

Morphometric Characterization of a Population of *Tetrapedia diversipes* in Restricted Areas in Bahia, Brazil (Hymenoptera: Apidae)

by

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ABSTRACT

Tetrapedia species are solitary bees which collect floral oils, being restricted to tropical regions of the Americas. Information on forms of nesting has been little researched in the literature, requiring studies on the diversity and variability of species to obtain better management and conservation strategies for their populations. Morphometry is a efficient technique and has been used to detect variation and for identification of species of bees in order to detect changes in quantitative traits within and among populations of bees. This study aimed to compare the variability of the population of *Tetrapedia diversipes* in artificial nests located in orchards and their surroundings (other fruit) of acerola in a restricted area of the Reconcavo region of Bahia, Brazil. Right wings were extracted from 155 individuals of the *T. diversipes* species, to perform the morphometric analysis. In conventional morphometry, 9 variables contributed significantly to the sexual dimorphism in the study areas ($\alpha = 0.05$). The geometric morphometric analysis revealed low gene flow in populations of *T. diversipes* demonstrating loss of genetic diversity, requiring proper management of this bee for its conservation and maintenance of the associated flora.

KEY WORDS: Solitary Bees, Tetrapediini, Morphometry.

INTRODUCTION

Solitary bees represent approximately 330 species in the Neotropical region (Michener 2000) including the genus *Tetrapedia* (Tetrapediini) with 18

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described species in Brazil (Silveira *et al.* 2002), presenting different morphological and behavioral characters in their nesting (Cordeiro *et al.* 2010).

The *Tetrapedia* species use preexisting cavities as nesting sites which allows the sampling of these bees by means of trap-nests or artificial nests (Camillo 2005), and this solitary behavior is another hallmark of the Tetrapediini tribe characterized by independence of the female in the construction and supply their nest (Alves-dos-Santos *et al.* 2007).

The study of oil-collecting bees and their importance in the pollination of many plant groups is an important tool to contribute to the knowledge of their ecology, economic importance (Imperatriz-Fonseca 2010) and morphological variation of bee populations in their natural environment (Francoy & Imperatriz-Fonseca 2010). Research for the evaluation of morphological diversity is important in ecological and genetic studies, and one of the techniques used in these studies are morphometric tools (Rohlf 1990).

In morphometric analysis variation is studied through the covariation between pairs of linear measurements, and geometric morphometry is able to more clearly describe and locate the regions of changes and rebuild and reconstruct these differences graphically (Francoy *et al.* 2011).

Morphometry has been widely applied in many studies with social bees using tools to detect or describe genetic patterns in colonies, species identification, geographical variations and phylogenetic relationships among and within populations of bees (Peruquetti 2003, Mendes *et al.* 2007; Nunes *et al.* 2008, Souza *et al.* 2009, Carvalho *et al.* 2011; Ferreira *et al.* 2011; Francoy *et al.* 2011, Souza *et al.* 2010), and variability in shape and size of wings of social bees (Nunes *et al.* 2007; Quezada-Euán *et al.* 2007). However, there are few morphometric studies on solitary bees (Bosch 2008), with the exception of a study on the patterns of wing venation and geographical differences between populations of the *Centris* genus (Ferreira *et al.* 2011) and one study on the morphology of *Tetrapedia diversipes* applying linear morphometric analysis to length and width of the wings, head width and cephalic and mouth appendages (Smith *et al.* 2011).

In this context, considering the importance of morphometric studies in populations of bees, it is necessary to collect data on solitary bees and offer suggestions for future research aimed at the morphological variability within and among populations, diversity and biology of nesting. The study aimed to compare the degree the morphological variability of *Tetrapedia diversipes*

residing in artificial nests located in orchards and their surroundings (other fruit) of acerola in a restricted area in the Bahia Reconcavo region, Brazil.

MATERIAL AND METHODS

The experiment was conducted in Embrapa (Brazilian Agricultural Research Corporation) Cassava and Tropical Fruits in the period between March/2008 and August/2009 in the Municipality of Cruz das Almas (12° 40' 12" S, 39° 06' 07" W, 220 m), located in the Reconcavo of Bahia, Brazil. The nests were installed in four areas: Area I - Active Germplasm Bank of *Malpighia emarginata* DC; Area II - Other Orchards (*Citrus* spp., *Spondias* sp., *Musa* spp. and *Mangifera indica*); Area III - Transition area (the area between the forest and other orchards) and Area IV - Forest Fragment. The artificial nests were made of colored paper timber of 5 mm in diameter with a length of 15 cm (Machado 2011), and the species collected from the Tetrapediini tribe was *Tetrapedia diversipes*.

The sealed nests were transferred to the Laboratory of the Center for the Study of Insects (INSECTA) of the Federal University of Bahia Reconcavo (UFRB), and placed in BOD (*Biologic Oxygen Demand*) chambers at a temperature of 25 ± 1 °C, humidity $80\% \pm 1\%$ with a photoperiod of 12 hours. Right wings of *T. diversipes* were removed and placed between two plates to capture images using the program MotiC 2.0 ML with a digital camera coupled to a stereomicroscope, with 7.5 X magnification (Carvalho *et al.* 2011). The veins and cells wings were classified according to the methodology described by Silveira *et al.* (2002), and biological material has been identified, and stored in Eppendorf tubes of 1.5 mL and deposited in the collection of the Laboratory of the Center for the Study of Insects (*Núcleo de Estudos dos Insetos-INSECTA*), at the Center of Agricultural, Environmental and Biological Sciences (*Centro de Ciências Agrárias, Ambientais e Biológicas*) of the Federal University of Bahia Reconcavo (UFRB).

Geometric Morphometric Analysis

18 landmarks were defined and recorded using intersections of the veins of the forewings of *Tetrapedia diversipes* specimens (Fig. 1) using software tpsDig2 version 2.12 (Rohlf 2008a). The alignment of the x and y coordinates of each line of the wings (alx aly) and the centroid size of the wings can be

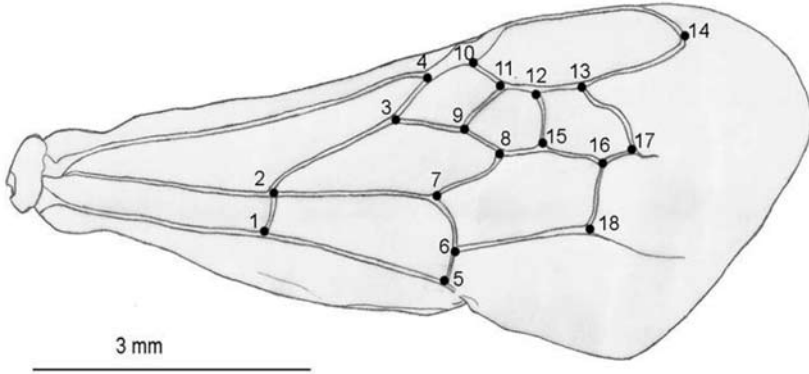


Fig. 1 – Landmarks used for geometric morphometric analysis of the forewing *Tetrapedia diversipes*.

used as a data matrix (matrix W) in order to perform multivariate analysis (Rohlf 2008b).

The coordinates of the landmarks of the wings and centroid size were used in principal component analysis (PCA), the matrix W was used in multivariate analysis of variance (MANOVA) and cluster analysis by UPGMA (Unweighted Pair-Group Method using Arithmetic Average).

Conventional Morphometric Analysis

In this analysis we used 24 linear measurements in the right wings (Figs. 2, 3) of 155 individuals measured by Motic software 2.0 ML. Measurements of the veins and cells were applied according to the methodology of de Souza *et al.* (2010), which used 17 measures on the forewing (length - C and Width - L of the wing, width and length of 1st, 2nd and 3rd submarginal cells, and 1st and 2nd Medial cells (M), length of the Subcostal veins + Radial (Sc + R) and Anal-1 vein; length of the cells: Marginal (M) and the 2nd cubital (Cu); distance from the intersection of the Anal-1 vein with Cubital-anal (Cu-a) until the intersection of the Cubital vein with 1st Middle-Cubital (m-Cu) and the hindwings with 7 measurements (length and width of the wing; length of the ribs sector Radial (R), Anal-1 and Cubital-anal veins; distance from the intersection of the rib Anal -1 with cubital-anal until the intersection of the median-Radial sector (Rs-m) with the Radial sector (Rs) and Radial (R) cell width.

The differences between the sexes of the individuals of the species *T. diversipes* were analyzed by multivariate variance (MANOVA), canonical variables (AVC), principal components (PCA) and cluster analysis by the UPGMA method.

RESULTS AND DISCUSSION

Conventional Morphometrics

For the multivariate analysis of variance (MANOVA), there was a significant difference between groups of individuals (Wilks's $\lambda = 0.65109$, $p < 0.00001$),

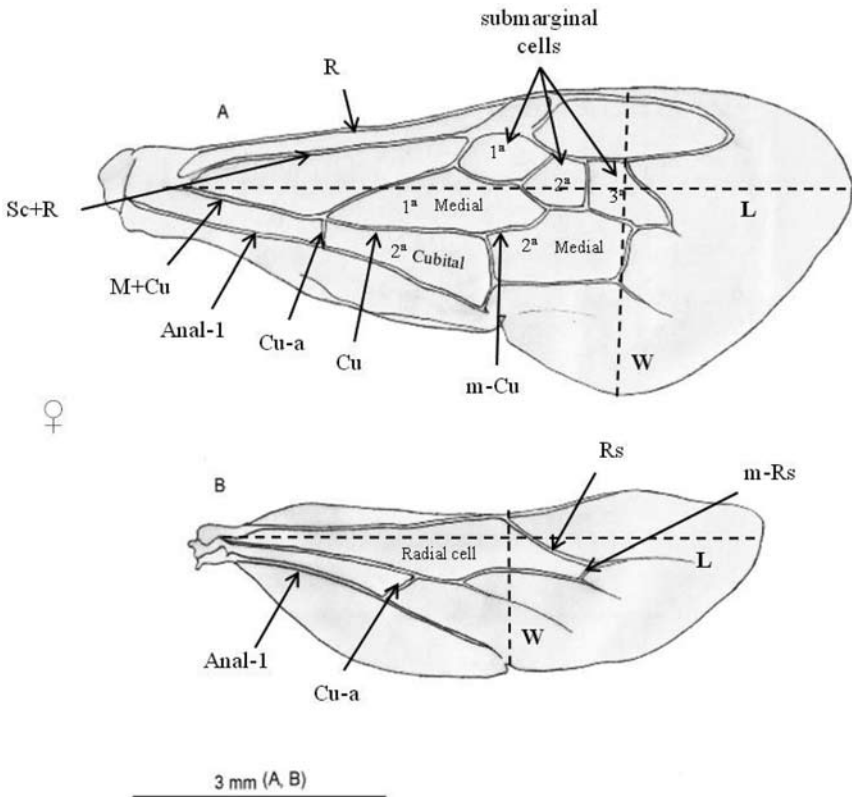


Fig. 2 – Linear measurements of morphological characters of the forewing (A) and hindwing (B) female *Tetrapedia diversipes* by conventional morphometric. Legend: Length (L), width (W), Radial + Subcostal veins (Sc + R), Cubital vein (Cu), Cubital-anal (Cu-a), Middle-Cubital or median-Cubital (m-Cu), Radial (R), Radial sector (Rs), median-Radial sector (m-Rs).

with only 10 variables contributing significantly to the separation of individuals ($\alpha = 0.05$), confirming the existence of sexual dimorphism of this species in relation to the size of the wings. This separation is represented graphically by principal component analysis (Fig. 4).

In principal component analysis (PCA) performed to test the existence of sexual dimorphism of *T. diversipes* the first three variables explained 74.40% of the variation, with the first component explained 63.28%, the second and the third 6.70%, and 4.42% (Table 1), and the variables that contributed most to the first component were length and width of forewings (Table 2).

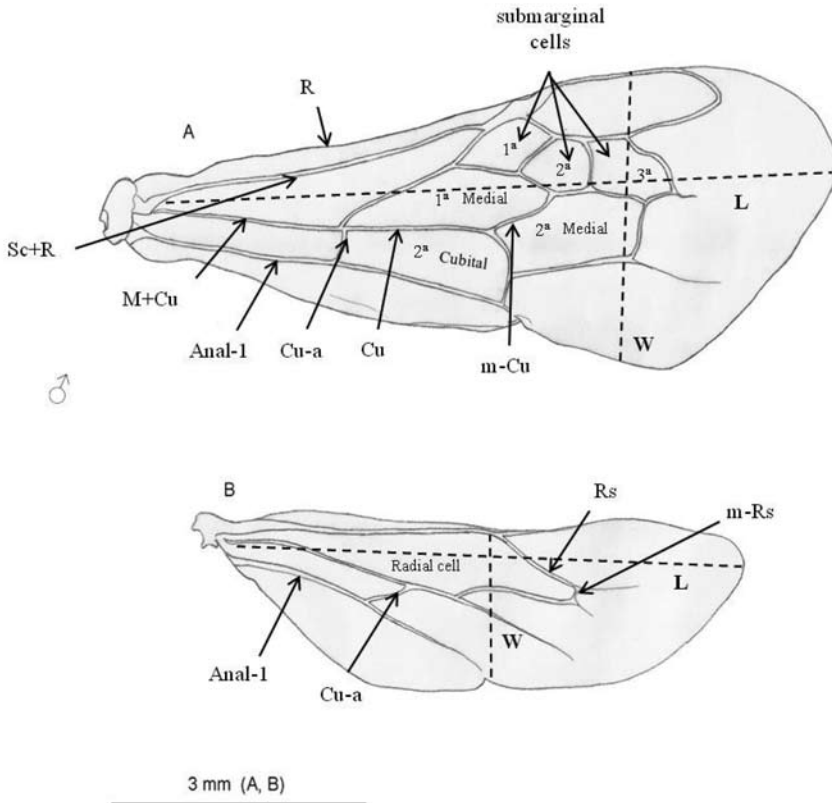


Fig. 3 – Linear measurements of morphological characters of the forewing (A) and hindwing (B) male *Tetrapedia diversipes* by conventional morphometric. Legend: Length (L), width (W), Radial + Subcostal veins (Sc + R), Cubital vein (Cu), Cubital-anal (Cu-a), Middle-Cubital or median-Cubital (m-Cu), Radial (R), Radial sector (Rs), median-Radial sector (m-Rs).

To evaluate the differences between the areas another PCA was performed (Fig. 5), where an overposition between the three areas of the population occurred, demonstrating the existence of low morphometric divergence between individuals of the three areas. This can be explained by the vicinity of the sampled areas, which contributes to gene flow between the population and therefore, maintains a similar phenotypic pattern.

The analysis of canonical variables showed morphometric differences between individuals in Area I and II, and area III formed a group with characteristics that are similar to areas I and II (Fig. 6). Multivariate analysis of variance (MANOVA) for the three areas showed a significant difference between individuals collected in areas I, II and III (Wilk's $\lambda = 0.57864$, $p < 0.00001$), where only 9 variables corresponding to the forewings contributed significantly to the separation of individuals in each area ($\alpha = 0.05$).

Considering the Mahalanobis D^2 distances (Table 3) between the areas, it appears that individuals of the areas I (Active Germplasm Bank of *M. emar-*

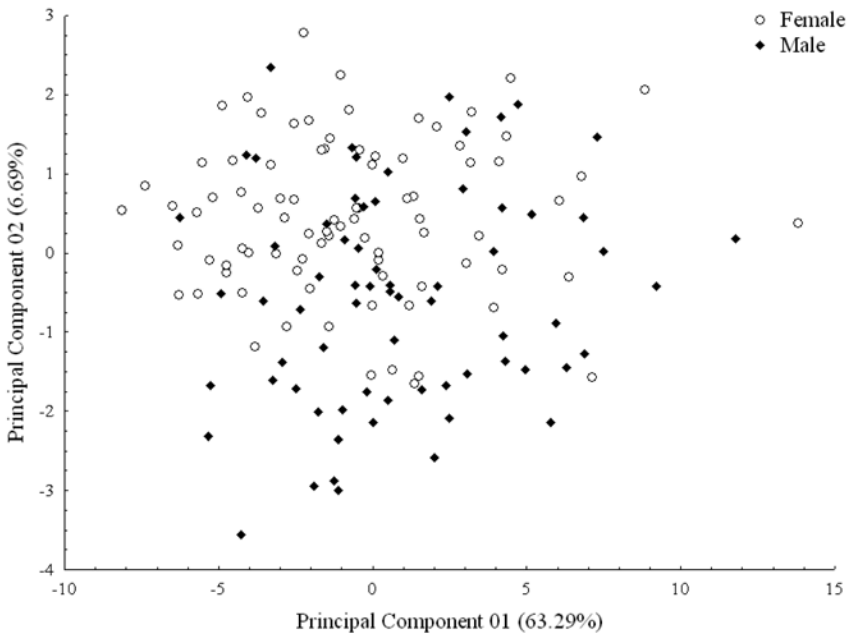


Fig. 4 - Graphic dispersion between males and females *Tetrapedia diversipes* in relation to Cartesian axes established by the first two principal components (PC1, PC2).

Table 1 - Eigenvalues, Total Variation (%), Proportion (%) and Cumulative Proportion of the 24 variables obtained with principal component analysis of *Tetrapedia diversipes* wings in the areas studied, Bahia of Reconcavo region, Brazil.

Principal Component	Eigenvalue	Total Variation (%)	Proportion (%)	Cumulative Proportion
1	15.1888	63.2868	15.1888	63.2868
2	1.6056	6.6901	16.7944	69.9769
3	1.0622	4.4257	17.8566	74.4026
4	0.8999	3.7494	18.7565	78.1520
5	0.6954	2.8976	19.4519	81.0497
6	0.6015	2.5064	20.0535	83.5560
7	0.5423	2.2597	20.5958	85.8158
8	0.4739	1.9747	21.0697	87.7905
9	0.4454	1.8560	21.5152	89.6465
10	0.4061	1.6922	21.9213	91.3387
11	0.3427	1.4279	22.2640	92.7666
12	0.2890	1.2041	22.5530	93.9706
13	0.2375	0.9898	22.7905	94.9604
14	0.2114	0.8809	23.0019	95.8413
15	0.1979	0.8245	23.1998	96.6659
16	0.1609	0.6702	23.3607	97.3361
17	0.1494	0.6224	23.5101	97.9586
18	0.1162	0.4840	23.6262	98.4425
19	0.0985	0.4103	23.7247	98.8528
20	0.0695	0.2896	23.7942	99.1424
21	0.0636	0.2651	23.8578	99.4075
22	0.0609	0.2537	23.9187	99.6612
23	0.0464	0.1935	23.9651	99.8547
24	0.0349	0.1453	24.0000	100.00

ginata) and II (Other Orchards) showed highly significant morphological differences ($p < 0.00001$). However, comparing areas I and III (Transition area: between the forest and other orchards), and II and III there was no significant difference between the total sample, demonstrating morphometric similarity, which can be attributed to the similarity of available resources between these areas.

Table 2. Principal component analysis between males and females of *Tetrapedia diversipes*, Bahia of Reconcavo region, Brazil.

Variable	Principal Component	
	PC01	PC02
L1	0.6028	0.0439
L2	0.7141	0.0823
L3	0.8419	0.0173
L4	0.8128	0.1221
L5	0.7525	0.3258
L6	0.7385	0.3392
L7	0.7719	0.3525
L8	0.7364	0.2207
L9	0.9338	0.1445
L10	0.8580	0.2261
L11	0.7232	0.1663
L12	0.8485	0.3841
L13	0.8553	0.3358
L14	0.7541	0.1905
L15	0.7733	0.3139
L16	0.9335	0.1209
L17	0.9340	0.1748
L18	0.8054	0.1504
L19	0.7467	0.3711
L20	0.8668	0.1418
L21	0.4813	0.6517
L22	0.8023	0.0295
L23	0.7515	0.2033
L24	0.8975	0.0174

Legend: L1: Width of 1st submarginal cell; L2: Length of the 1st submarginal cell; L3: length of marginal cell; L4: width of the 2nd submarginal cell; L5: length of the 2nd submarginal cell; L6: width of the 3rd submarginal cell; L7: length of the 3rd submarginal cell, L8: width of the 1st Medial cell; L9: length of the 1st Medial cell; L10: width of the 2nd Medial cell; L11: length of the 2nd medial cell; L12: Intersection of anal 1 vein with Cu-a (anal 1-Cubital) until intersection of Cu (Cubital vein) with 1st m-Cu (1st median-Cubital); L13: length of the 2nd Cubital cell; L14: length of Sc + R (Subcostal + Radial); L15: length of anal 1; L16: length of forewing; L17: width of the forewing; L18: length of the radial sector (Rs); L19: radial cell width; L20: width of the hindwing; L21-length of the Cu-a; L22: Intersection of anal 1 with Cu-a until intersection of m-Rs (median-Radial sector) with Radial sector (Rs); L23: the length of the anal 1 vein; L24: length of hindwing.

Table 3 - Mahalanobis D^2 distances (superior part) and statistical significance (p) for each distance (inferior part) between areas, by conventional morphometry analysis: I - Active Germplasm Bank of *Malpighia emarginata*; II - Other Orchards (*Citrus* spp., *Spondias* sp., *Musa* spp. and *Mangifera indica*) and III - Transition area (the area between the forest and other orchards).

Area	I	II	III
I	-	2.646967	2.137111
II	0.00001	-	5.670577
III	0.758967	0.086292	-

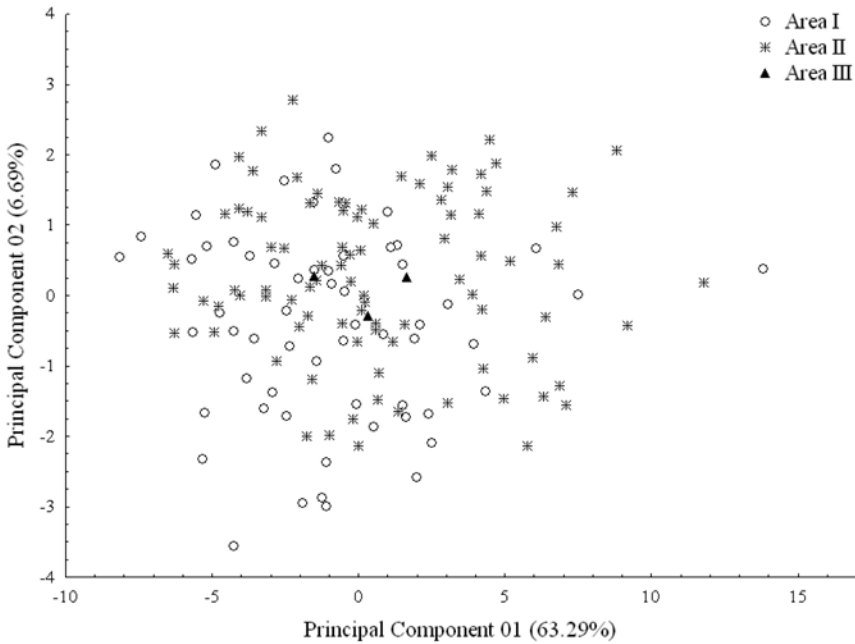


Fig. 5 - Graphic dispersion of the population *Tetrapedia diversipes* in relation to Cartesian axes established by the first two principal components (PC1, PC2).

Table 4 - Mahalanobis D^2 distances (superior part) and statistical significance (p) for each distance (inferior part) between areas, by geometric morphometry analysis: I- Active Germplasm Bank of *Malpighia emarginata*; II - Other Orchards (*Citrus* spp., *Spondias* sp., *Musa* spp. and *Mangifera indica*) and III - Transition area (the area between the forest and other orchards).

Area	I	II	III
I	-	3.83154	6.76865
II	0.00001	-	10.84870
III	0.239981	0.017491	-

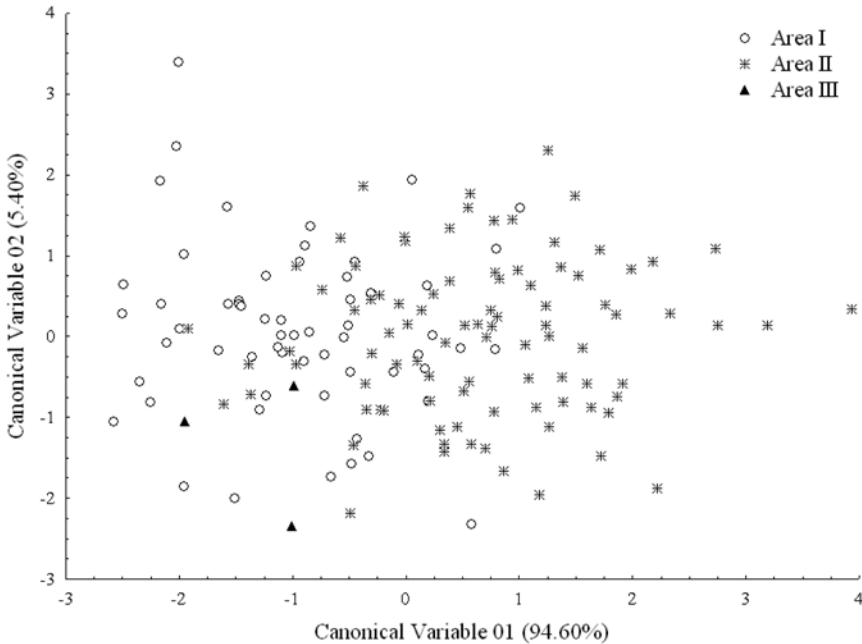


Fig. 6- Graphic dispersion of the population *Tetrapedia diversipes* in relation to Cartesian axes established by canonical variables (CV1, CV2) obtained from morphological characteristics of wings.

Ferreira *et al.* (2011) studied the degree of differentiation of populations in areas of *Centris aenea* in acerola orchards, where they observed significant morphometric differences between individuals, corroborating the present study. It is very important to consider that there are fluctuations of trophic resources over time, and the phenotypic expression of the bees may vary according to availability of resources.

Geometric Morphometrics

Multivariate analysis of variance (MANOVA) with the matrix W to observe the difference between the areas, also found significant differences (Wilk's $\lambda = 0.459$, $p < 0.0001$) between individuals of *T. diversipes* collected in the three areas, and all variables of the matrix W contributed significantly ($\alpha = 0.05$) to discriminate between the groups in the areas.

With the triangular matrix of Mahalanobis D^2 distances from the data coming from the matrix W , we found again significant differences between

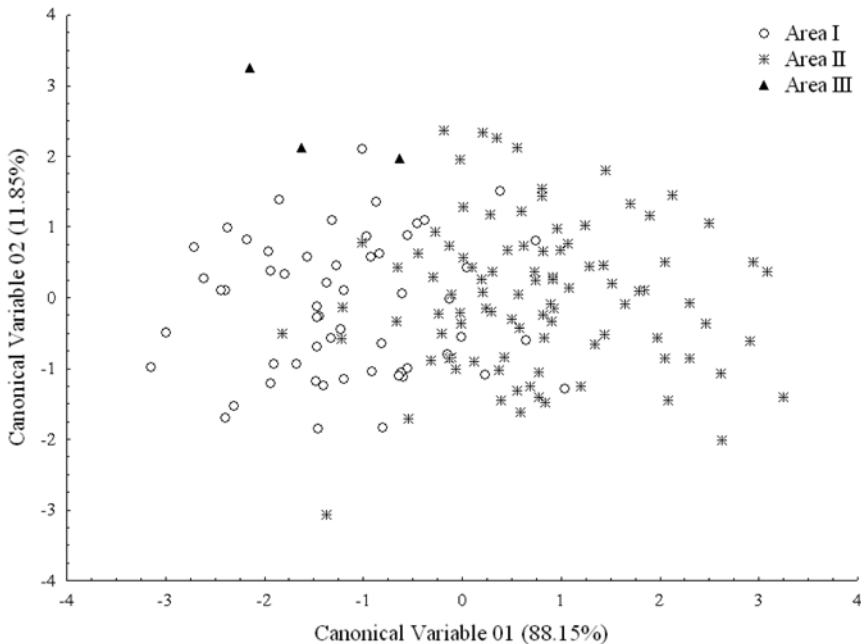


Fig. 7 - Graphic dispersion between the bees *T. diversipes* in each area, in relation to Cartesian axes established by canonical variables (CV1, CV2).

area I and areas I and II. However, among areas I and III, there was no significant difference (Table 4). Ghaderi *et al.* (1984) found that when several characteristics are analyzed at once, the squared Mahalanobis distances can be used as estimates of genetic diversity among the groups or areas where this variability is the result of morpho-physiological characteristics and ecological differences.

The analysis of canonical variables produced a difference between individuals in areas I, II and III (Fig. 7). The individuals of *T. diversipes* in areas I and III showed similar morphometrics, so there is probably a greater genetic interaction between the bees in these two areas. This similarity can be attributed to a low level of gene flow, with the assumption of the occurrence of inbreeding. According to Breda *et al.* (2004) inbreeding occurs among individuals related by descent, or is the union of individuals more closely related than the average population, increasing the homozygosity and phenotypic effect of expressing the recessive genes. It is possible that the fragmentation of the environment caused by anthropogenic activities and is mainly favoring the isolation of the population of *T. diversipes*, increasing the rate of inbreeding, weakening the genetic diversity and therefore favoring the disappearance of bees in these areas.

Similar data were found by Mendes *et al.* (2007). Analyzing the population *Nannotrigona testaceicornis* collected in two urban areas and an area of natural vegetation, they found that in urban areas there were more similarities when compared with areas of natural vegetation, affecting gene flow of these bees. However, Gonçalves (2010) noted that the geometric morphometry of wings was not sensitive enough to separate samples of *Frisieomelita varies* in natural and disturbed areas and diverged from the present work.

The conservation of native flora and reducing human disturbance around natural and agricultural areas can contribute to the maintenance of intrapopulation genetic material of *T. diversipes*, increasing their genetic variability and ensuring survival, demonstrating the need for further studies on the evolutionary variations of this species.

CONCLUSION

A conventional morphometric analysis revealed the existence of sexual dimorphism of the species *Tetrapedia diversipes*. However, geometric mor-

phometrics indicated the existence of low levels of gene flow, facilitating the isolation and weakening of the genetic diversity in the population. The study of morphometric characterization has provided important data on the degree of intrapopulational differentiation of *T. diversipes*, facilitating future research on other species of *Tetrapedia*, supporting strategies for the conservation and maintenance of this bee.

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