Article

Elytron shape variability among selected synanthropic beetles from Southern Philippines

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Abstract
The shape of biological structures is frequently studied using qualitative features, although this methodology can produce ambiguities and score mistakes. In addition, the lack of homologous locations and the challenges in comparing linear measurements limit the ability of standard morphometrics to quantify shape. Geometric Morphometrics (GM) is an alternate method for examining an organism's shape that addresses the shortcomings of conventional morphometrics and qualitative features. This study used GM to investigate the phenetic relationships of selected synanthropic Coleopteran species based on the shape of their elytron, which is an autapomorphic trait of beetles. Using Principal Component Analysis (PCA), it was possible to see how the average shape of these structures changed over time, and the results showed considerable shape differences between species. The sampled elytra displayed a broad shape with outward displacement on the anterior part, and a concave shape with a displacement on the mid-lateral section (PC1 = 84%, PC2 = 6.79%). These structures represent adaptations seen in Coleopteran species, which offer insightful information about the morphological evolution of elytron and to understand diversification of beetles.

Keywords autapomorphic; coleopterans; diversity; geometric morphometrics; phenetic relationships.

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1 Introduction

The phenetic relationship of beetles (Insecta: Coleoptera) is mainly evaluated through qualitative traits. However, using qualitative descriptions to represent biological shape structures generates inconsistencies, especially when grading character states. Even with multivariate morphometrics, quantitative morphology, and statistical analysis, quantifying shapes using conventional morphometrics presents difficulties. These issues are brought on by the difficulty in determining the homology of linear measures when homologous points are absent over some distances. If the same distance measurements were gathered from several shapes, it might also reduce the statistical power in different forms.

Additionally, the ability to discern between various quantifiable shapes may be hampered when employing the same distance measurements in multiple configurations. Finally, because geometric relationships may only be partially captured by linear distances, pictorial representations of structures frequently lack precision (Adams et al., 2004). Considering this, measuring shapes with linear measures is subject to misunderstanding and may result in taxonomic discrepancies, mainly when working with cryptic species.

A valuable alternative for efficiently examining organismal morphology is geometric morphometrics (GM). Multiple elements of morphological adaptation in Coleopterans have been studied through GM analysis. For instance, Eldred et al. (2016) considered interspecific and intersexual differences while examining size and shape variations among stag beetles. Additionally, this method was utilized to assess the morphological adaptations of ground beetles that live in caves (Chen et al., 2021). Additionally, Hernández et al. (2011) employed GM to investigate the competitive interspecific interactions among neotropical dung beetles, while Ren and colleagues (2017) studied the functional and morphological changes in leaf beetles. GM was used to investigate the interspecific and inter-sexual variation within the *Colophon* species (Coleoptera: Lucanidae) (Eldred et al., 2016). This research highlights how GM has been applied to enhance our knowledge of Coleopteran morphology, adaptability, and evolution.

While earlier GM research mainly concentrated on analyzing certain Coleopteran families or species, few published data use GM analysis of several shapes among a wider variety of Coleopteran species. To fill this gap, the current work used GM analysis to look at the links between Coleopteran species using multivariate shape data. This study used GM analysis to look at the shape variation of elytron among selected synanthropic beetles that were taken from anthropogenic habitats in Southern Philippines. Additionally, the present study established a link between trait size and shape of their autapomorphic feature. This study used GM methods to further our understanding of Coleopteran morphology and its ecological implications in landscapes that have undergone human influence.

2 Study Area and Methodology

2.1 Study site

This investigation obtained 148 beetle specimens from 12 families and 47 species (Table 1) from different sampling sites in Southern Philippines (Fig. 1). In order to study the elytron shape variation among synanthropic beetles, 149 individuals were collected and preserved from the sampling site. The preserved specimens were carefully positioned dorsally under the stereomicroscope, with the head directed towards the anterior portion of the plane each beetle's elytron were painstakingly examined and removed. Then, using a digital camera, every piece of the dissected body was captured in high resolution.

2.2 Landmark digitization

Using tpsUtil version 1.74, the elytron images were transformed into a tps (thin-plate spline) file (Rohlf, 2015). Using the tpsDig software version 2.30, the transformed image files were then submitted to image digitization (Rohlf, 2015). For each specimen, the study used 100 semi-landmarks around the elytron (Fig. 2.). To ensure their application across all beetle species, semi-landmarks rather than anatomical landmarks were used. This is because some beetles may lack specific homologous features, which could result in errors or incorrect interpretations. The digitized landmarks were subjected to Generalized Procrustes-based analysis (GPA). This is used to standardized the size of the structure and optimize their rotation and translation, thus effectively showing variations in the entire wing shapes.

Table 1 List of Coleopteran species included in this study.

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
Onitis phartopus (Lansberge, 1875)	Scarabaeidae	2
Onthophagus hielkemai (Meindert, 2019)	Scarabaeidae	1
Oryctes rhinoceros (Linnaeus, 1758)	Scarabaeidae	14
Otiorhynchus pauxillus (Rosenhauer, 1847)	Curculionidae	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

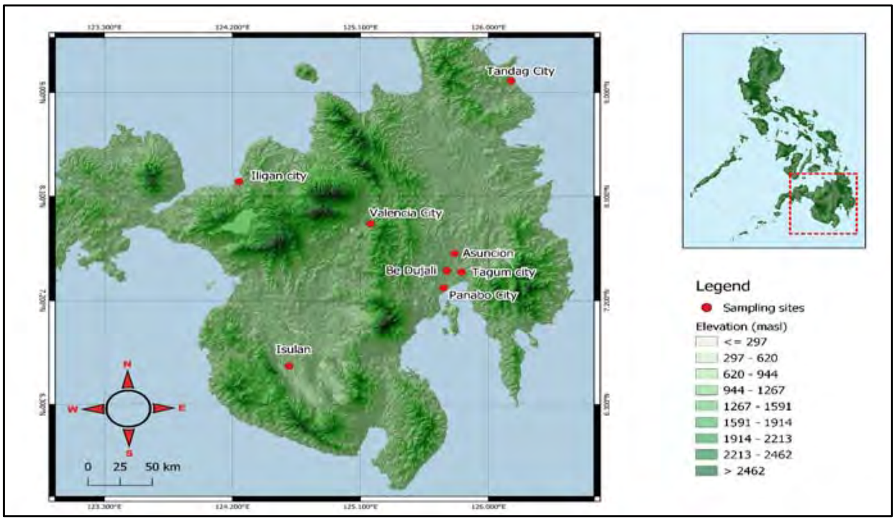

Fig. 1 Map of the Southern Philippines and location of collection sites.

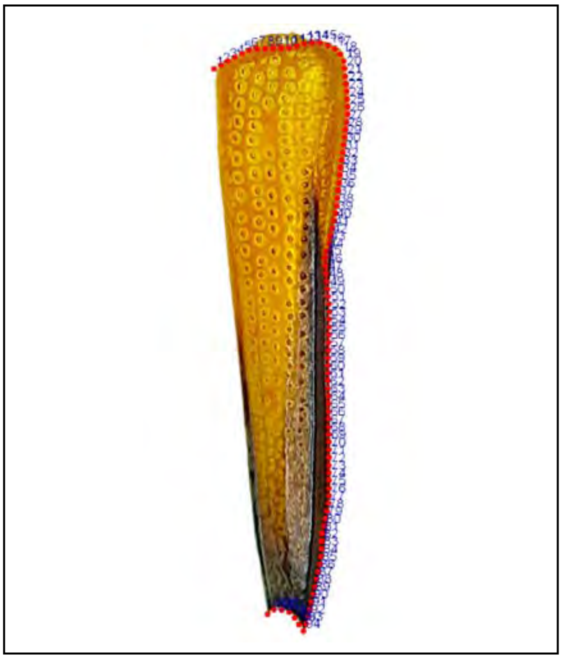

Fig. 2 Semi-landmark configurations of the right side of elytron.

2.3 Data analyses

The position of landmark configurations given by 2- or 3-dimensional Cartesian coordinates was summarised in this research using landmark-based GM analysis (Mitteroecker and Gunz, 2009; Webster and Sheets, 2010). The digitized images were loaded into R statistical programming version 3.2.5 (R Core Team, 2022) and MorphoJ software version 1.06d (Klingenberg, 2011).

Using the gpagen() function in the R geomorph package, the General Procrustes Analysis (GPA) was carried out (Adams et al., 2018). To eliminate variations brought on by scale, position, and orientation variations, GPA aligned and superimposed the landmark coordinates (Mitteroecker and Gunz, 2009).

Procrustes superimposition was used to arrange the images in a tangent space or standard coordinate system while describing shape variables as multivariate data along the axes (Adams et al., 2004). Procrustes superimposition plots, however, could not show covariation among landmarks, a crucial component of morphometric variation (Klingenberg, 2013). In order to visualize shape variation among Coleopterans, the stacked coordinates acquired by GPA were analyzed using Principal Component Analysis (PCA). Additionally, a 90% probability ellipse was created on the PCA scatterplot, covering roughly 90% of the data points (Ren et al., 2017; Chen et al., 2021).

The MorphoJ 1.06d program's first two principal component (PC) scores were also used to perform a thin-plate spline (TPS) analysis (Klingenberg, 2011), which resulted in a deformation grid of the extreme points of the coordinate origin arrangement where differences in the landmarks were graphically represented (Chen et al., 2021).

To evaluate and quantify the shape variations that exist among various Coleopteran species, Procrustes Analysis of Variance (ANOVA) was used. In addition, MorphoJ (Klingenberg, 2011) was used to execute multivariate regression with a permutation test consisting of 10,000 iterations to evaluate the impact of size on the morphology of the elytron depending on species subgroups. The log-transformed centroid size defined the elytron size data (Klingenberg, 2013).

3 Results and Discussion

3.1 Elytral shape variation and functional morphology

The variation among the Coleopterans is explained by the first two PC scores of the elytron of 149 distinct beetles, with the first PC accounting for 84% of the variation and the second PC for 7.69% (Fig. 3). The significant and distinctive shape variance among the beetle elytra is suggested by the high proportion of shape variation described by PC1. Furthermore, PC1 probably represents the Coleopterans' most distinct and consistent elytra shape distinctions. In contrast, PC2 shows a relatively tiny part of the beetle elytra total variance. Moreover, the second PC axis captures further, though less significant, shape variations among the elytra of Coleopterans. The scatterplot displays unique data points, especially for *Metiorrhynchus* species, *O. hielkemai*, *O. rhinoceros*, *O. phartopus*, and *P. fusca*, demonstrating apparent variations in their elytron shapes.

However, *P. coeruleipennis*, *C. fulminans*, and *C. attricornis* also share similarities in elytron shape characteristics. In contrast to other groups, these three species constitute a distinct and separate cluster. Similar to *C. concolor* and *P. orientalis*, these species form distinct clusters apart from the others, but *A. aphetor*, *B. magica*, *C. lacteus*, *E. crassicornis*, *E. plorator,* and *P. bankii* all belong to the same cluster, indicating that their elytra are similar in shape. On the other hand, species *C. discreta elaphroides*, *C. minuta*, *M. sulciperfectus*, and *P. flavilabris* form a different cluster. In addition, a cluster is formed by *E. griseus, M. adspersus*, *O. pauxillus*, *and P. quatuordecimpunctata*. However, the data points for *M. adspersus* are dispersed widely, indicating variability within this species. The *Anomala* species, together with *C. atlas*, *C. sulcipennis*, *Leucopholis* species, and *C. sexmaculata* group have distinct elytron shape from other Coleopteran groupings as a cluster.

Fig. 4 displays the deformation grid for the top two PC scores. According to PC1, based on the graphical reconstruction of the elytra shape, the landmarks' displacements from the typical elytra shape extend outward, giving the elytra a larger look. Since PC1 accounts for 84% of the diversity in elytra shape, a sizable fraction of the sampled beetles had explanatory elytra. On the other hand, the deformation grid of PC2 shows an inward displacement, especially in the middle of the lateral half of the elytra, giving it a concave shape. This shape, however, only adequately describes a small fraction of the beetles, as PC2 only accounts for 7.69% of the diversity in elytra shape among Coleopterans.

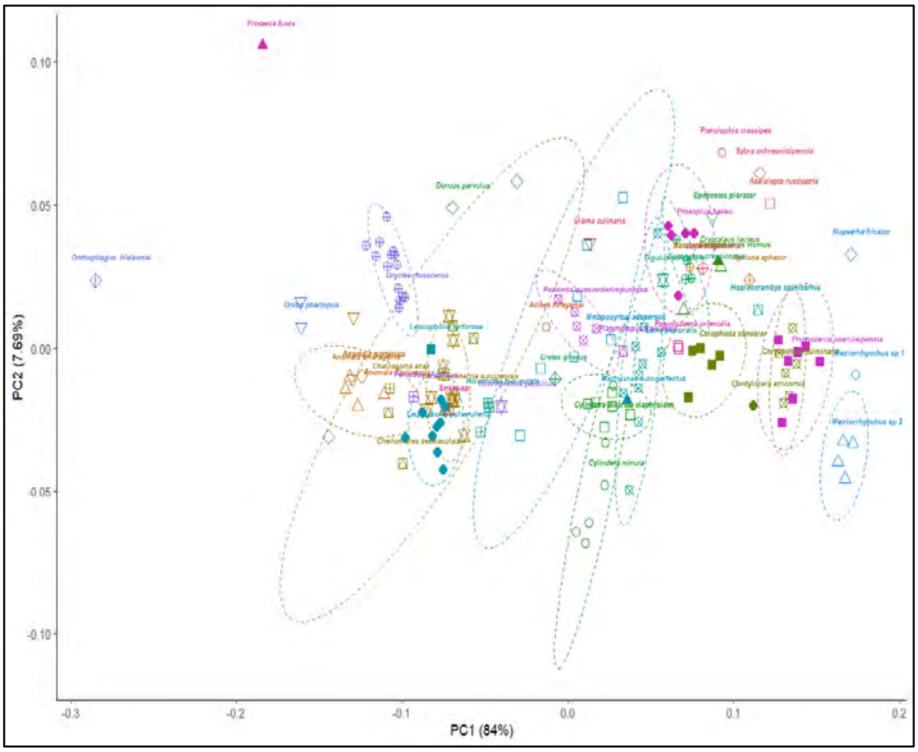

Fig. 3 Scatterplot of the first two PC scores of elytron shape data.

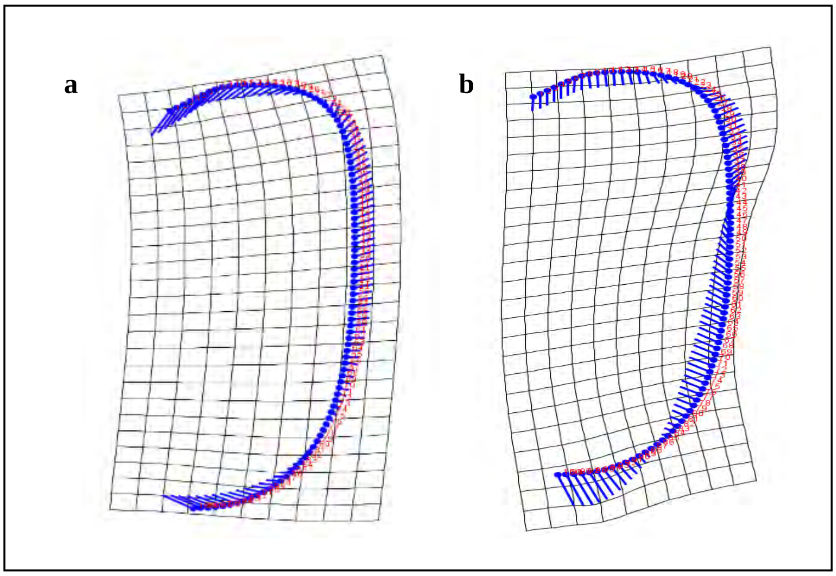

Fig. 4 Graphical reconstruction of the elytron. (a) deformation grid of PC1; (b) deformation grid of PC2.

The size of the elytron of different Coleopteran species differs significantly (F = 20.89, df = 47, p<0.0001), according to Procrustes ANOVA. The research also reveals that different species of Coleopterans have significantly diverse elytra shapes (F = 76.28, df = 9212, p<0.0001). Although size only accounts for 1.64% of the variation in elytron shape, regression analysis shows that size does not significantly alter elytra shape (Tab. 2).

Table 2 Shape variation and allometry in Coleopteran elytra

Parameter	***F* ratio**	***df***	***p*-value**
Size	20.89	47	<0.0001
Shape	76.28	9212	<0.0001
Allometry	**% Expl. Var**	***p-value***	
Centroid size	1.264%	0.1501	

Coleoptera's distinctive and defining feature is the elytron, a hardened shield-like structure. It developed from their forewings during the early and late Permian periods (Ponomarenko, 2004). According to Goczał et al. (2018), this conversion of the forewings into elytra is regarded as a critical adaptation that has greatly aided the evolutionary success of the Coleopteran species.

In the current study, deformation grids created from multivariate shape data were used to shed light on the variety in elytra among Coleopterans. The two main shapes from these grids were broad-shaped and mid-laterally reduced elytra. Their wings may also get smaller due to the change in elytra shape (Goczał et al., 2018). The exact causes of the drop in elytra morphology in beetles are still unknown; however, current suggestions point to mimicry, energy conservation, or improved maneuverability as the possible drivers of the elytral decline (Goczał and Beutel, 2023). As a decreased elytron relates to higher horn growth in male *Gnatocerus cornutus* (Fabricius, 1798) (Tenebrionidae) (Eldred et al., 2016), lower elytra shape may potentially be related to sexual dimorphism. The results of the study by Goczał and Beutel (2023) can be used to explain the slight variance in the reduced elytra as seen in the current data. The morphology of elytra in beetles demonstrates considerable variety, including more extensive, extended shapes. This is consistent with their hypothesis that short elytra have undergone numerous independent evolutionary events throughout different lineages of Coleoptera. Chrysomelidae, Tenebrionidae, and some species of Carabidae may exhibit a broader or explanate elytral shape that gives them a turtle-like look because of numerous selective pressures (Goczał and Beutel, 2023). Shinohara and Yasuoki (2020) found that extended elytra were excellent defenses against various predators, while De Souza and Alexander (1997) found that laterally extended elytra also produced passive aerodynamic stabilization during flight.

Even while research on the functional elements of elytra is still lacking and dispersed among several studies (Goczał et al., 2018), research on contemporary beetles has produced strong arguments. By providing defense against environmental stressors and protecting their hindwings, which enable flight, elytra have enabled beetles to exploit a wide range of ecological niches (Linz et al., 2016). Elytra also aid in mimicry and camouflage, help with desiccation tolerance, and lessen the effects of sudden temperature changes (Goczał et al., 2018). For instance, the elytra of darkling beetles (Coleoptera: Tenebrionidae) can repair and develop sub-elytral canals on the dorsal side of the abdomen. They also aid in flight and protect the delicate tissues on the pronotum. There is a lot of variety in elytral size and shape across Coleopteran species. However, this study's results do not suggest significant allometry in beetle elytra, demonstrating that changes in elytral size have little to no impact on shape variation. The overlapping and distinct data points and ellipses seen in the PCA scatterplot for Coleopteran elytra shape data suggest that the apparent similarities among them are primarily impacted by factors other than elytral size, which contribute to the similarity in elytral shape. Therefore, the shape of the elytra can be seen as a more suitable indicator for expressing the biodiversity of beetle taxa due to its stable morphology and straightforward function (Cheng et al., 2022).

3.2 Geometric morphometrics concerning phenetic relationships of beetles

When examining the variation of shape changes in diverse anatomical structures, Geometric Morphometric (GM) analysis has shown to be an invaluable tool. In addition, GM offers precise and accurate descriptions, making it crucial for deciphering and effectively presenting study findings (Bai et al., 2014). Furthermore, it offers strong visualization tools and a wide range of shape factors, allowing for a special exploratory study method. This method allows previously unknown shape features to be easily identified and quantified (Mitteroecker and Gunz, 2009).

According to Cheng et al. (2022), ecological convergence in Tenebrionid (Coleoptera: Tenebrionidae) beetles is caused by a resemblance in their foraging behaviors and morphologies. This similarity leads to similar functional adaptations. Convergent evolution is evidenced by the multiple independent colonizations and parallel morphological adaptations observed in *Trechini* species (Coleoptera: Carabidae) that have established themselves in cave environments over the course of their evolutionary history (Chen et al., 2021). Hence, the noted resemblances among the beetles in this investigation reflect the corresponding characteristics found within the habitats where these species reside.

4 Conclusions

This study used GM analysis to examine the elytra of Coleopterans. The results show that these features are important for Coleopteran species' biodiversity and adaptive success. However, the PCA of shape variables reveals that a few distantly related species group together, indicating that they share some shape characteristics. The outcome indicates that both species have experienced comparable changes, maybe in response to similar environmental stresses or functional requirements.

The preliminary results of this study provide essential views and theoretical underpinnings for understanding the morphological evolution of beetle elytra. This study also recommends combining morphological, ecological, and molecular data to investigate their relationships to the shape variations in these structures. In a similar vein, to fully understand the evolutionary processes that underlie shape variation, additional aspects such as food, flying prowess, and other behaviors must be considered. These elements may substantially impact how an organism looks and can shed light on the adaptive value of particular features and how they relate to ecological and behavioral adaptations. Such a strategy would improve our comprehension of their importance for adaptation and environmental relevance.

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