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Variation in wings shape of the common Neotropical stingless bee *Tetragonisca angustula* (Hymenoptera: Meliponini) across an urbanization gradient

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Abstract: *Tetragonisca angustula* bees are widely distributed throughout Brazil and nesting in various substrates, including urban areas. This study aims to investigate the environmental effects on wing morphometry in *T. angustula* bees along an urban gradient, determining if and where these variations occur and whether asymmetry increases in urbanized environments. We evaluated three distinct locations: an urban area, a semi-natural urban area, and a rural area. The results revealed different morphometric patterns related to both wing shape and asymmetry supporting the hypothesis that urban areas generate more biological stress in individuals compared to the other two areas. Additionally, the findings suggest that urbanization affects the wing morphometrics of the species' anatomical landmarks, indicating possible influences of food resources, temperature, and ecological pressures on these variations. Our study enhances the understanding of morphological variations in urban pollinators as a proxy for environmental quality and can help devise strategies to mitigate the negative effects associated with increased urbanization.

Key words: Geometric morphometrics, Native bee, Morphological plasticity, Urban Ecology.

INTRODUCTION

The development of intensive agriculture and animal production has raised concerns about bees' exposure to substances they previously did not encounter, such as fertilizers and pesticides, resulting in contaminant residues (Rundlöf et al. 2015). Another significant factor negatively affecting bees is urbanization, which leads to habitat loss, scarcity of food resources, changes in land use, air pollution, and the introduction of invasive species, all of which adversely impact urban bees (Freitas et al. 2007, Migdał et al. 2018, Birdshire et al. 2020). This process contributes to the decline of bee species due to the reduction of natural vegetation and its replacement by urban structures, posing significant challenges to the health and survival of these vital pollinators (Hostetler & McIntyre 2001, Hoffmann et al. 2005, Nunes et al. 2015, Banaszak-Cibicka et al. 2018, Peil & Aranda 2021).

Although urban areas are not natural habitats, they can serve as refuges for wildlife, enabling some species to persist despite human-induced modifications—provided that certain abiotic and biotic conditions support their survival (Hernandez et al. 2009, Ayers & Rehan 2021, Berger et al. 2024). Urban habitat alterations directly impact bees, influencing not only their abundance but also their morphology, including bilateral symmetry, which can be affected by stressors agents (Nunes et al. 2015, Banaszak-Cibicka et al. 2018, Benítez et al. 2020, Helm et al. 2021).

Bilateral symmetry reflects symmetrical development under favorable environmental conditions, as both sides originate from the same genes (Benítez et al. 2020). In insects like bees, variations in symmetry can indicate ecological stress, with increased asymmetry observed under adverse conditions such as food scarcity, environmental stress, or disruptions in normal development (Hoffmann et al. 2005, Nunes et al. 2015, Banaszak-Cibicka et al. 2018, Benítez et al. 2020, Helm et al. 2021, Tommasi et al. 2022, Zakharov & Trofimov 2022). Fluctuating asymmetry (FA) is widely recognized as a key indicator of developmental stability, reflecting an organism's ability to withstand environmental and genetic disturbances during ontogeny (Graham et al. 2010, Graham 2020). This subtle variation in bilateral symmetry arises from stochastic processes influencing development and is shaped by factors such as environmental stress, genetic load, and mutations (Zakharov & Trofimov 2022). Research consistently links increased FA to adverse conditions, suggesting greater developmental instability with potential consequences for an organism's fitness (Graham 2020). As a result, FA serves as a valuable tool for assessing the impact of environmental stressors and gaining insights into the underlying mechanisms of developmental stability across biological systems. Urban factors like pollution, temperature, and food scarcity may contribute to this increase in asymmetry (Banaszak-Cibicka et al. 2018).

Geometric morphometrics is the study of variation in the size and shape of an organism, using the statistical evaluation of the variation of predetermined points in two- or three-dimensional space (Daly 1985). Geometric morphometrics offers a method to assess changes in morphology and asymmetry, providing a deeper understanding of structural development, particularly in insect wings (Daly 1985, Benítez et al. 2020, Vaca-Sánchez et al. 2023). This approach is particularly useful in insects due to their rapid life cycles and the ease with which precise measurements can be obtained (Daly 1985). In the context of bees, geometric morphometrics is a fundamental tool for analyzing the impacts of environmental changes on populations. In stingless bees, changes in wing asymmetry were observed in colonies developed in rational boxes, when compared with natural nests in trees (Lima et al. 2016) or during the transport of managed colonies to other areas, indicating a stress response during the process (Andrade et al. 2020). Bees depend on environmental factors, such as the availability of floral resources and nesting sites, changes in these aspects can significantly influence body size and wing morphology (Chole et al. 2019). Understanding these impacts is essential for developing conservation strategies and ensuring the survival of these crucial pollinators, thereby maintaining the stability of urban ecosystems (Benítez et al. 2020).

Tetragonisca angustula (Latreille, 1811) (Hymenoptera: Apidae: Meliponini). commonly known as Jataí, has a broad geographical distribution, ranging from southern Brazil to Mexico (Engel et al. 2023). Due to this extensive range, the species exhibits diverse behaviors in foraging and shelter selection (Roubik 2023) and demonstrates remarkable adaptability in nesting habits, utilizing various substrates (Braga et al. 2012). It is commonly found in tree trunk cavities, concrete blocks, and walls, taking advantage of the ease of constructing aerial nests (Braga et al. 2012).

This study aims to investigate the effects of an urban gradient on the wing shape of *T. angustula* bees, determining if, where and to what extent these variations occur. We tested whether an increase in wing asymmetry will be greater in unfavorable urbanized environments. We hypothesized that greater morphometric differences would be observed in urban areas, where wing morphometry will be different and asymmetric differences being higher in these environments.

MATERIALS AND METHODS

Study area

The study was conducted in the state of Mato Grosso do Sul, Brazil. The study areas were selected and categorized into urban, semi-natural urban and rural areas (Figure 1a–e). For the study region in Campo Grande, Mato Grosso do Sul, approximately 13 species of stingless native bees belonging to the genera *Paratetrapedia*, *Paratrigona*, *Partamona*, *Plebeia*, *Scaptotrigona*, *Tetragona*, *Tetragonisca* and *Trigona* are reported (Aranda & Graciolli 2015).

The areas were categorized according to the type of land occupation and activities developed in their surroundings. The characterization of urban areas was based on the vegetation

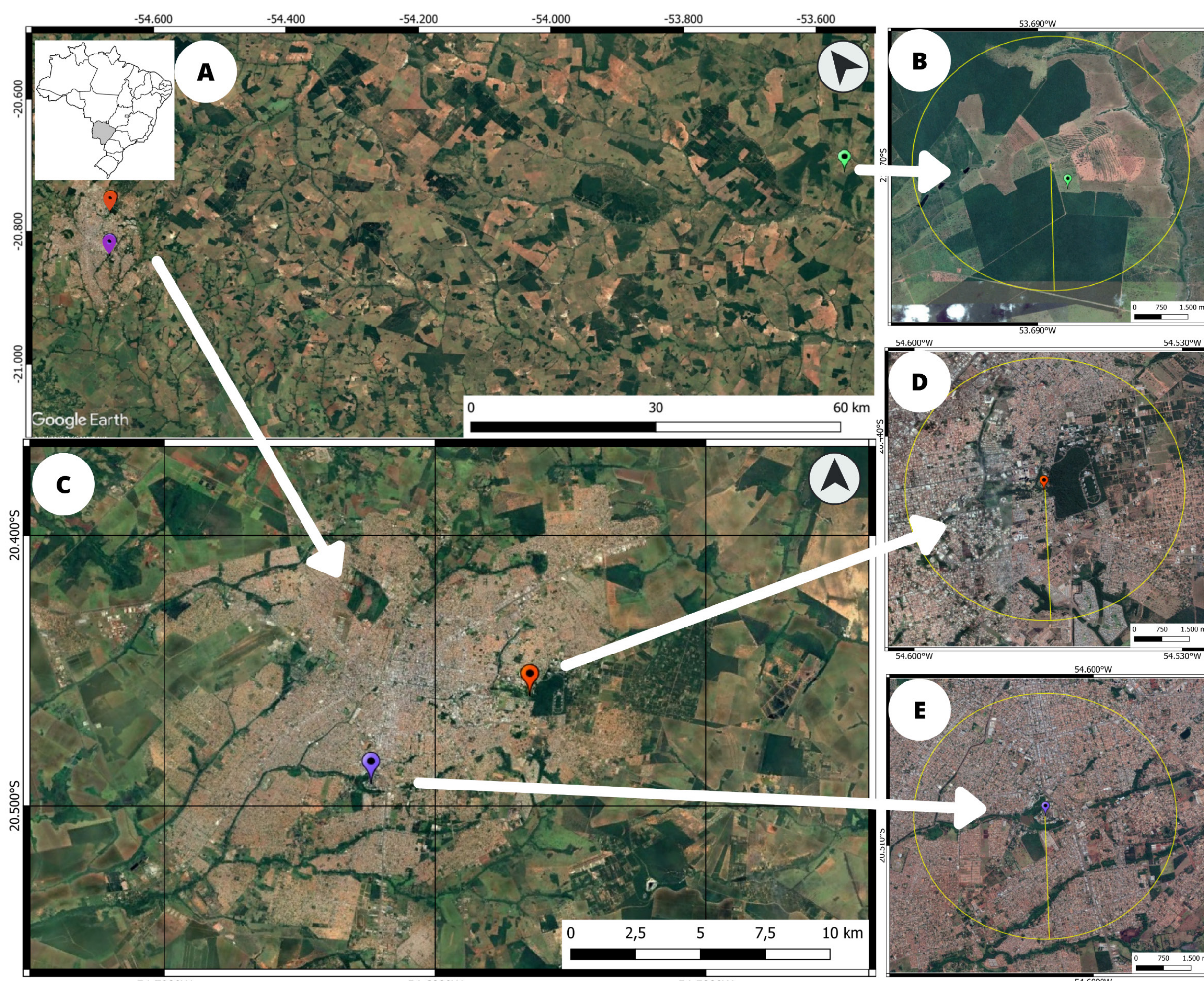

Figure 1. **(a) Sampling locations. The three study areas include natural urban (purple dot) urban (red dot) and rural areas (green dot); (b) Rural area located on a farm in Ribas do Rio Pardo (n = 54); (c) Natural Urban (n = 55) and Urban (n = 54) areas in Campo Grande, Mato Grosso do Sul; (d) Natural urban area located in Parque das Nações Indígenas; and (e) Urban area located in the University Campus at the Universidade Federal de Mato Grosso do Sul.**

cover in the surroundings of the sampling sites, considering an approximate 3 km radius. This radius was chosen based on the average foraging distance of Jataí colonies (*Tetragonisca* spp.) workers, thus ensuring that there is independence between sampling locations, which typically ranges from 600 to 650 meters (van Nieuwstadt & Iraheta 1996, Kaehler et al. 2024). This approach ensures that the assessed landscape encompasses potential foraging areas beyond the species' mean foraging range The categorization of urban areas was based on the vegetation cover present in the surroundings of the sampling area, considering the average foraging distance of Jataí colonies within an approximate radius of 3 km. The distance between urban areas is greater than the foraging area of Jatais (*Tetragonisca* spp, ~ 600 – 650 meter).

To approximate the green area, the satellite image was used, and the green area was calculated. Considering the area of the yellow circle on the maps (~28.2 km^2), the area with and without vegetation cover was calculated. To characterize the green areas, present in the analyzed images, the ImageJ software was used to quantify the vegetation cover. Initially, the images were opened in the program and converted to an 8-bit scale. Then, the "Threshold" adjustment was applied to highlight and define the limits of the vegetated areas. The scale calibration was performed based on a known reference (a 3 km radius), allowing the definition of the real distance in the image. The areas of interest were delimited using the "Wand Tool", which allowed the precise selection of the vegetated regions. Finally, the areas were measured using the "Measure" function, and the values obtained were recorded to calculate the percentage.

The rural area is located on a farm in the municipality of Ribas do Rio Pardo (Figure 1b - 21°04'15.60"S. 53°41'11.64"W) and is characterized by successive stages of Cerrado vegetation. This includes areas designated for livestock activities. intermediate regeneration areas where cattle grazing has been absent for the past 10 years, and preserved areas with native vegetation, including a legally protected reserve. It was estimated that the total vegetation cover in the image is approximately 75%. This estimate includes both dense forests and open fields and agricultural areas.

The semi-natural urban area is "Parque das Nações Indígenas." a city park in Campo Grande (Figure 1d - 20°27'84"S. 54°34'26"W), covering an area of 1.163.876.98 m². This environment features leisure and sports infrastructure along the shores of a lake formed by the headwaters of the Prosa stream. and it borders the Prosa State Park, thereby expanding the contiguous area of native vegetation. It was estimated that the total vegetation cover in the image is approximately 35%. This estimate includes both dense vegetation and sparse trees and undergrowth.

The urban area is located within the Universidade Federal de Mato Grosso do Sul (UFMS) (Figure 1e - 20°30'11.67"S. 54°36'40.77"W) and is characterized as an urbanized zone. Within the campus, there are patches of native vegetation, as well as a lake connected to the Cabaça and Bandeira streams, forming the Bandeira basin, an integral part of the Paraná River basin. The surroundings of UFMS are predominantly composed of residential areas with significant urban density. It was estimated that the total vegetation cover in the image is approximately 20%. This estimate includes both dense vegetation and sparse trees and undergrowth. The presence of riparian vegetation also increases the total estimate.

Collection and measurements

Tetragonisca angustula specimens (Figure 2), all female workers, were collected from the three study areas between November 2021 and March 2022, a period corresponding to the time of year when bees are most active. During this time, bees need to cover greater distances and explore larger foraging areas to obtain food and water compared to other times of the year (Couvillon et al. 2014). Collections in the rural area were conducted in November 2021 from 9 a.m. to 4 p.m., with bees being collected while foraging on the farm's flowers. In the city, collections took place from December 2021 to March 2022. In the semi-natural urban area, bees were collected while foraging between 9 a.m. and 3 p.m. during December 2021 and January 2022. In the urban area, approximately 5 individuals were collected from 10 previously identified (to avoid pseudoreplication by sampling from only one nest) natural nests across the campus, between 9 a.m. and 4 p.m. during February and March 2022. The sampling of bees that were returning from foraging activity ("campeiras") was ensured so that the sampling in the nests would be similar to the sampling carried out in the areas where the individuals were collected foraging.

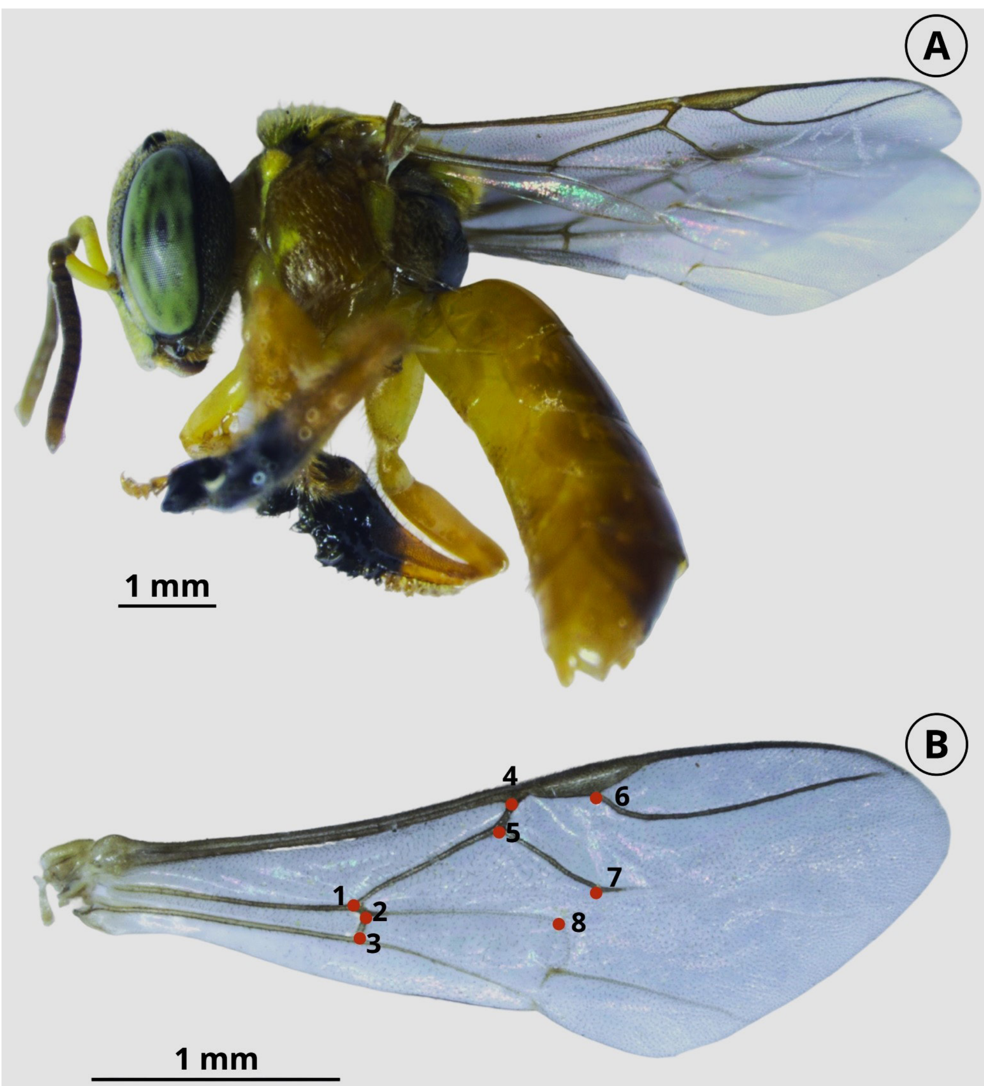

Figure 2. (a) Lateral view of *Tetragonisca angustula* worker, a Jataí bee. (b) Anatomical landmarks (red dots) used for analyzing the size and shape of the forewing (in the image, the right wing).

At the end of each collection, a small amount of cotton with ether was placed in the tube to anesthetize the bees. The bees were then transferred to 90% alcohol for preservation. A total of 163 individuals and 326 wing samples were prepared for analysis (considering both left and right anterior wings). The collections were authorized by the permanent license for collecting biological material. MMA-ICMBio-SISBIO No. 61938-1 (Authentication code: 86896465).

For the analysis, both the right and left anterior wings were removed from the 163 specimens (rural area: n = 54, natural urban area: n = 55, and urban area: n = 54) using scissors and forceps. For geometric morphometric characterization analyses, only the right wings were used to describe variations between areas. To evaluate asymmetry, both wings were utilized. Using the anterior wing is a standard protocol for comparisons in studies involving Hymenoptera (Owen 2012). Slides containing 10 wings (5 pairs per slide) were prepared and the wings were photographed using a Stereo Discovery V.20 stereomicroscope with an attached Zeiss camera and ZEN lite 2011 software (Zeiss) to capture images at standardized scales.

The morphometric analyses focused on assessing variations in wing venation landmarks among individuals from the three study areas (Figure 2). The methodology described by Francoy et al. (2012) was used for image processing. To this, 8 anatomical landmarks were manually plotted at the venation intersections on each wing of the *T. angustula* specimens. Landmark markings were consistently performed by the same person. These landmarks represent positional coordinates on a Cartesian plane (X and Y). The landmarks were numbered according to their distribution along the intersections of the wing cells (Francoy et al. 2012). To ensure that there was no gross measurement error, the repeatability of the distance (Lessells & Boag 1987), a measure of the proportion of variance between individuals not due to measurement error, was calculated from a subsampling corresponding to 20 individuals for each area, where 3 replicates for each wing were performed, indicating the reliability of the measurements (Supplementary Material - Table SI).

Data analysis

To obtain the anatomical landmarks takes for geometric morphometric analyses, the software ImageJ (Rasband 2011) was utilized. Once the landmarks were defined, their values on the Cartesian planes (X and Y axes) were recorded. To assess asymmetry, the left wings underwent an image mirroring process to ensure that the data obtained for the left wings were corresponding and comparable with those for the right wings.

Landmark values were used to perform Procrustes alignment, a technique that adjusts and aligns datasets by applying transformations such as scaling, translation, and rotation, thereby standardizing the anatomical landmarks across all wings relative to their centroids (Rohlf & Marcus 1993). This procedure eliminates the effect of size, allowing the analysis to focus on shape variations. The alignment and rotation of data were performed using all intersection points in the PAST® software (Hammer & Harper 2001).

Additionally, an analysis was conducted to determine if there was variation in the overall shape of the wings among the different areas. For the geometric morphometric comparison, Procrustes-transformed data of only the right wings from all individuals across the three areas were used. Principal Component Analysis (PCA) was performed to ascertain the amount of variance explained by each principal component (anatomical landmarks).

To test for significant differences in geometric morphometrics among the areas, a Multivariate Analysis of Variance (MANOVA - Pillai's trace test - since the data has the assumptions for application; Supplementary material - Table SII) was employed to analyze differences between groups (areas) in multiple dependent variables (anatomical landmarks). Based on the results, a Thin Plate Spline (TPS) deformation grid was generated, where the values in the Principal Strains represent the magnitudes and directions of local deformations occurring during the TPS transformation from the centroid. These Principal Strain values describe the alterations in the original shapes of the objects. The areas with high values in Principal Strains indicate the points of the wing that underwent greater changes, and the regions with high positive values represent areas where there was greater variation. The direction of the deformation vectors indicates how the shape of the wing is changing, with vectors pointing outwards (black line), there was expansion of the centroid expanding in that direction and if they point inwards (red line), there was a contraction in that direction. All statistical analyses were conducted using the free software PAST® 4.0.

The difference between the measurements from the left and right sides of each landmark was calculated and indicated the fluctuating asymmetry of the wing and we termed "Degree of asymmetry" (asymmetry = left - right). In this case, asymmetry can be negative (indicating greater variation in the right wing), positive (indicating greater variation in the left wing) or zero (representing perfect symmetry). The degree of asymmetry was calculated for each individual. For multiple anatomical traits, we used the F11 index provided by Palmer (1994), which involves calculating the difference between the left and right sides of each attribute pair (in this case, the landmarks), summing these differences and dividing by the number of samples (for more details, see Palmer 1994). The mean values of wing asymmetry among the areas were compared using Analysis of Variance (ANOVA), given that the data followed a normal distribution.

RESULTS

Morphology of the wings

In total, 5376 landmark annotations were made on 336 wings (Supplementary material - Table SIII). The left wing was evaluated with the same protocols as the right wing and presents the same general pattern of differences found between the study areas (Supplementary material - Figure S1), therefore we present in greater detail and discuss the results of the right wings. Differences in right wing shape were observed across the study areas (Table I, Figure 3a). Principal Component Analysis (PCA) revealed the direction and magnitude of the loadings with respect to the principal components (Figure 3b) for each of the landmarks, which helped in differentiating the groups. Landmarks with higher magnitudes (positive or negative) were those that contribute most to the variation explained by the principal component, for example, LX1, LY3 and LY7 have high loadings, indicating that these coordinates are important in the variation of the shape (Figure 3b). Landmarks with positive loadings (e.g.: LX1, LX3, LX5) are associated with an increase in the values of the principal component, while those with negative loadings (e.g.: LY1, LY3, LY7) indicate the opposite direction of variation. This suggests that there is a specific relationship between these coordinates in the variation of the wings. The significance of variations between the collection areas was assessed through Multivariate Analysis of Variance (MANOVA), showing significant differences in wing shape between rural and urban populations of *T.*

Table I. Mean and standard deviation of the landmarks in the X and Y positions in the Cartesian measurement plane of the right and left wings of *Tetragonisca angustula* in three different areas.

	Rural area				Urban natural area				Urbanized area			
	Left		Right		Left		Right		Left		Right	
LX1	598.09	±80.42	650.43	±82.75	618.98	±72.48	683.82	±70.34	612.37	±69.31	673.75	±77.51
LX2	613.17	±81.44	665.35	±82.87	633.97	±72.54	699.57	±69.86	626.41	±68.89	689.01	±78.02
LX3	584.95	±81.50	638.39	±82.48	607.41	±71.55	673.65	±69.50	601.53	±68.59	664.89	±77.80
LX4	988.84	±73.89	1040.04	±81.94	1031.41	±69.09	1095.97	±68.18	1023.75	±73.58	1086.45	±74.05
LX5	950.08	±74.29	1001.52	±82.19	989.24	±68.71	1053.61	±69.20	983.39	±73.38	1045.16	±74.21
LX6	1177.46	±70.61	1227.58	±83.94	1219.21	±67.03	1286.11	±71.37	1215.18	±77.09	1279.25	±73.54
LX7	1146.51	±73.26	1175.61	±166.66	1177.61	±65.73	1244.19	±70.46	1174.71	±77.55	1239.41	±74.12
LX8	1049.54	±73.81	1099.29	±83.28	1079.52	±66.27	1144.59	±72.32	1069.38	±75.23	1136.39	±74.53
LY1	782.50	±66.96	810.93	±79.97	778.35	±58.73	825.42	±78.75	782.08	±73.70	789.92	±80.99
LY2	804.07	±67.33	832.75	±80.31	800.29	±59.56	847.30	±77.73	803.98	±73.40	813.73	±81.32
LY3	854.32	±67.32	882.57	±80.98	848.26	±58.70	894.82	±79.23	851.64	±74.84	860.91	±80.01
LY4	609.14	±70.61	638.04	±76.91	599.42	±61.51	645.48	±74.61	602.25	±73.02	608.30	±83.21
LY5	668.93	±68.93	697.33	±76.12	662.97	±61.13	708.30	±75.37	665.81	±72.16	672.89	±82.26
LY6	636.52	±69.56	666.06	±74.73	632.84	±65.58	674.87	±74.32	633.45	±73.32	639.01	±83.42
LY7	841.57	±66.03	870.37	±76.49	846.38	±60.03	892.45	±74.93	852.75	±76.05	857.08	±82.67
LY8	901.27	±65.75	930.48	±76.09	903.13	±60.12	949.86	±74.42	908.27	±75.96	914.96	±82.14

angustula (Pillai's trace = 0.61; F = 4.53. p < 0.01). Specifically, bees from urban environments exhibited greater modifications in wing variation compared to those from rural areas. Although no significant difference was found between the two urban areas (p = 0.18), the rural area differed significantly from both the natural urban area (p < 0.01) and the urban area (p < 0.01). Consequently, the results suggest that morphological changes in wing structure were more pronounced in urban areas.

Variations in Anatomical Landmarks

The direction and magnitude of the shape variations are visualized through deformation plates that depict the displacement of each landmark relative to the mean configuration (i.e., the average of all landmark coordinates) (Figure 3c-f). In general, an expansion in landmark positions was observed, with expansion being more pronounced than retraction, both when evaluating all areas together (Figure 3c) and when examined separately (Figure 3d–f). Moreover, a clear separation of individuals from the rural area compared to those from natural urban and urban areas is evident (Figure 3a). The deformation plot of wing landmarks (Figure 3c) further illustrates how local variations in wing shape alter the positions of the landmarks. Specifically, for the rural context (Figure 3d), minimal variation was observed, with a predominance of minor local shortening, notably at landmark 6. In the natural urban area (Figure 3e), the deformation pattern is characterized by relative expansions at landmarks such as 5 and 8, while in the urban area (Figure 3f) significant deformations are observed particularly at landmarks 1, 4, and 5.

Asymmetry

The differences in wing asymmetry among the areas were significant (F = 23.38. p < 0.001), with

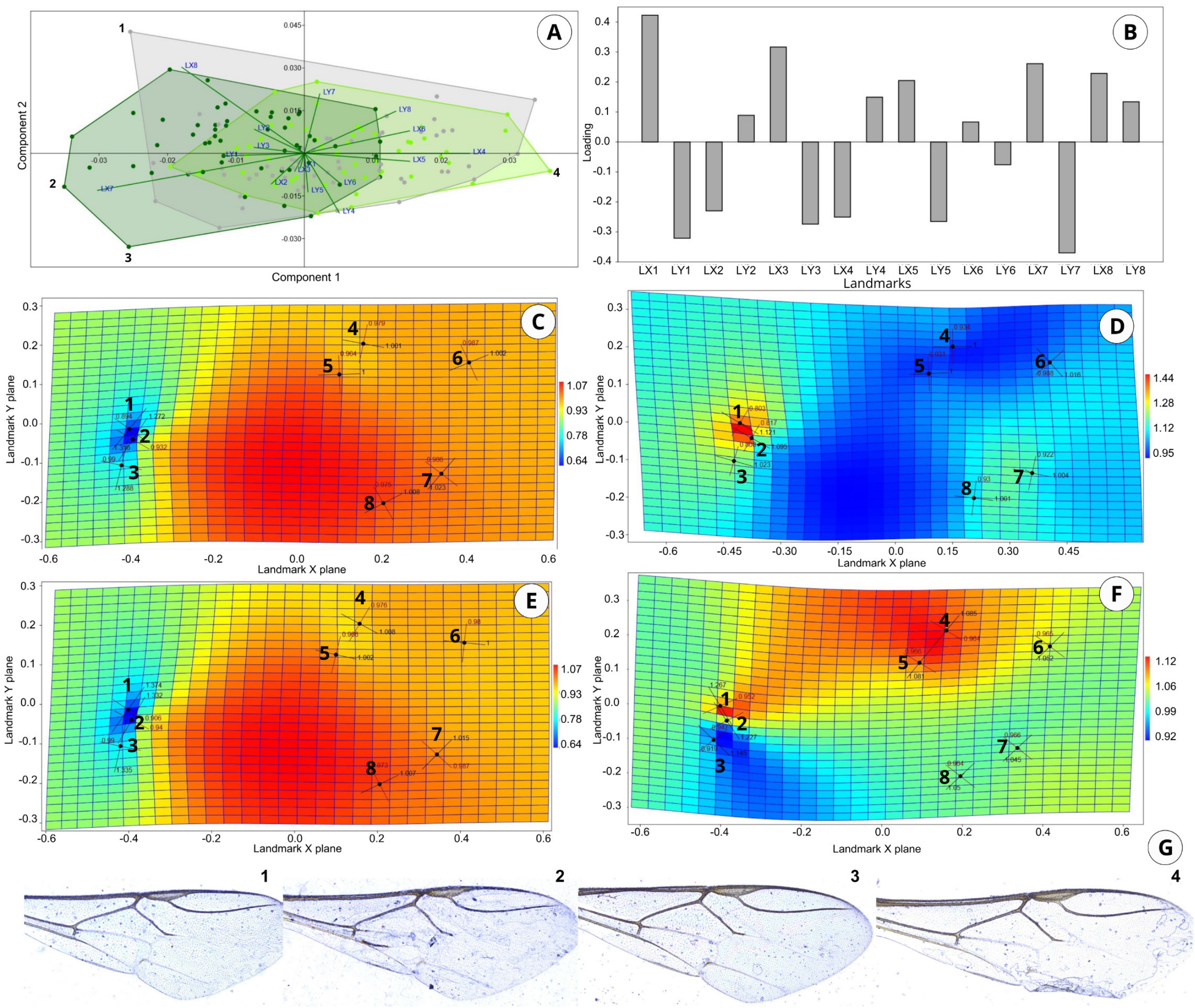

Figure 3. (a) Principal Component Analysis (PCA) result and (b) vector values representing the direction and magnitude of each landmark's contributions. Morphometric variation in the right wings of *Tetragonisca angustula* compared across three collection points: Rural area (dark green), natural urban area (light green) and urban area (gray). (c) Deformation plot illustrates the overall deformation of reference points in all locations; (d) Deformation in the rural area; (e) in the natural urban area and (f) urban area. Warm colors represent regions where there was an increase in extension (lengthening), while cool colors indicate a decrease in extension (shortening). (g) Representation of wings at each of the most extreme points on axes 1 and 2 of PCA indicated in figure a.

no significant difference between the rural area and the natural urban area ($p = 0.93$). However, the urban area differed significantly from the others ($p < 0.001$) (Figure 4). This indicates a higher concentration of individuals with positive asymmetry in the urban area, while the rural and natural urban areas tend to have individuals closer to the line of symmetry.

The urban area is notable for its higher asymmetry, exhibiting considerable variations in the landmark values on both sides of the wings. Although the rural and natural urban areas also show changes in the values on both sides of the wings, these variations are less pronounced. When examining the variation in the degree of asymmetry, the natural urban area had a higher number of individuals with

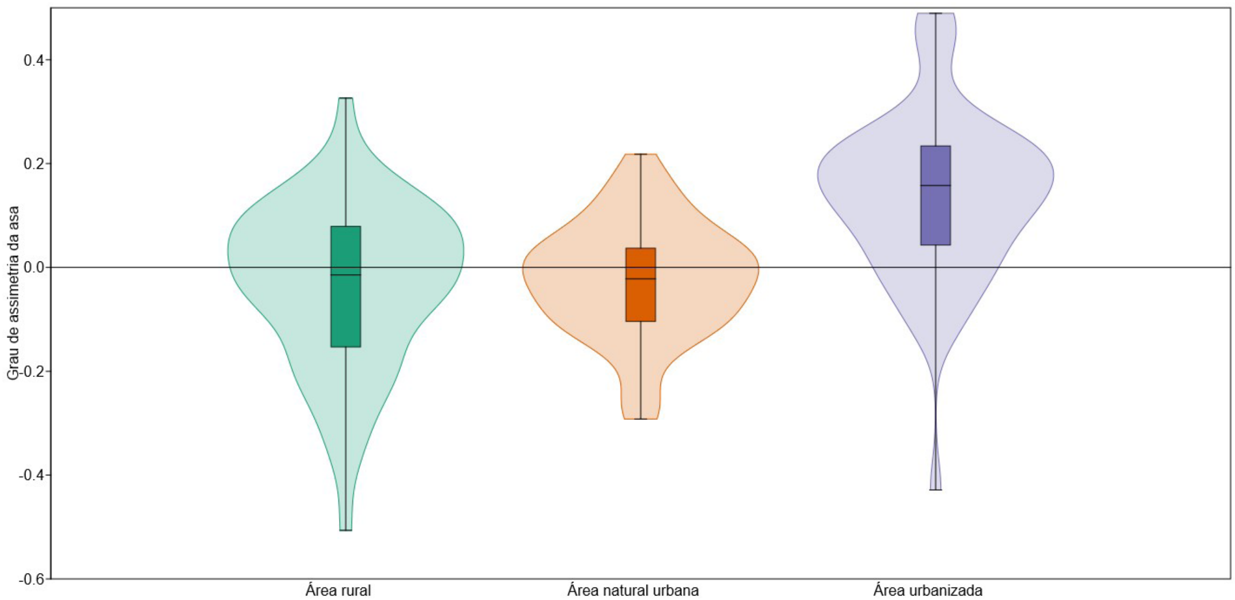

Figure 4. Wing asymmetry degree of *Tetragonisca angustula* in relation to different degrees of urbanization. Different letters indicate statistically significant (General: F = 23.38, p<0,01; Urban *vs* rural area: F = 8.594, p<0,01; Urban *vs* natural urban area: F = 8.151, p<0,01).

less symmetry compared to the rural area, with a slightly greater distance from the reference line. The comparison between the rural and natural areas showed no significant difference, suggesting that the degrees of asymmetry in these two areas are similar.

DISCUSSION

Studies using geometric morphometrics and asymmetric fluctuation in native stingless bees have helped to understand ecological pressures on the group, such identification, (Francoy et al. 2009, Ndungu et al. 2017) evolutionary intra-population variation (Mendes et al. 2007, Oi et al. 2022) or evaluating stress response in managed colonies (Lima et al. 2016, Andrade et al. 2020). These methodological advances expand the possibilities of morphological investigation in stingless bees, allowing a more detailed understanding of the environmental influences on their morphology.

The morphology of bees, such as *Tetragonisca angustula*, is influenced by environmental and nutritional factors. Principal component analysis indicates that bees from rural areas occupy a distinct region in the morphological space compared to urban and natural urban areas. The loadings of landmarks reveal which regions of the wing undergo greater variation, reflecting possible adaptations or morphological plasticity in the face of different environmental conditions. In more preserved environments, greater complexity and diversity of plants may favor structural variations associated with flight and foraging, while urbanized areas, with less diverse resources, tend to present more marked changes in specific points of the wing.

The main difference between the areas studied is in the type and quantity of vegetation cover and the conversion of land use. In rural areas, we have the presence of native species from different plant strata (trees, shrubs, herbaceous plants and grasses). While in the urban natural area and urban area, the presence and diversification of native species decreased proportionally with the increase in urbanization. In the case of the Cerrado vegetation, differences in the stratification of plant physiognomies and greater structural complexity are observed in more preserved areas, which tend to become more homogeneous in modified environments (Mota et al. 2018). The urban scenario directly reflects this simplification of plant resources.

Urbanization exerts a complex influence on bee populations, affecting not only the availability of floral resources but also the morphology of these insects. Recent studies indicate that the relationship between the degree of urbanization and fluctuating asymmetry in bee wings may vary according to species and environmental context. For example, Arce et al. (2023) observed that, over the last century, environmental stress signatures in bumblebee wings have increased, suggesting that factors associated with urbanization may contribute to this phenomenon. In contrast, Ferrari et al. (2024) reported that, although urbanization has reduced the body size of bees and wasps, the flight performance of these insects may have been potentially improved in urban environments. These findings highlight the need for further analysis of how different levels of urbanization influence bees' morphology, considering the particularities of each species and habitat.

Since bees depend on floral resources for nutrition, changes in the abundance and composition of these resources are crucial factors determining body size and wing morphology (Chole et al. 2019). Environments with lower food quality, morphological variations are more pronounced (Quezada-Euán et al. 2011, Banaszak-Cibicka et al. 2018, Chole et al. 2019, Vaca-Sánchez et al. 2023).

In addition to the challenges posed by urbanization, it is important to recognize that urban environments can offer benefits to some bee species. The presence of green areas, such as parks and gardens, provides year-round food resources, reducing the need for extensive foraging and potentially decreasing levels of nutritional stress (Hernandez et al. 2009, Ayers & Rehan 2021, Berger et al. 2024). The predominance of exotic plant species or the lack of floral diversity can limit available resources, negatively affecting bee populations. Therefore, urban planning that prioritizes the creation and maintenance of diverse green spaces rich in native species can mitigate the adverse effects of urbanization on these pollinators (Berger et al. 2024).

These findings highlight the complexity and importance of interactions between diet, environment, and morphological characteristics in bee populations, especially in urban environments. In this way, the increase of asymmetry observed in urban areas indicates that urban environments, even with surrounding vegetation like in natural urban areas, significantly influence wing shape. These factors are crucial for insect development, including bees (Vaca-Sánchez et al. 2023, Chávez-Galarza et al. 2013).

Although our results indicate an association between urban environments and morphological changes in the wings of *Tetragonisca angustula*, we cannot state that urbanization is the direct cause of these changes. Factors such as availability of food resources, exposure to pollutants and microclimatic variations may also play significant roles. In addition, it is necessary to consider the limitations of the study, such as the representativeness of the selected areas and the degree of urbanization, which may influence the results obtained. Cities with greater or lesser degrees of urbanization and urban connectivity may exhibit different responses.

Our results offer valuable insights into the responses of *Tetragonisca angustula* to varying environmental conditions, highlighting a significant impact of urbanization on wing shape. Notably, we observed more pronounced deformations in the urban population. Asymmetry has proven to be a sensitive indicator of environmental stress, reflecting the bees' exposure to fluctuating conditions. Inadequate nutrition, food scarcity, and the quality of resources have emerged as critical factors influencing both wing symmetry.

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SUPPLEMENTARY MATERIALS

Table SI-SIII.
Figure S1.

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