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Color Variability of Cosmopolitan Beetles in Mindanao, Philippines

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Abstract

Beetles (Insecta: Coleoptera) stands out as one of the most diverse insect groups, showcasing various color variations. These evolved color patterns present a fascinating trait crucial for understanding their evolution. However, studying these patterns poses challenges, given the intricate nature of colors in the natural world. While past researchers have explored beetle color patterns, quantifying colors requires costly equipment and sophisticated software. This paper introduces an alternative approach, utilizing digital images to examine color variability among Coleopterans. Forty-eight specimens from Mindanao, Philippines, were collected and photographed under consistent conditions. Subsequently, the images were calibrated and processed in R software to calculate image distances through cluster analysis. The results reveal that beetle color patterns fall into two categories: a dark color with deeper shades of gray and a moderately bright tone featuring a slight reddish hue with noticeable yellow highlights. *Chrysochroa fulminans* is the most distinct beetle across Coleopteran species due to its vibrant green coloration. Family-specific examination of color patterns revealed species with distinct color, *Anomala flavipennis* and *A. smaragdina* (Scarabaeidae), *Otiorhynchus* pauxillus (Curculionidae), *Uloma culinaris* (Tenebrionidae), and *Nupserha fricator* (Cerambycidae). The study's findings offer valuable insights into the evolution of Coleopterans, mainly their color patterns, serving as a valuable tool for classification.

Keywords

Digital images, cluster analysis, coleoptera, color variability, evolution

INTRODUCTION

Color patterns in beetles, which result from the interplay of pigment and structural-based coloration, are pivotal in ensuring their survival. The enormous variety of this outstanding trait across Coleopterans has been shown to have evolved repeatedly, making it a remarkable trait to understand their diversification. Coloration patterns are a notable and accessible trait for study and experimental manipulation, consisting of multiple interacting components with measurable functions, making it an excellent model for investigating evolutionary processes (Endler & Mappes, 2017). Moreover, colors provide information that may be used to identify and classify species, recognize individual characteristics, and indicate ecological or evolutionary elements of an organism's life (Badejo et al., 2020).

Beetles, constituting over 40% of all insect species, exhibit a vast diversity exceeding 450,000 species (Lawrence et al., 2011; McKenna et al., 2019; Sha'ari & Arumugam, 2019), with coloration patterns evolving to provide concealment from or deterrence of potential predators. These patterns serve various

functions, including crypsis, masquerade, motion dazzle, motion camouflage, defense, signaling, and physiological adaptations (Stevens & Merilaita, 2008; Badejo et al., 2020).

The study of color patterns in organisms encompasses a broad range of ecological and evolutionary topics, such as plasticity, habitat choice, and color polymorphism (Boyle & Start, 2019; Stanbrook et al., 2021; Martín-Vega & Baz, 2013). However, categorizing Coleopterans based on color patterns is challenging due to the extensive diversity and intricacy of these patterns. Attempts to quantify colors often require expensive equipment, sophisticated software, and expertise (Van den Berg et al., 2020; Badiane et al., 2017; Weller & Westneat, 2019). Nonetheless, using digital cameras presents a more practical and efficient method for analyzing coloration in organisms (Kendal et al., 2013).

This paper adopts an alternative and practical approach to analyze beetle color patterns in the anthropogenic areas of Mindanao, Philippines. Moreover, this investigation provided a comprehensive overview of Coleopteran color patterns and compared them according to their family, genus, and species.

METHODS

Sampling Sites

The beetles were collected from eight anthropogenic areas of Mindanao, Philippines (Figure 1). Using opportunistic sampling techniques, pitfall entrapment, insect netting, and manual handpicking were imposed to collect beetle samples. Then, the specimen was placed in a container with 70% ethanol before being transported to the laboratory for digital profiling.

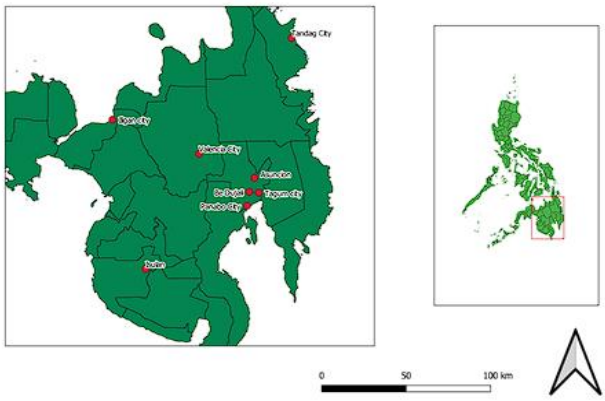


Figure 1. Map of the Collection Sites

Sample Preparation

Forty-eight (48) beetles, representing 12 different families, were gathered from various sampling sites included in the study. Table 1 provides the corresponding image codes assigned to each species, serving as labels for the subsequent graphs in this paper. The beetle samples were carefully positioned dorsally on a stereo microscope with a constantly aligned camera at a specific distance from the microscope eyepiece. Moreover, photographing the specimens took place in an enclosed dark room with exclusive illumination provided by the stereomicroscope, prohibiting other external light sources.

Table 1. Beetle species used in the study

Code	Species
SP01.jpg	*Acalolepta rusticatrix*
SP02.jpg	*Aclees hirayamai*
SP03.jpg	*Anomala flavipennis*
SP04.jpg	*Anomala marginata*
SP05.jpg	*Anomala smaragdina*
SP06.jpg	*Apriona aphetor*
SP07.jpg	*Batocera magica*
SP08.jpg	*Carlschoenherria sulcipennis*
SP09.jpg	*Chalcosoma atlas*
SP10.jpg	*Cheilomenes sexmaculata*
SP11.jpg	*Chrysochroa fulminans*
SP12.jpg	*Cordylocera atricornis*
SP13.jpg	*Colophotia concolor*
SP14.jpg	*Cryptalaus lacteus*
SP15.jpg	*Cylindera discreta elaphroides*
SP16.jpg	*Cylindera minuta*
SP17.jpg	*Derosphaerus vicinus*
SP18.jpg	*Dorcus parvulus*
SP19.jpg	*Epepeotes plorator*
SP20.jpg	*Eretes griseus*
SP21.jpg	*Eucorynus crassicornis*
SP22.jpg	*Figulus sulcicollis*
SP23.jpg	*Holotrichia bipunctata*
SP24.jpg	*Hoplocerambyx spinicornis*
SP25.jpg	*Lema pectoralis*
SP26.jpg	*Leucopholis furforosa*
SP27.jpg	*Leucopholis pulverulenta*
SP28.jpg	*Macrolinus sulciperfectus*
SP29.jpg	*Metapocyrtus adspersus*
SP30.jpg	*Metriorrhynchus sp.* (species 1)
SP31.jpg	*Metriorrhynchus sp.* (species 2)
SP32.jpg	*Nupserha fricator*
SP33.jpg	*Onitis phartopus*
SP34.jpg	*Onthophagus hielkemai*
SP35.jpg	*Oryctes rhinoceros*
SP36.jpg	*Otiorhynchus pauxillus*
SP37.jpg	*Pentodon algerinus*

SP38.jpg	*Platymetopus flavilabris*
SP39.jpg	*Podontia quatuordecimpunctata*
SP40.jpg	*Prionocerus coeruleipennis*
SP41.jpg	*Prosoplus bankii*
SP42.jpg	*Protaetia fusca*
SP43.jpg	*Pseudozaena orientalis*
SP44.jpg	*Pterolophia crassipes*
SP45.jpg	*Serica* sp.
SP46.jpg	*Sybra ochreovittipennis*
SP47.jpg	*Uloma culinaris*
SP48.jpg	*Zophobas morio*

Image Calibration

The digital images were processed using GIMP software version 2.13 (The GIMP Development Team, 2019). These images were calibrated through a simple white-balance correction and normalization. Moreover, the background of the photos was removed and replaced with a uniform white color. The resolution was adjusted to 700 pixels before saving the images as 24-bit Joint Photographic Experts Group (JPEG) files (Lenhert et al., 2011). These processed images were then organized into a separate folder for further analysis.

Colorimetric Analysis

Color quantification involves a three-step process: pixel binning, color conversion, and clustering (Figure 2). In pixel binning, the images were imported to R software (R Core Team, 2022), in which the plotPixels function of the colordistance package performed data reduction and normalization (Weller, 2021). In this process, the white background and photographic glares were excluded by setting the upper limit of RGB (Red, Green, and Blue) triplet colors to 0,0,0, the RGB triplet code for white color. This procedure eliminates unwanted elements from the images.

Then, the images were converted from RGB color space to Commission on Illumination 1976 L* a* b* (CIELab) using the LoadImage function. In the CIELab color space, Lightness (L*) is quantified on a scale from 0 (representing black) to 100 (representing white). Therefore, a higher L* value corresponds to a lighter color, while a lower value indicates a darker shade. The* channel spans from green to red on a scale of -120 to 120, where positive values represent red hues and negative values indicate green hues. On the other hand, the b* channel covers the range from blue to yellow on the same scale of -120 to 120, with positive values denoting yellow tones and negative values indicating blue tones (Ly et al., 2019).

A distance matrix was computed using the getColorDistanceMatrix function. This approach generated a heatmap, facilitating the comparison of each image based on the quantified color values. Then, the dominant color was determined by selecting the highest percentage of the quantified color that contributed to the image and then compared by beetle family, genus, and species.

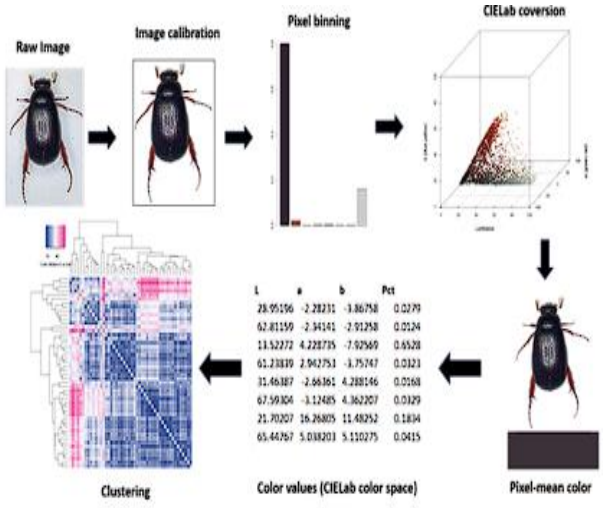


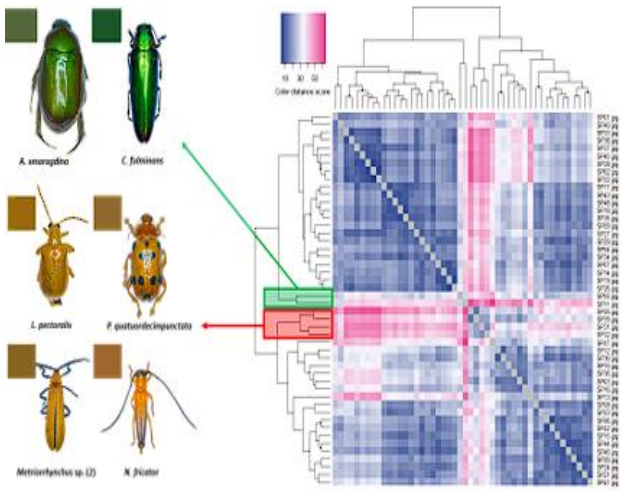
Figure 2. Colorimetric Analysis Workflow

RESULTS AND DISCUSSION

Overview of Coleopteran Coloration

The clustered heatmap shows the overall color variability of Coleopterans sampled in this study, with blue cells indicating a high degree of similarity (low color distance scores) and pink cells showing low similarity (high color distance scores). Based on the cluster heatmap, the color patterns of the Coleopterans collected from the anthropogenic environments are divided into two distinct clusters (Figure 3-A).

(A)



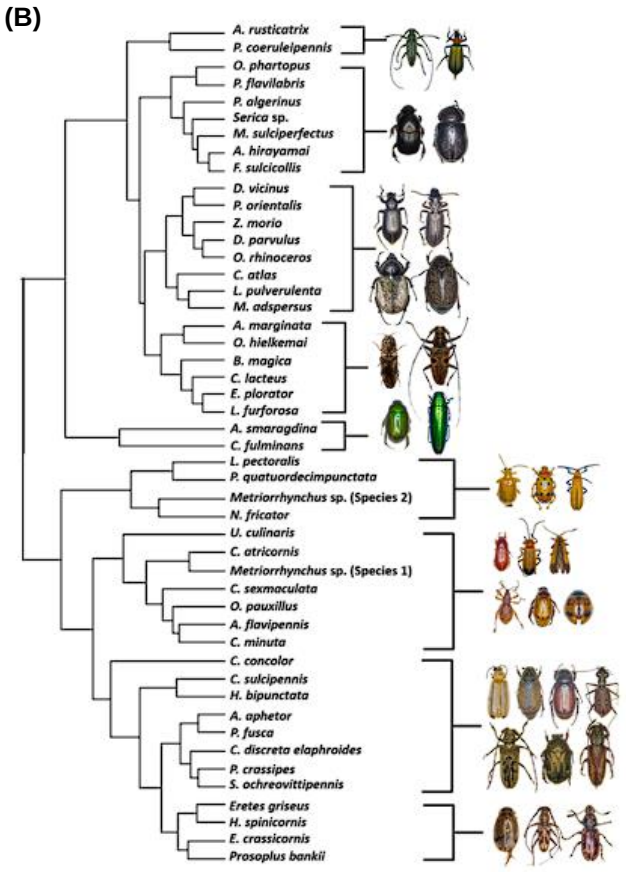


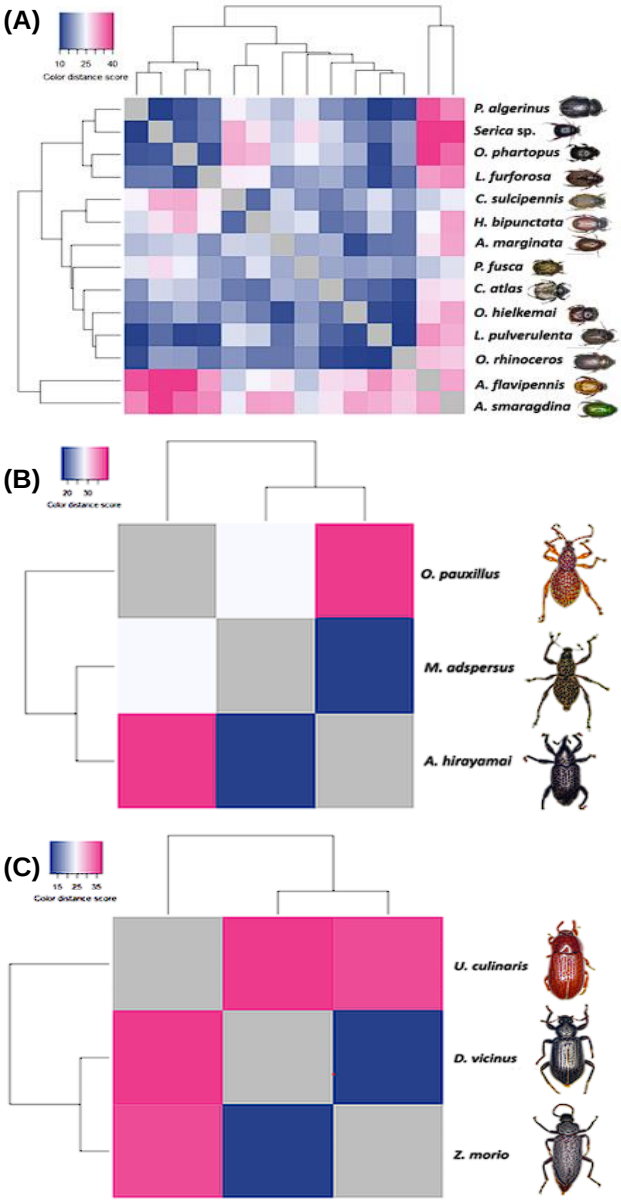
Figure 3. Color variability of Coleopterans (A). Overview of Coleopteran Coloration, (B) Clustering of Coleopterans based on their Body Coloration (Images not drawn to scale)

The first cluster is composed of darker beetles, with colors ranging from gray to black, while the second group is characterized by light-colored beetles ranging from red to yellow and orange. In the first cluster, the species *A. smaragdina* and *C. fulminans* are the most distinct beetles in this group, showing a distinctive greenish coloration. Furthermore, *C. fulminans* is the species that is distinct across Coleopteran species, as indicated by low similarity scores (pink cells). On the other hand, *L. pectoralis*, *P. quatuordecimpunctata, Metriorrhynchus* sp. (species 2), and *N. fricator* are beetles with distinct colors compared to the first cluster. The beetles in their respective clusters are depicted in Figure 3-B.

Color Variability Between Species Within Family

A comparative analysis of beetle species within their families was conducted to explore color variations. The Scarabaeidae, Curculionidae, Tenebrionidae, and Cerambycidae were specifically analyzed, as these families had more than three species in the study.

In the Scarabaeidae family, the results identified *A. flavipennis* and *A. smaragdina* as distinct species within the Scarab samples. Another group of scarab beetles exhibited darker colors, featuring prominent dark gray to red elements in their bodies in contrast to *A. flavipennis* and *A. smaragdina* (Figure 4-A). Within the Curculionidae family (weevils), *O. pauxillus* stood out as distinct from other weevils in this study, displaying a reddish coloration with a hint of yellow. Conversely, *M. adspersus* and *A. hirayamai* formed a group with darker colors *than O. pauxillus* (Figure 4-B). *U. culinaris* emerged as a distinct species among Tenebrionidae (darkling beetles) when colors were compared with *D. vicinus* and *Z. morio* (Figure 4-C). Furthermore, *N. fricator* emerged as a distinct Cerambycidae beetle species (Longhorn beetles) among the ten beetles sampled in the present study (Figure 4-D).



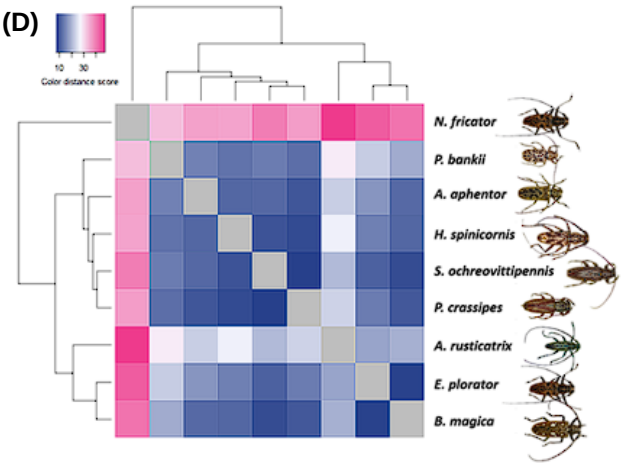


Figure 4. Color Variation of Beetle Species Based on Their Family Groups. (A) Scarabaeidae, (B) Curculionidae, (C) Tenebrionidae, (D) Cerambycidae (images are not drawn to scale)

Discussion

This paper presents a comprehensive classification of bettle coloration, highlighting two distinct categories: (1) deeper shades of gray and dark colors and (2) moderately bright tones with a slight reddish hue and noticeable yellow highlights. Moreover, the cluster heatmap revealed the occurrence of analogous individuals among Coleoptera, which denotes shared coloration patterns. The diverse coloration patterns observed and parallelism among Coleopterans are ascribed more to their functional morphology and evolutionary significance than their taxonomic position.

The emergence of these color patterns can be attributed to the presence of melanins, encompassing a spectrum ranging from deep black to rich reddish-brown, as well as the contributions of pterins, ommochromes, and carotenoids that infuse vibrant red, orange, and yellow hues (Badejo et al., 2020). These compounds can be transferred to the beetles through ingestion, causing them to express body coloration.

In leaf beetles (Chrysomelidae), those with a diverse range of host plants generally exhibit reduced red coloration compared to their counterparts with a more specialized diet. This implies that beetles with versatile dietary preferences may adopt a subdued red hue as a strategic measure to lower their visibility while navigating different ecological environments (Tan et al., 2017). This observation aligns with the coloration of the studied Chrysomelid beetles, *L. pectoralis* and *P. quatuordecimpunctata*, both recognized as polyphagous species, displaying a subtle red-orange tint in their bodies.

Interestingly, the polyphagous Melolonthinae beetles (*C. sulcipennis* and *H. bipunctata*) and *A. flavipennis* exhibit a prominent red element in their bodies. This coloration pattern is likely attributed to their shared dietary source, as insects obtain carotenoids (the pigments responsible for red, orange, and yellow hues) from their food (Badejo et al., 2020). These beetles gain a significant advantage from this coloration strategy, as the combination of red, orange, yellow, and white hues contributes to aposematism (Badejo et al., 2020). In the case of the Asian ladybird beetle *Harmonia axyridis* (Coccinellidae), the presence of red-orange elytra coloration indicates the conveyance of details regarding chemical defense to potential mates or predators (Bezzerides et al., 2007).

The diversity in color patterns among beetles is also attributed to their aposematic functions. Badejo and colleagues (2020) underscored that aposematic coloration reduces predation risk. For instance, *Geotrupes* beetles, *G. auratus* and *G. laevistriatus*, have evolved similar bright colorations, acting as a warning signal, especially for visually-oriented predators (Watanabe et al., 2022). Additionally, color patterns hold evolutionary significance for thermoregulation (Badejo et al., 2020). Insects with darker colors gain enhanced protection against ultraviolet radiation and excessive heat (True, 2003). For example, in *Harpalus affinis* (Carabidae), a dark bronze shade conferred selective advantages during periods of heightened pollution in urban areas, suggesting that varying degrees of urbanization could influence the prevalence of distinct color patterns in beetles (Keinath et al., 2020).

This study suggests that beetle coloration has undergone evolutionary changes over time, with these body colorations holding distinct evolutionary significance for the survival of beetles. Moreover, the observed variability in body coloration among Coleopterans indicates that species classification cannot rely solely on a single trait but requires the consideration of multiple characters.

CONCLUSIONS

This study employed digital images to explore the coloration patterns of beetles in Mindanao, Philippines. The analysis yielded a comprehensive examination of Coleopteran color variability, contributing to our understanding of the evolutionary processes that led to their diversification. The research results suggest that specific Coleopteran groups can be effectively classified through

quantitative color analysis, presenting a potential tool for the systematic classification of Coleopterans.

Utilizing digital image-based color profiling presents a non-invasive methodology; however, it is imperative to acknowledge that some beetle species exhibit iridescent color patterns. These patterns can considerably influence the results, particularly when observing the beetle from various angles. Furthermore, it is also essential to delve into the underlying color mechanisms to investigate how distinct non-homologous structures can contribute to chromatic effects. Recognizing this limitation and addressing it in subsequent research endeavors is imperative.

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