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When Shape Defines: Geometric Morphometrics Applied to the Taxonomic Identification of Leaf-Footed Bugs of the Genus *Acanthocephala* (Hemiptera: Coreidae)

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Abstract

The study of qualitative morphological variation is essential for taxonomists and professionals involved in the identification and diagnosis of species of agricultural importance. This becomes particularly critical when quarantine decisions depend on the accurate identification of species belonging to highly diverse genera, poorly reviewed taxonomic groups, or sets of morphologically similar species that lack comprehensive identification keys. Geometric morphometrics has proven to be a powerful tool for resolving taxonomic uncertainties and distinguishing economically significant pest insects, even in the absence of formal taxonomic keys. In this study, we applied geometric morphometrics to analyze pronotum shape variation across 11 species of the genus *Acanthocephala*, representing nearly half of the currently recognized diversity in the genus, including several species of quarantine relevance to the United States. Our results indicate that principal component analysis accounted for 67% of the total shape variation and identified shape patterns that are useful for distinguishing between several species. Discriminate analysis further supported the differentiation among species, with significant differences confirmed through Mahalanobis distances. Although some species exhibited morphological overlaps, particularly among closely related taxa, most comparisons yielded statistically significant results. These findings demonstrate that the shape of the pronotum is a reliable and informative characteristic for species delimitation within the *Acanthocephala* group. We propose the use of geometric morphometrics as a reproducible, cost-effective, and robust method for species-level identification in taxonomically complex groups, which has valuable applications in quarantine inspection, pest monitoring, and agricultural biosecurity.

Keywords: Heteroptera; Acanthocephalini; phytophagous insect; shape analysis; morphometrics

1. Introduction

The order Hemiptera, Linnaeus, 1758 with approximately 80,000 described species, is the fifth most diverse among insects; nearly half of its species belong to the suborder Heteroptera. All hemipterans share piercing–sucking mouthparts adapted for feeding. Within this group, the family Coreidae, Leach, 1815 commonly known as leaf-footed bugs or squash bugs is an important family of sap-feeding insects comprising four currently recognized subfamilies, 37 tribes, nearly 270 genera, and more than 2800 described species [1–3]. Although Coreidae are globally distributed, most species inhabit tropical and subtropical regions.

The subfamily Coreinae Leach, 1815 is the largest within the family, including 32 tribes and exhibiting a predominantly tropical distribution [4]. Within it, the Neotropical tribe Acanthocephalini Stål, 1870 includes 19 genera and more than 104 species [5–7], with *Acanthocephala* Laporte, 1833 being the most diverse genus. This complex group, comprising about 32 species, is widely distributed across the United States (with 15–16 species), Mexico, Central America, the Greater Antilles, and South America, excluding Chile [7].

Species in the genus *Acanthocephala* can be recognized by distinctive morphological features such as a compressed tylus (the median lobe of the clypeus that projects between the genae), spiny and strongly incrassate metafemora (more developed in males), and metatibial expansions in both sexes [8], while these traits are taxonomically informative, they also play a role in intraspecific competition. They may function as sexually selected weapons or visual signals in mate choice and rival assessment. Recent revisions have highlighted the presence of numerous synonyms, misidentified records, and undescribed species, particularly in tropical regions [9]. Despite these diagnostic traits, species identification within the genus remains challenging, especially outside geographically restricted regions. Furthermore, phylogenetic analyses have revealed the genus to be monophyletic yet internally diverse [9]. This has led to the proposal of multiple subgenera based on combined morphological characters (discrete and continuous). However, identification tools are limited or absent altogether for many species, particularly those outside North America. This highlights the urgent need for alternative methods, such as geometric morphometrics, to complement traditional taxonomy and enable precise species-level identification in ecological, agricultural, and biosecurity contexts. Previous studies on other Hemipteran taxa like *Triatoma* have shown that this approach is effective for more complex morphological questions [10,11]. It has helped uncover hidden diversity and improve diagnostic accuracy in other insect species [12,13]. By capturing variation in a statistically robust and reproducible framework, geometric morphometrics (GM) not only enhances taxonomic resolution but also supports broader applications in pest management, quarantine regulation, and ecological monitoring, where rapid and accurate identification is crucial [14].

In recent years, geometric morphometrics has been successfully applied to identify true bugs of medical importance, such as triatomines (Reduviidae: Triatominae) and bed bugs (Cimicidae) [15–17]. However, its use in agriculturally important groups has been limited, with some advances in genera such as *Eysarcoris* and in the tribe *Nezarini* (Pentatomidae) [18,19]. Galindo-Malagón et al. [20] showed that geometric morphometrics (GM), when combined with traditional morphometric data and discrete traits, can effectively resolve taxonomic ambiguities within the species complex of *Rhagovelia angustipes*

(Hemiptera: Veliidae). This group has historically experienced significant intraspecific variation and overlapping morphological features. Therefore, by applying GM to key body structures such as the head, abdomen, and pronotum, this method has revealed distinct shape-based differences that traditional techniques often fail to detect. Building on this approach, the present study evaluates the potential of geometric morphometrics of the pronotum to discriminate among 11 species of the genus *Acanthocephala*, some of which are of quarantine concern in the United States due to their absence in the national fauna. Additionally, several species in this genus are known to cause economic damage to crops, such as tomatoes, peaches, and pecans, due to their phytophagous behavior [21]. We also assess whether pronotum shape varies to the species' biogeographic origin, categorized as Nearctic Subtropical, Tropical Subtropical, or Tropical, thereby contributing to the refinement of taxonomic frameworks in agriculturally significant coreid bugs through morphometric analysis.

2. Materials and Methods

2.1. Sampling and Data Collection

For this study, 11 of the 32 currently recognized species of *Acanthocephala* were selected, including taxa of quarantine concern frequently intercepted at U.S. ports of entry, native North American crop pests, and less commonly encountered species from Central and South America. The selection was also constrained by the availability of verified high-resolution images. Consequently, some species recorded in the United States, such as *A. confraterna* (Say, 1832) and *A. thomasi* Uhler, 1872, could not be included due to the absence of suitable photographic material. Species identification within this group remains challenging when based solely on external morphology of either sex, as reflected by the high number of synonyms reported for some species (Table 1).

Table 1. Species and abbreviations used in this study. Species selection was based on the availability and quality of photographic data.

Species	Abbreviature
A. alata	alat
A. bicoloripes	bico
A. declivis	decl
A. distanti	dist
A. femorata	femo
A. latiantennata	lati
A. latipes	laty
A. mercur	merc
A. nigra	nigr
A. terminalis	term

2.2. Image Processing and Landmarking

To study the shape of the pronotum, high-definition images were selected from the ImageID database of the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ).Online photographs were verified by experts in the taxonomic group, and the identification of specimens in ImageID was performed by USDA specialists in true bugs. Once the images were processed, 40 landmarks were digitized on the pronotum of the species using the software TPSDig2 v2.17 [22] (Figure 1).

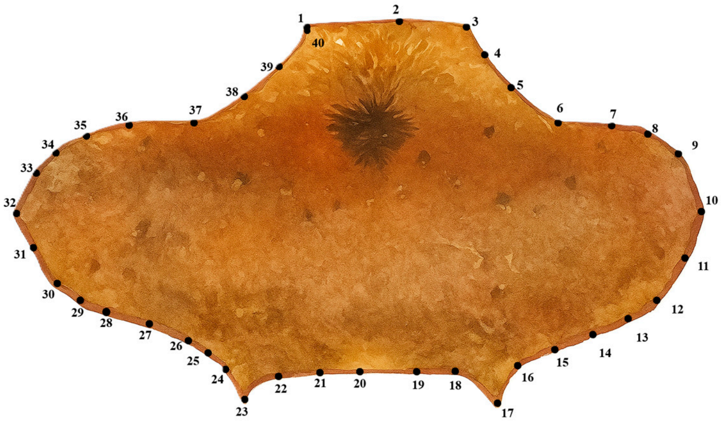

Figure 1. Dorsal view of the pronotum of *Acanthocephala* species showing the distribution of 40 landmarks along its contour.

2.3. Multivariate Shape Analyses

To analyze pronotum shape differences between species, 54 specimens (males and females) were examined. However, interspecific comparisons were performed using only female specimens. Generalized Procrustes Analysis (GPA) was applied to standardize shape data by removing variation related to size, rotation, and orientation [23]. This alignment allowed shape variation to be analyzed independently of these factors. To explore pronotum shape differences among species of the genus *Acanthocephala*, Principal Component Analysis (PCA) was performed on the covariance matrix of the dataset [24]. PCA served as a multivariate ordination method to explore shape variation in morphospace [25]. And results were visualized using the two principal components that accounted for the greatest variance. Procrustes ANOVAs were conducted using species identity as the explanatory variable to assess whether shape and size differences (centroid size and Procrustes distances) were structured by species. To assess whether size has an influence on shape (allometry), we performed a multivariate regression with shape as the dependent variable and centroid size as the independent variable, allowing us to detect potential allometric effects on shape [26]. To enhance group discrimination, Canonical Variate Analysis (CVA) was applied, maximizing morphological separation by identifying axes of greatest interspecific variation. The resulting CVA scatterplot illustrated the degree of morphological overlap among species. Additionally, Mahalanobis distances were calculated to quantify morphological divergence, both relative to the mean shape and among individuals, across species [27]. All analyses were conducted using MorphoJ v1.06d [28] and the geomorph package in R [29].

3. Results

The first three principal components explained 67% of the total variation in pronotum shape (PC1 = 37.28%; PC2 = 19.90%; PC3 = 10.79%), providing a robust estimate of its morphological variability. The principal component analysis (PCA) revealed shape patterns among the analyzed species; although many shapes overlap, it also allows for the differentiation of *alat*, *lati*, and *decl* within the morphospace (Figure 2).

Figure 3 shows the superimposition of the mean pronotum shapes for the evaluated species, allowing the visualization of notable shape differences. Clear variation is observed in the convexity of the dorsal margin and in the lateral expansion of the pronotum. *A. alat* and *A. decl* (blue and yellow lines, respectively) exhibit a more laterally expanded shape, with a dorsoventrally flattened structure and a more curved dorsal region. *A. dist* also shows some lateral widening, although to a lesser extent. In contrast, the remaining species display narrower pronotum, with straighter lateral margins and less expansion.

Among them, *A. mercur* (black line) stands out for having the narrowest pronotum laterally, although with a slight elevation in the dorsal region. The base of the pronotum remained relatively flat and conserved among most of the analyzed species.

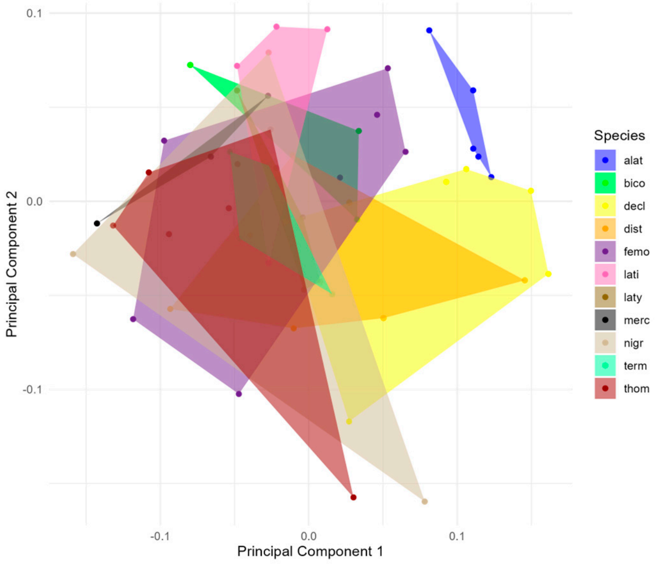

Figure 2. Principal Component Analysis (PCA) of pronotum shape among 11 species of the genus *Acanthocephala*.

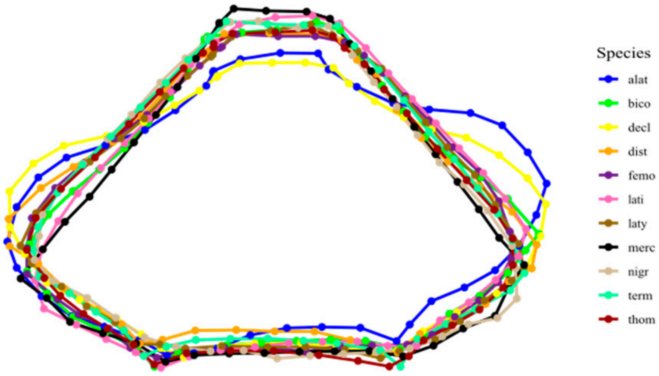

Figure 3. Superimposition of the mean pronotum shapes of the 11 evaluated species of the genus *Acanthocephala*.

The comparison between the overall consensus and the mean pronotum shape of each species (Figure 4) allowed the identification of landmarks with the highest deformation index, which contribute to explaining the main differences among the evaluated species. Species-specific variations were evident, particularly in lateral expansion as seen in *alat* (A), *bico* (B), *laty* (G), and *nigr* (I), and in the dorsal curvature of the pronotum, as observed in *decl* (C) and again in *laty* (G). Additionally, differences were noted in the ventral base of the pronotum in species such as *dist* (D) and *femo* (E).

The canonical variate analysis (CVA) revealed a clear morphological differentiation among the evaluated species based on pronotum shape (Figure 5). The species alat, decl, dist, merc, and thom exhibited marked shape separation, demonstrating distinctive patterns compared to the other species. In contrast, species such as bico, femo, latí, laty, nigr, and term showed a higher degree of shape overlap, indicating morphological similarities in the pronotum. These differences were statistically significant following the permutation test using Mahalanobis distances (Table 2).

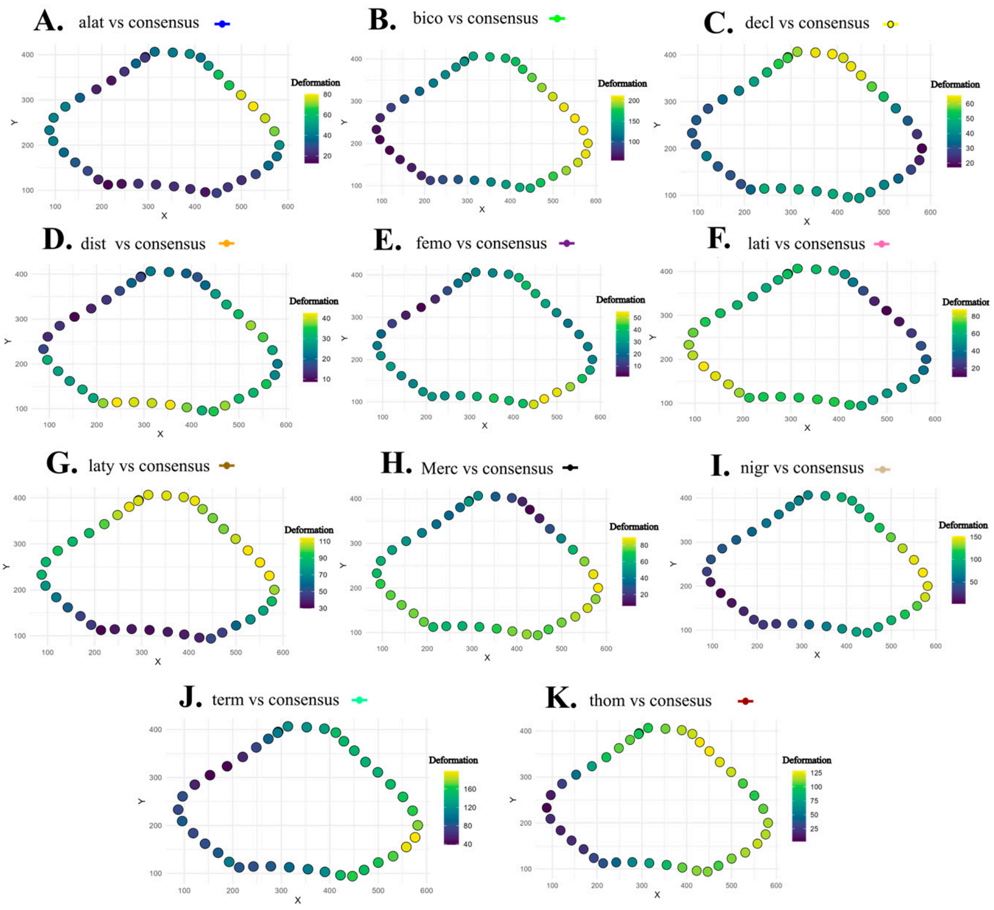

Figure 4. Comparison of the mean pronotum shapes of the 11 evaluated species with respect to the general consensus of the genus *Acanthocephala*. Each panel (**A**–**K**) shows the deviation of a species' mean pronotum shape from the overall morphological consensus derived from the complete dataset. Circles represent landmarks, and the color scale indicates the magnitude of Procrustes deviation at each anatomical point, ranging from lower to higher distances from the consensus.

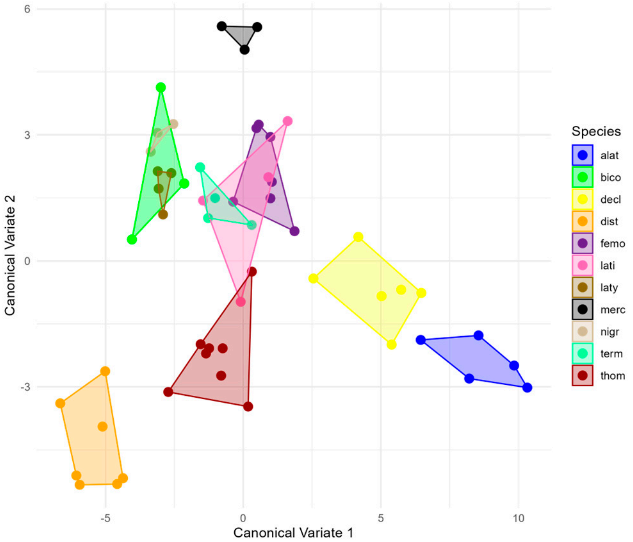

Figure 5. Canonical variate analysis of pronotum shape in the 11 evaluated species.

The Mahalanobis distance matrix (Table 2) revealed morphological differences in pronotum shape among most of the evaluated species. Comparisons such as alat vs. bico (DM = 13.96; p-value = 0.05), alat vs. dist (DM = 11.86; p-value < 0.0001), and decl vs. dist (DM = 11.86; p-value < 0.0001) highlight significant differences in pronotum shape. Overall, most distances were statistically significant ($p < 0.05$), indicating clear differentiation among species. However, some cases, such as lati vs. nigr (p-value = 0.058) and lati vs. term (p-value = 0.1999), showed non-significant differences, supporting the CVA results in revealing similar morphological patterns.

The multivariate regression of centroid size on pronotum shape revealed a weak allometric effect, explaining only 4% of the total shape variation (Figure 6). At the interspecific level, significant differences in average pronotum size were observed: *thom* and *term* displayed the largest pronota; *alat*, *dist*, *lati*, *femo*, *decl*, and *merc* showed intermediate-sized pronota; while *bico*, *nigr*, and *laty* exhibited the smallest pronota.

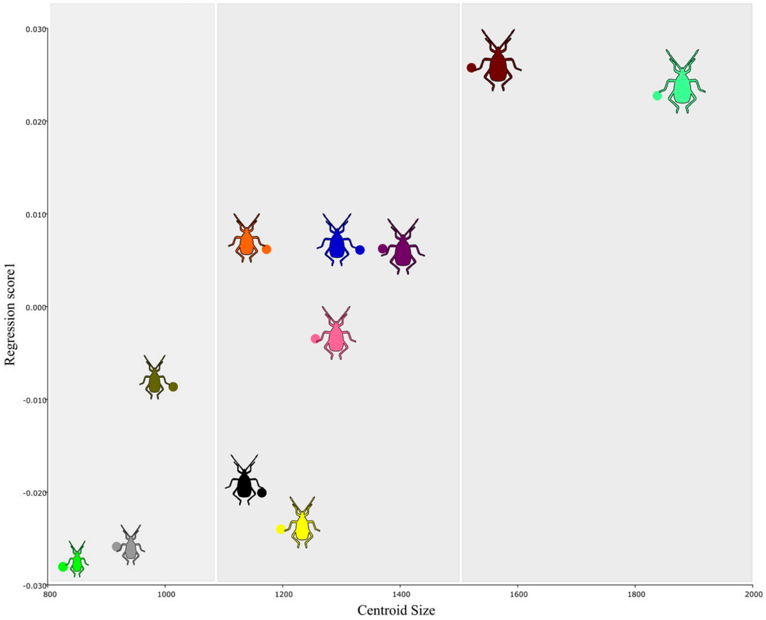

Figure 6. Multivariate regression of mean pronotum shape (dependent variable) by species against centroid size (independent variable).

Table 2. Permutation test of Mahalanobis distances and associated p-values for interspecific pronotum shape comparisons.

	alat	**bico**	**decl**	**dist**	**femo**	**lati**	**laty**	**merc**	**nigr**	**term**
bico	13.96									
***p*-value**	0.005									
decl	7.5888	9.724								
***p*-value**	0.0002	0.0078								
dist	14.4782	8.6504	11.8618							
***p*-value**	<0.0001	0.0036	<0.0001							
femo	9.8279	8.3621	8.7298	9.9523						
***p*-value**	0.0002	0.0009	0.0002	0.0001						
lati	9.9435	6.8861	7.6388	8.8345	5.0822					
***p*-value**	0.0025	0.0506	0.0031	0.0003	0.019					
laty	13.4195	8.2347	10.4011	8.6758	6.5717	6.903				
***p*-value**	<0.0001	0.0167	0.0005	0.0007	0.0005	0.0194				
merc	12.6694	6.8178	9.732	11.844	7.5256	7.0322	9.1256			

Table 2. *Cont.*

	alat	bico	decl	dist	femo	lati	laty	merc	nigr	term
***p*-value**	0.0018	0.046	0.007	0.0027	0.0007	0.0241	0.0266			
nigr	13.9692	7.3218	10.69	9.8336	8.185	6.7722	7.8795	8.2783		
***p*-value**	0.0003	0.0099	0.0042	0.0074	0.0045	0.058	0.0231	0.0125		
term	11.0042	7.6838	8.8747	8.4728	6.0205	5.0588	6.4682	6.8912	7.126	
***p*-value**	0.0009	0.0461	0.0049	0.0014	0.0018	0.1999	0.021	0.0393	0.0431	
thom	10.6407	7.8757	8.6237	7.4011	6.9412	6.6104	7.6667	9.4855	7.7883	6.6333
***p*-value**	0.0009	0.0006	0.0001	<0.0001	<0.0001	0.0009	<0.0001	0.0016	0.0003	0.0004

4. Discussion

Our research reaffirms the value of geometric morphometrics as an effective diagnostic tool for analyzing and quantifying the morphological variation in biological traits, providing concise and structured results that enable precise discrimination among closely related taxa [12]. This methodology is particularly useful in groups with high species diversity and subtle morphological differences, where taxonomic delimitation and accurate identification can be complex. In this regard, its application is especially relevant in practical scenarios such as border entry points, where determining whether a species poses a quarantine risk is crucial.

According to Withrow et al. [30], the rapid identification of quarantine or potentially quarantinable species is essential for timely decision-making in areas such as biosecurity and invasive species management. The use of accessible and low-cost tools such as geometric morphometrics allows for fast, appropriate, and scientifically grounded responses, thereby reducing potential negative impacts on ecosystem health, the economy, and human health.

In this context, our study confirms that pronotum morphology is a reliable diagnostic trait for interspecific delimitation within the genus *Acanthocephala*, enabling clear differentiation among all evaluated species, as evidenced in Figure 4 and the CVA results. However, this analysis also revealed a certain degree of morphological overlap in pronotum shape among some taxa, particularly between *A. bicoloripes*, *A. nigra*, and *A. latipes*, as well as between *A. femorata*, *A. latiantennata*, and *A. terminalis*. Despite this overlap, Mahalanobis distances (Table 2) indicate significant differences among these groups. As suggested by Smith-Pardo, Lingafelter, Laroze, Piñeiro-Gonzalez and Benítez [12], such morphological convergence may result from adaptive convergence or recent differentiation not yet fully reflected in external morphology, highlighting the need to explore additional structures to complement these results and enhance taxonomic resolution. It is also worth noting that the analyzed species exhibited significant differences not only in the morphological configuration of the pronotum but also in its size. This variability was supported by multivariate regression analysis, which revealed a consistent relationship between shape and size, suggesting that both attributes jointly contribute to interspecific differentiation within the genus *Acanthocephala*.

The pronotum has become established as a key diagnostic trait in taxonomic studies, supported by multiple studies across different groups. Zhang et al. [31] used it, along with structures such as the head, elytra, and spermatheca, to delimit subgenera within the genus *Chaetocnema* (Coleoptera). In another study, Zhang, et al. [32] demonstrated its usefulness not only for differentiating species of stag beetles but also for inferring evolutionary history and morphological diversification. A notable advancement is its application to quarantine species: Smith-Pardo et al. (2025) (In press) applied geometric morphometrics to maize stink bugs of the tribe Nezarini (Pentatomidae), evaluating shape variation in the pronotum and head. The landmark-based analysis of high-resolution images enabled the discrimination of cryptic species that could not be distinguished using traditional morphological characters.

Applying geometric morphometrics to the pronotum identified it as a highly informative structure for interspecific delimitation within the genus *Acanthocephala*. The results not only facilitated the discrimination of most evaluated species but also revealed subtle morphological overlaps among certain taxa, underscoring the need for integrative approaches to achieve more robust delimitation. In this direction, several studies have shown that integrating ecological and molecular data further strengthens species delimitation, particularly in taxonomically complex groups. Studies by Cáceres et al. [33], Jaramillo-O et al. [34] and Cruz et al. [35] demonstrate that ecological variables (distribution, biotic interactions, or habitat preferences) provide functional context to morphological differences, resolving cases of cryptic species. Molecular tools such as DNA barcoding (COI) or whole genome analysis have also effectively complemented morphological identification, providing additional taxonomic robustness [36–38].

The findings of this study have relevant practical implications for the monitoring and surveillance of species of agricultural or quarantine concern. Geometric morphometrics stands out as an accessible and effective diagnostic tool for distinguishing morphologically similar species, particularly in contexts where specialists and updated taxonomic keys are lacking, such as ports, border crossings, and agricultural zones. Its usefulness has been supported by previous studies in which landmark-based approaches allowed for highly accurate discrimination of closely related taxa [39,40].

This work, the first in the genus *Acanthocephala* and among the few in the family Coreidae, expands the potential of GM for identifying agriculturally important insects with subtly variable morphology. By demonstrating that data obtained from high-resolution images can accurately identify species even in the absence of diagnostic keys, this study strengthens the case for applying GM in rapid diagnostic protocols. These results support recent proposals advocating for the incorporation of GM into biosecurity and pest management workflows [30,41]. Finally, beyond its direct utility for pest management and quarantine inspection, the methodological framework presented here also contributes to classical taxonomy. Several studies have demonstrated the effectiveness of GM in resolving species boundaries, defining generic limits, and informing phylogenetic hypotheses across diverse insect groups [33,39]. Our findings reinforce that GM should not only be considered as an applied diagnostic tool but also as a methodological complement to traditional taxonomy.

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