Zoological Studies **42**(1): 173-178 (2003)

Zoological Studies

Erythrocyte Size and Morphology of Some Tortoises and Turtles from Turkey

İsmail Hakkı Uğurtaş *, Murat Sevinç and Hikmet Sami Yıldırımhan

Science and Art Faculty, Department of Biology, Uludag University, Bursa,Turkey

(Accepted October 5, 2002)

İsmail Hakkı Uğurtaş, Murat Sevinç and Hikmet Sami Yıldırımhan (2003) Erythrocyte size and morphology of some tortoises and turtles from Turkey. *Zoological Studies* **42**(1): 173-178. In this study, erythrocyte size and morphology of the turtles, *Emys orbicularis hellenica* and *Mauremys rivulata* (Emydidae), and tortoises, *Testudo hermanni hermanni*, and *T. graeca ibera* (Testudinidae), living in Turkey were examined. Twenty-five specimens were used in the study. There were 6 of *E. orbicularis hellenica*, 5 of *M. rivulata*, 5 of *T. h. hermanni*, and 9 of *T. graeca ibera*. Erythrocyte morphology of these examined species was described using Wright's technique. The sizes of erythrocytes and their nuclei were measured using an ocular micrometer at a magnification of 1600x. Results of this study were compared with previous work on the other reptile species. The largest and the widest erythrocytes were found in *E. orbicularis hellenica* and the smallest in *T. graeca ibera*. In terms of the studied species, the nuclear and erythrocyte sizes were found to be correlated (Testudinidae: $r = 0.494$, $p < 0.001$; Emydidae: $r = 0.668$, $p < 0.001$). http://www.sinica.edu.tw/zool/zoolstud/42.1/173.pdf

Key words: Testudinidae, Emydidae, Erythrocyte size, Erythrocyte morphology.

The 1st studies on the blood of reptiles described the structures, often comparing them with those of the other vertebrates. The literature on hematology of reptilian blood is based on a few studies, with most concerned with European species (Saint Girons 1970).

Various authors have described the various circulating blood cells of different reptile species (Ryerson 1949, Taylor and Kaplan 1961, Heady and Rogers 1963, Hartman and Lessler 1964, Szarski and Czopek 1966, Duguy 1970, Saint Girons 1970, Mateo et al. 1984, Canfield and Shea 1988, Cannon et al. 1996, Alleman et al. 1999, Sevinç et al. 2000, Sevinç and Uğurtaş 2001). Some authors have studied seasonal (Hutton 1960, Cline and Waldman 1962, Haggag et al. 1966) or sexual (Altland and Thompson 1958) variations in the number of blood cells of different reptile species. In addition, researchers have studied the number of blood cells of different reptile species (Baker and Kline 1932, Charipper and Davis 1932, Altland and Thompson 1958, Hutton 1961, Hutchinson and Szarski 1965, Engbretson and Hutchinson 1976, Mateo et al. 1984). Furthermore, authors have studied hemoglobin and hematocrit contents of blood and hematopoiesis of different reptile species (Altland and Thompson 1958, Hutton 1961, Goin and Jackson 1965, Engbretson and Hutchinson 1976, Newlin and Ballinger 1976, Mateo et al. 1984, Alleman et al. 1999). Finally, some researchers have studied on a single species (Mateo et al. 1984, Canfield and Shea 1988, Cannon et al. 1996, Alleman et al. 1999, Sevinç et al. 2000, Sevinç and Uğurtaş 2001).

In Turkey, hematological studies have generally been conducted on humans and some economically important animals. There are few hematological studies of the reptiles living in this country (Sevinç et al. 2000, Sevinç and Uğurtaş 2001). Studies on Turkish reptiles are usually restricted to morphology and systematics.

*To whom correspondence and reprint requests should be addressed. Fax: 90-224-4428022. E-mail: hakki@uludag.edu.tr

In the current study, our aim was to describe and measure erythrocytes of *Emys orbicularis hellenica*, *Mauremys rivulata*, *Testudo hermanni hermanni*, and *T. graeca ibera* which live in Turkey. This study is the 1st of its kind on Turkish species of tortoises and turtles.

MATERIALS AND METHODS

In this study, 6 (3♂♂, 3♀♀) individuals of *Emys orbicularis hellenica* (Emydidae), 5 (2♂♂, 3♀♀) of *Mauremys rivulata* (Emydidae), 5 (3♂♂, 2♀♀) of *Testudo h. hermanni* (Testudinidae), and 9 (4♂♂, 5♀♀) of *T. graeca ibera* (Testudinidae) were examined. Twelve specimens examined were male, and 13 were female. The study was carried out between Apr. and June 2000. *Testudo h. hermanni* specimens which live only in this region in Turkey were collected from different regions of Edirne, while other specimens were collected from different regions of Bursa (Table 1; Fig. 1). Blood was obtained by cardiac puncture of the turtles and tortoises (Gandal 1958). Immediately after blood was obtained in heparinized capillary tubes, blood smears were prepared. Three or 5 blood smears were prepared per individual. The smears were air-dried and stained with Wright's stain (Hartman and Lessler 1964). Twelve drops of Wright's stain were dropped to the slides and allowed to remain on the slide 90 s before rinsing with phosphate buffer (pH 6.5). The slides were allowed to stand for 10 min at room temperature, were washed with distilled water, and allowed to dry.

On each slide 50 mature erythrocytes and their nuclei were measured by means of an ocular micrometer at a magnification of 1600x. In this way 50 erythrocyte sizes were calculated from these measurements. Erythrocyte and nuclear measurements of examined species are given in tables 2-5. Erythrocyte and nuclear sizes were respectively calculated according to the formulas [(EL x EW x π) / 4] and [(NL x NW x π) / 4]; where EL is the erythrocyte length, EW is the erythrocyte width, NL is the nucleus length and NW is the nucleus width.

RESULTS

The erythrocytes or red blood cells of turtles and tortoises are nucleated, oval cells, and their nuclei are also oval and centrally located like those of the other reptile species. The cytoplasm of mature erythrocytes appeared both light and dark pink and was homogeneous under Wright's stain. The nuclei of mature erythrocytes are chromophilic (Figs. 2-5).

Because there were no significant differences between the erythrocyte sizes of female and male turtles and tortoises, the data from the females and males of individual species were pooled.

The largest and widest erythrocytes were found in *Emys orbicularis hellenica* (Table 2; Figs. 6, 7). The largest and widest nuclei were also found in *E. orbicularis hellenica* (Table 2; Figs. 6, 7). Erythrocyte and nuclear sizes and length/width ratios of *E. orbicularis hellenica* are given in table 2.

The erythrocyte length of *Mauremys rivulata* was smaller than that of *E. orbicularis hellenica* but larger than that of *Testudo h. hermanni* and *T. graeca ibera* (Table 3; Fig. 6). The erythrocyte width of *M. rivulata* was smaller than that of *E.*

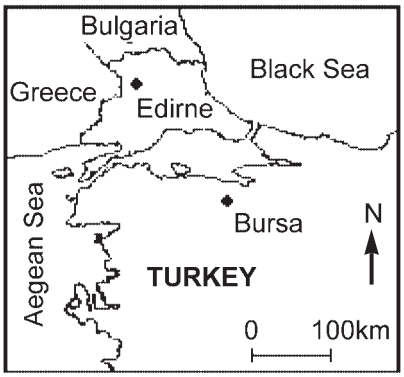

Fig. 1. Collection localities (◆).

Table 1. Materials list

Species	NM	NF	CD	CL	Museum
Emys orbicularis hellenica	3	3	15-18 June	Bursa	Zoology Museum in Uludag
Mauremys rivulata	2	3	3-7 June	Bursa	University
Testudo h. hermanni	3	2	2-5 May	Edirne	Science and Art Faculty
T. graeca ibera	4	5	10-20 April	Bursa	Department of Biology

NM: number of male, NF: number of female, CD: collection date, CL: collection locality.

orbicularis hellenica and *T. h. hermanni*, but larger than that of *T. graeca ibera* (Table 3; Fig. 7). The nuclear length of *M. rivulata* was smaller than that of *E. orbicularis hellenica* and *T. graeca ibera*, but larger than that of *T. h. hermanni* (Table 3; Fig. 6). The nuclear width of *M. rivulata* was smaller than that of *E. orbicularis hellenica* and *T. h. hermanni*, but larger than that of *T. graeca ibera* (Table 3; Fig. 7). Erythrocyte and nuclear sizes and length/width ratios of *M. rivulata* are given in table 3.

The erythrocyte length of *Testudo h. hermanni* was smaller than that of *E. orbicularis hellenica* and *M. rivulata*, but larger than that of *T. graeca ibera* (Table 4; Fig. 6). The erythrocyte width of *T. h. hermanni* was smaller than that of *E. orbicularis hellenica*, but larger than that of *M. rivulata* and *T.*

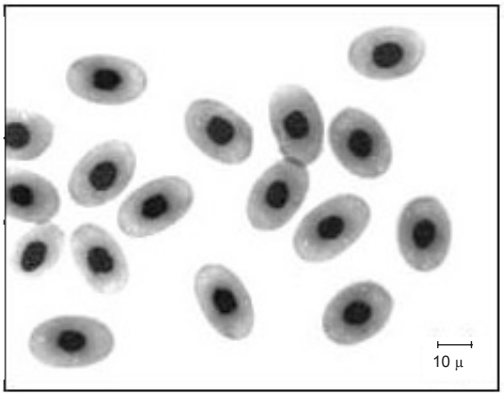

Fig. 2. Erythrocyte and nuclear sizes of *Emys orbicularis hellenica*.

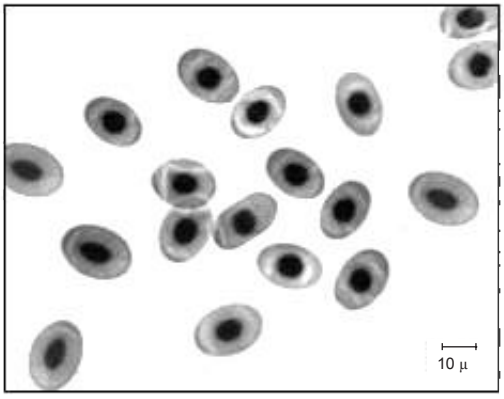

Fig. 4. Erythrocyte and nuclear sizes of *Testudo hermanni hermanni*.

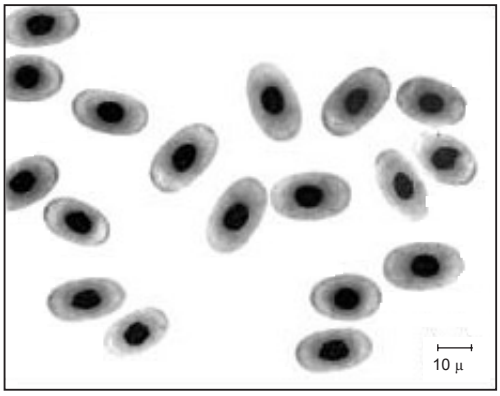

Fig. 3. Erythrocyte and nuclear sizes of *Mauremys rivulata.*

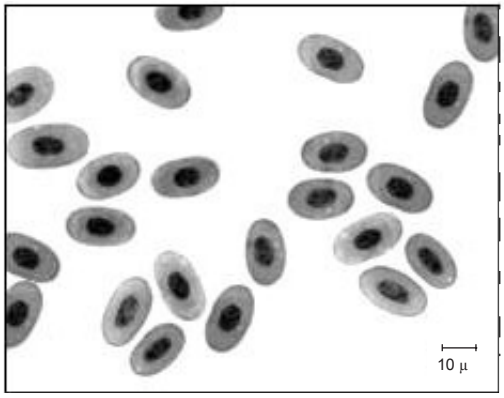

Fig. 5. Erythrocyte and nuclear sizes of *Testudo graeca ibera*.

Table 2. Erythrocyte dimensions of *Emys orbicularis hellenica* with standard deviations

	EL (µ)	EW (µ)	EL/EW	ES (µ²)	NS/ES
Maximum	23.18 ± 1.01	14.64 ± 0.98	2.05 ± 0.12	266.40 ± 22.80	0.22 ± 0.01
Minimum	19.52 ± 1.01	10.98 ± 0.98	1.50 ± 0.12	173.50 ± 22.80	0.11 ± 0.01
Mean	21.73 ± 1.01	12.53 ± 0.98	1.74 ± 0.12	214.00 ± 22.80	0.15 ± 0.01
	NL (µ)	**NW (µ)**	**NL/NW**	**NS (µ²)**	
Maximum	8.54 ± 0.54	6.71 ± 0.47	1.75 ± 0.15	44.98 ± 3.74	
Minimum	6.10 ± 0.54	4.88 ± 0.47	0.90 ± 0.15	25.70 ± 3.74	
Mean	7.53 ± 0.54	5.67 ± 0.47	1.33 ± 0.15	33.58 ± 3.74	

EL: erythrocyte length, EW: erythrocyte width, ES: erythrocyte size, NL: nucleus length, NW: nucleus width, NS: nucleus size.

graeca ibera (Table 4; Fig. 7). The nuclear length of *T. h. hermanni* was the smallest of the all (Table 4; Fig. 6). The nuclear width of *T. h. hermanni* was smaller than that of *E. orbicularis hellenica*, but larger than that of *M. rivulata* and *T. graeca ibera* (Table 4; Fig. 7). Erythrocyte and nuclear sizes and length/width ratios of *T. h. hermanni* are given in table 4.

The smallest erythrocyte length and width were found in *Testudo graeca ibera* (Table 5; Figs. 6, 7). The nuclear length of *T. graeca ibera* was smaller than that of *E. orbicularis hellenica*, but larger than that of *M. rivulata* and *T. h. hermanni* (Table 5; Fig. 6). The mean width of the nucleus of *T. graeca ibera* was the smallest of all (Table 5; Fig. 7). Erythrocyte and nuclear sizes and length/width ratios of *T. graeca ibera* are given in table 5.

DISCUSSION

Investigations carried out by various authors (Hartman and Lessler 1964, Szarski and Czopek 1966, Saint Girons 1970, Sevinç et al. 2000, Sevinç and Uğurtaş 2001) reported that the sizes of erythrocytes vary in members of the 4 orders of reptiles. Within the class Reptilia, the largest erythrocytes are seen in *Sphenedon punctatus*, turtles, and crocodilians (Hartman and Lesler 1964, Saint Girons 1970, Alleman et al. 1984). The smallest erythrocytes are found in the Lacertidae family (Hartman and Lessler 1964, Saint Girons 1970, Sevinç et al. 2000, Sevinç and Uğurtaş 2001).

The cryptodiran turtles have larger erythrocytes than do other orders of reptiles (Saint Girons 1970).

Szarski and Czopek (1966) reported that the largest erythrocytes were found *Chelydra serpentina* (22.5 μ), *Trionyx spinifer* (22.2 μ), and *Emys blandingi* (21.0 μ). Taylor and Kaplan (1961) measured the erythrocytes of *Pseudemys scripta elegans* (20.5 μ). Hartman and Lessler (1964) measured the erythrocytes of *Gopherus polyphemus* (19.1 μ) and *Pseudemys ornata* (18.6 μ). Heady and Rogers (1963) measured the erythrocytes of *Pseudemys elegans* (18.5 μ).

There are smaller numbers of erythrocytes in reptiles than in mammals or birds. Lizards have more erythrocytes than snakes, while turtles have the fewest. Since lizards have the smallest erythrocytes of all reptiles, and turtles the largest, there may be an inverse correlation between the

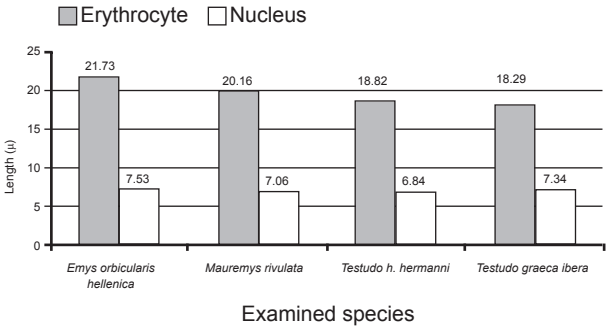

Fig. 6. Erythrocyte and nuclear lengths of examined species.

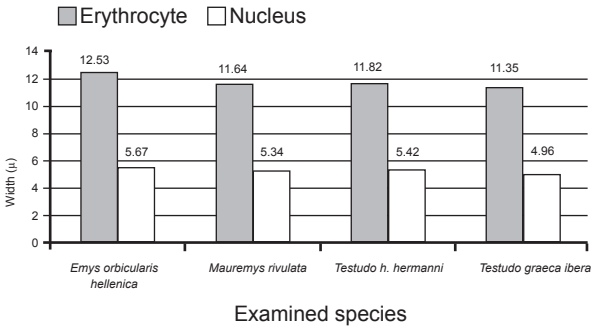

Fig. 7. Erythrocyte and nuclear widths of examined species.

Table 3. Erythrocyte dimensions of *Mauremys rivulata* with standard deviations

	EL (μ)	EW (μ)	EL/EW	ES (μ²)	NS/ES
Maximum	21.96 ± 0.84	13.42 ± 0.63	1.94 ± 0.09	231.30 ± 14.81	0.21 ± 0.01
Minimum	18.30 ± 0.84	10.37 ± 0.63	1.52 ± 0.09	158.90 ± 14.81	0.13 ± 0.01
Mean	20.16 ± 0.84	11.64 ± 0.63	1.73 ± 0.09	184.30 ± 14.81	0.16 ± 0.01
	NL (μ)	**NW (μ)**	**NL/NW**	**NS (μ²)**	
Maximum	9.15 ± 0.61	6.10 ± 0.46	1.71 ± 0.13	43.81 ± 4.22	
Minimum	6.10 ± 0.61	4.27 ± 0.46	1.00 ± 0.13	23.37 ± 4.22	
Mean	7.06 ± 0.61	5.34 ± 0.46	1.32 ± 0.13	29.74 ± 4.22	

EL: erythrocyte length, EW: erythrocyte width, ES: erythrocyte size, NL: nucleus length, NW: nucleus width, NS: nucleus size.

number of erythrocytes and their size; this hypothesis was advanced by Ryerson (1949) (Duguy 1970). Duguy (1970) reported that the number of erythrocytes was 260 000 - 680 000 no./mm^3 in *Emys orbicularis* and 362 000 - 730 000 no./mm^3 in *T. graeca ibera*.

In our study, the largest and the widest erythrocytes and nuclei were found in the turtles, *E. orbicularis hellenica* and *M. rivulata*; the smallest ones in the tortoises, *T. graeca ibera* and *T. h. hermanni*. We did not count the number of erythrocytes in these turtles and tortoises. However according to Ryerson's (1949) and Duguy's (1970) hypothesis, our results (Tables 2-5; Figs. 6, 7) are in agreement with the results of other researchers (Taylor and Kaplan 1961, Heady and Rogers 1963, Hartman and Lessler 1964, Szarski and Czopek 1966). Since turtles have fewer erythrocytes (260 000 - 680 000 no./mm^3 in *E. orbicularis*, Duguy 1970) than tortoises (362 000 - 730 000 no./mm^3 in *T. graeca ibera*), they should have larger erythrocytes.

REFERENCES

Alleman AR, ER Jacopson, ER Raskins. 1992. Morphologic, cytochemical staining and ultrastructural characteristics of blood cells from eastern diamondback rattlesnake (*Crotalus adamanteus*). Am. J. Vet. Res. **60:** 507-514.

Altland PD, EC Thompson. 1958. Some factors affecting blood formation in turtles. Proc. Soc. Exp. Biol. Med. **99:** 456-459.

Baker EGS, LE Kline. 1932. Comparative erythrocyte count of representative vertebrates. Proc. Indian Acad. Sci. **41:** 417-418.

Canfield PJ, GM Shea. 1988. Morphological observations on the erythrocytes, leukocytes and thrombocytes of blue tongue lizards (Lacertilia: Scincidae, *Tiliqua*). Anat. Histol. Embryol. **17:** 328-342.

Cannon MS, DA Freed, PS Freed. 1996. The leukocytes of the roughtail gecko *Cyrtopodion scabrum*: a bright-field and phase-contrast study. Anat. Histol. Embryol. **25:** 11-14.

Charipper HA, D Davis. 1932. Studies on the arneth count. XX. A study of the blood cells of *Pseudemys elegans* with special reference to the polymorphonuclear leukocytes. Q. J. Exp. Physiol. **21:** 371-382.

Cline MJ, TA Waldmann. 1962. Effect of temperature on red cells in the alligator. Proc. Soc. Exp. Biol. Med. **111:** 716-718.

Duguy R. 1970. Numbers of blood cells and their variation. *In* C Gans, ed. Biology of the reptilia. Vol. 3, Morphology C. New York: Academic Press, pp. 93-104.

Engbretson GA, VH Hutchinson. 1976. Erythrocyte count, hematocrit and haemoglobin content in the lizard.

Table 4. Erythrocyte dimensions of *Testudo h. hermanni* with the standard deviations

	EL (μ)	EW (μ)	EL/EW	ES (μ^2)	NS/ES
Maximum	20.13 ± 0.80	13.42 ± 0.59	1.77 ± 0.08	212.10 ± 13.58	0.20 ± 0.01
Minimum	17.08 ± 0.80	10.98 ± 0.59	1.40 ± 0.08	125.50 ± 13.58	0.12 ± 0.01
Mean	18.82 ± 0.80	11.82 ± 0.59	1.59 ± 0.08	174.90 ± 13.58	0.16 ± 0.01
	NL (μ)	**NW (μ)**	**NL/NW**	**NS (μ^2)**	
Maximum	7.93 ± 0.52	6.71 ± 0.46	1.62 ± 0.13	38.56 ± 3.52	
Minimum	6.10 ± 0.52	4.88 ± 0.46	0.90 ± 0.13	23.37 ± 3.52	
Mean	6.84 ± 0.52	5.42 ± 0.46	1.27 ± 0.13	29.19 ± 3.52	

EL: erythrocyte length, EW: erythrocyte width, ES: erythrocyte size, NL: nucleus length, NW: nucleus width, NS: nucleus size.

Table 5. Erythrocyte dimensions of *Testudo graeca ibera* with the standard deviations

	EL (μ)	EW (μ)	EL/EW	ES (μ^2)	NS/ES
Maximum	20.13 ± 0.76	12.81 ± 0.54	1.83 ± 0.08	202.40 ± 11.84	0.22 ± 0.02
Minimum	15.86 ± 0.76	10.37 ± 0.54	1.42 ± 0.08	129.10 ± 11.84	0.13 ± 0.02
Mean	18.29 ± 0.76	11.35 ± 0.54	1.61 ± 0.08	163.00 ± 11.84	0.17 ± 0.02
	NL (μ)	**NW (μ)**	**NL/NW**	**NS (μ^2)**	
Maximum	9.15 ± 0.56	6.10 ± 0.54	2.14 ± 0.20	39.43 ± 3.73	
Minimum	6.10 ± 0.56	4.27 ± 0.54	1.10 ± 0.20	20.45 ± 3.73	
Mean	7.34 ± 0.56	4.96 ± 0.54	1.49 ± 0.20	28.60 ± 3.73	

EL: erythrocyte length, EW: erythrocyte width, ES: erythrocyte size, NL: nucleus length, NW: nucleus width, NS: nucleus size.

Liolaemus multiformis. Copeia **1976:** 86.
Gandal CP. 1958. A practical method of obtaining blood from anaesthetized turtles by means of cardiac puncture. Zoologica **43:** 93-94.
Goin CJ, CG Jackson. 1965. Haemoglobin values of some amphibians and reptiles from Florida. Herpetologica **21:** 145-146.
Haggag G, KA Raheem, F Khalil. 1966. Hibernation in reptiles II, changes in blood glucose, haemoglobin, red blood cells count, protein and nonprotein nitrogen. Comp. Biochem. Physiol. **17:** 335-339.
Hartman FA, MA Lessler. 1964. Erythrocyte measurements in fishes, amphibians and reptiles. Biol. Bull. **126:** 83-88.
Heady JM, TE Rogers. 1963. Turtle blood cell morphology. Proc. Iowa Acad. Sci. **69:** 587-590.
Hutchinson VH, H Szarski. 1965. Number of erythrocytes in some amphibians and reptiles. Copeia **1965:** 373-375.
Hutton KE. 1961. Blood volume, corpuscular constants and shell weight in turtles. Am. J. Physiol. **200:** 1004-1006.
Hutton KE. 1960. Seasonal physiological changes in the red-eared turtle *Pseudemys scripta elegans*. Copeia **1960:** 360-362.
Mateo MR, ED Roberts, FM Enright. 1984. Morphologic, cytochemical and functional studies of peripheral blood cells from young healthy American alligators (*Alligator mississippiensis*). Am. J. Vet. Res. **45:** 1046-1053.
Newlin ME, RE Ballinger. 1976. Blood haemoglobin concentration in four species of lizards. Copeia **1976:** 392-394.
Ryerson DL. 1949. A preliminary survey of reptilian blood. J. Ent. Zool. **41:** 49-55.
Saint Girons MC. 1970. Morphology of the circulating blood cells. *In* C Gans, ed. Biology of the reptilia. Vol. 3. Morphology C. New York: Academic Press, pp. 73-91.
Sevinç M, IH Uğurtaş. 2001. The morphology and size of blood cells of *Lacerta rudis bithynica* (Squamata, Reptilia), Turkey. Asiatic Herpetol. Res. **9:** 122-129.
Sevinç M, IH Uğurtaş, HS Yıldırımhan. 2000. Erythrocyte measurements in *Lacerta rudis* (Reptilia, Lacertidae). Turk. J. Zool. **24:** 207-209.
Szarski H, G Czopek. 1966. Erythrocyte diameter in some amphibians and reptiles. Bull. Acad. Pol. Sci. Cl. II Ser. Sci. Biol. **14:** 437-443.
Taylor K, HM Kaplan. 1961. Light microscopy of the blood cells of pseudemyd turtles. Herpetologica **17:** 186-196.