

An Investigation on the Blood Cells of the Leopard Gecko, *Eublepharis angramainyu* (Reptilia: Sauria: Eublepharidae)

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Abstract. - In this study, blood cell counts and sizes in three adult *Eublepharis angramainyu* specimens (one male, two female) collected from SE Anatolia (Sanlurfa – Birecik). The number of erythrocytes in 1 mm³ ranged between 870,000 and 950,000 (average 910,000). The mean total length of erythrocytes was calculated as 20.35 µm, the width as 10.59, the size as 169.68 µm²; the mean nucleus length as 7.50 µm, the width as 4.15 and the size as 24.47 µm². Small lymphocytes had a mean diameter of 9.78 µm, big lymphocytes 13.71 µm, monocytes 15.37 µm, neutrophils 16.56 µm, eosinophiles 17.59 µm, and basophiles 12.87 µm. The mean length of thrombocytes was measured at 8.82 µm, and the width at 5.93 µm.

Key words. - *Eublepharis angramainyu*, Sauria, blood cell count, blood smears, erythrocytes, leucocytes, thrombocytes.

Introduction

Eublepharis angramainyu Anderson and Leviton, 1966, also known as the “leopard gecko”, was first found between Masjid Soleyman and Batsvand in the Khuzestan province of Iran. The species was reported to range in the western foothills of the Zagros Mountains and Mesopotamia in Iraq and Iran, and NE Syria with a vertical distribution of 300 to 1000 meters (Anderson and Leviton, 1966; Leviton et al., 1992; Disi and Böhme, 1996; Anderson, 1999). Studies conducted in recent years (Göçmen et al., 2002) established that the species also inhabited SE Anatolia (Sanlurfa-Birecik). Although there are a number of studies on the distribution, morphology and ecology of the species (Anderson and Leviton, 1966; Leviton et al., 1992; Disi and Böhme, 1996; Anderson, 1999; Göçmen et al., 2002), a literature review has not revealed any detailed haematological studies.

Most studies on the haematology of different species are related to blood cell counts (Alder and Huber, 1923; Hutchison and Szarski, 1965; Duguy, 1970; Arıkan, 1989) and blood cell sizes (Szarski and Czopek, 1966; Hartman and Lessler, 1964; Atatürk et al., 1998, 1999). The number haematological studies related to amphibian and reptile species living in Anatolia has been increasing in recent years (Arkan, 1989; Atatürk et al., 1998, 1999, 2001; Sevinç et al., 2000). In this study, the number and sizes of blood cells of *Eublepharis*

angramainyu were determined and photographs of their blood cells presented.

Materials and Methods

Three adult specimens (one male, two female) examined in this study were collected near Çiçekalan Village between 2200-2400 hours at an altitude of 400 m during the species breeding season (01 July, 2002). Blood samples were taken within the first three days after the specimens were collected live in the wild and brought to the laboratory.

Blood cell counts were carried out by means of Neubauer hemocytometer. Hayem solution was used to dilute the erythrocytes. Wright-Stained blood smears were made use of in the measurement (erythrocytes, leucocytes and thrombocytes) and computation of blood cells. The necessary blood samples were obtained by cardiac (ventriculus) puncture, via heparinized hematocrit capillaries. Blood cell measurements were taken by means of a MOB-1-15x ocular micrometer. On each blood smear, measurements related to 40 randomly chosen erythrocytes (total erythrocyte length, total erythrocyte width, nucleus length and nucleus width) were made, and the nucleus size was calculated according to the formula $EL.EW./4$ and the nucleus size according to the formula $NL.NW./4$ (Duguy, 1970; Atatürk et al., 2001). Moreover, micrometric measurements were made on leucocytes and thrombocytes. Photographs of

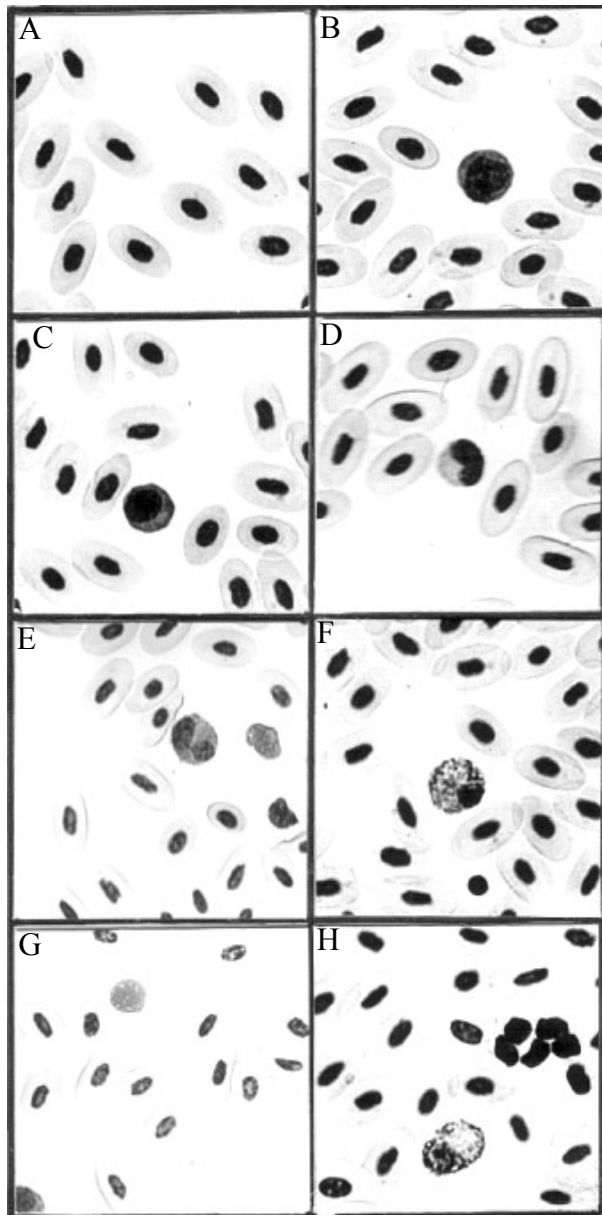


Figure 1: The blood cells of *Eublepharis angramainyu*. A- erythrocytes, B- small lymphocyte, C- large lymphocyte, D- monocyte, E- neutrophil, F- eosinophil, G- basophil, H- a cluster of thrombocytes.

blood cells were taken using a Carl Zeiss Jena microscope at 40x magnification.

Results

Male and female specimens were assessed together as there were no significant differences between them with respect to the number and size of blood cells. As in other lizards, the erythrocytes belonging to *Eublepharis angramainyu* are also ellipsoidal cells with nuclei. The nuclei are also ellipsoidal, somewhat regular and centrally

located (Figure 1A). The mean total length of the erythrocytes (L) was calculated as 20.35 μm the width (W) as 10.59 μm , the size (S) as 169.68 μm^2 ; the mean nucleus length (NL) as 7.50 μm , the width (NW) as 4.15 μm , and the size (NL) as 24.47 μm^2 . The number of erythrocytes in 1 mm^3 of blood ranged between 870,000 and 950,000 (average 910,000) (Table 1).

Lymphocytes have a spherical shape. Both small and large lymphocytes were examined in the blood smears prepared (Figure 1B, C). Large lymphocytes were 13.71 μm in diameter and had a large cytoplasmic zone and a centrally-located, large, round nucleus. The cytoplasm is stained pale blue and the nucleus purplish blue using the Wright Stain. No granule formation was observed in the cytoplasm. Small lymphocytes had a mean diameter of 9.78 μm (Table 1). The large nucleus covers the majority of the cell's area; the cytoplasm is in the shape of a thin ring. Lymphocytes are the most commonly seen leucocytes in the preparates.

Although resembling the large lymphocytes in size, monocytes are easily distinguished from the shape of the nucleus (Figure 1D). They have a mean diameter of 15.37 μm . Granule formation was observed in the cytoplasm (Table 1). The nucleus is not oval, but depressed on one side and occupies at least half of the cell. The cytoplasm is stained light purple and the nucleus dark blue. They are the second most common leucocytes after lymphocytes and neutrophils.

Neutrophils are spherical cells with a mean diameter of 16.56 μm (Table 1). Using the Wright Stain, the cytoplasm is stained light blue and the nucleus dark blue. There are very fine granules in the cytoplasm (Figure 1E). The nucleus is a structure with lobes and segments. They are the most common leucocytes second to lymphocytes.

Eosinophils have a diameter of 17.59 μm (Table 1). The cytoplasm is stained light blue and the nucleus dark blue. Large, round, bright red granules within the cytoplasm strike eye as the most distinctive characteristic of these cells (Figure 1F). The nucleus was seen to have two lobes. These cells take the fourth place in the preparation of smears after lymphocytes, monocytes and neutrophils.

Basophils are oval-shaped with a mean diameter of 12.87 μm (Figure 1G and Table 1). When stained by means of the Wright Stain, dark bluish purple granules within the light blue cytoplasm are in a position marking the dark blue nucleus. These cells are rarely seen in the preparates.

Thrombocytes are spindle-shaped cells with a mean length of 8.82 μm , a width of 5.93 μm (Figure 1H and Table 1). In the Wright-Stained preparates, dark stained cells with large oval nuclei and small irregular cytoplasmic zones form groups of two or more.

Table 1: The established counts, measurements and sizes concerning the blood cells of *Eublepharis angramainyu* (in m and m²). N: Number of specimens; n: Number of measurements/computings in each specimen; Ext: extreme values; SD and SE: standard deviations and the standard errors of the means, respectively.

Blod Cells	N	n	Ext	Mean	SD	SE
Number of Erythrocytes	2	3	870,000-950,000	910,000	-	-
Total Erythrocyte Length	2	40	15.00-22.50	20.35	2.15	0.24
Total Erythrocyte Width	2	40	7.50-12.50	10.59	1.00	0.11
Total Erythrocyte Size	2	40	91.26-220.78	169.68	26.87	3.00
Nucleus Length	2	40	6.25-8.75	7.50	0.41	0.05
Nucleus Width	2	40	3.25-5.25	4.15	0.52	0.06
Nucleus Size	2	40	17.86-31.40	24.47	3.44	0.38
Lymphocyte (big) Diameter	2	20	12.50-16.25	13.71	1.21	0.19
Lymphocyte (small) Diameter	2	20	7.50-11.25	9.78	1.11	0.17
Monocyte Diameter	2	40	11.25-20.00	15.37	2.00	0.31
Neutrophile Diameter	2	40	15.00-20.00	16.56	1.61	0.36
Eosinophile Diameter	2	20	15.00-20.00	17.59	1.56	0.24
Basophile Diameter	2	5	10.00-15.20	12.87	1.81	0.40
Thrombocyte Length	2	40	7.25-11.25	8.82	1.16	0.18
Thrombocyte Width	2	40	5.00-8.25	5.93	0.77	0.12

Table 2: The number of erythrocytes in 1 mm³ of blood in different lizard species.

Researchers	Species	Number of Erythrocytes
Present study	<i>Eublepharis angramainyu</i>	870,000-950,000
Duguy (1970)	<i>Hemidactylus turcicus</i>	866,000
	<i>Chalcides ocellatus</i>	806,000
	<i>Agama atra</i>	1,250,000
	<i>Lacerta agilis</i>	945,000-1,420,000
	<i>Lacerta viridis</i>	840,000-1,600,000
	<i>Anguis fragilis</i>	466,000-1,615,000

Table 3: Sizes of erythrocytes and nuclei in different lizard species according to various researchers (EL/EW: Erythrocyte Length/Erythrocyte Width Ratio, ES: Erythrocyte Size (μm^2), NL/NW: Nucleus Length/Nucleus Width Ratio, NS: Nucleus Size (μm^2), N/C: Nuclear surface/Cell surface ratio).

Researchers	Species	EL/EW	ES	NL/NW	NS	N/C
Present study	<i>Eublepharis angramainyu</i>	1.92	169.68	1.80	24.47	0.14
Atatür, et al. (2001)	<i>Ablepharus chernovi</i>	1.87	84.12	2.45	12.01	0.14
	<i>Chalcides ocellatus</i>	1.86	91.33	1.98	10.70	0.12
	<i>Mabuya aurata</i>	1.90	84.88	2.01	10.02	0.12
	<i>Ophiomorus punctatissimus</i>	1.96	92.08	2.30	12.70	0.14
	<i>Eumeces schneideri</i>	1.97	92.31	2.81	14.20	0.15
Sevinç, et al. (2000)	<i>Lacerta rudis</i>	1.63	87.46	1.64	16.66	0.19
	<i>Lacerta viridis</i>	1.86	125.00	1.94	14.6	0.11
Duguy (1970)	<i>Eumeces algeriensis</i>	1.61	154.80	2.10	26.4	0.17
	<i>Anguis fragilis</i>	1.88	143.90	1.62	22.5	0.14

Discussion

As stated in the 'Results' section, *E. angramainyu* specimens were collected during the mating season and did not display sexual dimorphism with respect to the number of erythrocytes and sizes. It has been found that there are significant differences among lizard families with respect to the number and size of erythrocytes, and the members of Gekkonidae have the highest number of erythrocytes among lizards (Alder and Huber, 1923; Hutchison and Szarski, 1965; Duguy, 1970). Values belonging to the number of blood cells determined in different lizard species (Table 2), by various researchers were compared with those we obtained for *E. angramainyu* in the present study, and it was established that the number of erythrocytes in 1 mm³ of blood was very close to that of *Hemidactylus turcicus* (Gekkonidae) species, but different from that of other lizard species.

Values we obtained in *E. angramainyu* with respect to the sizes of erythrocytes and nuclei were compared with those determined for some lizard species (Table 3), and it was found that values concerning the sizes of erythrocytes and nuclei were very close to those of species belonging to Gekkonidae family. When compared with the other lizard species, *E. angramainyu* can be said to have the largest erythrocytes with respect to the sizes of erythrocytes and nuclei.

Literature Cited

- Alder, A. and E. Huber. 1923. Untersuchungen über Blutzellen und Zellbildung bei Amphibien und Reptilien. Folia Haematologica 29:1-22.
- Anderson, S. C. and A. E. Leviton. 1966. A new species of *Eublepharis* from Southwestern Iran (Reptilia: Gekkonidae). Occasional Papers of the California Academy of Science 53:1-5.
- Anderson, S. C. 1999. The Lizards of Iran (Contributions to Herpetology Vol. 15). Society for the Study of Amphibians and Reptiles, Missouri, USA. 422 pp.
- Arıkan, H. 1989. Anadolu'daki *Rana ridibunda* (Anura: Ranidae) populasyonlarının kan hücrelerinin sayısı bakımından incelenmesi. Turkish Zoology 13:54-59.
- Atatür, M. K., H. Arıkan, and A. Mermer. 1998. Erythrocyte sizes of some Urodeles from Turkey. Turkish Journal of Zoology 22:89-91.
- Atatür, M. K., H. Arıkan, and İ. E. Çevik. 1999. Erythrocyte sizes of some Anurans from Turkey. Turkish Journal of Zoology 23:111-114.
- Atatür, M. K., H. Arkan, İ. E. Çevik, and A. Mermer. 2001. Erythrocyte measurements of some Scincids from Turkey. Turkish Journal of Zoology 25:149-152.

- Disi, A. M., and W. Böhme. 1996. Zoogeography of the amphibians and reptiles of Syria, with additional new records. *Herpetozoa* 9(1/2):63-70.
- Duguy, R. 1970. Numbers of blood cells and their variation. Pp. 93-109. In C. Gans and F. H. Pough (eds.), *Biology of Reptilia*, Volume 3. Academic Press, London and New York.
- Göçmen, B., M. Tosunoğlu, and D. Ayaz. 2002. First Record of the Leopard Gecko, *Eublepharis angrainyu* (Reptilia: Sauria: Eublepharidae) from Anatolia. *Herpetological Journal* 12(2):79-80.
- Hartman, F. A. and M. A. Lessler. 1964. Erythrocyte measurements in Fisches, Amphibia and Reptiles. *Biological Bulletin* 126:83-88.
- Hutchison, H. V. and H. Szarski. 1965. Number of erythrocytes in some Amphibians and Reptiles. *Copeia* 3:373-375.
- Leviton, A. E., S. C. Anderson, K. Adler, and S. A. Minton. 1992. Handbook to Middle East Amphibians and Reptiles. In *Contributions to Herpetology*, Vol. 8., Society for the Study of Amphibians and Reptiles. Missouri, USA.
- Sevinç, M., İ. H. Uğurtaş, and H. S. Yıldırımhan. 2000. Erythrocyte measurements in *Lacerta rudis* (Reptilia, Lacertidae). *Turkish Journal of Zoology* 24:207-209.
- Szarski, H. and G. Czopek. 1966. Erythrocyte diameter in some amphibians and reptiles. *Bulletin of the Polish Academy of Sciences Biological Sciences* 14(6):433-437.