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Research Article

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Comparison of Leukocyte Profiles and Cytomorphometry in Wild-Caught and Captive Morph Reticulated Pythons (*Malayopython Reticulatus*)

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ABSTRACT

Background: Indonesia, with its high biodiversity, is home to the reticulated python (*Malayopython reticulatus*), one of Southeast Asia's endemic reptile species. The health status of this species can be influenced by environmental conditions, hydration, diet, and stress, particularly when comparing wild-caught individuals and captive morphs.

Aims: This study aimed to compare total leukocyte counts, leukocyte differentials, and leukocyte morphometric characteristics between wild-caught and captive morph *M. reticulatus.*

Methods: Blood samples were collected from wild-caught and captive morph reticulated pythons. Total leukocyte counts and leukocyte differentials, including heterophils, lymphocytes, monocytes, and azurophils, were evaluated. Morphometric analysis was conducted to measure cytoplasmic and nuclear diameters of leukocyte cells. Statistical analysis was performed to determine differences between the two groups.

Results: The average total leukocyte counts in wild-caught and captive morph pythons were 11.20 × 10^3/µL and 8.50 × 10^3/µL, respectively. Mean heterophil counts were 3.23 × 10^3/µL in wild-caught and 1.99 × 10^3/µL in captive morphs, while lymphocyte counts were 2.95 × 10^3/µL and 2.24 × 10^3/µL. Monocyte counts were 3.28 × 10^3/µL and 2.88 × 10^3/µL, and azurophil counts were 1.75 × 10^3/µL and 1.38 × 10^3/µL. No statistically significant differences were observed in total leukocyte counts or leukocyte differentials. However, significant differences were found in the cytoplasmic diameter of heterophils and lymphocytes, as well as in the nuclear diameter of all leukocyte types, which were larger in wild-caught pythons.

Conclusion: Although leukocyte counts were comparable, wild-caught *M. reticulatus* exhibited larger leukocyte cell dimensions, suggesting physiological differences potentially associated with environmental and stress-related factors.

INTRODUCTION

The reticulated python is one of Indonesia's endemic snake species, known for its beautiful colors and patterns. The stunning skin of this snake has become an attraction for exotic animal enthusiasts globally. *M. reticulatus* continues to be crossbred to produce new skin variations called morphs. Unfortunately, these breeding efforts have led to the emergence of certain genetic diseases.

The hematology profile of *M. reticulatus* has been studied previously. Vet Sulham Sunusi found differences in the total leukocyte count between male and female snakes. However, the leukocyte profile of wild-caught and captive morph *M. reticulatus* has not been investigated before. A study in 2012 by Bryant *et al.* also mentioned that factors such as season, temperature, humidity, hydration, and diet can influence the hematology profile of snakes.

By conducting an examination and evaluation of the leukocyte count and cytomorphometry, the researcher hopes to compare and conclude the immune response capabilities of wild-caught and captive morph *M. reticulatus*. This will also help assess stress levels and the susceptibility to diseases. The data obtained can be used as a reference in the future and can help design appropriate care strategies for *M. reticulatus*.

There are differences between mammalian and reptilian blood. Leukocytes in reptiles are classified into granulocytes and agranulocytes. Granulocytes include heterophils, eosinophils, basophils, and azurophils, while agranulocytes include lymphocytes and monocytes.

Each type of leukocyte in reptiles has distinct functions and morphologies. Heterophils, or neutrophils in mammals, are 15–25 µm in size and are responsible for bacterial infections, as well as being indicators of an individual's stress level. Heterophils are round-shaped, with clear cytoplasm containing eosinophilic orange granules and a small round to oval nucleus. Eosinophils are 5–8 µm in size and are responsible for parasitic infections and are correlated with weather adaptations. Eosinophils are not always found in every reptile species, such as snakes, but when present, they are round-shaped with light blue cytoplasm containing eosinophilic and basophilic granules and a large, slightly indented nucleus. Basophils are 20–25 µm in size and are responsible for immunoglobulins and parasitic or viral infections. Basophils are rarely found or are present in very low numbers and are round-shaped with basophilic blue-purple granules and a non-lobed nucleus. Lymphocytes are 5–10 µm in size and are responsible for immune responses, hematopoietic growth factor production, and viral infections. Lymphocytes are round-shaped with clear cytoplasm and a large basophilic nucleus with slight indentation. Monocytes are 12–25 µm in size and are responsible for granulomatous inflammation. Monocytes have varying shapes with pale blue cytoplasm and a nucleus that can vary in shape. Lastly, azurophils, are 15–25 µm in size, but their clinical function is not yet clearly defined. Azurophils resemble monocytes in morphology but contain granules of varying sizes and have darker cytoplasm.

MATERIALS AND METHODS

Ethical Approval

This study acquires ethical approval Number: B/85/UN14.2.9/PT.01.04/2025)

Study Period and Location

Wild caught samples were obtained through rescue in Bali meanwhile, the captive morphs samples were obtained through a consented collaboration with a reticulated python breeder, "*Weird Retic Indonesia*" in Yogyakarta. The study period, including sample collection and evaluation, spanned four months. Sample evaluation was done at Brawijaya University's Animal Hospital Laboratory and Udayana University's Immunology Laboratory.

Sample Parameters

The reticulated python used as samples must be within the weight range of 3 – 12 kg, with a length range of 200 – 400 cm. The number of reticulated pythons used in this study are 4 wild-caught and 4 captive morph individuals. The morphs recorded and used in this study include: *Golden Child Albino* (CF1), *Orange Glow* (CF2), *Jaguar/Harlequin* (CM1), and *Lavender Sunfire* (CM2). The limitation in sample size was due to the limited availability of samples that met the specified parameters and the low occurrence of samples in the wild.

Clinical Examinations and History

Physical examinations were conducted on the reticulated pythons prior to blood sampling to ensure the general health of each individual, as it could affect the results of the study. The physical examination included checking for ectoparasites, as well as examining the mouth, eyes, and scale condition. The captive morph reticulated python samples were recorded to be fed live chickens every week, while the wild caught python samples have a history of eating local chicken livestock, although it is also possible for them to consume rats, monkeys, birds, and other animals in the wild. Each captive morph reticulated python samples were kept individually in a ventilated wooden enclosure with constant temperature and humidity, and the enclosures were routinely cleaned. On the other hand, the wild caught reticulated python samples were found in the wild, where temperature and humidity varied.

Sampling Methods

All samples, both wild caught and captive morph reticulated pythons, were treated the same. The reticulated pythons were restrained, and blood samples were taken from their ventral coccygeal vein. The amount of blood collected from each individual was 3 ml, which was then placed into a labeled heparin tube. All samples were evaluated within 24 hours after blood collection. After blood collection, each individual was injected with the blood-boosting vitamin *Hematodin* via intramuscular injection.

Total Leukocyte Examination

The total leukocyte count was performed at the Veterinary Hospital of Universitas Brawijaya, Malang City, East Java. The total leukocyte count was carried out using a hemocytometer. The blood sample was diluted 20 times using a Thoma leukocyte pipette by aspirating the blood up to the 0.5 mark, then adding the modified Rees-Ecker reagent solution (Dixon's modification) up to the 11 marks. The mixture was homogenized by gently shaking the Thoma leukocyte pipette. Discard the first three drops onto a tissue before placing the sample into the Neubauer counting chamber groove, and let it sit for 3-5 minutes. Sample evaluation was performed using a Nikon EclipseEi light microscope with 400× magnification and the total leukocyte count was calculated from the four Neubauer chamber using the appropriate formula: $n\,sel \times 50 = (mm^3)$.

Differential Leukocyte Count and Cytomorphometry Examination

The differential count and cytomorphometry of each leukocyte type were performed at the Immunology Laboratory of Udayana University, Denpasar, Bali. The differential count and cytomorphometry were conducted using blood smear preparation. The blood smear preparation begins by placing a small drop of blood sample at the end of a microscope slide, then making a single push at a 30° angle. The blood smear is allowed to dry and then fixed using Diff-Quick staining: methanol fixative for 2 seconds, eosin stain for 20 seconds, and methylene blue stain for 15 seconds. The stained sample is then rinsed with distilled water (aquades) and thoroughly dried.

The evaluation of the differential leukocyte count was performed using an Olympus CX 33 microscope with 1000× magnification and the *WBC Counter* application with results expressed in percentage (%). The interpretation was based on the absolute counts of each leukocyte differentials, which were obtained by multiplying it by the total leukocyte count ($n\text{sel} \times 10^3 / \mu L$).

The evaluation of leukocyte cytomorphometry was done using the same microscope, equipped with *Optilab Viewer* software. Three cells were examined for each differential count, and the mean value was calculated. All pictures and measurements were taken using the *Image J*, with measurements compatible to the Neubauer chamber in micrometers (µm).

Data Analysis

The research results were tabulated and compared using an independent T-test in SPSS for Windows with a 95% confidence level.

RESULTS AND DISCUSSION

The normal leukocyte range in M. *reticulatus* is 1.32–15.8 × 10^3/µL (Carpenter et al., 2023), and all individuals examined in this study fell within this range. Although the mean leukocyte count was higher in wild-caught M. *reticulatus* compared to captive morphs, statistical analysis revealed no significant difference, despite the numerical values suggesting a notable trend. Higher leukocyte counts in wild populations are commonly associated with parasitic infestations and environmental challenges compared to captive conditions (Salakij et al., 2002; Rangel-Mendoza et al., 2009; Lisičić et al., 2013). Environmental factors such as habitat type, temperature, humidity, handling, and feeding frequency may further influence reptilian hematological values (Campbell, 2006), while seasonal variation has also been reported, with leukocyte counts increasing in summer and decreasing in winter (Stacy et al., 2011).

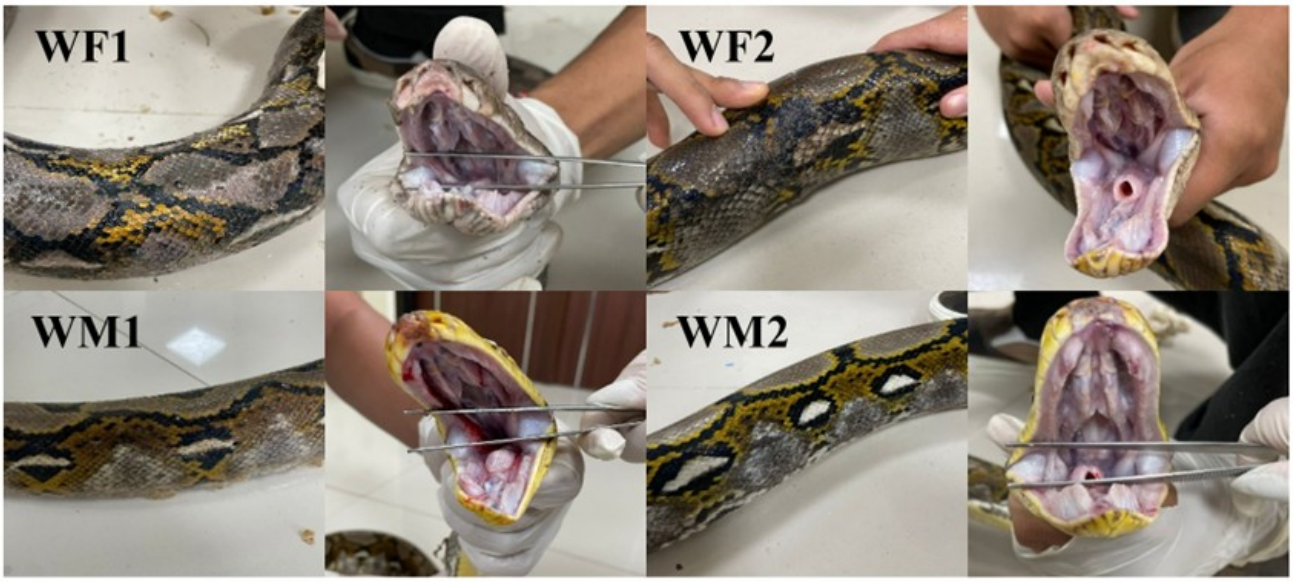

Figure 1. Documentation of Wild Caught M. *reticulatus* Samples Used in this Study.

Leukocytes in snakes consist of heterophils, eosinophils, basophils, lymphocytes, monocytes, and azurophils (Grego, 2006). Heterophils, comprising 20–40% of total leukocytes, are associated with stress and bacterial infection (Quandrini, 2018; Carvalho et al., 2013; Dervas *et al.*, 2023), while eosinophils account for 7–20% but are absent in some species, including pythons (Stacy *et al.*, 2011; Carpenter, 2010). Basophils (0–4%) vary with species, season, and pathogen exposure (Carvalho et al., 2013), whereas lymphocytes, the most common leukocytes (15–89%), can decrease with malnutrition or severe stress (Quandrini, 2018; Carvalho et al., 2013). Monocytes represent 0.5–3% (Stacy et al., 2011) and azurophils up to 35% of leukocytes (Quandrini, 2018). The differential leukocyte counts of wild-caught and captive morph M. *reticulatus* in this study were consistent with Carpenter *et al.* (2023), validating the sampling and analysis methods and suggesting that geographic origin does not significantly influence leukocyte differentials. Although statistical analysis revealed no significant differences between wild-caught and captive morphs M. *reticulatus*, wild-caught individuals tended to have higher heterophil

Figure 2. Documentation of Captive Morph M. *reticulatus* Samples Used in this Study
CF1. *Golden Child Albino: Reduced body pattern with albino coloration and red eyes*
CF2. *Orange Glow: Characteristic contrasting colors of orange and grayish purple*
CM1. *Jaguar/Harlequin: Dorsal motif extending into the lateral motif*
CM2. *Lavender Sunfire: Purple-colored lines and thicker circular borders*
(Personal Documentation, 2024).

percentages, likely due to subclinical infection or tissue regeneration processes/ecdysis (Campbell, 2006; Carvalho et al., 2013). Elevated heterophil counts in wild-caught M. *reticulatus* may also reflect adaptation to more challenging environments (Salakij et al., 2002; Rangel-Mendoza et al., 2009; Lisičić et al., 2013; Claunch et al., 2022). Furthermore, Claunch et al. (2022) reported that pythons gradually adapt their physiology to captivity, a process that varies among individuals and may influence leukocyte composition and activity.

The enlargement of heterophil and lymphocyte cells in wild-caught M. *reticulatus* is likely associated with undetected subclinical bacterial infections, reflecting a reactive state characterized by increased metabolic activity and organelle proliferation to support leukocyte differentiation into effector cells capable of producing antimicrobial enzymes and proteins (Cano et al., 2013). Wild-caught M. *reticulatus*, being more frequently exposed to diverse pathogens and parasites than captive morphs in controlled environments, thus exhibit larger and more active immune cells, particularly heterophils and lymphocytes (Cano et al., 2013). Additionally, heterophil enlargement in wild-caught M. reticulatus may be influenced by the ecdysis process (Carvalho et al., 2013). Overall, the larger leukocyte size observed in wild-caught M. reticulatus represents an adaptive response to biologically challenging natural environments, supporting the view of Claunch et al. (2022)

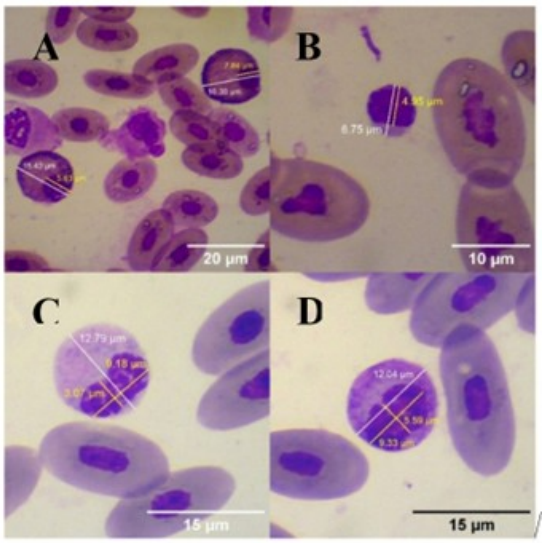

Figure 3. Example of Cytoplasmic and Nuclear Diameter Measurements of Leukocyte Cells in Wild Caught and Captive Morphs *M. Reticulatus.*
A. Heterophil; **B.** Lymphocyte; **C.** Monocyte; **D.** Azurophil.
Stain: Diff-quick; 1000×(Personal Documentation, 2024)

Table 1. Wild Caught and Captive Morph M. *reticulatus* Leukocyte Differential Results

Parameters		Wild Caught (n = 4)	Captive Morph (n = 4)	*Sig.*	Carpenter et al., 2023
Total Leukocyte (10^3/µL)		11.20±2.10	8.50±3.00	0.19	7.48 (1.32-15.8)
Differential Leukocyte	Heterophil	3.23±0.51	1.99±0.91	0.06	1.96 (0.08 – 4.83)
	Eosinophil	0.00±0.00[a]	0.00±0.00[a]	-	0.68 (0.04 – 1.95)
	Basophil	0.00±0.00[a]	0.00±0.00[a]	-	0.06 (0.06 – 0.7)
	Lymphocyte	2.95±0.76	2.24±0.73	0.22	2.24 (0.12 – 7.47)
	Monocyte	3.28±0.10	2.88±.56	0.51	1.22 (0.02 – 5.50)
	Azurophil	1.75±1.06	1.38±1.14	0.65	0.10 (0.01 – 4.30)

Table 2. Wild Caught and Captive Morph M. *reticulatus* Cytoplasm Diameter Results (µm)

Parameters	Wild Caught (n = 4)	Captive Morph (n = 4)	*Sig.*
Heterophil	15.64±0.79	11.10±0.92	<.001*
Eosinophil	0.00±0.00[a]	0.00±0.00[a]	-
Basophil	0.00±0.00[a]	0.00±0.00[a]	-
Lymphocyte	8.46±0.93	6.48±0.611	.01*
Monocyte	12.45±0.91	10.19±1.64	.05
Azurophil	11.18±0.63	10.30±1.51	.33

Note: [a] : *t* could not be calculated because the standard deviations of both groups are 0; * : Significant.

Table 3. Wild Caught and Captive Morph M. *reticulatus* Nucleus Diameter Results (µm)

Parameters	Wild Caught (n = 4)	Captive Morph (n = 4)	*Sig.*
Heterophil	7.09±1.13	4.62±0.33	.01*
Eosinophil	0.00±0.00[a]	0.00±0.00[a]	-
Basophil	0.00±0.00[a]	0.00±0.00[a]	-
Lymphocyte	6.47±0.60	5.14±0.66	.03*
Monocyte	8.28±0.40	5.48±0.77	<.001*
Azurophil	7.24±0.73	5.90±0.79	.05*

Note: [a] : *t* could not be calculated because the standard deviations of both groups are 0; * : Significant.

that pythons can adjust their physiological functions in response to environmental changes.

The nucleus of leukocytes plays a critical role in cellular functions, including protein and enzyme synthesis, genetic information storage, regulation of cell activity, and control of leukocyte migration (Li et al., 2022). Nuclear size and flexibility influence diapedesis, as larger or stiffer nuclei slow leukocyte passage through blood vessel walls, thereby delaying immune responses (Li et al., 2022). In this study, the nuclear diameters of heterophils, eosinophils, basophils, lymphocytes, monocytes, and azurophils in wild-caught M. reticulatus were significantly larger than those in captive morphs, consistent with cytoplasmic measurements and the observation by Cano et al. (2013) that reactive cells are typically larger. This suggests that wild-caught M. *reticulatus* possess more active leukocytes, potentially experiencing slower diapedesis due to enlarged nuclei, reflecting a constantly engaged immune defense against pathogens. In contrast, leukocytes of captive morphs exhibited smaller nuclei, likely due to lower pathogen exposure, resulting in less active nuclear organelles (Claunch et al., 2022; Cano et al., 2013). The smaller nuclear size in captive morphs may also be influenced by management factors such as feeding frequency and handling (Campbell, 2006), representing a form of cellular efficiency under less immunologically challenging conditions. Since reference data for normal nuclear size in M. *reticulatus* are unavailable, interpretation remains limited. It should also be noted that the small sample size (n = 4 per group) represents a significant limitation, potentially reducing statistical power and increasing the likelihood of Type II errors. Consequently, this limitation should be considered when discussing the findings.

CONCLUSION

Based on the research conducted, it can be concluded that the total leukocyte counts of wild-caught and captive morph *Malayopython reticulatus* did not show a significant difference statistically. Similarly, the leukocyte differential between the two groups was not significantly different statistically. However, the cytoplasmic diameter of heterophil and lymphocyte cells in wild-caught M. *reticulatus* was larger compared to the captive morph. In addition, the nuclear diameter of leukocytes in wild-caught M. *reticulatus* was also larger than that of the captive morph. The use of *Diff-Quick* staining was less effective in clearly visualizing cell granules; therefore, Wright-Giemsa staining would be more appropriate. It should be remembered that there are notable sample limitations (n = 4 per group) that can influence the statistical results and preliminary observations. Future studies should incorporate larger sample sizes to enhance the validity of the findings. Complementary evaluations (e.g., parasitology, stress hormones, or inflammatory markers) of the samples are recommended to exclude the presence of other pathological conditions that could affect the results. Moreover, further research examining variations in leukocyte profiles, leukocyte cytomorphometry, and genetic traits across different morphs of M. *reticulatus* is strongly advised.

AUTHORS CONTRIBUTION

S.A. designed, conceptualized, performed sample collection, analyzed data and wrote the manuscript, I.G.A.A.S. conceptualized, supervised, wrote the manuscript, I.N.M.A. conceptualized, supervised, and analyzed data. All authors have read and approve the final manuscript.

"The author declares that there is no conflict of interest with parties involved in this research".

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