Geometric Morphometrics as a Diagnostic Tool for Cryptic Agricultural Pests: Insights from Nezarini Stink Bugs (Hemiptera: Heteroptera: Pentatomidae)[1]

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Abstract Understanding morphological variation is crucial for clarifying taxonomic uncertainties and enhancing the identification of economically important pest insects. In this research, geometric morphometrics (GM) was applied to analyze the head and pronotum shape of cryptic species from 3 genera within the tribe Nezarini (*Acrosternum*, *Chinavia*, and *Nezara*) (Hemiptera: Heteroptera: Pentatomidae), which include significant agricultural pests. By quantifying shape variation, we assessed the efficacy of GM in genus-level discrimination and explored its ability to distinguish morphologically cryptic species within *Acrosternum*, which are often intercepted in imported commodities outside their native range (Europe and Western Asia). For species level, GM effectively distinguishes 4 frequently intercepted *Acrosternum* species, emphasizing its applicability in biosecurity. This study highlights GM as a reliable, reproducible, and statistically robust tool for taxonomic resolution, especially in insect groups with subtle morphological differences that are relevant to agriculture and quarantine monitoring.

Key Words Heteroptera, Pentatomoidea, true bugs, stink bugs, quarantine significant

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The taxonomic order Hemiptera ranks fifth in terms of number of species among the insect orders, with nearly 80,000 described species, after Coleoptera, Hymenoptera, Diptera, and Lepidoptera. Within the Hemiptera, which all have a piercing-sucking apparatus for feeding, the suborder Heteroptera (38,000 species), or true bugs, is monophyletic and includes 91 families (Rider et al. 2018). The family Pentatomidae or stink bugs, with nearly 940 genera and 5,000 species, is 1 of the 3 largest among the Heteroptera, including 9 subfamilies (Schuh 1995). Apart from the subfamily Asopinae, whose species are all predators of other insects (De Clercq 2000, Lupoli 2019), the other subfamilies are phytophagous. Phytophagous stink bugs feed on all

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above-ground plant tissue from stems to petioles, leaves, flowers, fruits, and seeds; however, they prefer feeding on developing shoots, fruits, and seeds (McPherson 2018, Todd and Herzog 1980).

Pentatomidae is considered a monophyletic family based on morphological and molecular data (Grazia et al. 2008). However, recent molecular studies show that the Cyrtocorinae, considered a subfamily of Pentatomidae should be considered another family (Roca-Cusachs et al. 2022). They also show that the subfamilies Podopinae and Pentatominae are not monophyletic (Jia et al. 2024, Lian et al. 2022). The subfamily Pentatominae is the largest, with over 660 genera and more than 3,484 species (Grazia et al. 2015). The assignment of genera in the different tribes of Pentatominae has been the subject of much discussion, particularly in the tribe Nezarini Atkinson. The green stink bugs were first attached to it, all initially classified in the genus *Nezara* Amyot & Serville, whose origin is African. Later, a group of paler, less punctuated, brighter, and smaller species of stink bugs, present in Africa, the Middle East, southern Europe, dry and arid regions, and in the New World, was separated from the genus *Nezara* and placed in the genus *Acrosternum* Fieber.

However, in the New World, particularly in humid tropical environments, larger species of a more vibrant and green coloration have been observed and have been classified as such in the genus *Chinavia* Orian. Henceforth, in the New World, all species that previously belonged to the genus *Acrosternum* are now classified in the genus *Chinavia*. More recently, molecular studies have confirmed this and shown that other genera belonging to other tribes on the basis of morphological criteria can be attached to the Nezarini tribe, such as the genera *Palomena* or *Chlorochroa*, previously classified in the tribe Carpocorini Mulsant & Rey (Roca-Cusachs et al. 2022). The Nezarini tribe, therefore, now includes 26 genera and 272 species (Rider et al. 2018).

Some species belonging to 3 genera of the Nezarini: *Acrosternum*, *Chinavia*, and *Nezara* are crop pests. The genus *Nezara* includes 12 species described today (Ferrari et al. 2010). The species with the most significant agricultural importance is the southern green stink bug, *Nezara viridula* (L.), which is native to Africa, but has invaded many countries in the world to the point of now being a cosmopolitan species. It is the only species of this genus in the United States (about 3,200 observations in iNaturalist). It is found primarily on the west and east coasts of the United States, extending from Mexico to Canada. It does not appear to occur in areas with an average annual temperature below 55°F. *Nezara viridula* is a highly polyphagous feeder observed on 145 genera of plants belonging to 39 families, and it attacks many important food crops (Esquivel et al. 2018). Saliva damage from its piercing mouth parts is evident by hard brownish or black spots; these punctures affect the quality of plants and fruits and lower their market value. In addition, it can transmit the tomato bacterial spot. (Squitier 2011).

The genus *Chinavia* on the other hand, includes 85 described species, which are Nearctic, Neotropical, and Afrotropical (Servino and Schwertner 2020). Three species of the genus *Chinavia* are found in the United States: *Chinavia hilaris* (Say), *Chinavia marginata* (Palisot de Beauvois), and *Chinavia pensylvanica* (Gmelin). The green stink bug, *C. hilaris* (almost 40,000 observations in iNaturalist, versus 170 for *C. marginata* and 125 for *C. pensylvanica*), is one of the most common damaging native stink bug species in the United States. It is a pest of economic importance in a variety of commodities, including cotton [*Gossypium hirsutum* (L.)], soybeans [*Glycine max* (L.)], and tree fruits such as peaches (Capinera 2020).

The genus *Acrosternum* includes 13 described species, with 2 species having an important role from an agricultural point of view: *Acrosternum heegeri* Fieber is a polyphagous herbivore that feeds on fruits and vegetables. It is considered a significant pest, particularly in the Mediterranean region, where its polyphagous feeding habits can damage crops like pistachios and *Acrosternum millierei* (Mulsant & Rey) which is vector of *Eremothecium coryli* Kurtzman, a plant pathogen that causes stigmatomycosis (a fungal disease) in pistachio and other crops (Mehrnejad 2020).

Multiple references and tools exist for identifying species (Servino and Schwertner 2020), genera, and other suprageneric categories in the family Pentatomidae (Schuh 1995). Still, they are far from covering the high diversity of this family of true bugs. In addition, the limited number of resources is scattered throughout the literature, geographically limited (Falcon-Brindis and Villanueva 2024, Lupoli and Dusoulier 2015, McPherson 1982, Paiero et al. 2013, Salini and Viraktamath 2015, Sites et al. 2012), focused on individual genera (Frey-da-Silva and Grazia 2001, Rolston 1983, Schwertner and Grazia 2007), or only for taxa associated with particular crops (Esquivel et al. 2018, Koch et al. 2017).

In addition, in recent decades, morphometrics has shifted from traditional analyses based on lengths and proportions to more complex geometric morphometrics (GM) that analyzes organisms' shapes and structures (Adams et al. 2013, 2004; Klingenberg 2002). This quantification, in turn, allows for the analysis of the evolution of shape using phylogenetic comparative methods or for the discrimination of taxa based on repeatable means with a system that contains information and which generates traditional character-based methods (Catalano et al. 2010, Catalano and Torres 2017). Furthermore, GM is a valuable tool for inferring the correlation with a species' shape and the different factors that affect it, and helps us understand the diversification of the shape in organisms and discriminating taxa (Lawing and Polly 2010, Rohlf and Marcus 1993, Zelditch et al. 2012).

Studies using GM for the identification of true bugs have been performed with groups of medical importance, such as triatomines (Reduviidae: Triatominae) and bed bugs of the family Cimicidae (Alvarez et al. 2024, Deku et al. 2022, Lázari Cacini et al. 2022), and only a couple of others on groups of agricultural importance, such as the genus *Eysarcoris* (Li and Zhang 2017, Li et al. 2017). This study aims to assess the effectiveness of geometric morphometrics in identifying shape variation, particularly in the head and pronotum, among 3 agriculturally important genera of the tribe Nezarini (*Acrosternum*, *Chinavia*, and *Nezara*). These genera are of economic relevance due to their phytophagous habits and potential role as disease vectors, including viruses. Additionally, we investigate species-level differentiation within *Acrosternum*, mainly focusing on taxa frequently encountered at U.S. ports of entry, either as hitchhikers or as pests associated with agricultural commodities. By applying geometric morphometric analyses, we aim to distinguish subtle morphological differences, especially among cryptic species, and evaluate the power of this approach for resolving taxonomic challenges in morphologically similar groups.

Materials and Methods

For this study, the most intercepted genera of true bugs at U.S. ports of entry, belonging to the tribe Nezarini, were selected. Species identification within these genera is challenging when based solely on the external morphology of either males or females.

To study the shape of the head and pronotum, high-definition images were selected from the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) image database *ImageID*, along with high-quality images sourced from the world wide web (e.g., GBIF and iNaturalist). All photos were verified by an expert in the group, with most identifications provided by one of the authors (RL), while specimens in *ImageID* were identified by USDA specialists in the group. Once the photos were processed, landmarks were digitized for 2 different traits. The first trait, the head, was analyzed using 10 landmarks distributed across its entire morphology (Fig. 1A). The second trait, the pronotum, was digitized with 9 landmarks placed along its contour and specific anatomical sections (Fig. 1B). The species were digitized using the software TPS Dig2 v2.17 (Rohlf 2015). For the study on the differences in the shape of the 3 genera in the tribe Nezarini, we used a total of 40 specimens (12 specimens of 11 species of *Acrosternum*, 17 specimens of 14 species of *Chinavia*, and 11 specimens of 2 species of *Nezara*) for the head and 57 (19 specimens of 11 species of *Acrosternum*, 16 specimens of 12 species of *Chinavia*, and 22 specimens of 3 species of *Nezara*) for the pronotum. An important digitization procedure was conducted in which a subset of 50 specimens was digitized twice to assess measurement error. This was verified by performing Procrustes ANOVA, testing whether the mean square (MS) for individuals was greater than the MS for digitizing error. Generalized Procrustes Analysis (GPA) was then applied to the dataset to standardize shape by eliminating non-shape variation, including differences in size, rotation, and orientation (Rohlf and Slice 1990). This alignment process ensured that shape variation among specimens was analyzed independently of these confounding factors. To visualize shape variation among the 3 genera of Nezarini and the different species of *Acrosternum*, datasets for the head and the pronotum were first analyzed using principal component analysis (PCA) (Jolliffe 2002) based on the covariance matrices of the datasets. PCA was used as an ordination multivariate analysis to better reflect shape morphospace (Klingenberg 2013). These analyses were visualized graphically based on the 2 components (PC) explaining the highest variation. Procrustes ANOVAs with the genera and species as the explanatory variables were used to determine whether or not this variation was partitioned according to genera or species by size (as indicated by centroid) or shape (as indicated by Procrustes Distances). To identify if there is any allometric influence in the data a multivariate regression, was conducted using the centroid size as an independent variable and shape as a dependent variable. To enhance the visualization of shape variation among cryptic *Acrosternum* species, a Canonical Variate Analysis (CVA) was performed. This analysis maximizes group separation by identifying axes of greatest morphological differentiation, allowing for a more apparent distinction among species. The resulting CVA scatterplot highlighted the degree of morphological overlap between groups. Procrustes distances (a measure of the absolute magnitude of shape deviation from the centroid) and Mahalanobis distances (which indicate how unusual an individual is relative to others in the sample) were computed and compared across genera and species (Klingenberg 2015). Finally, all analyses were performed using MorphoJ v1.06d (Klingenberg 2011) and the geomorph package in R (Adams and Otárola-Castillo 2013).

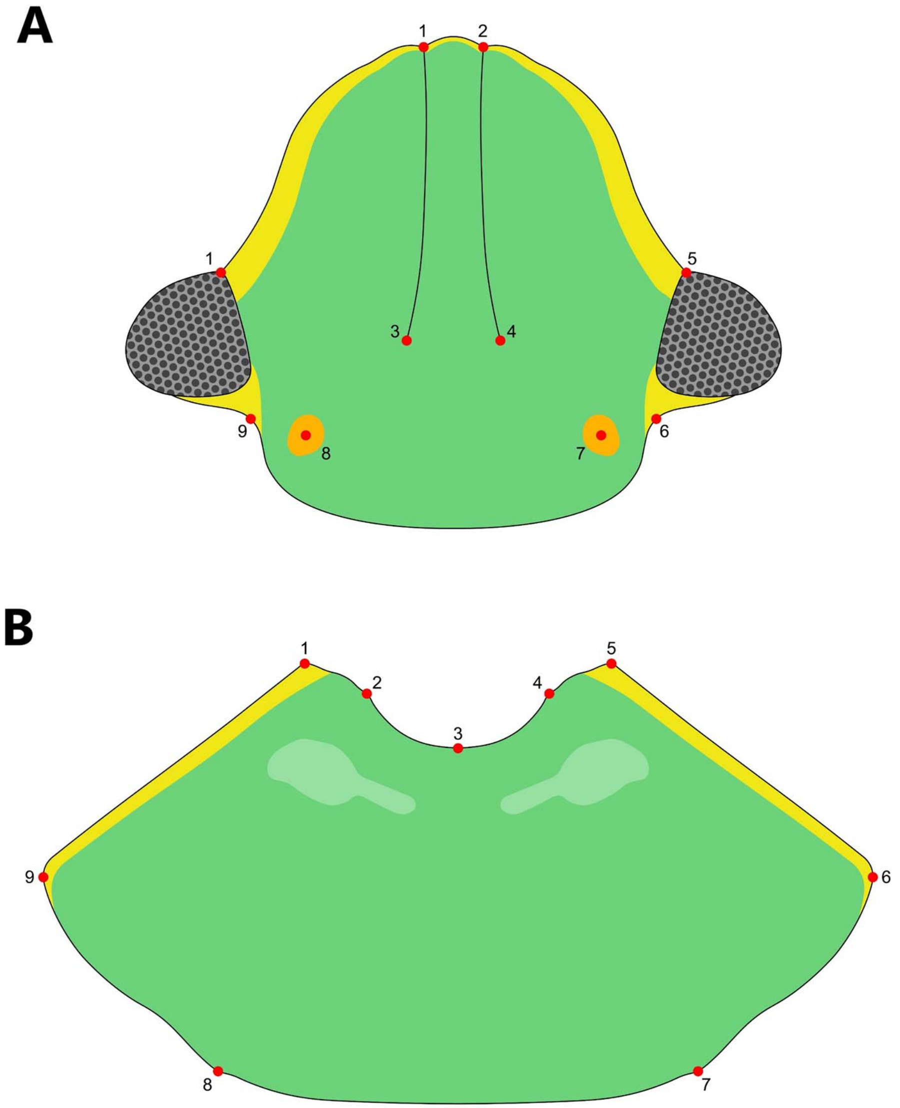

Fig. 1. Representation of both traits used in this study with landmarks in (A) head of the general Hemiptera of the Nezarini tribe, with 10 landmarks and (B) pronotum of the general Hemiptera of the Nezarini tribe with 9 landmarks.

Results

Differences in head shape among the 3 genera of the tribe Nezarini. As a verification procedure, we confirmed that there was no measurement error in the data, as the Procrustes ANOVA showed that the mean square (MS) for individuals

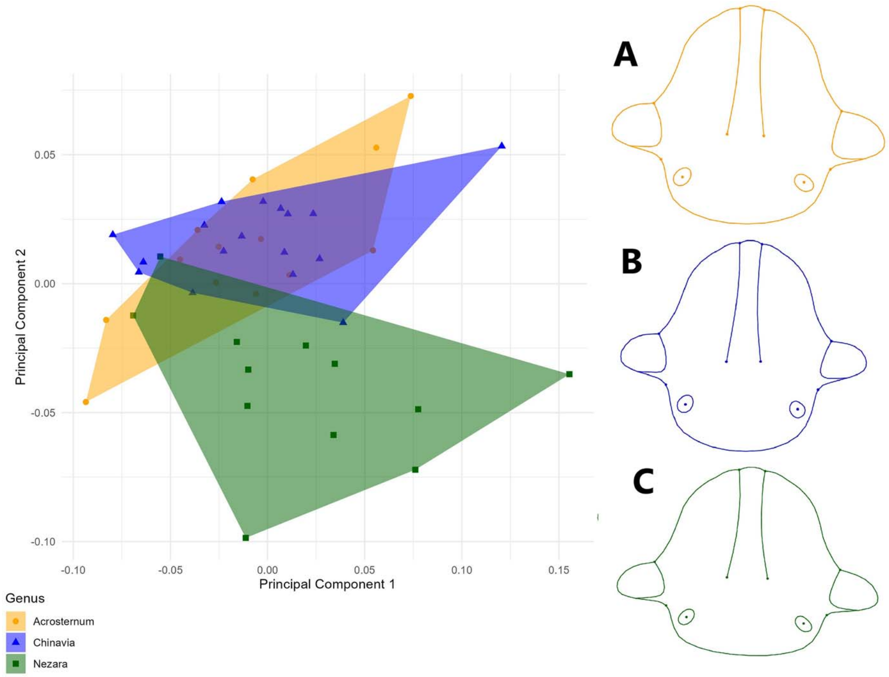

Fig. 2. Principal Component Analysis (PCA) of head shape among the 3 genera of the tribe Nezarini. The symbols represent different genera: yellow circles for *Acrosternum*, blue triangles for *Chinavia*, and green squares for *Nezara.* The average shape of the genera was shown by letters: (A) *Acrosternum*, (B) *Chinavia*, (C) *Nezara*.

were greater than the MS for digitizing error. A PCA of the covariance matrix of the individual shape showed that the head shape followed a clear pattern associated with the genus, where *Acrosternum* shape showed a cylindrical elongated shape similar to *Chinavia*, but where differences were found in the area of the ocular cavities that have a shape slightly wider, but not as wide as the species of *Nezara*. The first 3 PCs accounted for more than 77% (PC1 = 47.82%; PC2 = 22.02%; PC3 = 7.62%) of the total head shape variation and provided a sound estimate of the total amount of head shape variation. Examining the morphospace, a clear overlap occurred between *Acrosternum* and *Chinavia* but little among these 2 genera and the genus *Nezara* in the shape space constructed by the first 2 PCs; these results support the fact that the first 2 genera are often confused with one another if no information about the origin is known (Fig. 2). It is important to mention that the multivariate regression found a small but significant influence of size in the head data as 10.6% ($P = 0.0162$) and for Pronotum 5.3% of size influence ($P = 0.015$). ANOVA analyses support significant differences in the size of the head among the 3 genera (as measured by centroid: $F = 6.76$, $P < 0.0032$) and shape (as measured by Procrustes: $F = 5.07$, $P < 0.0001$) and justified further statistical analyses. The largest Procrustes distances found were between *Acrosternum* and *Nezara* and for Mahalanobis distances between *Chinavia* and

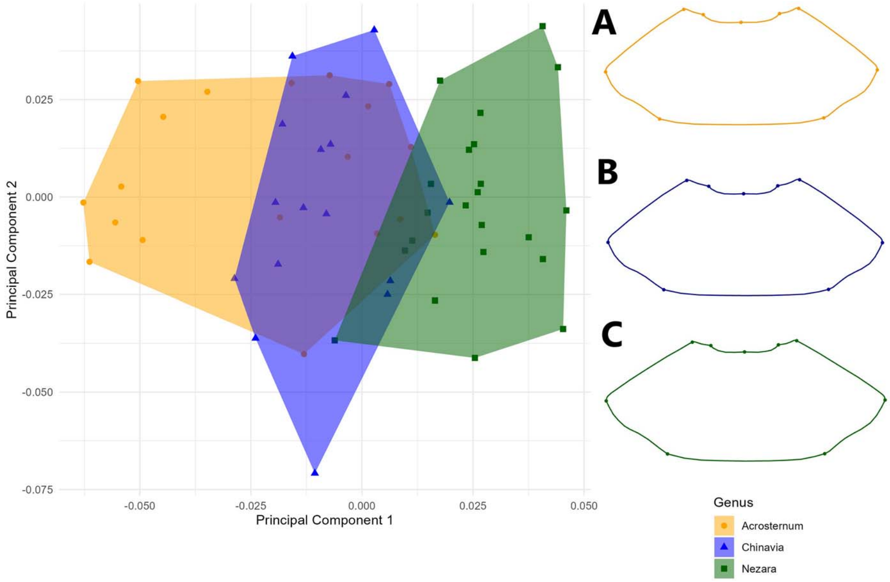

Fig. 3. Principal Component Analysis (PCA) of pronotum shape among the 3 genera of the tribe Nezarini. The symbols represent different genera: yellow circles for *Acrosternum*, blue triangles for *Chinavia*, and green squares for *Nezara.* The average shape of the genera was shown by letters: (A) *Acrosternum*, (B) *Chinavia*, (C) *Nezara*.

Nezara, respectively, while the shortest Procrustes and Mahalanobis distances, as expected, were between *Acrosternum* and *Chinavia*. The P values for the Mahalanobis distances were statistically significant between all genera ($P < 0.0001$). In contrast, the P values for the Procrustes distances were significant between *Nezara* with either *Chinavia* or *Acrosternum* ($P < 0.0003$ and $P < 0.0004$, respectively) but not significant between the last 2 genera ($P < 0.1466$).

Differences in pronotum shape among the 3 genera of the tribe Nezarini. A PCA of the covariance matrix of the average shape showed that the pronotum shape followed a clear pattern associated with the genus, where in contrast to the head the shape was most similar among genera, where *Acrosternum* and *Chinavia* share a wider pronotum but *Chinavia* was more elongated than *Acrosternum.* Nevertheless, the more elongated and pointed in both extreme pronotum were the specimens from *Nezara*. The first 3 PCs accounted for more than 81% ($\text{PC1} = 39.74\%$; $\text{PC2} = 27.45\%$; $\text{PC3} = 13.98\%$) of the total pronotum shape variation and provided a sound estimate of the total amount of pronotum shape variation. Pronotum shape variation was clearly distributed along PC1, with many individuals overlapping with *Chinavia* specimens. Nevertheless, the overlap was less evident than that observed for head shape, indicating more significant variation across the genera (Fig. 3).

ANOVA analyses support significant differences in the shape of the pronotum among the 3 genera (as measured by Procrustes: $F = 10.67$, $P < 0.0001$) but not for the centroid size (as measured by centroid: $F = 0.24$, $P = 0.7853$). The largest

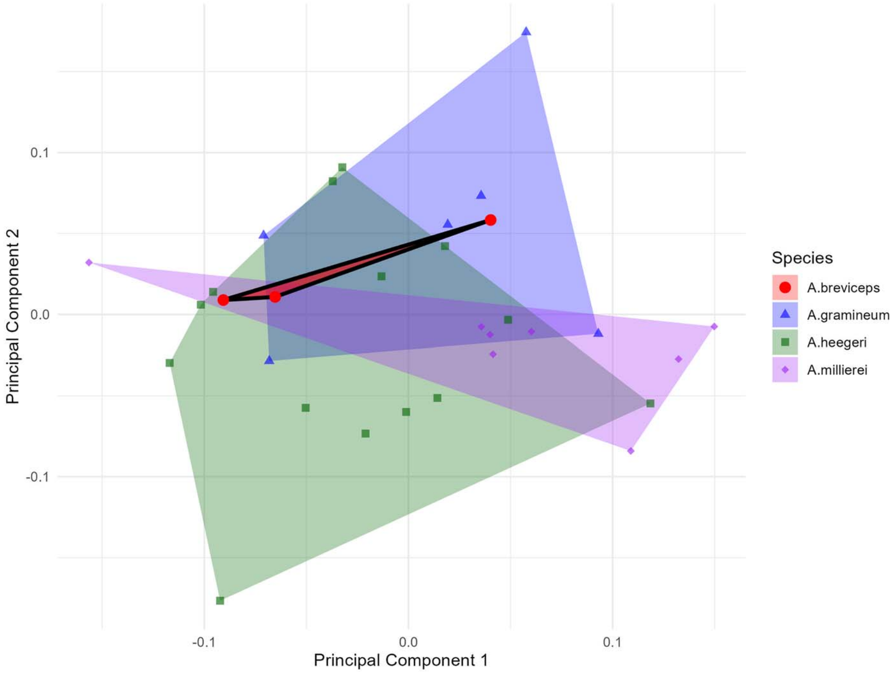

Fig. 4. Principal Component Analysis (PCA) of head shape among species of the genus *Acrosternum*. The color symbols represent the following species: red circles (*A. breviceps*), blue triangles (*A. gramineum*), green squares (*A. heegeri*), and purple diamonds (*A. millierei*). The black line between *A. breviceps* specimens was added to better represent the overlap within this group.

Procrustes distances found were between *Acrosternum* and *Nezara* and for Mahalanobis distances between *Chinavia* and *Nezara*, while the shortest Procrustes and Mahalanobis distances, as expected, were between *Acrosternum* and *Chinavia* all similar results to what was observed with the shape of the head (previous analysis herein). The P values for the Mahalanobis distances were statistically significant among all genera ($P < 0.0001$). In contrast, the P values for the Procrustes distances were significant between *Nezara* with either *Chinavia* or *Acrosternum* (both $P < 0.0001$) but not significant between the las 2 genera ($P < 0.0138$).

Differences in head shape among 4 species of *Acrosternum*. The first 3 PCs accounted for more than 82% (PC1 = 44.16%; PC2 = 27.74%; PC3 = 11.1%) of the total head shape variation and provided a sound estimate of the total amount of its variation. A clear overlap of all 4 species confirms the cryptic morphology of this group of species, nevertheless *A. heegeri* and *A. gramineum*, showed to have more variance in the head morphology contrary to *A. breviceps* which overlaps completely in the cloud of points of *A. gramineum,* and *A. millierei* which showed a head variation with part of its shape distributed by the PC1, being in the half of PC2 (Fig. 4).

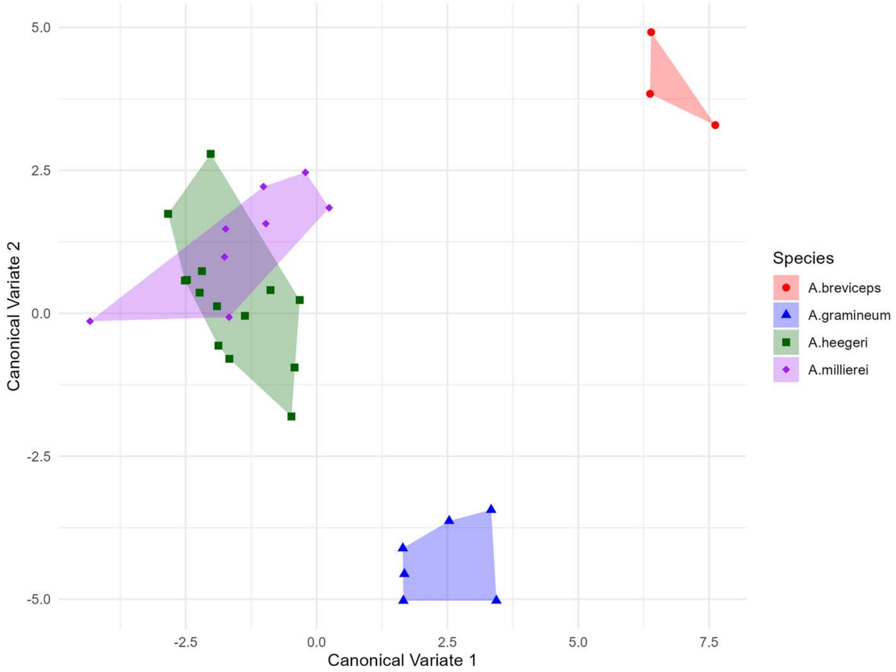

Fig. 5. Canonical Variate Analysis (CVA) of head shape among species of the genus *Acrosternum*. The color symbols represent the following species: red circles (*A. breviceps*), blue triangles (*A. gramineum*), green squares (*A. heegeri*), and purple diamonds (*A. millierei*).

ANOVA analyses support significant differences in the head shape among the species of *Acrosternum* (as measured by Procrustes: $F = 2.31$, $P < 0.0008$) as well as the centroid size (as measured by centroid: $F = 10.45$, $P = 0.0002$). Mahalanobis distances were more considerable between *A. heegeri* and *A. millierei* (= 53.15) and the shortest between *A. breviceps* and *A. gramineus* (= 7.26). On the other hand, Procrustes distances were also more considerable between *A. breviceps* and *A. millierei* (= 0.1115) and the shortest between *A. gramineus* and *A. heegeri* (= 0.0779).

A CVA was performed to magnify the differences between groups; using the new axes of maximum variation, a clear differentiation was observed, particularly between *A. breviceps* and *A. gramineum*. In contrast, *A. heegeri* and *A. millierei* showed considerable overlap, indicating few shape differences between these highly cryptic species (Fig. 5). Procrustes, Mahalanobis distances, and associated permutation tests (corrected for multiple comparisons) revealed significant relationships among the 4 *Acrosternum* species evaluated in this study. The greatest Mahalanobis distances for head shape were observed in pairwise comparisons between *A. breviceps* and *A. gramineum*. In contrast, the largest Procrustes distances for head shape were found between *A. breviceps* and *A. millierei*. The *P*-values for Mahalanobis distances were statistically significant for all species comparisons ($P < 0.05$). However, for Procrustes distances, significance was only observed between

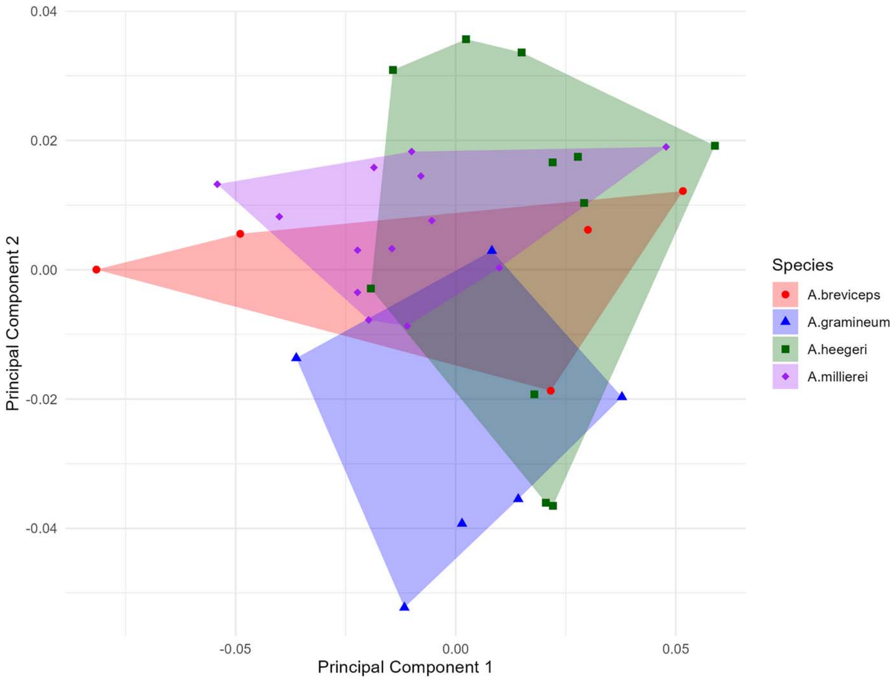

Fig. 6. Principal Component Analysis (PCA) of pronotum shape among species of the genus *Acrosternum*. The color symbols represent the following species: red circles (*A. breviceps*), blue triangles (*A. gramineum*), green squares (*A. heegeri*), and purple diamonds (*A. millierei*). The black line between *A. breviceps* specimens was added to better represent the overlap within this group.

A. breviceps and *A. heegeri* ($P < 0.05$), while for the other species pairs, P values exceeded 0.05.

Differences in pronotum shape among 4 species of *Acrosternum*. The first 3 PCs accounted for more than 78% (PC1 = 49.2%; PC2 = 23.47%; PC3 = 6.25%) of the total pronotum shape variation and provided a sound estimate of the total amount of pronotum shape variation. Even when the variation seems to be accumulated on the PC1 mostly, the variation occurs on the PC2, where a clear overlap of the 4 species is more evident in the center of the morphospace; *A. heegeri* was the species with more variance across the genus and, on the contrary, *A. millierei* was the species with less variance and overlapped with *A. heegeri* and *A. breviceps.* In addition, *A. gramineum* was the most different species in the genus morphospace (Fig. 6).

ANOVA analyses support significant differences in the shape of the pronotum shape among the 4 studied species of *Acrosternum* (as measured by Procrustes: $F = 2.99$, $P < 0.0001$) as well as the centroid size (as measured by centroid: $F = 24.13$, $P < 0.0001$). The most considerable differences between Procrustes and Mahalanobis distances were found between *A. gramineum* and *A. heegeri*. In contrast, the shortest Procrustes distances were found between *A. breviceps* and *A. millierei,* and the shortest Mahalanobis distances between *A. heegeri* and *A. millierei.*

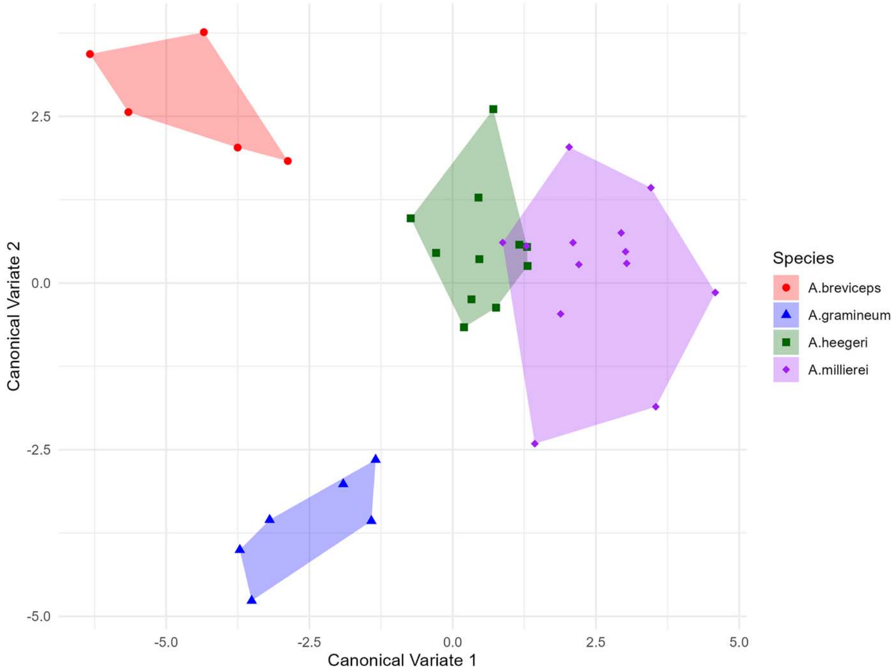

Fig. 7. Canonical Variate Analysis (CVA) of pronotum shape among species of the genus *Acrosternum*. The color symbols represent the following species: red circles (*A. breviceps*), blue triangles (*A. gramineum*), green squares (*A. heegeri*), and purple diamonds (*A. millierei*).

Fig. 7 shows the CVA graph, which reveals a similar shape variation between *A. heegeri* and *A. millierei* as observed in the head shape, but with more significant variance and no overlap between them. Additionally, a clear distinction in pronotum shape was found between *A. breviceps* and *A. gramineum*, demonstrating that these species exhibit distinct morphological differences. In contrast, *A. heegeri* and *A. millierei* form a more cryptic group.

The Procrustes and Mahalanobis distances and associated permutation tests (corrected for multiple comparisons) showed significant relationships among the species. The most considerable Mahalanobis distances for the pronotum shape were found between *A. breviceps* and *A. millierei* for pairwise comparisons. On the other hand, the Procrustes distances for the pronotum shape were more considerable between *A. gramineum* and *A. millierei*, showing a more or less distinctive pronotum shape for *A. millierei* compared to that of the other species. The P values for the Mahalanobis distances were statistically significant between all species ($P < 0.001$). In contrast, the P values for the Procrustes distances were only significant between *A. gramineum* and *A. millierei* ($P = 0.0009$), between *A. millierei* and *A. heegeri* ($P = 0.0046$), and between *A. gramineum* and *A. heegeri*.

Discussion

Herein, we confirm the power of geometric morphometrics as a tool for analyzing and quantifying the morphological variability of biological traits giving clear, well-structured results to discriminate closely related taxa (Villalobos-Leiva and Benítez 2020). This is particularly useful in groups where subtle morphological differences pose taxonomic challenges, such as in the Nezarini tribe of Pentatomidae. Our results confirm that head and pronotum shapes partially differentiate between *Acrosternum*, *Chinavia*, and *Nezara* genera. The PCA, revealed morphological patterns, with *Nezara* forming a distinct morphospace, while *Acrosternum* and *Chinavia* exhibited more significant overlap. This is consistent with their taxonomic history, as these last 2 genera were once classified together before being formally separated. This similarity makes separating specimens of these 2 genera difficult when there is no information about the locality or when we deal with a species introduced outside its range and in the range of the other "sister" genus. In cases like this, geometric morphometrics can be highly useful for help in species identification. This approach does not require the examiner to be an expert in the group, as a high-quality image of the entire body in dorsal view or a close-up of the head or pronotum is sufficient for analysis. Smith-Pardo et al. (2025) applied geometric morphometrics to analyze head and thorax shape variation in 8 species of thrips, including both common and quarantine significant pests in the United States. Using landmark-based analysis on high-resolution images, the results revealed clear species differentiation, confirming GM as a valuable tool for distinguishing closely related species, even when traditional morphological characters are insufficient for identification. Other studies have integrated additional tools to improve taxonomic identification, with ecological data providing valuable insights for improved species descriptions (Cáceres et al. 2023, Jaramillo-O et al. 2015, Sagastume-Espinoza et al. 2024, Viscosi and Cardini 2011). For example, Cruz et al. (2023) applied geometric morphometrics and ecological niche modeling to analyze the same traits examined in this study but in *Triatoma pallidipennis* Stål. Their findings revealed significant head shape differences among haplogroups, while pronotum shape showed more significant overlap. These results suggest cryptic species variation and highlight the importance of combining morphometric and ecological approaches for improved taxonomic resolution. One of the most noticeable results of this study was the ability of GM to discriminate among the 4 commonly captured species of *Acrosternum*. Despite their cryptic morphology, both PCA and CVA revealed clear patterns of variation that allowed the separation of *A. breviceps* and *A. gramineum*, while *A. heegeri* and *A. millierei* showed greater overlap. These results highlight the potential of GM to resolve subtle interspecific differences, even in groups where traditional taxonomy struggles to provide reliable diagnostic characters. The use of GM for identification of closely related species has been very useful for taxonomy and systematics studies where landmarks have been used as useful traits (Catalano et al. 2010, Catalano and Torres 2017).

Hemiptera species have been used for different examples in the use of geometric morphometrics, even for agronomic or health problem-based. Belintani et al. (2022) used geometric morphometrics to analyze female genitalia in Triatominae, revealing shape-based taxonomic differentiation. Their study highlighted operational homology and its role in phylogenetic relationships. These findings reinforce geometric morphometrics as a valuable tool for species identification and vector surveillance. Thanks to this work (the first in the tribe Nezarini or the genera in it), we can now explore more profound applications of GM for the identification of groups of insects that are not only important in agriculture but also are highly diverse and with slight variation in the family Pentatomidae. The ability to distinguish between species using morphometric data extracted from images that indirectly apply to biosecurity and agricultural pest monitoring. Many intercepted species lack comprehensive taxonomic revisions or user-friendly identification keys, making visual identification challenging, especially for non-experts. This study opens the possibility for integrating GM into rapid diagnostic workflows at quarantine stations and border inspections by demonstrating that high-resolution images and landmark-based GM analyses can accurately differentiate species.

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Data will be made available on request.

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