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Morphometry Analysis of Stingless Bee *Tetragonula iridipennis* Smith (1854)

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A B S T R A C T

The present studies were carried out to know the diversity in morphology of stingless bee *Tetragonula iridipennis* in Coimbatore, Erode and Tiruppur districts of Tamil Nadu, India. Adult worker bees were collected from identified feral nest of *T. iridipennis* in three different locations and bees were preserved in 70% ethanol. The preserved bees were dissected and fourteen morphometric measurements were studied using Leica M 165C stereo microscope. Morphometric analysis revealed that the bees collected from Coimbatore district were comparatively larger (Mean of HL= 1.53, HW 1.76, AL 1.87, HLL 3.46 and BTL 0.57 FL 4.00, WL2= 1.19, HTL=1.60 mm) compared to other two districts. The bees collected from Erode district are relatively small (Mean of HL= 1.23, HW= 1.62, AL 1.67, HLL 3.26 and BTL 0.44, FL 3.32, WL2= 0.89, HTL=1.31 mm). However there is no difference observed in number of hamuli (5) irrespective of the district from which bees are collected. The results of PCA showed that the cumulative variance of the three principal components reached 54% (PC 1), 29.3% (PC 2) and 6.2% (PC 3). The most discriminative characters recorded in the principle component analysis are FL, HTL, BTL, HW, FW, AL and WL2. These characters supported in forming three distinct clusters.

Introduction

Stingless bees belong to the family Apidae and subfamily Meliponinae. It consists of two genera *Melipona* and *Trigona*. Meliponinae includes eight genera, having 15 subgenera and more than 500 species (Wille, 1983). These bees are widely known as dammar bees in India (Rasmussen, 2013). As the name implies, these bees can't sting as their stingers are highly reduced, but they try to defend their colony from intruders using their mandibles (Michener, 2000). The stingless bees are important pollinators of various food crops and can be domesticated (Kishan *et al.*, 2017). Stingless bee species which were commonly seen in India are *Trigona* (*Tetragonula*) *iridipennis* (Raakhee and Devanesan, 2000; Swaminathan, 2000 and Danaraddi, 2007). Apart from *T. iridipennis*, seven other stingless bee species were also reported from India. Though stingless bees are abundant in southern part of India (Rasmussen, 2013). Earlier studies on stingless bees were conducted on biology, nesting behaviour, morphometric characters, foraging behaviour and mellisopalynology (Roopa, 2002; Batista *et al.*, 2003; Devanesan

et al., 2003; Franck *et al.,* 2004; Gajanan, 2005; Roubik, 2006; Bassindale and Harrison Matthews, 2009; Nayak Pavithra *et al.,* 2013 and Rahman and Das, 2013). However exclusive studies on species diversity of Trigona in different parts of India are lacking. Some research on distribution of *T. iridipennis* was done in southern part of India (Vijaykumar and Jeyaraj, 2014). Morphometric taxonomy is one of the most widely used classification of organisms. Some attempts were made earlier to classify stingless bees based on their body size, number of hamuli, length of fore wing, hind wing and cephalic characters etc. Apart from phylogeny, major part of the morphological variation in Meliponini occurs independently 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.

Earlier biometric investigations especially in India were based on few morphological characters and geographic samples which lacked proper statistical analysis of data (Kapil, 1956; Narayanan *et al.,* 1960 a, b; Kshirsagar, l976; Venkatasubbayya, 1938; Rahman and Singh, 1948; Jaggannadham and Goyal, 1980; Kshirsagar and Ranade, 1981; Kshirsagar, 1973; Maa, 1953; Sakai, 1958; Akahira and Sakagami, 1959; Bingham, 1897). Ruttner (1988) for the first time used factorial, principal component and multivariate discriminant analysis methods to study the variation among the population of *Apiscerana* (Indian honey bee).

Ruttner, using morphological characters and computer assisted standard statistical methods distinguished the following four different subspecies of *Apiscerana (Apiscerana cerana*, *Apiscerana himalaya*, *Apiscerana indica* and *Apiscerana japonica*)

Hepburn *et al.,* (2001) re-examined the earlier list of morphometric parameters used by Ruttner (1988) and Alpatov (1929) performing a factor analysis, revealed that there are four morphoclusters of Indian honey bee. Studies on combination of morphometry and statistical analysis for Stingless bees are scarce in India and so the present study was taken.

Materials and Methods

Standard morphometrics

Adult worker bees were collected from identified feral nest of *T. iridipennis* in three different locations (Table 1) and bees were preserved in 70% ethanol. The preserved bees were dissected and fourteen morphometric measurements were studied (Table 2) by using Leica M 165C stereo microscope (Fig. 1-3). Some of the parameters are represented in numbers while 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).

Data analysis

The statistical procedure was to perform a factor analysis by using 14 morphometric characters for 30 bees collected from three districts of 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 was 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. 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 and Discussion

Standard morphometrics analyses were performed to find out population variation within *Tetragonula iridipennis* collected from three different localities representing in Coimbatore, Erode and Tiruppur districts of Tamil Nadu, India. A total of 14 morphometric characters was measured and analyzed the morphometric variations within *T. iridipennis*. The mean values and standard deviations of the 14 morphometric characters are shown in table 4.

Cephalic region

Head length, head width, distance between two lateralocelli, ocello - ocular distance and antennal length were the characters measured in cephalic region and found that the head length varied from1.23 (Erode) to 1.53mm (Coimbatore). Head width ranged between 1.62 mm in Erode samples to 1.76mm in Coimbatore. Mean antennal length of the bees were in the range of 1.67mm in bees collected from Erode, 1.78mm in samples collected from tirupur and 1.87mm from Coimbatore. However ocello - ocular distance was 0.21mm in Coimbatore and Erode districts and 0.22 mm in Tiruppur district. Our results are in concurrence with Danaraddi (2007) who reported that the width of the head including eyes in *T. iridipennis* ranged from 1.52 to 1.61 mm. Franck *et al.,* (2004) reported that head length and width was 1.40 ± 0.04 mm and 1.72 ± 0.05mm in *T. mellipes*, 1.36 ± 0.04 mm and 1.73 ± 0.03 mm in *T. carbonaria*, 1.50 ± 0.04 mm and 1.86 ± 0.05 mm in *T. hockingsi* and 1.41 ± 0.01 mm and 1.72 ± 0.03 mm in *T. davenportii.*

Table.1 Sampling locations and its geographical positions

S.No	Location	Collection site	Geographical position
1	Coimbatore	TNAU Botanical garden	11.0148° N, 76.9315° E
2	Erode	ADP Apiary	11.1179^{o} N, 77.7888^{o}E
3	Tiruppur	Jain irrigation- Mango orchard	10.4764^{o} N, 77.2931^{o}E

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

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

Table.3 Mean values of morphometric characters measured for *T. iridipennis* from three districts

Characters/ Location	Coimbatore*	Trippur*	Erode*
HL	1.53±0.05	1.35±0.07	1.23±0.06
HW	1.76±0.03	1.63±0.04	1.62±0.04
AL	1.87±0.02	1.78±0.04	1.67±0.05
DBO	0.35±0.01	0.41±0.01	0.32±0.01
OOD	0.21±0.01	0.22±0	0.21±0.01
FL	4±0.07	3.38±0.07	3.32±0.07
FW	1.53±0.02	1.52±0.03	1.17±0.03
WL2	1.19±0.01	1.14±0.03	0.89±0.01
NH	5±0.00	5±0.00	5±0.00
HLL	3.46±0.05	3.4±0.04	3.26±0.06
HTL	1.62±0.03	1.34±0.03	1.31±0.04
HTW	0.49±0	0.43±0.01	0.49±0.01
BTL	0.57±0.01	0.42±0.01	0.44±0.01
BTW	0.26±0.01	0.21±0.01	0.23±0.02

*measurments in mm

Fig.1 *T. iridipennis* head under Leica M 165C stereo microscope

Fig. 2 *T. iridipennis* hind wing with hamulai under Leica M 165C stereo microscope

Fig.3 *T. iridipennis* hind leg under Leica M 165C stereo microscope

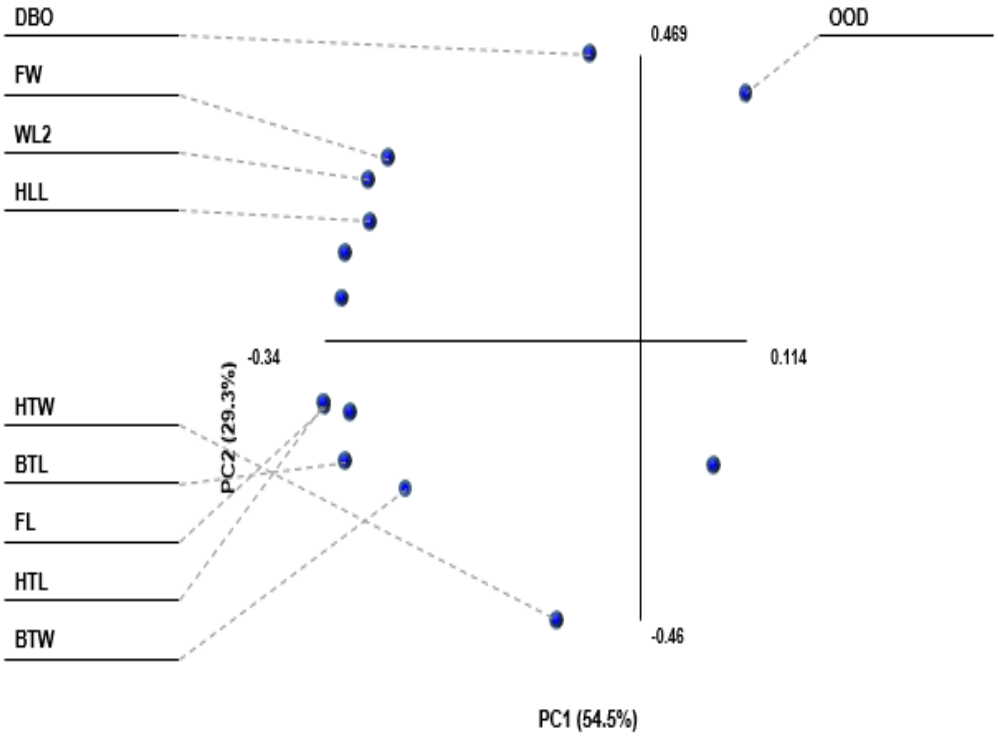

Fig.4 Discriminative characters recorded through principle component analysis

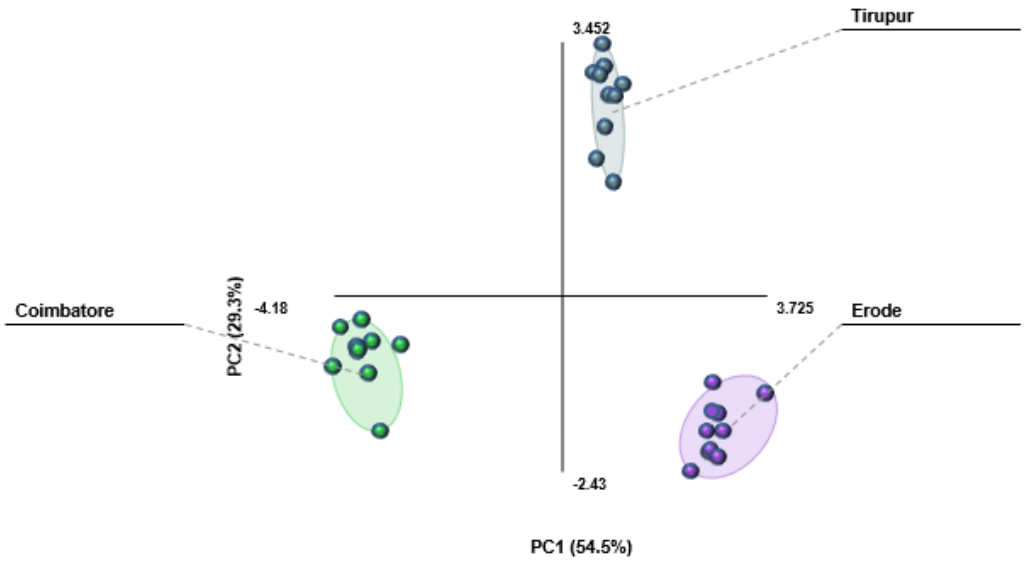

Fig.5 Samples forming three clusters through PCA analysis

Thoracic region

Hind leg length (HLL), hind tibial length(HTL), hind tibial width (HTW), hind basitarsus length (HBL), fore wing length (FL), fore wing width (FW), bifurication between veins M and Cu (WL2) and number of hamuli in hind wing(NH) were the characters studied in the thoracic region. Among the parameters, the average HLL was highest in samples of Coimbatore district (3.46mm) compared to 3.40mm in Tiruppur district and 3.26mm (Table 3) in Erode district. Similarly HTL was highest in Coimbatore (1.62 mm) and lowest in Erode district. However, there was no difference observed in number of hamuli (5) irrespective of the district from which bees are collected. Our study is in concurrence with Danaraddi, 2007; reported that the number of hamuli recorded was 5 in *Tetragonula* collected from different places in Karnataka. Danaraddi, 2007also stated that the length of tibia in *T. iridipennis* varied from 1.32 to 1.39 mm whereas the width varied from 0.47 to 0.50 mm. Rasmussen, 2013stated thatthe length and width of tibia were 1.43 and 0.57 mm in *Lepidotrigona arcifera*, 0.86 and 0.31 mm in *Lisotrigonacacciae*, 1.55 and 0.54 mm in *Tetragonula iridipennis*, 1.47 and 0.54 mm in *T. praeterita*, 1.43 and 0.52 mm in *T. ruficornis*and1.51 and 0.54 mm in *T. bengalensis*. Swaminathan (2000) recorded the hind wing length of 2.24 mm in *T.iridipennis* collected from Tamilnadu. Rahman and Das (2013) reported that the length and width of head of *T. iridipennis* ranged from 0.96 – 1.12 mm and 1.16 – 1.26 respectively. The sample means yielded 3 factors with high eigenvalues. Analysis of variance of morphometric characters showed that all the 14 morphometric variables studied, displayed statistically significant differences among groups ($P > 0.05$)

Principal component anaysis

Measurments were made for 30 Stingless bees collected from Coimbatore, Erode and Tirupur districts (10 bees from each location). Each individual bee have been measured in respect of 14 potentially important characters (Table 2). Finally, the data matrix consisted of 420 measurements was constructed

(comprised of 14 characters of 30 individuals belonging to three districts). The results of PCA showed that the cumulative variance of the three principal components reached 54.% (PC 1), 29.3% (PC 2) and 6.2% (PC 3). The most discriminative characters recorded in the principle component analysis are FL, HTL, BTL, HW, FW, AL and WL2 (Fig. 4). These characters supported in forming three distinct clusters (coimbator, Erode and Tirupur) Figure 5.

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