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Blood Cytomorphometry and Cytomorphology of Red-tailed Green Viper (*Trimeresurus insularis*) On the Bali Island

(SITOMORFOMETRI DAN SITOMORFOLOGI DARAH ULAR HIJAU EKOR MERAH (*TRIMERESURUS INSULARIS*) BERBISA DI PULAU BALI)

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ABSTRACT

Snakes are becoming increasingly popular among reptile enthusiasts due to their uniqueness and exoticism. Snakes can be classified into venomous and non-venomous. Venomous snakes exhibit unique colors, body shapes, and behavior. Snake bites are common in Indonesia, and many cases go unreported. The red-tailed green viper (*Trimeresurus insularis*/*T. insularis*), a venomous snake, is frequently found on the island of Bali. However, research on this snake is limited. Blood morphology is an indicator of an animal's health status and can provide comprehensive information about the animal. The study was aimed to know the cytomorphology and cytomorphometry of Red-tailed Green Viper (*Trimeresurus insularis*) on the Bali Island. Knowing the health status of *T. insularis* through the morphology of its blood cells can assist veterinarians in diagnosing and treating sick animals. This study used nine healthy *T. insularis* snakes, comprising five males and four females from Bali. Blood was collected from the coccygeal vein smeared on a glass slide, fixed with 90% ethanol, and stained with 10% Giemsa. The blood smear was then observed under a microscope to measure cytomorphometry. The Veterinary Immunology Laboratory at Udayana University and the Malang Healthy Animal Clinic conducted cell measurements using an Olympus CX33 microscope, analyzing data with SPSS via the Analysis of variance test. The study found significant differences in erythrocyte nucleus width between male (25.92 µm) and female (30.44 µm) *T. insularis* snakes, as well as in basophil cytoplasm length (males: 6 µm, females: 5.37 µm). No differences were observed in heterophils, lymphocytes, or azurophils, and eosinophils were absent in these snakes on Bali.

Key words: *T. insularis*; erythrocytes; leukocytes; cytomorphometry

ABSTRAK

Ular kini semakin populer di kalangan pecinta reptil karena keunikan dan eksotismenya. Ular dapat diklasifikasikan menjadi ular berbisa dan tidak berbisa. Ular berbisa menunjukkan warna, bentuk tubuh dan perilaku yang unik. Gigitan ular merupakan hal yang umum terjadi di indonesia, dan banyak kasus yang tidak dilaporkan. Ular hijau ekor merah (trimeresurus insularis/t. Insularis) yang berbisa merupakan ular yang banyak ditemukan di pulau bali. Namun, penelitian tentang ular ini masih terbatas. Morfologi darah merupakan indikator status kesehatan suatu hewan dan dapat memberikan informasi yang komprehensif tentang hewan tersebut. Penelitian ini bertujuan untuk mengetahui sitomorfologi dan sitomorfometri ular hijau ekor merah (*Trimeresurus insularis*) yang berbisa di pulau bali. Mengetahui status kesehatan*T. insularis* melalui morfologi sel darahnya dapat membantu para dokter hewan dalam mendiagnosis dan mengobati hewan yang sakit. Penelitian ini menggunakan sembilan ekor ular *T. insularis* sehat asal bali yang terdiri atas lima jantan dan empat betina. Darah diambil dari vena koksigeus, kemudian diulas pada kaca objek, difiksasi dengan etanol 90%, dan diwarnai dengan giemsa 10%. Apusan darah kemudian diamati di bawah mikroskop untuk mengukur sitomorfometri. Pengukuran dilakukan di laboratorium imunologi veteriner universitas udayana dan klinik hewan sehat malang menggunakan mikroskop olympus cx33, dan analisis data dilakukan dengan spss melalui uji sidik ragam. Studi ini menemukan adanya perbedaan yang signifikan dalam lebar inti eritrosit antar ular *T. insularis* jantan (25,92 µm) dan betina (30,44 µm), serta panjang sitoplasma basofil (jantan: 6 µm, betina: 5,37 µm). Tidak ada perbedaan yang diamati pada heterofil, limfosit, atau azurofil, dan eosinofil tidak ditemukan pada ular hijau ekor merah di bali.

Kata-kata kunci: *T. insularis*; erithrosit; leukosit; sitomorphometri

INTRODUCTION

Data from the Indonesian Central Statistics Agency as of July 2020 shows that the trend of raising exotic animals as pets in Indonesia has increased significantly (Steven, 2021), marked by an increase in imports of exotic animals (such as reptiles, mammals and poultry) which has reached more than $ 52 million US. Reptiles are one of the exotic animals whose number of enthusiasts has increased dramatically. Snakes are one of the reptiles experiencing an increasing number of enthusiasts due to their uniqueness and exoticism, with content creators on social media contributing a major role by influencing people to keep these exotic animals. Known for the uniqueness of their bodies, such as color, head shape, and their rarity in nature, contributes to its growing number of enthusiasts. More venomous snakes generally have more beautiful colors and body shapes than non-venomous snakes (Landova *et al*., 2018).

Indonesia is a country geographically located in two biogeographic zo-nes, namely Asia and Australo-Papua. Bet-ween this region is bounded by the Wallace Line, Weber Line and Lydeker Line which includes Sulawesi, Nusa Tenggara and Maluku. This unique geographical condition accommodates Indonesia's unique and rich flora and fauna. Snake species in the western part of Indonesia tend to be similar to snake species in the Asian region, meanwhile, in the east, they tend to be related to the Australasian snake species.

Currently, Indonesia is known to have around 350-370 species of snakes, 77 of which are venomous. According to reports from the Indonesia Toxinology Society over the last decade, there have been approximately 135,000 cases of snake bites each year. It's estimated that the death rate from these bites is around 10% per year,

which means that one person dies every hour due to snake bites. However, this data does not fully reflect the actual concern since it is based solely on reports from clinicians in the field, including hospitals and Community Health Centers, as well as from the community, and has not been officially collected by the Ministry of Health (Maharani, 2021).

The island of Bali is known for its extraordinary natural beauty. Apart from having natural beauty, Bali is also a habitat for various Herpetofauna species. Amarasinghe (2021) stated that in West Bali National Park, 30 species of herpetofauna could be found in just 10 days of observation. One of them is the red-tailed green viper (*T. insularis*) which is commonly found on the island of Bali.

Reptiles are animals with a very wide range of species. To date, it is estimated that there are more than eight thousand species of reptiles living in the world. Reptiles have unique blood characteristics and are different from mammals, for example, red blood cells in reptiles have nuclei, whereas in mammals, they do not. The increasing popularity of reptiles as exotic pets makes information regarding blood morphology important. This snake generally lives in trees (arboreal) and is found widely distributed in central to eastern Indonesia, especially on the east side of the island of Java to the Lesser Sunda Islands (Bali, Flores, Lembata, Lombok, Pantar, Sumba, Sumbawa, Timor, and Wetar).

Blood morphology is a very important component of data in assisting in establishing a diagnosis or health status of an animal. Apart from that, information related to reptile blood morphology is also important as material for studies assessing the health status of an ecosystem in the wild (Stacy *et al*., 2011).

Snakes are animals that were previously rarely kept by the general public, so data related to health checks, such as blood morphology, is still minimal and does not cover all existing snake species. Hematological analysis of blood can be significantly important for studying the biology of a species and determining the health or reproductive status of animals both in the wild and in captivity (Duje Lisičić *et al*., 2013).

Until now, the blood morphology of the red-tailed green viper (*T. insularis*) on the island of Bali has never been studied, even though this data is very important as reference data to determine the health status of the *T. insularis*. Based on the study above, it is hoped that through this research, data related to the blood morphology of *T. insularis* will be obtained to enrich data related to the blood morphology of reptiles, especially *T. insularis* on the island of Bali.

RESEARCH METHODS

Sample Collection and Materials

In this study, samples were used in the form of blood from the snake *T. insularis* found on the island of Bali. Samples were taken from nine snakes, five males and four females, which were rescued or found directly in nature. In this study, snakes were coded, in order of sex (M (male) and F (female), individual number, and cage (alphabet).

The materials used in this research were whole blood from the snake *T. insularis* as a sample, 0.5 inch acrylic pipe, 90% ethanol, 10% Giemsa dye, and a 70% alcohol swab. In this research we use 1 mL syringe, 26G needle, 70% alcohol swab, glass slide, and microscope (Olympus CX33®, Olympus, Tokyo, Japan) with an Optilab Plus digital camera.

Research Design and Procedures

This study involved the use of nine *T. insularis* snakes. Each snake underwent a month-long quarantine period before the blood collection procedure was carried out. The snakes were housed in groups of 2-3 in cages. Prior to being placed in the cage, each snake's mouth was checked for inflammation, and their skin was inspected for subcutaneous worms. After being placed in their cages for two2 weeks, each snake was fed to ensure their appetite. When it was confirmed that they were healthy, the blood collection procedure was performed. For each sample, three blood samples were taken and observed using 10 fields of view through an Olympus CX33 microscope. The cells were then directly measured using Optilab Viewer 2.0 image processing software, recording the cytoplasm length (µm), cytoplasm width (µm), nucleus length (µm), and nucleus width (µm). Once the measurements were complete, the data was processed using a completely randomized design via the Analysis of Variance test using Statistical Package for the Social Sciences (SPSS) Software. The snakes were coded based on sex (M [male] and F [female]), individual number, and cage (alphabet). In addition, approximately 0.05 mL of blood was taken from the coccygeal vein.

This study involved nine *T. insularis* snakes – five males and four females - that were caught in the wild. Blood samples were collected from the coccygeal vein of each snake using a 1 mL syringe. The snakes were first placed in an acrylic tube with a diameter of 0.5 inch, and then the caudal ventral part of the snake was cleaned with a 70% alcohol swab for 5 cm from the cloaca caudally. The needle was then inserted under the scales to extract blood from the coccygeal vein. Once the blood collection procedure was completed, the blood sample was immediately reviewed using a glass slide. The part of the snake where the blood was taken was wiped with a 70% alcohol swab, and the snake was then returned to its cage.

The blood samples that were reviewed were air-dried for seven minutes, fixed with 90% ethanol for five minutes, air-dried again, and then stained with 10% Giemsa for five minutes. The samples were then rinsed with distilled water and air-dried before being stored in the preparation storage box for later observation (Suartini *et al.*, 2015).

Data Analysis

The research produced images of blood cells from the snake *T. insularis*, including erythrocytes, leukocytes, and platelets. Morphometric data for these cells was collected using an Olympus CX33 microscope, paired with an Optilab Plus digital camera and Optilab Viewer 2.0 software for image processing. The data was then analyzed using SPSS (Statistical Product and Service Solutions) and subjected to the Analysis of Variance test.

Location and Time

The sampling for the research was done in Denpasar, and the blood samples were examined at the Veterinary Immunology Laboratory, Faculty of Veterinary Medicine, Udayana University. To validate the data, the samples were sent to the Malang Healthy Animal Clinic Research and Diagnostic Laboratory, which has the expertise to measure reptile blood cells. The sampling was conducted in December 2023.

RESULTS AND DISCUSSION

Research on blood cytomorphometry of *T. insularis* snakes on the island of Bali is crucial and has not been explored before. By analyzing the blood of these animals, we can better understand their health status and overall condition. The results of this study can be valuable for veterinarians, helping them to diagnose and plan appropriate therapy for animals that may be experiencing health problems.

Erythrocyte Cytomorphometry

In male *T. insularis* snakes, the length of the erythrocyte cytoplasm is (104.58 ± 24.39 μm) with the width of the cytoplasm (66.21 ± 12.46 μm), while the nucleus is long (41.94 ± 9.97 μm) in width (25.92 ± 6.28 μm). In female *T. insularis* snakes, the length of the erythrocyte cytoplasm is (111.78 ± 30.79 μm) with the width of the cytoplasm (65.16 ± 12.95 μm), while the nucleus is long (47.14 ± 15.50 μm). in width (30.44 ± 9.22 μm). The average *T. insularis* snake erythrocyte cell has a cytoplasm length (107.78 ± 27.49 μm) with a cytoplasm width (65.75 ± 12.62 μm), while the erythrocyte nucleus has a length of (44.27 ± 12.92 μm) in width (27.93 ± 8 μm) (Table 1). Based on the results of the Anova test, the width of the nucleus of male and female *T. insularis* erythrocytes was significantly different ($P < 0.05$), with a significance of 0.007. Meanwhile, the length and width of the cytoplasm and the length of the nucleus were not significantly different between males and females.

Mature erythrocytes in *T. insularis* snakes show an oval shape with cytoplasm that tends to be acidophilic (Fig. 1). The nucleus of these red blood cells is ellipsoidal to irregular in shape, highly basophilic, and located in the center of the cell. Nuclei with irregular shapes are very common in snake erythrocytes (Dissanayake *et al*., 2017). Meanwhile, erythrocytes that have not yet reached maturity have smaller dimensions than mature ones, with a rounder cytoplasm and a nucleus that is also basophilic.

Trimeresurus insularis on the island of Bali (Fig. 2) shows significant differences in erythrocyte nucleus width based on sex, where the width of the erythrocyte nucleus in males (25.92 μm) is smaller than in females (30.44 μm). This difference has statistical significance with a value of less than 0.05 (0.007), so it can be categorized as a real or significant difference. The difference in erythrocyte size between male and female *T. insularis* is most likely influenced by the higher oxygen requirements of female snakes compared to male snakes. When a female *T. insularis* snake is pregnant, the snake will need more oxygen due to heavier metabolic processes so oxygen requirements also increase (Penman *et al*., 2022). When compared with the cytomorphometry of *Vipera ammodytes* snake erythrocytes which have cytoplasm length (18.36 ± 1.21 μm), cytoplasm width (12.65 ± 0.67 μm), nucleus length (8.04 ± 0.73 μm), and nucleus width (4.69 ± 0.44 μm) (Lisičić *et al*., 2013). The snake *T. insularis* has a larger erythrocyte size.

The microcytic form was found in sample F6E. Microcytic erythrocyte abnormalities (Fig. 3; Table 2) indicate microcytic anemia, characterized by red blood cells that are smaller than normal due to decreased hemoglobin production (DeLoughery, 2014). The teardrop shape (Fig. 4) is found in samples F2A, F9D, M3E, M12F, and M13F. Teardrop-shaped erythrocyte abnormalities are poikilocytes (erythrocytes with an irregular shape) caused by changes in blood formation. Teardrop-shaped erythrocytes have blunt rounded sides and thin, leaky tips, similar to a tear. This shape can occur because the blood smear is technically lower so that it points in one direction in the field of view. There must be more than one teardrop erythrocyte on the blood smear to warrant microscopic assessment (Gütgemann *et al*., 2014). Hypochromic cell color (Fig. 5) was found in the M (TNBB) sample. A hypochromic color may indicate hypochromic anemia (or hemoglobin synthesis deficit) caused by a block of protoporphyrin synthesis (sideroblastic anemia), a disorder of iron metabolism (iron deficiency anemia, simple

chronic anemia), or a disorder of globin synthesis (thalassemia) (Rada *et al*., 2018).

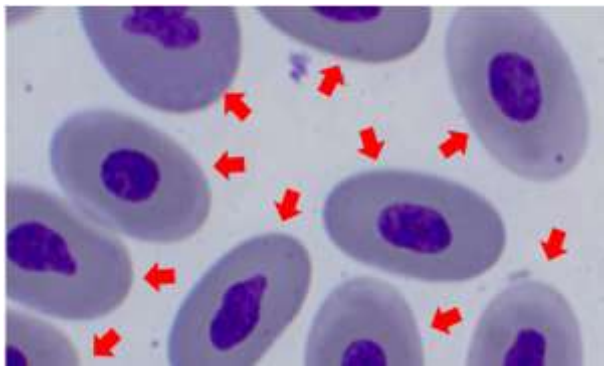

Figure 1. Erythrocytes (arrow) from the *Trimeresurus Insularis* on Bali Island with 10% Giem sastaining and 1000× magnification using immersion oil

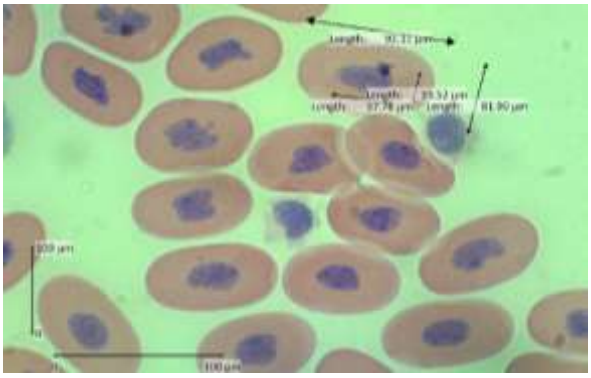

Figure 2. Erythrocytes from the *Trimeresurus Insularis* snake on the island of Bali with Diff Quick staining.

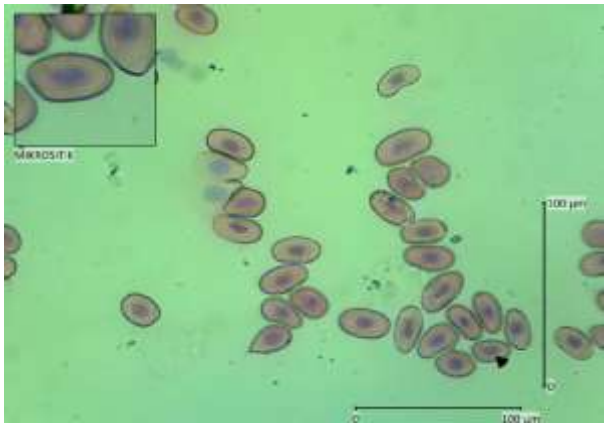

Figure 3**.** Microcytic Erythrocyte Abnormalities from *Trimeresurus insularis* snakes on Bali Island with Diff Quick staining and 1000× magnification using immersion oil.

Figure 4. Teardrop Erythrocyte abnormality from the snake *Trimeresurus insularis* on the island of Bali with Diff Quick staining and 1000× magnification using immersion oil.

Table 1. *Trimeresurus insularis* erythrocyte abnormalities

Variable	Cytomorphometry	N	**Sex**	Mean ± SD	Sig.
Erythrocyte	Cytoplasm Length (µm)	50	Male	104,58 ± 24,39	0,219
		40	Female	111,78 ± 30,79	
	Cytoplasm Width (µm)	50	Male	66,21 ± 12,46	0,698
		40	Female	65,16 ± 12,95	
	Nucleus Length (µm)	50	Male	41,94 ± 9,97	0,056
		40	Female	47,17 ± 15,50	
	Nucleus Width (µm)	50	Male	25,92 ± 6,28	0,007*
		50	Female	30,44 ± 9,22	

Table 2. *Tremeresurus insularis* erythrocyte morphometry

Sample	Microcytic	Teardrop	Hypochromic
F2A		√	
F6E	√		
F9D		√	
F11F			
M(TNBB)			√
M3E		√	
M10D			√
M12F		√	
M13F		√	

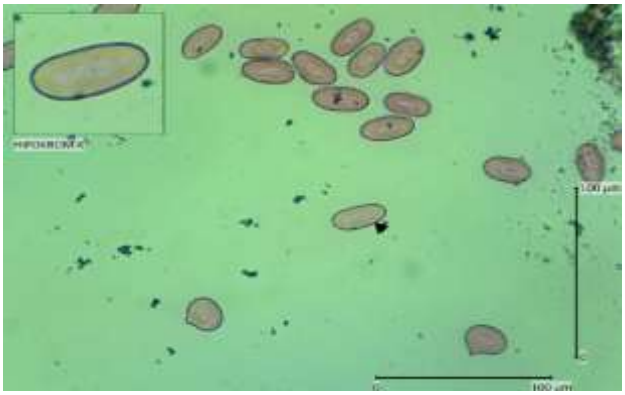

Figure 5. Hypochromic Erythrocyte Disorders from Trimeresurus insularis snakes on Bali Island with Diff Quick staining and 1000× magnification using immersion oil.

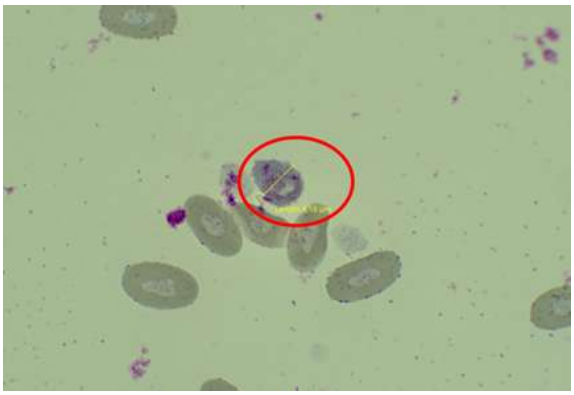

Figure 6. Basophils (red circles) from the snake *Trimeresurus insularis* on the island of Bali with 10% Giemsa staining and 1000× Magnification using immersion oil.

Basophil Cytomorphometry

Basophils from *T. insularis* snakes have various shapes ranging from round to oval, and can even have irregular shapes. The basophil nucleus is eccentric (not in the center of the cell) which is identified in the area of the cell membrane that appears violet in color and is hidden in the nucleus and is full of basophilic granules (Fig. 6).

In male *T. insularis* snakes, the length of the basophil cytoplasm is (6 ± 0.21 µm) with the width of the cytoplasm (4.67 ± 0.31 µm), while for the nucleus it has a length (3.26 ± 0.29 µm) with a width of (3.05 ± 0.29 µm). In female *T. insularis* snakes, the length of the basophil cytoplasm is (5.37 ± 0.48 µm) with the width of the cytoplasm (4.61 ± 0.54 µm), while for the nucleus it is long (3.24 ± 0.67 µm) with wide (2.86 ± 0.33 µm). The average basophil cell of *T. insularis* snakes has a cytoplasm length (5.72 ± 0.47 µm) with a cytoplasm width (4.64 ± 0.40 µm), while the nucleus has a length of (3.25 ± 0.46 µm). in width (2.96 ± 0.30 µm).

Based on the Anova test carried out, the results showed that the length of the cytoplasm of male and female *T. insularis* basophil cells was significantly different. Meanwhile, the width of the cytoplasm and,

the length and width of the nucleus were not significantly different.

The length of the basophil cytoplasm in male snakes (6 µm) is greater than in females (5.37 µm). When compared with the cytomorphometry of the basophils of *V. ammodytes* snakes which have cytoplasm diameter (10.21 ± 1.07 µm), nucleus length (8.56 ± 0.46 µm), and nucleus width (6.09 ± 0.52 µm) (Lisičić *et al*., 2013). The snake *T. insularis* has a smaller basophil size.

Heterophil Cytomorphometry

Heterophyl of the snake *T. insularis* have round cell shapes with granular and colorless cytoplasm or sometimes slightly acidophilic or basophilic. The nucleus in heterophils is located eccentrically (not exactly in the middle) with a partially ellipsoidal and basophilic shape (Fig. 7).

In male *T. insularis* snakes, the diameter of the heterophyll cytoplasm is (8 ± 1.19 µm), and the nucleus is long (3.69 ± 0.52 µm) and wide (2.59 ± 0.27 µm). In female *T. insularis* snakes, the diameter of the heterophyll cytoplasm is (8.72 ± 0.88 µm), while the nucleus is long (3.81 ± 0.79 µm) and wide (2.77 ± 0.32 µm). The average diameter of the cytoplasm of *T. insularis* snake heterophyll cells is (8.32 ± 1.07 µm), while the nucleus is long (3.74 ± 0.61 µm) and wide (2.67 ± 0.29 µm).

The results of the Anova test on cytoplasm diameter, length, and width of the nucleus showed that the results were not significantly different between male and female *T. insularis* snakes.

When compared with the cytomorphometry of the heterophyll snake *V. ammodytes* which has a cytoplasm diameter (16.87 ± 1.41 µm), nucleus length (7.97 ± 1.18 µm), and nucleus width (6.4 ± 0.99 µm) (Lisičić *et al*., 2013). The snake *T. insularis* has a smaller heterophyll size.

Lymphocytes Cytomorphometry

Lymphocytes from *T. insularis* snakes (Fig. 8) have irregularly shaped cytoplasm with basophilic properties and a large nucleus (which is basophilic).

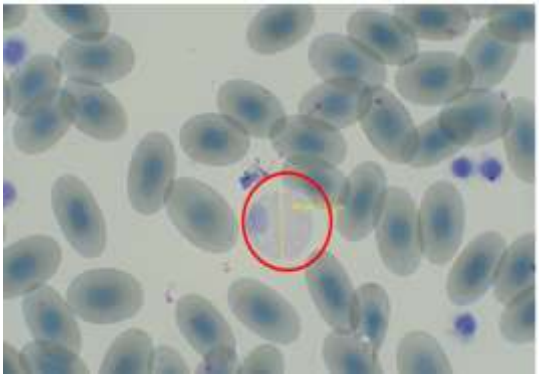

Figure 7. Heterophyll (red circles) from the snake *Trimeresurus insularis* on the island of Bali with 10% Giemsa staining and 1000× magnification using immersion oil.

In male *T. insularis* snakes, the diameter of the lymphocyte cytoplasm was (4.30 ± 0.87 µm), and the nucleus was (3.15 ± 0.87 µm). In female *T. insularis* snakes, the diameter of the lymphocyte cytoplasm was (4.61 ± 0.59 µm), while the diameter of the nucleus was (2.98 ± 0.66 µm). Mean cell morphometry *T. insularis* snake lymphocytes have a cytoplasm diameter of (4.24 ± 0.72 µm), while the nucleus diameter is (3.07 ± 0.74 µm) (Table 3).

The results of the Anova test carried out can be interpreted as all data not being significantly or significantly different, because they have a significance of > 0.05.When compared with the cytomorphometry of *V. ammodytes* snake lymphocytes which have a cytoplasm diameter (10.15 ± 2.46 µm), nucleus length (8.81 ± 1.46 µm), and nucleus width (7.27 ± 1.07 µm) (Lisičić et al., 2013). *T. insularis* snakes have smaller lymphocyte sizes.

Figure 8. Lymphocytes (red circles) from the snake *Trimeresurus insularis* on the island ofBali with 10% Giemsa staining and 1000× magnification using immersion oil.

Azurophil Cytomorphometry

Azurophil of the snake *T. insularis* have cells with a round to slightly oval shape with an eccentrically located nucleus. The azurophilic cytoplasm of the snake *T. insularis* appears to contain many azurophilic granules. In addition, azurophils can contain vacuoles in the cytoplasm (Fig. 9).

In male *T. insularis* snakes, the diameter of the azurophil cytoplasm is (7.92 ± 0.85 μm), while the nucleus is long (4.82 ± 0.48 μm) and wide (3.80 ± 0.55 μm). In female *T. insularis* snakes, the diameter of the azurophil cytoplasm is (6.98 ± 0.65 μm), while the nucleus is long (3.92 ± 0.83 μm) and wide (3.02 ± 0.49 μm). So the mean morphometry of *T. insularis* snake azurophil cells has a cytoplasmic diameter of (7.50 ± 0.88 μm), while the nucleus has a length of (4.42 ± 0.77 μm) and a width of (3.45 ± 0.77 μm). .64 μm).

Based on the Anova test carried out, it can be interpreted that all data is not significantly or significantly different, because it has a significance of >0.05. When compared with the cytomorphometry of the azurophil snake *V. ammodytes* which has a cytoplasm diameter (15.54 ± 3.71 μm), nucleus length (9.41 ± 0.85 μm), and nucleus width (7.81 ± 0.72 μm) (Lisičić *et al*., 2013). The snake *T. insularis* has a smaller azurophil size.

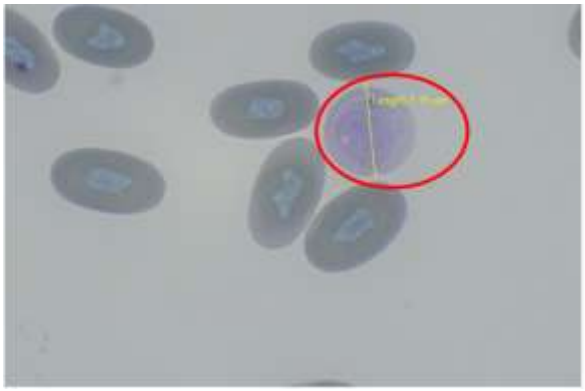

Figure 9. Azurophil (red circles) from the snake *Trimeresurus insularis* on the island of Bali with 10% Giemsa staining and 1000× magnification using immersion oil.

Table 3. *Trimeresurus insularis* Leukocytes morphometry

Variable	Cytomorphometry	N	Sex	Mean ± SD	Sig.
Basophil	Cytoplasm Length (μm)	5	Male	6,00 ± 0,21	0,031*
		4	Female	5,37 ± 0,48	
	Cytoplasm Width (μm)	5	Male	4,67 ± 0,31	0,827
		4	Female	4,61 ± 0,54	
	Nucleus Length (μm)	5	Male	3,26 ± 0,29	0,95
		4	Female	3,24 ± 0,67	
	Nucleus Width (μm)	5	Male	3,05 ± 0,29	0,393

		4	Female	2,86 ± 0,33	
Heterophil	Cytoplasmic Diameter (µm)	5	Male	8,00 ± 1,19	0,347
		4	Female	8,72 ± 0,88	
	Nucleus Length (µm)	5	Male	3,69 ± 0,52	0,796
		4	Female	3,81 ± 0,79	
	Nucleus Width (µm)	5	Male	2,59 ± 0,27	0,383
		4	Female	2,77 ± 0,32	
Lymphocytes	Cytoplasmic Diameter (µm)	5	Male	4,30 ± 0,87	0,801
		4	Female	4,16 ± 0,59	
	Nucleus Width (µm)	5	Male	3,15 ± 0,87	0,757
		4	Female	2,98 ± 0,66	
Azurophil	Cytoplasmic Diameter (µm)	5	Male	7,92 ± 0,85	0,111
		4	Female	6,98 ± 0,65	
	Nucleus Length (µm)	5	Male	4,82 ± 0,48	0,079
		4	Female	3,92 ± 0,83	
	Nucleus Width (µm)	5	Male	3,80 ± 0,55	0,063
		4	Female	3,02 ± 0,49	

Note: * = Significantly different (P < 0.05)

Platelets

Platelets have oval-shaped cytoplasm and a centrally located nucleus with strong basophilic properties. Platelets are also smaller in size compared to erythrocytes. Based on the observations made (Fig. 10) , an image was obtained of a cell from sample F2A which can be suspected to be a platelet from the snake *T. insularis*, because it has a morphology that matches the morphology of platelets from other viper snakes (*V. ammodytes*), namely having an oval-shaped cytoplasm and a nucleus located in the middle with strong basophilic properties (Lisičić *et al*., 2013).

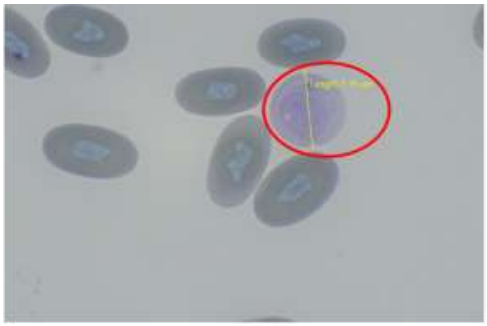

Figure 10. Platelets (red circles) from the snake *Trimeresurus insularis* on the island of Bali with 10% Giemsa staining and 1000× magnification using immersion oil.

CONCLUSION

The research findings on *T. insularis* snakes on the island of Bali can serve as a valuable data reference for their identification and blood morphometry. The study revealed significant differences in the width of the erythrocyte nucleus between male and female snakes, with males measuring 25.92 µm and females measuring 30.44 µm. Similarly, the length of the basophil cytoplasm varied significantly, with males measuring 6 µm and females measuring 5.37 µm. However, there were no significant differences in the cytomorphometry of heterophils, lymphocytes, and azurophylls between males and females. Notably, the study did not find any eosinophils in *T. insularis* snakes.

SUGGESTION

This research is focused on identifying the morphology of blood cells in *T. insularis* snakes on the island of Bali. However, the study used a small number of samples due to the susceptibility of *T. insularis* snakes to stress and their highly venomous nature. Therefore, further

research is needed to obtain a more comprehensive understanding of the blood cells of *T. insularis* snakes on the island of Bali through complete blood tests. In the future, it is recommended to consider an intracardiac blood collection procedure to obtain a larger number of blood samples. This will enable researchers to cover a wider geographical area and obtain a more comprehensive blood picture.

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