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The morphology and size of Erythrocytes of six reptiles species from the Syrian coastal region

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Abstract

This study aimed to check the erythrocyte and nucleus sizes of six species of reptiles, one species of Agamidae (*Laudakia stellio*), one species of Testudinidae (*Testudo graeca*), one species of Chamaeleonidae (*Chamaeleo chamaeleon recticrista*), and three species of Lacertidae (*Ophisops elegans*, *Lacerta media*, *Phoenicolacerta laevis*) from Coastal region in Syria using blood smears stained with Wright's stain. The longest, widest and largest erythrocytes and the widest nuclei were found in *T. graeca*. The shortest and narrowest erythrocytes and nuclei were found in *O. elegans*. The longest, largest nuclei were found in *C. chameleon*. The shortest and narrowest nuclei were found in *P. laevis*.

Keywords: Erythrocyte, nucleus size, comparative biology, Syria

Introduction

Blood analyzes is useful, widely used as a tool aids in monitoring animal health and diagnosis of disease (Christopher et al., 1999). Checking blood parameters in reptiles may help in the evaluation of physiological and health conditions of populations. It may also be used as an indicator determining environmental conditions (Dickinson et al., 2002). These techniques are used with several wildlife species, especially for threatened or endangered populations, and help to indicate ecosystem health (Deem et al., 2006). It is, therefore important to examine blood parameters in different species of reptiles to find the

possible findings associated with the environmental variables. There may be many internal (species, sex, age, and physiological state) and external (season, temperature, habitat nutritional pattern, and captivity) factors that affect blood parameters. Therefore, it is difficult to determine the reference interval of blood values (Lopes-olivera et al., 2003).

No previous studies are dealing with hematology and blood in Syrian reptiles. We aimed to describe erythrocyte morphology and measure the erythrocyte and nucleus sizes of six species of reptiles that live In the Syrian coastal region.

Material and methods

Individuals of 6 species belonging to sexually mature reptiles were collected for the study which was carried out between July and August. Specimens were collected from Lattakia and Tartus province in Northwestern Syria (Table 1). Blood samples were obtained from the caudal vein of turtles via a heparinized injector (Szarski and Czopek, 1966), and from postorbital sinuses of lizard individuals via heparinized glass capillaries according to MacLean et al. (1973). Four blood smears were prepared per individual. Blood smears were air-dried, stained with Wright's stain for 2 minutes, washed in running tap water for 2 minutes. Blood smears per individual animal were randomly selected and were measured under a microscope. From each blood smear, 40 erythrocytes were randomly chosen for the measurements of their lengths (L), widths (W), nuclear lengths (NL), and nuclear widths (NW). Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed from the formula:

ES = ELEWπ/4 and EN= NLNWπ/4

Cells and nuclear shapes were compared with L/W and NL/NW ratios and nucleus/cytoplasm with NS/ES ratio.(Uğurtaş et al., 2003; Metin et al., 2006).

Results

The erythrocytes or red blood cells of reptiles 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 light yellowish pink and was homogeneous and the chromophilic nuclei were dark purplish-blue under Wright’s stain.

The blood smears of the examined species demonstrated interspecific and even intraspecific variations in terms of the lengths, widths, and sizes of the erythrocytes and nuclei.

The erythrocyte measurements (lengths and widths), sizes, L/W ratios, nuclear measurements, and nucleocytoplasmic ratios are given in Table 2.

Table 1: Collecting localities of 6 species of reptiles from Northwestern Syria (n: number of individuals).

Species	n	Latitude, Longitude
P. laevis	12	(35.450488,36.041282)/(34.911781,36.151173)/ (35.372821,36.067674)/(34.904414,36.140439)/ (34.858259,35.910116)/(35.374084,35.930162)/ (35.507224,35.836712)/(35.107551,36.156439)/ (35.088684,36.157892)/(35.564160,35.741904)/ (35.522773,35.804106).
O. elegans	5	(35.450488,36.041282)/(35.371744,36.067304)/ (35.377546,36.053836)/(35.088684,36.157892)/ (35.411964,35.913058).
L. media	5	(35.372821,36.067674)/(35.107551,36.156439)/ (35.564160,35.741904)/(35.486501,36.229521)/ (35.597279,36.182308).
T. graeca	5	(35.451591,36.040670)/(35.370137,36.061160)/ (34.911781,36.151173)/(35.374084,35.930162)/ (35.507224,35.836712).
L. stellio	4	(35.461847,36.045862)/(35.370353,36.055991)/ (34.858259,35.910116)/(35.522773,35.804106).
C. chameleon	4	(35.452399,36.044004)/(34.904414,36.140439)/ (35.377546,36.053836)/(35.093940,36.161546).

Table 2: Erythrocyte and nuclei measurements (± standard deviation) of six reptiles species from Northwestern Syria (L: Erythrocyte length, W: Erythrocyte width, EL/EW: Erythrocyte length/Erythrocyte width, ES: Erythrocyte size, NL: Nucleus length, NW: Nucleus width, NL/NW: Nucleus length/Nucleus width, NS: Nucleus size, NS/ES: Nucleocytoplasmic ratio, n: number of individuals).

		Erythrocytes				**Nuclei**				
Species	n	L	W	L/W	ES	NL	NW	NL/NW	NS	NS/ES
P. laevis	12	15.14±1.23	8.84±0.52	1.71±0.16	104.57±6.35	5.19±0.41	3.94±0.11	1.27±0.09	16.17±1.61	0.15±0.02
O. elegans	5	13.33±1.22	7.74±0.43	1.27±0.12	81.29±6.82	7.34±0.91	4.13±0.51	1.55±0.23	20.65±0.13	0.25±0.02
L. media	5	15.92±0.81	8.23±0.44	1.95±0.18	102.85±8.97	6.78±0.62	4.42±0.25	1.53±0.11	22.51±1.78	0.22±0.02
T. graeca	5	17.94±0.25	12.15±0.54	1.49±0.10	170.17±6.55	6.23±0.21	5.02±0.09	1.24±0.03	24.55±0.72	0.14±0.02
L. stellio	4	16.52±0.36	9.47±0.31	1.74±0.32	122.87±3.16	7.25±0.42	4.19±0.17	1.74±0.06	25.84±0.91	0.21±0.01
C. chameleon	4	16.23±0.53	9.83±0.27	1.67±0.08	125.24±2.64	7.60±0.36	4.71±0.65	1.61±0.08	27.91±1.42	0.22±0.01

The longest, widest and largest erythrocytes were found in *T. graeca*. The mean length and width of mature erythrocyte of *T. graeca* was 17.94 µm±0.25 and 12.15µm ±0.54, respectively (Table 2). The shortest and narrowest erythrocytes were found in *O. elegans*. The mean length and width of mature erythrocytes of *O. elegans* were 13.33µm ±1.22 and 7.74µm ±0.43, respectively (Table 2). The longest and largest nuclei were

found in *C. chameleon*. The mean length and width of mature nuclei of *C. chameleon* were 7.60µm ±0.36 and 4.71µm ±0.65, respectively (Table 2). The widest nuclei were found in *T. graeca*. The mean width of mature nuclei of *T. graeca* was 5.02µm ±0.09, the shortest and narrowest nuclei were found in *P. laevis*. The mean length and width of mature nuclei of *P. laevis* were 5.19 µm ±0.41 and 3.94 µm ±0.11, respectively (Table 2).

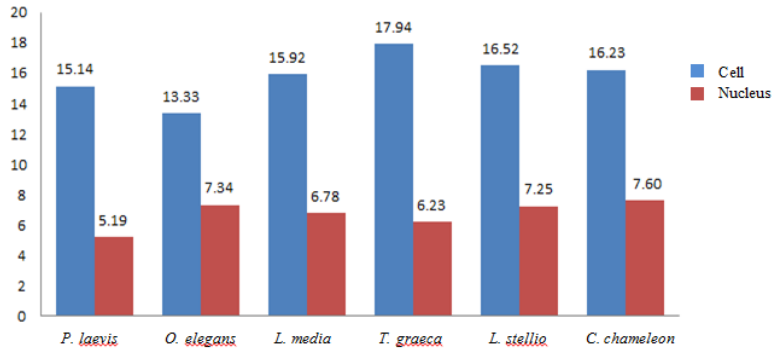

Figure 1: Average and one standard deviation of the lengths of erythrocytes and nuclei obtained from 40 randomly chosen specimens.

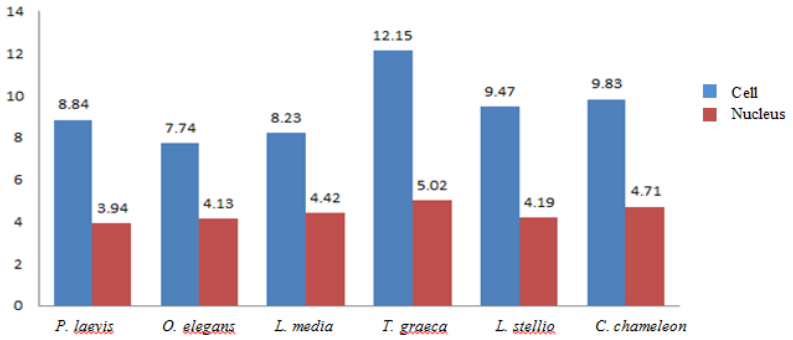

Figure 2: Average and one standard deviation of the width of erythrocyte and nucleus obtained from 40 randomly chosen specimens.

Discussion

Reptiles are considered a heterogeneous group among vertebrates in terms of their blood cell morphology, and demonstrated considerable variations among orders, even within the same family members according to many researchers (Campbell, 2004; Strik et al., 2007; Sykes and Klaphake, 2008; Arikan et al., 2009a, b; Claver and Quaglia, 2009; Vaissi et al., 2013). The sizes of erythrocytes in lizards varied not only among families but sometimes even among different species in the same family (Sevinç et al., 2000; Atatür et al., 2001; Arikan et al., 2009a, b; Çiçek and Arikan, 2010; Arikan and Cicek, 2014). And according to (Arikan et al., 2009b) study, it was confirmed that erythrocyte size difference not only among species but also in different blood smears of the same species in 16 lacertid lizards from Turkey, The largest erythrocyte was observed in *Acanthodactylus harranensis*, the smallest in *O. elegans*. In *O. elegans*, erythrocyte had a length 12.43 µm and width 7.51 µm; nucleus length 6.51 µm and width 3.84 µm. In another study, among

the 68 species of reptiles investigated by (Çiçek and Arikan, 2010), the largest erythrocytes were observed in *Varanus griseus* and the smallest in *O. elegans*. The erythrocyte and nucleus measurements of *L. stellio*, were: the length 16.85 µm; the width 9.12 µm; the nucleus length 7.84 µm and the width 4.40 µm, while in *C. chameleon*, were: the length 15.97 µm and the width 9.75 µm; the nucleus length 7.72 µm and the width 4.35 µm, and in *T. graeca* were: the length 17.35 µm and the width 11.96 µm; the nucleus length 6.09 µm and the width 4.91 µm. The size of erythrocytes showed significant differences among families and in some cases even within species of the same family. The study of (GüL and Tosunoğlu, 2011), entitled "Hematological reference intervals of four Agamid species in Turkey", it was found that the measurements of erythrocyte In *L. stellio*, were: the length is 17.91 µm and the width is 9.89 µm; the nucleus length 7.23 µm and the width 3.98 µm. And according to (Mohammed, 2013) in Libya, Erythrocyte measurements of *Agama sellio* for females were (length 17.69 µm, width 8.34 µm) and for males were (length 17.26 µm, width 8.02 µm). The nucleus was centrally located. The measurements of the nucleus for males were (length 4.64, width 2.93 µm) and for females were (length 4.81 µm, width 3.28 µm).

Our results demonstrate the presence of some differences in erythrocyte sizes among six reptiles species (*O. elegans,P. laevis, L. media, T. graeca,L. stellio, C. chameleon*) from Syria. In this study, the longest, widest and largest erythrocytes were found in *T. graeca*. The shortest and narrowest erythrocytes were found in *O. elegans*. The longest and largest nuclei were found in *C. chameleon*. The widest nuclei were found in *T. graeca*. The shortest and narrowest nuclei were found in *P. laevis*. In the present study, erythrocyte morphology and the results of erythrocytes and nuclei sizes correspond with the results of previous studies.

It is necessary to expand the scope of hematological studies in Syria and combine them with environmental studies, where, according to the study of (Al-Masri et al., 2009a,b), *P.laevis* is considered endangered due to the loss of habitat in some areas in the Syrian coastal region, and *L. media* is threatened with extinction by habitat loss and animal trafficking. This finding should be taken into consideration when comparing the results of studies. And considering that this study was conducted in a balanced ecosystem, these values can be considered as a base reference for subsequent studies, and that any modification in it might indicate a modification in the health of the ecosystem.

Conclusion

The morphology of erythrocytes differed in terms of cell size and nucleus size in the reptile species studied. The largest cell size was in *T. graeca* and the smallest in *O. elegans*, the largest was the nucleus size in C. chameleon and the smallest in *P. laevis*. The difference also appeared in the same erythrocytes belonging to the same type, and this can be explained by the different environmental conditions and different levels of activity.

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