SALAMANDRA 48(4) | 193–197 | 30 December 2012 | ISSN 0036–3375

# Variations in the size of erythrocytes in the blood of *Neurergus kaiseri* and *Neurergus microspilotus* from Iran

Somaye Vaissi [1], Paria Parto [1], Mozafar Sharifi [1,2] & Zahra Minoosh Siavash Haghighi [1]

[1)] Department of Biology, Razi University, Baghabrisham, 6714967346, Kermanshah, Iran
[2)] Razi University Center for Environmental Studies, Baghabrisham, 6714967346, Kermanshah, Iran

Corresponding author: Mozafar Sharifi, e-mail: sharifimozafar2012@gmail.com

Manuscript received: 1 June 2012

**Abstract.** We studied the variation in the sizes of erythrocytes and their nucleus in two closely related species of mountain newts, *Neurergus kaiseri* and *Neurergus microspilotus,* living in two different environments, using blood smears stained with Giemsa. The erythrocytes were found to be more elongated and bulky in *N. kaiseri* with a broader nucleus, whereas in *N. microspilotus*, the erythrocytes were smaller with a relatively elongated nucleus. The nuclear membrane showed crenation in both species, which is located central or eccentric in both *N. kaiseri* and *N. microspilotus*.

Key words. Amphibia, Caudata, Salamandridae, blood smears, erythrocyte size.

## Introduction

Blood analyses are useful, widely used tools that aid in the diagnosis and monitoring of animal health, respectively disease, and in the differentiation of physiological processes (Chiristopher et al. 1999). These techniques are used for several wildlife species, especially for threatened or endangered populations, and may also help to assess ecosystem health (Deem et al. 2006). However, very little has been published with regard to amphibian haematology. The majority of these works were concerned with blood cell counts of different anurans, however only a small percentage of them were on the haematology of urodeles, although the similarity in function of amphibian blood cell types and those of other species is largely unknown (Claver & Quaglia 2009).

In Iran, the family Salamandridae is represented by three genera, *Triturus*, *Neurergus* and *Salamandra* (Baloutch & Kami 1995). The genus *Neurergus* has a relatively wide geographic distribution, ranging from the southern Zagros Mountains to the central Zagros range and extending into Iraq and southern Turkey (Cope 1862, Baloutch & Kami 1995). Sharifi & Assadian (2002) demonstrated that *N. microspilotus* occurs in several highland streams in the central Zagros Mountains. *N. kaiseri* is endemic to Iran and occurred only in the southern parts of the Zagros Mountains (Sharifi et al. 2008). They also showed that *N. kaiseri* was highly vulnerable to the changes occurring in the area, including the presence of exotic fish as a result of the expansion of the Dez Dam reservoir. Available information showed us that no haematological studies have so far been conducted on *N. microspilotus* and *N. kaiseri.*

The aim of the present work was to establish baseline information on the haematology of these two species and to compare data for two closely related species of urodeles living in Iran. The protocols and haematological results obtained in this study could be incorporated into future monitoring activities and programs associated with a Captive Breeding Facility established at Razi University for a possible re-introduction plan.

## Materials and methods

Four specimens were used in this experiment, two of *N. microspilotus* that had been collected from Kavat Stream (34°53' N, 46°31' E) in western Zagros and two specimens of *N. kaiseri* from Bozogab Stream in the southern Zagros Mountains (32°56' N, 48°28' E). Permits for collections were issued on the grounds of scientific use by the regional Office of Environment in Kermanshah Province for *N. microspilotus* and in Khoramabad Province for *N. kaiseri*. Blood samples were obtained 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 Dinocapture 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 = ELEWπ/4 and NS = NLNWπ/4 (Arikan & Cicek 2010). Cells and nuclei shapes were compared as to their EL/EW and NL/NW ratios and nucleus/cytoplasm as to its NS/ES ratio. Correlation between erythrocyte sizes were analysed with a student t-test.

## Results

The erythrocytes of both *N. kaiseri* and *N. microspilotus* are oval. They have an ellipsoid nucleus, which is located central or eccentric in the erythrocyte. On smears stained with Giemsa, the cytoplasms were light yellowish and the chromophilic nuclei dark purplish blue. The blood smears of the examined species demonstrated inter- and intraspecific variation 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. Amongst the two species, the more elongated erythrocyte was observed in *N. kaiseri* (Table. 1). Mean lengths, widths and sizes of erythrocytes in *N. kaiseri* were found to measure 21.49–37.73 µm, 14.79–17.82 µm, and 261.66–503.51 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 1.28–2.06, 7.91–11.49 µm, 5.21–7.08 µm, 32.35–54 µm$^2$, 1.52–1.90 and 0.09–0.17 in this species.

Mean length, width and size of erythrocytes in *N. microspilotus* ranged from 20.04–24.71 µm, 12.06–14.98 µm and 192.64–289.80 µm$^2$. The EL/EW ratio, mean nucleus length, mean nucleus width, mean nucleus size, NL/NW ratio and

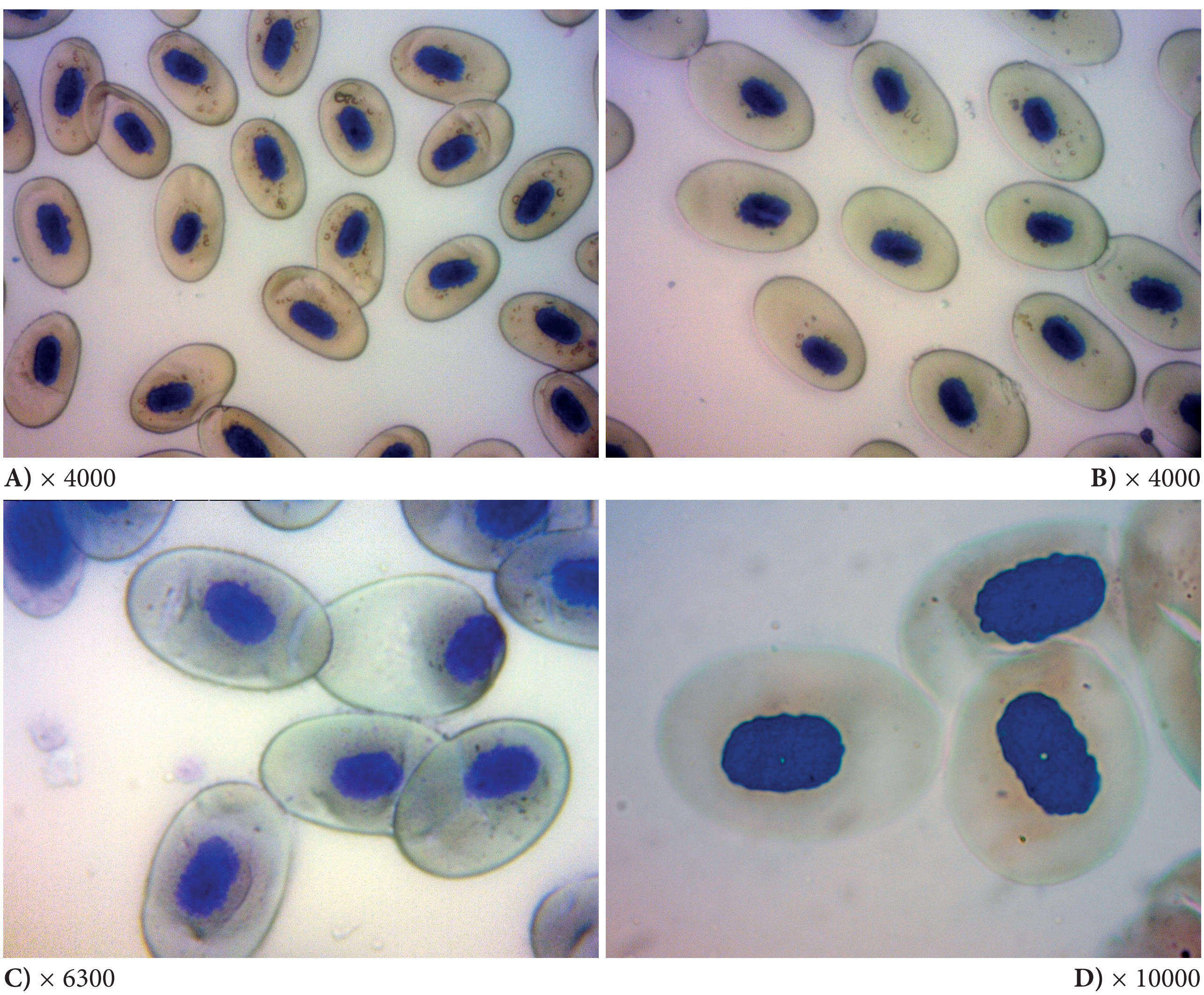


Figure 1. Photomicrographs of erythrocytes from two species of newts from Iran. The nucleus membrane shows crenation in both species (C, D). *N. microspilotus* (A, C) and *N. kaiseri* (B, D).

Table 1. Erythrocyte and nuclei measurements (± standard deviation) of two *Neurergus* species from Iran (L: erythrocyte length, W: erythrocyte width, ES: erythrocyte size, NL: nucleus length, NW: nucleus width, NS: nucleus size, NS/ES: nucleocytoplasmic ratio).

| Species | L (μm) | W (μm) | L/W | ES (μm²) | NL (μm) | NW (μm) | NL/NW | NS (μm) | NS/ES |
|---|---|---|---|---|---|---|---|---|---|
| *N. kaiseri* | 27.41 ±3.17 | 16.48 ±0.87 | 1.67 ± 0.20 | 355.71 ±47.60 | 9.78 ±0.71 | 6.01 ±0.42 | 1.63 ± 0.14 | 45.92 ± 5.85 | 0.13 ±0.003 |
| *N. microspilotus* | 22.15 ± 1.43 | 13.68 ± 1.14 | 1.63 ± 0.15 | 238.30 ±29.05 | 9.91 ± 0.64 | 5.26 ± 0.20 | 1.88 ± 0.14 | 41.005 ±0.32 | 0.17 ±0.019 |

nucleocytoplasmic ratio (NS/ES) were found to vary between 1.08 and 2.2, 8.3 and 10.84 μm, 5.01 and 7.07 μm, 37.21 and 76.64 μm², 1.09 and 2.13, and 0.09 and 0.22, respectively. Comparing erythrocyte lengths in the two species, those in *N. kaiseri* are significantly more elongated than those of *N. microspilotus* (t-test, $p < 0.002$). Similarly, the most strongly ellipsoidal erythrocytes ($p < 0.002$) were observed in *N. kaiseri*. The nucleus was more elongated in *N. microspilotus* (Table 1).

## Discussion

*Neurergus microspilotus* is slightly larger than *N. kaiseri* (Table 2) and lives under two different climatic regimes. Although both species of *Neurergus* occur in highland streams of the first order, these two areas (central Zagros and southern Zagros) are distinctively different ecologically. In the southern Zagros Range, where *N. kaiseri* occurs, the cold and wet highland climate with its pronounced seasonal variations infiltrates the warm and dry lowlands of the Persian Gulf littoral. Within the relatively short distance from the highlands of the Iranian plateau to the southern lowlands, there are many deep valleys that receive one of the highest amounts of precipitation in the country, giving rise to many streams that eventually discharge their into the Mesopotamian marshlands (Sharifi et al. 2008). Several first-order streams, which originate from the highlands of the southern Iranian plateau, have very narrow and short upper reaches that are cool enough to support *N. kaiseri*. Rapid decrease in elevation and the dynamic interaction of the two different climatic systems in the south and north provide warmer climatic conditions with no periods of frost. In contrast to the southern Zagros Mountains, the climatic conditions in the central Zagros Mountains, where *N. microspilotus* occurs, are characterized by long freezing periods (Sharifi & Assadian 2002).

One important function of the erythrocytes is to carry oxygen and carbon dioxide through the blood stream. In transforming these vital gases, the surface to volume ratio has been proved to be a determining factor (Niknmaa 1997). Thus, a small erythrocyte offers the possibility of a higher rate of gas exchange than a larger one. Moreover, the size of an erythrocyte reflects the position of a species on the evolutionary scale (Atatur et al. 2001). In lower vertebrates, such as cyclostomes, elasmobranches and urodeles, the erythrocytes are large, but in higher vertebrates, such as mammals, these cells are smaller and do not contain nuclei (Atatur et al. 2001). Several authors have clearly demonstrated that urodeles had the largest blood cells (erythrocyte, leucocytes and thrombocytes) amongst amphibians and reptiles (e.g., Hartman & Lessler 1964, Kuramoto 1981, Claver & Quaglia 2009). These studies have also shown that blood smears display considerable

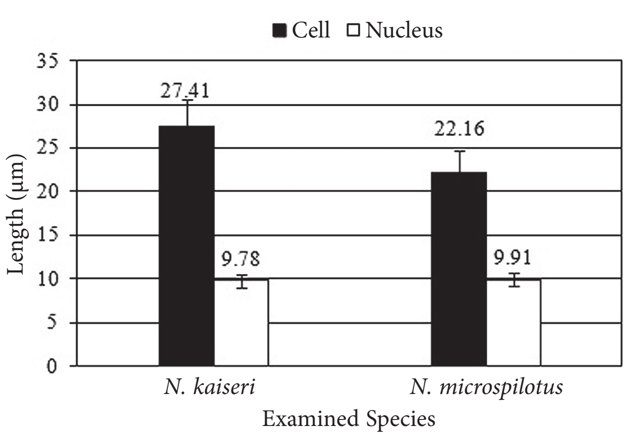


Figure 2. Average and standard deviation of the lengths of erythrocytes and nuclei obtained from 50 randomly chosen specimens.

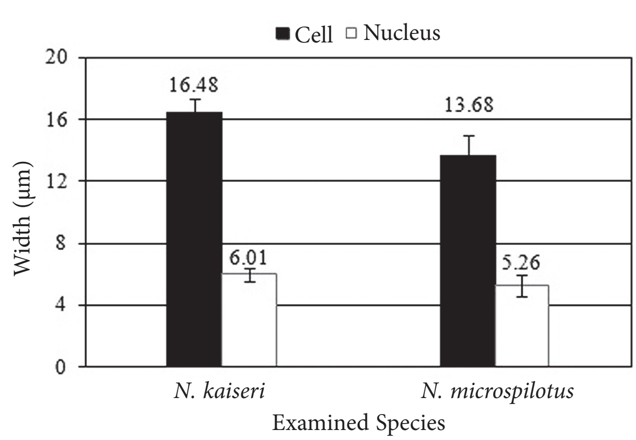


Figure 3. Average and standard deviation of the width of erythrocyte and nucleus obtained from 50 randomly chosen specimens.

Table 2. Morphological characteristics *Neurergus microspilotus* from Kavat Stream and *N. kaiseri* from Bozorgab Stream. Differences indicate significant differences at alpha 0.05. [SVL: snout–vent length; TL: tail length; HW: head width; FLL: fore limb length; HLL: hind limb length].

| Morphological characteristics | *N. microspilotus* | *N. kaiseri* | p-value |
|---|---|---|---|
| SVL (mm) | 63.12 ± 7.34 | 60.37 ± 4.28 | 0.24 |
| TL (mm) | 129.23 ± 13.07 | 113.54 ± 31.22 | 0.05 |
| HW (mm) | 16.06 ± 17.52 | 10.21 ± 0.35 | 0.08 |
| FLL (mm) | 17.55 ± 1.45 | 17.33 ± 1.49 | 0.69 |
| HLL (mm) | 20.62 ± 1.93 | 17.63 ± 1.35 | 0.00 |

interspecific and even intraspecific variations in terms of cell sizes. Within the examined amphibians, no important differences were observed between the erythrocyte and nucleus sizes of urodelanand anuran species (Arikan & Cicek 2010). Such differences, however, do not show any correlations with body size (Arikan & Cicek 2010).

Some authors have established associations between blood cell characteristics and environmental conditions such as temperature or barometric pressure (Ruiz et al. 1983, 1989) and/or various activity levels such as hibernating, foraging and breeding and other daily activities (e.g., Campbell 2004, Allander & Fry 2008, Sykes & Klaphake 2008). These views are congruent with the conclusions drawn by Harris (1963), Atatur et al. (1998, 1999) and Gul & Tok (2009). In a similar study conducted by Kuramoto (1981), the EL/EW ratio ranged between 1.63 and 1.80 in urodeles and from 1.38 to 1.69 in anurans. Consequently, the erythrocyte shape was more ellipsoidal in urodeles than in anurans. The NL/NW ratio ranged from 1.55 to 1.69 in urodeles and from 1.57 to 2.35 in anurans. This information indicates that, contrary to the situation in EL/EW, anurans have more ellipsoidal nuclei than urodeles, which is compatible with our findings. Arikan & Cicek (2010) have also found that the nucleocytoplasmic ratio had a range of 0.22–0.34 in urodeles and 0.10–0.16 in anurans. This indicates that anurans have a larger cytoplasmic surface area than urodeles in terms of the nuclear surface area of erythrocytes. Our results obtained from the current study are in accordance with those demonstrated for urodeles by Kuramoto (1981) and Arikan & Cicek (2010).

This study establishes baseline information for erythrocyte and nuclear size and nuclear membrane characteristics for two critically endangered species. Due to small sample sizes, we cannot quantitatively attribute the differences in erythrocyte and nuclear size to size, age, or environmental factors. Further studies focusing on factors that contribute to differences in blood parameters amongst and within populations may be able to clarify these questions. Ongoing and future monitoring studies may find these data helpful for assessing the physiological and immunological parameters of individual newts. Of particular interest will be to find out if and how parameters change in captive breeding facilities for conservation initiatives as compared to free-ranging populations.

## References

Allander, M. C. & M. M. Fry (2008): Amphibian haematology. – Veterinary Clinics of North America: Exotic Animal, **11**: 463–480.

Arikan, H. & K. Cicek (2010): Morphology of peripheral blood cells from various species of Turkish herpetofauna. – Acta Herpetologica, **5**: 179–198.

Atatur, M. K., H. Arikan & İ. E. Cevik (1999): Erythrocyte sizes of some anurans from Turkey. – Turkish Journal Zoology, **23**: 111–114.

Atatur, M. K., H. Arikan & A. Mermer (1998): Erythrocyte sizes of some urodeles from Turkey. – Turkish Journal Zoology, **22**: 89–91.

Atatur, M. K., H. Arikan & A. Mermer (2001): Erythrocyte sizes of some scincids from Turkey. – Turkish Journal Zoology, **25**: 149–152.

Baloutch, M. & H. G. Kami (1995): Amphibians of Iran. – Tehran University Publications, **177**: 91–99.

Campbell, T. W. (2004): Haematology of lower vertebrates. – pp. 1214 in: ACVP & ASVCP (eds.): 55th Annual Meeting of the American College of Veterinary Pathologists (ACVP) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP). – Middleton, WI, American College of Veterinary Pathologists & American Society for Veterinary Clinical Pathology.

Cope, E. D. (1862): On *Neurergus crocatus* from Iran and Iraq. – Proceedings of the Academy of Natural Sciences Philadelphia, **1862**: 343.

Christopher, M. M., K. H. Berry, I. R. Wallis, K. A. Nagy, B. T. Henen & C. C. Peterson (1999): Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert. – Journal of wildlife diseases, **35**: 212–238.

Claver, J. A. & A. I. E. Quaglia (2009): Comparative morphology, development, and function of blood cells in non-mammalian vertebrates. – The Journal of experimental medicine, **18**: 87–97.

Deem, S. L., E. S. Dierenfeld, G. P. Sounguet, A. R. Alleman, C. Cray, R. H. Poppenga, T. M. Norton & W. B. Karesh (2006): Blood values in free-ranging nesting leatherback sea turtles (*Dermochelys coriacea*) on the coast of the Republic of Gabon. – Journal of zoo and wildlife medicine, **37**: 464–471.

Gul, C. & C. V. Tok (2009): Blood cell counts and sizes of some anurans from Turkey. –Russian Journal Herpetology, **16**: 119–125.

Harris, J. (1963): The red cell. – Cambridge, MS, Harward University Press.

Hartman, F. A. & M. A. Lessler (1964): Erythrocyte measurements in fishes, amphibians, and reptiles. – The Biological Bulletin, **126**: 83–88.

Kuramoto, M. (1981): Relationships between number, size, and shape of red blood cells in amphibians. – Comparative Biochemistry and Physiology, **69**: 771–775.

Nikinmaa, M. (1997): Oxygen and carbon dioxide transport in vertebrate erythrocytes: an evolutionary change in the role of membrane transport. – The Journal of Experimental Biology, **200**: 369–380.

Ruiz, G., M. Rosenmann & A. Veloso (1983): Respiratory and hematological adaptations to high altitude in *Telmatobius* frogs from the Chilean Andes. – Comparative Biochemistry and Physiology, **76A**: 109–113.

Ruiz, G., M. Rosenmann & A. Veloso (1989): Altitudinal distribution and blood values in the toad, *Bufo spinulosus* Wiegmann. – Comparative Biochemistry and Physiology, **94A**: 643–646.

Sharifi, M. & S. Assadian (2002): Distribution and conservation of *Neurergus microspilotus*. – Asiatic Herpetologica Research, **10**: 224–229.

Sharifi, M., T. Papenfuss, N. Rastegar-Pouyani, S. Anderson & S. Kuzmin (2008): *Neurergus kaiseri*. – in: IUCN (ed.): IUCN Red List of Threatened Species. Version 2009 – IUCN, Gland (http://www.iucnredlist.org, accessed 22 October 2009).

Sykes, I. V. & E. Klaphake (2008): Reptile Hematology. – Veterinary Clinics of North America: Exotic Animal Practice, **11**: 481–500.