

Erythrocyte Sizes of Some Snake Species from West of Iran (*Platyceps najadum najadum*, *Malpolon insignitus insignitus* and *Eirenis collaris*) After Hibernation

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Abstract: In this study, erythrocyte of the snakes, *Platyceps najadum najadum*, *Malpolon insignitus insignitus* and *Eirenis collaris*, living in Iran were examined. Smears stained with Gimsa were utilized. The sizes of erythrocytes and their nuclei were measured using an ocular micrometer at a magnification of 6300x. The largest erythrocytes were found in *Eirenis collaris* while the widest and the smallest erythrocytes were found in *Malpolon i. insignitus*. The largest and widest nucleuses were found in *Malpolon i. insignitus* and the smallest nucleuses in *Platyceps n. najadum*.

Key words: Snakes · Blood Smears · Erythrocytes Size

INTRODUCTION

Blood analyses is useful as widely used tool aids in the diagnosis of disease and monitoring of animal health in different physiologic states [1]. The previous studies done on the blood of reptiles described the structures, often comparing them with those of the other vertebrates. The literature on hematology of reptilian blood is based on a few studies, with most concerned with European species [2]. Various authors have studied the morphology of circulating blood cells of reptiles, the number of red blood cells and seasonal or sexual variations in the number of blood cells of different reptile species [3,4]. Checking blood parameters in reptiles may guide the evaluation of physiological and health conditions of populations and may be used as an indicator in determining environmental conditions, since species are very sensitive to changes of habitat [5]. Thus, it is important to examine blood parameters in reptiles and determine the changes in these species from environmental change. 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. Recently, the majority of the hematologic studies have been carried out on different reptile species have

dealt with blood composition, as well as blood cell counts and sizes [6,7]. Hematologic studies on different snake species are quite high in number. Particularly, the hematologies of venomous species of snakes have been studied by many researchers [8,9]. In Iran, hematological studies have generally been conducted on humans and some economically important animals. In the current study, our aim was to describe and measure blood cells of *Platyceps n. najadum*, *Malpolon i. insignitus* and *Eirenis collaris*, which live in Iran. This study is the 1st of its kind on this species of snakes in Iran.

MATERIALS AND METHODS

Ten (five♂, five♀) *Platyceps n. najadum* (Colubridae), ten (five♂, five♀) *Malpolon i. insignitus* (Colubridae) and ten (five♂, five♀) *Eirenis collaris* (Menetries, 1832) (Colubridae) were used in this study (Fig. 1). This research was carried out from March to April 2011 and 2012. *Platyceps n. najadum* (Eichward, 1831) specimens were collected from Siahe-Dareh village, *Malpolon i. insignitus* (Geoffroy Saint-Hilaire, 1827) from Kasra foothills and *Eirenis collaris* (Menetries, 1832) collected from regions of Islam-Abad, Kermanshah province western Iran (Schatti & McCarthy, 2001). Blood samples from specimens were collected from the dorsal coccygeal vein. Three or four blood smears were prepared



Fig. 1: Photomicrographs of A: *Eirenis collaris* (MÈnÈtriÈs, 1832), B: *Malpolon i. insignitus* (Geoffroy Saint-Hilaire, 1827) and C: *Platyceps n. najadum* (Eichward, 1831).

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 using photographed and measurements under a camera microscope (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 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 $ES = \frac{L \times W}{4}$. Cells and nuclear shapes were compared with L/W and NL/NW ratios and nucleus/cytoplasm with NS/ES ratio.

RESULT

The erythrocytes or red blood cells of snake's 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 blue and was homogeneous under Gimsa stain. The nuclei of mature erythrocytes are basophilic. (Figs.2). The erythrocyte measurements (lengths and widths), sizes, L/W ratios, nuclear measurements and nucleocytoplasmic ratios are given in Table 1. The largest erythrocytes were found in *Eirenis collaris* while the widest erythrocytes were found in *Malpolon i. insignitus* and the smallest in *Malpolon i. insignitus*. The largest and widest nucleuses were found in *Malpolon i. insignitus* and the smallest nucleuses in *Platyceps n. najadum* (Fig. 3, 4).

Table 1: Erythrocyte measurements (means ± standard errors) of 3 snakes species

Species	No.	L (µm)	W (µm)	L/W	ES (µm ²)	NL (µm)	NW (µm)	NL/NW	NS (µm ²)	NS/ES
<i>Platyceps n. najadum</i>	4	11.02±0.06	8.07±0.00	1.38±0.01	70.44±0.41	4.65±0.00	2.19±0.00	1.91±0.031	8.87±0.15	0.13±0.002
<i>Malpolon i. insignitus</i>	3	11.05±0.00	8.47±0.00	1.31±0.00	73.45±0.00	4.91±0.00	3.20±0.00	1.81±0.024	10.55±0.24	0.14±0.003
<i>Eirenis collaris</i>	3	11.22±0.00	6.41±0.00	1.75±0.00	56.04±0.00	4.84±0.00	1.93±0.00	2.60±0.036	7.48±0.099	0.13±0.002

L: Erythrocyte length, W: Erythrocyte width, ES: Erythrocyte size, NL: Nucleus length, NW: Nucleus width, NS: Nucleus size ; NS/ES: Nucleocytoplasmic ratio.

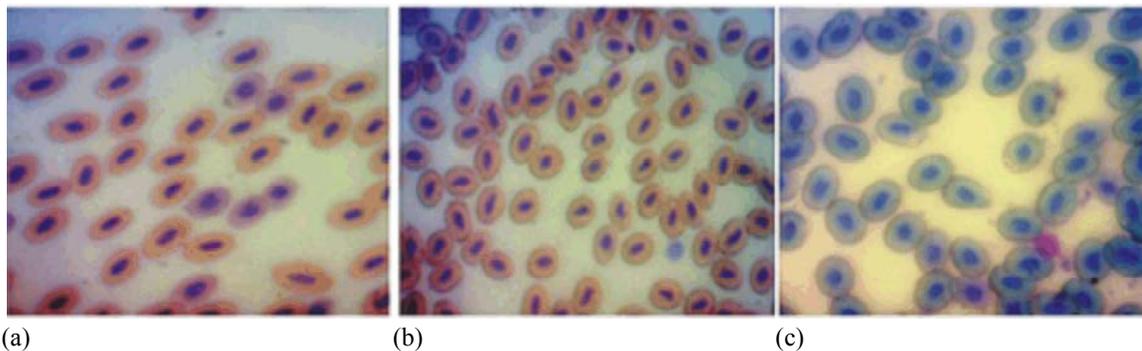


Fig. 2: Photomicrographs of erythrocytes of 3 species belong to Iran Herpetofauna. A: *Eirenis collaris* (MÈnÈtriÈs, 1832), B: *Malpolon i. insignitus* and C: *Platyceps n. najadum*. (Gimsa staining, '630).g 2

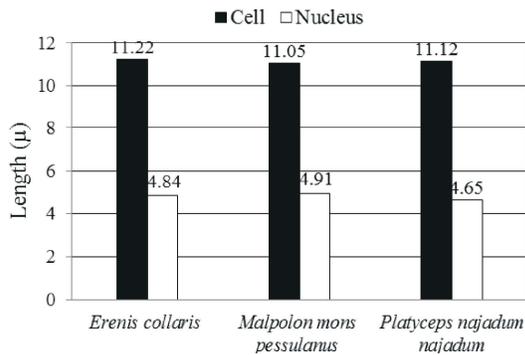


Fig. 3: Average and one standard error (error bars) of the length of erythrocyte and nucleus obtained from 100 randomly chosen specimens.

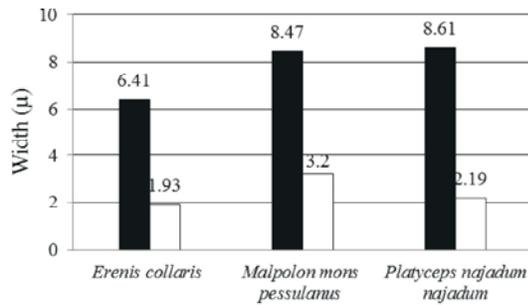


Fig. 4: Average and one standard error (error bars) of the width of erythrocyte and nucleus obtained from 100 randomly chosen specimens.

DISCUSSION

In cyclostomes, elasmobranches and urodeles, the erythrocytes are large, but in higher vertebrates (mammals) the same cells are smaller and do not contain nuclei. The erythrocyte size of these species reflects the position of them in lower vertebrates. Investigations carried out by various authors [10,11] reported that the sizes of erythrocytes vary in members of the 4 orders of reptiles. Different researchers [12-14] reported that reptiles constitute a heterogeneous group among vertebrates in terms of their blood cell morphology and demonstrated considerable variations among orders, even within the same family members. Also, within the class Reptilia, erythrocyte sizes vary greatly. The cryptodiran turtles have larger erythrocytes. The erythrocytes of *Sphenodon punctatus* differ from those of all other reptiles by their large size. The erythrocytes of lizards vary greatly in size depending on the family and sometimes even within one family. The snakes, except for *Typhlops vermicularis*, form a relatively homogenous group. There are smaller

numbers of erythrocytes in reptiles than in mammals or birds. Lizards have more erythrocytes than snakes, while turtles have the fewest. Since lizards have the smallest erythrocytes of all reptiles and turtles the largest, here may be an inverse correlation between the number of erythrocytes and their size; this hypothesis was advanced by Ryerson [15]. Lizards generally have higher erythrocytes counts than snakes, but turtles have the lowest. Many researchers have stated that there are great intraspecific and interspecific variations of blood cell count in snakes [2, 15-17]. Hematologic measurements may vary depending on factors such as sex, age, pregnancy, physical exercise, weather, stress, altitude and captivity [18, 19]. In our study, the largest erythrocytes were found in *Erenis collaris* while the widest erythrocytes were found in *Malpolon i. insignitus* and the smallest in *Malpolon i. insignitus*. The largest and widest nuclei were found in *Malpolon i. insignitus* and the smallest nuclei in *Platyceps n. najadum*. We did not count the number of erythrocytes in these snakes.

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