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Research Article

Geometric morphometry analysis of three species of stingless bees in India

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Abstract

Geometric morphometry is applied to discriminate the three species of stingless bees (*Trigona* (*Tetragonula*) *iridipennis, Trigona* (*Tetragonula*) aff. *laeviceps* and *Trigona* (*Lepidotrigona*) *ventralis* var. *arcifera*). The present study characterized the three species by using the relative wrap analysis, the three species forms three distinct groups and the Principal Component Analysis (PCA) explained that there are 90.29% of the variations and forms three distinct clusters within these three groups. The multivariate analyses of variance (MANOVA) are showed that the stingless bee forewings indicate significant differences (P < 0.0001).

Keywords: Geometric morphometery, relative wrap analysis, species variation and insect forewing

Introduction

Geometric morphometrics is a relatively new technique that has generated valuable results in many fields of classic morphometry. A major advantage of the geometric framework is a comprehensive use of information about shape, available from a set of landmarks (Bookstein, 1998). Insect wings have been the subject of geometric morphometric analysis in the past (Rohlf and Slice, 1990), they are especially attractive because they can be treated with biological realism in only two dimensions. Wing morphometrics can help to characterize populations within a species and also analysis of geographic variation in populations. Wings also proved useful to study complexes of species, for example in Diptera (De La Riva *et al.,* 2001), or examine the effects of hybridization, such as in *Apis melifera* subspecies (Smith *et al.,* 1997). The wing venation pattern of Odonata has long provided students of Odonata with a rich source of diagnostic characters at all taxonomic levels (Rehn, 2003).

Geometric morphometry has recently been applied to the identification of stingless bees is a geometric morphometric analysis of the forewings and also used to resolve taxonomic problems in bumble bees (Aytekin *et al.,* 2007). Identification of honey bee subspecies and to examine changes in the morphometric profile of some Africanized honey bee populations through the geometric morphometric analysis (Francoy *et al.,* 2008). The technique was introduced on two populations of *Plebeia remota* collected from various regions of Brazil and maintained (Francisco *et al.,* 2008). Relative warp analysis of the forewings was also found to be efficient for differentiating sub-populations of *Nannotrigona testaceicornis* from a single locality, attaining 74% accuracy in identifying these sub-populations (Mendes *et al.,* 2007).

The forewing of *Nannotrigona testaceicornis*, *Melipona quadrifasciata*, *Frieseomelitta varia*, and *Scaptotrigona* aff. *depilis*, the venation patterns of males and females from the same species were

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more similar than the patterns of individuals of the same sex from different species. Francoy *et al.* (2009). The present study reports that stingless bee species variation in India through geometric morphometry analysis.

Materials and Methods

The materials utilized and methodologies followed in the present study are described below in detail. The adult worker bee samples from three species (17 nests) were collected from different locations in India during March 2011 to December 2011. Based on the nest architecture and morphology according to Sakagami (1978) and Schwarz (1948) the species identity was confirmed. Twenty bees in each location were collected and bees were preserved in 70 percent ethanol for further studies. The right forewing of each stingless bee species (N=20 for each species) was removed, wet mounted, and photographed.

The species variation in stingless bees was studied by using geometric morphometrics method. Photographic images of the wings were produced using Leica M 165C stereo microscope with image analyzer. Photographs were first input to tps-UTIL 1.40 (Rohlf, 2004). Two dimensional Cartesian coordinates of nine landmarks from the front wings (Fig.-1) was digitized by types-DIG02 (Rohlf, 2005a). The Tps-Dig program provides X and Y coordinates (in Euclidean space in units of imaging pixels) between specified points. Once the digitized points were acquired the data were saved in an NTS text file format and opened with Microsoft Excel. These distance measurements were then used in the statistical analysis. The Cartesian coordinates of the landmarks were then aligned and a partial warps analysis was done using the tpsRelw version 1.42 software (Rolhf, 2005b). A total of 340 worker bee forewings of stingless bee samples collected from 17 nests of three species from various locations were analyzed.

Mean configuration of the 9 landmarks plotted of the stingless bee worker forewing. Multivariate statistical analysis *i.e.,* analysis of variance, factor analysis, principal components analysis and discriminant function analysis were used to detect variations. Analysis of variance was carried out to find out the impact of geometric morphometry used in the study of species variations in stingless bees. Principal components analyses using colony data were done to detect the presence of possible clusters among the scatter scores from a plotted plane graph of the first two high loading factors. Stepwise discriminant analysis using principal component clusters was carried out to determine the most discriminatory variables to enter into the discriminant functions. The discriminant functions were used to classify the subgenuses. The multivariate analysis of variance (MANOVA) was executed using the MANOVA function that investigates if a significant difference among groups is present in a particular data set. All statistical analyses were done by using SPSS 16.0 statistical package.

Results

Geometric morphometrics analyses were performed to find out the species variations in 340 forewings of three species of stingless bees from in India. Principal Component Analysis (PCA), the Cartesian coordinates extracted from the fore wings of stingless bees gave three eigenvalues greater than one. Cartesian coordinates extracted from the wings of three species of stingless bee workers samples from different locations in India and explained a total of 90.20% of the total variability in the data. The first principal component explained 74.56% of the data in the variability and the second principal component explained 9.78% of the variability of the relative positions of the landmarks. The relative positions of landmarks that contributed most of the first components where the Cartesian

Table - 1. Mean configuration of the 9 landmarks plotted of the three species of stingless bees

Coordinates	*T.iridipennis*	*T.laeviceps*	*T.v. arcifera*
1x	-0.43692	-0.45367	-0.39253
1y	-0.25623	-0.21779	-0.29619
2x	-0.41677	-0.42584	-0.41483
2y	-0.16054	-0.11759	-0.19746
3x	-0.07429	-0.05288	-0.06768
3y	0.13158	0.16073	0.15924
4x	-0.01385	0.02505	-0.01354
4y	0.28771	0.29433	0.30575
5x	0.02373	-0.03005	0.13716
5y	0.34454	-0.3325	0.32247
6x	0.23294	0.18648	0.21896
6y	-0.28645	-0.32355	-0.2739
7x	0.18257	0.15864	0.17119
7y	-0.09003	-0.11203	-0.1051
8x	0.26819	0.29224	0.25718
8y	0.02581	-0.0007	0.0414
9x	0.25812	0.26997	0.24125
9y	0.34815	0.31659	0.36626

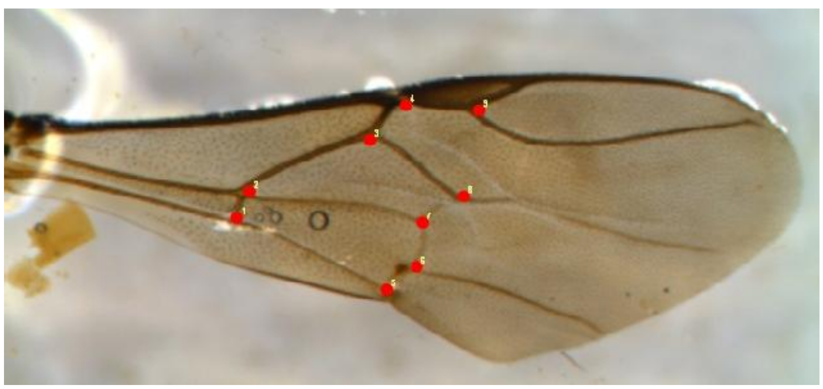
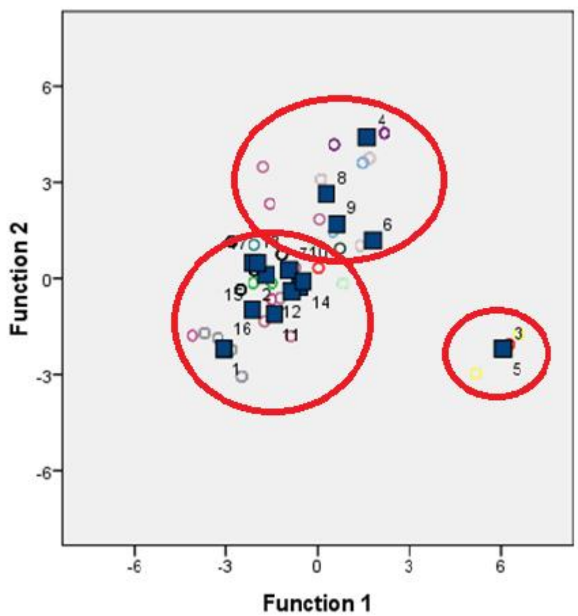


Fig.- 1. Distribution of 9 landmarks plotted on fore wing of stingless bees (*Tetragonula* and *Lepidotrigona*)

coordinate 'x' position in the landmarks 3,4,5,6 and 9 and 'y' position in the landmarks 1,2,7,8 and 9 and the first component alone explained 74.56% percent of the variability among the groups. The relative positions of landmarks that contributed the second component where the Cartesian coordinate 'x' position in the landmarks 2,7 and 8 and 'y' position in the landmarks 3 and 5. The relative positions of landmarks that contributed the third component where the Cartesian coordinate 'x' position in the landmark 1 alone. Mean configuration of nine landmarks plotted in stingless bee forewings is presented in Table - 1. Based on the positions of the groups in the Discriminant Function Analysis (DFA) (Fig.-2), it is clear that the three species of stingless bees were well distinguished.

The MANOVA of the measures indicated that the three species are statistically different (Wilk's λ = 4.763 $P < 0.0001$ and partial $\varepsilon^2 = 1.000$). A graphical representation of the discriminant analysis shows there are existence of three species in the present study, within the three groups, South Indian common species *T.*

iridipennis formed first group, *T. laeviceps* formed the second group. In the Eastern part of India, *T. ventralis* var. *arcifera* formed the third group.



Trigona (Tetragonula)iridipennis- No.1,2,7,10,11,12,13,14,15,16& 17.
Trigona (Tetragonula) laeviceps- No.4,6,8 &9
Trigona (Lepidotrigona) ventralis var.*arcifera*- No.3&5

Fig.- 2. The species variation through discriminant function analysis

Discussion

Geometric morphometry revealed itself a valuable tool to examine the overall variation in the stingless bee forewings. As our samples were collected from different places in India, we were able to show the differentiation of these groups. With the evolution of computational morpho-metrics identification systems, it is now possible to identify species of various groups of insects using only wing features (Mendes *et al.,* 2007). Francoy *et al.* (2009) examined the forewing venation patterns of males and females of five stingless bee species and reports that the patterns of males and females from the same species were more similar than the patterns of individuals of the same sex from different species. They suggest that the features extracted from the wings of males and females were very informative in discriminating the five species.

The greater variability of stingless bees can be the result of the greater number of colonies and greater availability of mate choices, consequently increasing the genetic variability of the *Nannotrigona testaceicornis* from Uberlândia (Mendes *et al.,* 2007). In this case, the Mahalanobis square distances show a greater proximity of the within the species group. The results showed that geometric morphometrics yielded better discrimination of stingless bee species than standard morphometry. The differences in the discrimination were found only when it was based on individual forewings. Stingless bee species diversity was directly affected by the fluctuation in populations of individual species and several factors influenced the activity of the bees such as temperature, relative humidity, transmittance, body size and flight range. The geometric morphometry is a simple and quick technique, with low costs and a very good discriminatory power.

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