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The Effect of Colony Translocation on Meliponaries of *Melipona scutellaris* (L.) (Hymenoptera: Apidae) from Northeastern Brazil by Morphogeometric Analysis

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Abstract

This study investigated the morphometric variation in rational hive boxes of *Melipona scutellaris* stingless bees from five states, inserted into the natural range of species occurrence in northeastern Brazil. Cluster analysis (UPGMA) revealed three distinct groups by determining the cutoff point. Multivariate analysis was performed using multivariate analysis of variance (MANOVA) and canonical variate analysis (CVA) for shape. One-way analysis of variance (ANOVA) was used to assess differences in centroid size. The results showed population divergence between localities limited by geographical barriers or great distances, corroborating previous observations by UPGMA. Variation between inter-state colonies was higher than intra-state colonies and, in most cases, Geometric Morphometrics was effective in identifying the origin of colonies transported to other states, as observed in the cross-validation analysis that showed more than 70% of classification accuracy. Geometric Morphometrics can be used to evaluate the introduction of colonies within the kindred meliponary. Natural populations of *Melipona scutellaris* exhibit significant variation, which has important implications for meliponiculture and conservation strategies for this endangered species.

Introduction

Ensuring genetic diversity is indispensable for improving beekeeping, particularly for stingless eusocial bees. Honey farmers maintain a minimum of 44 colonies to reduce inbreeding depression and the occurrence of diploid males (Kerr & Vencovsky, 1982). Another way to avoid homozygosity, or its increase, is to introduce colonies of meliponaries from different locations, thereby decreasing the kinship between bees (Villas-Bôas, 2012).

However, meliponine beekeepers do not always know the origin of the population colony; thus, kin-related colonies are often introduced in meliponaries, which may increase inbreeding and decrease the population of meliponaries (Cook & Crozier, 1995). Currently, there is no efficient control over the translocation of colonies; therefore, techniques are necessary to identify the origin of bee species transported

to other regions (Villas-Bôas, 2012). This control is also important for preventing environmental damage in native populations (Jensen et al., 2005). The decrease in genetic diversity is worrying for several eusocial stingless bees, including *Apis mellifera*, because it can potentially increase the vulnerability of bees to ecological and anthropogenic stressors (Themudo et al., 2020).

Genetic diversity of bees is a major concern in the scientific community. For instance, the project of a European network of research groups (Prevention of Honey Bee COLony LOSSes – COLESS) aimed to characterize the population of colonies of commercial apiaries to fight the collapse of domestic swarms by preventing the parental crossing of these colonies (Neumann & Carreck, 2010). These groups have published several papers on *A. mellifera* populations in different European countries. These studies, in addition to characterizing the genetic populations of apiaries,

have sought to standardize methodologies and techniques to easily and reliably collect population information, as well as create a homogeneous database for comparative studies (Bouga et al., 2011). One of the techniques tested by COLOSS, which has generated robust results, is geometric morphometrics (Francis et al., 2014), which also stands out for its easy applicability and efficiency.

Geometric morphometric methods (GM) have answered numerous questions related to Hymenoptera, enabling the identification of intra-and interspecific variations by grouping subspecies and populations based on landmarks (Silva et al., 2019; Porrini et al. 2019). Geometric morphometrics has been an effective instrument in the evaluation of population identity in bee groups, and it can be associated with variations in environmental factors, such as geographical distance and differences between ecoregions (Souza et al., 2018; Carneiro et al., 2019; Ribeiro et al., 2019). Geometric morphometrics can also be used as a technique of choice with bees of the genus *Melipona*, as it is efficient and fast to trace colonies geographically (Francoy et al., 2011; Miguel et al., 2016), and also allows the identification of morphological variations caused by pesticides (Prado-Silva et al., 2018).

This study analyzed the population morphometric structure of Uruçu bees (*Melipona scutellaris*), evaluating variations in meliponaries with zootechnical records of the origin of their colonies from five different states of the natural occurrence of this species in northeastern Brazil to identify the geographical origin and colony translocation effect through GM.

Material and Methods

Workers of *M. scutellaris* were collected from the states of Bahia, Sergipe, Alagoas, Paraíba, and Rio Grande do Norte, sites of natural occurrence of this species in northeastern Brazil (Table 1, Fig 1). We collected 646 specimens from 111 colonies, in the period 2015-2019, which were placed in tubes containing 70% alcohol under refrigeration at -20°C. Information was also collected on the meliponary, individuals collected, colonies where the samples were obtained, meliponary location, geographic origin of lineages, and the relationship structure between colonies of the meliponary.

The right forewings of the bee workers were removed, mounted on microscope slides, and photographed under a stereomicroscope using a Leica image capture system (Leica

Table 1. Information *Melipona scutellaris* samples with data on the state, locality, location of meliponaries (latitude and longitude), numbers of colonies, and origin of colonies.

State	Locality	Latitude / Longitude	Colony/ Specimens	Geographical Origin
Alagoas	Barra de Santo Antônio	09°24'18" S/ 35°30'25" W	28/258	Alagoas
Bahia	Cruz das Almas	39º06'26"S/ 12º40'39"W	11/105	Bahia
Paraíba	Lagoa Seca	07° 10' 15" S/ 35° 51' 13" W	33/106	Paraíba
			03/26	Rio Grande do Norte
Rio Grande do Norte	Macaíba	5° 51' 36" S/ 35° 20' 59" W	04/19	Pernambuco
			07/36	Bahia
Sergipe	São Cristóvão	10°55'35"S/ 37°6'14"W	11/32	Bahia
	Malhador	10º 39' 28" S/ 37º 18' 17" W	14/64	Sergipe

Fig 1. Map of the northeastern region of Brazil, with emphasis on the municipalities of *Melipona scutellaris* meliponaries.

Microsystems®). The images allowed the creation of a TPS extension using tpsUtil (Rohlf, 2008a). We then recorded ten landmarks at the intersection positions of the venations using tpsDig2 (Fig 2) (Rohlf, 2008b).

Based on the Cartesian coordinates obtained from the landmarks, we carried the Procustes superimposition, analysis of variance (MANOVA), and canonical variate analysis (CVA) using the MorphoJ software (Klingenberg, 2011). Subsequently, cross-validation analysis was performed to verify the correct classification of individuals in their respective locations, using R 3.0.1.

UPGMA cluster analysis was performed using the PAST software (Hammer et al., 2001). The cut-off point for the cluster

analysis was established using the method of Calinski & Harabasz (1974) and calculated using the following formula:

$$CH(q) = \frac{trace\ (B_q)/(q - 1)}{trace\ (W_q)/(n - q)}$$

Where:

$W_q = \Sigma q_k = 1 \Sigma i \epsilon C_k (x_i - C_k)(x_i - C_k)T$ is the dispersion matrix within the group for the grouped data.

$B_q = \Sigma q_k = 1\ n_k * (C_k - C)(C_k - C)T$ is the dispersion matrix between the groups for the grouped data.

x_i = p-dimensional vector of object observations in set k

C_k = centroid of set k

C = data center barycenter

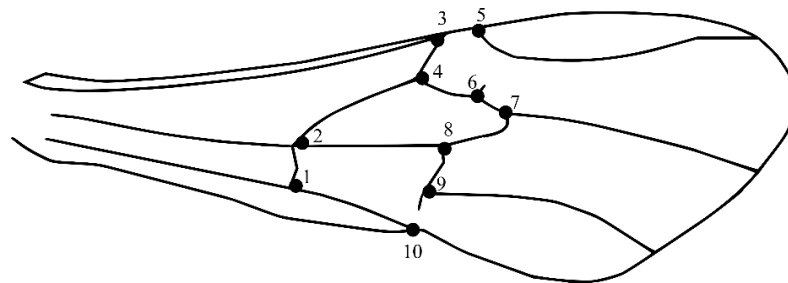n_k = number of objects in cluster C_k

Fig 2. Illustration of the forewing of *Melipona scutellaris* showing ten intersections of wing veins used as landmarks in the analysis of shape and size.

A Mantel test was performed, with 5000 permutations, to compare the variation of wing shape in relation to geographic distances depending on the place of collection and origin. In addition, in PAST, a One-Way ANOVA and Tukey's test were performed to compare means for the size analysis using the centroid size.

Results

The MANOVA showed significant differences between groups (p < 0.01) when comparing the locations where *M. scutellaris* occurred naturally. Samples collected in the state

of Alagoas showed the greatest differences in wing shape between the colonies. There were no significant differences between the colonies sampled in Bahia and Sergipe (p > 0.05), or between the states of Paraíba and Rio Grande do Norte (p > 0.05). In addition, no significant differences (p > 0.05) were found between the wing shapes of bees from Rio Grande do Norte and those from Bahia.

The first two canonical variables explained roughly 91.8% of the total variation in the sampled groups. The first CVA explained 70.3%, followed by 21.5% of the total variation between the colonies (Fig 3). The results show that colonies from Alagoas formed a group separated from those in other
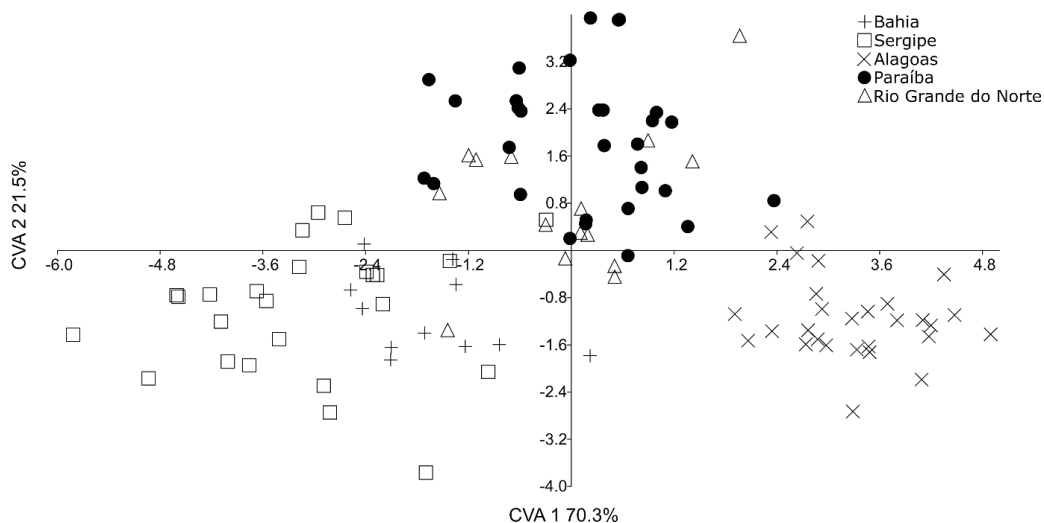

Fig 3. Graphical dispersion by the Canonical Variables method with the colonies of *Melipona scutellaris* in their area of natural occurrence.

locations, while Bahia and Sergipe showed greater proximity, as well as Paraíba and Rio Grande do Norte. Cross-validation showed that 76.57% of the colonies were classified correctly to their respective states, except for colonies classified in the state of Alagoas, which obtained 100% correspondence.

Similar CVA data were obtained from UPGMA cluster analysis. The UPGMA resulted in the formation of three groups, according to Calinski and Harabasz (1974). The former is composed of colonies of the states of Bahia and Sergipe, and the latter is composed of colonies of the states of Paraíba and Rio Grande do Norte. The third group was formed only

by colonies from Alagoas (Fig 4). The cross-validation test showed 92.72% accuracy in classification, considering the groups formed in the cluster analysis (UPGMA). According to the Mantel test, considering the geographic distances in relation to the shape of the wing, significant values were found ($p < 0.05$), indicating that the variation in the shape of the wings was related to the origin or location of the colonies.

Regarding wing size, the ANOVA showed significant results ($p < 0.01$). Bee colonies from the state of Alagoas differed significantly from those in Bahia ($P = 0.0001$) and Rio Grande do Norte. Colonies from the state of Sergipe showed a
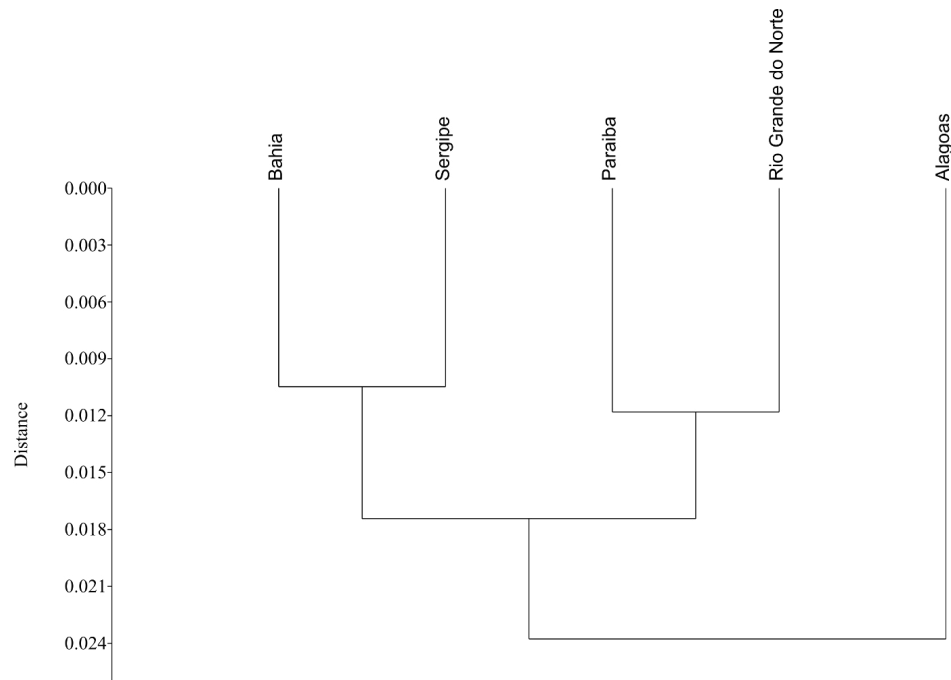

Fig 4. The UPGMA cluster analysis comparing the average wing shapes of *Melipona scutellaris* between states in northeastern Brazil.
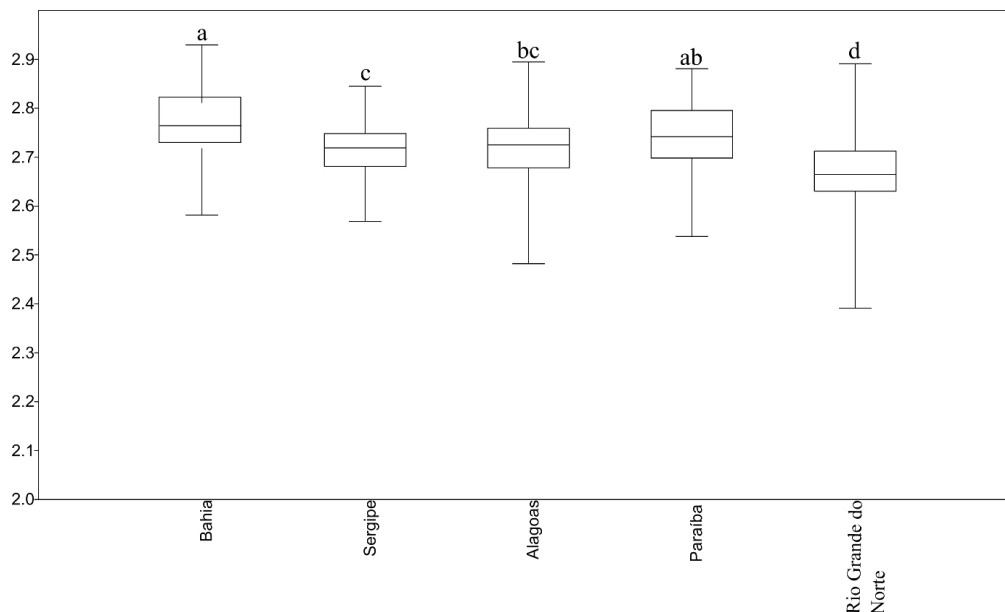

Fig 5. Boxplot with the variation of the centroid size of wings of *Melipona scutellaris* from the states of the northeastern region of Brazil. In the Tukey test, equal letters do not differ statistically.

significant difference (P < 0.05), although they were similar to those in the states of Bahia and Paraíba (P < 0.03). However, the colonies did not show a significant difference from the colonies in the state of Alagoas. Tukey's test showed that only colonies of Rio Grande do Norte differed from colonies of other locations, with smaller sizes (Fig 5).

Discussion

Analyses of *Melipona scutellaris* colonies with geographical origins attested through zootechnical questionnaires, showed that stingless bees from the state of Alagoas (left bank of the São Francisco River) present differences in wing shape, despite their proximity to the states of Bahia and Sergipe (right bank of the São Francisco River). This difference may be attributed to the strong geographical barrier represented by the São Francisco River, which is the borderline between the states of Alagoas and Sergipe. The width of the São Francisco River ranges from to 500-1000 m and, although the maximum flight distance of *Melipona scutellaris* bees can reach 2000 m (Araújo et al., 2004), stingless bees of daughter colonies are highly dependent on the mother colony during swarming (Wille, 1983), which restricts colony dispersion. Therefore, the São Francisco River is an effective barrier to geographical isolation. A similar finding was observed in the same region in mandaçaia bee populations (*Melipona quadrifasciata*), in which the São Francisco River functioned as an effective geographical barrier. This feature may extend to other species of the genus *Melipona* (Araújo et al., 2016). The study conducted by Prado-Silva et al. (2016) on *M. mandacaia* also suggests that the São Francisco River, in the state of Bahia, acts as a geographical barrier for this species. In the state of Bahia, *M. scutellaris* has significant morphological variations due to the occurrence of geographical barriers, even in nearby locations (Nunes et al., 2007). The presence of the São Francisco River as an effective geographical barrier may explain this population divergence.

The fact that bees from the state of Alagoas formed a separate group could be explained by the greater geographical distance from the states of Paraíba and Rio Grande do Norte (to the north), hindering the gene flow between these populations, in addition to the São Francisco River barrier (to the south). Anthropic effects, such as fragmentation of the Atlantic Forest and extensive sugarcane plantations, could also represent limiting factors for the nesting and dispersion of *Melipona* bees.

Colonies in Alagoas were collected from meliponine beekeepers in nearby municipalities despite being from the same state (Table 1). Thus, meliponaries provide a good representation of the bees in this region. Similarly, the presence of colonies only from the state of Alagoas contributed to preserving the characteristics of local populations, influencing the differentiation of other groups.

The similarity in wing shapes between bees collected in the states of Sergipe and Bahia could be explained by the proximity between the two states and the absence of limiting geographical barriers, which allows free gene flow between bee populations. Additionally, the introduction of colonies from Bahia was recorded in the meliponaries of the state of Sergipe (Table 1). Likewise, the similarity of wing shape found between the colonies of the states of Paraíba and Rio Grande do Norte could be explained by the proximity between states and low restriction for natural dispersion of colonies.

The lack of differences in wing shape between colonies of the states of Rio Grande do Norte and Bahia could be understood when the zootechnical data of meliponaries are associated with the origin of these colonies. Table 1 shows that half of the colony samples collected in the state of Rio Grande do Norte originated from Bahia. This explains the morphometric similarity between these colonies, despite a distance of approximately 1000 km, which reinforces the need for a zootechnical record of colonies in meliponaries, which could otherwise compromise the maintenance of meliponaries and prevent inbreeding.

Regarding bee size, only bees in the state of Rio Grande do Norte were smaller, whereas bees in the state of Bahia were larger. This size difference was expected because the size presents less evolutionary constraints than wing shape variation, as inter-population differences appear first in size variations and then in shape variations (Nunes et al., 2012).

The morphometric variation within the groups studied, such as colonies from the state of Alagoas, may have been due to greater genetic variability. This high variability rate between colonies in this state can be explained by the handling adopted by melipone beekeepers, who usually introduce two new colonies every year for every 15 colonies already existing in the meliponary. This favors genetic variability and maintains a large number of colonies (> 50), which is higher than the number recommended by Kerr and Vencovsky (1982) to ensure genetic variability between rearing colonies. Colonies from Bahia also showed large intra-population differences of 85.4% and 88.6% in shape and size, respectively, which may also be due to genetic variation. The meliponary at the Federal University of Recôncavo da Bahia (UFRB), where bees were collected in Bahia, is representative of this region, as it has colonies from different locations in Bahia, the largest state in the northeastern region.

Meliponaries in Sergipe showed high variation among colonies, except for the meliponaries from the Federal University of Sergipe (UFS). In one of the meliponaries in the municipality of Malhador (SE), where colonies come from different matrices, there was a great difference in the shape and size of bees between colonies, indicating great genetic diversity within the meliponary. In the municipality of São Cristóvão (SE), where the meliponary of the Federal University of Sergipe is located, 36.6% of colonies showed a significant difference in size and only 12.7% in shape.

This extremely small number of colonies with a significant difference could be explained by the fact that all *M. scutellaris* colonies in the meliponary were derived from the division of a few mother colonies from the state of Bahia. This structure leads to low variability within the meliponary and may be responsible for future loss of colonies (Gerloff & Schmid-Hempel, 2005; Hedrick et al., 2006), which could be avoided with management care involving the exchange of colonies.

Our data demonstrate that the GM is an instrument with high accuracy for identifying the origin of colonies. The correlation between the colonies allows the verification of whether colonies introduced in the meliponaries are related to those that are already allocated there and consequently contribute to increasing genetic variability. Regarding the population variation in *M. scutellaris* in its distribution area in northeastern Brazil, the design of the studies carried out is important, as it is not important to evaluate the origin of colonies in meliponaries. Thus, following the recommendation to exchange colonies between meliponine beekeepers is not enough, since meliponaries that are very distant geographically could in fact share the same geographical origin of matrices. In addition, in the case of natural *M. scutellaris* populations, there is significant population genetic variability, with at least three distinct groups at the site of occurrence, which has important implications for conservation strategies for this endangered species.

The results of this study make it possible to develop strategies for managing stingless bee species more in line with the conservation of native populations in their geographic regions of occurrence, provided that meliponicultors carry out zootechnical bookkeeping of their colonies. Although our study addressed the morphometric variation and population structure of *Melipona scutellaris* colonies in different states of northeastern Brazil, we acknowledge that there are some important considerations that may have impacted our results.

Firstly, it is important to note that our sample was based on colonies available in meliponaries and may not necessarily represent all the wild colonies present in each state. This could introduce a possible selection bias since the colonies maintained in meliponaries may not fully represent the genetic diversity of wild populations. Additionally, the active exchange of colonies between states may have influenced the observed population structure and morphometric variation. Considering the exploratory strategy of this study, more accurate values can be obtained in future studies using planned experimental designs to validate this method for large-scale applications.

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Authors' Contribution

EDA: Conceptualization, methodology, formal analysis, writing-review and editing;
HASP: Conceptualization, methodology, investigation, formal analysis, writing-initial draft, writing-review and editing;
WRTS: writing-initial draft, writing-review and editing;
LAN: formal analysis, writing-initial draft, writing-review and editing.

Conflicts of Interest

On behalf of all the authors, the corresponding author states that there are no conflicts of interest.

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