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Morphometrical Analysis of Blood Cells of Yellow-Bellied House Gecko, *Hemidactylus flaviviridis* Ruppell, 1835 with Sexual Dimorphism

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Abstract: ***The study was held to investigate the size of blood cells of Hemidactylus flaviviridis (Gekkonidae) in Odisha. Blood smears stained with Giemsa were analysed. The measured blood cells were erythrocytes and leucocytes (lymphocytes, heterophils, eosinophils and monocytes). The morphometry regarding the dimension (mean length and breadth) of cell and nucleus show no significance difference (t – value) in two different sexes. The cells show a difference in their mean values.***

Keywords: blood cell, Gecko, morphometry, sex

1. Introduction

Geckos are small to average sized lizards belonging to Class-Reptilia, Order-Squamata, Suborder-Sauria and Family-Gekkonidae found in warm climatic region throughout the world [1]. Fluctuations regarding hematological parameters of reptiles depend on age, sex and throughout the life, these parameters can vary [2], [3]. The blood cells of reptiles also vary with respect to the physiological conditions, mode of diet intake, environment, climatic conditions, age, sex and state of reproduction [4] - [7]. According to [8] some morphometrical parameters of blood cells of house gecko are taken. The blood cells have been identified [9] - [14]. Blood volume of reptiles in accordance with their body weight is thought to be between 5-8% [15] - [17]. The hematological data interpretation is still challenging in reptiles in comparison with small animals, because of the lower number of studies and the inadequate of reference values for the majority of species. Manual counting techniques and evaluation of blood smears are necessary only due to the presence of nucleated erythrocytes in reptile blood which limits the use of electronic cell-counting devices for leucocytes [18].

2. Materials and Methods

2.1 Study area and animal collection

The lizard *Hemidactylus flaviviridis* Ruppell, 1835 is common to Odisha. These were collected from the walls of Pandit Nilakantha Chhatrabas, Ph. D. Hostel of Utkal University and P. G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar-751 004, Odisha located in 20˚18'14.26" N latitude and 85˚ 50'22.73" E longitude. They were caught at night and transferred to laboratory. To acclimatize with the laboratory condition they were kept in the animal house. Animals were clinically healthy and in good condition. A maximum of ten adult specimens of each sex was selected for conducting this experiment.

2.2 Collection of blood samples

The coccygial vein (tail vein) was made aseptic by rectified sprit (Qualigens Product No.34457, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Maharastra, India). An insulin syringe (BD Ultra-Fine^{TM} Needle 12.7 mm x 30 G) of 1ml/cc capacity was used to collect blood between the scales on ventral mid line of tail vein [19], [20]. After blood collection the lizards were released to their natural habitat.

2.3 Blood smear preparation

Smears were prepared on microscopic slides (BLUE STAR, PIC 2, Polar Industrial Corporation, Mumbai, India) just after venipuncture. Anticoagulants may interfere with morphometrical changes in blood cells and can cause hemolysis and thus avoided [21], [17], [15], [16]. Slides were marked and precisely identified according to their respective sexes.

2.4 Morphometrical examination

The prepared blood smears were air dried, fixed with methanol (Qualigens Product No.34457, Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Maharashtra, India) and stained with Giemsa stain prepared from Giemsa powder (Qualigens CAS NO.51811- 82-6 Product NO. 39382, scientific India Pvt. Ltd., Mumbai, Maharashtra, India) as protocol cited by Lillie [22].The sizes of blood cells were measured by using an ocular and stage micrometer. The entire data (10 observations) per sex were subjected for morphometrical analysis by standardizing the ocular micrometer against the stage micrometer (ERMA TOKYO, Japan made) using a standard light microscope (LABOSCOPE MICROSCOPES Research microscope M. No. BD-08 B, S. No. 21320 Mfg. by B.D. INSTRUMENTATION, Ambala Cantt, India) under 40X objective.

2.5 Statistical analysis

Each morphometrical parameter is expressed as Mean±SE for male and female and Microsoft Office Excel 2007 was used for statistical analyses. Student t - test was used to determine statistical significance and was done with the help of Paleontological Statistics (PAST) version 2.17 [Natural History Museum, University of Oslo].

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3. Results and Discussion

3.1 Results

3.1.1 Size of Erythrocytes

The average length and breadth of erythrocytes and their nuclei between the two sexes of *Hemidactylus flaviviridis* are recorded in micron meter (µm) (Table 1) and analyzed. The findings of the study revealed about the size of erythrocytes of *Hemidactylus flaviviridis* with sexual dimorphism. The difference in length and breadth of cells and nuclei of erythrocytes are not significant in between male and female.

Table 1: Size of erythrocytes of male and female of *Hemidactylus flaviviridis* (Mean ± SE expressed in µm)

Type of cell	Cell/ Nucleus	Parameters (µm)	Male (n=10)	Female (n=10)	t-value
Erythrocyte	Cell	Length	14.71± 0.22	16.26±0.64	0.55
		Breadth	10.43 ±0.56	11.65±0.42	0.12
	Nucleus	Length	7.72 ±0.24	7.96 ±0.26	0.52
		Breadth	4.96 ±0.28	5.91 ±0.34	0.06

3.1.2 Size of Leucocytes

The measured leucocytes were large lymphocytes, small lymphocytes, heterophils, eosinophils and monocytes (Table 2). Leucocytes are generally round in shape. So their length and breadth of cell and nucleus do not vary significantly. Agranulocytes (lymphocytes and monocytes) do not show significant difference between males and females of same species. The mean values in their length and breadth of cell and nucleus varies to some extent. In agranulocytes (eosinophils and heterophils) no significant difference is observed in length and breadth of cell and nucleus. The mean value regarding length and breadth of leucocytes and their nuclei between the two sexes of *Hemidactylus flaviviridis*, show sexual dimorphism.

Table 2: Size of different leucocytess of male and female of *Hemidactylus flaviviridis* (Mean ± SE expressed in µm)

Type of cell	Cell/ Nucleus	parameters	Male (n=10)	Female (n=10)	t- value
Large lymphocyte	Cell	Length	18.38±0.27	19.06±0.53	0.29
		Breadth	13.65±0.40	17.15±0.63	0.002
	Nucleus	Length	9.25±0.57	10.78±0.41	0.06
		Breadth	7.55±0.18	9.13±0.38	0.006
Small lymphocyte	Cell	Length	15.73±0.41	17.06±0.5	0.07
		Breadth	14.06±0.06	14.65±0.03	0.10
	Nucleus	Length	6.02±0.09	6.71±0.36	0.10
		Breadth	5.8±0.05	5.98±0.14	0.27
Heterophil	Cell	Length	12.34±0.16	15.03±0.41	0.0003
		Breadth	10.84±0.29	12.92±0.27	0.0009
Monocyte	Cell	Length	14.2 ±0.09	14.27±0.09	0.66
		Breadth	13.77±0.09	13.77±0.99	0.69
	Nucleus	Length	7.75±0.07	7.75±0.07	0.69
		Breadth	7.29±0.07	7.29±0.07	0.02
Eosinophil	Cell	Length	14.12±0.85	13.16±0.29	0.06
		Breadth	12.85±0.10	11.65±0.42	0.19

3.2 Discussion

3.2.1 Erythrocytes

The length of erythrocytes varies between the families and also within the family. It has been seen that in Gekkonidae and Pygopodidae, the red blood cells are usually large in size [23]. The data about mean length of erythrocytes and nucleus corroborates with the data of some geckos like *A. elisae, A. nasrullahi and C. scrabum* [24]. But the mean breadth value of erythrocytes and their nucleus of *H. flaviviridis* are somehow greater than the above said three species. The mean value regarding length and breadth of cells of erythrocytes in male *H. flaviviridis* shows greater value in comparison to

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wild keeled Mabuya, *Eutropis carinata* but the mean value regarding length and breadth of nucleus agree with the data [3]. It was found that the length and breadth of erythrocytes and their nucleus in case of *H. flaviviridis* agree with the morphometrical values of erythrocytes in *H. turcicus, C. heterocereum and C. scabrum* [25]. The data regarding mean length of cell and nucleus of erythrocytes in male *H. flaviviridis* is smaller and in case of female it is greater than the data in case of *P. blanfordanus* [2]. Cell breadth of erythrocytes and nuclear length and breadth is greater but cell length is smaller in case of *H. flaviviridis* in comparison to some Agamidae [26]. Cell length of erythrocytes also matches with the greatest diameter of some species of reptiles [27].

3.2.2 Leucocytes

Lymphocytes are the most important class of leucocytes. According to their size they show variations and are classified into large and small lymphocytes it is greatly seen in *Crodylus vittifer* [4]. In case of *H. flaviviridis* the data regarding the mean length and breadth of large lymphocytes falls in the reference range but in case of small lymphocyte it is greater [4]. Lymphocyte size regarding length and breadth is greater in *H. flaviviridis* than some agamides [26].The neutrophils show morphological variants in their nucleus and are named as heterophils [28]. Length and breadth of heterophils agree with the value of some agamides [26].The monocytes are generally rare. Their nucleus shows polymorphism [4]. Length and breadth of monocytes corroborate with the value of some agamides [26] and fall in reference ranges of agamides [3]. The data regarding the size of eosinophils vary between the species and also within a species with sexual dimorphism. Eosinophil corroborates with the data of agamides [3], falls in the reference value of some agamides [26] and length of eosinophils matches with some species of reptiles [27].

4. Conclusion

Morphometrical analysis of different blood cell in case of *Hemidactylus flaviviridis* Ruppell, 1835 in both male and female do not show any striking significance but there is a little difference in mean values of blood cells. In conclusion, it may be said that hemocytomrphometrical parameters can serve as a means to evaluate the physiological conditions and health status of lizard populations, which may be a useful indicator of their status of environment. The present data has also established a base line to which further studies may be compared.

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