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Coat Color Variation and Hair Color Banding in the Papuan Bandicoot (*Echymipera kalubu*, Marsupialia: Peramelidae)

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ABSTRACT

Coat color in mammals plays important adaptive roles, including camouflage, communication, and thermoregulation. Intraspecific variation in coat color is often associated with local environmental conditions. This study investigates coat color and hair color banding in the common spiny bandicoot (*Echymipera kalubu*), which exhibits ventral color polymorphism. We examined twenty individuals from Manokwari, West Papua, categorizing them into red-ventral and white-ventral groups. Coat color was quantified from digital photographs using CIE Lab* values, and hair color banding types were analyzed microscopically from body areas: dorsal, lateral, and ventral. Significant differences were found between the two groups and among the body areas. Red-ventral individuals exhibited higher a (redness) and b (yellowness) values, especially in the ventral area, whereas white-ventral individuals showed higher L (Lightness) values and reduced chromaticity. Seven hair color banding types were identified, with red-ventral individuals displaying a more diverse hair color banding type across body areas, particularly in lateral and ventral areas. Habitat substrate color analysis revealed that red-ventral individuals inhabited darker, red-yellow environments, whereas white-ventral individuals occupied lighter, less saturated habitats. The dorsal-to-ventral gradient in pigmentation and banding in *E. kalubu* is predicted to have a countershading function to avoid predators.

1. Introduction

Coat color in mammals serves multiple adaptive functions, including camouflage, communication, sexual selection, and thermoregulation. These functions help individuals survive and reproduce by enhancing concealment, signaling social status, and regulating body temperature (Caro 2005; Caro and Mallarino 2020). Countershading is a common form of camouflage in animals, where the dorsal area is darker and the ventral area lighter, helping to reduce visual detection by predators (Caro 2005).

Intraspecific coat color variation is frequently shaped by local habitat conditions. For example, deer mice (*Peromyscus maniculatus*), populations with light coat color inhabit light sandy dunes, whereas populations with black coat color are found in darker vegetated environments (Linnen *et al.* 2009). In Budin's grass mouse (*Akodon budini*), populations with black coat color inhabiting darker habitats exhibit lower lightness (L) values, whereas those with orange or lighter coat color in more open or brighter habitats show significantly higher L and b (yellowness) values (Sandoval *et al.* 2017).

Variation in coat color across body areas in mammals is often attributed to differences in the type and frequency of hair color bands. Mammalian hair coat color is typically non-uniform, with many species exhibiting alternating bands of different pigments along individual hair shafts. These banding patterns, when integrated across thousands of hairs, determine the

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overall appearance of the coat (Caro 2005; Hoekstra 2006). These pigmentation bands are typically produced by varying distributions of eumelanin (black/brown pigment) and pheomelanin (red/yellow pigment) deposited during hair growth cycles (Slominski *et al.* 2004).

Studies on intraspecific coat color variation in marsupials remain limited. In species such as the brushtail possum (*Trichosurus vulpecula*) and the koala (*Phascolarctos cinereus*), coat color differences have been observed across populations inhabiting different environments, including open woodlands and closed forests. However, most of these reports are descriptive and do not investigate the underlying ecological or genetic mechanisms driving pigmentation variation at the intraspecific level (Menkhorst and Knight 2011).

Despite extensive research on placental mammals, similar morphological investigations in marsupial coat colors are scarce, particularly in the common spiny bandicoot (*E. kalubu*). This species exhibits clear intraspecific variation in ventral pigmentation, with individuals categorized into red-ventral and white-ventral groups (Menzies 1991; Flannery 1995; Wilson and Mittermeier 2015; Maker *et al.* 2016). From an evolutionary perspective, bandicoots have been isolated from placental mammals for over 120 million years and have independently adapted to diverse environments in Australasia (Meredith *et al.* 2008; Jackson and Groves 2015). This long divergence and ecological adaptability make *E. kalubu* a promising model to explore how pigmentation patterns evolve in response to environmental conditions.

This study aims to document the morphological characteristics of coat color and hair color banding in *E. kalubu*, with a focus on intraspecific variation between coat phenotypes (red-ventral vs. white-ventral). It also investigates whether the type of hair color banding across body areas (dorsal, lateral, and ventral) correlates with the overall coat phenotype. This study provides the first fine-scale morphological description of hair color banding in marsupial *E. kalubu*, offering insights into adaptive coat color in isolated environments.

2. Materials and Methods

2.1. Animals

Twenty individual bandicoots (10 individuals were red ventral group, and 10 individuals were white ventral group) were collected from the forests around Manokwari, West Papua, Indonesia (0°52′0″ S, 134°5′0″ E) (Figure 1). All samples were identified based on morphology characteristics and morphometric

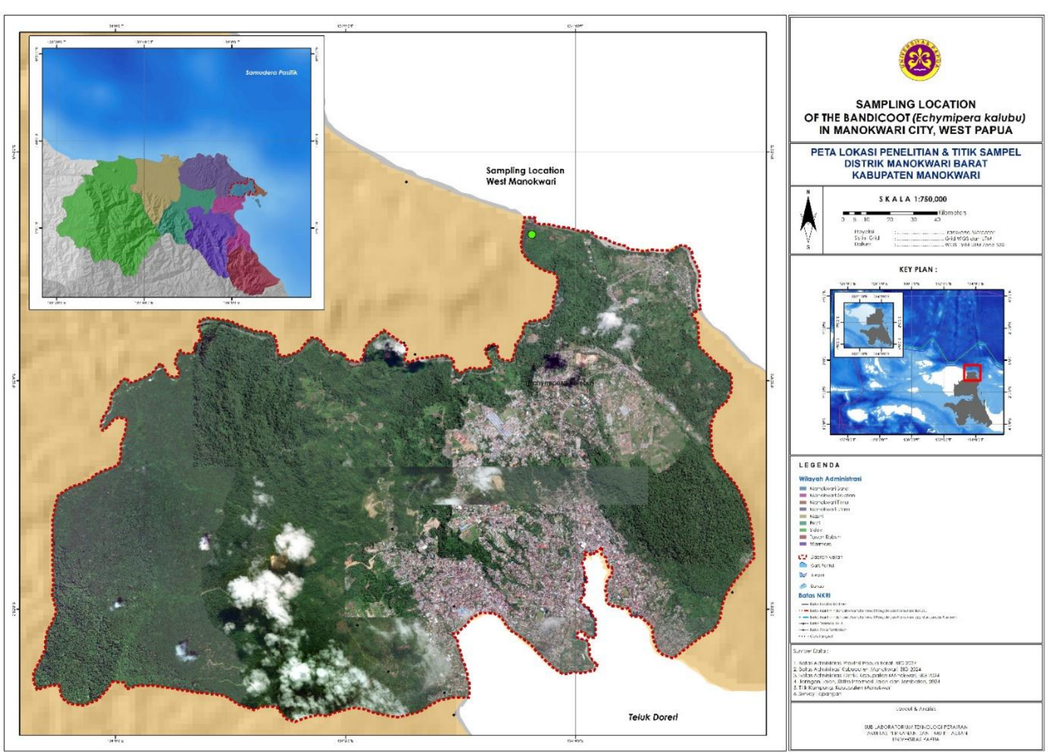


Figure 1. Geographic sampling locations of the Papuan bandicoot (*E. kalubu*) in Manokwari City, West Papua, Indonesia

measurements (Menzies 1991; Flannery 1995; Wilson and Mittermeier 2015). Bandicoots were anesthetized via intramuscular injection with a combination of ketamine hydrochloride (50 mg/kg body mass) and xylazine (10 mg/kg body mass) (Maker *et al.* 2016). The sample collection protocol was approved by the Animal Ethics Commission of IPB University (Permission number 207-2021).

2.2. Coat Color Phenotype in the Body Areas

Quantitative coat color data were obtained from standardized digital photographs of twenty individuals. For each individual, digital photographs were taken from three anatomical areas: dorsal, lateral, and ventral. Four sampling points were selected to represent variation within each body area, as illustrated in Figure 2 (modified from Potash *et al.* 2020). All photographs were taken using a Color-Checker Classic (X-Rite, USA) for color calibration under natural daylight conditions at midday (11:00 AM-1:00 PM) to ensure consistent and uniform illumination. Outdoor photo sessions were conducted in open areas with minimal shading to reduce variability caused by directional or diffused sunlight.

Before digital photographs were analyzed for quantitative color measurement, each image was calibrated using a color chart included in the photo. This calibration step aimed to standardize color and brightness across all images, ensuring that the resulting color measurements (CIE Lab* values) were accurate, consistent, and comparable. By correcting for variations in lighting conditions and camera settings during image capture, calibration allowed the recorded color data to better reflect the true appearance of the subject.

Color calibration and subsequent CIE Lab* analysis were conducted using ImageJ software (version 1.5). Colors were interpreted in the CIE Lab* color space, which provides numerical values for L* (lightness, ranging from 0 to 100), a* (green to red, –60 to 60), b* (blue to yellow, –60 to 60), and Chroma (Ch), calculated as $\sqrt{(a^2 + b^2)}$, representing the intensity or saturation of the color. This method of color analysis followed the guidelines established by Haeghen and Naeyaert (2006), Koyabu *et al.* (2008), and Hamada *et al.* (1988).

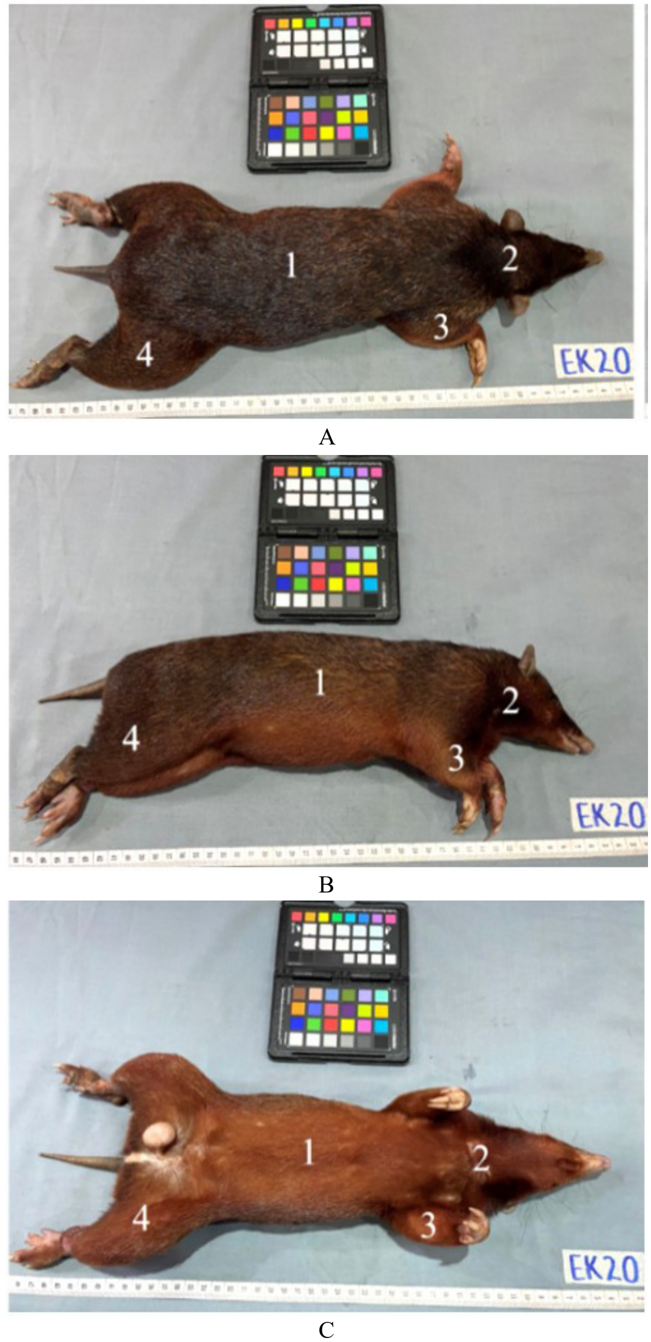


Figure 2. Photographs of *E. kalubu* illustrating body areas and color sampling areas. (A) The dorsal, (B) lateral, and (C) ventral. Number positions (1–4) indicate the areas of measurement. Color chart as reference for standardized calibration

2.3. Hair Color Bands

To analyze hair color banding, we collected hairs from the dorsal, lateral, and ventral body areas. Approximately 30 hairs were collected per body area using anatomical tweezers and stored in labeled plastic bags. Detailed examination of each hair color banding was performed under a VHX 7000 digital microscope (magnification: 100x–<500x), and images were captured at 2,000 × 500 pixels resolution. Hair color banding types were grouped using modified criteria derived from previous studies (Koyabu *et al.* 2008; McRobie *et al.* 2009; Hoekstra 2006) (Table 1).

Table 1. Description of hair color banding types in *E. kalubu*

Hair color band types	Description	References
black	shows completely saturated black hair without any agouti bands, when there are wider areas of black bands than of other colors. This band potentially contains eumelanin, with the pigment distributed uniformly along the hair shaft	Koyabu *et al.* 2008; McRobie *et al.* 2009
brown; bright brown; dark brown	There are wider areas of brown bands than black ones When there are wider areas of brown bands than of black ones, when the black area prevails	Koyabu *et al.* 2008
red	shows completely red. This band potentially contains pheomelanin, with the pigment distributed uniformly along the hair shaft	Koyabu *et al.* 2008
yellowish	shows completely yellowish. This band potentially contains pheomelanin, with the pigment distributed uniformly along the hair shaft	Hoekstra 2006
grey	completely grey, or there are wider areas of grey bands than of other colors	McRobie *et al.* 2009
white	may not contain melanin pigments with white color in the basal to shaft area of hair, with cream color at the top of the hair	Hoekstra 2006
white/unpigmented/ albino	shows a completely white color, or solid colored/unbanded, because it does not contain pigments	Hoekstra 2006; McRobie *et al.* 2009

2.4. Habitat Substrate Color Measurement

We adapted the soil imaging and color analysis methods based on Kirillova & Sileva (2017) and Haeghen & Naeyaert (2006), with modifications for field conditions. Soil samples were collected from locations where *E. kalubu* individuals were observed (Figure 3). Each sample was photographed under diffuse natural lighting during overcast conditions to minimize variability. A Color Checker Classic (X-Rite, USA) was included in each frame for calibration. Images were processed using ImageJ v1.5; calibration was performed based on the reference colors of the Color Checker. Color values were extracted from representative, shadow-free areas and averaged across three replicates. These values were then converted to the CIE Lab* color space for analysis.

The interpretation data of absolute substrate color differences (Δ): Δ 0-1: is generally not perceptible to the human eye, as the color difference is extremely minimal. Δ of 1-3: indicates a very slight difference, often detectable only by trained observers under controlled conditions. Δ 3-6: the difference becomes noticeable, though still relatively subtle. Δ 6-10: represents a visible color difference for most people. Δ >10 is considered very strong, indicating a visually striking and easily distinguishable color contrast (Mahy *et al.* 1994).

2.4. Statistical Analysis

All statistical analyses were conducted using R version 4.4.3 (R Core Team 2025). The normality of the Lab* and chroma values in each dataset was first assessed using the Shapiro-Wilk test.

Since most variables were not normally distributed, we applied non-parametric tests. Coat color values between red-ventral and white-ventral groups at each body area (dorsal, lateral, and ventral) were compared using the Mann–Whitney U test ($P<0.05$). To evaluate variation across body areas within each group (red-ventral and white-ventral), the Kruskal-Wallis test was applied ($P<0.05$). When significant differences were found, pairwise comparisons were conducted using Dunn's post hoc test.

Substrate color data were analyzed descriptively. Interpretations were limited to direct numerical comparisons of Lab* values between the red-ventral and white-ventral groups. Absolute differences (Δ) were reported to illustrate visual contrasts between groups (Mahy *et al.* 1994).

A B

Figure 3. Habitat types and substrate color at the location where *E. kalubu* individuals were found. (A) A representative habitat of the red-ventral group, typically occurring in dense, shaded forest areas with darker, reddish, and yellowish substrate colors. (B) The habitat of the white-ventral group, typically found in more open areas with greater light exposure but substrates that are less red and less yellow. Substrate color was calibrated using color charts to allow standardized measurements under natural daylight conditions

3. Results

3.1. Coat Color Variation between Red-Ventral and White-Ventral Groups

E. kalubu individuals could be grouped into two categories based on the ventral coat color and habitats. The first category, the red-ventral group, consisting of 10 individuals (six males and four females), displayed reddish and yellowish color in their ventral area (Table 2). The second category, a white-ventral group consisting of 10 individuals (seven males and three females), displayed a grey ventral coat color (Table 3). Dorsal and lateral areas differed between the groups: the red-ventral group showed dark-brown color, whereas the white-ventral group exhibited dark-black color.

The coat color of dorsal, lateral, and ventral body areas was quantitatively measured, and significant differences in color variables Lab* value were found between the red-ventral and white-ventral groups (Mann-Whitney U Test, $P<0.05$; Table 4). Significant differences in lightness (L) were observed between the red ventral and white ventral groups in the ventral area ($P<0.05$), with the red ventral group being darker. The red-ventral group exhibited higher redness (a), yellowness (b), and chroma (Ch) values than the white-ventral group across all body areas ($P<0.05$).

3.2. Differences in Coat Color Lab* Values between Body Areas in the Red-Ventral and White-Ventral Groups

Significant differences in coat color Lab* values were observed between body areas: dorsal, lateral, and ventral, within both the red-ventral and white-ventral groups (Table 5).

In the red-ventral group, significant differences in coat color lightness (L) were observed between the dorsal-lateral areas ($P<0.05$), with the dorsal area having a darker color than the lateral. A significant difference was also found between the dorsal-ventral areas ($P<0.05$), with the dorsal area appearing darker. For redness (a), significant differences were detected between the dorsal-ventral areas ($P<0.05$), with the ventral area exhibiting higher redness. Significant differences were also observed between the lateral and ventral areas ($P<0.05$), with the ventral area showing greater redness than the lateral. For yellowness (b), significant differences were found between the dorsal and lateral areas ($P<0.05$), with the lateral area showing higher yellowness. A significant difference was also observed between the dorsal and ventral areas ($P<0.05$), with the ventral area exhibiting higher yellowness than the dorsal.

In the white-ventral group, significant differences in lightness (L) were found between the dorsal and ventral areas ($P<0.05$), with the dorsal area being significantly darker. A significant difference was also observed between the lateral and ventral areas, where the lateral area was darker than the ventral area. No significant differences in redness (a) were found between body areas. A significant difference in yellowness (b) was observed between the dorsal and ventral areas ($P<0.05$), with the ventral area exhibiting higher yellowness.

Table 2. Coat color of body areas: dorsal, lateral, and ventral of each individual in the red-ventral group *E. kalubu*

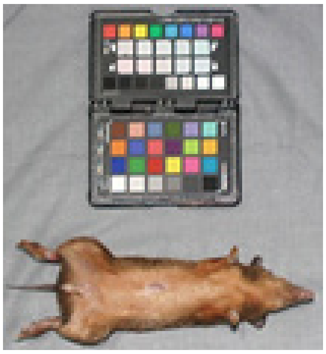
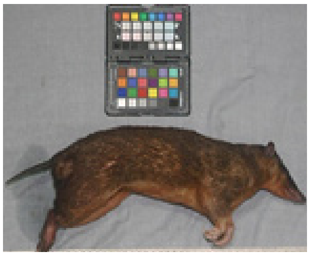
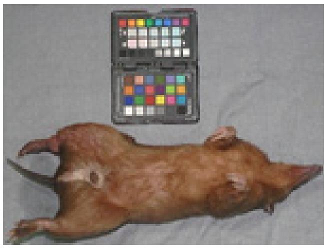
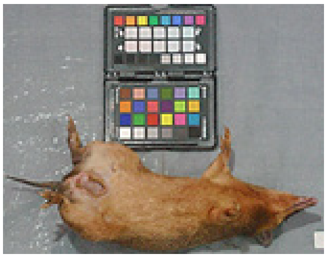
Specimen	Sex	Dorsal	Lateral	Ventral
M1	male			
M2	male			
M3	female			
M6	female			
M11	female			

Table 2. Continued

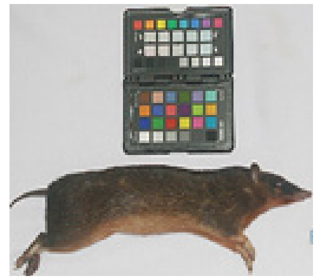
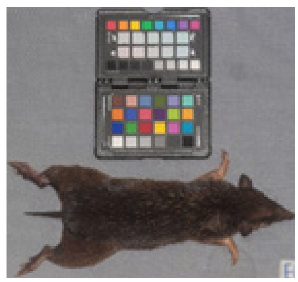
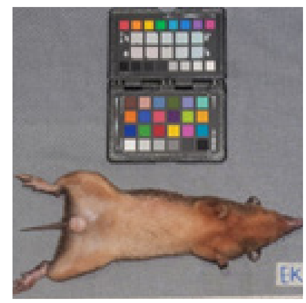
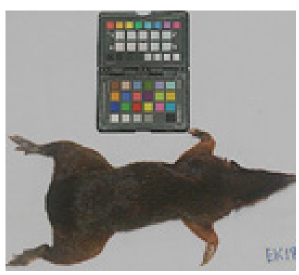
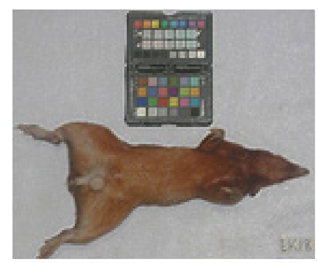
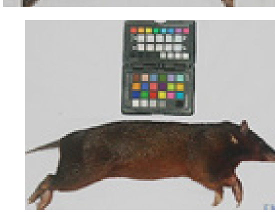
Specimen	Sex	Dorsal	Lateral	Ventral
M12	female			
M15	male			
M18	male			
M19	male			
M20	male			

Table 3. Coat color of body areas: dorsal, lateral, and ventral of each individual in the white-ventral group *E. kalubu*

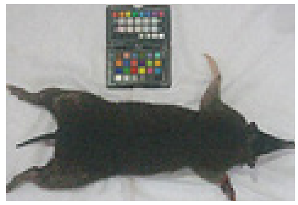
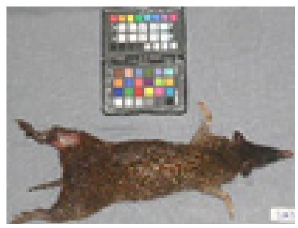
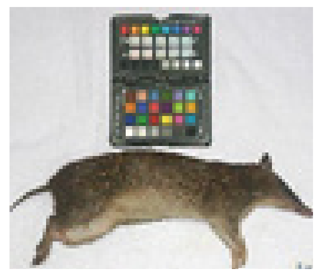
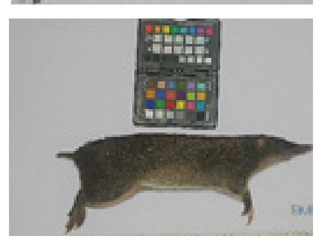
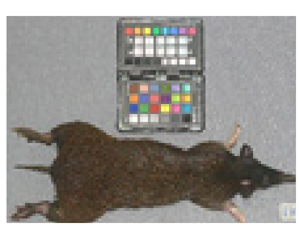
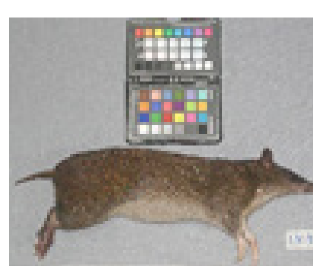
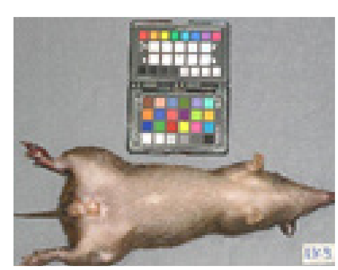
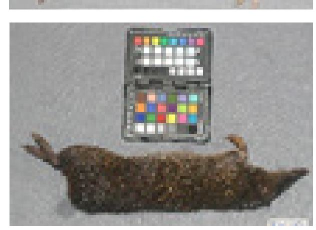
Specimen	Sex	Dorsal	Lateral	Ventral
M4	male			
M5	female			
M7	female			
M8	female			
M9	male			
M10	male			

Table 3. Continued

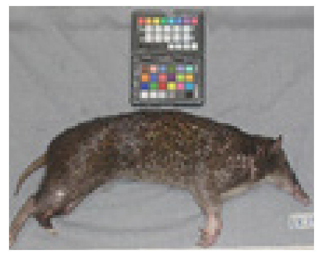
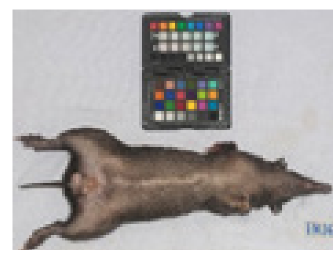
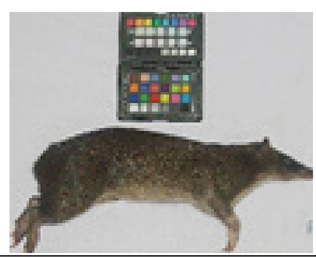
Specimen	Sex	Dorsal	Lateral	Ventral
M13	male			
M14	male			
M16	male			
M17	male			

Table 4. Mean (± SD) values of lightness (L), redness (a), yellowness (b), and chroma (Ch) in the dorsal, lateral, and ventral areas of red-ventral and white-ventral *E. kalubu*. In general, the white-ventral group showed significantly higher lightness values, especially in the ventral area. Red-ventral group had higher redness, yellowness, and brightness in all areas

Body areas/groups	Coat color value							
	L (Lightness)		a (Redness)		b (Yellowness)		Ch (Brightness)	
	mean ± SD	P-value	mean ± SD	P-value	mean ± SD	P-value	mean ± SD	P-value
Dorsal:								
Red-ventral	9.01±4.4	0.22	3.32±0.5	*0.00	6.59±1.4	*0.04	7.43±1.4	*0.03
White-ventral	7.01±3.61		2.14±0.6		4.85±1.9		5.39±2	
Lateral:								
Red-ventral	16.1±4.89	0.91	4.96±1.2	*0.00	11.3±3.4	*0.00	12.4±3.3	*0.00
White-ventral	15.8±4.37		2.18±0.9		6.42±1.6		6.9±1.6	
Ventral:								
Red-ventral	25.3±11.8	*0.00	8.72±1.5	*0.00	18.3±4.1	*0.00	20.5±3.3	*0.00
White-ventral	43.1±3.53		2.57±0.5		9.05±1.9		9.48±1.7	

P-values are based on the Mann–Whitney U test; asterisks (*) indicate statistically significant differences (P<0.05)

Table 5. Mean (± SD) values of lightness (L), redness (a), and yellowness (b) across the dorsal, lateral, and ventral areas of red-ventral and white-ventral *E. kalubu*. In the red-ventral group, lightness, redness, and yellowness were significantly higher in the ventral than the dorsal region; lightness and yellowness were also higher in the lateral than the dorsal region; and redness was higher in the ventral than the lateral region. In the white-ventral group, lightness and yellowness were higher in the ventral than the dorsal region, and lightness was also higher in the ventral than the lateral region

Groups	Body areas	Coat color value					
		L (Lightness)		a (Redness)		b (Yellowness)	
		mean ± SD	P-value	mean ± SD	P-value	mean ± SD	P-value
Red-ventral	Dorsal Lateral	8.82±4.22 15.72±4.83	*0.047	3.22±0.56 4.71±1.45	0.054	6.44±1.38 10.7±3.74	*0.038
	Dorsal Ventral	8.82±4.22 27.05±12.61	*0.000	3.22±0.56 8.2±2.21	*0.000	6.44±1.38 17.27±5.14	*0.000
	Lateral Ventral	15.72±4.83 27.05±12.61	0.612	4.71±1.45 8.2±2.21	*0.035	10.7±3.74 17.27±5.14	0.087
White-ventral	Dorsal Lateral	7.03±3.84 16.26±4.38	0.058	2.14±0.66 2.19±0.9	1.00	4.85±2 6.59±1.64	*0.064
	Dorsal Ventral	7.03±3.84 43.56±3.16	*0.000	2.14±0.66 2.51±0.49	0.25	4.85±2 9.28±1.88	*0.000
	Lateral Ventral	16.26±4.38 43.56±3.16	*0.024	2.19±0.9 2.51±0.49	0.16	6.59±1.64 9.28±1.88	0.062

P-values are based on Dunn's test ($P<0.05$); asterisks (*) indicate statistically significant differences ($P<0.05$)

3.3. Variation of Seven Hair Color Band Types

We examined each hair shaft under a VHX 7000 digital microscope and identified seven hair color banding types (Figure 4; Table 6). Type 1 (T1) was fully uniformly black, Type 2 (T2) exhibited a color gradient: grey in the basal, black in the middle, and red at the top. Type 3 (T3) was entirely red, Type 4 (T4) was uniformly yellowish, and Type 5 (T5) was uniformly cream colored. Type 6 (T6) exhibited a two-pattern, with a light white base and a cream-colored top. Type 7 (T7) was entirely light white.

3.4. Differences in Hair Color Banding between Red-Ventral and White-Ventral Groups

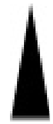
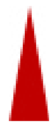
The hair color banding types (T1–T5) in red-ventral *E. kalubu* varied across different areas of the body (Table 7). The back (dorsal) area mostly had darker bands, T1, T2, and T3, while the belly (ventral) area mainly showed lighter bands, T4 and T5, which were cream-colored. Some individuals had all five banding types on their bodies, which suggests that the way pigments are added during hair growth may be quite complex. On the dorsal area, the distribution of hair color banding types was uniform across all individuals. All ten individuals shared the same three band types: T1, T2, and T3. The dominance of T1 and T2 made the dorsal area appear darker compared to other areas of the body.

On the lateral area, the distribution of hair color banding types in each individual was more variable compared to the dorsal area. In general, four band types (T1–T4) were observed on the lateral area. Some individuals exhibited only a single type, while others showed two or three types. For example, individual M1 had only one type, T1. Individuals M11 and M12 also had only one type, which was T3. Five individuals showed two different types. Among them, M3, M18, and M20 shared the same combination of T3 and T4. Individual M6 had a different pair: T2 and T4, while M19 showed T2 and T3. Two individuals (M2 and M15) displayed three different band types. M2 had T1, T2, and T3 matching the distribution found on the dorsal area. In contrast, M15 exhibited T2, T3, and T4. Among these ten individuals, only M2 had T1 present on the lateral region. The dominance of T3 and T4 made the lateral area appear reddish and yellowish compared to other dorsal areas.

On the ventral area, three hair color banding types were observed: T3, T4, and T5. The most dominant type was T4 (yellow). Each individual exhibited either one or two band types on the ventral area. Among the ten individuals, eight had only one type, while the remaining two individuals had the same two types. Of the eight individuals with a single type, five individuals, i.e., M2, M3, M6, M12, and M19, shared the same T4. The other three individuals, i.e., M11, M18, and M20, shared the same T3. The two individuals (M1 and M15) with the two types had

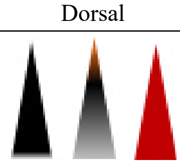
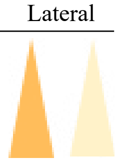
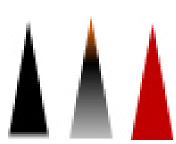
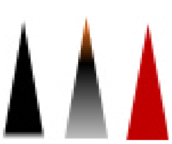
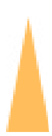
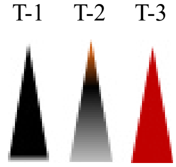
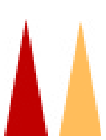
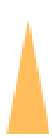
Figure 4. Hair color banding observed under a VHX-7000 digital microscope (magnification: 100× to <500×). Triangular icons represent the pigmentation of classified hair color banding types: T-1 (black), T-2 (grey-dark), T-3 (red), T-4 (yellow), T-5 (cream), T-6 (white), and T-7 (white light). Scale bar = 1 mm

Table 6. Description and phenotype of diagrammatic hair color band types (T1–T7) representation in *E. kalubu*

No of Type	Diagrammatic of hair color band types	Description	Phenotype
T1		Completely black dark color	black
T2		Combined three colors: dominant grey from the basal until the middle, black in the top of the middle, and reddish on the top	grey dark
T3		Completely reddish color	red
T4		Completely yellownish color	yellow
T5		Completely cream color	cream
T6		Combined two colors: dominand white light from the basal until the middle, cream in the top	white
T7		Completely white light color	white

These hair color bandings are central to understanding coat color and its ecological significance. Triangular icon showing the pigmentation. T were several hair color banding types (T1=Type 1, T2=Type 2, T3=Type 3, T4=Type 4, T5=Type 5, T6=Type 6, and T7=Type 7). Each hair color banding type is characterized based on the arrangement and combination of pigmentation along the hair shaft and is associated with specific visual phenotypes

Table 7. Distribution of hair color banding types (T1–T5) in the body areas: dorsal, lateral, and ventral of the red-ventral group of *E. kalubu* individuals

Specimen	Sex	Dorsal	Lateral	Lateral
M1	male	 T-1 T-2 T-3	 T-2	 T-4 T-5
M2	male	 T-1 T-2 T-3	 T-1 T-2 T-3	 T-4
M3	female	 T-1 T-2 T-3	 T-3 T-4	 T-4

Triangular icons represent individual hair color banding types coded as follows: T-1: black; T-2: grey-dark; T-3: red; T-4: yellow; T-5: cream

Table 7. Continued

Specimen	Sex	Dorsal	Lateral	Lateral
M6	female	T-1 T-2 T-3	T-2 T-4	T-4
M11	female	T-1 T-2 T-3	T-3	T-3
M12	female	T-1 T-2 T-3	T-3	T-4
M15	male	T-1 T-2 T-3	T-2 T-3 T-4	T-4 T-5
M18	male	T-1 T-2 T-3	T-3 T-4	T-3
M19	male	T-1 T-2 T-3	T-2 T-3	T-4
M20	male	T-1 T-2 T-3	T-3 T-4	T-3

Each banding type (T1–T7) is represented by a triangular icon showing the pigmentation pattern: T-1: black; T-2: grey-dark; T-3: red; T-4: yellow; T-5: cream; T-6: white; T-7: white light

the same T4 and T5. These two individuals were also unique compared to the others, as they were the only ones to possess T5, which distinguished the ventral from the dorsal and lateral areas, where T5 was not observed. The dominance of T4 made the ventral area appear yellow compared to other areas of the body.

The hair color banding types (T1–T2; T6-T7) in white-ventral *E. kalubu* varied across different areas of the body (Table 8). The dorsal and lateral areas were consistently present in all individuals, except for T1 and T2. In contrast, the ventral area exclusively displayed T6 and T7, lacking any darker hair color bands. Across individuals, this pattern remained consistent, with minimal variation, highlighting the uniformity of pigment expression within this phenotype. No clear sex based pattern was observed in banding distribution, indicating that sexual dimorphism does not influence hair pigmentation in both groups.

On the dorsal area, the distribution of hair color banding types was uniform across all individuals. Each individual had the same two band types: T1 and T2. The presence of T1 and T2 contributed to the darker appearance of the dorsal coat color compared to the ventral side.

Table 8. Distribution of hair color banding types (T1–T2; T6-T7) in body areas: dorsal, lateral, and ventral of the white-ventral group of *E. kalubu* individuals

Specimen	Sex	Dorsal	Lateral	Lateral
M4	male	T-1 T-2	T-1 T-2	T-6
M5	female	T-1 T-2	T-2	T-6
M7	female	T-1 T-2	T-1 T-2	T-6
M8	female	T-1 T-2	T-1 T-2	T-6 T-7
M9	male	T-1 T-2	T-2	T-6 T-7
M10	male	T-1 T-2	T-1 T-2	T-6 T-7
M13	male	T-1 T-2	T-1 T-2	T-6 T-7
M14	male	T-1 T-2	T-1 T-2	T-6 T-7
M16	male	T-1 T-2	T-1 T-2	T-6
M17	male	T-1 T-2	T-1 T-2	T-6 T-7

Triangular icons represent individual hair color banding types coded as follows: T-1: black; T-2: grey-dark; T-6: white; T-7: white light

On the lateral area, the same two band types: T1 and T2, were also observed. However, there were two individuals, M5 and M9, who had only one band type: T2, while the remaining eight individuals exhibited both T1 and T2, identical to the distribution observed on the dorsal area. As a result, the coat color on the lateral area appeared similar to the dorsal area due to the dominance of T1 and T2.

On the ventral area, two hair color banding types were found: T6 and T7. Some individuals had only one band type, while others had two band types. Four individuals (M4, M5, M7, and M16) exhibited only T6, whereas the remaining six individuals had both band types: T6 and T7. The distribution of T6 and T7 distinguished the ventral area from the other body areas. These types also contributed to the ventral area appearing lighter or a whiter coat color compared to the dorsal and lateral areas.

The hair color banding types between red and white ventral groups varied across different areas of the body (Table 9). Hair color banding types T1 (black) and T2 (dark grey) were predominantly in the dorsal area of both groups, indicating a higher presence of eumelanin. The lateral area displayed a more diverse combination. T1 and T2 were also found on the lateral area in both groups, along with T3 (red) and T4 (yellow), especially in the red-ventral group. In contrast, the ventral area differed between the two groups. The red-ventral group exhibited three band types: reddish (T3) to yellowish (T4, T5), whereas the white-ventral group was dominated by T6 (white) and T7 (light white).

3.5. Substrate Color

The red-ventral group was associated with a darker substrate (L = 14.9) compared to the white-ventral group (L = 17.5), indicating a less reflective or dimmer background. In terms of chromatic components, the red-ventral group's substrate exhibited significantly higher redness (a = 32.4) and yellowness (b = 26.7) than that of the white-ventral group (a = 22.3; b = 8.9). These differences (Δa = 10.1; Δb = 17.8) suggest a visually reddish and yellowish environment for the red-ventral group. Although no statistical test was applied due to limited data, the observed color contrast may indicate a potential correspondence between coat color and substrate background, supporting ecological adaptation via background matching (Table 10).

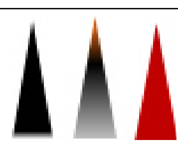
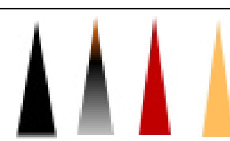
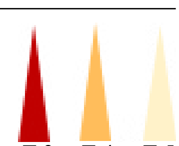
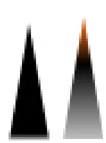
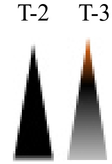
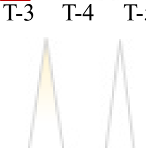
4. Discussion

The hair color banding type in *E. kalubu* shows clear variation between body areas and between ventral coat color. In the red-ventral group, the dorsal area is dominated by dark bands (T1–T2) which indicate high content of eumelanin, while the ventral area more frequently exhibits cream-yellow bands (T4–T5) which indicate high content of pheomelanin. These types align with pigment distribution observed in other mammals, where eumelanin is concentrated

Table 10. CIE Lab color values (L, a, b) of soil substrates collected from habitats of red-ventral and white-ventral *E. kalubu* individuals. ΔL, Δa, and Δb indicate the difference in color values between the two groups, showing that substrates color associated with red-ventral individuals tend to be darker, redder, and more yellow compared to those of white-ventral individuals

Substrate color of groups	Substrate color value					
	L	ΔL	a	Δa	b	Δb
Red-ventral	14.9	2.6	32.4	10.1	26.7	17.8
White-ventral	17.5		22.3		8.9	

Table 9. Distribution of hair color banding types in body areas: dorsal, lateral, and ventral between the red and white-ventral groups of *E. kalubu*

Phenotype color group	Dorsal	Lateral	Lateral
Red-ventral group	 T-1 T-2 T-3	 T-1 T-2 T-3 T-4	 T-3 T-4 T-5
White-ventral group	 T-1 T-2	 T-1 T-2	 T-6 T-7

Hair color banding types (T1–T7) are represented by a triangular icon showing the pigmentation: T-1: black; T-2: grey-dark; T-3: red; T-4: yellow; T-5: cream; T-6: white; T-7: white light dorsally to provide camouflage, and pheomelanin in the ventral area supports countershading (Caro 2005). In contrast, in the white-ventral group, the dorsal and lateral areas consistently feature dark bands (T1–T2), whereas the ventral area contains only light bands (T6–T7), indicating reduced eumelanin production. The absence of sex-based differences suggests that sexual dimorphism does not influence this banding distribution. Such variation indicates complex pigment regulation and potential adaptation to habitat background (Hoekstra 2006; Rowland 2009).

4.1. Differences in Coat Color Lab* Value between Red-Ventral and White-Ventral Groups with Habitat

We found that there are differences in coat color Lab* value between red-ventral and white-ventral groups. These findings align with previous studies examining intraspecific coat color variation using both visual assessments and quantitative measurements (Lab* value). Similar intraspecific coat color variation has been documented in other mammals, such as small rodents, *A. budini*. Significant differences in color parameters (Lab* value) were found between dark and orange coat color variants. The black-coated individuals exhibited lower L value and higher b values compared to the orange ones (Sandoval *et al.* 2017). We found that *E. kalubu* appears to follow similar patterns observed in placental mammals.

Our results showed that the red-ventral bandicoots inhabit environments with red and yellowish substrates. In contrast, white-ventral individuals are found in habitats with reduced red and yellow pigmentation in the background. The red-ventral and white-ventral groups were found inhabiting the same general area of former garden lands. However, the red-ventral group was typically observed in more closed vegetation, often located on sloped or hilly terrain. The white-ventral group was commonly found in more open habitats, often near stream edges or areas with sparser vegetation cover (Figure 2). These habitat preferences are consistent with previous observations of *E. kalubu* ecology in Papua New Guinea, where individuals are known to occupy a variety of forested environments, including secondary growth and disturbed habitats (Menzies 1991; Flannery 1995).

We predict that the observed coat color variation in *E. kalubu* may reflect ecological adaptation to background substrate color, aligning body coat color with local substrate color to enhance camouflage or reduce visual detection, consistent with patterns observed in other mammalian species (Caro 2005; McRobie *et al.* 2009; Caro and Mallarino 2020).

4.2. The Differences in Coat Color Lab* Values between Body Areas in the Red-Ventral and White-Ventral Group Correlated with Countershading

Significant differences in coat color Lab* values were observed between body areas within each group. The differences were most pronounced in all body areas, especially between dorsal-ventral areas, where the dorsal was consistently darker, and the ventral area lighter. These results suggest that the different coat colors in the body area may function as countershading. This classical adaptive strategy involves a darker dorsal area and a lighter ventral area, which reduces visual shadowing and flattens body contours under natural light, aiding in predator avoidance (Caro 2005; Rowland 2009). Although *E. kalubu* is primarily nocturnal, camouflage may still be important during daytime resting periods when individuals are exposed to potential diurnal predators or incidental disturbances. Moreover, many nocturnal predators such as wild boars and snakes possess good night vision (local people, personal communication), which could render the bandicoot visually conspicuous even under low-light or moonlit conditions. Therefore, dorsoventral pigmentation differences may serve a countershading function that protects both diurnal and nocturnal predators (Caro 2005; Rowland 2009; Howell *et al.* 2021).

Comparative evidence from small mammals further supports this interpretation. In deer mice (*Peromyscus maniculatus*), gradual transitions from dark dorsal to light ventral coat color improve camouflage by disrupting body outlines (Hoekstra *et al.* 2006; Linnen *et al.* 2009). Similarly, in the eastern grey squirrel (Sciurus carolinensis), dorsoventral pigmentation contrast aids in predator avoidance (McRobie *et al.* 2009). These parallels suggest that the variation in hair color banding and pigmentation across body areas in *E. kalubu* enhances crypsis through mechanisms comparable to countershading strategies observed in other mammals (Caro 2005; Howell *et al.* 2021).

4.3. Variations of Hair Color Banding between Body Areas in Red-Ventral and White-Ventral Groups

Fine-scale morphological analyses revealed that coat color in each body area was related to the composition of the hair color banding types, with some hair color band types only existing in particular body areas. These results suggest that each body area exhibits a characteristic pigmentation pattern, likely controlled by area-specific regulation of melanogenesis during hair growth. Melanogenesis, the biological process of melanin synthesis within melanocytes, is regulated by key genes such as MC1R and ASIP, which control the type and amount of melanin produced, eumelanin (dark brown/black) or pheomelanin (reddish-yellow) (Slominski *et al.* 2004; Hoekstra 2006; and Wolf *et al.* 2016). The MC1R gene promotes eumelanin production, while ASIP acts as its antagonist by inhibiting MC1R signaling, thereby shifting pigment synthesis toward pheomelanin and contributing to adaptive body coat color variation (Nachman *et al.* 2003; Steiner *et al.* 2007). Interestingly, our preliminary results of the amino acid sequences of the MC1R gene of *E. kalubu* suggested the different amino acid residues in the coding region of the individuals between red-ventral and white-ventral animals (Marker et al, unpublished results). Further research on these genes will provide insight into the mechanisms of adaptation in different genetic backgrounds.

This intraspecific variation in hair color banding type reflects similar findings in other mammals. For example, McRobie *et al.* (2009) reported area-specific hair color banding in grey squirrels (*Sciurus carolinensis*), with unbanded eumelanin-rich hairs dominating the dorsal area, and more varied or white band types present ventrally. Similarly, agouti banding in Peromyscus was shown to differ along the body axis, with implications for pigment regulation at the follicular level (Steiner *et al.* 2007). The banding differences observed in *E. kalubu* thus provide a morphological basis for investigating differential pigment deposition mechanisms among body areas.

Differences in hair color banding in Lab* values across body areas reflect area-specific pigmentation patterns that align with ecological adaptations, including countershading and background matching. Red-ventral individuals exhibited more diverse hair color banding and greater chromatic intensity ab value, especially in the ventral area, which correlated with darker, red-yellow substrates in their habitats.

Moreover, the observed hair color banding patterns offer a valuable morphological framework to investigate pigment regulation in marsupials. *E. kalubu* thus represents a promising model species for understanding the ecological and evolutionary mechanisms underlying coat color variation in non-placental mammals.

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