Article

Phenetic relationships among selected synanthropic beetles inferred from morphometric analysis

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Abstract
Changes in the size of insect morphology, notably in beetles (Insecta: Coleoptera), may come from disturbances happening in anthropogenic contexts. Beetles living in anthropogenic situations may evolve similar sizes in their physical characteristics, which can lead to systematic discrepancy. This study examined how synanthropic beetles' morphometric characteristics varied within and between species. The present investigation collected 149 individual beetles from 18 families, and 11 morphological parameters were measured. Adonis and Similarity Percentage (SIMPER) analyses were performed after an NMDS (non-metric multidimensional scaling) plot was created to evaluate the similarities between various groups. The length of the body, elytra, antennae, and pronotum width were the most distinctive characteristics among beetles, which all share similar shorter character attributes. This work provides insights into the morphometrics of synanthropic beetle species in Mindanao, Philippines. The findings not only corroborate prior studies but also emphasize how the environment may influences the size and adaptability of beetles.

Keywords anthropogenic; Coleopterans; sisparity; morphometry; Philippines.

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1 Introduction

For characterizing a taxon, qualitative evaluations of biological structures are frequently used. However, this approach has several drawbacks, especially when evaluating characteristics that can be measured. Morphological qualities, such as body size, length, and shape, are quantifiable characteristics that majorly impact an organism's fitness (Soto et al., 2019). It is difficult to categorize and describe resilient beetles in anthropogenic contexts when these features change in response to anthropogenic disturbances, as there may be similarities among, within, and across beetles due to evolutionary mechanisms.

The natural environment of beetles (Insecta: Coleoptera) is significantly impacted by urbanization and the conversion of forest lands, which alters their geographic range. Although some beetles may thrive in urban and agricultural contexts, these anthropogenically created habitats also bring about disturbances that might affect the phenotypic features of Coleopteran populations. According to Armijos et al. (2023), conventional farming methods and human habitation introduce a variety of stressors that can lead to microevolutionary events. The traits of organisms capable of adapting to environmental change modify, making these events visible. Hernández and colleagues (2011) emphasized the significance of the environment in creating variations in morphological features.

In this research, we investigate whether there are morphological parallels or differences between beetles found in anthropogenic environments. This study specifically determined the morphological similarities across synanthropic beetles and identified the physical characteristics contributing to the differences between beetle species. In order to evaluate beetles' adaptability and foretell their ecological interactions, it is crucial to comprehend how their morphology reacts to anthropogenic surroundings.

2 Materials and Methods

2.1 Sampling sites

The collection sites were established in anthropogenic habitats or human-disturbed ecosystems throughout urban and agricultural regions of Mindanao, Philippines (Fig. 1). These sampling locations were Asuncion, Davao del Norte; B.E. Dujali, Davao del Norte; Iligan City; Isulan, Sultan Kudarat; Panabo City; Tagum City; Tandag City; and Valencia City. Residences and privately held agricultural grounds were the collection sites. The beetles were gathered in the present investigation using convenience and opportunistic sampling methods. Additionally, several trapping techniques were used, such as pitfall trapping (for the collection of ground beetles), insect netting (for the collection of flying beetles), and handpicking (for the collection of large beetles) (Torrejos et al., 2020). The collection period lasted from August 2022 to January 2023, from 0700H to 2100H. The collected samples were then kept in a jar filled with 70% ethanol before being transported into the lab for morphological analysis.

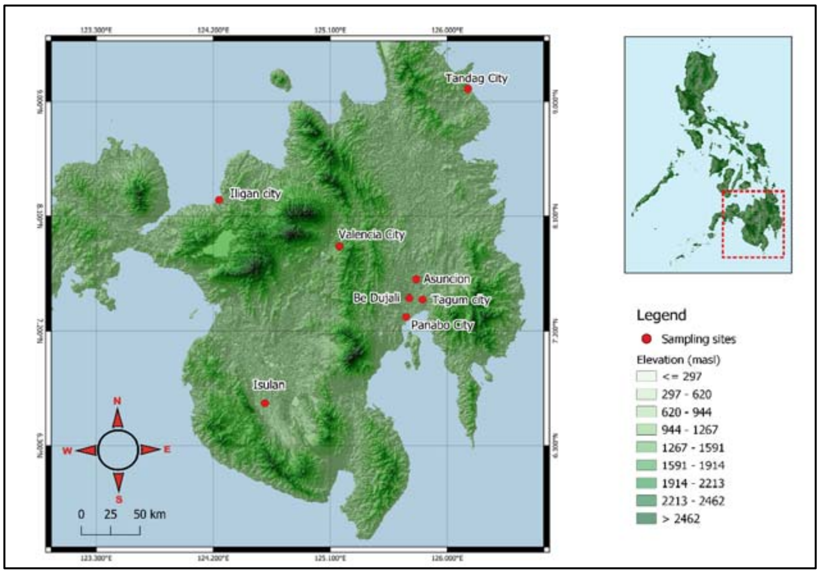

Fig. 1 Map of Mindanao, Philippines and the location of collection sites.

2.2 Sample preparation

The gathered beetles underwent meticulous preservation and air-drying before being photographed alongside a ruler beneath a stereomicroscope. The specimens were oriented dorsally, with their heads directed towards the anterior region of the plane. The Image J software (Schneider et al., 2012) was employed to quantify the beetles' character traits by aligning the measurements with the calibrated scale in the digital images. The head, pronotum, elytra, antennae, tibia, and total body length, which runs from the anterior to the posterior end of the specimen, are among these morphometric feature traits (Jaskula et al., 2021). Due to their inherent bilateral symmetry, average dimensions were calculated for the paired morphological attributes, such as the left and right antennae, elytra, and appendages (Fig. 2).

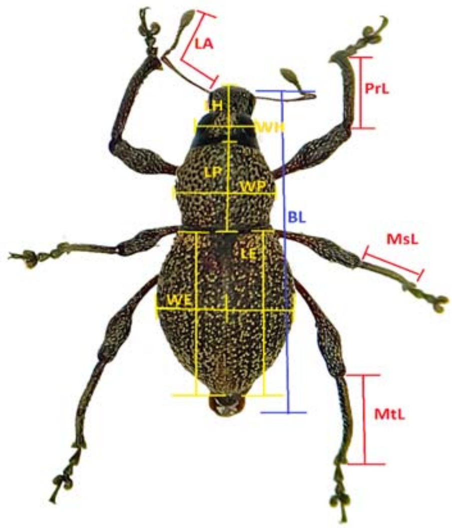

Fig. 2 Character trait description of the 11 morphometric traits in beetles: length of antennae (LA), length of the head (LH), width of the head (WH), length of pronotum (LP), width of pronotum (WP), length of elytra (LE), width of elytra (WE), protibial length (PrL), mesotibial length (MsL), metatibial length (MtL), and total body length (BL).

2.3 Statistical analyses

Using the Bray-Curtis similarity index in the non-metric multidimensional scaling (NMDS) plot, it was possible to compare the quantitative morphological attributes of Coleopteran species in anthropogenic environments. According to how similar they were, the specimens were grouped together using this ordination technique, which also ordered them into various variables that were condensed into a two- or three-dimensional space (Jaskula et al., 2021). As a result, the data points in the NMDS plot that are ordinated close together are probably more comparable than those that are ordinated far away.

A nonparametric Adonis analysis was performed to investigate the differences between two or more groups established in the NMDS plot based on their distinctive characteristics because the assumptions of the normal data were not satisfied (Armijos et al., 2023). Additionally, the body characteristics in the multivariate data that most strongly influence group dissimilarity were identified using the Similarity Percentage (SIMPER). The R programming language was used for all data analysis (R Core Team, 2022). For NMDS, Adonis, and SIMPER studies, the R *vegan* package (Oksanen et al., 2020) was used, while the *ggplot2* package (Wickham, 2016) was utilized for data visualization.

3 Results and Discussion

3.1 Frequency distribution of beetles

The current study found 149 individual beetles from 18 different families, 118 of which were gathered from urban areas and 31 from agricultural settings (Table 1).

Table 1 Frequency of Coleopterans and respective families and collection sites.

Species	Family	Frequency
Acalolepta rusticatrix (Fabricius, 1801)	Cerambycidae	1
Aclees hirayamai (Kôno, 1933)	Cucurlionidae	1
Anomala flavipennis (Burmeister, 1844)	Scarabaeidae	4
Anomala marginata (Fabricius, 1792)	Scarabaeidae	2
Anomala smaragdina (Eschscholtz, 1822)	Scarabaeidae	2
Apriona aphetor (Newman, 1842)	Cerambycidae	1
Batocera magica (Thomson, 1859)	Cerambycidae	1
Carlschoenherria sulcipennis (Laporte, 1840)	Scarabaeidae	13
Chalcosoma atlas (Linnaeus, 1758)	Scarabaeidae	1
Cheilomenes sexmaculata (Fabricius, 1781)	Coccinilidae	4
Chrysochroa fulminans (Fabricius, 1787)	Buprestidae	6
Cordylocera atricornis (Guérin-Méneville, 1838)	Cantharidae	1
Colophotia concolor (Olivier, 1886)	Lampyridae	5
Cryptalaus lacteus (Candeze, 1857)	Elateridae	1
Cylindera discreta elaphroides (Doktouroff, 1882*)*	Cicindelidae	6
Cylindera minuta (Olivier, 1790)	Cicindelidae	6
Derosphaerus vicinus (Pic, 1923)	Tenebrionidae	2
Dorcus parvulus (Hope & Westwood, 1845)	Lucanidae	2
Epepeotes plorator (Newman, 1842)	Cerambycidae	1
Eretes griseus (Fabricius, 1781)	Dytiscidae	1
Eucorynus crassicornis (Fabricius, 1802)	Anthribidae	4
Figulus sulcicollis (Hope, 1845)	Lucanidae	1
Holotrichia bipunctata (Brenske, 1892)	Scarabaeidae	3
Hoplocerambyx spinicornis (Newman, 1842)	Cerambycidae	1
Lema pectoralis (Baly, 1867)	Chrysomelidae	10
Leucopholis furforosa (Chevrolat, 1841)	Scarabaeidae	1
Leucopholis pulverulenta (Burmeister, 1855)	Scarabaeidae	9
Macrolinus sulciperfectus (Kuwert, 1891)	Passalidae	1
Metapocyrtus adspersus (Schultze, 1925)	Curculionidae	7
Metriorrhynchus sp. (sp1)	Lycidae	1
Metriorrhynchus sp. (sp2)	Lycidae	4
Nupserha fricator (Dalman, 1817)	Cerambycidae	1

Table 1 (cont.) Frequency of Coleopterans and respective families and collection sites.

Species	Family	Frequency
Onitis phartopus (Lansberge, 1875)	Scarabaeidae	2
Onthophagus hielkemai (Meindert, 2019)	Scarabaeidae	1
Oryctes rhinoceros (Linnaeus, 1758)	Scarabaeidae	14
Otiorhynchus pauxillus (Rosenhauer, 1847)	Curculionidae	1
Pentodon algerinus (Fuessly, 1788)	Scarabaeidae	1
Platymetopus flavilabris (Fabricus, 1798)	Carabidae	1
Podontia quatuordecimpunctata (Linnaeus, 1767)	Chrysomelidae	5
Prionocerus coeruleipennis (Perty, 1831)	Prionoceridae	7
Prosoplus bankii (Fabricius, 1775)	Cerambycidae	5
Protaetia fusca (Herbst, 1790)	Scarabaeidae	1
Pseudozaena orientalis (Klug, 1831)	Carabidae	2
Pterolophia crassipes (Weidemann, 1823)	Cerambycidae	1
Serica sp.	Scarabaeidae	1
Sybra ochreovittipennis (Breuning, 1964)	Cerambycidae	1
Uloma culinaris (Linnaeus, 1758)	Tenebrionidae	1
Zophobas morio (Fabricius, 1776)	Tenebrionidae	1

The morphometric character features of beetles are positively skewed to the right, with the tail of the distribution extending towards the higher values on the x-axis, as can be seen by taking a closer look at the histogram. Therefore, fewer beetles have more extended character traits, and a greater number of beetles with shorter body lengths, which include the length of antennae (LA), length of the head (LH), the width of the head (WH), length of pronotum (LP), the width of pronotum (WP), length of elytra (LE), the width of elytra (WE), protibial length (PrL), mesotibial length (MsL), metatibial length (MtL), and total body length (BL) (Fig. 3).

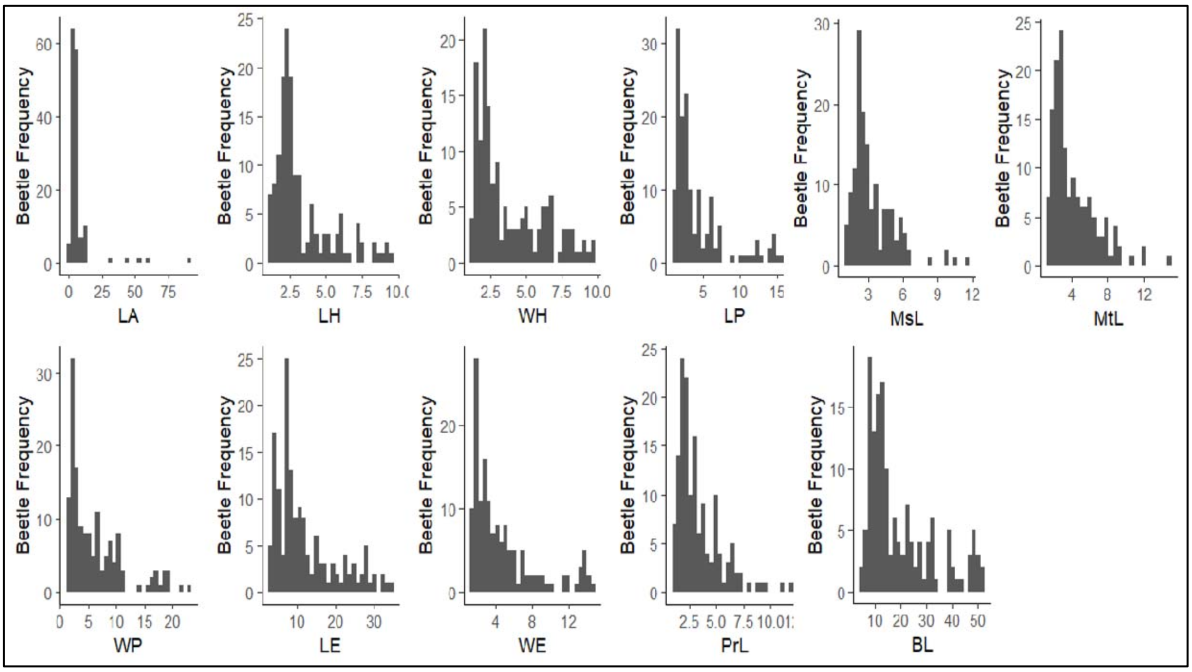

Fig. 3 Frequency distribution of beetles according to morphometric traits.

3.2 Phenotypic variations among beetle species

As can be seen from the ordination plot in Fig. 4, there is a slight separation within *Cylindera* species, *C. discreta elaphroides* and *C. minuta*, indicating subtle similarities between them. Additionally, the *C. discreta elaphroides* population is more dispersed than the *C. minuta* population, suggesting that *C. discreta* has more varied body characteristics than *C. minuta.* Together, these species form a pattern that denotes a family grouping for the Cicindelidae. On the other hand, *O. phartopus* and *O. hielkemai* differ from other Scarabaeidae yet share certain similarities. Likewise, despite both species belonging to the Chrysomelidae family, *L. pectoralis* and *P. quatuordecimpunctata* share only a passing resemblance. Individual data points closely cluster together within *L. pectoralis, C. sulcipennis*, and *A. smaragdina*, indicating a high degree of similarity of the measured variables within these species.

The data points are not compact within *E. crassicornis*, indicating differences in the assessed body features within the species. In the Curculionidae cluster, *M. adspersus*, *O. pauxillus*, and *A. hirayamai* are separate species. Individuals of *P. bankii* and *C. concolor* also have this pattern, suggesting that these species vary, even though the data points are not nearly similar.

The species *C. sulcipennis*, *L. pulverulenta*, *H. bipunctata*, and *A. flavipennis* are also known to overlap. *N. fricator* shares space with the *Metriorrynchus* sp. (species 2) cluster. As opposed to other Coleopterans, *E. griseus*, *C. lacteus*, *O. pauxillus*, *O. hielkemai*, and *A. hirayamai* are separate species. The NMDS figure demonstrates distinct clustering and separation of several beetle species.

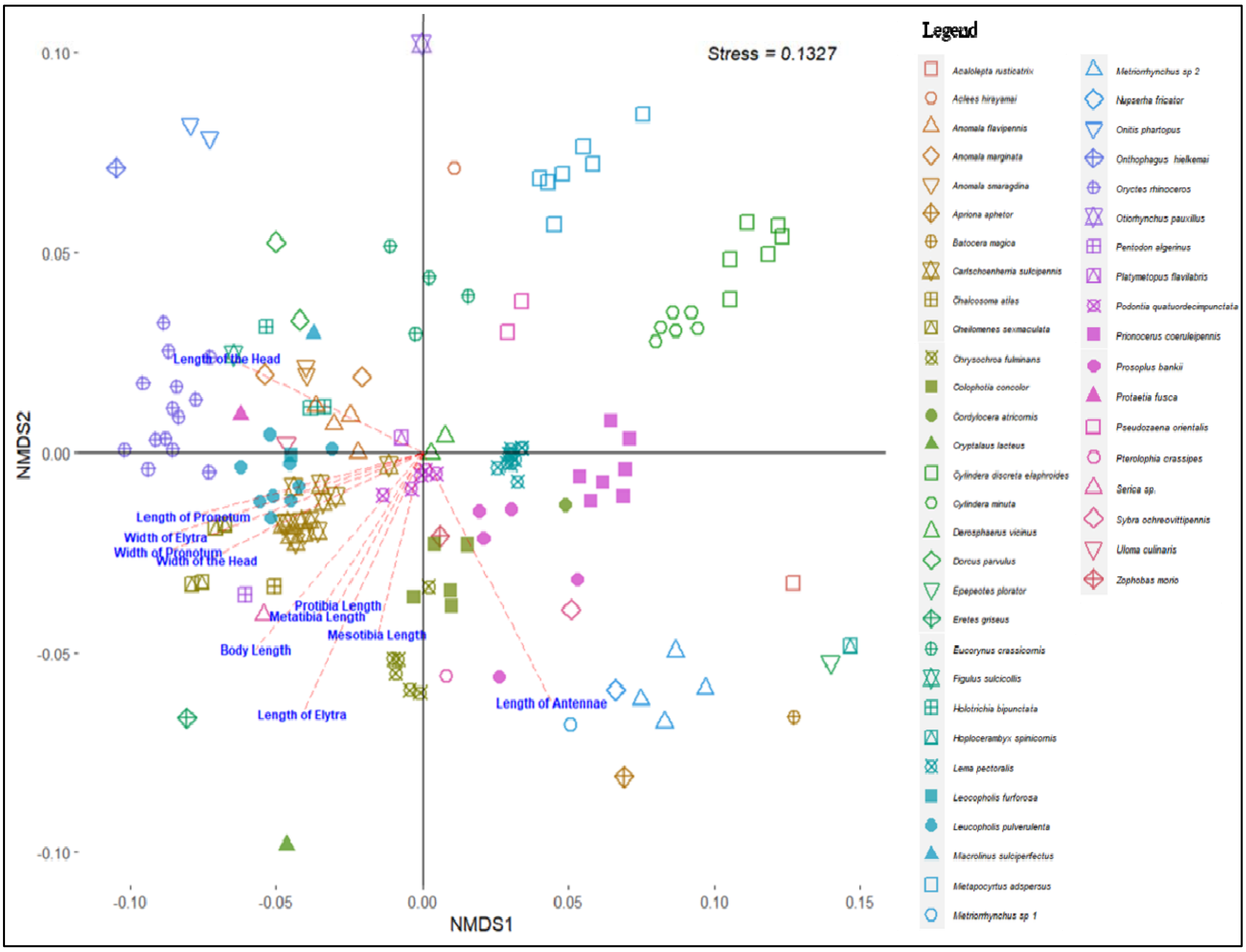

Fig. 4 NMDS plot of the 11 body parameters among synanthropic beetles.

The SIMPER analysis shows that BL had an average disparity of 8.94, followed by LE, with an average dissimilarity of 5.50. The average distinctions for LA and WP are 3.73 and 3.37, respectively (Table 2). These morphological factors explained the discrepancy seen among species when pooled together in 64.46% of cases. The overall dissimilarity index is calculated to be 33.42, meaning that, on average, the species showed a 33.42% dissimilarity concerning these body parameters. According to the findings, the sampled species are highly similar when grouped.

Table 2 SIMPER analysis of body parameters pooled by species.

Body parameters	Average Dissimilarity	Contrib. %	Cumulative %	Overall dissimilarity
BL	8.935	26.73	26.73	33.42
LE	5.501	16.46	43.19	
LA	3.732	11.17	54.36	
WP	3.371	10.09	64.44	
WE	2.386	7.14	71.58	
LP	2.191	6.555	78.14	
WH	1.695	5.072	83.21	
MtL	1.677	5.018	88.23	
PrL	1.4	4.188	92.42	
LH	1.299	3.886	96.3	
MsL	1.236	3.697	100	

Legend: LA = Length of Antennae; LH = Length of Head; WH = Width of the Head; LP = Length of Pronotum; WP = Width of Pronotum; LE = Length of Elytra; WE = Width of Elytra; PrL = Protibial Length; MsL = Mesotibia Length; MtL = Metatibia Length; BL = Body Length

3.3 Disparities in body parameter composition

The outcomes of the Adonis analysis, which used 999 permutations based on the distance matrix of 11 observable morphological body traits, are shown in Table 3. The R-square (R^2) score quantifies the proportion of the anticipated variability in the distance matrix that can be ascribed to the examined factor, which is the grouping based on species. Results indicate that the differences in the distance matrix of the body parameters are significantly explained by species (R^2 = 0.985, p = 0.000999). Furthermore, the substantial R^2 value underscores that species classifications account for 98.5% of the observed variance in the measured body parameters. Therefore, 98.5% of the differences in their body parameters of synanthropic beetles, can be attributed to the different species present in the dataset.

Table 3 Adonis results based on Bray-Curtis similarity index

Factor	*df*	SumSq	R^2	*F* ratio	*p*-value
Species	47	10.05	0.985	141.51	0.000999

3.4 Assessing the degree of similarity between species within families

To assess the extent of similarity among species within their corresponding families, a SIMPER (Similarity Percentage) analysis was conducted (Tab. 4). Among the groups with fewer species, those with a dissimilarity value below 15 were considered; however, the comparison considered overall dissimilarity values lower than 10 for the Scarabaeidae and Cerambycidae families. A lower dissimilarity value suggests comparability among the samples, whereas a higher value signifies distinctness among the sampled groups.

Table 4 The similarity percentage of Coleopteran species within their respective families (Values in parentheses represent the percentage contributions to the dissimilarity of the morphometric traits.).

Scarabaeidae	*L. pulverulenta*	*A. flavipennis*	*P. algerinus*	*A. marginata*
A. marginata	27.67 (BL, LE, WE, WP = 72.3%)	6.949 (BL, LE, WP, PrL = 68.06%)	59.87 (BL, LE, WP, WE = 66.62%)	N/A
C. atlas	31.14 (BL, LE, WP, MtL = 62.67%)	61.43 (BL, LE, WP, MtL = 62.8%)	3.714 (LA, MtL, LP, WH = 70.58%)	62.01 (BL, LE, WP, MtL = 63.28%)
H. bipunctata	8.32 (BL, LE, WE, WP = 65.18%)	31.99 (BL, LE, WP, WH = 63.25%)	33.6 (BL, LE, WP, LP = 67.45%)	32.84 (BL, LE, WP, WH = 66.19%)
L. furforosa	6.823 (BL, LE, WE, LH = 61.17%)	42.44 (BL, LE, WP, MtL = 71.6%)	22.75 (BL, LE, WP, PrL= 67.56%)	43.21 (BL, LE, WP, WE = 66.67%)
P. fusca	37.92 (BL, LE, WE, WP = 65.83%)	7.809 (BL, LP, WP, LA = 74.88%)	59.69 (BL, LE, WP, WE = 65.78%)	5.426 (LP, WP, BL, LH = 51.5%)
Cucurlionidae	*M. adspersus*			
O. pauxillus	12.2 (WE, LA, MsL, PrL = 66.83%)			
Chrysomelidae	*L. pectoralis*			
P. quatuordecimpunctata	10.79 (BL, LE, WP, WE = 66.4%)			
Cicindelidae	*C.discreta elaphroides*			
C. minuta	5.381 (BL, LE, LA, WE = 60.86%)			
Cerambycidae	*B. magica*	*S. ochreovittipennis*	*P. bankii*	
A. aphetor	5.46 (LA, MsL, MtL, BL = 69.4%)	63.05 (LA, BL, LE, WP = 74.22%)	56.6 (LA, BL, LE, WP = 75.53%)	
P. crassipes	66.93 (LA, BL, LE, MtL = 72.62%)	8.279 (LA, LH, BL, LE = 80.3%)	13.97 (LA, BL, LE, MtL = 64.25%)	
N. fricator	61.47 (LA, BL, LE, PrL = 71.23%)	8.706 (LA, BL, WE, MtL = 76.05%)	9.435 (LA, WE, WP, BL = 63.82%)	

Species *L. pulverulenta* and *H. bipunctata* are found to be comparable to one another within the Scarabaeidae family, having an overall dissimilarity of 8.32. Body Length (BL), Length of Elytra (LE), Width of Elytra (WE), and Width of Pronotum (WP), which accounted for 65.18% of their observed dissimilarity, are the defining characteristics between these two species. The average discrepancy between *L. pulverulenta* and *L. furforosa*, which is 6.82, also shows similarities. The BL, LE, WE, and Length of Head (LH) are the distinctive bodily characteristics that separate them and account for 61.17% of their dissimilarity.

With an overall dissimilarity of 6.95, *A. flavipennis* and *A. marginata* among the tested *Anomala* species exhibit the most similarity. They differ from one other by BL, LE, WP, and Protibial Length (PrL), which together account for 68.96% of their differences. *A. flavipennis* also shows similarities to *P. fusca*, with a dissimilarity score of 7.89 overall. Their differences in BL, Length of Pronotum (LP), WP, and Length of Antennae (LA) account for 74.88%. With a total dissimilarity of 5.426, *A. smaragdina* and *P. fusca* are also comparable to one another. They differed in LP, WP, BL, and LH, accounting for 51.5% of their observed dissimilarity. Last, the Dysnastinae subfamily members *C. atlas* and *P. algerinus* show similarities with a dissimilarity score of 3.71. LA, Metatibial Length (MtL), LP, and Width of Head (WH) are the body parameters that separates them, accounting for 70.58% of the difference.

Despite the NMDS plot showing only slight similarities between these two species, *L. pectoralis* and *P. quatuordecimpunctata* share characteristics with the Chrysomelidae family and have an average dissimilarity of 10.79. Body Length (BL), Length of Elytra (LE), Width of Pronotum (WP), and Width of Elytra (WE), which together account for 66.4% of their dissimilarity, are the main elements that distinguish them. Only *O. pauxillus* and *M. adspersus* of the Cucurlionidae family share similarities, with an overall disparity of 12.2. They differ 66.83% from each other in terms of the body characteristics WE, Length of Antennae (LA), Mesotibial Length (MsL), and Protibial Length (PrL). The average disparity between *C. minuta* and *C. discreta elaphroides* in the Cicindelidae family is 5.381. The defining characteristics, which accounted for 60.86% of their distinctiveness, were BL, LE, LA, and WE.

On the other hand, the overall dissimilarity between *B. magica* and *A. aphetor* of the Cerambycidae is 5.46, indicating similarities. The body parameters LA, MsL, MtL, and BL are responsible for 69.4% of the observed dissimilarity and contribute to it. The similarities between *S. ochreovittipennis* and *P. crassipes* and *N. fricator* are 8.279 and 8.706 overall, respectively. LA, LH, BL, and LE make up the differences between *S. ochreovittipennis* and *P. crassipes*, accounting for 80.3% of the differences. In contrast, the differences between *S. ochreovittipennis* and *N. fricator* can be attributed to LA, BL, WE, and MtL, which account for 76.05% of the disparity. With an overall dissimilarity of 9.43, *P. bankii* and *N. fricator* show considerable similarity. They are different in LA, WE, WP, and BL, accounting for 63.82% of their difference.

4 Discussion

The patterns of grouping and separation seen in this study support the hypothesis that different physical characteristics are connected to distinct taxonomic groups. The similarities between *O. phartopus* and *O. hielkemai* and their distinction from other Scarabaeidae support previous studies' findings that both species belong to the Scarabaeinae subfamily, which includes coprophagous species (Latha and Sabu, 2018). *L. pectoralis* and *P. quatuordecimpunctata* are grouped under the family of leaf beetles known as Chrysomelidae because of their similarities. Nevertheless, there is a discrepancy between the findings obtained from NMDS and SIMPER analyses. The SIMPER analysis resembles these species; however, the NMDS plot shows different data points for *L. pectoralis* and *P. quatuordecimpunctata*. The various primary clades within the Chrysomelidae family may be responsible for this contradictory result. Specifically, *L. pectoralis* falls within the Criocerinae subfamily in the "sagrine" clade, whereas *P. quatuordecimpunctata* is classified under the separate Alticinae subfamily within the "chrysomeline" clade (Nie et al., 2020).

The observed variation between *C. discreta elaphroides* species, and the similarity between *C. minuta* species in tiger beetles may indicate that their interaction impacts them. The diversity in body characteristics seen in *C. discreta elaphroides* may indicate the species' strategy for avoiding competing with *C. minuta* for resources, given that both *C. discreta elaphroides* and *C. minuta* were taken from the same environment. According to an experiment by Brosius and Higley (2013), co-occurring tiger beetles used various thermoregulatory behaviors to reduce competition with other tiger beetle species. Due to variations in body size and physical traits, including wing loading and insulation, changes in thermoregulatory behaviors have been noted (Merrick and Smith, 2004). The similarities between *C. sulcipennis*, *L. pulverata*, *H. bipunctata*, and *A. flavipennis*, phytophagous beetles, align with the cladistics findings in this paper's Chapter 2. Additionally, these species are supported by Calcetas and colleagues (2017; 2021) as Melolonthinae members, which view them as economically significant and severely destructive pest species in the Philippines.

Insights into the phylogeny, ecology, and selective pressures influencing the development of a particular taxon can be gained through studying morphology, which is influenced by both genotype and phenotype (Losos and Miles, 1994). Insects' morphological characteristics are greatly influenced by their environment, allowing them to adapt and fill unique ecological niches (Armijos et al., 2023). The present data's positively skewed distribution implies that more beetles have shorter body characteristics than average, which may result from particular biological or environmental variables that encourage the evolution of smaller bodies. Because larger body parts take longer to grow and are therefore more vulnerable to predation, reduced size, for example, promotes early reproduction and lowers the chance of mortality (Suárez et al., 2011). Additionally, a smaller size allows beetles to use less energy, which frees up more energy for vital tasks, including feeding, reproduction, and immunological responses (Mamantov and Sheldon, 2021).

Armijos et al. (2023) speculate that global warming may result in smaller bodies. The sample's beetles are probably smaller since they were mostly gathered from urban and agricultural regions. This result validates the findings of a study by Armijos and colleagues from the year 2023, which found that *Dichotomius problematicus* (Coleoptera: Scarabaeidae: Scarabaeinae) had longer body parts in forested areas than in grasslands as a result of the availability of resources. In addition, mature dung beetles experience a reduction in body size due to rising temperatures. To preserve energy for vital processes, including feeding, reproduction, and immune responses, the beetles have shrunk in size (Carter and Sheldon, 2020). The NMDS analysis result on the measured body parameters of beetles gathered from urban and agricultural settings suggests that these body characteristics are comparable. In light of this, it is possible that the beetles would experience similar environmental pressures, which may cause the formation of related traits in several lineages (Washburn et al., 2016).

The length of the body, elytra, antennae, and pronotum width were found to be the body parameters contributing to the dissimilarity among Coleopterans in the current analysis. These characteristics might be essential for allowing beetles to carry out their particular tasks in a habitat unrelated to their common ancestry. For instance, as the antennae are typically shorter in visual hunters than in tactile hunters, the length and form of the antenna are related to habitat preference and hunting skill (Talarico et al., 2007). Additionally, species with larger eyes, broader heads, and longer pronota are thought to have evolved specializations for visual hunting. As opposed to this, species with smaller eyes, a flatter pronotum, and smaller antennae are better suited to interstitial behaviours and digging activities (Pacheco et al., 2022). To avoid desiccation in arid environments, flightless species exhibit rounder, shorter elytra (Stanbrook et al., 2021).

5 Conclusion

This study offers interesting information on the phenetic links among beetle species in anthropogenic contexts based on multivariate data on the magnitude of morphological features. The findings support earlier research and highlight how the environment influences beetle form and adaptation. Additional investigation is required to determine the additional factors that affect morphological variation and to appreciate the ecological significance of these characteristics for the evolution and diversity of beetles.

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