World Journal of Zoology 9 (2): 80-85, 2014
ISSN 1817-3098

DOI: 10.5829/idosi.wjz.2014.9.2.83244

Evaluating the Size of Erythrocytes in the Blood of *Acanthodactylus nilsoni* (Sauria: Lacertidae) from Iran

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Abstract: Studying and evaluating the sizes of erythrocytes and their nuclei in the nilson's fringe-toad lizard, *Acanthodactylus nilsoni* from Qasr-e-shirin, Kermanshah Province, western Iran, using blood smears stained with Giemsa for the assessment of morphology and size parameters of the blood cells. The erythrocytes were then photographed using a camera microscope (Leica with Dinocapture 2.0).

Key words: Reptilia · Squamata · Lacertidae · *Acanthodactylus nilsoni* · Blood smears · Erythrocyte size

INTRODUCTION

Blood analyses are very useful and widely used in the monitoring and diagnosis of animal health, disease and also in the differentiation of physiological processes [1]. These techniques are used for numerous wildlife species, especially for endangered or threatened taxa and may also help to assess ecosystem health [2]. The basic hematology of birds is very similar to that of reptiles, although reptiles placed in one of the most heterogeneous classes in animals thus it may be more difficult to draw a conclusion between species [3].

Erythrocytes of birds are similar in appearance and function to those of reptiles but those of reptiles can vary in size, being relatively large in some Chelonia, Crocodilia and Rhynchocephalia and smaller in Sauria [4]. Lizards generally have a greater erythrocyte population than do snakes. There appears to be an inverse correlation between erythrocyte number and cell size [5, 6]. The lifetime of a reptile erythrocyte has been reported to be between 600 to 800 days, the high lifetime of a reptile erythrocyte being attributed to the low metabolic rate in these animals [7].

In Iran, the family Lacertidae is represented by five genera, *Acanthodactylus, Lacreta, Eremias, Mesalina* and *Ophisops* [8]. *Acanthodactylus* is commonly referred to as fringe-fingered or fringe-toed lizards. They are native to a wide area in Africa and southern Europe and Western Asia, across the Sahara-Desert and up to the Iberian Peninsula. Though the lizards prefer dry and sparsely vegetated regions, it is not strictly tied to an arid terrain, so it is not uncommon to come across it in various environments. The *Acanthodactylus* coloration and the pattern of its spots is extremely variable, so it is unsurprising that zoologists have, at one time or another, classified every variety as a separate species [9]. *Acanthodactylus nilsoni* is endemic to Iran occurring only in western regions of Kermanshah Province [8]. Available information showed us that no hematological studies have so far been conducted on *Acanthodactylus nilsoni.* The aim of the present work is to establish baseline information on the hematology of this species.

MATERIALS AND METHODS

Six (Three males and three females) of *Acanthodactylus nilsoni* (Figure 1) were used in this study. The studied specimens collected from 5km south of Qasr-e-shirin (34°27' N, 45°37' E), Kermanshah Province, western Iran (Figure 2: Type locality). Permission for collecting was issued on the grounds of scientific use by the Regional Office of the Department of the Environment in Kermanshah Province (DOE) for *Acanthodactylus nilsoni.* Blood samples were obtained

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Fig. 1: *Acanthodactylus nilsoni*

Fig. 2: Type locality- 5km south of Qasr-e-shirin (34°27' N, 45°37' E), Kermanshah Province, western Iran

by means of heart puncture. For each individual, approximately six to eight blood smears were prepared and stained with Giemsa for the assessment of the morphology and size parameters of their blood cells. The erythrocytes were then photographed using a camera microscope (Leica with Dino-capture Lite 2.0). Lengths (EL) and widths (EW) of 50 randomly chosen erythrocytes as well as nuclei lengths (NL) and nuclei widths (NW) were measured in each blood smear. Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed for $ES = ELEW\pi/4$ and $NS = NLNW\pi/4$ [10]. Cells and nuclei shapes were compared as to their EL/EW and NL/NW ratios and nucleus/cytoplasm as to its NS/ES ratio. Erythrocyte sizes and nuclei sizes were analyzed with a student T-test.

RESULTS

The erythrocytes of *Acanthodactylus nilsoni* are oval in shape and they have an ellipsoid nucleus, which is located in the central part of erythrocyte (Figure 3a-d). On smears stained by Giemsa, the cytoplasm was light gray and the chromophilic nuclei dark purplish blue due to their heterochromatin. Reticulocyte (Immature RBCs) was seen 1% of RBCs in blood smears (Figure 3 d). The blood smears of the examined specimens calculated in terms of length, width and size of the erythrocytes and also in nuclei dimensions. The erythrocyte measurements, their ratios, nuclei measurements and nucleocytoplasmic ratios are given in Table 1. Mean lengths, widths and sizes of erythrocytes in *Acanthodactylus nilsoni* were

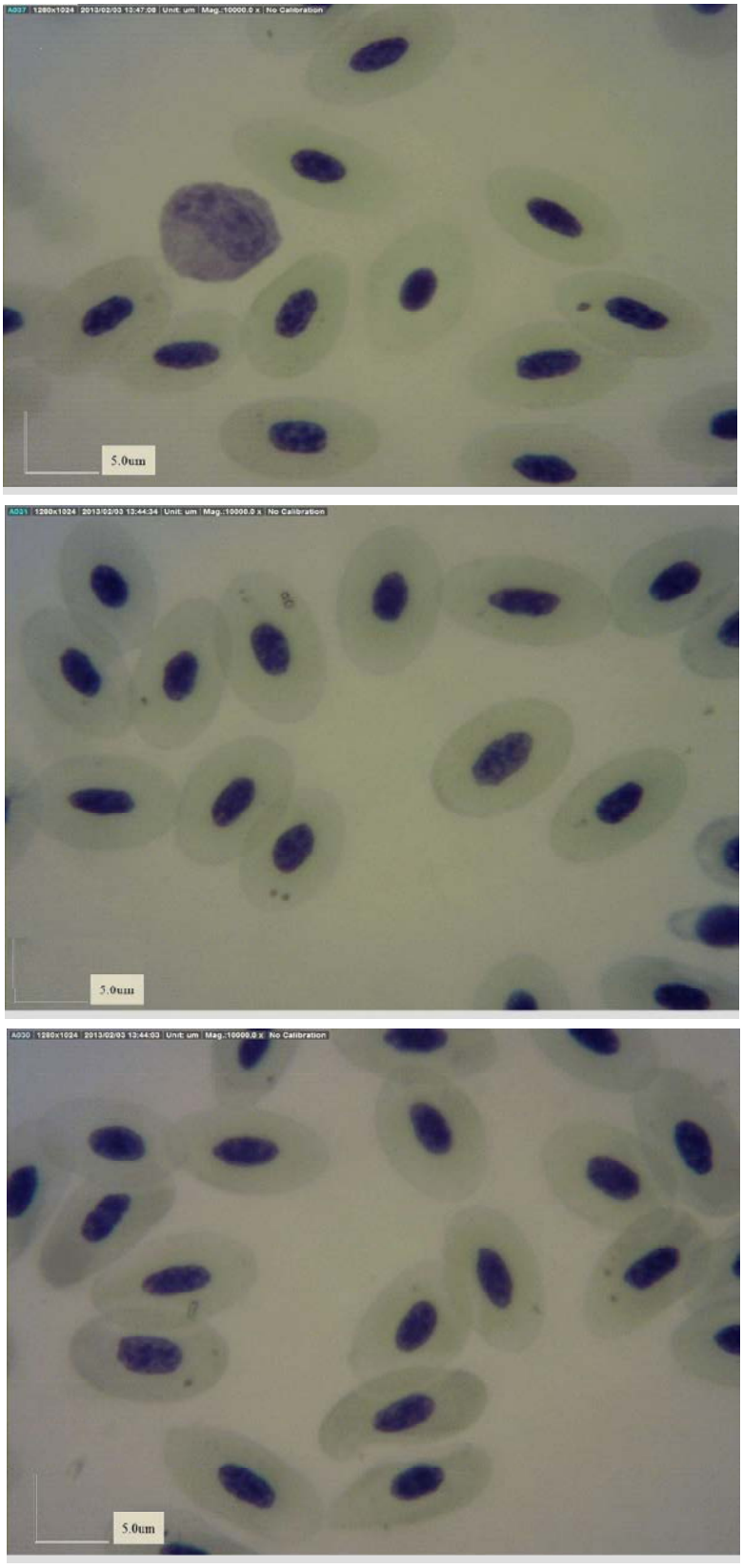

Fig. 3(A-C): Blood smears stained with Giemsa for the assessment of morphology and size parameters, R-Reticulocyte.

Table 1: Erythrocyte and nuclei measurements (± standard deviation) of *Acanthodactylus nilsoni* from Iran

Species	EL(µm)	EW(µm)	EL/EW	ES(µm^2)	NL(µm)	NW(µm)	NL/NW	NS(µm^2)	NS/ES
A. nilsoni	11.14± 0.58	6.44±0.41	1.73±0.14	55.79±5.46	4.41±0.33	2.18±0.23	2.10±0.32	7.56±1.12	0.135±0.021

(EL: Erythrocyte Length, EW: Erythrocyte Width, ES: Erythrocyte Size, NL: Nucleus Length, NW: Nucleus Width, NS: Nucleus Size, NS/ES: Nucleocytoplasmic Ratio)

Table 2: Morphological characteristics (± standard deviation) of *Acanthodactylus nilsoni* from Iran

Species	SVL(mm)	TL(mm)	HL(mm)	HW(mm)
A. nilsoni	65.0±3.94	123.6±9.65	17.8±0.91	10.12±0.59

[SVL: Snout-Vent Length; TL: Tail Length; HL: Head Length; HW: Head Width]

found to measure 10.10-12.22 µm, 5.23-7.17 µm and 45.04-67.99 µm^2, respectively. In addition, EL/EW ratio, mean nucleus length, mean nucleus width, mean nucleus size, NL/NW ratio and nucleocytoplasmic ratio (NS/ES) were found to measure 1.51-2.15 µm, 3.69-5.21 µm, 1.66-2.79 µm, 5.47-11.02 µm^2, 1.02-2.83 µm, 0.098-0.18 µm in this species. The mean values for snout-vent length (SVL), tail length (TL), head length (HL) and head width (HW) are listed in Table 2.

DISCUSSION

According to a general rule for reptiles, the blood volume is expressed to be between 5-8% of the total body weight. Thus, lizard with body weight up to 100g will have 5-8 ml of total blood volume [11]. One of the most important physiological functions of erythrocytes in blood is to carry O_2 and CO_2 through the blood stream, also surface area to size ratio in transforming these vital gases has been proved to be a determining factor [12]. Thus, large erythrocytes offer the possibility of a lower rate of gas exchange than smaller ones. Moreover, the erythrocyte size reflects the position of a species on the evolutionary scale [13]. Thus in higher vertebrates, such as mammals, the erythrocytes are smaller than lower vertebrates. For instance, cyclostomes, elasmobranches and urodeles the erythrocytes are larger and contain nuclei but in the higher vertebrates these cells are smaller and do not bear nuclei [13].

Some factors such as sex, age, pregnancy, physical exercise, weather, stress, altitude and captivity on hematologic and biochemical measurements may affect the size and number of the erythrocytes [6, 14]. The erythrocyte number of reptiles is smaller than that of birds or mammals. Within the class Reptilia, lizards generally have higher erythrocytes numbers than snakes, whereas turtles have the lowest erythrocytes numbers [15-17]. Research conducted by several authors showed that the size of red blood cells vary in members of the four existing orders of reptiles. The largest erythrocytes are found in *Sphenodon punctatus*, then in turtles and crocodiles and the smallest ones in lacertid lizards [18]. Research conducted by several authors have shown that there is a close relation between blood cell characteristics and environmental conditions such as temperature or barometric pressure and various activity levels such as hibernating, foraging and breeding and other daily activities [19-21].

Erythrocytes of various species of reptiles are similar in terms of morphological characteristics [22]. The reptile erythrocytes have an oval to round nucleus that is located in the center of the cells. The nuclei in this cell have a dense coarse chromatin and their cytoplasm is homogeneously eosinophilic [23]. Depending on the age of the cells, the dense chromatins are more or less visible which permits the demonstration of granules within the cytoplasm and of the Golgi apparatus by the most critical staining methods [15, 24, 25].

Acanthodactylus nilsoni is an endemic species for Iran and found in the western regions of Kermanshah Province. The habitat of this species is characterized by soft- sandy alluvial substrate covered by desert-adapted vegetation including *Tamarix, Astragalus, Zygophyllum, Artemisia, Euphorbia* and *Alhagi* as well as grasses. The habitat lies in a lowland area at the foot of the western Zagros Mountains. Other lizard species found in this area include *Laudakia n. nupta, Trapelus lessonae, Ophisops elegans, Trachylepis aurata, Uromastyx loricatus* and *Varanus g. griseus. Acanthodactylus nilsoni* is diurnal being active during the day, foraging among the bushes searching for insects and arachnids [9].

In this study, we faced with high activity of *Acanthodactylus nilsoni* in its habitat. In other words this species moves too fast for hunt or escape from enemies. Thus, because the high activity in this species and also ability to camouflage and hide under the soil, erythrocytes in this species should be able to transfer appropriately the vital gases for providing oxygen for the tissues. This problem is solved by small erythrocytes in this species. The smallest erythrocytes are found in the

Lacertidae family [18]. According to our investigation, *Acanthodactylus nilsoni* has one of the smallest erythrocytes among reptiles; hence erythrocyte count in this species is higher than the other species. These two features (Small erythrocyte and high erythrocyte count) make *A. nilsoni* to be able to provide enough oxygen needed in its tissues.

To summarize, this study establishes baseline information for erythrocyte and nuclear size in *Acanthodactylus nilsoni*. Ongoing and future monitoring studies may find these data helpful for assessing the physiological and immunological parameters in *A. nilsoni.* The results of this study add new information to our knowledge of *A. nilsoni* physiology and provide an important database for veterinarians, scientists and biologists assessing lizards' medicine, ecology and survival.

ACKNOWLEDGMENTS

This work was supported by the Faculty of Science, Razi University, Kermanshah, Iran.

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