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Morphometric analysis of *Tetragonula iridipennis* (Hymenoptera: Apidae) in Coimbatore district, Tamilnadu, India

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Abstract

This study investigated the level of morphometric variability among the stingless bee populations in Coimbatore district, Tamilnadu, India. A total of 16 morphometric characters was measured and analyzed to detect the morphometric variations among *T. iridipennis.* Data were subjected to multivariate analysis to evaluate population variation within *T. iridipennis*. The variables in the present study displayed statistically significant differences among groups (P > 0.05). The present study find out the three distinct morphocluster within *T. iridipennis* based on principal component analysis.

Keywords: Meliponini, *Tetragonula,* morphometry, multivariate analysis.

INTRODUCTION

During the last twenty years, the systematic relationships among species was studied by using morphometry analysis (Kerr and Cunha**,** 1976) and sex determination and caste differentiation are confirmed by using this analysis (Hartfelder and Engel, 1992).

The stingless bees composed of more than 600 species and live in perennial colonies (Michener, 2013). Stingless bees are an alternative to the domestic honey bees for agricultural pollination purposes (Michener, 2000). These large numbers of species are also characterized by behaviour and morphology (Michener, 2007) leading in some cases to the recognition of distinct ecotypes (Camargo *et al*., 1988; Ayala, 1999). Stingless bees showed a wide range of worker body size variation, ranging from smaller species in the *Plebeia* group to larger species of the genus *Melipona* (Wille, 1983). The genus *Liotrigona* is one of the smallest stingless bees

in the world. The total body length of *Liotrigona bitika* is less than 2 mm (Brooks and Michener, 1988).

Sakagami (1978) distinguished *T. pagdeni* from "*iridipennis*" species group based on the morphometric variation on male and female bees and reported *T. pagdeni* is only statistically different from *T. iridipennis.* May-Itza *et al* (2012) reported that the strong divergence in morphological, mitochondrial and nuclear data between geographically isolated populations of the *Melipona yucatanica* in Yucatan and Guatemala. The worker body weight and size were highly correlated with the protein content of the larval food (Quezada-Euan *et al*., 2011). Worker bee size reflects an adaptation to environmental conditions (Ruttner, 1988). A major part of the morphological variation in Meliponini occurs independently of phylogeny due to the fact that, for social bees, worker body size has been generally considered as an adaptation to foraging activity and floral resource exploitation (Baumgartner and Roubik, 1989).

In India, Akum *et al.* (2012) reported that the variation between two populations of stingless bees in Nagaland, India and showed the morphometric variation between the two populations. Danaraddi and Viraktamath (2009) studied 15 morphological characters of *Trigona iridipennis* collected from eight places of Karnataka and observed that the body length varied from 3.93 to 4.12 mm. According to Devanesan *et al.* (2009) the body length of *T. iridipennis* was 4.07 mm and they used the morphometric characters for differentiating the queen and workers bees of *T. iridipennis*. Recently, Vijayakumar and Jeyarraj (2014) used the various morphometric characters of *T. iridipennis, viz.,* length of the body, head width, forewing length, bifurcation between vein m and cu, hind tibial length for separation of this species from *iridipennis* species group.

In this study, we investigated morphometric variability among *T. iridipennis* females from different locations. We obtained morphometric data from 140 female *T. iridipennis* from seven different locations. Data were subjected to multivariate analysis to evaluate population variation within *T.iridipennis*.

MATERIALS AND METHODS

Standard morphometrics

Adult worker bees were collected from identified feral nest of *T. iridipennis* in seven different locations (Table 1) and bees were preserved in 70% ethanol. The preserved bees were dissected and sixteen morphometric measurements were studied (Table 2) by using Leica M 165C stereo microscope. The measurements represent in numbers, lengths or widths of morphometric characters and are reported in millimeter. The morphological characters were measured for each worker bee based on previous literature (Ruttner, 1988 and Sakagami, 1978).

Table 1: Sampling locations and its geographical positions

S.No	Location	Collection site	Geographical position
1	Sambaravalli	Arboreal	11°.20' N / 77°.41' E
2	Sirumugai	Arboreal	11°.18' N / 77°.18' E
3	Chickadasampalayam	Arboreal	11°.19' N / 76°.58' E

4	Nellithurai	Arboreal	11°.16' N / 76°.53' E
5	Pungampalayam	Arboreal	11°.14' N / 76°.55' E
6	Velliankadu	Terrestrial	11°.07' N / 76°.95' E
7	Vellakinaru	Terrestrial	11°.04' N / 76°.58' E

Data analysis

The statistical procedure was to perform a factor analysis by using 16 morphometric characters for 140 bees collected from seven different locations in Coimbatore district, Tamil Nadu, India. Multivariate statistical analysis, i.e., analysis of variance, factor analysis, principal components analysis and discriminant function analysis were used to detect population variation within *T. iridipennis*. The morphometric variation in female bee samples were studied using principal component analysis (PCA) based on covariance matrices. The first principal component explains the major part of the variance present in the original characters (Reis *et al.*, 1988). Stepwise discriminant analysis using principal component clusters was carried out to determine the most discriminatory variables to enter into the discriminant functions. All statistical analyses were done by using SPSS 16.0 statistical package.

RESULTS

Standard morphometrics analyses were performed to find out population variation within *Tetragonula iridipennis* collected from seven different localities representing in Coimbatore district, Tamil Nadu, India. A total of 16 morphometric characters was measured and analyzed the morphometric variations within *T. iridipennis*. The mean values and standard deviations of the 16 morphometric characters are shown in table 3.

Table 2: List of morphological characteristics of worker stingless bee studied

S. No.	**Characteristics** (mm)	**Abbreviation**
I. Head		
1	Total body length	BL
2.	Head length	HL
3	Head width	HW
4	Proboscis length	PL
5	Distance between two lateral ocelli	DBO
6	Ocello - ocular distance	OOD
7	Antennal length	AL
II. Thorax		
8	Hind leg length	HLL
9	Hind tibial length	HTL
10	Hind tibial width	HTW
11	Hind basitarsus length	HBL
12	Hind basitarsus width	HBW
13	Fore wing length	FL
14	Fore wing width	FW
15	Bifurication between veins M and Cu	WL2
16	Number of hamuli in Hind wing	NH

The bees collected from Nellithurai are comparatively larger (Mean of BL = 3.84, HL= 1.35, HW= 1.79, FL=3.36, WL2= 1.09, HTL=1.53 mm) than bees collected from other locations. The bees collected Sambaravalli are the smallest bees (Mean of BL = 3.25, HL= 1.19, HW= 1.38, FL=3. 28, WL2=0. 93, HTL=1. 31 mm) than bees from other locations.

Table 3: The mean values and standard deviations of morphometric characters

S. No.	Characters (in mm)	Sambaravalli	Sirumugai	Chickadasam palayam	Nellithurai	Pungam palayam	Velliankadu	Vellakinaru
		m±sd	m±sd	m±sd	m±sd	m±sd	m±sd	m±sd
1	BL	3.25±0.05	3.45±0.01	3.52±0.08	3.84±0.06	3.65±0.12	3.55±0.09	3.65±0.12
2	HL	1.19±0.01	1.29±0.03	1.31±0.04	1.35±0.10	1.30±0.06	1.27±0.07	1.30±0.12
3	HW	1.38±0.10	1.53±0.11	1.69±0.10	1.79±0.06	1.63±0.01	1.53±0.11	1.61±0.06
4	PL	0.61±0.05	0.65±0.02	0.66±0.05	0.79±0.04	0.67±0.08	0.62±0.09	0.71±0.02
5	AL	1.19±0.07	1.26±0.05	1.24±0.06	1.31±0.05	1.22±0.07	1.27±0.10	1.21±0.09
6	DBO	0.44±0.01	0.47±0.04	0.45±0.02	0.55±0.07	0.47±0.06	0.51±0.01	0.47±0.05
7	OOD	0.30±0.05	0.33±0.06	0.29±0.05	0.39±0.08	0.28±0.03	0.25±0.04	0.31±0.10
8	FL	3.28±0.07	3.37±0.07	3.32±0.08	3.41±0.07	3.31±0.08	3.35±0.01	3.29±0.05
9	FW	1.18±0.04	1.28±0.01	1.22±0.04	1.36±0.06	1.27±0.04	1.29±0.06	1.27±0.04
10	WL2	0.93±0.05	0.99±0.03	0.95±0.06	1.09±0.11	1.00±0.07	0.98±0.05	0.93±0.07
11	NH	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
12	HLL	3.12±0.09	3.45±0.09	3.49±0.11	3.53±0.04	3.41±0.13	3.34±0.09	3.28±0.13
13	HTL	1.31±0.12	1.41±0.10	1.41±0.07	1.53±0.06	1.42±0.09	1.46±0.02	1.40±0.07
14	HTW	0.41±0.03	0.49±0.10	0.48±0.05	0.55±0.08	0.43±0.12	0.47±0.09	0.45±0.11
15	BTL	0.65±0.07	0.73±0.09	0.69±0.07	0.81±0.05	0.67±0.01	0.71±0.03	0.66±0.04
16	BTW	0.35±0.02	0.41±0.10	0.38±0.03	0.51±0.02	0.39±0.06	0.37±0.10	0.41±0.03

The sample means yielded 3 factors with high eigenvalues. Analysis of variance of morphometric characters showed that 16 out of the 16 morphometric variables displayed statistically significant differences among groups ($P > 0.05$) (Table 4).

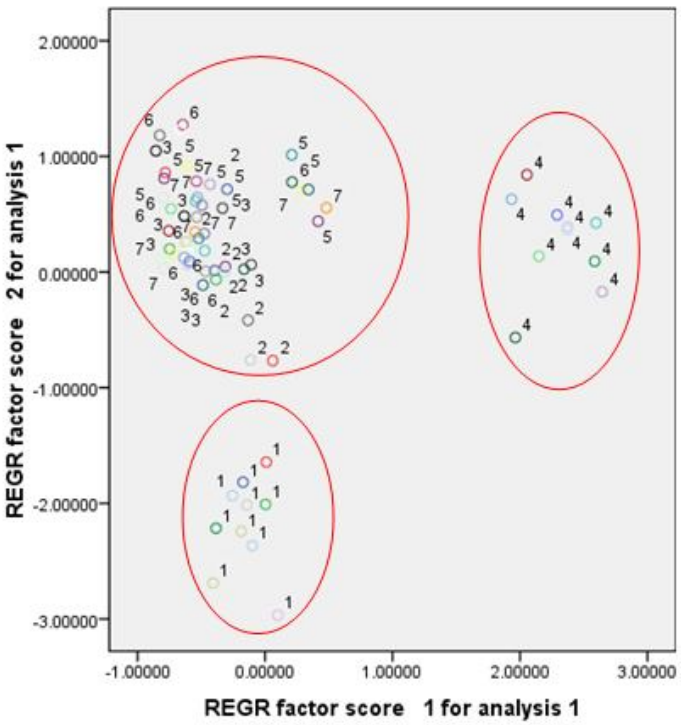
Stingless bees collected from different locations were classified into three distinct morphoclusters based on principal component analysis (PCA). In this analysis, bees from Nellithurai are formed first cluster. The bees from Samparavalli formed a second cluster and bees from remaining locations (Sirumugai Chickadasampalayam, Pungampalayam, Velliankadu, Vellakinaru) formed the third cluster. The morphometric analysis reveals that there is the existence of three morphoclusters from three morphocluster within *Tetragonula iridipennis*.

Table 4: Test of equality of means of 18 morphometric characters

Tests of Equality of Group Means					
	Wilks' Lambda	F	df1	df2	Sig.
HL	0.631	6.128	6	63	0.000*
HW	0.446	13.030	6	63	0.000*
PL	0.265	29.071	6	63	0.000*
DBO	0.221	36.940	6	63	0.000*
OOD	0.053	188.451	6	63	0.000*
AL	0.168	51.971	6	63	0.000*
FL	0.085	112.443	6	63	0.000*
TL	0.031	329.461	6	63	0.000*
LMT	0.120	76.677	6	63	0.000*
WMT	0.380	17.105	6	63	0.000*
FWL	0.025	414.760	6	63	0.000*
FWB	0.125	73.240	6	63	0.000*
TERL	0.413	14.929	6	63	0.000*
STEL	0.293	25.277	6	63	0.000*
STEW	0.132	69.165	6	63	0.000*
WPL	0.473	11.681	6	63	0.000*
WPW	0.363	18.393	6	63	0.000*
DBWPL P	0.740	3.682	6	63	0.003*

#(P > 0.05)

Three principal components with eigenvalues greater than one were isolated. The three principal components accounted for a total of 68.53% of the variation in the data PC1: with morphological character including head width, total body length, hind leg length, forewing width, head length, hind tibial length, antennal length, proboscis length and the bifurcation between vein *m* and *cu* with component loadings between 0.55 and 0.92 accounting for 47.56% of the variation; PC 2: with morphological character including distance between two oeclli, forewing length, ocello-ocular distance and hind tibia width with component loadings between 0.56 and 0.87 accounting for 13.23% of the variation; PC 3: with morphological character including hind basitarsus length and hind basitarsus width with component loadings between 0.55 and 0.85 accounting for 7.74% of the variation. The discriminant function analysis (DFA) displayed the similar results as in principal components analyses (PCA) (Figure 1).



1. Sambaravalli, 2.Sirumugai, 3.Chickadasampalayam, 4. Nellithurai, 5.Pungampalayam

6. Velliankadu, 7.Vellakinaru

Figure 1: A scatter plot of worker bees of *Tetragonula iridipennis* from 7 different locations based on PCA analysis

The test for equality of the group means for the characters used in the discriminant function, Wilks' lambda approximated by the F statistic was determined. A significant difference between the means of the three groups was established ($\Lambda = 0.201$, $F = 11.52$, $P < 0.0001$) partial $\varepsilon^2 = 0.383$. The graph of the principal component scores shows that there are existences of three morphoclusters of within this species in Coimbatore district and morphocluster one (Nellithurai) having the total body length (3.84±0.06) and head width (1.79±0.06) are comparatively larger in size than the bees from other locations. The morphocluster two (Samparavalli) is the smaller than other locations.

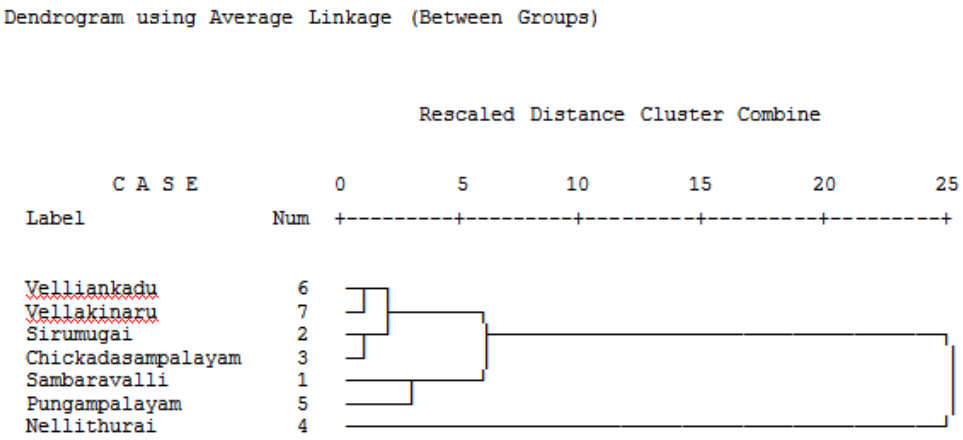


Figure 2: Dendrogram for population variation within *T.iridipennis*

The dendrogram shows that the three morphogroups of *T. iridipennis* were clumped into two groups, that is the bees from Nellithurai was found to be a separate isolated branch and while the other six locations (Sambaravalli, Sirumugai, Chickadasampalayam, Pungampalayam, Velliankadu, Vellakinaru) were split into sub-branches (Figure 2). Mahalanobis distances comparing the size of average distances between samples from seven locations are differed ($P < 0.0001$) from each other. The shortest Mahalanobis distance ($D2 = 0.134$) was between samples from Chickadasampalayam and Vellakinaru, whereas the longest distance was between samples from Nellithurai and Samparavalli ($D2 = 0.969$).

DISCUSSION

The present study used 16 morphometric characters of *T.iridipennis* in seven different locations in Coimbatore district, Tamil Nadu, India and revealed the existence of three distinct morphoclusters. The bees collected from Nellithurai are formed the first morphocluster and the bees from these regions were comparatively larger (total body length 3.84mm; head width 1.79 mm) than bees collected from other locations. The bees collected from five other locations are formed the second morphocluster. The bees from these regions were comparatively intermediate between morphocluster one and three. The bees collected from Sambaravalli are formed the third morphocluster. Similarly, Sakagami (1978) reported that the *T. iridipennis* having a head width is about 1.69 mm and Rasmussen (2013) reported that the total body length of *T. iridipennis* is about 3.55 mm, head width 1.60 mm, hind leg length is about 1.55 mm, forewing length, including tegulae, 3.8mm bifurcation between vein *m* and *cu* is 1.01mm. Danaraddi (2007) studied the morphometric variation between *T. iridipennis* collected from different places of Karnataka, India. The variations among *T. iridipennis* were not statistically significant and summarized the existence of *T. iridipennis* throughout Karnataka. The total body length of *T. iridipennis* worker bees ranging from 3.93 to 4.12 mm, head width ranging from 1.52 to 1.61mm, length of hind tibia ranging from 1.32 to 1.39 mm and forewing length including tegulae ranging from 3.54 to 3.78 mm. In the present study, we documented three morpho groups within *Tetragonula iridipennis* in Coimbatore district, Tamilnadu, India based on morphometry analysis.

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