punctatolineatus              | $16.22 \pm 0.15$ | 9.58±0.07        | $1.70 {\pm} 0.01$ | 122.07±1.78           | 7.63±0.08        | $4.52 \pm 0.03$ | $1.69 \pm 0.02$ | $27.06\pm0.31$        | $0.22 \pm 0.01$ |
|                                       |                  |                  |                   |                       |                  |                 |                 |                       | (continued).    |

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| (bentinned) | communda). |
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| Table 2 /   | Taulo J.   |

|                               |                  | Erythre          | ocytes          |                       |                    |                 | Nuclei            |                       |                   |
|-------------------------------|------------------|------------------|-----------------|-----------------------|--------------------|-----------------|-------------------|-----------------------|-------------------|
| species                       | L (µm)           | W (µm)           | L/W             | ES (μm <sup>2</sup> ) | NL (µm)            | NW (µm)         | NL/NW             | NS (μm <sup>2</sup> ) | NS/ES             |
| Eirenis rothii                | $14.77\pm0.15$   | 8.73±0.06        | $1.69 \pm 0.02$ | $101.24\pm 1.44$      | $7.84 \pm 0.12$    | $4.14 \pm 0.02$ | $1.90 {\pm} 0.03$ | $25.45\pm0.38$        | $0.25 \pm 0.01$   |
| Hemorrhois numnifer           | $15.61 \pm 0.10$ | 9.33±0.04        | $1.68 \pm 0.01$ | $114.30\pm0.90$       | 6.92±0.08          | $4.52 \pm 0.01$ | $1.53 \pm 0.02$   | $24.53\pm0.24$        | $0.21 \pm 0.01$   |
| Colubridae                    |                  |                  |                 |                       |                    |                 |                   |                       |                   |
| Hemorrhois ravergieri         | $14.76\pm0.16$   | 9.95±0.11        | $1.49 \pm 0.02$ | 115.38±1.91           | $7.49\pm0.09$      | $4.91 \pm 0.04$ | $1.53 \pm 0.02$   | $28.90 \pm 0.50$      | $0.25 \pm 0.01$   |
| Malpolon monspessulanus       | $15.24\pm0.13$   | $11.16\pm0.09$   | $1.37 \pm 0.01$ | $133.60\pm1.77$       | $7.42 \pm 0.09$    | $4.84{\pm}0.03$ | $1.54 \pm 0.02$   | $28.17 \pm 0.38$      | $0.21 \pm 0.02$   |
| Natrix natrix                 | $16.87 \pm 0.18$ | $10.15\pm0.08$   | $1.67 \pm 0.02$ | $134.46\pm 1.66$      | 7.95±0.07          | $4.56 \pm 0.02$ | $1.74 \pm 0.02$   | $28.49\pm0.30$        | $0.21 \pm 0.01$   |
| Natrix tessellata             | $15.98 \pm 0.21$ | 7.92±0.09        | $2.02\pm0.03$   | 99.61±2.13            | $10.21 \pm 0.09$   | $5.04 \pm 0.04$ | 2.03±0.02         | $40.46 \pm 0.61$      | $0.41 \pm 0.01$   |
| Platyceps collaris            | $14.40\pm0.12$   | $10.04 \pm 0.08$ | $1.44 \pm 0.01$ | $113.63\pm1.54$       | 7.29±0.07          | $4.62 \pm 0.02$ | $1.58 \pm 0.02$   | $26.42 \pm 0.26$      | $0.23 \pm 0.02$   |
| Platyceps najadum             | $15.47\pm0.14$   | $10.23 \pm 0.13$ | $1.52 \pm 0.01$ | $124.50\pm 2.41$      | $8.44{\pm}0.23$    | $5.03 \pm 0.05$ | $1.69 \pm 0.05$   | 33.32±0.97            | $0.27 \pm 0.01$   |
| Platyceps ventromaculatus     | $15.94 \pm 0.17$ | $10.67 \pm 0.11$ | $1.50 \pm 0.02$ | $133.60\pm 2.14$      | $6.91 \pm 0.06$    | $4.49 \pm 0.03$ | $1.54 \pm 0.02$   | $24.33\pm0.22$        | $0.18 \pm 0.01$   |
| Rhynchocalamus melanocephalus | $17.96 \pm 0.20$ | 9.85±0.07        | $1.83 \pm 0.02$ | $138.88 \pm 1.90$     | $7.95\pm0.08$      | $4.47\pm0.02$   | $1.78{\pm}0.02$   | 27.88±0.28            | $0.20 {\pm} 0.01$ |
| Spalerosophis diadema         | $15.74\pm0.18$   | 9.52±0.13        | $1.66 \pm 0.02$ | $118.10\pm 2.69$      | $6.81 \pm 0.10$    | $4.67 \pm 0.02$ | $1.46 \pm 0.02$   | $24.98 \pm 0.42$      | $0.21 \pm 0.02$   |
| Telescopus fallax             | $18.33 \pm 0.23$ | $10.33 \pm 0.10$ | $1.78 \pm 0.02$ | $148.80\pm 2.57$      | 7.53±0.06          | $5.06 \pm 0.04$ | $1.49 \pm 0.02$   | 29.87±0.34            | $0.20 \pm 0.02$   |
| Telescopus nigriceps          | $18.55 \pm 0.20$ | $10.43 \pm 0.11$ | $1.78 \pm 0.02$ | $152.14\pm 2.79$      | $7.96\pm0.10$      | $4.60 \pm 0.02$ | $1.73 \pm 0.02$   | $28.73\pm0.37$        | $0.19 \pm 0.02$   |
| Zamenis hohenackeri           | $17.66 \pm 0.24$ | $9.91 \pm 0.09$  | $1.79 \pm 0.03$ | $137.55\pm 2.51$      | $8.49 \pm 0.13$    | $4.44 \pm 0.02$ | $1.92 \pm 0.03$   | $29.53 \pm 0.41$      | $0.22 \pm 0.01$   |
| Zamenis longissimus           | $12.71 \pm 0.15$ | 7.38±0.07        | $1.72 \pm 0.02$ | 73.83±1.38            | $6.61 \pm 0.08$    | $4.56 \pm 0.03$ | $1.45\pm0.02$     | $23.63 \pm 0.31$      | $0.32 \pm 0.01$   |
| Viperidae                     |                  |                  |                 |                       |                    |                 |                   |                       |                   |
| Macrovipera lebetina          | $17.21 \pm 0.25$ | $9.83 \pm 0.10$  | $1.75 \pm 0.02$ | $133.11\pm 2.61$      | 6.68±0.12          | $4.74{\pm}0.05$ | $1.41 \pm 0.03$   | $24.87\pm0.51$        | $0.19 \pm 0.03$   |
| Montivipera albizona          | $17.16\pm0.26$   | 9.67±0.13        | $1.78{\pm}0.03$ | $130.72\pm 3.31$      | $7.39\pm0.14$      | $4.36 \pm 0.05$ | $1.70 \pm 0.01$   | $25.32\pm0.62$        | $0.20 \pm 0.01$   |
| Montivipera wagneri           | $17.63 \pm 0.20$ | $7.62 \pm 0.10$  | $2.32\pm0.03$   | $105.71\pm 2.22$      | $10.61 {\pm} 0.07$ | $4.70 \pm 0.05$ | $2.27\pm0.02$     | $39.19\pm0.55$        | $0.38 \pm 0.01$   |
| Montivipera xanthina          | $17.08 \pm 0.16$ | $7.20{\pm}0.10$  | $2.38 \pm 0.03$ | 96.78±2.08            | ı                  | ·               | ı                 | ı                     | ı                 |
| Vipera eriwanensis            | $16.98 \pm 0.17$ | 7.58±0.08        | $2.25\pm0.03$   | $101.16\pm 1.65$      | $10.58 \pm 0.06$   | $4.91 \pm 0.04$ | $2.16\pm0.02$     | $40.77 \pm 0.45$      | $0.41 \pm 0.01$   |
| Elapidae                      |                  |                  |                 |                       |                    |                 |                   |                       |                   |
| Walterinnesia morgani         | $16.20\pm0.15$   | $10.14 \pm 0.10$ | $1.60 \pm 0.02$ | 129.12±2.05           | $7.53\pm0.08$      | $4.82 \pm 0.04$ | $1.57\pm0.02$     | 28.52±0.41            | $0.22 \pm 0.01$   |

ocytoplasmic ratio in *M. caspica* and the smallest in *T. graeca*. Nuclei were found more spherical in turtles than amphibians.

In the lizard species examined; mean length, width and size of erythrocytes ranged respectively between 12.43  $\mu$ m-16.85  $\mu$ m, 7.51  $\mu$ m-10.21  $\mu$ m and 73.27  $\mu$ m<sup>2</sup>-130.33  $\mu$ m<sup>2</sup>; on the other hand, L/W ratio between 1.56-1.99 (Fig. 1F, G, H, I). In this regard, the longest erythrocytes were observed in *Laudakia stellio*; the widest and largest in *Varanus griseus*; the shortest, narrowest and smallest in *Ophisops elegans* (Fig. 1F). And in terms of L/W ratios, the most strongly ellipsoidal cells were determined in *Lacerta pamhylica* and the least ellipsoidal cells in *Anatololacerta danfordi* (Table 3). The longest nuclei were found in *L. stellio*, the widest and largest in *Chamaeleo chamaeleon*, the shortest and smallest in *Trachlepis vittata*. Considering NL/NW ratios, the most strongly ellipsoidal nuclei were found in *Eumeces schneideri*, and the least ellipsoidal in *Darevskia uzzelli*. The highest nucleocytoplasmic ratio was determined in *O. elegans*, and the smallest in *T. aurata* and *Chalcides ocellatus* (Table 3).

In the snake species examined; mean length, width and size of erythrocytes ranged respectively between 14.13 µm-18.55 µm, 7.20 µm-11.16 µm and 84.78 µm<sup>2</sup>-152.14 µm<sup>2</sup>; and L/W ratio between 1.37-2.38 (Fig. 1J, K, L). In this regard, the longest and largest erythrocytes were observed in Telescopus nigriceps; the widest in Malpolon monspessulanus; the shortest in Eirenis eiselti; the narrowest in M. xanthina and the smallest in Eirenis modestus. In terms of L/W ratio; the most strongly ellipsoidal cells were found in M. xanthina, and the least ellipsoidal in M. monspessulanus (Table 3). Because of the irregular nuclei shapes of erythrocytes in M. xanthina, measurements of nuclei were not given in Table 3. Among the examined species, the longest nuclei were observed in Montivipera wagneri; the widest in Telescopus fallax; the largest in Dolichopis jugularis; the shortest in Macrovipera lebetina; the narrowest in Eirenis rothi, and the smallest in Platyceps ventromaculatus. In terms of NL/NW ratio; the most strongly ellipsoidal nuclei were determined in *M. wagneri*; the least ellipsoidal in *M. lebetina*; the highest nucleocytoplasmic ratio in Vipera eriwanensis, and the smallest in Zamenis longissimus (Table 3). According to the data obtained in the study, there was no correlation between body size and their erythrocytes size (kendall  $\tau$  test, r = 0.024, P  $\leq$  0.845).

Regarding leucocytes, both small and large lymphocytes were observed as the dominant cells in blood smears of all species in herpetofauna. Lymphocytes and monocytes were formed by 80% in leucocytes of the examined species. In small lymphocytes, chromophilic nuclei almost filled the whole cell. Cytoplasm was pushed to a small zone (Fig. 2A). The biggest mean diameter of small lymphocytes was observed in urodeles (14.92  $\mu$ m), and the smallest (7.79  $\mu$ m) in lizards (Table 4). Spherical nuclei were more chromophilic in large lymphocytes, and localized in a certain cell zone. Cytoplasm covered larger area than small lymphocytes and was stained a pale blue, and nuclei was stained a purplish blue with Wright's stain (Fig. 2B). The biggest mean diameter in large lymphocytes was observed in urodeles (20.73  $\mu$ m), and the smallest (11.63  $\mu$ m) in lizards (Table 4).

Monocytes were similar to large lymphocytes; however, could easily be differentiated by kidney shaped nuclei. Cytoplasm was stained a light gray, and the nuclei was stained a dark purplish blue with Wright's stain (Fig. 2C, D). The biggest mean diameter in monocytes was observed in urodeles (21.00  $\mu$ m), and the smallest (12.20  $\mu$ m) in turtles (Table 4). No monocyte was observed in *M. lebetina* (a snake species).

The biggest mean diameter in heterophils of granulocytes, spherical cells, was observed in urodeles ((22.78  $\mu$ m), and the smallest (11.49  $\mu$ m) in turtles (Table 4). Their cytoplasms were stained a light blue, and the nuclei, consisting of 2-3 lobes, was stained a red to brown with Wright's stain (Fig. 2C, E, F). The granules are eosinophilic, elongated, or spindle shaped and could be numerous.

Cytoplasms of eosinophils were stained a light yellowish color with Wright's stain. Since nucleus was masked by the large and bright red granules in cytoplasm, its shape couldn't be fully distinguished (Fig. 2G, H). The biggest mean diameter in eosinophils was observed in urodeles (21.13  $\mu$ m), and the smallest in lizards (Table 4). No eosinophil was observed in *W. morgani*.



Fig. 2. Photomicrographs of leucocytes and thrombocytes of some species belong to Turkish Herpetofauna. A: Small lymphocyte (*L. trilineata*); B: Large lymphocyte (*Z. hohenackeri*); C: Monocyte and Heterophile (*O. elegans*); D: Monocyte (*P. najadum*), E: Heterophile (*N. strauchi*), F: Heterophile (*E. modestus*), G: Eosinophile (*P. najadum*), H: Eosinophile (*P. caralitanus*), I: Basophile (*A. cappadocica*), J: Basophile (*S. diadema*), K: A group of thrombocytes (*O. elegans*), L: A group of thrombocytes (*P. najadum*). Horizontal bar: 20 μm.

|            | Lymphocyte       | Lymphocyte       | Monocyte        | Heterophile      | Eosinophil       | Basophil         | Throm             | bocytes         |
|------------|------------------|------------------|-----------------|------------------|------------------|------------------|-------------------|-----------------|
|            | (small) (µm)     | (Large) (µm)     | (μm)            | (µm)             | (μm)             | (μm)             | TL (µm)           | TŴ (μm)         |
| Amphibia   |                  |                  |                 |                  |                  |                  |                   |                 |
| Urodela    | $14.92 \pm 0.20$ | 20.73±0.48       | $21.0 \pm 0.18$ | $22.78 \pm 0.14$ | 21.13±0.18       | 19.41±0.67       | 24.13±0.64        | 12.88±0.23      |
| Anura      | $9.68 \pm 0.14$  | 12.46±0.23       | 14.75±0.34      | 13.63±0.19       | 11.61±0.16       | 11.77±0.20       | $8.82 {\pm} 0.18$ | 5.93±0.12       |
| Reptilia   |                  |                  |                 |                  |                  |                  |                   |                 |
| Testudines | 8.52±0.16        | 11.91±0.18       | 12.20±0.14      | $11.49 \pm 0.71$ | $11.80 \pm 0.32$ | 10.73±0.10       | 13.68±0.35        | 6.27±0.25       |
| Squamata   |                  |                  |                 |                  |                  |                  |                   |                 |
| Sauria     | 7.79±0.13        | 11.63±0.25       | 13.52±0.28      | $12.39 \pm 0.21$ | $10.47 \pm 0.14$ | 9.99±0.10        | 7.13±0.16         | $5.02 \pm 0.06$ |
| Ophidia    | 7.99±0.11        | $12.48 \pm 0.20$ | 12.85±0.12      | 11.87±0.31       | $11.00 \pm 0.12$ | $10.52 \pm 0.08$ | $10.17 \pm 0.42$  | 5.95±0.13       |

 Table 4. Leucocytes and thrombocytes size (± with their standard errors) in the peripheral bloods of Turkish amphibians and reptiles [TL: Thrombocyte length, TW: Thrombocyte width].

The biggest mean diameter determined in basophils which was smaller than other granulocytic cells was observed in urodeles (19.41  $\mu$ m), and the smallest (9.99  $\mu$ m) in lizards (Table 4). Their cytoplasms were filled with black granules, and the nucleus was masked by granules just like in the eosinophils (Fig. 2I, J). No basophile was observed in *W. morgani*.

Thrombocytes were observed as spindle shaped in some species (Fig. 2L), and as nearly spheroidal in others (Fig. 2K). Chromophilic nuclei were found to fill nearly the whole cell. The longest and largest thrombocytes were observed in urodeles (TL = 24.13  $\mu$ m, TW = 12.88  $\mu$ m), and the shortest and narrowest in lizards (TL = 7.13  $\mu$ m, TW = 5.02  $\mu$ m, Table 4).

#### DISCUSSION AND CONCLUSIONS

As stated in literature (Wintrobe, 1933; Foxon, 1964; Hartman and Lessler, 1964; Kuramoto, 1981; Claver and Quaglia, 2009), findings of the study clearly demonstrated that urodeles had the biggest blood cells (erythrocyte, leucocyte, thrombocyte) among the amphibians and reptiles of herpetofauna, and blood smears displayed considerable interspecific and even intraspecific variations in terms of cell sizes (Fig. 1, 2; Table 2, 3).

No important difference was observed between both the erythrocyte and nucleus sizes of urodele and anuran species of the examined amphibians. However, it is impossible to attribute these differences to the correlation regarding body weight and size, defined by Vernberg (1955). More probably, these differences were derived from various environmental conditions (e.g. temperature, air pressure) (Ruiz et al. 1983, 1989) and/or various activity levels (e.g. healthy, breeding, hibernating, foraging, and daily activity) (e.g. Wojtaszek et al., 1997; Campbell, 2004; Allander and Fry, 2008, Sykes and Klaphake, 2008), for erythrocytes were found larger in aquatic species than terrestrials, and smaller in more active species. This view is compatible with the conclusions of Haden (1940), Altman and Dittmer (1961), Harris (1963), Atatür et al. (1998, 1999) and Gül and Tok (2009). L/W ratio ranged between 1.63-1.80 in urodeles, and 1.38-1.69 in anurans (Table 2); consequently, erythrocyte shape was more ellipsoidal in urodeles than anurans. NL/NW ratio ranged between 1.55-1.69 in urodeles, and 1.57-2.35 in anurans (Table 2); that is, contrary to the situation in L/W, anurans were found to have more ellipsoidal nucleus than urodeles, which was compatible with the findings of Kuramoto (1981). Nucleocytoplasmic ratio ranged between 0.22-0.34 in urodeles, and 0.10-0.16 in anurans (Table 2); that is, anurans had wider cytoplasmic surface area than urodeles in terms of the nuclear surface area in erythrocytes. Therefore, erythrocytes in anurans were more convenient for gas exchange than urodeles.

Wintrobe (1933) stated that erythrocyte size reflected the place of a species in the evolutionary scale where the lower vertebrate and the species which were unsuccessful from evolutionary aspect had larger and nucleated erythrocytes; on the other hand, the higher vertebrates had small and enucleated erythrocytes. From this respect, reptiles are regarded as intermediate between amphibians and birds (Szarski and Czopek, 1966; Szarski, 1968). Results of the study indicate that erythrocyte size reflected the place of a species in the evolutionary scale in higher taxa.

Different researchers (Hartman and Lessler, 1964; Szarski and Czopek, 1966; Saint Girons and Saint Girons, 1969; Saint Girons, 1970; Arıkan et al., 2004; Frye, 1991; Mader, 2000; Campbell, 2004; Strik et al., 2007; Sykes and Klaphake, 2008; Arıkan et al., 2009a, b; Claver and Quaglia, 2009) reported that reptiles constitute a heterogeneous group among vertebrates in terms of their blood cell morphology, and demonstrated considerable variations among orders, even within the same family members. Among reptiles, the largest erythrocytes were observed in *Sphenodon punctatus*, in turtles and crocodiles; and the smallest in lacertid lizards (Hartman and Lessler, 1964; Saint Girons and Saint Girons, 1969; Saint Girons, 1970; Sevinç et al., 2000).

Among the turtle species examined, aquatic ones had larger erythrocytes and nuclei than terrestrial *T. graeca* (Table 3). Aquatic species had more ellipsoidal erythrocytes than *T. graeca* regarding L/W ratio; however, *T. graeca* had more ellipsoidal nuclei than aquatic species regarding NL/NW ratio (Table 3). This confirms the findings of Uğurtaş et al. (2003). Nucleocytoplasmic ratio was found smaller in *T. graeca* than aquatic species (Table 3). Consequently, it can be concluded that *T. graeca* had more convenient erythrocytes for gas exchange than aquatic ones.

Among the 30 lizard species examined, the largest erythrocytes were observed in *V. griseus* and the smallest in *O. elegans.* Erythrocyte size demonstrated great variations among the families, and in some cases even within the species of the same family, which we believe were caused by different activity levels (e.g., healthy, breeding, hibernating, foraging, and other daily activities). Regarding L/W ratio, the most ellipsoidal erythrocytes were observed in *L. pamhylica*, and the least or nearly spheroidal ones in *A. danfordi.* Regarding NL/NW ratio, scincid lizards had more ellipsoidal nucleus than others (Table 3). Generally, there was a positive correlation between erythrocyte and nucleus sizes in lizards. Nucleocytoplasmic ratio ranged between 0.12-0.15 in Scincidae family, and 0.19-0.27 in others (Table 3). In this regard, we could deduce that Scincidae had more convenient erythrocytes for gas exchange than other lizards.

Saint Girons and Saint Girons (1969) reported that except for *Typhlops vermicularis* with their relatively small erythrocytes and large nuclei, the snakes formed a homoge-

nous group regarding their erythrocyte sizes. However, in this study, great variations were found among families, and even within the same family members regarding their erythrocyte sizes. Among the 34 species examined, the largest erythrocytes were observed in *T. nigriceps*, and the smallest in *Z. longissimus*. Regarding L/W ratio, the most ellipsoidal erythrocytes were in *M. xanthina*, and the least ellipsoidal or nearly spheroidal ones in *M. monspessulanus*. Results of the study indicated that small sized species (e.g., *T. vermicularis*'s mean size is 25 cm) don't have smaller erythrocytes than the biger ones (e.g., *Z. long-issimus*'s mean size is 150 cm).

Some researchers (Gulliver, 1875; Saint Girons and Saint Girons, 1969; Arıkan et al., 2004) reported the presence of somewhat irregular nuclei in the erythrocytes of viperid and elapid species. Similar results were found especially in *M. xanthina*. Regarding NL/NW ratio, the most ellipsoidal nuclei were observed in *M. wagneri*, and the least ellipsoidal in *M. lebetina*. Contrary to lizards, there was no correlation between the erythrocyte and nuclei sizes in snakes. Regarding nucleocytoplasmic ratio, snakes formed a heterogeneous group, and this ratio ranged between 0.19 and 0.46.

Lymphocytes are generally dominant leucocytes in amphibians and reptiles (Frye, 1991; Mader, 2000; Campbell, 2004; Strik et al., 2007; Allander and Fry, 2008; Sykes and Klaphake, 2008). Saint Girons (1970) and Arıkan et al. (2004, 2009a) reported that small and large lymphocytes were the dominant cells in blood smears of different reptile species, and the nuclei were not easily be distinguished, for they were masked by dense granulations in the cytoplasms of both eosinophils and basophils. In this study investigating both amphibian and reptile species of herpetofauna; the largest leucocytes were found in urodeles (Table 4); small and large lymphocytes were the dominant cells in the blood smears; and the shapes of the nuclei were not distinguished because of the dense granulations in the cytoplasms of both the eosinophils and basophils, which were all compatible with the literature (e.g., Claver and Quaglia, 2009). Though monocytes, heterophils, and eosinophils were present in amphibians and reptiles (Allander and Fry, 2008; Sykes and Klaphake, 2008), Cannon et al. (1996) reported that the heterophils were not observed in Cyrtopodion scabrum. Besides, eosinophils were observed in Crocodilia and Chelonia, but their existence in Squamata is controversial (Claver and Quaglia 2009). Even inside a genus of snakes, eosinophils were found in some species and not found in some others (Alleman et al., 1992; Troiano et al., 1997). Number and kind of leucocytes could be change environmental and physiological activity (Allander and Fry, 2008; Sykes and Klaphake, 2008). However, blood cells of reptiles still completely unknown (e.g., Frye, 1991; Mader, 2000; Campbell, 2004; Strik et al., 2007), five types of leucocytes are observed (lymphocyte, monocyte, heterophile [neutrophile], eosinophile and basophile) (Sykes and Klaphake, 2008).

Thrombocytes were defined by some researchers as spindle shaped cells with centrally localized extremely chromophilic nuclei (Saint Girons, 1970; Canfield and Shea, 1988; Arıkan et al., 2004, 2009a; Allander and Fry, 2008; Sykes and Klaphake, 2008). Spindleshaped thrombocytes were observed in some species of herpetofauna, and nearly spheroidal ones were found in some other species. In this study, the largest thrombocytes were observed in amphibians and the smallest in lizards (Table 4).

In conclusion, the findings of the study presented basic data comprising cytomorphological structure of peripheral blood cells (Table 2, 3, 4) of some Turkish amphibians and reptiles. According to the results, morphology and size of erythrocytes have showed great variations among species and even among the preparations of the same species. The largest blood cells were found in urodeles and aquatic and semiaquatic species had larger erythrocytes than terrestrials; in addition, the largest erythrocytes were in turtles among the reptile species examined. Lymphocytes were predominant cells among leucocytes in blood smears of the species.

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