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Morphology of Erythrocytes and Leukocytes of the Lesser Sunda Islands Pit Viper (*Trimeresurus insularis*): The Green Coloration from Bali Island and the Blue Coloration from Komodo Island

Morfologi Eritrosit dan Leukosit Ular Beludak Sunda (Trimeresurus insularis) yang Berwarna Hijau Asal Pulau Bali dan Berwarna Biru Asal Pulau Komodo

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Abstrak

Indonesia merupakan negara beriklim tropis yang memiliki keanekaragaman herpetofauna yang tinggi, salah satunya adalah ular berbisa endemik *Trimeresurus insularis*. Spesies ini tersebar luas melintasi Pulau Jawa, Bali, Kepulauan Nusa Tenggara, hingga Pulau Wetar. Menariknya, ular ini menampilkan variasi warna tubuh geografis yang sangat khas: populasi yang ditemukan di Pulau Bali umumnya berwarna hijau, sedangkan populasi dari Pulau Komodo didominasi warna biru, meskipun keduanya tetap memiliki ekor berwarna merah. Penelitian ini bertujuan untuk mengkaji morfologi dan morfometri eritrosit serta leukosit pada kedua populasi tersebut guna memahami potensi adaptasi fisiologis dan lingkungan. Preparat ulas darah dari enam individu *T. insularis* sehat (tiga spesimen hijau asal Bali dan tiga spesimen biru asal Komodo) dianalisis secara mikroskopis. Dimensi sel dan nukleus diukur menggunakan perangkat lunak ImageJ, dan data dianalisis menggunakan uji *Independent-Samples T-Test*. Hasil penelitian menunjukkan tidak ada perbedaan bentuk atau morfologi pada sel darah dari kedua wilayah. Namun, terdapat perbedaan yang signifikan secara morfometrik pada panjang dan lebar nukleus eritrosit, serta pada diameter sitoplasma dan nukleus limfosit. Parameter lainnya, termasuk dimensi keseluruhan eritrosit serta ukuran heterofil, basofil, eosinofil, dan monosit, tidak menunjukkan perbedaan yang bermakna. Variasi morfometrik spesifik pada nukleus eritrosit dan limfosit ini mengindikasikan secara kuat bahwa faktor lingkungan lokal sangat memengaruhi profil hematologi *T. insularis*. Kondisi ekologis yang sangat kontras—khususnya lingkungan Bali yang lembap dan kaya sumber daya dibandingkan dengan habitat Komodo yang lebih panas dan kering—kemungkinan besar mendorong adaptasi metabolik, respirasi, dan imunologis yang spesifik untuk kelangsungan hidup populasi.

Kata kunci: *blood*; morfologi eritrosit; morfologi leukosit; Pulau Bali; Puluau Komodo; *Trimeresurus insularis*

Abstract

Indonesia is a tropical country endowed with a highly diverse herpetofauna, including the endemic venomous pit viper, *Trimeresurus insularis*. This species is widely distributed across Java, Bali, the Lesser Sunda Islands, and Wetar. Interestingly, *T. insularis* exhibits distinct geographic color variations: populations found in Bali are typically green, whereas those from Komodo Island are predominantly blue, though both share a characteristic red tail. This study aims to examine the morphology and morphometry of erythrocytes and leukocytes in these two distinct populations to understand potential physiological and environmental adaptations. Blood

smear preparations from six healthy *T. insularis* individuals (three green specimens from Bali and three blue specimens from Komodo) were microscopically analyzed. Cell and nuclear dimensions of both erythrocytes and leukocytes were measured using ImageJ software. The morphometric data were statistically analyzed using the Independent-Samples T-Test. The results indicated no structural or morphological differences in the blood cells between the two localities. However, significant morphometric differences were observed in the length and width of the erythrocyte nucleus, as well as in the cytoplasmic and nuclear diameters of lymphocytes. Other parameters, including overall erythrocyte dimensions and the sizes of heterophils, basophils, eosinophils, and monocytes, showed no significant differences. These specific morphometric variations strongly suggest that localized environmental factors profoundly influence the hematological profile of *T. insularis*. The contrasting ecological conditions—specifically Bali's humid, resource-abundant environment compared to Komodo's hotter, arid habitat—likely drive targeted metabolic, respiratory, and immunological adaptations essential for population survival.

Keywords: Bali Island; erythrocyte morphology; Komodo Island; leukocyte morphology; *Trimeresurus insularis*

Introduction

Indonesia has a rich diversity of herpetofauna, including approximately 600 reptile species (LIPI, 2021). Among these, around 350–370 species are identified as snakes, with 77 species classified as venomous. These venomous snakes pose significant health risks to humans, particularly in areas adjacent to their natural habitats. One venomous snake of particular concern is *Trimeresurus insularis*, a species endemic to the Sunda Islands of Indonesia.

Trimeresurus insularis is known as the red-tailed green pit viper or "lipi gadang" in Bali, and the Sunda pit viper in Indonesia. This snake is characterized by varying body colors depending on its region. *T. insularis* found on Bali Island is generally green, while those found on Komodo Island are blue. However, both share the same distinctive red coloration on their tails. These differences in body color are thought to be influenced by environmental variations between Bali and Komodo Islands. Bali has an average temperature of 26.4–28.9°C and high rainfall, supporting lush tropical forests. In contrast, Komodo Island has a drier environment with temperatures ranging from 33–35°C and vegetation dominated by grasslands and savannas.. Environmental factors such as temperature, humidity, and food availability can influence color variation within a single species of snake (Jones *et al.,* 2022).

Hematological evaluation in reptiles is a complex process influenced by numerous variables. External factors such as season, ambient temperature, habitat, diet, disease, and stress can significantly affect blood parameters (Campbell, 2006). Additionally, internal factors—including species, sex, age, and physiological condition—play crucial roles in shaping a reptile's hematological profile. This variability can influence leukocyte distribution and the immune response to pathogens (Nardini, 2013). Understanding reptile blood morphology is also essential for evaluating the health status of ecosystems in the wild (Stacy *et al.,* 2011).

This study aims to examine potential differences in the morphology and morphometry of leukocytes in *Trimeresurus insularis* individuals with green coloration from Bali Island and those with blue coloration from Komodo Island. This investigation is significant, as differences in geographic location are suspected to influence blood morphology within a single species. The results of this study are expected to contribute to the fields of herpetology and reptile health, and to support future efforts in the conservation and habitat management of this species.

Materials and Methods

Ethical approval

This study was approved the ethical commission of Faculty of Veterinary Medicine, Udayana University with Letter No. B/245/UN14.2.9/PT.01.04/2024.

Study Period and Location

This study was conducted from November 2024 to January 2025. Blood sampling was carried out in Badung and Denpasar. Blood smear examinations were performed at the Veterinary Immunology Laboratory, Faculty of Veterinary Medicine, Udayana University.

Experimental Design

This study is a descriptive observational study involving six *Trimeresurus insularis* snakes. The samples consisted of three snakes originating from

Bali Island and three snakes from Komodo Island, all owned by hobbyists residing in Denpasar and Jimbaran. The *T. insularis* specimens originating from Komodo Island had been maintained in captivity by their owners for at least one year. Prior to blood collection, each snake underwent a clinical examination to ensure the absence of inflammation, infection, and other physical conditions, confirming that the samples were healthy. After the clinical examination confirmed normal health status, blood was collected, and blood smear samples were prepared for further analysis. The blood smear preparations were observed under a microscope, photographed using the Optilab Viewer device, and cell measurements were performed using ImageJ software.

Evaluation

Observation of the blood smear preparations was carried out using an Olympus CX33 microscope at 1000× magnification with the aid of immersion oil. Cell images were captured using the Optilab Viewer for documentation and further analysis. The morphology of erythrocytes and leukocytes was examined in detail, including their shape, color, and characteristics of the cytoplasm and nucleus. Subsequently, the diameters of the cells and nuclei were measured using ImageJ software running on a computer. For each blood smear sample, three cells with the best visualization were selected for analysis. The parameters observed included the morphology and morphometry of erythrocytes as well as six types of leukocytes: heterophils, basophils, eosinophils, lymphocytes, monocytes, and azurophils.

Blood Samples Collection Procedure

Blood was collected from the coccygeal vein using a 1 ml syringe equipped with a 26-gauge (26G) needle. The process began by restraining the snake, focusing primarily on securing the head. The ventral side of the snake's tail was disinfected with 70% ethanol. The needle was inserted beneath the scales approximately fifteen scales distal from the cloaca at a 45° angle to access the coccygeal vein for blood withdrawal. After blood collection, the puncture site was cleansed again with 70% ethanol, and the snake was returned to its enclosure.

Preparation of Blood Smears

Three blood smear preparations were made from each individual to increase the data volume, improve accuracy, and minimize technical errors. The prepared blood smears were air-dried for 7 minutes, then fixed with methanol for 5 minutes, followed by an additional 7 minutes of air drying. Subsequently, the smears were stained using Diff-Quik staining, rinsed with distilled water, dried, and stored in a slide box until ready for microscopic examination.

Morphological and Morphometric Observation

The stained blood smears were observed using an Olympus CX33 microscope at 1000× magnification, with immersion oil applied to enhance image clarity. Cell images were captured using the Optilab Viewer device for documentation and further analysis. The morphology of erythrocytes and leukocytes was examined in detail, including cell shape, coloration, and the characteristics of the cytoplasm and nucleus. The diameters of the cells and nuclei were measured using ImageJ software on a computer. From each blood smear sample, three cells with the best visual quality were selected for analysis. Observational parameters included the morphology and morphometry of erythrocytes and six types of leukocytes: heterophils, basophils, eosinophils, lymphocytes, monocytes, and azurophils.

Data Analysis

Cell morphology data were analyzed descriptively, while the morphometric data of blood cells between Trimeresurus insularis—the green coloration from Bali Island and the blue coloration from Komodo Island—were analyzed using the Independent Samples T-Test with SPSS software.

Results and Discussion

Erythrocytes

The erythrocyte morphology of *Trimeresurus insularis* with green coloration from Bali Island (Figure 1A) and blue coloration from Komodo Island (Figure 1B) showed no differences. The cytoplasm in both populations appeared pink to dark purple and exhibited an ellipsoid shape. The nucleus was dark purple, centrally located, and typically ellipsoid, although some cells displayed variations in the form of round or irregular shapes. These variations in erythrocyte nuclear morphology were observed in both the green *T. insularis* from Bali and the blue *T. insularis* from Komodo.

Table 1. Results of Cell and Nucleus Length and Width Diameters of *T. insularis* Erythrocytes

Erythrocytes	Green - Bali Island (n=3)		Blue - Komodo Island (n=3)		Sig. (Two-sided p)
	Mean (µm)	Std. Deviation	Mean (µm)	Std. Deviation	
Cell Length Diameter	21.86	1.14	20.57	1.19	0.032
Cell Width Diameter	12.68	0.87	12.27	0.60	0.262
Nucleus Length Diameter	8.76	0.37	7.97	0.55	0.002*
Nucleus Width Diameter	4.70	0.27	4.23	0.30	0.003*

Legend: **n**: Number of Individuals
*: Significant

The erythrocytes of green *Trimeresurus insularis* from Bali Island had a mean cell length and width of (21.86 ± 1.14 µm) and (12.68 ± 0.87 µm), respectively, while the mean nuclear length and width were (8.76 ± 0.37 µm) and (4.70 ± 0.27 µm), respectively. In contrast, the erythrocytes of blue *T. insularis* from Komodo Island had a mean cell length and width of (20.57 ± 1.19 µm) and (12.27 ± 0.60 µm), respectively, while the mean nuclear length and width were (7.97 ± 0.55 µm) and (4.23 ± 0.30 µm), respectively. The erythrocyte morphological data (Table 1 and Figure 1) showed significant differences in nuclear length (p = 0.002) and nuclear width (p = 0.003), while no significant differences were found in cell length (p = 0.032) and cell width (p = 0.262).

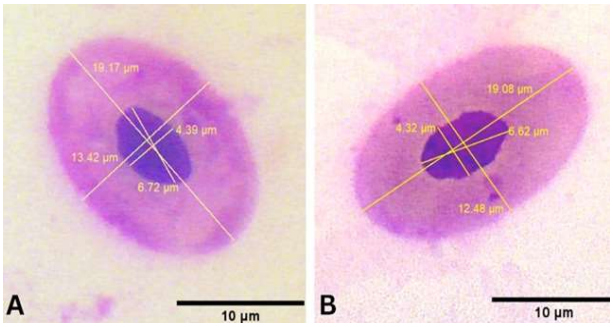


Figure 1. Erythrocytes of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

Leucocyte

Based on Table 2, the statistical analysis results indicated a significant difference in the cytoplasmic diameter of lymphocytes (P < 0.01), whereas no significant differences were observed in heterophils, basophils, eosinophils, azurophils, and monocytes (P > 0.01).

Based on Table 3, the statistical analysis results showed a significant difference in nuclear diameter (P < 0.01), while no significant differences were found in heterophils, basophils, eosinophils, azurophils, and monocytes (P > 0.01).

Heterophil

Heterophils of Trimeresurus insularis with green coloration from Bali Island (Figure 2A) and blue coloration from Komodo Island (Figure 2B) showed no morphological differences. Under Diff-Quik staining, the cytoplasm of both appeared colorless, round in shape, and contained purple granules. The nucleus was dark purple, eccentrically located, and ranged from ellipsoid to round in shape. Variations in heterophil morphology were observed

Table 2. Results of *T. insularis* Leukocyte Cell Diameter

Leukocyte Cell Diameter	Green - Bali Island (n=3)		Blue - Komodo Island (n=3)		Sig. (Two-sided p)
	Mean (µm)	Std. Deviation	Mean (µm)	Std. Deviation	
Heterophil	19.45	1.20	19.31	1.02	0.787
Basophil	13.13	1.10	12.75	0.96	0.443
Eosinophil	16.24	0.99	15.98	0.77	0.541
Azurophil	16.53	0.91	15.69	1.09	0.094
Lymphocyte	9.87	0.54	8.69	0.81	0.002*
Monocyte	19.55	1.34	18.61	1.06	0.117

Legend: n: Number of Individuals
*: Significant

Table 3. Results of *T. insularis* Nucleus Diameter

Leukocyte Nucleus Diameter	Green - Bali Island (n=3)		Blue - Komodo Island (n=3)		Sig. (Two-sided p)
	Mean (µm)	Std. Deviation	Mean (µm)	Std. Deviation	
Heterophil	7.12	0.46	7.12	0.35	0.996
Basophil	8.87	0.95	9.35	0.54	0.208
Eosinophil	8.48	0.52	8.35	0.54	0.615
Azurophil	8.63	0.74	8.77	1.11	0.754
Lymphocyte	7.85	0.46	6.84	0.75	0.003*
Monocyte	10.12	0.92	9.79	1.28	0.531

Legend: n: Number of Individuals
*: Significant

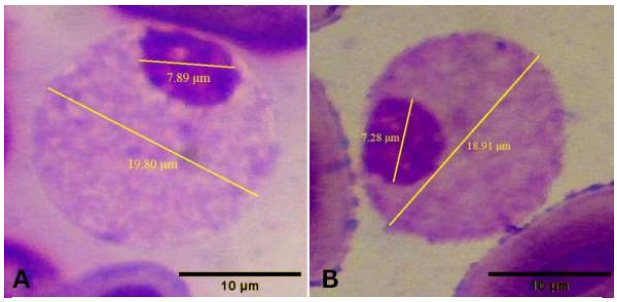


Figure 2. Heterophils of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

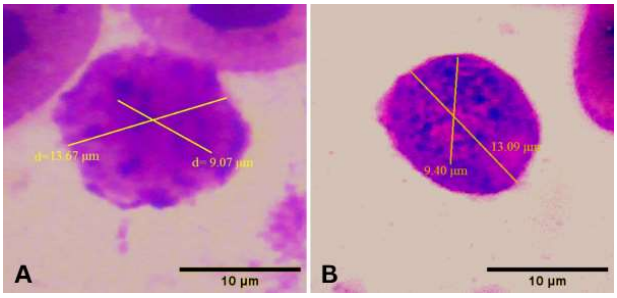


Figure 3. Basophils of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

in both the green T. insularis from Bali and the blue T. insularis from Komodo.

Morphometric analysis of heterophils in T. insularis revealed no significant differences in cell diameter (Table 2) with p = 0.787, nor in nuclear diameter (Table 3) with p = 0.996. In green T. insularis from Bali, heterophils had a mean cell diameter of (19.45 ± 1.20 µm) and a mean nuclear diameter of (7.12 ± 0.46 µm). In blue T. insularis from Komodo, heterophils had a mean cell diameter of (19.31 ± 1.02 µm) and a mean nuclear diameter of (7.12 ± 0.35 µm).

Basophil

Basophils of Trimeresurus insularis with green coloration from Bali Island (Figure 3A) and blue coloration from Komodo Island (Figure 3B) showed no morphological differences. Under Diff-Quik staining, the cytoplasm of both appeared acidophilic, ranging from round to irregular in shape, and contained basophilic granules. The nucleus was purple, eccentrically located, round to irregular in shape, and often obscured by basophilic granules. Morphological variations in basophils were observed in both the green T. insularis from Bali and the blue T. insularis from Komodo.

According to the data analysis, basophils of T. insularis showed no significant differences in cell diameter (p = 0.443) (Table 2) or nuclear diameter (p = 0.208) (Table 3). In the green T. insularis from Bali, basophils had a mean cell diameter of (13.13 ± 1.10 µm) and a mean nuclear diameter of (8.87 ± 0.95 µm). In contrast, in the blue T. insularis from Komodo, basophils had a mean cell diameter of (12.75 ± 0.96 µm) and a mean nuclear diameter of (9.35 ± 0.54 µm).

Eosinophil

Eosinophils in Trimeresurus insularis with green coloration from Bali Island (Figure 4A) and blue coloration from Komodo Island (Figure 4B) showed no morphological differences. Under Diff-Quik staining, the cytoplasm of both appeared colorless, ranging from round to irregular in shape, and contained acidophilic granules. The nucleus was also acidophilic, eccentrically located, round to irregular in shape, and partially obscured by acidophilic granules. Morphological variations in eosinophils were observed in both the green T. insularis from Bali and the blue T. insularis from Komodo.

Based on the data analysis, the morphometric measurements of eosinophils in T. insularis showed

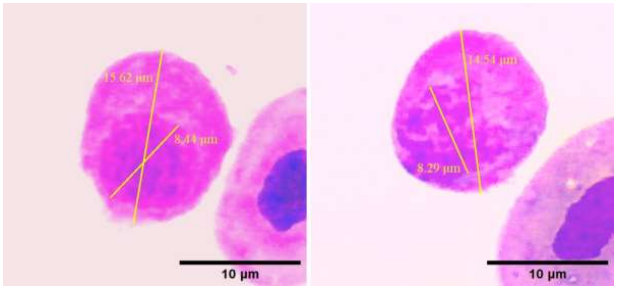


Figure 4. Eosinophils of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

no significant differences in cell diameter (p = 0.541) (Table 2) or nuclear diameter (p = 0.615) (Table 3). In the green T. insularis from Bali, eosinophils had a mean cell diameter of (16.24 ± 0.99 µm) and a mean nuclear diameter of (8.48 ± 0.52 µm). In the blue T. insularis from Komodo, eosinophils had a mean cell diameter of (15.98 ± 0.77 µm) and a mean nuclear diameter of (8.35 ± 0.54 µm).

Azurophil

Azurophils can be identified by the presence of characteristic colorless vacuoles in their cytoplasm (Limanto, 2024). In *Trimeresurus insularis* with green coloration from Bali Island (Figure 5A) and blue coloration from Komodo Island (Figure 5B), no morphological differences in azurophils were observed. Under Diff-Quik staining, the cytoplasm of both appeared purple and round in shape, while the nucleus was acidophilic, ranged from round to irregular in shape, eccentrically located, and partially covered by basophilic granules. Morphological variations in azurophils were observed in both the green *T. insularis* from Bali and the blue *T. insularis* from Komodo.

Based on the data analysis, the morphometric measurements of azurophils in *T. insularis* showed no significant differences in cell diameter (p = 0.094) (Table 2) or nuclear diameter (p = 0.754) (Table 3).

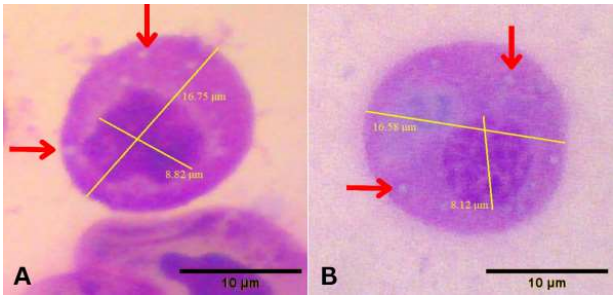


Figure 5. Azurophils of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

In green *T. insularis* from Bali, azurophils had a mean cell diameter of (16.53 ± 0.91 µm) and a mean nuclear diameter of (8.63 ± 0.25 µm). In blue *T. insularis* from Komodo, azurophils had a mean cell diameter of (15.69 ± 1.09 µm) and a mean nuclear diameter of (8.77 ± 0.37 µm).

Lymphocytes

Lymphocytes in Trimeresurus insularis with green coloration from Bali Island (Figure 6B) and blue coloration from Komodo Island (Figure 6A) showed no morphological differences. Under Diff-Quik staining, the cytoplasm of both appeared purple and round in shape, while the nucleus was purple, large, eccentrically located, and ranged from round to irregular in shape. Morphological variations in lymphocytes were observed in both the green T. insularis from Bali and the blue T. insularis from Komodo.

Based on the data analysis, the morphometric measurements of lymphocytes showed significant differences in both cell diameter (p = 0.002) (Table 2) and nuclear diameter (p = 0.003) (Table 3). In green T. insularis from Bali, lymphocytes had a mean cell diameter of (9.87 ± 0.54 µm) and a mean nuclear diameter of (7.85 ± 0.46 µm). In blue T. insularis from Komodo, lymphocytes had a mean cell diameter of (8.69 ± 0.81 µm) and a mean nuclear diameter of (6.84 ± 0.75 µm).

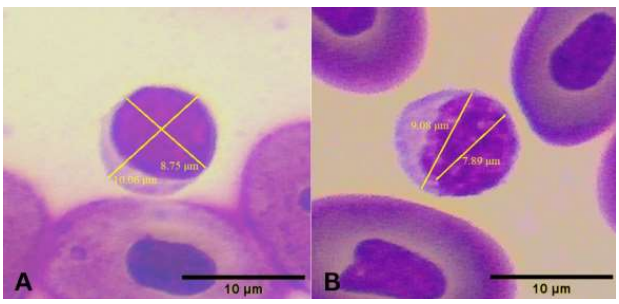


Figure 6. Lymphocytes of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

Monocytes

Monocytes of *Trimeresurus insularis* with green coloration from Bali Island (Figure 7A) and blue coloration from Komodo Island (Figure 7B) showed no morphological differences. Under Diff-Quik staining, the cytoplasm of both appeared colorless and round in shape, while the nucleus was purple, ranging from ellipsoid to irregular in shape, and centrally located. Morphological variations in monocytes were observed in both the green *T.*

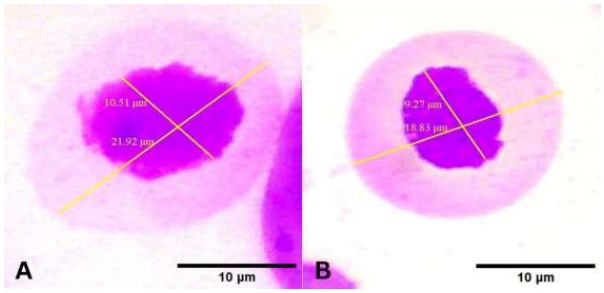


Figure 7. Monocytes of T. insularis, with green coloration from Bali Island (A) and blue coloration from Komodo Island (B), 1000× magnification, Diff-Quik staining.

insularis from Bali and the blue *T. insularis* from Komodo.

Based on the data analysis, monocytes showed no significant differences in cell diameter ($p = 0.117$) (Table 2) or nuclear diameter ($p = 0.531$) (Table 3). In green *T. insularis* from Bali, monocytes had a mean cell diameter of (19.55 ± 1.34 µm) and a mean nuclear diameter of (10.12 ± 0.92 µm). In blue *T. insularis* from Komodo, monocytes had a mean cell diameter of (18.61 ± 1.06 µm) and a mean nuclear diameter of (9.79 ± 1.28 µm).

Result and Discussion

The morphological observation of *T. insularis* erythrocytes, both the green-colored individuals from Bali and the blue-colored individuals from Komodo Island, showed no distinct differences when stained with Diff-Quik. The erythrocytes of *T. insularis* are ellipsoid in shape, with cytoplasm ranging from pink to dark purple. Their nuclei are densely stained purple, centrally located, and ellipsoid in shape, although some variations such as round or irregular nuclei were also observed.

The variation in nuclear shape in the erythrocytes of *T. insularis* from both localities may be attributed to physiological and environmental factors that affect hematopoiesis and red blood cell maturation. A key factor is cellular plasticity in reptiles; unlike mammalian erythrocytes, reptilian erythrocytes retain their nuclei throughout their lifespan (Stacy *et al.,* 2011). Furthermore, erythropoiesis in reptiles is continuous, resulting in a circulation that contains erythrocytes at various developmental stages—from young cells with large nuclei to mature cells with condensed, centralized nuclei (Campbell, 2015). Other influencing factors include stress, metabolic rate, and environmental adaptations. Snakes living in low-oxygen environments tend to have erythrocytes with larger nuclei to enhance oxygen transport efficiency (Dissanayake et al., 2017). Additionally, the presence of morphologically altered or deformed cells may indicate regeneration processes following injury or infection (Dessauer, 1970). Hence, nuclear variation in *T. insularis* erythrocytes can be considered a natural physiological adaptation to internal and external conditions. This aligns with Dissanayake *et al.* (2017), who stated that irregularly shaped nuclei are commonly found in snake erythrocytes.

Morphometric statistical analysis of the erythrocytes revealed significant differences in cell length ($p < 0.05$), and highly significant differences in nuclear length and width ($p < 0.01$), while no significant difference was found in cell width ($p > 0.05$). This indicates that nuclear size varies between *T. insularis* populations from different localities. According to Lisičić (2013), the erythrocytes of *Vipera ammodytes* measure 18.36 ± 1.21 µm in length, 12.65 ± 0.67 µm in width, with nuclei measuring 8.04 ± 0.73 µm in length and 4.69 ± 0.44 µm in width. Comparatively, *T. insularis* erythrocytes are larger in size.

The distinct phenotypic and hematological variations observed between the T. insularis populations are fundamentally intertwined with the contrasting climatic and ecological conditions of their respective habitats. Bali Island exhibits a wet tropical climate with high annual rainfall that supports lush, dense tropical forests, while maintaining a relatively stable and moderate average annual temperature ranging from 26.4°C to 28.9°C. In stark contrast, the Komodo region in West Manggarai Regency features a significantly arid environment dominated by savannas and open grasslands, where dry-season ambient temperatures frequently soar to 33°C–35°C, creating a notably harsher microclimate. These profound differences in temperature, humidity, and vegetation density serve as the primary ecological drivers for the physiological, morphological, and metabolic adaptations seen in the blood profiles of these two snake populations.

The observed difference in erythrocyte size between the green-colored individuals from Bali and the blue-colored individuals from Komodo—particularly the larger nuclei found in the Bali population—may be attributed to both physiological and environmental factors. One notable factor is the variation in ambient oxygen availability between habitats. Bali, characterized by denser vegetation and a more humid climate, likely provides higher oxygen levels compared to the drier, rockier Komodo Island.

In low-oxygen environments, organisms often develop larger erythrocytes with prominent nuclei to enhance oxygen-carrying capacity. Conversely, *T. insularis* from Komodo may have adapted by producing smaller erythrocytes, a morphological adjustment that facilitates more efficient oxygen diffusion and supports metabolic demands under challenging environmental conditions. This is consistent with the statement by Arikan and Çiçek (2014), who noted that erythrocyte size and nuclear morphology in reptiles may vary in response to ecological and physiological pressures.

Environmental temperature differences may also influence erythrocyte size. Higher temperatures on Komodo Island may accelerate the snakes' metabolism, leading to faster erythropoiesis and smaller erythrocytes. Conversely, the more humid and stable climate of Bali may support slower erythropoiesis and the development of erythrocytes with larger nuclei. This is consistent with findings by Stacy *et al.* (2011), who noted that reptiles living in hotter environments tend to have smaller erythrocytes as an adaptation to increased metabolic rates.

From a genetic perspective, natural selection may also shape erythrocyte size across populations. For example, active metabolic demands and habitat pressures have been shown to influence erythrocyte morphology in reptiles — small erythrocytes are often associated with high body temperature and active foraging, while larger erythrocytes are favored in oxygen-poor conditions (Bury, Kratochvíl & Starostová, 2023). Thus, the *T. insularis* population in Bali may have experienced different selective pressures compared to those in Komodo, leading to genetic regulation of erythropoiesis that favors smaller red blood cells in Komodo individuals. Furthermore, differences in diet and nutrient availability may affect erythropoiesis. The more diverse and abundant food resources in Bali could support the production of erythrocytes with larger nuclei due to greater nutrient availability, whereas the limited food supply in Komodo may favor more energy-efficient erythropoiesis, resulting in smaller erythrocytes (Urry *et al.,* 2018).

These differences in erythrocyte size suggest potential variations in oxygen transport efficiency and physiological adaptation between the two populations. Snakes in Bali may benefit from transporting more oxygen per erythrocyte, enhancing endurance during high activity. In contrast, Komodo snakes with smaller erythrocytes may exhibit more efficient energy metabolism to survive in harsher environments with limited resources. Therefore, these variations reflect population-specific adaptations to local geographic and ecological conditions.

In terms of leukocyte morphology observed using Diff-Quik staining, *T. insularis* from both Bali (green-colored) and Komodo (blue-colored) showed variation in cell and nuclear shape, but no notable difference in leukocyte types was observed. Statistical analysis indicated that only lymphocytes exhibited significant differences in both cell and nuclear diameter. Specifically, lymphocytes from the Bali population were larger than those from Komodo. Other leukocytes—heterophils, basophils, eosinophils, azurophils, and monocytes—showed no significant differences in cell or nuclear dimensions.

According to Lisičić et al. (2013), *T. insularis* heterophils are larger than those of *V. ammodytes* (16.87 ± 1.41 μm). Similarly, basophils of *T. insularis* are larger than those of *V. ammodytes* (10.21 ± 1.07 μm), and the same holds true for azurophils (15.54 ± 3.71 μm). Conversely, *T. insularis* eosinophils are smaller than those of *V. ammodytes* (17.3 ± 1.36 μm), and *T. insularis* lymphocytes are also smaller than *V. ammodytes* lymphocytes (10.15 ± 2.46 μm).

Variations in leukocyte morphology in reptiles—especially snakes—arise due to environmental adaptation, differences in immune function, and cell development stages. Reptilian leukocytes display diverse morphology: heterophils are oval or round with large granules, eosinophils are elongated with dense granules, and monocytes are irregular with broad cytoplasm (Stacy *et al.,* 2011). Such diversity is tied to specific immune functions—e.g., heterophils change shape during phagocytosis, and lymphocytes enlarge and morphologically change upon immune activation (Campbell, 2015). Morphological changes in leukocytes can indicate health status, with larger and more variable forms suggesting active immune response, whereas abnormal shapes may reflect physiological stress or pathological conditions like infection or malnutrition (Dissanayake *et al.,* 2017).

Leukocyte size in snakes also varies significantly, influenced by physiological, metabolic, and environmental factors. Immature leukocytes tend to be larger due to extensive cytoplasm and uncondensed nuclei, while mature leukocytes become smaller as they specialize functionally (Dessauer, 1970). Environmental conditions such as temperature and nutrition also play roles: lower

temperatures slow metabolism, leading to slower leukocyte production and larger cells, while stress may result in smaller leukocytes due to accelerated maturation (Campbell, 2015). Thus, snakes in hotter environments tend to have smaller leukocytes due to faster metabolism and early maturation, while those in cooler habitats may have larger leukocytes due to slower development (Stacy *et al.,* 2011).

This study revealed significant differences in lymphocyte size: *T. insularis* from Bali (green-colored) had larger lymphocytes, whereas those from Komodo (blue-colored) had smaller ones. These differences are likely influenced by environmental factors and adaptation to geographic conditions. Bali's humid climate and richer food sources may support more stable metabolism and the production of larger lymphocytes. Conversely, Komodo's dry and extreme climate may induce physiological stress and elevate glucocorticoid hormones, leading to smaller leukocytes as a metabolic adaptation (Dessauer, 1970).

Geographic conditions and food availability also contribute to differences in leukocyte size. Snakes from Bali, with greater food abundance, can maintain optimal nutrition for producing larger leukocytes. Meanwhile, Komodo snakes must adapt to limited food availability and a drier ecological setting, likely resulting in different metabolic strategies for leukocyte production and maturation (Stacy *et al.,* 2011). These differences imply that Bali snakes may mount faster and stronger immune responses, while Komodo snakes might rely on more energy-efficient immune strategies with smaller but still functional lymphocytes. Hence, this study demonstrates that geographic and environmental factors significantly influence leukocyte morphology—especially lymphocytes—in *T. insularis*.

Finally, the observed morphological variation in erythrocytes and leukocytes of *T. insularis* may also be affected by human error during sampling, slide preparation, and microscopic analysis. Fixation and staining errors—such as inconsistent immersion time or pH imbalance—can alter cell staining intensity (Stacy *et al.,* 2011). Technical issues during slide making, such as smear thickness and applied pressure, may cause cell deformation unrelated to physiological factors (Campbell, 2015). Moreover, microscope quality, lighting, and observer interpretation can introduce inconsistencies in analysis. Therefore, standardized and consistent methods are crucial to minimize human-induced bias.

Conclusion

The erythrocyte morphology of green *Trimeresurus insularis* from Bali Island and blue *T. insularis* from Komodo Island showed no differences in cell and nuclear shape or structure. However, significant morphometric differences were observed in the nuclear length and width. In contrast, no morphometric differences were found in the cell length and width.

The leukocyte morphology of green *T. insularis* from Bali Island and blue *T. insularis* from Komodo Island also showed no differences in cell and nuclear shape or structure. Nevertheless, significant morphometric differences were observed in the cell and nuclear diameters of lymphocytes. Meanwhile, no morphometric differences were found in the cell and nuclear diameters of heterophils, basophils, eosinophils, azurophils, and monocytes.

These statistically significant variations in erythrocyte nuclear dimensions and lymphocyte sizes strongly indicate that environmental factors play a critical role in shaping the hematological profiles of *T. insularis.* The contrasting ecological conditions—specifically the cooler, humid, and resource-rich tropical environment of Bali versus the hotter, arid, and resource-limited savanna of Komodo—likely drive distinct physiological demands. Consequently, these hematological differences reflect population-specific metabolic, respiratory, and immunological adaptations required to survive and thrive in their respective native habitats.

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