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Variations in the Size of Erythrocytes and Morphology of Four Lizard Species (*Laudakia nupta*, *Trapelus lessonae*, *Mabuya aurata* and *Ophisops elegans*) from Western Iran

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Abstract: The aim of this study was to determine the erythrocyte and nucleus sizes of two species of Agamidae (*Laudakia nupta* and *Trapelus lessonae*), one species of Scincidae (*Mabuya aurata*) and one species of Lacertidae (*Ophisops elegans*) from Iran by means of blood smears stained with Giemsa. The sizes of erythrocytes and their nuclei were measured using an ocular micrometer at a magnification of 6300x. The longest, widest and largest erythrocytes were found in *Laudakia nupta*. The shortest and narrowest erythrocytes and nuclei were found in *Trapelus lessonae*. The longest, widest and largest nuclei were found in *Mabuya aurata.* The study revealed significant variation between Iranian species and others indicating the significant affect of environmental conditions.

Key words: Agamidae · Scincidae · Lacertidae · Erythrocyte Size · Iran

INTRODUCTION

Blood analyses is useful, widely used as a tool aids in monitoring of animal health and diagnosis of disease [1]. Checking blood parameters in reptiles may help in evaluation of physiological and health conditions of populations. It may also be used as an indicator determining environmental conditions [2]. It is, therefore, important to examine blood parameters in different species of reptiles in order 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 [3].

There are very scarce studies dealing with hematology of the Iranian reptiles. expect for 3 snake species [4] and 3 gecko species from west of Iran [5]. Our aim was to describe erythrocyte morphology and measure the erythrocyte and nucleus sizes two species of agamidae (*Laudakia nupta* and *Trapelus lessonae*), one species of Scincidae (*Mabuya aurata*) and one species of Lacertidae (*Ophisops elegans*) which live in Ilam province, Iran.

MATERIALS AND METHODS

In this study, 5 individuals of *Laudakia nupta* (Agamidae) and 6 individuals of *Trapelus lessonae* (Agamidae), 4 individuals of *Mabuya aurata* (Scincidae), 5 individuals of *Ophisops elegans* (Lacertidae) were collected for the study which was carried out between July and Septamber 2012. Geographic positions for the specimens collected are: *L. nupta* (47, 17, 41.92 E; 33, 01, 06.71 N; 1261 masl), *T. lessonae* (47, 17, 14.56 E; 33, 02, 18.23 N; 1326 masl), *M. aurata* (47, 22, 33.45 E; 33, 01, 00.56 N; 945 masl) and *O. elegans* (47, 17, 41.92 E; 33, 01, 06.71 N; 1261 masl) Specimens were collected from Abdanan - Ilam province in western Iran. Blood samples were obtained by heart puncture. Four or five blood smears were prepared per individual. Blood smears were air-dried, fixed in methanol and stained with Giemsa (diluted 1:10 in buffered water, pH 7) for 20 min washed in running tap water for 2 minutes. Blood smears per individual animal were randomly selected. One hundred erythrocytes were measured under a microscope camera (Dinocapture 2.0 and Olympus light microscope). The erythrocyte measurements were taken by means of a BBT Krauss ocular micrometer. Lengths (L) and widths (W) of 100

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randomly chosen erythrocytes as well as nuclear lengths (NL) and nuclear widths (NW) were measured for each blood smear. Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed from the formula ES= (ELEWπ)/4 and EN= (NLNWπ)/4 [12]. Cells and nuclear shapes were compared with L/W and NL/NW ratios and nucleus/cytoplasm with NS/ES ratio.

RESULTS

The erythrocytes or red blood cells of *Laudakia nupta*, *Trapelus lessonae*, *Mabuya aurata* and *Ophisops elegans* 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 and was homogeneous under Gimsa stain. The nuclei of mature erythrocytes are basophilic (Fig. 1).

The longest, widest and largest erythrocytes were found in *L. nupta*. The mean length and width of mature erythrocyte of *L. nupta* was 12.21µm ± 0.44 and 6.82µm ± 0.34, respectively (with a range of 12.05-12.38 µm and 6.69-6.95 µm, respectively) (Table 1; Fig. 2). The shortest and narrowest erythrocytes were found in *T. lessonae*. The mean length and width of mature erythrocytes of *T. lessonae* was 7.81µm ± 0.38 and 4.50µm ± 0.28, respectively (with a range of 7.66-7.95 µm and 4.39-4.60 µm, respectively) (Table 1; Fig. 2). The longest, widest and largest nuclei were found in *M. aurata*. The mean length and width of mature nuclei of *M. aurata* was 6.26 µm ±1.02 and 2.56 µm ±0.29, respectively (with a range of 5.87-6.64 mm and 2.45-2.67, respectively) (Table 1; Figs. 2, 3). The shortest and narrowest nuclei were found in *T. lessonae*. The mean length and width of mature nuclei of *T. lessonae* was 2.49 µm ±0.32 and 1.49 µm ±0.25, respectively (with a range of 2.37-2.60 µm and 1.40-1.60 µm, respectively) (Table 1; Figs. 2, 3).

Table 1: Erythrocyte and nuclei measurements (± standard deviation) of four lizard species from Iran (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).

Examined species	EL	EW	EL/EW	ES	NL	NW	NL/NW	NS	NS/ES
L. nupta	12.21±0.44	6.82±0.34	1.79±0.12	65.39±3.5	3.74±0.35	2.51±0.28	1.50±0.18	7.4±1.20	0.11±0.01
T. lessonae	7.81±0.38	4.50±0.28	1.74±0.12	27.60±2.37	2.49±0.32	1.49±0.25	1.70±0.30	2.93±0.67	0.10±0.02
M. aurata	11.60±0.37	6.53±0.38	1.78±0.09	59.55±4.48	6.26±1.02	2.56±0.29	2.46±0.42	12.65±2.82	0.21±0.04
O. elegans	12.08±0.43	6.66±0.42	1.81±0.11	63.25±5.23	4.33±0.36	2.45±0.31	1.78±0.23	8.37±1.45	0.13±0.02

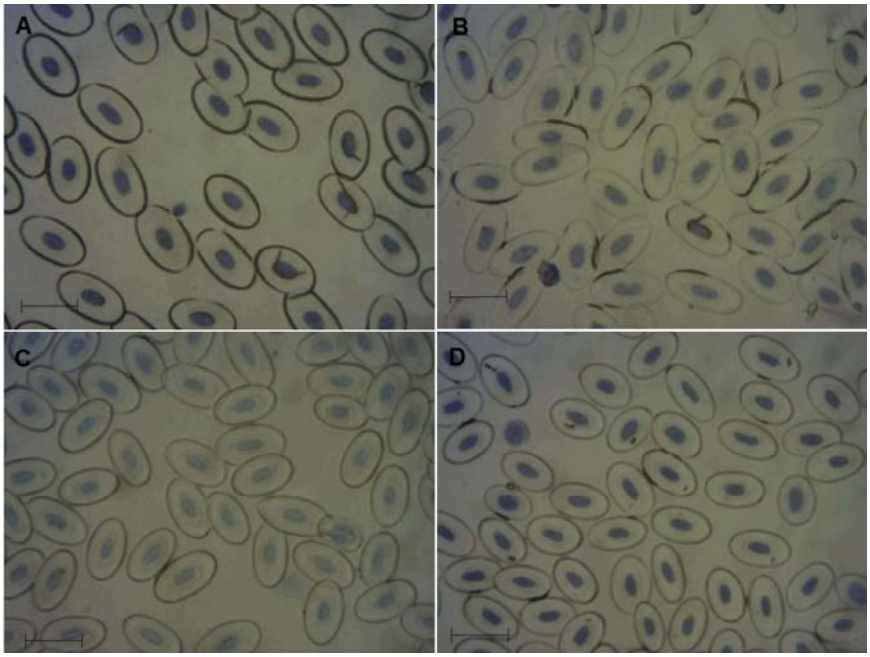

Fig. 1: Photomicrographs of erythrocytes from four species belonging to the Iranian herpetofauna. A) *Laudakia nupta*, B) *Trapelus lessonae*, C) *Mabuya aurata* and D) *Ophisops elegans*. Giemsa. Bar=10 µm.

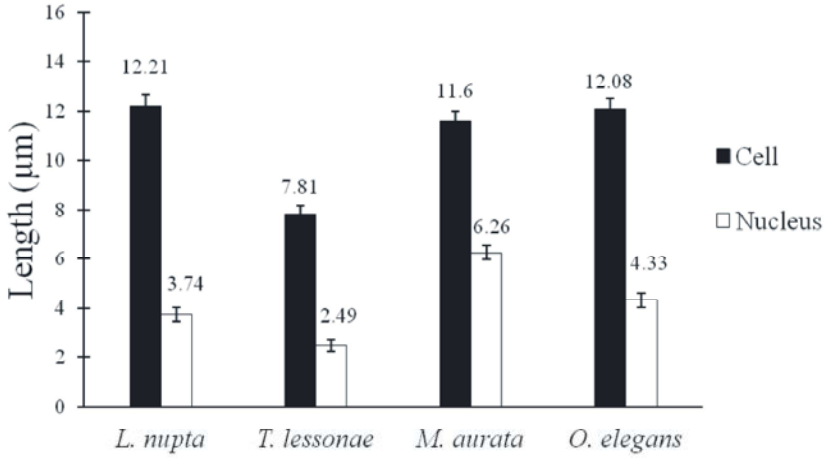

Fig. 2: Average and one standard deviation of the lengths of erythrocytes and nuclei obtained from 100 randomly chosen specimens.

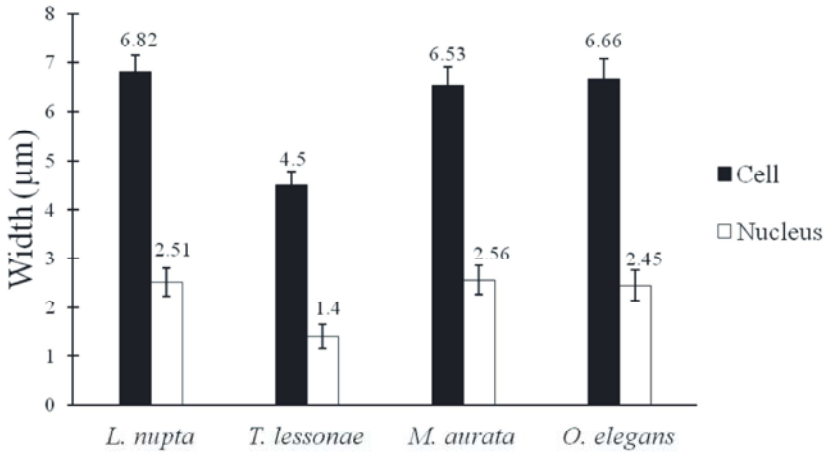

Fig. 3: Average and one standard deviation of the width of erythrocyte and nucleus obtained from 100 randomly chosen specimens.

DISCUSSION

Reptiles are heterogenous group of vertebrates with regard to their blood cell morphology. Hematological measurements may vary depending on factors such as gender, age, pregnancy, physical exercise, weather, stress, altitude and captivity [6]. Different researchers [7, 8] reported that reptile's blood cell morphology and demonstrated considerable variations among orders, even within the same family members. Among reptiles, the largest erythrocytes were observed in *Sphenodon punctatus*, in turtles and crocodiles; and the smallest in lacertid lizards [9]. Lizards generally have a greater erythrocyte population than do snakes [8]. The nucleus of the reptile erythrocyte is more rounded, particularly in turtles and often has irregular margins. Immature erythroid forms have basophilic cytoplasm, similar to those in avian species.

In a study conducted by Atatur *et al.* [10] erythrocyte and nucleus sizes of some scincids (*Ablepharus chernovi*, *Chalcides ocellatus*, *Eumeces schneideri*, *Mabuya aurata*, *Mabuya vittata* and *Ophiomorus punctatissimus*) from Turkey were examined. The largest erythrocytes were observed in the smears of *E. schneideri* and the smallest in *M. vittata*, while the largest nuclei were observed in *E. schneideri* and the smallest were in *M. aurata.* In other study, among the 30 lizard species examined by Arikan and Cicek [11], the largest erythrocytes were observed in *Varanus griseus* and the smallest in *Ophisops elegans*. Erythrocyte size demonstrated great variations among the families and in some cases even within the species of the same family.

Regarding L/W ratio, the most ellipsoidal erythrocytes were observed in *Lacerta pamphylica* and the least or nearly spheroidal ones in *Anatololacerta danfordi*. Regarding NL/NW ratio, scincid lizards had more ellipsoidal nucleus than others. Nucleocytoplasmic ratio ranged between 0.12-0.15 in Scincidae family and 0.19- 0.27 in others Scincidae had more convenient erythrocytes for gas exchange than other lizards. The work of GüL and Tosunoğlu [12], hematological reference intervals of 4 agamid species *Laudakia caucasia*, *Laudakia stellio, Phrynocephalus horvathi* and *Trapelus lessonae*, occurring in Turkey, examined. In *Laudakia stellio,* erythrocyte length is 17.91 µm and width is 9.89 µm; nucleus length is 7.23 µm and width is 3.98 µm. In *Laudakia caucasia,* erythrocyte length is 17.02 µm and width is 9.54 µm; nucleus length is 7.57 µm and width is 3.58 µm. In *Phrynocephalus horvathi,* erythrocyte length is 16.12 µm and width is 8.89 µm; nucleus length is 6.77 µm and width is 4.11 µm. In *Trapelus lessonae,* erythrocyte length is 15.89 µm and width is 8.33 µm; nucleus length is 7.03 µm and width is 3.40 µm.

Also, the work of Salamat *et al.* [5] provides examples of on morphological observations on the erythrocyte and erythrocyte size of some gecko species in Iran. The longest erythrocytes and nuclei were found in *A. elisae*, the shortest erythrocytes and nuclei in *C. scabrum*, the widest erythrocytes in *C. scabrum* and the narrowest erythrocytes in *A. elisae*. The widest nuclei were found in *A. elisae* and the narrowest *C. scabrum.* The largest erythrocytes were found in *A. nasrullahi* and the largest nuclei in *A. elisae*.

Our results demonstrate the presence of some differences in erythrocyte sizes among four lzard species (*Laudakia nupta*, *Trapelus lessonae, Mabuya aurata* and *Ophisops elegans*) from western Iran. In this study, the longest, widest and largest erythrocytes were found in *L. nupta*. The shortest and narrowest erythrocytes and nuclei were found in *T. lessonae*. The longest, widest and largest nuclei were found in *M. aurata*. In the present study, erythrocyte morphology and the results of erythrocytes and nuclei sizes are in agreement with the results of previous studies [11-14]. We believe more probably, these differences were derived from variation of environmental conditions (e.g. temperature, air pressure) [15] and/or various activity levels (e.g. healthy, breeding, hibernating, foraging and daily activity) [16-18]

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