

## SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

### Morphometric Analysis of Populations of *Centris aenea* Lepeletier (Hymenoptera: Apidae) from Northeastern Brazil

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#### Abstract

*Centris aenea* Lepeletier is a solitary bee that has raised interest in management to pollinate crops, such as acerola, *Malpighia emarginata*. This study investigated the level of morphometric variability among populations of *C. aenea* from Northeastern Brazil. Traditional and geometric morphometric analyses were used. Head length, leg length, wing length, and wing shape were measured in samples (5-10 females) from eight localities. We did not find statistically significant differences among the populations ( $P > 0.01$ ). The partial wing warps were similar in the populations and indicated that the bees were not morphometrically different. Our results suggest that *C. aenea* shows low population morphometric variability and highlight the need for further investigations on population variation in this species, preferably including populations sampled at the extremes of their geographic distribution. Significant insight into the population variation of *C. aenea* will probably require the use of molecular markers to allow a comparative approach between morphometric variability and genetic variability.

#### Introduction

*Centris aenea* Lepeletier is a solitary bee with occurrence restricted to Central and South America in the Neotropics (Moure *et al* 2007). In South America, *C. aenea* ranges from the Guianas to the state of São Paulo, Brazil (Moure 1969). In Brazil, *C. aenea* has been reported to nest in several habitats in the caatinga (Martins 1994, Aguiar & Gaglianone 2003), cerrado (Silveira & Campos 1995), and restinga (beach areas) (Ramalho & Silva 2002, Viana & Kleinert 2006, Albuquerque *et al* 2007). The females pollinate native vegetation in addition to cultivated plants (Teixeira & Machado 2000) such as guava (*Psidium guajava*) (Castro 2002) and acerola (*Malpighia emarginata*) (Freitas *et al* 1999, Vilhena & Augusto 2007). *Centris*

*aenea* shows sexual dimorphism, as well as size polymorphism among males (Gaglianone MC personal communication). Actually, *C. aenea* shows a pronounced size polymorphism among males, however, there are no reports of size variation among females.

Size and shape variability of several parts of the bee body can be studied using traditional and geometric morphometry, and these techniques are widely applied to the study of social bees. Traditional morphometry has been employed to investigate differentiation among species (Nunes *et al* 2007, Quezada-Euán *et al* 2007), population structure and geographic variation (Ruttner *et al* 1978, Hadisoesoilo *et al* 2008), subspecies discrimination (Radloff *et al* 2005a,b), phylogeny (Hernandez *et al* 2007), and also to establish a correlation between body size and flight distance

(Araújo *et al* 2004). Geometric morphometry has been applied to systematics (Aytakin *et al* 2007), to the identification of subspecies (Francoy *et al* 2006, 2009, Mendes *et al* 2007), and to the differentiation of species (Francisco *et al* 2008).

In this study, we investigated whether there is also morphometric variability among *C. aenea* females from different populations and tested what, if any, role geographic distance may play in population differentiation of this species. This is the first morphometric study of bees in the genus *Centris* and it will likely guide future investigations of population variation in this bee species such as studies on genetic variability. Considerable morphometric variability between distinct populations indicates the urgency to investigate population genetic structure prior to proceeding with any translocation of females for orchard pollination.

## Material and methods

### Sampling

Females ( $n = 74$ ) of *C. aenea* were collected from eight localities in the states of Bahia, Paraíba, and Pernambuco, in the Northeastern region of Brazil (Tables 1, 2) where the species is abundant and frequently collected (e.g. Martins 1994, Madeira-da-Silva & Martins 2003, Aguiar & Zanella 2005, Viana & Kleinert 2006). Sampled areas included natural vegetation and also acerola orchards usually visited by *C. aenea* (Tables 1, 2). Adults were collected using an entomological net and killed with ethyl acetate vapors or in 100% ethanol. Some specimens were deposited at the Coleção Entomológica Prof. Jonhan Becker of the Museu de Zoologia da Universidade Estadual de Feira de Santana (MZUEFS). Individuals preserved in 100% ethanol were stored in a freezer (-20°C) at the Laboratório de Entomologia at the Universidade Estadual de Feira de Santana (Feira de Santana, Bahia) for future molecular analyses.

### Traditional morphometry

The head, right hind leg, and the right forewing of each female were dissected with the aid of a stereomicroscope (Zeiss Stemi SV6). The wings were mounted between microscope slides (Francoy *et al* 2006, 2009, Mendes *et al* 2007, Francisco *et al* 2008). Twenty one characters were measured using a Zeiss Stemi SV6 stereomicroscope with an ocular micrometer: head length, head width, maximum interorbital distance, clypeus width, forewing width, femur length, tibia length, tibia width, basitarsus width (following Quezada-Euán *et al* 2007), distance between antennal sockets, distance between lateral ocelli, distance between medium ocellus and upper clypeus, distance between medium ocellus and right lateral ocellus, distance between the lateral ocellus and the composed eye (following Hurd & Moure 1963), marginal cell length (following Francoy TM, unpublished data), head length (diagonal), secondary basitibial plate length, and primary basitibial plate length, primary basitibial plate width (new characters proposed herein).

### Geometric morphometry

The right forewing of each individual was dissected, mounted between microscope slides, and photographed with a digital camera attached to a Nikon Eclipse 50i stereomicroscope (Francoy *et al* 2006, 2009, Mendes *et al* 2007, Francisco *et al* 2008). First, a TPS file was created from the image file in the software tpsUtil version 1.4 following Rohlf (2008a). Eighteen homologous landmarks were manually plotted at the vein intersections (Fig 1) using the software tpsDig version 2.12 following Rohlf (2008b). The centroid size was calculated for all anatomic landmark configurations for each wing. The vein intersections were then aligned using a Procrustes superimposition, which generated a matrix of partial warp scores (matrix W) using the software tpsRelw version 1.45 (Rohlf 2007). All software programs used for this analysis are freely available for academic use at <http://life.bio.sunysb.edu/morph/>.

Table 1 Number of *Centris aenea* females, sampled localities, and geographical coordinates.

Locality	Vegetation	State	Latitude	Longitude	n
Feira de Santana	Caatinga	Bahia	12° 11' 88.3"	38° 58' 95"	10
Mucugê	Campo rupestre	Bahia	12° 50' 45"	41° 31' 04"	9
Morro do Chapéu	Caatinga	Bahia	11° 34' 29"	41° 11' 00"	10
Mata de São João	Restinga	Bahia	12° 27' 20.4"	37° 56' 05"	10
Palmeiras	Cerrado	Bahia	12° 32' 44"	41° 43' 31"	10
Cruz das Almas	Acerola orchard	Bahia	12° 40' 39"	39° 40' 23"	10
Petrolina	Acerola orchard	Pernambuco	09° 09'	40° 22'	10
Santa Teresinha	Acerola orchard	Paraíba	07° 00'	37° 23'	5

Table 2 Distance (km) among sampled localities based on latitude and longitude.

	FSA	MU	MC	MSJ	ST	PE	PAL
MU	277.8						
MC	256.4	145.9					
MSJ	115	391.2	366.6				
ST	628.9	792.1	657.7	609.6			
PE	394.2	429.7	284	453.5	406.2		
PAL	296.9	175.1	123	411.6	778.7	405.7	
CA	78.82	200.9	204.9	190.3	679.3	399.5	223.2

FSA, Feira de Santana; MU, Mucugê; MC, Morro do Chapéu; MSJ, Mata de São João; ST, Santa Teresinha; PE, Petrolina; PAL, Palmeiras; CA, Cruz das Almas

### Statistical analysis

Traditional morphometric data, centroid size, and partial warp scores were analyzed using Kruskal-Wallis analysis of variance and differences among populations were assessed by Dunn's test using InStat 3.0 software (Graphpad 1999). Traditional morphometric data and partial warp scores were analyzed together using MANOVA and canonical variate analysis (CVA) using PAST software (PALaeontological STatistics, version 1.81, <http://life.bio.sunysb.edu/morph/>) (Hammer *et al* 2008).

### Results

There were no significant differences for most traditional morphometric variables analyzed among populations (Kruskal-Wallis,  $P > 0.05$ ) except for head width and tibia length (Kruskal-Wallis,  $P < 0.05$ ), marginal cell length, and forewing width (Kruskal-Wallis,  $P < 0.001$ ). These four morphometric characters showed significant differences (Dunn's test,  $P < 0.05$  and  $P < 0.01$ ) among three population pairs that were compared (Mucugê and Cruz das Almas, Mata de São João and Cruz das Almas, Morro do Chapéu and Cruz das Almas). In all characters analyzed by traditional

morphometry, the Cruz das Almas bees were significantly different from the other populations (Dunn's test,  $P < 0.05$  and  $P < 0.01$ ). The populations from Petrolina, Palmeiras, and Santa Teresinha were not statistically different from any other studied population (Dunn's test,  $P > 0.05$ ). The populations from Chapada Diamantina (Mucugê, Palmeiras, and Morro do Chapéu) were not statistically different among themselves (Dunn's test,  $P > 0.05$ ).

Centroid size analysis showed extremely significant statistical differences among populations (Kruskal-Wallis,  $P < 0.001$ ). The population pairs from Mucugê and Cruz das Almas and Morro do Chapéu and Cruz das Almas were significantly different (Dunn's test,  $P < 0.05$  and  $P < 0.001$ , respectively), indicating that these populations harbor individuals with significant differences regarding wing size.

The MANOVA of the traditional morphometric data indicated that the different populations were not statistically different (Wilk's  $\lambda = 0.5436$ ;  $P > 0.05$ ). The two first canonical variables were able to explain 31.37% and 21.27% of the data variability (Fig 2a). A total of 32 partial warps was generated ( $k = 2n - 4$ , where  $k$  represents the number of partial warps and  $n$  the number of anatomic landmarks). None of the 32 warps contributed significantly to the discrimination of populations (Kruskal-Wallis,  $P > 0.05$ ). The compared population pairs revealed no significant differences among samples (Dunn's test,  $P > 0.05$ ).

The MANOVA/CVA of partial warps showed no significant differences among populations (Wilk's  $\lambda = 1$ ;  $P > 0.05$ ), which means that there were no notable differences in wing shape among the studied populations. The two first canonical variables were able to explain 34.12% and 20.19% of the data variability (Fig 2b).

### Discussion

The MANOVA indicated that the differences observed among the studied populations were not statistically significant in either traditional or geometric

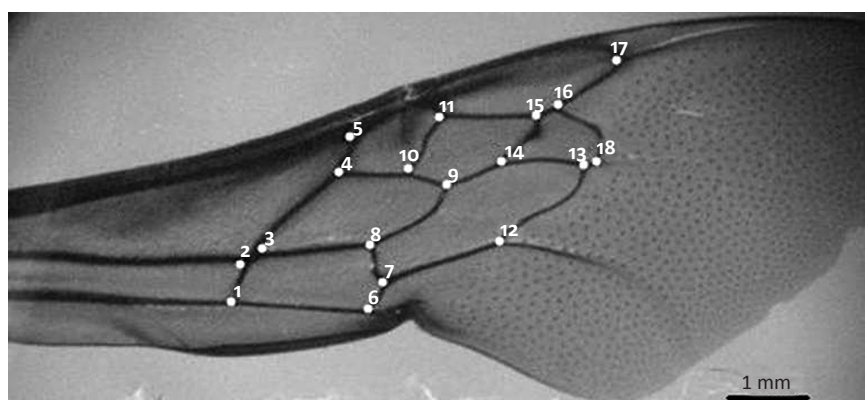


Fig 1 Anatomic landmarks manually plotted at the veins intersections of the right forewing of *Centris aenea*.

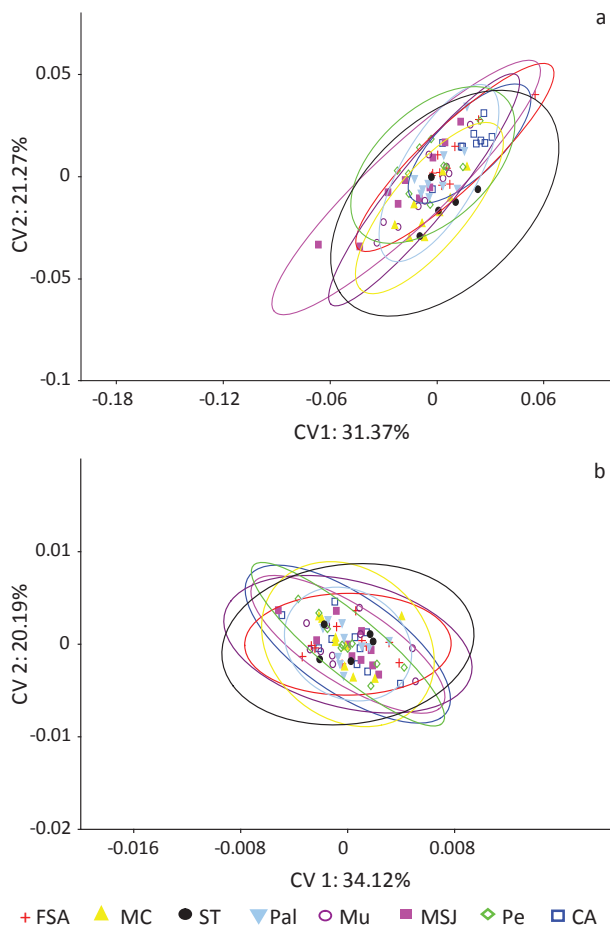


Fig 2 Scatter plots of the canonical variate analysis (CVA) of the grouped matrices for traditional morphometric data (a) and geometric morphometric data (b). The scores of the first canonical variable (CV 1) are on the x axis and the scores for the second canonical variable (CV2) are on the y axis. The ellipses represent the limits of each population. FSA. Feira de Santana (BA); MC. Morro do Chapéu (BA); ST. Santa Teresinha (PB); PAL. Palmeiras (BA). MU. Mucugê (BA); MSJ. Mata de São João (BA); PE. Petrolina (PE); CA. Cruz das Almas (BA).

morphometrics. The canonical variate analysis revealed that all samples were grouped together (Figs 1, 2a,b). Our results differ from those reported for the social bee *Melipona quadrifasciata anthidioides* Lepeletier for which the morphological variation among populations resulted from variation in wing width and length (Nunes *et al* 2008).

In *C. aenea*, as well as in other related species in this genus, a pronounced variation in size among males has been reported (Gaglianone MC, unpublished data). In some species such as *Centris pallida* Fox, larger males (metandric) have an enhanced mating success and exhibit reproductive strategies that are distinct from those exhibited by smaller males (Alcock 1979). There are no reports of such polymorphism among *C. aenea* females. Our results show that there was no morphometrical

divergence among populations based on the 21 characters measured, even though some populations were far apart, e.g. Santa Teresinha and Palmeiras which are 778.7 km apart (Table 2). The possible explanations for the absence of morphometric differentiation among the populations of *C. aenea* studied are: (1) the amplitude of the geographic sampling area was insufficient to reflect variation along the geographic distribution of this species and/or (2) the sample size studied, from five to ten individuals from each population, may have been insufficient to detect phenotypic variation.

Quezada-Euán *et al* (2007) and Nunes *et al* (2008) found morphometric variation in populations of *Melipona beecheii* Bennett and *M. quadrifasciata anthidioides*, respectively, using larger sample sizes of 10 to 30 individuals from each population. However, it should be emphasized that the sample size is usually limited in studies focusing on solitary bees due to the inherent difficulties in collecting a large number of individuals in the same locality, where females are always dispersed and frequently occur at low densities. This contrasts with studies on social bees, especially those of managed colonies.

The wing warp analysis takes into consideration solely the structure shape and not linear measurements. Thus wing warp is a useful analysis as it allows for the identification of bees using anatomic landmarks in one single marginal cell of the forewing (Francoy *et al* 2006). In our study on *C. aenea*, the partial warp analysis of the forewings did not differ among populations. This result was similar to that reported for *M. quadrifasciata anthidioides* (Nunes LA, unpublished data). In subspecies of *Apis mellifera* L., the relative wing warps were not adequate to identify the geographical origin of Africanized populations; however, the analysis was useful to demonstrate that in Brazil these bee populations have a unique morphometric profile for the forewing (Francoy TM, unpublished data). The relative wing warp analysis was also useful to investigate intrapopulation variation in *Nannotrigona testaceicornis* Lepeletier (Mendes *et al* 2007). Therefore, warp analysis of the forewings generates consistent results for the discrimination of populations in certain taxa, but not in others, as the current study of *C. aenea*.

Despite the rather conspicuous morphometric variability found among some bee populations, morphometric differences among animal populations attributed to size variables are questionable (Rohlf & Marcus 1993, Adams *et al* 2004). Some studies on bees show that intraspecific and intrasexual size polymorphisms are highly dependent on fluctuation in food availability (Kim 1999, Peruquetti 2003, Bosch 2008). Studies on females of *C. aenea* generated in different periods of the reproductive season could provide information whether there are intrapopulation

morphometric differences and, if so, whether they could be ascribed to availability variation of floral resources.

*Centris aenea* plays a relevant role in the pollination of acerola orchards; therefore, population studies are of paramount importance for the Northeastern region of Brazil. Both methods employed in this study generated similar results revealing no differences among *C. aenea* populations from the eight localities. However, an ongoing study by our research group with molecular markers (PCR-RFLP of the mitochondrial DNA) has revealed evidence of differentiation among the same populations from the current investigation. Further genetic studies on the reproductive biology and flight range of this species are necessary in order to better evaluate variations in *C. aenea* populations.

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