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RESEARCH ARTICLE

ERYTHROCYTES COUNTS AND MORPHOLOGY OF ACANTOCERCUS ADRAMITANUS AND CHAMELEO CALYPTRATUS CALYPTRATUS FROM YEMEN

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Abstract

The aim of this study was to determine the erythrocyte and nucleus morphology of *Acantocercus adramitanus* and *Chameleo calyptratus calyptratus* from Yemen by means of blood smears stained with Giemsa stain. The longest and largest erythrocytes and their nucleus were observed in the smears of C. *c. calyptratus*, while the narrowest and smallest in *A. adramitanus*. In terms of the studied species, the nucleus and erythrocyte sizes were found to be correlated. No significant difference between two species lizard's in erythrocyte count was determined. The relationship between the raw erythrocyte measurements and erythrocyte length was determined as positive correlation.

Keywords: Yemen, Blood smears, Erythrocyte and nucleus morphology.

1. Introduction

The reptiles in Yemen include 105 species of lizards. Acantocercus adramitanus (Anderson, 1896) and *Chameleon calyptratus calyptratus* (Dumeril, 1851) are two common species in the southwest of Arabia, furthermore, *A. adramitanus* is distributed from southwest of Yemen into Dhofar in the east. In Yemen, *A. adramitanus* is widespread and abundant in the range of the Yemen mountains up to 2300 m, it is observed at Al Nabi Shuaib mountain (30 km west of Sana'a), which represent the highest elevation in Arabia. The etymology of this species was related to Hadhramaut [10,13,14,48,50].

Currently 12 genera and over 200 species are recognized within the family Chamaeleonidae [37,40,44,49], with members of this family distributed across Africa into the Middle East, southern Europe, India, and across a few small islands in the Indian Ocean [24]. In Yemen, the veiled or Yemen Chameleon, C. *calyptratus calyptratus*, may be the form of the Yemen mountains, is abundant in the Taiz. It is native to the southwestern Arabian peninsula in western Yemen and southwestern Saudi Arabia [11,12,14,15,31,46]. This species has recently been introduced into Hawaii and Florida, where it thrives and appears to be flourishing [23,46].

In the living organisms, the size, shape and action are very closely linked together [35]. So, movement through the environment, locomotion, is the behavior that most dictates the morphology and physiology of animals. Biologists have long been attracted to locomotor extremes because they provide an especially clear example from which determine structure-function relationship [16]. The gait an animal selects depends on the rate of travel; obstructions in the terrain, maneuverability sought and body size of the animal [20].

One of the most important functions of erythrocytes is to carry oxygen and carbon dioxide and also its surface area to size ratio is a determining factor in the exchange of oxygen and carbon dioxide in the tissues. Thus, a small erythrocyte offers a possibility of greater rate of exchange than a larger one [5,17].

Because the differential blood cell count is important in determining the health status of animals, normal blood morphology needs to be described for representative species of the four major orders of reptiles [21]. Various authors have studied the morphology of blood cells of reptiles and their count [1,5,9,17,18,28,29,33,34,38,39, 42,45]. we did not found any studies on blood reptiles in Yemen. So, the purpose of our investigation was to descriptions of the morphology of erythrocytes in *A. adramitanus* and *C. c. calyptratus* from Yemen.

2. Materials and Methods

A total of 38 specimens, 24 *A. adramitanus* and 14 *C. c. calyptratus*, caught from Taiz (Southern Sana'a, Yemen) from October- December 2018 were used for the present study. The blood samples were obtained by cutting off the tail of the specimens [8].

For each individual, three blood smears were prepared and stained with Giemsa stain and then were utilized to measure the morphology and size of erythrocytes. The measurements of erythrocytes and its nuclei were done by an ocular micrometer. On each blood smear preparation, the erythrocyte length (L), erythrocyte width (W), nucleus length (NL) and nucleus width (NW) were measured according to Arikan & Çiçek [41]. The erythrocyte sizes and their nuclear sizes were calculated according to the formulae (LWπ)/4 and (NLNWπ)/4. The cellular and nuclear shapes were compared using the L/W and NL/NW ratios, while the comparison of nucleus/cytoplasm ratio was made according to the N/C ratio. The erythrocyte count was conducted as a diluting solution with Neubauer hemocytometer by using standard Hayem solution [41].

For elucidating interspecific – intraspecific variations, the morphometric data of erythrocytes and nucleus were statistically analyzed and described by RMA regression (reduced major axis), one way ANOVA test and unpaired t-test using software PAST package release 3.25 [51].

3. Results

The erythrocyte count in 1 mm^3 blood (RBC) was calculated as 1526500 (range= 190000 - 4390000) in *A. adramitanus* and as 1248333 (330000-2270000) in *C. c. calyptratus*. There is no significant difference between the two species lizard's in erythrocyte count (t_{30} =0.66, *P*= 0.51).

Differences in the erythrocyte count - size relationship between two species was observed, there is a positive correlation between the erythrocyte counts and size in *A. adramitanus* (b= 7.396, r =0.490 and *P*= 0.028). On the other hand, there is no significant correlation between the number of erythrocytes and cell size (b= -5.200, r =-0.276 and *P*= 0.385) in *C. c. calyptratus*.

In both two species, the typical shape of erythrocytes is oval, and their nuclei were located centrally. The nucleus is stained deep bluish violet, the cytoplasm is stained light pink (Fig. 1a & b). In *A. adramitanus*, the mature erythrocytes were oval cells (15.8µm x 8.6µm) with rounded poles. The nucleus (6.3µm × 3.7µm) was elongated. Whereas in *C. c. calyptratus*, the average length of erythrocytes was calculated as 18µm, its width as 9.9µm, the average length of the nucleus was calculated as 7.8µm, its width as 4.1µm.

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, their ratios and nucleocytoplasmic ratios were given in Table 1.

In the investigated species, the longest and largest erythrocytes were observed in *C. c. calyptratus*, while the narrowest and smallest in *A. adramitanus*. The longest and largest nuclei were again observed in *C. c. calyptratus*, with the narrowest in *A. adramitanus*. In terms of NL/NW ratio; the most ellipsoidal cells were those of *C. c. calyptratus* while the least ellipsoidal ones were observed in *A. adramitanus* (Table 1, Figure 1a).
The erythrocyte characteristics of two species exhibited intraspecific variations with responsible differences. Variations in erythrocyte length, width and sizes were found within the individuals of the same species (Table 2). On the contrary, no important difference was detected among the *C. c. calyptratus* individuals in nucleus length.

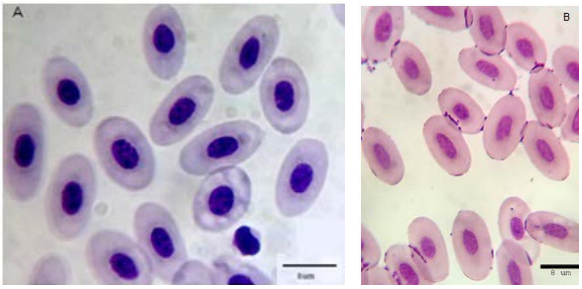

Figure 1. The Erythrocytes of *A. adramitanus* (A) and *C. c. calyptratus* (B) from Yemen. Horizontal bar: 8 µm.

Table 1: The erythrocyte and their nuclei measurements in the blood of *A. adramitana* and *C. c. calyptratus.*

Species		L (µm)	W (µm)	L/W	ES (µm²)	NL (µm)	NW (µm)	NL/NW	NS (µm²)	NS/ES
A. adramitana	N	24 (216)	24 (216)	24 (216)	24 (216)	24 (216)	24 (216)	24 (216)	24 (216)	24 (216)
	Mean	15.8 a	8.6 a	1.9 a	106.8 a	6.3 a	3.7 a	1.7 a	18.3 a	0.2 a
	SE	0.116	0.115	0.021	1.694	0.059	0.048	0.027	0.29	0.003
	Min	11	6	0.8	56.5	3	3	0.7	7.1	0.1
	Max	20	18	3.2	268.5	9	6	3	31.4	0.3
	SD	1.708	1.695	0.315	24.901	0.869	0.707	0.403	4.26	0.05
C. c. calyptratus	N	14 (123)	14 (123)	14 (123)	14 (123)	14 (123)	14 (123)	14 (123)	14 (123)	14 (123)
	Mean	18 a	9.9 a	1.8 b	141.3 a	7.8 a	4.1 a	2 a	24.9 a	0.2 b
	SE	0.178	0.150	0.026	2.96	0.158	0.061	0.045	0.703	0.005
	Min	12	5	1	62.8	5	3	1.2	14.1	0.1
	Max	25	17	3.2	293.6	19	6	4.3	74.6	0.4
	SD	1.973	1.665	0.293	32.823	1.752	0.681	0.495	7.795	0.055

The Means with different letters in same column indicate non- significant difference (p > 0.05)

The erythrocyte characteristics of the two species were described in terms of the parameters of reduced major axis (RMA) equations that describe the relationship between the raw erythrocyte measurements and erythrocyte length (Table 3). Examination of Person correlation and *P* – values between variables clarifies these relationships. Except of L–WN relationship, a positive correlation was evident between the raw erythrocyte measurements and erythrocyte length of the two species investigated (Table 4).

Table 2: Results from the one-way ANOVA on the six biometric measures. Given are the degrees of freedom, *F*-values and significant levels.

Trait and source of variation	df	*F*	*P- value*
Erythrocyte length			
A. adramitana	23,192	3.849	0.000
C. calyptratus	13,109	5.618	0.000
Erythrocyte width			
A. adramitana	23,192	1.866	0.012
C. calyptratus	13,109	2.334	0.009
Erythrocyte size			
A. adramitana	23,192	3.477	0.000
C. calyptratus	13,109	3.093	0.001
Nucleus length			
A. adramitana	23,192	3.391	0.000
C. calyptratus	13,109	1.667	**0.078**
Nucleus width			
A. adramitana	23,192	2.362	0.000
C. calyptratus	13,109	6.143	0.001
Nucleus size			
A. adramitana	23,192	3.925	0.000
C. calyptratus	13,109	3.693	0.000

Non - significant *P*- values are in bold

Table 3: Intercept (a) and the regression coefficient (b) of reduced major axis regression (RMA) and Pearson correlation coefficient values that describe the relationship between the raw erythrocyte and their nuclei measurements and erythrocyte length of *A. adramitana* and *C. c. calyptratus*.

Species	Erythrocyte characters	a	b	r	*p- value*
A. adramitana	LN	-0.748	1.288	0.313	0.000
	WC	-0.932	1.556	0.205	0.003
	WN	2.561	-1.670	-0.023	**0.737**
	ES	-0.39	2.015	0.654	0.000
	NS	-1.263	2.105	0.174	0.011
C. c. calyptratus	LN	-1.212	1.672	0.344	0.000
	WC	-0.755	1.394	0.338	0.000
	WN	2.542	-1.548	-0.094	**0.300**
	ES	-0.331	1.972	0.747	0.000
	NS	-1.662	2.428	0.177	0.051

Non - significant *P*- values are in bold

Table 4: Intercept (a) and the regression coefficient (b) of reduced major axis regression (RMA) and Pearson correlation coefficient values that describe the relationship between the erythrocyte width and nucleus width; cell size and nucleus size of *A. adramitanus* and *C. c. calyptratus*.

Species	a	b	r	*p- value*
A. adramitana				
W - WN	-0.430	1.073	0.183	0.007
ES - NS	-0.890	1.061	0.130	0.050
C. c. calyptratus				
W - WN	-0.499	1.110	0.240	0.007
ES - NS	-1.255	1.231	0.297	0.001

4. Discussion

The differential blood cell count is important in determine the health condition of animals. In the present work, the erythrocyte count for *A. adramitanus* are 1526500, while for *C. c. calyptratus* is 1248333. These values are similar to those reported in *Agama atra* [4], *Sitana ponticeriana* [25], *Laudakia caucasia* and *Laudakia stellio* [31] and *Furcifer pardalis* [47].

Several authors [9,17,18,19,26,32,45] studied the blood of reptiles and reported species-specific variations in erythrocyte counts, however, our results did not show any differences in erythrocyte counts between *A. adramitanus* and *C. c. calyptratus*.

There is correlation between the number of erythrocyte and cell size [3,4,5]. Our results support this hypothesis in *A. adramitanus*. Contrary to this hypothesis, there is no significant correlation between the number of erythrocytes and cell size (b= -5.200, r =-0.276 and *P*= 0.385) in *C. c. calyptratus*.

The shape and size of erythrocytes are variable for different vertebrates and morphologically similar among various species of reptiles [9]. Erythrocyte nuclei become condensed, stain darker as the cells age, and it is centrally located [21]. The shape of erythrocytes is oval or elongated or elliptical as in *Lacerta rudis* [17], some Scincid species [18], *Agrionemys horsfieldi* [21], *Tupinambis merianae* [27], Turkish snakes [28], sea turtles [30], *Elaphe sauromates* [34], four species from agamidae [32], *Psammophilus Blanfordanus* [33], three species of turtle from Iran [38], *Naja naja* [43], *Neurergus kaiseri* , *Neurergus microspilotus* [36], *Emys orbicularis*, *Mauremys rivulata* [45] and *Furcifer pardalis* [47], whereas the present study reported the oval or elliptical shape of erythrocyte and their nuclei in the examined species.

Arikan & Çiçek [29] recorded interspecific and even intraspecific variations in terms of the lengths, widths and sizes of the erythrocytes and nuclei in 87 species belonging to amphibians and reptiles. The blood smears

of the *Neurergus kaiseri* and *Neurergus microspilotus* demonstrated inter- and intraspecific variation of the erythrocytes and also in nuclei dimensions [36] and in snakes [28]. Differences in terms of erythrocyte morphology between *Emys orbicularis* and *Mauremys rivulata* were observed [45]. In the present study, variations were observed interspecies and even in the blood smears of the same species in terms of erythrocyte, nucleus measurements and ratios.

In the present work, the average erythrocyte length was established as 15.8µm and the width as 8.6µm, with the average nucleus length as 6.3µm and the width as 3.7µm of *A. adramitanus*, but in *C. c. calyptratus*, the average length of erythrocytes was calculated as 18µm and its width as 9.9µm, the average length of nucleus was calculated as 7.8µm and its width as 4.1µm, which is in accordance with the findings of Sevinç *et al.* [17] in *Lacerta rudis* (13.45µm x 8.28µm cell axes and 5.87µm x 3.61µm nucleus axes), as well as in Knotkova *et al.* [22] in *Agrionemys horsfieldi* (19.5 x 9.2 µm cell axes and 6.4 x 3.6 µm for nucleus), Uğurtaş *et al.* [22] in *Emys orbicularis* and *Mauremys rivulata* (21.73 x 12.53 µm cell axes and 20.16 x 11.64 µm cell axes respectively), Gül and Tosunoğlu [32] in *Laudakia caucasia*, *Laudakia stellio*, *Phrynocephalus horvathi* and *Trapelus lessonae* (17.02µm x 9.54µm axes cell and 7.57µm x 3.85µm nucleus axes, 17.91µm x 9.89µm axes cell and 7.23µm x 3.98µm nucleus axes, 16.12µm x 8.89µm axes cell and 6.77µm x 4.11µm nucleus axes and 15.89x 8.33µm cell axes and 7.03 x 3.40 µm for nucleus respectively), *Parida et al.* [33] in *Psammophilus blanfordanus* (15.37µm x 10.75µm cell axes and 7.0 x 5.1 µm nucleus axes in males, while 12.83 µm x 7.98µm cell axes and 6.13 x 4.0µm nucleus axes in females), Javanbakht *et al.* [38] in *Emys orbicularis* (15.18µm x 9.3µm cell axes and 4.8 x 3.51 µm nucleus axes), in *Mauremys caspica* (14.28 µm x 8.5µm cell axes and 4.4 x 3.33µm nucleus axes) and in *Testudo graeca* (12.47 µm x 6.85µm cell axes and 3.79 x 3.01µm nucleus axes), Lisičić, *et al.* [39] in *Vipera Ammodytes* (18.36 µm x 12.65µm cell axes and 8.04 x 4.69µm nucleus axes), Arizza *et al.* [42] in *Trachemys scripta elegans and Emys trinacris* (19.2µm x 13.6µm cell axes and 22.5µm x 14.1µm axes cell respectively) and *Çiçek et al.* [45] in *Emys orbicularis* and *Mauremys rivulata (*20.1µm x 12.7µm cell axes and 22.5µm x 14.1µm axes cell respectively).

The erythrocytes of lizards vary greatly in size depending on the family and sometimes even within one family. In reptiles, 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 smallest erythrocytes are found in the Lacertidae family [9]. Hartman and Lessler [5] stated that the lizard families have the smallest erythrocytes in reptiles.

Acantocercus adramitanus inhabits rocky and mountainous areas. Commonly found on rocky surfaces or walls of buildings. It is arboreal when large trees are available. *C. c. calyptratus* entirely arboreal found on trees and bushes throughout its range. It come to the ground only to move from one bush to another. It is entirely diurnal feeding on insects that happen to come within the range of its sticky tongue. In nature, the *A. adramitanus* is more active than *C. c. calyptratus*.

In the present study, the erythrocyte and nucleus sizes of *A. adramitanus* are smaller than *C. c. calyptratus*. This is may be due to the difference in activity levels (e.g. health, reproduction, hibernation, foraging and other daily needs). These findings is supported by many authors including Hartman & Lessler [5], Sevinç *et al.* [17], Arikan & Çiçek [29,41] and *Parida et al.* [33]. There is no correlation between body size and erythrocyte size in *A. adramitanus* and *C. c. calyptratus*. ($r = 0.187$, $P \leq 0.382$ and $r = -0.087$, $P \leq 0.767$ respectively), this result is in agreement with those of Arikan & Çiçek [41].

In the current work, the parameters of the reduced major axis equations that exhibited additional patterns of interspecific relationships. Except of L–WN relationship, such analysis emphasized on high correlation between the erythrocyte and their nucleus measurements with erythrocyte length. Furthermore, nucleus width is closely linked to cell width. A positive correlation between the erythrocyte and nucleus sizes was also recorded in the present study. Atatür *et al.* [18] reported positive correlation between the erythrocyte and nucleus sizes of the Scincids. Arikan & Çiçek [29] stated that there was a positive correlation between erythrocyte and nucleus sizes in lizards and no correlation between the erythrocyte and nuclei sizes in snakes.

5. Conclusion

In conclusion, the findings of the study presented basic data comprising shape, size, the numbers of erythrocytes and their variation of *A. adramitanus* and *C. c. calyptratus* from yemen. According to the results, morphology and size of erythrocytes have showed some variations among two species. This is may be due to the difference in activity levels and even microhabitat.

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مقالة بحثية

شكل وعدد كريات الدم الحمراء للوحر الصخري والحرباء المحجبة من اليمن

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الملخص

الهدف من البحث الحالي هو تحديد شكل كريات الدم الحمراء والأنوية للوحر الصخري والحرباء المحجبة من اليمن بواسطة عمل مسحة من الدم وصباغتها بواسطة صبغة جيمزا. أظهرت الدراسة الحالية أن أكبر وأطول كريات دم حمراء وأنويتها توجد في الحرباء المحجبة بينما أصغر كريات دم حمراء لوحظت في الوحر الصخري. وقد أكدت النتائج على الارتباط بين حجم الخلية ونواتها. كذلك أوضحت الدراسة الحالية عدم وجود اختلاف بين كلا النوعيين في عدد كريات الدم الحمراء. كذلك النتائج أكدت وجود ارتباط بين طول الخلية الدموية وصفاتها.

الكلمات الرئيسية: اليمن، مسحة دم، شكل كريات الدم الحمراء وأنويتها.