

Morphological Observations on the Erythrocyte and Erythrocyte Size of some Gecko Species, Iran

Mohammad Ali Salamat, Somaye Vaissi, Farajolah Fathipour, Mozafar Sharifi and Paria Parto

Department of Biology, Razi University, Baghabrisham, 6714967346, Kermanshah, Iran

Abstract: The study was held to investigate erythrocyte size and morphology of the three gecko species *Asaccus elisae*, *A. nasrullahi* and *Cyrtopodion scabrum* (Gekkonidae) from Iran were examined. Blood smears stained with Giemsa were examined. 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*.

Key words: Gekkonidae • Iran • Erythrocyte Size

INTRODUCTION

Blood analyses is useful in the diagnosis and monitoring of animal health and disease and in the differentiation of physiologic processes [1]. 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 [2]. Thus, it is important to examine blood parameters in reptiles and determine the changes in these species in different from conditions. 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].

Erythrocyte sizes have not been measured for all reptile species. In Iran, many papers on reptiles were carried out but there were no haematological studies on reptiles except for 3 snake species from west of Iran [4]. Our aim was to describe erythrocyte morphology and measure the erythrocyte and nucleus sizes of some Gekkota lizards (*Asaccus elisae*, *A. nasrullahi* and *Cyrtopodion scabrum*), which live in Ilam province, Iran. This study is the first of its kind on Iranian species.

MATERIALS AND METHODS

In this study, 4 individuals of each *Asaccus elisae* (Gekkonidae), *A. nasrullahi* (Gekkonidae), *Cyrtopodion scabrum* (Gekkonidae) (Figure 1). The study was carried out between July and September 2012. Geographic positions for the specimens collected are: *A. elisae* (47, 24, 14. 46 E; 32, 58, 31. 86 N; 915 m), *A. nasrullahi* (47, 22, 36013 E; 33, 01, 33.82 N; 1142 m) and *C. scabrum* (47, 25, 00.38 E; 32, 59, 46.90 N; 891 m) specimens were collected from Khuzestan- Ilam province. Blood samples were obtained by heart puncture. Four 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 using measurements under a microscope camera (Dinocapture 2.0 and Olympus light microscope). The erythrocyte measurements were taken by means of a BBT Krauss ocular micrometer. Length (L) and width (W) of 100 randomly chosen erythrocytes as well as nuclear length (NL) and nuclear width (NW) were measured for each blood smear. Erythrocyte sizes (ES) and their nuclei sizes (NS) were computed from the formula $ES = (ELEW\pi)/4$ and $EN = (NLNW\pi)/4$. Cells and nuclear shapes were compared with L/W and NL/NW ratios and nucleus/cytoplasm with NS/ES ratio [5].

RESULTS

The erythrocytes (RBC) of *Asaccus elisae* (Fig. 1A), *A. nasrullahi* (Fig. 1B) and *Cyrtopodion scabrum* (Fig. 1C) 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 Giemsa stain. The nuclei of mature erythrocytes are basophilic (Figs. 2A, B, C).

The longest erythrocytes and nuclei were found in *A. elisae*. The mean length of mature erythrocyte and nuclei of *A. elisae* was 12.46 ± 0.44 and 4.55 ± 0.33 μm , respectively (with a range of 12.29-12.62 μm and 4.42-4.67 μm , respectively) (Table 1; Fig. 3) and also erythrocyte size, nuclei size and length/width ratios of *A. elisae* are given

in table 1. The shortest erythrocytes and nuclei were found in *C. scabrum*. The mean length of mature erythrocytes and nuclei of *C. scabrum* was $11.70 \mu\text{m} \pm 0.35$ and $3.73 \mu\text{m} \pm 0.50$, respectively (with a range of 11.57-11.84 μm and 3.55-3.93 μm , respectively) (Table 1; Fig. 3). Erythrocyte size, nuclei size and length/width ratios of *C. scabrum* are given in table 1. The widest erythrocytes were found in *C. scabrum*. The mean width of mature erythrocytes of *C. scabrum* was $7.20 \mu\text{m} \pm 0.43$ (with a range of 7.04-7.37 μm) (Table 1; Fig. 4). Erythrocytes size and length/width ratios of *C. scabrum* are given in table 1. The narrowest erythrocytes were found in *A. elisae*. The mean width of mature erythrocytes of *A. elisae* was $26.67 \mu\text{m} \pm 0.33$ (with a range of 6.55-6.80 μm) (Table 1; Fig. 4). Erythrocytes size and length/width ratios of *A. elisae* are given in table 1.

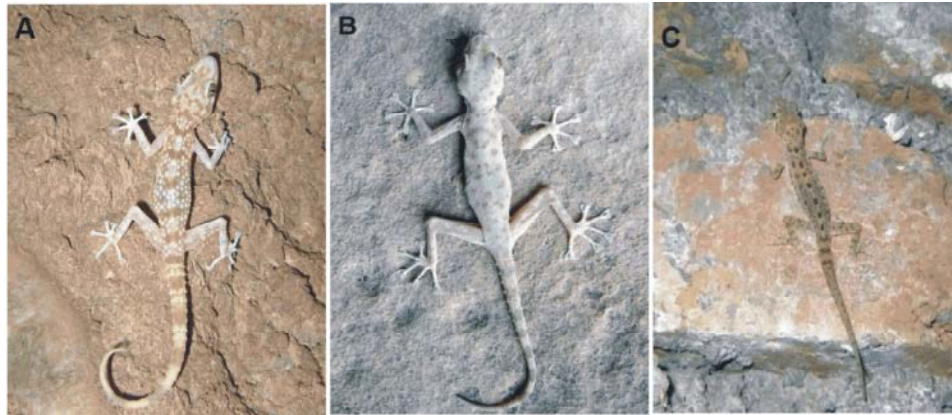


Fig. 1: A) *Asaccus elisae*, B) *Asaccus nasrullahi*, C) *Cyrtopodion scabrum*.



Fig. 2: Photomicrographs of erythrocytes from three species belonging to the Iranian herpetofauna. A) *Asaccus elisae*, B) *Asaccus nasrullahi*, C) *Cyrtopodion scabrum*. Giemsa. Bar=10 μm .

Table 1: Erythrocyte and nuclei measurements (\pm Standard Deviation) of three 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	L /W	ES	NL	NW	NL/NW	NS	NS/ES
<i>A. elisae</i>	12.46 ± 0.44	6.67 ± 0.33	1.87 ± 0.09	65.37 ± 4.53	4.55 ± 0.33	2.62 ± 0.30	1.76 ± 0.24	9.34 ± 0.22	0.14 ± 0.02
<i>A. nasrullahi</i>	11.98 ± 0.64	7.1 ± 0.56	1.70 ± 0.14	66.83 ± 7.06	3.76 ± 0.43	2.50 ± 0.26	1.51 ± 0.21	7.40 ± 1.26	0.11 ± 0.01
<i>C. scabrum</i>	11.70 ± 0.35	7.20 ± 0.43	1.63 ± 0.10	66.20 ± 4.88	3.73 ± 0.50	2.45 ± 0.27	1.53 ± 0.018	7.25 ± 1.54	0.11 ± 0.02

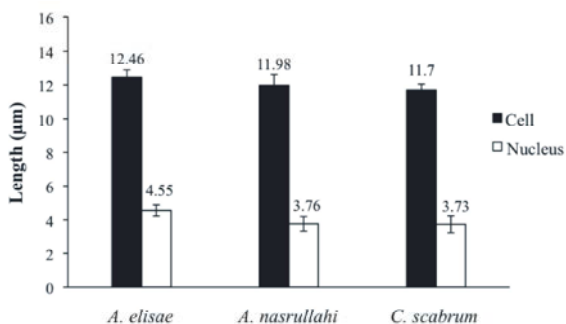


Fig. 3: Average and one standard deviation of the lengths of erythrocytes and nuclei obtained from 100 randomly chosen specimens.

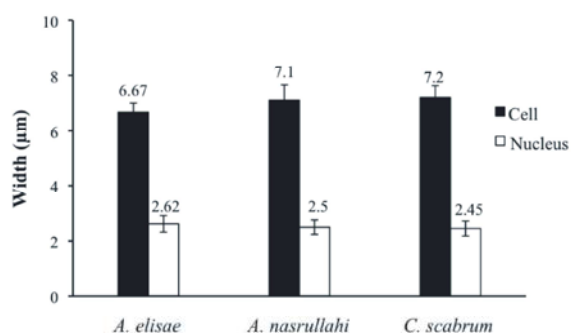


Fig. 4: Average and one standard deviation of the width of erythrocyte and nucleus obtained from 100 randomly chosen specimens.

The widest nuclei were found in *A. elisae*. The mean width of mature nuclei of *A. elisae* was $2.62 \mu\text{m} \pm 0.30$ (with a range of $2.50\text{-}2.73 \mu\text{m}$) (Table 1; Fig. 4). Nuclei size and length/width ratios of *A. elisae* are given in table 1. The narrowest nuclei were found in *C. scabrum*. The mean width of mature nuclei of *C. scabrum* was $2.45 \mu\text{m} \pm 0.27$ (with a range of $2.35\text{-}2.55 \mu\text{m}$) (Table 1; Fig. 4). Nuclei size and length/width ratios of *C. scabrum* are given in table 1. The largest erythrocytes were found in *A. nasrullahi* and the largest nuclei in *A. elisae* (Table 1).

DISCUSSION

According to previous studies [6,7] erythrocyte size vary among lizard families, sometimes even between species within a family. Within the class Reptilia, the largest erythrocytes are seen in *Sphenodon punctatus*, turtles and crocodilians [8]. Cryptodiran turtles have the largest erythrocytes from all previously studied reptiles [9]. The shortest erythrocytes are found in the Lacertidae family [6]. Lizards have more

erythrocytes than snakes and turtles have the fewest. Several authors [7,10] studied the blood of lizards and reported species-specific variations concerning both erythrocyte and leukocyte counts. Saint Girons [9] and Sevinç *et al.* [11] reported erythrocytes and nuclei measurements of some gecko species. In *Coleonyx variegatus*, erythrocyte length is $18.9 \mu\text{m}$ and width is $9.6 \mu\text{m}$; nucleus length is $7.3 \mu\text{m}$ and width is $3.7 \mu\text{m}$. In *Gehyra variegata*, erythrocyte length is $17.2 \mu\text{m}$ and width is $11.5 \mu\text{m}$; nucleus length is $6.3 \mu\text{m}$ and width is $3.8 \mu\text{m}$. In *Heteronota binoei*, erythrocyte length is $21.4 \mu\text{m}$ and width is $10.7 \mu\text{m}$; nucleus length is $8.1 \mu\text{m}$ and width is $3.4 \mu\text{m}$. In *Hemidactylus turcicus*, erythrocyte length is $16.98 \mu\text{m}$ and width is $9.69 \mu\text{m}$; nucleus length is $6.41 \mu\text{m}$ and width is $3.53 \mu\text{m}$. In *Asaccus elisae*, erythrocyte length is $14.96 \mu\text{m}$ and width is $9.26 \mu\text{m}$; nucleus length is $6.06 \mu\text{m}$ and width is $3.62 \mu\text{m}$. In *Cyrtopodion scaber*, erythrocyte length is $16.20 \mu\text{m}$ and width is $10.26 \mu\text{m}$; nucleus length is $6.81 \mu\text{m}$ and width is $3.65 \mu\text{m}$. In *Cyrtopodion heterocercus*, erythrocyte length is $15.65 \mu\text{m}$ and width is $9.18 \mu\text{m}$; nucleus length is $6.56 \mu\text{m}$ and width is $3.78 \mu\text{m}$. Cannon *et al.* [12] reported the leukocyte morphology and size of the rougtail gecko *Cyrtopodion scabrum*.

Our results demonstrate the presence of some differences in erythrocyte sizes among three gecko species *Asaccus elisae*, *A. nasrullahi* and *Cyrtopodion scabrum* (Gekkonidae) from Iran. In this study, the longest erythrocytes were found in *A. elisae*, the shortest in *C. scabrum* and the largest in *A. nasrullahi*. In the present study, erythrocyte morphology and the results of erythrocytes and nuclei sizes are in agreement with the other results carried out by [9,12,13]. these differences may be attributed to the different environmental conditions (e.g. temperature, air pressure) of their habitat [14] and/or various activity levels (e.g. healthy, breeding, hibernating, foraging and daily activity) [15,16].

REFERENCES

1. Christopher, M.M., K.H. Berry, I.R. Wallis, K.A. Nagy, B.T. Henen and C.C. Peterson, 1999. Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert, J. Wild. Dis., 35: 212-238.
2. Dickinson, V.M., J.L. Jarchow and M.H. Trueblood, 2002. Hematology and plasma biochemistry reference range values for free-ranging desert tortoises in Arizona. J. Wild. Dis., 38: 143-153.

3. Lopez-Olivera, J.R., J. Montane, I. Marco, A. Martinez Silvestre, J.S. oler and S. Lavin, 2003. Effect of venipuncture site on hematologic and serum biochemical parameters in marginated tortoise (*Testudo marginiata*). J. Wild. Dis., 39: 830-836.
4. Parto, P., N. Rastegar Pouyani, S. vaissi, F. Zarei and R. Karamiani, 2013. Erythrocyte Sizes of Some Snake Species from West of Iran (*Platycephs najadum najadum*, *Malpolon insignitus insignitus* and *Eirenis collaris*) After Hibernation. World Journal of Zoology, In Press.
5. Sevinç, M., H.U. Urta and H.S. Yldrlmhan, 2000. Erythrocyte measurements in *Lacerta rudis* (Reptilia, Lacertidae). Turk. J. Zool., 24: 207-209.
6. Sevinç, M. and H.U. Urta, 2001. The morphology and size of blood cells of *Lacerta rudis bithynica* (Squamata, Reptilia) Turkey. A.H.R., 9: 122-129.
7. Atatür, M.K., H. Arkan, E. Çevik and A. Mermer, 2001. Erythrocyte measurements of some scincids from Turkey. Turk. J. Zool., 25: 149-152.
8. Alleman, A.R., E.R. Jacobson and R.E. Raskin, 1992. Morphological and Cytochemical characteristics of blood cells from the desert tortoise (*Gopherus agassizii*). Am. J. Vet. Res., 53: 164-51.
9. Saint Girons, M.C., 1970. Morphology of the circulating blood cells. pp: 73-91 In Gans (ed.), Biology of the Reptilia, Vol. 3, Morphology C. Academic Press, New York.
10. Ponsen, S., N. Narkkong, S. Pamok, K. Sappaso and W. Aengwanich, 2008. Hematological values and morphological observation of blood cells in ballong frog, *Glyphogloossus molossus*. J. Micro. Soci. Thail., 22(1-2): 71-75.
11. Sevinç, M., I.H. Ugurta and H.S. Yldrlmhan, 2004. Morphological Observations on the Erythrocyte and Erythrocyte Size of Some Gecko Species, Turkey. A.H.R., 10: 217-223.
12. Cannon, M.S., D.A. Freed and P.S. Freed, 1996. The leukocytes of the roughtail gecko *Cyrtopodion scabrum*: a bright-field and phase-contrast study. Anat. Histol. Embryol., 25: 11-14.
13. Arikan, H. and K. Cicek, 2010. Morphology of peripheral blood cells from various species of Turkish Herpetofauna. Acta Herpetol., 5(2): 179-198.
14. Ruiz, G., M. Rosenmann and A. Veloso, 1983. Respiratory and hematological adaptations to high altitude in *Telmatobius* frogs from the Chilean Andes. Comp. Biochem. Physiol., 76A: 109-113.
15. Allander, M.C. and M.M. Fry, 2008. Amphibian haematology. Vet. Clin. Exo. Anim., 11: 463-480.
16. Sykes, I.V. and E. Klaphake, 2008. Reptile Hematology, Vet. Clin. Exot. Anim., 11: 481-500.