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Haemocytomorphometry of Leschenault's Leaf Toad Gecko, *Hemidactylus leschenaultii* Dumeril and Bibron, 1836

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Abstract: ***The present study aims to investigate and provide a reference range on haemocytomorphometry of Hemidactylus leschenaultii in Odisha in a stipulated geographical region. Blood smears are stained with Giemsa's stain. The blood cells measured were erythrocytes and leucocytes. The mean value of length and breadth of cell and nucleus of different blood cells shows significant difference (p<0.05, p<0.01, and p<0.001) with sexual dimorphism. A comparative picture in form of histograms shows a clear idea on differences in size of blood cells.***

Keywords: Haemocytomorphometry, *Hemidactylus leschenaultii*, Significant difference, Sexual dimorphism

1. Introduction

Leschenault's leaf toad geckos are found in warm climatic region throughout the world [1]. The use of geckos in traditional medicine [2], biology, husbandry and reproduction [3] are considered. In order to assess the health condition of lower vertebrates such as fishes, amphibians, and reptiles hematological parameters are used as an essential diagnostic tool, which may be influenced by a number of internal and environmental factors [4],[5][6],[7],[8]. Haematological parameters of reptiles show fluctuations depending on age and sex and these parameters can vary throughout the life [9],[10],[11],[12]. A thorough knowledge of reptilian physiology is becoming more imperative due to diagnostic evaluation of reptiles [13]. Economic importances of reptiles, their role as pets, need for conservation actions [14] and comparative study [15]. A comparative study on morphology of blood cells of reptiles has been taken according to [16].

Information about the reptile blood composition especially Sauria are studied according to[17],[18],[19],[20],[9]. Earlier workers on reptiles are principally confined to the European species [18].These works describe the cell composition and the chemistry of the blood. [17] proposed a nomenclature of the circulating blood cells of reptiles, which was modified by [18]. Different research publications demonstrated that the population of different kinds of cells in the blood of reptiles varies with respect to the environment, season, sex, age, reproductive state, disease, nutrition, and physiological conditions [17],[21], [22],[9]. According to [23] Olayemi, 2011 some hematological parameters of house gecko are taken. The circulating blood cells of different reptiles have been described [24],[25],[26],[27],[28],[29]. [7] and [13] reported total erythrocyte and leukocyte counts in reptiles fail to account for the nuances and the fact that all the cells in the peripheral blood are nucleated. Hematology of green iguanas has been a subject of interest for number of authors. The results of trials showed a significant degree of variation due to different animal selection methods and technical differences in blood sample treatment [30], [31], [8]. Erythrocytes are measured and the diameter is taken [32].

2. Materials and Methods

2.1 Research site

The study is conducted between 2014 and 2017. *Hemidactylus leschenaultii* Dumeril and Bibron 1836 is common to Odisha. The lizards are collected from the geographical area located on 20° 20' N to 20° 37' N latitude and 86° 14' E to 87° 01' E longitude, is the coastal area of Rajnagar block of Kendrapara- 754 225, Odisha. They are captured at night from the light sources using nets and kept in a wooden cage. They are acclimatized with laboratory conditions inside the animal room. A maximum of fifteen (n=15) adult healthy individuals of both sexes are selected for collection of blood.

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™ 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. After collection of blood the lizards were released to their natural habitat.

2.3 Preparation of Blood Smear

Smears are 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], [13], [31].

2.4 Morphometrical examination

The prepared blood smears are 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 .The sizes of blood cells are measured by using an ocular and stage micrometer. The entire data (15 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 in Mean and Standard Error of mean for both sexes and Microsoft Office Excel 2007 was used for statistical analyses. Student's t-test is used to determine the statistical significance and was done with the help of Paleontological Statistics (PAST) version 2.17 [Natural History Museum, University of Oslo].

3. Results and Discussion

3.1Results

Table 1: Morphometrical analysis of blood cells of *Hemidactylus leschenaultii*

Sl No	Types of Blood Cells	Parameter	Male		Female		P Value
			Mean	SE	Mean	SE	
1	Erythrocyte (RBC)	L	15.19	0.41	16.21	0.63	2.85*
		B	10.31	0.31	10.03	0.35	3.92**
		L'	7.33	0.27	8.58	0.42	5.43***
		B'	4.73	0.35	5.39	0.27	3.74**
2	Large Lymphocyte	L	16.83	0.44	15.94	0.61	2.87*
		B	14.06	0.31	14.34	0.52	0.22 **NS**
		L'	6.41	0.16	6.17	0.32	1.32 **NS**
		B'	5.84	0.13	5.83	0.23	0.04 **NS**
3	Small Lymphocyte	L	13.91	0.40	14.49	0.57	0.86 **NS**
		B	12.78	0.55	12.74	0.52	0.21 **NS**
		L'	9.25	0.19	9.52	0.25	2.15*
		B'	6.23	0.14	6.21	0.15	0.35 **NS**
4	Monocyte	L	17.41	0.41	17.01	0.58	1.17 **NS**
		B	13.02	0.43	15.91	0.54	4.15***
		L'	10.50	0.56	11.57	0.65	4.91***
		B'	8.72	0.24	8.88	0.16	0.50 **NS**
5	Heterophil	L	12.76	0.61	13.06	0.61	1.08 **NS**
		B	10.73	0.56	11.63	0.45	2.62*
6	Eosinophil	L	12.56	0.45	13.09	0.71	1.34 **NS**
		B	11.18	0.54	11.84	0.78	0.71 **NS**

(The significant difference (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) for each haematological parameters and NS: Non significant values. L= Cell length, B= Cell breadth, L'= Nucleus length, B'= Nucleus breadth)

The values on morphometrical analysis of erythrocytes show significant difference between both sexes. The mean values of cell length is significant at $p<0.05$, cell breadth at $p<0.01$, nucleus length is at $p<0.001$ and nucleus breadth is at $p<0.01$. The cell length of large lymphocytes shows significance difference ($p<0.05$). The cell breadth of small lymphocyte is also significantly different at $p<0.05$. Monocytes show a significant difference on cell breadth and nucleus length at $p<0.001$. The cell breadth of heterophils is significant at $p<0.05$ (Table 1).

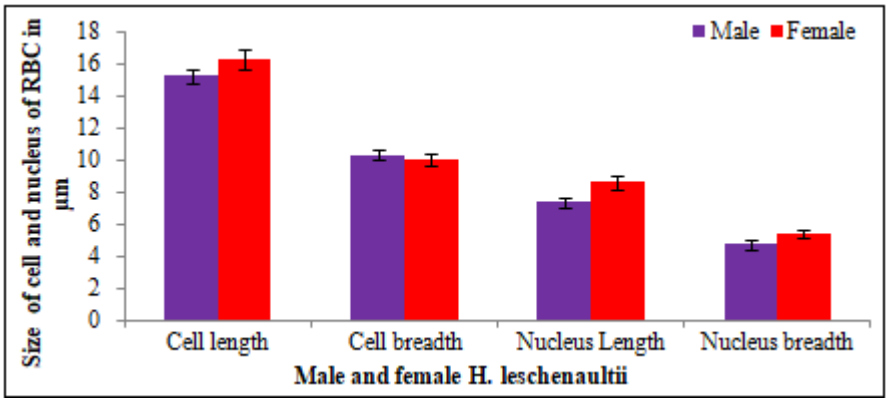

Figure 1 Average length and breadth of cell and nucleus of erythrocytes of *Hemidactylus leschenaultii*

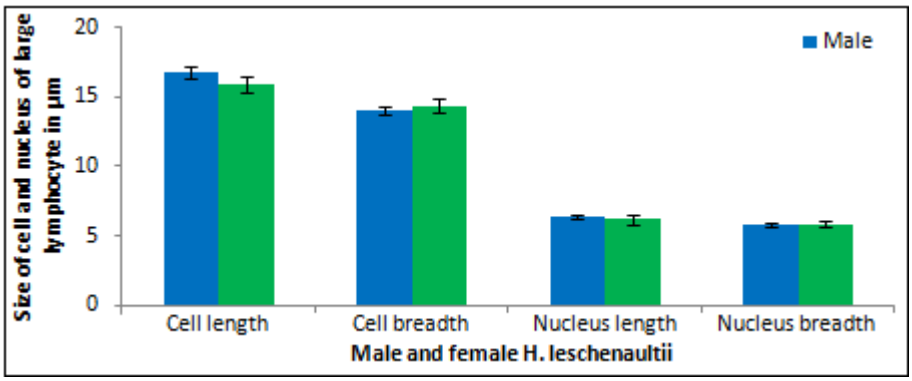

Figure 2: Average length and breadth of cell and nucleus of large lymphocytes of *Hemidactylus leschenaultii*

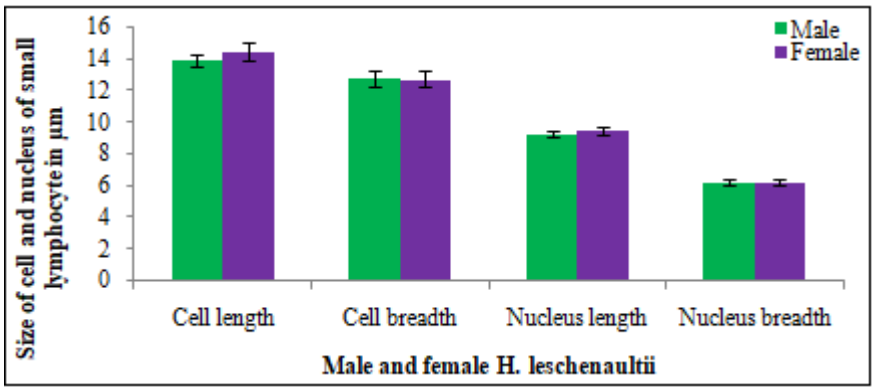

Figure 3: Average length and breadth of cell and nucleus of small lymphocytes of *Hemidactylus leschenaultia*

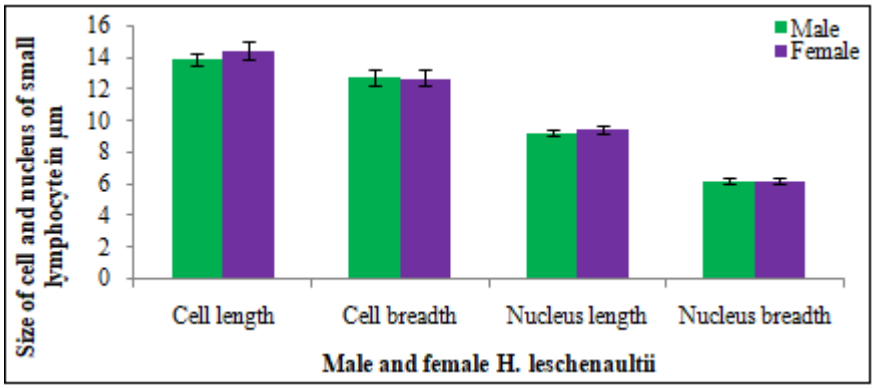

Figure 4: Average length and breadth of cell and nucleus of monocytes of *Hemidactylus leschenaultia*

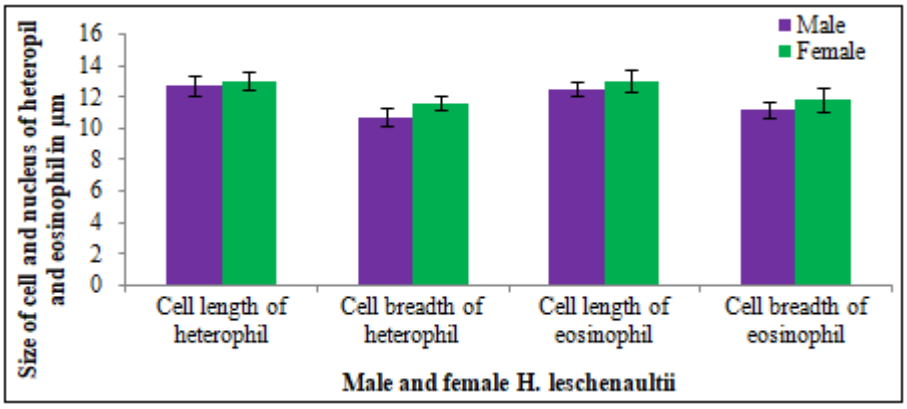

Figure 5: Average length and breadth of cell of heterophils and eosinophils of *Hemidactylus leschenaultia*

A comparative picture on average values of morphometry of erythrocytes shows that the cell length, nucleus length and nucleus breadth is higher in female and cell breadth is higher in male lizard (Fig.1). In large lymphocytes the average values of cell length, nucleus length and nucleus breadth is higher in male but cell breadth is higher in female (Fig.2). The cell length and nucleus length is higher in male (Fig.3). The cell length of monocytes is higher in male individuals and is the highest one among all cell types (Fig.4). The cell

length and breadth of both heterophils and eosinophils are higher in female in comparison to male (Fig.5).

3.2 Discussion

The present study is based on morphometry of blood cells include the measuremenr of length and breadth of cell as well as nucleus of erythrocytes and leucocytes. It has been seen that in Gekkonidae, the red blood cells are usually large in size [33]. The data about mean length of erythrocytes and nucleus corroborates with the data of some geckos like *A. elisae, A. nasrullahi and C. scrabum* [34] but the cell length and breadth of erythrocytes and their nucleus of male is higher than above species. The mean value regarding length and breadth of cells of erythrocytes in male *H. leschenaultii* shows greater value in comparison to wild keeled Mabuya, *Eutropis carinata* but the mean value regarding length and breadth of nucleus falls within the range [11]. It was found that the length and breadth of erythrocytes and their nucleus in case of *H. leschenaultii* agree with the morphometrical values of erythrocytes in *H. turcicus, C. heterocereum and C. scabrum* [35]. The data regarding mean length of cell and nucleus of erythrocytes in male *H. leschenaultii* is higher and in case of female it is lower than the data in case of *P. blanfordanus* [10]. Cell length and breadth of erythrocytes and nuclear length and breadth is greater in case of *H. leschenaultii* in comparison to some agamids [36]. Cell length of erythrocytes also matches with the greatest diameter of some species of reptiles [18]. Lymphocytes are the most important class of leucocytes followed by heterophils. They show variations in size and are classified into large and small lymphocytes, it is greatly seen in *Crodylus vittifer* [17]. In case of *H. leschenaultii* the data regarding the mean length and breadth of both lymphocytes fall in the reference range [17]. Size of lymphocytes regarding length and breadth is lower in *H. leschenaultii* than some agamides [36].The neutrophils show morphological variantions in their nucleus and are named as heterophils [37]. Length and breadth of heterophils agree with the value of some agamides and falls within the range values [36].The nucleus of monocytes shows polymorphism [17]. Length and breadth of monocytes is higher than some agamids [26] and fall in reference ranges of other agamids [11]. 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 [11], falls within the reference value of some agamides [36] and length of eosinophils matches with some species of reptiles [18].

4. Conclusion

The morphometrical analysis of different blood cells in case of *Hemidactylus leschenaultii* Dumeril and Bibron in both male and female show striking significant difference at 95% confidence level but there is also nonsignificant difference with a little difference in mean values of blood cells. It may say that, these haemocytomorphometric data can provide a thorough knowledge about the health status of lizards with their environments.

5. Acknowledgements

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6. Future Scope

These values may be used as references in future diagnostic purpose such as in case of outbreak of disease in reptiles. This can also provide the health status of reptiles with their environments.

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