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Haematology of amphibians and reptiles: a review

Hüseyin ARIKAN and Kerim ÇİÇEK*

Ege University, Faculty of Science, Biology Department, Zoology Section, TR-35100, Bornova-Izmir, Turkey.
*Corresponding author, K. Çiçek, E-mail's: kerim.cicek@hotmail.com, kerim.cicek@ege.edu.tr

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Abstract. In this review, function and composition of blood, haemopoiesis, morphology, number and size of blood cells (*erythrocytes*, *leukocytes* and *thrombocytes*), and intra/extracellular blood parasites of amphibians and reptiles are revised by utilizing the literature knowledge.

Key words: Amphibia, Reptilia, blood cells, plasma, haemopoiesis, blood parasites.

Introduction

In old Greek, *haemat* means blood and /*o*/-*logy* means investigation or examination. Thus, *haematology* refers to the examination of blood (Seiverd 1972). Multicellular animal organisms need the circulatory system to transport food particles and respiratory gases to the cells in tissues and to carry the wastes from cells to the necessary organs. Blood is the circulatory fluid in vertebrates. Keeping both the components and the amount of blood at a constant level is essential for the continuity of life.

1. Characteristics of Vertebrates Blood

Composition of Blood

Blood corpuscles, or blood cells (*erythrocytes*, *leukocytes* and *thrombocytes*), and the liquid intercellular substance are a type of specialized connective tissue of mesenchymal origin originating from the *blood plasma*. Plasma consists of water, food substances, wastes, hormones, antibodies and enzymes (Seiverd 1972).

Formation of Blood

Plasma originates from the intestines and different organs of the body. Water and food substances come from the intestines, whereas wastes, hormones, antibodies and enzymes come from different organs in the body (Seiverd 1972). Mature blood cells are relatively short-lived; thus, they are continually being renewed by the differentiation of stem cells produced in *hematopoietic organs*. The region of production blood cells depends on individual's age. The first blood cells are produced from mesenchymal cells in embryonic stages. In amphibian tadpoles, the blood cells get formed from mesonephros type kidney. Until the completion of metamorphosis, spleen is also taking on the task of creating new blood cells (Jordan 1933). Most of the erythrocytes, thrombocytes and leukocytes are formed in the bone marrow in adults. The most widespread sources of the red bone marrow are ribs, sternum and spine (Seiverd 1972).

Hematopoiesis in Amphibians

Haemopoiesis in amphibians has been studied by many researchers (e.g. Jordan 1938, Jordan & Speidel 1923, Maniatis & Ingram 1971, Hadji-Azimi et al. 1987, Allander & Fry 2008). The development of hematopoiesis in amphibians displays some similarities to those of birds and mammals (Allander & Fry 2008). There are great differences in erythropoiesis between juvenile and adult amphibians. Erythropoiesis takes place in the liver and kidney in juvenile amphibians, while it takes place in the bone marrow as well as in the spleen and the liver in adult aquatic and terrestrial frogs. Thyroxine application affects erythropoiesis in adults. In amphibians, ventral blood islets are the hematopoietic organs in the embryonic period. Kidneys are also main blood production centres during the larval periods of amphibian species. Thymus is the most important place of T-cell maturation; however, the development place of B-cells is not completely clear. In adult urodeles and anurans, the spleen is the greatest place for erythrocyte production. Nevertheless, the liver also serves as a secondary organ for this activity. In amphibians, metamorphosis or post-hibernation hematopoiesis typically occurs in the bone marrow. The bone marrow is haemopoietically active in some salamanders (Plethodontidae) and in species *Rana* (Schaefer 1935, Curtis et al. 1979, Turner 1988). Seasonal changes could affect activity rate of haemopoietic organ (e.g. spleen and bone marrow) in some species (Glomski et al. 1997).

Haemopoiesis in Reptiles

Very few studies have been made on the haemopoiesis of reptiles (Vasse & Beaupain 1981, Dessaurer 1970). In reptiles, the blood islets appear to be the only erythropoietic centre during the embryonic life. Although, like in birds, the liver and the spleen have a hematopoietic function in the early periods of development, erythropoiesis and granulopoiesis after birth mainly occur in the bone marrow. The lymphocytes that originate from the bone marrow pass through the thymus via circulation, where they get differentiated as T-cells. It is not exactly known where the B-cells develop. Lymphopoiesis takes place in the bone marrow, spleen and the other organs in the body. The mucosa-associated lymphoid tissue (MALT) is localized under the mucous membranes of the digestive, respiratory and urogenital organs.

2. Obtaining of Blood for Hematological Studies

Obtaining of Blood from Amphibians

Depending on species, season and health condition, many factors affect the volume of blood circulating in amphibians. The blood volume was reported to constitute 25% of the body weight in caecilians but 10% of the body weight in salamanders and tailless frogs.

Blood can be collected from many areas in amphibians (Hutchison & Szarski 1965, Szarski & Czopek 1966, Allender & Fry 2008, Heatley & Johnson 2009). In most salamander and anuran species, blood can be collected from the ventral abdominal vein, lingual plexus, femoral vein, facial (maxillary)/musculo-cutaneous vein, and the ventricle of the heart (Campbell 2004, Forzán et al. 2012). In salamanders, blood can be collected from the ventral tail vein. Amphibians generally have lymphatic vessels that are widely found along the blood vessels. The contamination of blood samples with lymph might cause erroneous results. Therefore, attention should be paid to lymphatic vessels when collecting blood. Some researchers recommend collecting blood with heparinized microhematocrit tubes. The blood samples should be taken to an anticoagulant tube immediately after they have been collected. Information about the animal and the date should be labeled onto the slides and blood tubes.

Obtaining of Blood from Reptiles

Before blood is collected, the maximum blood volume should be determined. When reptiles are compared with a mammalian of similar size, they have a lower blood volume, which constitutes 5 to 8% of the body weight. 10% of this volume can be collected from healthy reptile samples (e.g. 0.5-0.8 ml in an animal that weighs 100 g). Since it is reported that ethylenediaminetetraacetic acid (EDTA) causes haemolysis particularly in turtles, heparin should generally be preferred as an anticoagulant in reptiles (Tavares-Dias et al. 2008).

In lizards and snakes, there are three areas for blood collection by means of heparinized glass capillaries, namely the caudal tail vein, the postorbital sinuses and the heart (MacLean et al. 1973, Campbell 2006, Sykes & Klaphake 2008, Heatley & Johnson 2009). There are numerous areas for blood collection in turtles, including the heart and jugular, subcarapacial, femoral, branchial and occipital sinuses. In crocodiles, the ventral tail vein and the supravertebral sinus are the most common areas, from which blood is collected.

Obtaining the Bone Marrow

The bone marrow can be obtained from the femur or tibia in amphibians and most reptile species, like in mammalians (Selleri & Hernandez Divers 2006). In turtles, the bone marrow can be obtained from the area between external and internal bone plates (Frye 1991). In snakes, rib biopsy is the most realistic method to obtain the bone marrow.

3. Procedures for Counting Erythrocytes, Leukocytes and Thrombocytes

Blood Cell Counts

The erythrocytes were counted using a Neubauer haemocytometer. Standard Hayem's or Natt and Herrick's (1952) solution was most used as a dilution solution for erythrocytes. The results are expressed as the number of erythrocytes in 1mm^3 of blood.

The total leukocyte and thrombocyte count acquisition for amphibian and reptilian species was precluded by several factors, especially by the aggregation (Tavares-Dias et al. 2008). The leukocyte count could be performed by indirect or direct method (Campbell, 2004). The direct count using Natt & Herrick's (1952) solution and with no dye (Hawkey 1988) or an indirect method called as the eosinophil pipette using 0.1% phloxine B dye

(Campbell 2004) for total leukocytes.

The leukocytes were generally well counted by using the method of Jerrett and Mays (1973), a modified application of Blain's method, or Natt and Herrick's (1952) and with a Neubauer haemocytometer. According to Jerrett & Mays (1973), neutral red, diluted with 0.007 of physiological water at a ratio of 1:5000, and 12% formalin mixture that was prepared again with 0.007 of physiological water were mixed at a ratio of 1:1. Natt & Herrick solution (1952) contain 3.88 g of sodium chloride, 2.50 g of sodium sulfate, 1.74 g of sodium phosphate, 0.25 g of potassium phosphate, 7.5 ml of formalin (37%), 0.10 g of methyl violet. The mixture dilutes to 1000 ml and filter. The diluent for leukocytes count described by Natt and Herrick (1952) and generally it is suitable for all non-mammalian vertebrates. However, when this diluent is used for some turtle species, additional salts are required to adjust the osmolality of the stock solution (Arnold 2005).

In the eosinophil pipette method is counting only the cells containing eosinophilic granules (heterophils and eosinophils) and they stained orange to red with phloxine B. The total leukocyte number calculated based on the percent heterophils and eosinophils from the differential (ASVCP 2013). Main disadvantage of this method could not be work well for some species. The development of using of lysis methods to facilitate counting mammalian leukocytes by removing the non-nucleated erythrocytes occurred the distinction between mammalian and lower vertebrate haematology (Tavares-Dias et al. 2008).

The thrombocyte count and size were calculated from the blood smears prepared with Wright's stain. In addition, the direct method reported by Seiverd (1972) was also used. Accordingly, a Neubauer haemocytometer is used to count the thrombocytes and the Rees and Ecker or Natt and Herrick (1952) solutions were used as the dilution solution. Preparation of Rees and Ecker solution: 0.1 g of brilliant cresyl blue and 3.8 g of sodium citrate are weighed and placed into a 100 cc volumetric flask. 0.2 cc 40% formaldehyde is added to it and it is completed with 100 cc purified water and stored in the refrigerator.

Blood Smear Preparation

Blood cells are generally examined on the smears prepared in such a way that a drop of blood is dropped onto a slide, smeared as a thin layer and stained. Blood smears from amphibians and reptiles should be performed without any treatment with any anticoagulant following blood collection. After blood has been dropped onto a slide, it is smeared and left in order for it to dry in air (Seiverd 1972).

Blood smears are stained with polychrome stains which are the mixtures of acidic and basic stains. The original polychrome stain was invented by Russian scientist Romanowsky. Many modifications of the Romanowsky's stain include Wright's, Giemsa, Jenner's, May-Grünwaldand May-Grünwald-Giemsa stains. The most popular of them is the Wright's and May-Grünwald-Giemsa.

During staining, a buffer solution is used to check the acid-base balance of the stain. This is the most essential procedure. If the buffer solution is rather acidic, acidic staining will be quite bright and basic staining will be rather pale. If the buffer solution is rather basic, basic staining will be quite bright and acidic staining will be rather pale. In both cases, the result is a poorly stained smear.

The buffer solution used with Wright's stain should have a pH value between 6.4 and 6.8. These pH values provide the best contrast between acidic and basic staining. Wright's stain is the methanol solution of an acidic and a basic stain. The acidic stain is eosin in red, while the basic stain is methylene blue in blue.

Leukocytes are predominantly diagnosed with the presence of acidic and basic stains. In some cases, the cells are even called with the name of the stain. For instance, eosinophils mean "those which have affinity with eosin" - an acidic stain. The cells which prefer a basic stain are called basophils.

4. Morphology of Blood Cells

The peripheral blood cells of amphibians and reptiles consist of erythrocytes, leukocytes and thrombocytes. Granulocytic leukocytes can be subdivided as heterophils, eosinophils and basophils, whereas agranulocytes can be subdivided as lymphocytes and monocytes. Both heterophils and eosinophils show acidophilic granules in their cytoplasm; however, basophils have basophilic cytoplasmic granules (Arıkan & Çiçek 2011a). Azurophiles is a unique type of leucocytes, identified only in reptiles. However, it defined from snakes, lizards and crocodiles, whereas seldom observed in turtles and tortoises (Montali 1988, Dotson et al. 1995). It contains azurophilic granules and similar to monocytes. They also known as primary granules and some authors claim it is originated from differentiation of monocytes (Rosskopf 2000).

Erythrocytes or Red Blood Cells

Amphibians are cold-blooded vertebrates that are represented by about 7,173 species (AmphibiaWeb 2013). Amphibians are widely kept in captivity as model animals and pets for physiological and embryological studies. Erythrocytes are predominant cells in quantity. The ratio of erythrocytes to leukocytes (E/L) ranges from 20 to70 in amphibians; however, this ratio is 100 in reptiles (Stephan 1954). It might be stated that this ratio is gradually increasing depending on the phylogenetic line. Like in the adults of all vertebrates other than of mammalians, amphibians and reptiles typically have nucleated ellipsoidal, oblate and biconvex erythrocytes. Erythrocytes are cells which are specialized to carry respiratory gases (O_2 and CO_2) by means of haemoglobin (Foxon 1964).

Amphibians have the largest erythrocytes among vertebrates (Fig. 1). *Amphiuma tridactilum* is the species with the largest erythrocytes recorded (70×40 µm) (Vernberg 1955). Among the amphibian species living in Turkey, the largest erythrocytes (33.28-20.13 µm) were detected in *Salamandra infraimmaculata* (Table 1) (Atatür et al. 1998). The erythrocytes of amphibians and reptiles are ellipsoidal to some extent and located at the centre of the cell (Fig. 1A). In the blood smears prepared with Wright's stain, the cytoplasms appear light yellowish-pink and the chromophilic nucleus appears dark purplish blue (Atatür et al. 1998).

Anurans have smaller erythrocytes (22 × 15 µm) and nuclei than urodeles (Fig. 2 B) (Atatür et al. 1999, Arıkan et al. 2003a, Arıkan & Çiçek 2011a). On the contrary, the number of erythrocytes and the haemoglobin concentration are low in urodeles. Even though amphibians have nucleated erythrocytes, non-nucleated erythrocytes (erythroplastids) were observed in lungless salamander species *Batrachoseps attenuates* (family Plethodontidae) (Villolobos et al. 1988). Erythroplastids are not well known in anurans; however, few non-nucleated erythrocytes were found in the spleen and blood in *Rana pipiens* and *Rana catesbeiana* (Jordan & Speidal 1923). In amphibians, the lifetime of erythrocytes is longer than that in birds and mammalians (e.g. about 700-1,400 days in *Bufo marinus*) (Altland & Brace 1962). Among the

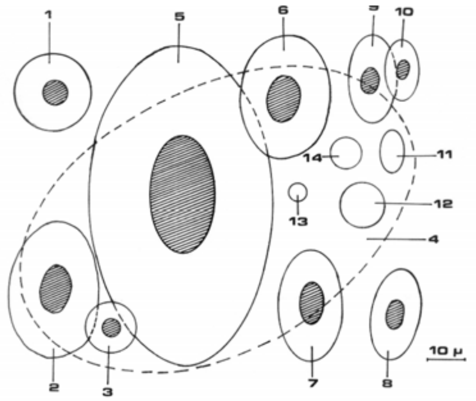

Figure 1. The erythrocyte sizes in various vertebrates (modified from Stephan, 1954). 1. *Petromyzon marinus*, 2. *Raja*, 3. *Pleuronectes solea*, 4. *Amphiuma*, 5. *Proteus anguineus*, 6. *Rana*, 7. *Testudo graeca*, 8. *Lacerta*, 9. *Struthio camelus*, 10. *Gallus gallus*, 11. *Lama*, 12. *Elephas*, 13. *Capra*, 14. *Homo*.

anuran species examined, the largest and the most ellipsoidal erythrocytes were observed in aquatic species *Pelophylax caralitanus* (Fig. 2B) and the smallest erythrocytes were observed in terrestrial species *Pelodytes caucasicus* (Fig. 2C) (Atatür et al. 1999, Arıkan et al. 2001, 2003a, 2003b, Dönmez et al. 2009, Arıkan et al. 2010, Arıkan & Çiçek 2011a).

Vernberg (1955) stated that these differences depended on body weight and body size. These differences are probably due to different environmental conditions (e.g. temperature and atmospheric pressure) (Ruiz et al. 1983, 1989) and/or different activity conditions (e.g. health, reproduction, hibernation, feeding and daily activity) (Wojtaszek et al. 1997, Campbell 2004, Allander & Fry 2008). Sykes & Klaphake (2008) found that erythrocytes were larger in aquatic species than in terrestrial species and that they were smaller in more active species. This view is in agreement with the results by Haden (1940), Altman & Dittmer (1961), Harris (1963), Atatür et al. (1998, 1999) and Gül & Tok (2009). The ratio of erythrocyte length to erythrocyte width (L/W) ranges from 1.63 to 1.80 in urodeles and from 1.38 to 1.69 in anurans (Table 1). Besides, the shape of an erythrocyte is more ellipsoidal in urodeles than in anurans. The ratio of nucleus length to nucleus width (NL/NW) ranges from 1.55 to 1.69 in urodeles and from 1.57 to 2.35 in anurans (Table 1). That is to say, contrary to the case of L/W, it was found that the anurans had more ellipsoidal nuclei than urodeles, which is in agreement with the findings by Kuramoto (1981).

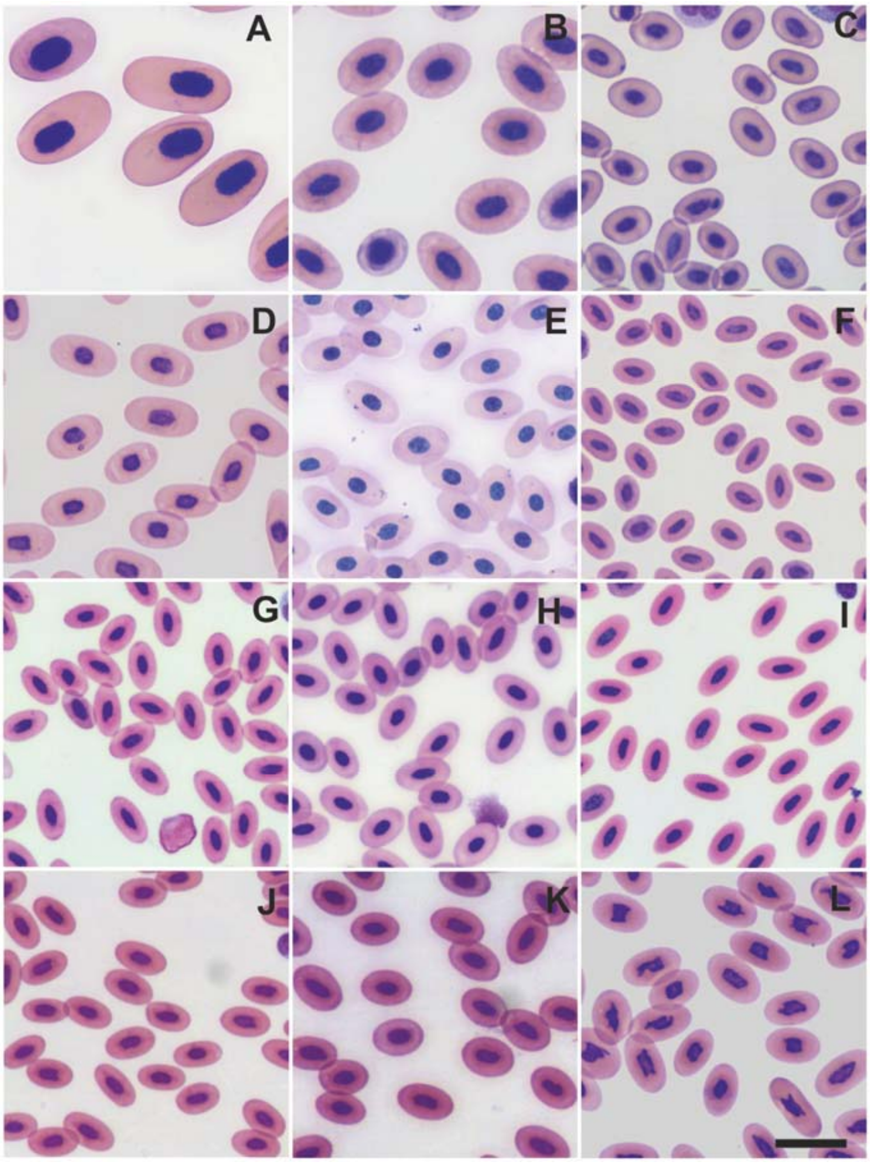

Figure 2. Photomicrographs of erythrocytes of some amphibian and reptile species (modified from Arıkan & Çiçek, 2011a). **A**: *O. vittatus,* **B:** *P. caralitanus*, **C**: *P. caucasicus*, **D**: *E. orbicularis*, **E**: *T. graeca*, **F**: *O. elegans*, **G**: *M. brevirostris*, **H**: *A. danfordi*, **I**: *L. trilineata*, **J**: *L. macrorhynchus*, **K**: *H. ravergieri*, **L**: *M. xanthina*. Horizontal bar: 20 µm.

The nucleocytoplasmic ratio ranges from 0.22 to 0.34 in urodeles and from 0.10 to 0.16 in anurans (Table 1). That is to say, anurans have a wider cytoplasmic surface area than urodeles (Arıkan et al. 2010). Hence, It seems that erythrocytes are moresuitable for the exchange of gases in anurans than in urodeles.

Reptiles are cold-blooded vertebrates represented by approximately 9,766 species (Uetz & Hošek 2013). Reptiles represent different animal groups (e.g. turtles, crocodiles, lizards and snakes). The erythrocytes of reptiles resemble those of birds in terms of function and appearance; however, they vary in size (Sypek & Borysenko 1988, Sykes & Klaphake 2008). The number of erythrocytes circulating in reptiles is lower than

Table 1. The erythrocyte and their nuclei measurements established in the peripheral bloods of various amphibian and reptile species. [L: Erythrocyte length, W: Erythrocyte width, ES: Erythrocyte size, NL: Nucleus length, NW: Nucleus width, NS: Nucleus size; NS/ES: Nucleocytoplasmic ratio, A&Ç- Arıkan & Çiçek 2010].

	Erythrocytes			Nuclei			References
	L (µm)	W (µm)	ES (µm²)	NL (µm)	NW (µm)	NS (µm²)	
Urodela							
Salamandridae							
Lissotriton vulgaris	30.02±0.16	17.81±0.08	419.44±3.11	13.86±0.13	8.53±0.07	92.85±0.68	A&Ç 2010
Lyciasalamandra atifi	33.28±0.17	19.44±0.09	507.54±3.35	14.99±0.11	9.44±0.09	111.14±0.64	A&Ç 2010
Mertesiella caucasica	31.69±0.29	17.69±0.29	440.44±5.79	16.64±0.16	9.84±0.09	128.60±0.92	A&Ç 2010
Neurergus strauchii	31.20±0.21	18.93±0.08	463.82±4.35	15.45±0.07	9.88±0.03	120.10±0.77	A&Ç 2010
Ommatotriton vittatus	28.06±0.16	16.63±0.12	367.05±3.82	16.03±0.14	9.86±0.09	124.14±0.70	A&Ç 2010
Salamandra infraimmaculata	33.10±0.20	20.13±0.13	523.44±4.79	16.86±0.19	10.46±0.11	138.51±0.84	A&Ç 2010
Triturus karelinii	29.50±0.16	18.14±0.09	420.37±2.96	14.98±0.11	9.44±0.07	111.06±0.74	A&Ç 2010
Anura							
Ranidae							
Pelophylax ridibundus	24.36±0.23	14.46±0.11	276.62±3.86	8.13±0.13	5.03±0.03	32.15±0.62	A&Ç 2010
Rana camerani	19.81±0.08	12.78±0.03	198.85±1.11	8.45±0.06	3.94±0.03	26.09±0.20	A&Ç 2010
Rana dalmatina	19.99±0.24	12.11±0.11	190.47±3.54	8.78±0.09	5.59±0.02	38.48±0.42	A&Ç 2010
Rana holtzi	19.10±0.12	12.80±0.06	192.81±1.83	7.84±0.04	4.13±0.04	25.46±0.27	A&Ç 2010
Rana macrocnemis	20.55±0.12	13.46±0.05	217.68±1.76	8.66±0.06	4.14±0.04	28.03±0.28	A&Ç 2010
Bufonidae							
Bufo bufo	20.85±0.10	13.45±0.07	221.22±1.90	7.81±0.10	4.34±0.10	26.60±0.63	A&Ç 2010
Bufotes variabilis	17.86±0.07	12.71±0.04	179.18±0.96	6.25±0.13	3.72±0.04	18.13±0.56	A&Ç 2010
Pelobatidae							
Pelobates syriacus	17.56±0.08	11.70±0.07	161.85±1.31	6.63±0.09	3.47±0.04	18.13±0.56	A&Ç 2010
Pelodititidae							
Pelodytes caucasicus	17.56±0.08	9.68±0.09	116.42±2.07	6.21±0.07	3.81±0.05	18.71±0.31	A&Ç 2010
Bombinatoridae							
Bombina bombina	21.80±0.12	15.05±0.08	258.14±2.36	9.59±0.16	4.88±0.06	36.66±0.82	A&Ç 2010
Hylidae							
Hyla orientalis	19.80±0.10	12.89±0.06	200.33±1.66	7.94±0.10	3.50±0.08	21.88±0.61	A&Ç 2010
Hyla savignyi	18.63±0.18	12.41±0.08	181.44±1.98	7.09±0.09	3.97±0.08	22.30±0.55	A&Ç 2010

that of birds. Within class Reptilia, the largest erythrocytes were found in *Sphenodon punctatus* (order Rhynchocephalia), turtles and crocodiles, while the smallest erythrocytes were found in lizards (Hartman & Lessler 1964, Saint Girons & Saint Girons 1969, Saint Girons 1970, Sevinç et al. 2000).

Wintrobe (1933) put forward that the size of erythrocytes reflected the position of a species on the evolutionary scale. Lower vertebrates and those which display unsuccessful evolutionary trials have large nucleated erythrocytes; however, higher vertebrates have small non-nucleated erythrocytes. In this sense, reptiles are intermediates between amphibians and birds (Szarski & Czopek 1966, Szarski 1968). Reptiles form a heterogeneous group among vertebrates with respect to blood cell morphology and show noteworthy variations among orders and even within the members of the same family (Hartman & Lessler 1964, Szarski & Czopek 1966, Saint Girons & Saint Girons 1969, Saint Girons 1970, Arıkan et al. 2004, Frye 1991, Mader 2000, Campbell 2004, Tok et al. 2006, Strik et al. 2007, Sykes & Klaphake 2008, Arıkan et al. 2009a,b, Claver & Quaglia 2009, Arıkan et al. 2010).

Among turtles, the largest erythrocytes were observed in aquatic species (e.g. 200.67 µm² in *Emys orbicularis*) (Fig. 2D) and the smallest erythrocytes were observed in terrestrial species (e.g. 163.81 µm² in *Testudo graeca*) (Fig. 2E) (Work et al. 1998, Uğurtaş et al. 2003. Yılmaz & Tosunoğlu 2010, Zhang et al. 2011a, Arıkan & Çiçek 2011a). In this sense, the longest, the widest and the largest erythrocytes were observed in *E. orbicularis* and the most ellipsoidal erythrocytes and the least ellipsoidal nuclei were observed in *Mauremys caspica*. Additionally, the smallest and the least ellipsoidal cells were found in *T. graeca* and the longest, the widest and the largest nuclei were found in *M. caspica*. Nevertheless, the shortest, the narrowest and the most ellipsoidal nuclei were detected in *T. graeca*, whereas the largest nucleocytoplasmic ratio was detected in *M. caspica* and the smallest nucleocytoplasmic ratio was determined in *T. graeca*. The nuclei were found more spherical in turtles than in amphibians.

The erythrocytes of lizards vary in size among families and sometimes even within a family. In lizard species, the mean length, width and erythrocyte size range from 12.43 µm to 16.85 µm, from 7.51 µm to 10.21 µm and from 73.27 µm² to 130.33 µm², respectively and the L/W ratio ranges from 1.56 to 1.99 (Fig. 2F, G, H and I) (Saint Girons & Saint Girons 1969, Atatür et al. 2001, Arıkan et al. 2009b, Arıkan & Çiçek 2011a). In this sense, the longest erythrocytes were found in *Stellagama stellio*, the widest and the largest ones in *Varanus griseus* and the shortest, the narrowest and the smallest ones in *Ophisops elegans* (Fig. 2F). In addition,

Table 2. The erythrocyte and their nuclei measurements (±with their standard errors) established in the peripheral bloods of various amphibian and reptile species. (A&Ç- Arıkan & Çiçek 2010, C&O- Casal & Orós 2007, Z&co- Zhang et al. 2011b, K&co- Kassab et al. 2009, G&co- Gillespie et al. 2000, Po&co- Ponsen et al. 2008, Pa&co- Parida et al. 2012, S&co- Salakij et al. 2002b).

	Erythrocytes			Nuclei			References
	L (µm)	W (µm)	ES (µm²)	NL (µm)	NW (µm)	NS (µm²)	
Testudines							
Chelonidae							
Caretta caretta juveniles	19.05 ± 1.35	12.85 ± 1.25	194.28 ±27.54				C&O 2007
Lepidochelys olivacea	20.35	12.55	201.65			19.79	Z&co 2011b
Emydidae							
Emys orbicularis	19.99±0.11	12.76±0.09	200.67±1.88	7.15±0.05	6.26±0.19	35.37±1.10	A&Ç 2010
Geoemydidae							
Mauremys caspica	18.99±0.09	11.90±0.07	177.64±1.42	7.15±0.05	6.31±0.04	35.64±0.41	A&Ç 2010
Mauremys rivulata	19.02±0.12	12.19±0.08	182.74±1.97	6.72±0.04	5.89±0.04	31.22±0.35	A&Ç 2010
Testudinidae							
Testudo graeca	17.35±0.14	11.96±0.11	163.81±2.34	6.09±0.05	4.91±0.04	23.60±0.34	A&Ç 2010
	18.21-19.77	9.08-10.34		5.98-6.49	4.27-4.97		K&co 2009
Squamata / Sauria							
Scincidae							
Ablepharus chernovi	14.13±0.07	7.58±0.03	84.12±0.57	6.12±0.05	2.50±0.00	12.01±0.10	A&Ç 2010
Chalcides ocellatus	14.68±0.06	7.92±0.04	91.33±0.61	5.15±0.03	2.64±0.03	10.70±0.13	A&Ç 2010
Eumeces schneiderii	15.17±0.06	7.74±0.04	92.31±0.56	7.11±0.04	2.54±0.02	14.20±0.13	A&Ç 2010
Ophiomorus punctatissimus	15.14±0.06	7.73±0.04	92.08±0.64	6.05±0.05	2.68±0.04	12.70±0.21	A&Ç 2010
Trachylepis aurata	14.27±0.08	7.56±0.02	84.88±0.54	5.06±0.02	2.52±0.01	10.02±0.07	A&Ç 2010
Trachylepis vittata	14.14±0.08	7.55±0.02	83.77±0.53	6.14±0.05	2.50±0.00	12.06±0.10	A&Ç 2010
Lacertidae							
Acanthodactylus boskianus	14.22±0.98	7.92±0.41	88.45±8.37	6.24±0.55	4.02±0.12	19.69±1.70	A&Ç 2010
Acanthodactylus harranensis	15.46±1.24	8.56±0.59	104.22±13.53	6.59±0.50	4.07±0.13	21.02±1.49	A&Ç 2010
Anatololacerta danfordi	14.14±1.17	9.09±0.51	101.13±12.10	6.73±0.61	4.41±0.13	23.32±2.30	A&Ç 2010
Apathya cappadocica	13.42±0.90	7.94±0.47	83.73±8.13	6.33±0.46	4.11±0.13	20.42±1.58	A&Ç 2010
Darevskia praticola	13.08±0.97	8.01±0.38	82.34±7.78	6.19±0.43	4.31±0.12	20.93±1.46	A&Ç 2010
Darevskia uzzelli	13.65±0.96	7.84±0.48	84.22±9.33	5.98±0.36	4.34±0.14	20.36±1.22	A&Ç 2010
Darevskia valentini	13.32±0.93	7.73±0.57	80.97±9.47	6.13±0.48	4.28±0.16	20.63±2.03	A&Ç 2010
Lacerta pamphylica	15.61±1.00	7.89±0.52	96.77±9.91	6.33±0.41	4.23±0.11	21.01±1.61	A&Ç 2010
Lacerta trilineata	14.39±1.01	7.63±0.49	86.31±10.22	6.93±0.52	3.93±0.11	21.38±1.56	A&Ç 2010
Lacerta viridis	14.94±1.04	8.16±0.56	96.03±11.78	6.64±0.52	4.36±0.13	22.68±1.73	A&Ç 2010
Mesalina brevirostris	14.06±0.91	8.07±0.42	89.09±7.52	6.46±0.49	3.84±0.15	19.48±1.34	A&Ç 2010
Ophisops elegans	12.43±0.65	7.51±0.25	73.27±4.88	6.51±0.34	3.84±0.15	19.63±1.22	A&Ç 2010
Parvilacerta parva	13.63±0.86	8.01±0.44	85.80±8.39	6.12±0.48	3.98±0.10	19.15±1.67	A&Ç 2010
Podarcis muralis	13.93±0.95	8.43±0.59	92.46±11.32	6.36±0.54	4.35±0.12	21.74±2.06	A&Ç 2010
Podarcis siculus	13.89±0.94	8.10±0.35	87.41±7.85	6.59±0.51	4.19±0.12	21.69±1.82	A&Ç 2010
Timon princeps	14.98±1.14	8.43±0.47	99.27±10.83	6.04±0.53	3.99±0.11	18.89±1.67	A&Ç 2010
Eublepharidae							
Eublepharis angramainyu	16.57±0.17	8.93±0.08	116.29±1.95	7.38±0.07	4.38±0.02	25.35±0.24	A&Ç 2010
Gekkonidae							
Cyrtopodion heterocercum	16.17±0.20	8.81±0.06	111.77±1.57	7.57±0.08	4.59±0.02	27.27±0.33	A&Ç 2010
Cyrtopodion scabrum	14.83±0.10	8.34±0.07	97.13±1.26	7.08±0.06	4.38±0.02	24.34±0.24	A&Ç 2010

Continued on next page

Table 2. (continued)

	Erythrocytes			Nuclei			References
	L (μm)	W (μm)	ES (μm²)	NL (μm)	NW (μm)	NS (μm²)	
Hemidactylus turcicus	16.56±0.21	8.91±0.06	115.89±1.93	7.44±0.09	4.40±0.02	25.71±0.32	A&Ç 2010
Agamidae							
Stellagama stellio	16.85±0.18	9.12±0.06	120.71±1.71	7.84±0.08	4.40±0.02	27.08±0.29	A&Ç 2010
Trapelus lessonae	14.75±0.16	8.69±0.08	100.78±1.67	6.91±0.06	4.58±0.02	24.83±0.25	A&Ç 2010
Leiolepis belliana rubritaeniata	15.35 - 15.90	9.59 - 9.88					Po&co 2008
Psammophilus blanfordanus	12.83 - 15.37	7.97 - 10.75	104.69-119.24	6.12 – 7.00	4.0 – 5.1	24.76-35.42	Pa&co 2011
Chamaeleonidae							
Chamaeleo chamaeleon	15.97±0.16	9.75±0.08	122.34±1.81	7.72±0.09	4.85±0.03	29.37±0.35	A&Ç 2010
Varanidae							
Varanus griseus	16.24±0.13	10.21±0.09	130.33±1.99	7.09±0.07	4.69±0.03	26.12±0.34	A&Ç 2010
Varanus komodoensis	21.0–27.9	11.2–15.8					G&co 2000
Squamata / Ophidia							
Leptotyphlopidae							
Leptotyphlops macrorhynchus	15.86±0.11	9.29±0.08	115.75±1.45	7.33±0.08	4.45±0.02	25.58±0.31	A&Ç 2010
Typhlopidae							
Typhlops vermicularis	16.57±0.17	9.13±0.06	118.76±1.60	7.27±0.08	4.54±0.02	25.93±0.29	A&Ç 2010
Boidae							
Eryx jaculus	16.36±0.19	8.77±0.07	112.83±2.01	7.16±0.09	4.39±0.02	24.67±0.33	A&Ç 2010
Colubridae							
Dolichophis caspius	14.91±0.16	7.64±0.12	89.88±2.21	10.01±0.08	4.84±0.04	38.08±0.52	A&Ç 2010
Dolichophis jugularis	16.29±0.18	7.48±0.07	95.81±1.69	10.57±0.06	4.98±0.04	41.27±0.37	A&Ç 2010
Dolichophis schmidti	16.21±0.14	9.88±0.07	125.82±1.71	7.67±0.08	4.27±0.02	25.70±0.31	A&Ç 2010
Eirenis barani	16.18±0.12	9.68±0.05	122.98±1.27	7.94±0.08	4.54±0.02	28.32±0.29	A&Ç 2010
Eirenis coronella	16.59±0.23	10.22±0.11	133.52±2.81	7.43±0.09	4.58±0.02	26.66±0.34	A&Ç 2010
Eirenis decemlineatus	14.75±0.14	10.03±0.07	116.25±1.55	7.68±0.10	4.50±0.03	27.12±0.37	A&Ç 2010
Eirenis eiselti	14.13±0.13	9.62±0.07	106.84±1.65	7.29±0.05	4.54±0.02	26.00±0.22	A&Ç 2010
Eirenis levantinus	16.60±0.15	10.04±0.09	130.84±1.77	8.12±0.08	4.47±0.02	28.47±0.29	A&Ç 2010
Eirenis modestus	14.47±0.15	7.45±0.08	84.78±1.53	10.05±0.06	4.92±0.07	38.80±0.55	A&Ç 2010
Eirenis punctatolineatus	16.22 ±0.15	9.58±0.07	122.07±1.78	7.63±0.08	4.52±0.03	27.06±0.31	A&Ç 2010
Eirenis rothii	14.77±0.15	8.73±0.06	101.24±1.44	7.84±0.12	4.14±0.02	25.45±0.38	A&Ç 2010
Hemorrhois nummifer	15.61±0.10	9.33±0.04	114.30±0.90	6.92±0.08	4.52±0.01	24.53±0.24	A&Ç 2010
Colubridae							
Hemorrhois ravergieri	14.76±0.16	9.95±0.11	115.38±1.91	7.49±0.09	4.91±0.04	28.90±0.50	A&Ç 2010
Malpolon insignitus	15.24±0.13	11.16±0.09	133.60±1.77	7.42±0.09	4.84±0.03	28.17±0.38	A&Ç 2010
Natrix natrix	16.87±0.18	10.15±0.08	134.46±1.66	7.95±0.07	4.56±0.02	28.49±0.30	A&Ç 2010
Natrix tessellata	15.98±0.21	7.92±0.09	99.61±2.13	10.21±0.09	5.04±0.04	40.46±0.61	A&Ç 2010
Platyceps collaris	14.40±0.12	10.04±0.08	113.63±1.54	7.29±0.07	4.62±0.02	26.42±0.26	A&Ç 2010
Platyceps najadum	15.47±0.14	10.23±0.13	124.50±2.41	8.44±0.23	5.03±0.05	33.32±0.97	A&Ç 2010
Platyceps ventromaculatus	15.94±0.17	10.67±0.11	133.60±2.14	6.91±0.06	4.49±0.03	24.33±0.22	A&Ç 2010
Rhynchocalamus melanocephalus	17.96±0.20	9.85±0.07	138.88±1.90	7.95±0.08	4.47±0.02	27.88±0.28	A&Ç 2010
Spalerosophis diadema	15.74±0.18	9.52±0.13	118.10±2.69	6.81±0.10	4.67±0.02	24.98±0.42	A&Ç 2010
Telescopus fallax	18.33±0.23	10.33±0.10	148.80±2.57	7.53±0.06	5.06±0.04	29.87±0.34	A&Ç 2010
Telescopus nigriceps	18.55±0.20	10.43±0.11	152.14±2.79	7.96±0.10	4.60±0.02	28.73±0.37	A&Ç 2010
Zamenis hohenackeri	17.66±0.24	9.91±0.09	137.55±2.51	8.49±0.13	4.44±0.02	29.53±0.41	A&Ç 2010
Zamenis longissimus	12.71±0.15	7.38±0.07	73.83±1.38	6.61±0.08	4.56±0.03	23.63±0.31	A&Ç 2010
Viperidae							
Macrovipera lebetina	17.21±0.25	9.83±0.10	133.11±2.61	6.68±0.12	4.74±0.05	24.87±0.51	A&Ç 2010
Montivipera albizona	17.16±0.26	9.67±0.13	130.72±3.31	7.39±0.14	4.36±0.05	25.32±0.62	A&Ç 2010
Montivipera wagneri	17.63±0.20	7.62±0.10	105.71±2.22	10.61±0.07	4.70±0.05	39.19±0.55	A&Ç 2010
Montivipera xanthina	17.08±0.16	7.20±0.10	96.78±2.08	-	-	-	A&Ç 2010
Vipera eriwanensis	16.98±0.17	7.58±0.08	101.16±1.65	10.58±0.06	4.91±0.04	40.77±0.45	A&Ç 2010
Elapidae							
Walterinnesia morgani	16.20±0.15	10.14±0.10	129.12±2.05	7.53±0.08	4.82±0.04	28.52±0.41	A&Ç 2010
Naja kaouthia	16.65 ± 0.12	9.45 ± 0.11					S&co 2002b
Naja siamensis	16.25 ± 0.18	10.10 ± 0.12					S&co 2002b
Naja sumatrana	16.70 ± 0.16	9.90 ± 0.09					S&co 2002b

regarding the L/W ratios, the most ellipsoidal cells were detected in *Lacerta pamhylica* and the least ellipsoidal cells were detected in *Anatolol-acerta danfordi* (Table 2). The longest nuclei were found in *S. stellio*, the widest and the largest ones in *Chamaeleo chamaeleon*, the shortest and the smallest ones in *Trachlepis aurata* and the narrow-est ones in *Ablepharus chernovi* and *Trachylepis vittata*. When the NL/NW ratios were considered, the most ellipsoidal nuclei were found in *Eumeces schneiderii* and the least ellipsoidal nuclei were found in *Darevskia uzzelli*. The highest nucleocyto-plasmic ratio was detected in *O. elegans* and the lowest nucleocytoplasmic ratio was determined in *T. aurata* and *Chalcides ocellatus* (Table 2).

In snake species, the mean length, width and erythrocyte size range from 14.13 µm to 18.55 µm, from 7.20 µm to 11.16 µm and from 84.78 µm² to 152.14 µm², respectively and the L/W ratio ranges from 1.37 to 2.38 (Fig. 2J, K and L) (Arıkan & Çiçek 2011a). In this sense, the longest and the largest erythrocytes were found in *Telescopus nigriceps*, the widest ones in *Malpolon insignitus*, the shortest ones in *Eirenis eiselti*, the narrowest ones in *M. xanthina* and the smallest ones in *Eirenis modestus*. Regarding the L/W ratio, the most ellipsoidal cells were found in *M. xanthina* and the least ellipsoidal cells were found in *M. insignitus* (Table 2). Due to the irregular nucleus shapes of erythrocytes in *M. xanthina*, the measurements of nuclei are not provided in Table 2 (Arıkan et al. 2004; Arıkan & Çiçek 2011a). Among the species under examination, the longest nuclei were observed in *Montivipera wagneri*, the widest ones in *Telescopus fallax*, the largest ones in *Dolichopis jugularis*, the shortest ones in *Macrovipera lebetina*, the narrowest ones in *Eirenis rothi* and the smallest ones in *Platyceps ventromaculatus*. Regarding the NL/NW ratio, the most ellipsoidal nuclei were determined in *M. wagneri*; the least ellipsoidal nuclei were determined in *M. lebetina*; the highest nucleocytoplasmic ratio was detected in *Vipera eriwanensis*; and the lowest nucleocytoplasmic ratio was found in *Zamenis longissimus* (Table 2). As a result, there is no correlation between body size and erythrocyte size (Kendall's τ test, r = 0.024, $P \leq 0.845$).

Different researchers reported that vertebrates were a heterogeneous group in terms of blood cell morphology (Hartman & Lessler 1964, Szarski & Czopek 1966, Saint Girons & Saint Girons 1969, Saint Girons 1970, Arıkan et al. 2004, Frye 1991, Mader 2000, Campbell 2004, Strik et al. 2007, Sykes & Klaphake 2008, Arıkan et al. 2009a, b, Claver & Quaglia 2009, Arıkan et al. 2010, Arıkan & Çiçek 2011a) and they stated that there were noteworthy variations among different orders and even within the same family. Among the turtle species under examination, the aquatic ones have larger erythrocytes and nuclei than those of terrestrial *T. graeca* (Table 2). In terms of the L/W ratio, aquatic species have more ellipsoidal erythrocytes than *T. graeca*. However, *T. graeca* has a more ellipsoidal nucleus than aquatic species in terms of NL/NW (Table 2). This is in agreement with the findings by Uğurtaş et al. (2003). The nucleocytoplasmic ratio was found smaller in *T. graeca* than in aquatic species (Table 2). In conclusion, it might be stated that terrestrial species (e.g. *T. graeca*) have more favourable erythrocytes for exchange of gases than aquatic ones (e.g. *Emys orbicularis*).

Among the 30 lizard species under examination, the largest erythrocytes were observed in *V. griseus* and the smallest ones were observed in *O. elegans*. The erythrocyte size displays great variations among families and, in some cases, even within the species of the same family. It might be stated that these variations result from different activity levels (e.g. health, reproduction, hibernation, food and other daily needs). In terms of the L/W ratio, the most ellipsoidal erythrocytes were observed in *L. pamhylica* and the least ellipsoidal or almost spherical ones were observed in *A. danfordi*. When the NL/NW ratio was considered, scincid lizards have a more ellipsoidal nucleus than the others (Table 2). There is generally a positive correlation between erythrocyte and nucleus sizes in lizards. The nucleocytoplasmic ratio ranges from 0.12 to 0.15 in family Scincidae and from 0.19 to 0.27 in the others (Table 2). In this sense, it might be stated that the members of family Scincidae have more favorable erythrocytes for exchange of gases than other lizards.

Saint Girons and Saint Girons (1969) reported that, except for *Typhlops vermicularis* with relatively small erythrocytes and large nuclei, snakes form a homogeneous group in terms of erythrocyte sizes. Nevertheless, great variations in erythrocyte size were found among families and even within the members of the same family. Among the 34 species under examination the largest erythrocytes were measured in *T. nigriceps* and the smallest ones were measured in *Z. longissimus*. In terms of the L/W ratio, the most ellipsoidal erythrocytes are found in *M. xanthina* and the least ellipsoidal or relatively almost spherical ones are found in *M. insignitus*.

Some researchers (Gulliver 1875, Saint Girons & Saint Girons 1969, Arıkan et al. 2004) reported the presence of nuclei which were irregular to some extent in the erythrocytes of some viperid and elapid species. Similar results were particularly found in *M. xanthina*. In terms of the NL/NW ratio, the most ellipsoidal nuclei were observed in *M. wagneri* and the least ellipsoidal nuclei were observed in *M. lebetina*. Contrary to lizards, there is no correlation between erythrocyte and nucleus sizes in snakes. When the nucleocytoplasmic ratio was considered, snakes formed a heterogeneous group and this ratio ranges from 0.19 to 0.46.

Lizards generally have larger erythrocytes than snakes. There is an inverse correlation between the number of erythrocytes and cell size (Pienaar 1962, Hartman & Lessler 1964).

<u>Leukocytes or White Blood Cells</u>

<u>*1. Agranulocytes*</u>

<u>*a. Lymphocytes*</u>

Lymphocytes are cells which are related to immune responses and the production of hematopoietic growth factors. Among leukocytes, both small and large lymphocytes were observed to be dominant cells in the blood smears of all species of amphibians and reptiles (Frye 1991, Mader 2000, Campbell 2004, Strik et al. 2007, Allander & Fry 2008, Sykes & Klaphake 2008, Saint Girons 1970, Arıkan et al. 2004, 2009a). Lymphocytes and monocytes constitute 80% of the leukocytes of the species under examination. The lymphocytes of reptiles morphologically resemble those of mammalians. In small lymphocytes, the nucleus that is quite chromophilic fills almost the whole cell. The cytoplasm is pushed aside in the form of a small zone (Fig. 3A). Small lymphocytes are often confused with thrombocytes. Regarding small lymphocytes, the largest mean diameter was observed in urodeles (14.92 µm) and the smallest mean diameter was observed in lizards (7.79 µm) (Table 3). In large lymphocytes, the nuclei which are spherical are more chromophilic and they are located in one region of the cell. The cytoplasm covers a larger area than that of small lymphocytes and is stained in pale blue and the nuclei are stained with Wright's stain in purplish blue (Fig. 3B). In large lymphocytes, the largest mean diameter was measured in urodeles (20.73 µm) and the smallest mean diameter was measured in lizards (11.63 µm) (Table 3).

<u>*b. Monocytes*</u>

Monocytes morphologically resemble large lymphocytes and are often confused with large lymphocytes. Therefore, it might be difficult to distinguish between monocytes and large lymphocytes; however, they are easily distinguished from large lymphocytes by their possessing of a kidney-shaped nucleus. With Wright's stain, the cytoplasm was stained in lightgreyish and the nucleus was stained in darkpurplish blue (Fig. 3C & D). Regarding monocytes, the largest mean diameter was measured in urodeles (21.00 µm) and the smallest mean diameter was measured in turtles (12.20 µm) (Table 3).

In most reptiles apart from lizards and snakes, monocytes contain azurophilic granules. These cells were reported as "azurophils" or "azurophilic monocytes" as a variation of a normal monocyte rather than as a different cell (Campbell 2006, Harr et al. 2001). On the contrary, the azurophils of snakes were defined as a distinct cell type with a function similar to that of the neutrophil (Alleman et al. 1999). The azurophilic monocytes of reptiles other than snakes have coarser granules and a lobed nucleus, whereas the azurophils of snakes have finer granules and a spherical nucleus (Alleman et al. 1992). As described Martínez-Silvestre et al. (2005); with May-Grünwald-Giemsa, azurophils have dispersed azurophilic

Table 3. Leukocytes and thrombocytes size (±with their standard errors) in the peripheral bloods of various amphibians and reptiles [TL: thrombocyte length, TW: thrombocyte width].

	Lymphocyte (small) (µm)	Lymphocyte (Large) (µm)	Monocyte (µm)	Heterophil (µm)	Eosinophil (µm)	Basophil (µm)	Thrombocytes	
							TL (µm)	TW (µm)
Amphibia								
Urodela	14.92±0.20	20.73±0.48	21.0±0.18	22.78±0.14	21.13±0.18	19.41±0.67	24.13±0.64	12.88±0.23
Anura	9.68±0.14	12.46±0.23	14.75±0.34	13.63±0.19	11.61±0.16	11.77±0.20	8.82±0.18	5.93±0.12
Reptilia								
Testudines	8.52±0.16	11.91±0.18	12.20±0.14	11.49±0.71	11.80±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±0.21	10.47±0.14	9.99±0.10	7.13±0.16	5.02±0.06
Ophidia	7.99±0.11	12.48±0.20	12.85±0.12	11.87±0.31	11.00±0.12	10.52±0.08	10.17±0.42	5.95±0.13

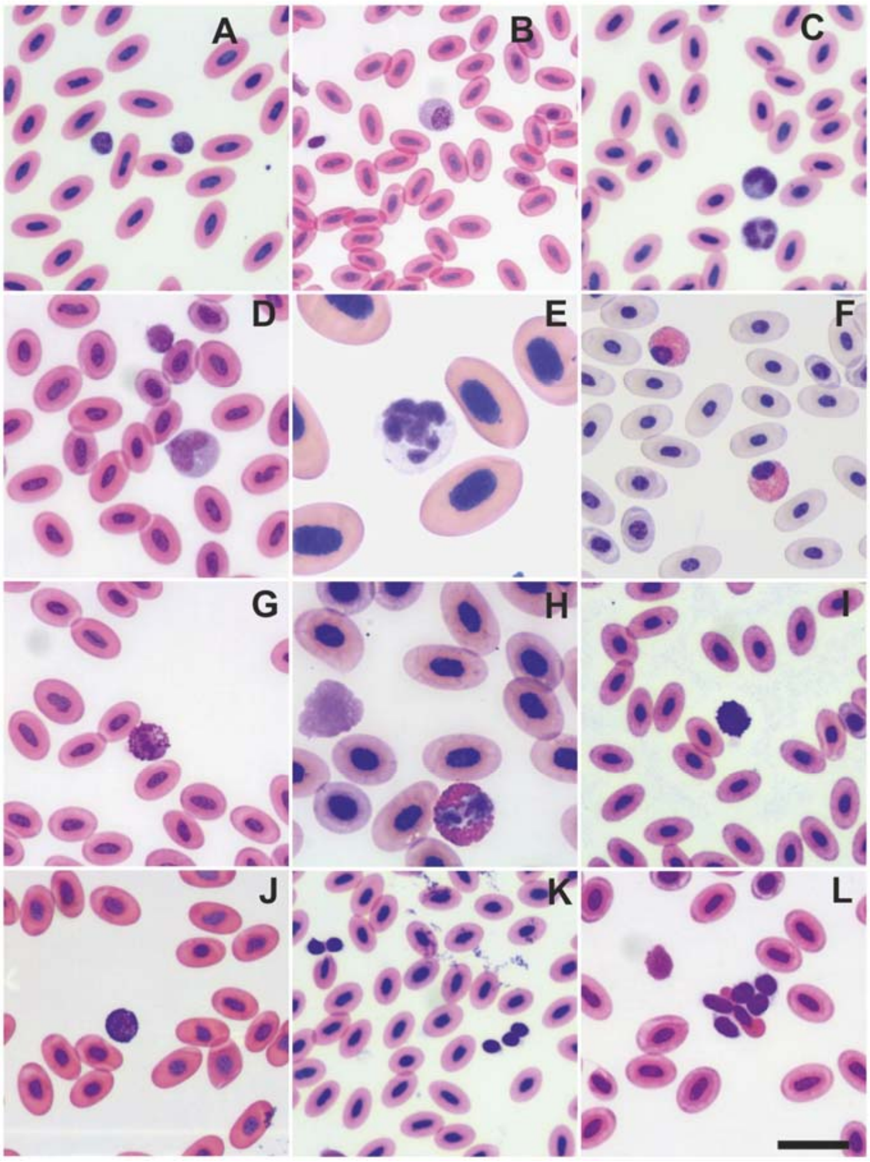

Figure 3. Photomicrographs of leukocytes and thrombocytes in some amphibian and reptile species (modified from Arıkan and Çiçek, 2011a). **A**: Small lymphocyte (*L. trilineata*); **B**: Large lymphocyte (*Z. hohenackeri*); **C**: Monocyte and Heterophil (*O. elegans*); **D**: Monocyte (*P. najadum*), **E**: Heterophil (*N. strauchi*), **F**: Heterophil (*T hermanni*), **G**: Eosinophil (*P. najadum*), **H**: Eosinophil (*P. caralitanus*), **I**: Basophil (*A. cappadocica*), **J**: Basophil (*S. diadema*), **K**: A group of thrombocytes (*O. elegans*), **L**: A group of thrombocytes (*P. najadum*). Horizontal bar: 20 µm.

granules and its cytoplasm with different size of vacuoles. Besides, azurophilic granules have not stained dark shaded than that of other granulocytes. The nucleus of azurophils is non-lobed and usually located in the center of cell. It is not located peripherally as in eosinophils and basophils Nucleus-to-cytoplasm ratio is quite low.

2. Granulocytes

The functions of amphibian granulocytesresemble those of the other vertebrate species and they are identified considering their morphological characteristics (Wright 2001).

a. Heterophils (neutrophils)

Neutrophils (heterophils) are cells which are related to chemotaxis and the killing of bacteria with phagocytosis. Regarding heterophils, the largest mean diameter was measured in urodeles (22.78 µm) and the smallest mean diameter was measured in turtles (11.49 µm) (Table 3). With Wright's stain, their cytoplasm was stained in light-blue and their nucleus, which comprised 2 to 3 lobes, was stained in reddish-brown (Fig. 3C, E & F). Their granules are eosinophilic, elongated or fusiform and abundant in quantity.

Heterophils are the most abundant granulocytic cells in the blood of reptiles and they are analogous to neutrophils (Frye 1991, Strik et al. 2007). Heterophils are spherical cells and have an open cytoplasm. The non-lobed nucleus is either spherical or ellipsoidal and located at the periphery of the cytoplasm. The granules are eosinophilic, elongated or fusiform and in a large number. Due to the staining characteristics of granules, the morphology of heterophils might change. It is believed that this difference is due to the different development stages of the heterophil. Toxic changes can be shown with the presence of a basophilic cytoplasm, abnormal granules and vacuoles.

The morphology of these cells might vary. Visible cytoplasmic granules are unavailable in some species; however, eosinophilic granules are always present in the other species. Cells with visible granules are often stated as heterophils (Campbell 2004). Heterophil granules are typically smaller and more elongated than those of eosinophils (Wright 2001).The type of the stain used might affect the morphology of a granulocyte (Martínez-Silvestre et al 2005). It was determined that Wright-Giemsa stain increased the staining of basophilic granules (Wright 2001). Most amphibian neutrophils probably have lobed nuclei. Besides, neutrophils with a scarcely lobed nucleus were also observed in clinically normal amphibian species.

b. Eosinophils

Amphibian eosinophils generally have less lobed nuclei than those of neutrophils and have heavily stained large granules. The cytoplasm of eosinophils is stained with Wright's stain in light-yellowish. The shape of the nucleus cannot be fully distinguished, for the nucleus is masked by the large and bright red granules in the cytoplasm (Fig. 3G & H). The largest mean diameter was measured in urodeles (21.13 µm) and the smallest mean diameter was measured in lizards (10.47 µm) (Table 3).

Eosinophils resemble heterophils in shape and size and have an eccentric nucleus. The fact that the eosinophilic granules of eosinophils are generally spherical is their morphological characteristic which distinguishes them from the heterophils with either ellipsoidal or elongated granules. The eosinophils of green iguanas (*I. iguana*) have bluish-green spherical granules, the function of which is not fully known. Eosinophils were not observed particularly in some snakes. Although eosinophils were present in the king cobra (*Ophiophagus hannah*) (Salakij et al. 2002a), they were not observed in the diamondback rattlesnake (*Crotalus adamanteus*) and yellow rat snake (*Elaphae obsoleta quadrivitatta*) (Dotson et al. 1995, Alleman et al. 1999). As stated in heterophils, individual variations in eosinophils were observed in green turtles as well (*Chelonia mydas*) (Work et al. 1998).

c. Basophils

Basophils are small granulocytes which have a centrally located non-lobed nucleus that cannot be fully recognized and which have dark basophilic granules. The largest mean diameter detected in basophils that were smaller than other granulocytic cells was determined in urodeles (19.41 µm) and the smallest mean diameter was detected in lizards (9.99 µm) (Table 3). Their cytoplasm was filled with purplish black granules and, as in eosinophils, the nucleus is masked by granules (Fig. 3I & J). No basophils were observed in *W. morgani* (Arıkan & Çiçek 2011a).

In amphibian basophils, basophilic cytoplasmic granules and non-lobed nuclei were heavily stained. Basophils which discharged their granules were encountered in the circulatory blood (Wright 2001). The size of basophils varies among species (Wright 2001). In some amphibians, basophils were observed to be the predominant cells of leukocytes (Campbell 2004).

Thrombocytes

Thrombocytes are nucleated fusiform cells that are considered to be functionally equal to mammalian platelets. Immature cells are spherical cells that have a round nucleus and a more basophilic cytoplasm. It was noted that adult thrombocytes were confused with small lymphocytes. Thrombocytes generally form clusters in blood smears; therefore, they are easily distinguished from small lymphocytes by this characteristic of theirs. Thrombocytes were observed to be fusiform in some species (Fig.

3L) and almost spherical in others (Fig. 3K). Their chromophilic nucleus filled almost the whole cell. The longest and the largest thrombocytes were measured in urodeles (TL = 24.13 µm, TW = 12.88 µm), while the shortest and the narrowest ones were measured in lizards (TL = 7.13 µm, TW = 5.02 µm) (Table 3).

5. Numbers and Variations of Blood Cells

Number of Erythrocytes

In amphibians and reptiles, the number of erythrocytes is smaller than those of birds and mammalians. When compared with the other amphibians, it might be stated that urodeles with very large erythrocytes have fewer cells in blood volume. Hence, the number of erythrocytes in 1 mm^3 of blood was determined as 51,000 in *Necturus maculosus* (the erythrocyte diameter is about 54 µm) (Harris 1963) and the values detected for *Pelophylax bedriagae* are 23.14 µm and 326,600 (Table 4) (Arıkan and Çiçek 2011b).

In reptiles, lizards generally have more erythrocytes than snakes; however, the fewest erythrocytes are found in turtles (Table 4). While lizards have the smallest erythrocytes among all reptiles, turtles have the largest erythrocytes. Therefore, according to this hypothesis suggested by Ryerson (1949), there is an inverse correlation between erythrocyte number and size.

Variations by sex, age, season and pathologi-

Table 4. Numbers of erythrocytes and leukocytes per mm^3 of blood in various amphibian and reptile species. (Cp– captivity, S– Summer, W- winter)

List of species	Erythrocyte	Leukocyte	Thrombocyte	References
Amphibia				
Urodela				
Ambystoma maculatum	52520			Vernberg 1955
Ambystoma tigrinum	81424			Vernberg 1955
Necturus maculosus	27912			Vernberg 1955
Neurergus strauchii	127000	2330		Arıkan et al. 2003
Plethodon cinereus	91078			Vernberg 1955
Plethodon glutinosus	55700			Vernberg 1955
Anura		179		
Bombina bombina	340000	9734 (♂) 7030 (♀)		Wojtaszek & Adamowicz 2003
Bombina bombina	251429	2114		Arıkan et al. 2010
Bombina orientalis	183100			Kuramoto 1981
Bufo bufo	900000 (♂) 870000 (♀)			Dönmez et al. 2009
Hyla japonica	596900			Kuramoto 1981
Pelodytes caucasicus	776000	2560		Arıkan et al. 2003
Pelophylax esculentus	324000-800000	4920		Klieneberger 1927
Pelophylax esculentus	166000-200000 (W)	1100-2100 (W)		Schermer 1954
Pelophylax ridibundus	326600	3142		Arıkan 1989
Rana catesbeiana	252000			Hutchison & Szarski 1965
Rana clamitans	275625			Hutchison & Szarski 1965
Rana japonica	563600			Kuramoto 1981
Rana pipiens	480000 (♂) 512000 (♀)	16134 (♂) 14131 (♀)		Kaplan 1951, 1952
Rana pipiens	319000	5500		Rouf 1969
Rana rugosa	288300			Kuramoto 1981
Rana temporaria	408000	25000		Alder & Huber 1923
Rana temporaria	531000			Arvy 1947
Rana temporaria	280000-626000 (S)	4900-7300 (S)		Schermer 1954
Rana temporaria	400000			Stephan 1954
Reptilia				
Testudines				
Caretta caretta	130000-520000	9022 - 11042		Deem et al. 2009
Chelonia mydas		13800		Work et al. 1998
Chelydra serpentina	154166-530000			Hutchison & Szarski 1965

Continued on next page

Table 4. (continued)

List of species	Erythrocyte	Leukocyte	Thrombocyte	References
Clemmys guttata	475000-750000			Hutchison & Szarski 1965
Clemmys japonica	442000			Mori 1940
Emys orbicularis	260000-680000			Duguy 1967
Emys orbicularis		8000		Alder & Huber 1923
Emys orbicularis		5965		Yılmaz & Tosunoğlu 2010
Emys orbicularis	444333-455555	4940-7433		Tosunoğlu et al. 2011a
Hieremys annandalii	275000	11660	10970	Chansue et al. 2011
Lepidochelys olivacea	230000	9250	1050	Hutchison & Szarski 1965
Mauremys leprosa		4580 (♂) 4400 (♀)		Hidalgo-Vila et al. 2007
Mauremys rivulata	197333 - 449333	3950 2479 - 5790		Yılmaz & Tosunoğlu 2010 Tosunoğlu et al. 2011a
Podocnemis expansa		3290		Tavares-Dias et al. 2008
Testudo graeca	362000-730000			Babudieri 1930; Graziadei 1954
Trionyx spiniferus	530000-960000			Hutchison & Szarski 1965
Crocodilia				
Alligator mississippiensis	618000-1480000			Altman & Dittmer 1961
Crocodylus palustris	800000 (Cp)	6970 (Cp)	2380 (Cp)	Stacy & Whitaker 2000
Crocodylus rhombifer	289000 (♂) 237000 (♀)			Carmena-Suero et al. 1979
Sauria				
Acanthodactylus erythrurus	846000			Salgues 1937
Agama atra	1250000			Pienaar 1962
Anguis fragilis	466000-1615000			Duguy 1963a
Basiliscus plumifrons		18700		Dallwig et al. 2011
Chalcides ocellatus	806000			Salgues 1937
Chamaeleo chamaeleon	737000	3603		Cuadro et al. 2003
Gallotia bravoana	954000	8000	4070	Martínez-Silvestre et al. 2004
Gallotia intermedia	925000	6500	2760	Martínez-Silvestre et al. 2004
Gallotia simonyi	10801000	6000	1940	Martínez-Silvestre et al. 2004
Heloderma horridum	860000	4610		Espinosa-Avilés et al. 2008
Heloderma suspectum	646000			Ryerson 1949
Heloderma suspectum	500000	4720		Cooper-Bailey et al. 2011
Hemidactylus turcicus	866000			Salgues 1937
Lacerta agilis	945000-1420000			Salgues 1937
Lacerta viridis	840000-1600000			Salgues 1937
Leiolepis belliana rubritaeniata	860000	7300		Ponsen et al 2008
Phyllodactylus europaeus	644000			Salgues 1937
Psammophilus blanfordanus	57000 – 83500	14225 - 20697		Parida et al. 2012
Podarcis muralis	960000-2050000			Duguy 1967
Varanus komodoensis		2300		Gillespie et al. 2000
Ophidia				
Agkistrodon piscivorus	468000-697000			Hutton 1958
Bothrops alternatus	660600 (Cp)	12050 (Cp)	5680 (Cp)	Troiano et al. 2000
Bothrops ammodytoides	489400	9420	4600	Troiano et al. 1999
Bothrops jararacussu	642300 (Cp)	10640 (Cp)	5200 (Cp)	Troiano et al. 2000
Bothrops moojeni	543100 (Cp)	10060 (Cp)	5200 (Cp)	Troiano et al. 2000
Bothrops neuwiedi diporus	667300 (Cp)	13520 (Cp)	6140 (Cp)	Troiano et al. 2000
Bungarus fasciatus	527000 ♂ 529000 ♀	14000 ♂ 16000 ♀		Salakij et al. 2003
Coluber viridiflavus	908000-1608000			Salgues 1937
Coronella austriaca	508000-140600			Salgues 1937
Crotalus durissus terrificus	1535000 (♂) 1530000 (♀)	12300 11000	7240 6950	Troiano et al. 1997
Crotalus durissus terrificus	503750	13,066		Santos et al. 2008
Crotalus horridus	1140000			Carmichael & Petcher 1945
Dolichophis caspius	918500	5750		Tosunoğlu et al. 2011b

Continued on next page

Table 4. (continued)

List of species	Erythrocyte	Leukocyte	Thrombocyte	References
Eirenis modestus	768196	5500		Tosunoğlu et al. 2011b
Elaphe longissima	622000-1410000			Salgues 1937
Elaphe scalaris	1181000			Salgues 1937
Malpolon monspessulanus	1442000			Salgues 1937
Malpolon insignitus	894444	5466		Tosunoğlu et al. 2011b
Naja kaouthia	616000	14000		Salakij et al. 2002b
Naja siamensis	576000	12000		Salakij et al. 2002b
Naja sumatrana	657000	10000		Salakij et al. 2002b
Natix natrix	668000-1302000			Salgues 1937
Natrix maura	378000-1070000			Duguy 1967
Natrix natrix	846166	3766		Tosunoğlu et al. 2011b
Natrix natrix	1748000 (♂) 1545000 (♀)	16710 (♂) 16030 (♀)	32940 (♂) 34230 (♀)	Wojtaszek 1991
Ophiophagus hannah		13530		Salakij et al. 2002a
Platyceps collaris	1156667	6080		Tosunoğlu et al. 2011b
Sistrus catenatus		8116		Allender et al. 2006
Telescopus fallax	681750	5530		Tosunoğlu et al. 2011b
Typhlops vermicularis	849333	4611		Tosunoğlu et al. 2011b
Vipera ammodytes	667000			Babudieri 1930
Vipera aspis	571000-1410000			Salgues 1937
Vipera berus	615000-1232000			Salgues 1937
Vipera ursini	1350000			Salgues 1937

cal factors occur in the number of erythrocytes. Variation by sex was reported in *Rana temporaria*, in which the mean number of erythrocytes in 1 mm^3 of blood was determined as 300,000 in females and as 450,000 in males (Arvy 1947). It was revealed that the number of erythrocytes was higher in males than in females in some reptile species such as *Terrapene carolina* (Altland & Thomson 1958), *Cordylus vittifer* (Pienaar 1962), *Vipera aspis* (Duguy 1963b, 1970), *Anguis fragilis* (Duguy 1963a) and *Natrix maura* and *Emys orbicularis* (Duguy 1967).

Variation by season was shown in *Bufo arenarum* that lived in Brazil. The number of erythrocytes in 1 mm^3 of blood was 1,000,000 in July and August, while there was a rapid reduction in the number of erythrocytes in September and October – the reproduction period (Varela & Sellarés, 1938). The observation of an increase in the number of erythrocytes before winter was stated for *Crotalus horridus* by Carmichael & Petcher (1945), for *Terrapene carolina* by Hutton and Goodnight (1957), for *Cordylus vittifer* by Pienaar (1962), for *Vipera aspis* (Duguy 1963b, 1970) and *Anguis fragilis* by Duguy (1963a), for *Natrix natrix* by Binyon & Twigg (1965) and for *Natrix maura* and *Podarcis muralis* by Duguy (1967).

It was found that the feeding status of an animal was effective on the blood structure. It was detected that in *Bufo viridis*, hunger led to an increase in the number of immature erythrocytes in blood (Chury 1952). It was revealed that in *Necturus*, hunger led to a decline in the number of erythrocytes (Harris 1963).

<u>Number of Leukocytes</u>

In amphibians and reptiles, the number of leukocytes in 1 mm^3 of blood varies. The variations in the number of leukocytes are due to sex, age, season, ecdysis, pregnancy, and ecological and pathological factors. The number of leukocytes in 1 mm^3 of blood in different amphibian and reptile species is provided in Table 4.

6. Composition of Plasma

Plasma is a fluid that carries all food substances. The food substances in the content are met from the digestive system, whereas the wastes are produced in tissues (Seiverd 1972). Plasma is a homogeneous and slightly alkaline fluid which contains matters with low and high molecular weights. In addition to water, dissolved gases, inorganic salts, carbohydrates and other organic compounds including amino acids, vitamins, hormones and lipoproteins found in the content, 7% of plasma is comprised of plasma proteins (Junqueira et al. 1998).

The plasma proteins include albumin, α-, βand γ-globulins and fibrinogen. Albumin is the main component of plasma proteins and has an important role in providing the osmotic pressure of blood (Junqueira et al. 1998). The percentage rate of albumin is gradually increasing from amphibians to higher vertebrates (birds and mammalians) (Ferguson 1980). Amphibians have two types of antibodies. Nevertheless, reptiles have three types of antibodies (Abbas et al. 1991).

7. Blood Parasites of Amphibians and Reptiles

<u>Blood Parasites of Amphibians</u>

Systemic parasite invasions rarely cause a disease in free-living amphibians. In captivity, these invasions generally cause many diseases and frequently eosinophilia. Furthermore, parasites might directly invade the circulatory system. Nematodes of order Filarioidea might be found in blood and the lymphatic system (Reichenbach-Klinke & Elkan 1965). The invasion of microfilariae out of these parasites causes lethargy; however, a severe invasion is required before systemic symptoms are seen. For the treatment of these microfilariae, the intermediate host (generally insects) should be kept away and its treatment with fenbendazole and the other anthelmintics is recommended (Poynton & Whitaker 2001). It was reported that erythrocytic protozoans were widely found in amphibians (Reichenbach-Klinke & Elkan 1965). The parasites reported in amphibians encompass *Haemogregarina*, *Plasmodium*, *Aegyptianella*, *Haemoproteus* and *Lankesterella* (Reichenbach-Klinke & Elkan 1965, Barta & Desser 1984, Readel & Goldberg 2010, Campbell & Ellis 2007). *Haemogregarina* spp. and *Aegyptianella* spp. are common intraerythrocytic organisms that are considered with low pathogenicity; nevertheless, they bring about anaemia in some animals and the rate of affected erythrocytes might influence prognosis (Wright 2001, Campbell & Ellis 2007). *Bartonella ranarum* has been reported in a frog and causes mortality within 6 months (Reichenbach-Klinke & Elkan 1965). The extracellular blood parasites found in amphibians cover trypanosomes and microfilaria; however, their clinical significance is unknown (Campbell & Ellis 2007, Readel & Goldberg 2010). The treatment of intraerythrocytic parasites is rarely compulsory as their clinical significance is unknown; however, a parasite-specific drug treatment might be applied (Poynton & Whitaker 2001). Foronda et al. (2007) showed the clinical efficiency of atovaquone-proguanil for treatments against haemogregarine (Apicomplexa) infection in *Gallotia caesaris*.

Blood parasites can be divided into two classes depending on whether they have infected blood without attacking the corpuscles or whether they have been settled in corpuscles (Saint Girons 1970).

<u>Blood Parasites of Reptiles</u>

The blood parasites of reptiles encompass haemogregarins, trypanosomes, microfilaria, piroplasmids and plasmodium. Haemogregarins are represented with three genera, namely *Haemogregarina* found in aquatic turtles, *Hepatozoon* found in snakes, and lizards (Roca & Galdòn 2010) and *Karyolysus* found in old world lizards and tree snakes. Haemogregarins are identified with their sausage-shaped gametocytes that impair the shape of the host cell by forming a projection in the cytoplasm. Piroplasmids such as *Sauroplasma* and *Serpentoplasma* are sometimes found in the blood of lizards and snakes. They are seen as small, round and seal ring-like vacuoles with no pigment in the cytoplasm of erythrocytes.

The blood corpuscles of reptiles are infected by a large number of telosporidian species of genera *Karyolysus*, *Hepatozoon* and *Haemogregarine*. Haemogregarins infect erythrocytes and, rarely, leukocytes. Telosporidians are also found in blood; however, they are widely found in the bladder and the intestine. *Haemoproteus* and particularly *Plasmodium* are the most important genera among the haemosporidians that infect the blood corpuscles of reptiles. Pienaar (1962) studied these parasites in detail and presented a table concerning hosts and descriptions for 19 species of *Plasmodium*. *Pyrhemocyton* was identified from lizards, snakes and turtles (Brumpt & Lavier 1935, du Toit 1937, Carpano 1939, Pienaar 1962).

Flagellates: Flagellates from genera *Trypanosoma*, *Leptomonas*, *Leishmania* and probably *Proteromonas* were found in the blood of reptiles (Doflein & Reichenow 1953).

Trypanosomes are found in both terrestrial and aquatic reptiles. Trypanosomes are particularly well studied in aquatic turtles (Pienaar 1962). *Leptomonas* was reported in the blood of lizard species (Hindle 1930, Reichenov 1953). *Leishmania* is also found in the blood of lizard species (Reichenov 1953, Heisch 1958).

Nematodes: Filarial worms were frequently reported in the blood of reptiles (Chabaud &

Frank 1961, Reichenbach-Klinke 1963, McKenzie & Starks 2008, Telford 1965, 2009).

Trematodes: Trematodes were noted in the blood vessels of aquatic turtles (Martin & Bamberger 1952).

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