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Population Structure of *Melipona subnitida* Ducke (Hymenoptera: Apidae: Meliponini) at the Southern Limit of its Distribution Based on Geometric Morphometrics of Forewings

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Abstract

Bees provide fundamental services to humanity, and many researchers have been concerned about the rapid loss of genetic diversity that these organisms have been suffering. The stingless bee *Melipona subnitida* Ducke is endemic to Northeastern Brazil and has high potential for the production of honey and wax; it is also an important pollinator in the Caatinga biome. Populations of *M. subnitida* have increasingly declined due to predatory extractivism and habitat destruction. However, knowledge about its population structure could give insights on strategies for monitoring and conservation of this species. Here we collected workers from nine sites located at the southern limit of the species distribution and employed geometric morphometric techniques on their forewings in search of covariance between sampling site and wing morphology. A very significant correlation between both variables was observed, indicating that the divergence among the sampled populations of *M. subnitida* was due to geographical distance among the sampling sites and, hence, suggesting the formation of different groups of populations along the studied geographical zone, each one with specific characteristics. Since *M. subnitida* habitat has been increasingly fragmented thus hindering the genetic flow among populations, our findings will contribute to the formulation of management and conservation plans for this species in order to preserve its genetic diversity and, therefore, to collaborate to the generation of income for beekeepers in meliponiculture programs.

Introduction

Stingless bees form an important group of pollinators in the Neotropics, playing a significant role on the maintenance of ecosystems and agricultural production (Heard, 1999; Slaa et al., 2006). The stingless bee *Melipona subnitida* Ducke, locally known as "jandaira", is endemic to the Caatinga (Zanella, 2000), one of the biomes found in the Northeastern Brazil, which is characterized by a semiarid climate and xerophylous vegetation (Andrade-Lima, 1981). *M. subnitida* is found mainly in the states of Bahia, Ceará, Rio Grande do Norte and Paraiba (Martins, 2002; Camargo & Pedro, 2007; Webbee, 2014), playing an important role on pollination of cultivated plants, as well as on honey and wax production (Cruz et al., 2005).

The predatory exploitation of nests of native bees by humans has become a major concern for scientists (Brown & Paxton, 2009), but deforestation, agriculture intensification and introduction/spread of exotic competing bee species are considered the main threats to most indigenous species (Freitas et al., 2009). On the other hand, knowledge about the population structure of these organisms has been considered critical for management and conservation plans of endangered species (Nunes et al., 2008; Bonatti et al., 2014).

The employment of geometric morphometrics on identification and assessment of population diversity/structure has been proven efficient (Breuker et al., 2006; Wappler et al., 2012). Geometric morphometrics allows detecting relationships between animal populations and their geographic locations through a rigorous analysis of variations in the

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shape of a model structure. The most common application of the geometric morphometrics is the identification of landmark configurations in several morphological characters (Klingenberg, 2002). Cartesian coordinates provide the relative positions of each point, and, therefore, they make possible the reconstruction of shape and the identification of shape variations (Rohlf & Marcus, 1993; Rohlf, 1998). This method is very sensitive to subtle variations and, hence being suitable for detection of groups and subgroups (Francoy et al., 2011).

Studies on population structure and geographic variation of bees have been carried out based on morphometric data comparing races or populations (Ferreira et al., 2011; Lima Junior et al., 2012; Nunes et al., 2013). Those analyses are usually based on wing characters due to their high heritability and because they are strongly affected by the environment (Diniz-Filho & Bini, 1994). A possible disadvantage of this technique is that the biological structures that will be studied need to be completely undamaged. However, such disadvantage is rarely a critical factor (Lyra et al., 2010).

Considering the ecological and economical importance of stingless bees for the Neotropics and the necessity of studies on the population structure of endemic species of the Caatinga biome, the aim of this study was to determine the population diversity of *M. subnitida* from sites located at the southern limit of its distribution based on geometric morphometrics of forewings.

Material and methods

We collected 630 workers from 63 colonies of *M. subnitida* in nine localities of Northeastern Brazil, from August to December 2012 and in July 2013 (Table 1).

We removed the bees' forewings, placed them between microscope slides, and photographed them with a digital camera coupled to a stereomicroscope for the analyses of venation. We transformed the photographs in the software tpsUtil 1.40 (Rohlf, 2008a). We inserted ten landmarks (Fig. 1) at the vein intersections of each forewing using tpsDig version 2.17 (Rohlf, 2008b). We used the data obtained as variables for multivariate analyses, such as principal component analysis (PCA), canonical correlation analysis, Mahalanobis distance (D^2), and Procrustes distance in the software MorphoJ 1.03 (Klingenberg, 2011). We also made cluster analyses based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and a Mantel test based on geographic distance, and size and shape of bee wings in the software Past 2.17.

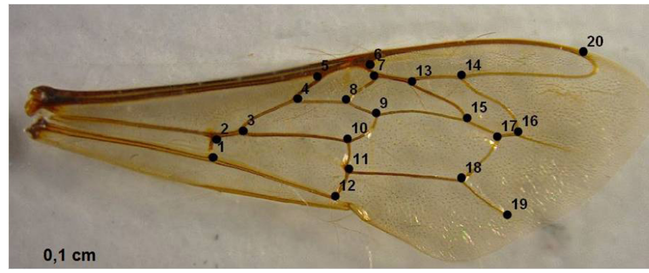

Fig 1 Right forewing of *Melipona subnitida* showing the 10 landmarks located at wing vein intersections, which were used in the morphometric analysis.

Results

In the PCA applied to the populations of *M. subnitida*, the first four principal components explained 62.96% of the total variation among individuals of different communities: PC1 = 20.61%, PC2 = 16.26%, PC3 = 14.20%, and PC4 = 11.88%. We represented the group distribution in a bidimensional space, using the scores of the first two components, to test for dispersal among groups.

Based on the Mahalanobis and Procrustes distances, the largest difference was observed between the populations from Exu and Água Branca (305 km apart) whereas the highest morphological proximity occurred between the populations from Água Branca and Mata Grande (30 km apart) (Table 2). Based on a dendrogram (UPGMA; Fig. 2), we observed morphological differences between the populations from Exu, Passira and Taquaritinga do Norte, which were grouped in a

Table 1. Origin and geographic location of the *Melipona subnitida* samples at the Southern Limit of its Distribution natural in Brazil. N- number of samples.

Sites/ State	N	Latitude (S)	Longitude (W)	Altitude (m)	Climate / Relief
Água Branca/Alagoas	9	9º10'24.7"	37°51'41.9"	380	Semiarid/ massive
Cumaru/Pernambuco	6	8°1'58.55"	35°45'3.11"	348	Semiarid/ low hills
Exu/Pernambuco	3	7°20'22.66"	39°54'58.54"	887	Semiarid/low mountains
Joá/Paulo Afonso/ Bahia	6	09º31'08.8"	38°25'36.7"	243	Semiarid/ upland
Mata Grande/Alagoas	12	09º11'09.3"	37°50'09.8"	424	Semiarid/ massive
Passira/Pernambuco	5	7°55'37.85"	35°30'14.01"	160	Semiarid/ high massive
Riacho das Almas/Pernambuco	6	8°3'40.79"	35°49'9.62"	413	Semiarid/ low hills
São José/ Paulo Afonso /Bahia	8	09º39'04.8"	38º22'43.2"	243	Semiarid/upland
Taquaritinga do Norte/Pernambuco	8	7°56'14.15"	36°7'05.7"	785	Tropical/massive

more isolated branch, standing out from populations of other regions, with a correlation index (P< 0.0001), which shows that the divergence between population is highly significant.

The correlation between shape, size, altitude, and geographic distance among 63 *M. subnitida* colonies compared with a Mantel test (Table 3) indicated a positive correlation between wing shape and geographic distance among all populations (P < 0.01). The other correlations had high P-values, which points to no relationship among variables.

There was variation in the shape of the forewings among *M. subnitida* populations, which is reflected in the formation of groups.

Table 2. Mahalanobis distances (lower half of the matrix) and Procrustes distance (upper half of the matrix) among populations calculated from the canonical correlation analysis. Sites: AB: Água Branca; CU: Cumaru; EX: Exu; JO: Joá; MT: Mata Grande; PA: Passira; RA: Riacho das Almas; SJ: São José; TN: Taquaritinga do Norte.

	AB	CU	EX	JO	MT	PA	RA	SJ	TN
AB		0.017	0.020	0.015	0.008	0.019	0.017	0.015	0.014
CU	2.93		0.015	0.012	0.014	0.015	0.010	0.013	0.016
EX	3.68	2.54		0.019	0.017	0.014	0.017	0.014	0.015
JO	2.96	1.78	3.08		0.012	0.019	0.013	0.011	0.016
MT	1.33	2.32	3.27	2.37		0.017	0.018	0.010	0.017
PA	3.37	2.17	2.84	2.52	2.77		0.017	0.012	0.017
RA	3.21	1.68	2.85	2.11	3.10	2.66		0.015	0.013
SJ	2.87	1.94	2.63	1.83	2.13	1.71	2.31		0.016
TN	2.47	2.65	2.48	2.87	2.72	3.10	2.41	2.70	

Discussion

The geometrical morphometrics of forewings was very efficient in detecting variability among the populations and, hence, in unveiling the population structure of *M. subnitida*. The results of the morphometric analysis pointed to morphological variability in wing shape among populations along their geographic distribution. This variation was associated with geographic distance among the sampling sites and is also probably related to the environmental variability among sampling localities.

The populations from Exu and Água Branca showed the highest index of morphometric divergence, with a dissimilarity value of 3.68, which may be explained by an interaction between geographic distance and climatic/relief differences between regions. Exu is characterized by a semi-arid climate: warm in the summer and cold in the winter; it is located between massifs and low mountain ranges (300 – 800 m height); on the other hand, Água Branca is characterized by a tropical semi-arid climate with summer rains, and it is located between massifs and high mountain ranges (650 – 1,000 m height) (CPRM, 2014). The less divergent populations were those of Água Branca and Mata Grande, both located in the state of Alagoas (dissimilarity value = 1.33). These areas are geographically close to each other (3 km) and have similar climate, vegetation, and relief.

Table 3. Mantel test used to compare matrices of shape, size, altitude, and geographic distance, based on wing measurements of Melipona subnitida with 5,000 permutations. NS not significant; * significant

COMPARED MATRICES	R²	P
Shape x Altitude	0.009	0.473^{NS}
Shape x Geographic distance	0.506	0.008*
Size x Shape	0.282	0.972^{NS}
Size x Altitude	0.072	0.537^{NS}
Size x Geographic distance	0.045	0.512^{NS}

In the cluster analysis, the formation of groups reflected morphological differences between the populations and, in general, we found a correlation between forewing morphology and geographical distance. However, the populations from Exu and Passira, which are more than 300 km apart, curiously were found to form a distinct group, indicating morphological similarities between the two far apart populations. These similarities might be a result of the similar environmental conditions found in Exu and Passira, which, despite the distance, present suchlike vegetation and relief (CPMR, 2014).

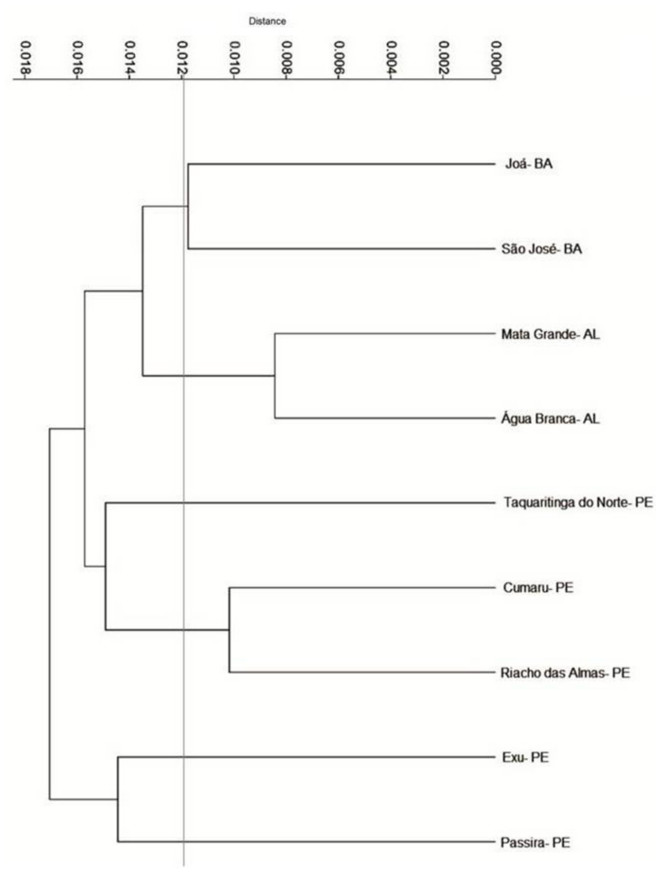

Fig 2 Dendrogram generated by UPGMA with the average morphometric distances between *Melipona subnitida* colonies from different localities.

We have previously found similar results when studying population diversity in *Melipona quadrifasciata anthidioides* Lepeletier from different regions of Bahia State (Nunes et al., 2008), observing that the populations that were located far from each other showed morphological divergence. Mendes et al. (2007) observed morphological differences between *Nannotrigona testaceicornis* (Lepeletier) groups from urban and rural areas in the municipality of Uberlândia, State of Minas Gerais, in the Southeast of Brazil. These authors associated these differences with selective pressures in the urban area and with a higher probability of genetic variability in the rural area.

Once the temperature is a factor that can influence the insect populations and distribution (Easterling et al., 2000; Damos & Savopoulou-Soultani, 2012), and considering the expected impacts of climate warming on stingless bees (Saraiva et al., 2012; Giannini et al., 2012), the characterization of population structures of *M. subnitida* will help to preserve its genetic diversity, increasing the likelihood of conserving lineages more adapted to the expected climatic changes, with potential impacts on pollination.

Melipona subnitida is narrowly distributed and it is currently found in fragmented and geographically-isolated environments (Zanella, 2000; Bonatti et al., 2014). The strong relationship between geographical distance and morphological variability found in our study indicates a lack of genetic flow among populations that are located far from each other or even among those that would be able to crossbreed if in favorable environmental conditions, corroborating the importance of conserving nesting sites and food sources. This finding will contribute to the formulation of management and conservation plans for *M. subnitida* in order to preserve its population diversity and, therefore, to collaborate to the generation of income in meliponiculture programs. In a broader sense, our results highlight the importance of characterizing the population structure of the stingless bees, because they help to identify differentiated populations, which need more human intervention as a way to decrease the rates of inbreeding.

Conclusion

There is a correlation between sampling site and wing shape in *M. subnitida* populations inhabiting the limit of the species distribution, indicating that the divergence among the sampled populations is due to geographical distance among the sampling sites and, hence, suggesting the formation of characterized groups of populations along the studied zone.

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