

MORPHOMETRY OF THE STINGLESS BEES OF THE GENUS *LEPIDOTRIGONA* (HYMENOPTERA: APIDAE: MELIPONINI) INDICATES OCCURRENCE OF MORE THAN ONE SPECIES IN INDIA

SHASHIDHAR VIRAKTAMATH*¹ AND ROJEET THANGJAM²

¹Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru - 560065

²Department of Entomology, Central Agricultural University (Imphal), Thenzawl - 796186

e-mail: shashiv777@gmail.com

ORCID ID: <http://orcid.org/0000-0001-7186-2589>

KEYWORDS

Lepidotrigona
Morphometry
Meliponini

Received on :

11.01.2023

Accepted on :

21.03.2023

*Corresponding author

ABSTRACT

As a precursor to understand the diversity of stingless bees of the genus *Lepidotrigona* we made an exhaustive study on the morphometry of 122 bees representing eight states of northeastern India. Bees from Manipur were larger measuring 4.31 mm in body length as against the body length of 4.70 mm in primary type of *Lepidotrigona arcifera*. The median ocellus was two times larger (0.15 to 0.16 mm in diameter) than the median ocellus of the primary type (0.08 mm). Bees from Arunachal Pradesh, Nagaland and Sikkim had longer and wider forewings (4.48 to 4.49 and 1.50 to 1.55 mm, respectively) but the forewings of the primary type measured 4.60 and 1.65 mm. Hind tibia and hind basitarsus were longer and wider (1.50 and 0.67 mm, respectively) in the bees from Nagaland than in the primary type of *L. arcifera* (1.43 and 0.62 mm, respectively). The ratios of different parts of the body differed in the bees from northeastern India and the primary type. Principal Component and Canonical Discriminant analysis resulted in formation of 5 and 4 clusters, respectively. In both the methods, some bees from Arunachal Pradesh, Sikkim and Tripura formed separate clusters while the bees from remaining states formed an overlapping cluster. Based on these results we conclude that Indian fauna of *Lepidotrigona* bees consists of 4 to 5 species of ventralis group including *L. arcifera*. Further detailed studies need to be focused on inclusion of both females and associated males in description of these species.

INTRODUCTION

Stingless bees (Hymenoptera: Apidae: Meliponini) are one of the most important beneficial insects yielding medicinally valued honey, propolis and contributing for pollination of several plant species including cultivated crops (Roubik, 1989; Heard, 1999). They are distributed in tropical and subtropical areas of America Australia, Africa and Asia (Michener, 2000; Rasmussen, 2013). Indian stingless bees belong to three genera namely *Tetragonula*, *Lepidotrigona* and *Lisotrigona* (Rasmussen, 2013; Viraktamath and Rojeet, 2021). Stingless bees of the genus *Tetragonula* are widely distributed in India but those of the genus *Lisotrigona* are rare. However, bees of the genus *Lepidotrigona* are very common but confined to northeastern states of India (Viraktamath and Rojeet, 2021).

The genus *Lepidotrigona* Schwarz is characterized by tessellate sculpturing on head, mesosoma and apical tergites of metasoma. Mesoscutum is usually bordered by short, thick, scale like, yellowish hairs. *L. nitidiventris* (Smith) is the type species of the genus. *Lepidotrigona* bees are classified into three groups namely, the small *L. ventralis*, medium *L. terminata* and the large *L. nitidiventris* groups based on the length of body and forewings (Schwarz, 1939; Rasmussen 2013). *L. arcifera* (Cockerell) which belongs to the small ventralis group, is the only species described from Sikkim, India by Cockerell in 1929 based on a single worker bee (Rasmussen, 2013).

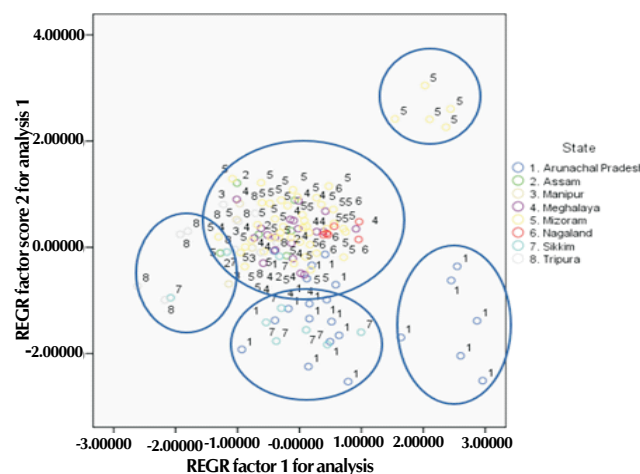
Morphometry is one of the important tools to identify and delineate species in Meliponini which includes several cryptic and complex species (Moure, 1961; Sakagami, 1978; Francoy et al., 2015; Halcroft et al., 2015). Since no new species of *Lepidotrigona* are reported from India for more than nine decades, we made an intensive collection of these bees in eight states of northeast region of India to verify whether a single species occurs in India. As a precursor to describe the diversity species-wise, we made an exhaustive study of the morphometry to understand the dimension of variation and to speculate the number of species among these bees. Our results of these studies are presented in this paper.

MATERIALS AND METHODS

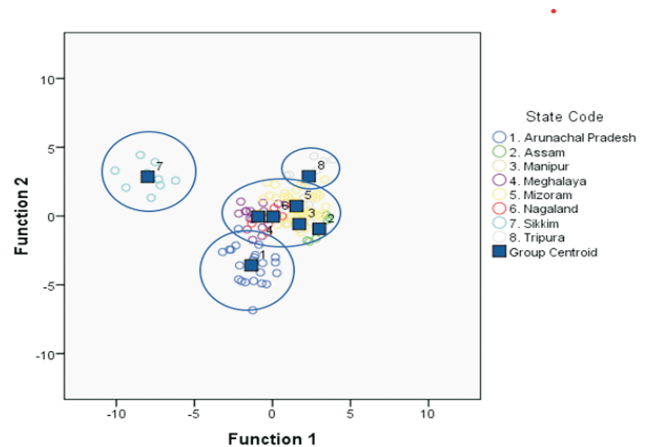
Materials and methods adopted were similar to those described by Viraktamath et al. (2021). We collected more than 1000 bees from 25 locations belonging to eight states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura) of northeastern India from 2017 to 2020. In each state, 5 to 10 *Lepidotrigona* bee colonies were examined which were either wild colonies or kept by the beekeepers in random places. Bee colonies of the genus were easily identified by their characteristic entrance tube made up of white very thin, soft, paper-like wax of various length.

Table 1: Morphological parameters selected for morphometry and their abbreviations

SN	Morphological Parameter	Abbreviation
1	Length of body	BL
2	Width of head including compound eyes	HW
3	Length of head	HL
4	Length of compound eye	EL
5	Width of compound eye	EW
6	Upper inter-orbital distance	UIOD
7	Diameter of median ocellus	DMO
8	Inter-ocellar distance	IOD
9	Ocello-ocular distance	OOD
10	Length of clypeus	CLL
11	Maximum width of clypeus	CLW
12	Length of malar space	MSL
13	Length of scape	SCL
14	Width of scape	SCW
15	Length of pedicel + flagellum	FL
16	Length of first flagellar segment	FFL
17	Length of second flagellar segment	SFL
18	Length of third flagellar segment	TFL
19	Width of third flagellar segment	TFW
20	Length of mandible	MNL
21	Width of mandible	MNW
22	Length of forewing	FWL
23	Width of forewing	FWW
24	Length of pterostigma	PTL
25	Length of marginal cell	MCL
26	Width of marginal cell	MCW
27	Diagonal length of forewing	FWD
28	Number of hamuli	HAM
29	Length of mesoscutum	MSCL
30	Maximum width of mesoscutum	MSCW
31	Length of mesoscutellum	SCTL
32	Maximum width of mesoscutellum	SCTW
33	Length of hind tibia	HTL
34	Width of hind tibia	HTW
35	Length of hind basitarsus	HBTL
36	Width of hind basitarsus	HBTW

**Figure 1. Factor analysis scatter plot showing clusters of female stingless bees of the genus *Lepidotrigona***

The opening of these tubes was usually funnel like at the apex. From each colony 20 to 50 outgoing bees were collected in a specimen tube containing a cotton swab having a few drops of ethyl acetate. Sample from each colony was transferred to a vial containing 95% ethyl alcohol and labeled indicating the place and date of collection. Each sample was

Canonical Discriminant Functions**Figure 2: Discriminant analysis scatter plot showing clusters of female stingless bees of the genus *Lisotrigona* from India**

later examined in the Systematic laboratory at the Department of Entomology, University of Agricultural Sciences, Bengaluru, under a stereoscopic binocular microscope. The genus identity was confirmed by using key characters enumerated by Rasmussen (2013). Though we collected large samples of bees from different places we used 122 female bees representing 25 locations from eight states for our study.

We selected 36 morphological parameters (modified from Sakagami, 1978 and Rasmussen, 2013) for morphometry studies (Table 1). These parameters that included various body parts of head, thorax and abdomen were measured under a stereoscopic binocular microscope fitted with ocular micrometer. The number of hamuli on the right wing were counted. All the measurements were expressed in millimeter. Mean and standard deviation were calculated for each parameter. All the data were subjected to log₁₀ transformation before further analysis.

We adopted two methods of statistical analysis by using SPSS software (version 16) to identify discrete morphological groups of bees from these eight states. The data were first subjected to factor analysis which included analysis of variation, principal component analysis (PCA) on a correlation matrix of all measured variables and a scatter plot by using regression factor score 1 and factor score 2. The second method of analysis was Canonical Discriminant analysis (CDA). A scatter plot was prepared by using the first two discriminate functions to study clustering of samples.

RESULTS AND DISCUSSION

Detailed morphometry of *Lepidotrigona* bees from eight states of northeastern India in comparison with the primary type of *L. arcifera* is presented in Table 2. Bees from all the eight states measured >4.00 mm in body length with longer bees (4.31 mm) recorded in Manipur followed by the bees from Mizoram (4.26 mm) as against the primary type of *L. arcifera* which measured 4.70 mm in body length. Bees from Manipur had narrower head width of 1.71 mm as compared to > 1.80 mm head width in the bees from other states including the primary type of *L. arcifera*. The median ocellus was two times larger

Table 2: Morphometry of female stingless bees of the genus *Lepidotrigona* from India

SN	Parameter/ Place	AP	AS	MN	MG	MZ	NG	SK	TR	PT
Morphometry (Mean in mm ± Standard deviation)										
1	BL	4.18 ± 0.15	4.10 ± 0.17	4.31 ± 0.1	4.20 ± 0.21	4.26 ± 0.26	4.20 ± 0.14	4.15 ± 0.07	4.07 ± 0.14	4.7
2	HW	1.80 ± 0.05	1.80 ± 0.05	1.71 ± 0.01	1.81 ± 0.02	1.80 ± 0.05	1.83 ± 0.02	1.82 ± 0.03	1.73 ± 0.02	1.89
3	HL	1.28 ± 0.03	1.30 ± 0.01	1.32 ± 0.03	1.31 ± 0.03	1.30 ± 0.04	1.33 ± 0.03	1.28 ± 0.05	1.24 ± 0.03	1.51
4	EL	1.07 ± 0.03	1.09 ± 0.03	1.11 ± 0.02	1.10 ± 0.03	1.11 ± 0.03	1.13 ± 0.03	1.10 ± 0.05	1.11 ± 0.04	1.13
5	EW	0.41 ± 0.02	0.42 ± 0.02	0.41 ± 0.01	0.40 ± 0.01	0.42 ± 0.03	0.44 ± 0.01	0.38 ± 0.02	0.43 ± 0.02	0.47
6	UIOD	1.19 ± 0.03	1.18 ± 0.04	1.15 ± 0	1.20 ± 0.03	1.17 ± 0.04	1.23 ± 0.02	1.18 ± 0.04	1.14 ± 0.03	1.23
7	DMO	0.15 ± 0.02	0.15 ± 0	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0	0.15 ± 0	0.15 ± 0	0.08
8	IOD	0.35 ± 0.11	0.35 ± 0.02	0.35 ± 0	0.35 ± 0.01	0.34 ± 0.01	0.35 ± 0	0.35 ± 0.01	0.32 ± 0.02	0.36
9	OOD	0.29 ± 0.01	0.30 ± 0.01	0.30 ± 0	0.29 ± 0.01	0.28 ± 0.02	0.30 ± 0	0.29 ± 0.01	0.29 ± 0.01	0.32
10	CLL	0.35 ± 0.03	0.34 ± 0.01	0.35 ± 0	0.36 ± 0.02	0.35 ± 0.02	0.34 ± 0.04	0.34 ± 0.01	0.33 ± 0.02	0.36
11	CLW	0.72 ± 0.04	0.75 ± 0.02	0.77 ± 0.03	0.79 ± 0.04	0.77 ± 0.05	0.75 ± 0.02	0.74 ± 0.02	0.76 ± 0.02	0.71
12	MSL	0.10 ± 0.02	0.13 ± 0.01	0.09 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	0.11 ± 0.01	0.10 ± 0	0.11 ± 0.01	0.13
13	SCL	0.66 ± 0.03	0.65 ± 0.03	0.59 ± 0.03	0.65 ± 0	0.65 ± 0.02	0.66 ± 0.02	0.65 ± 0.04	0.63 ± 0.02	0.6
14	SCW	0.10 ± 0.01	0.12 ± 0	0.10 ± 0	0.10 ± 0	0.10 ± 0.01	0.10 ± 0	0.10 ± 0.01	0.10 ± 0	0.13
15	FL	1.30 ± 0.07	1.38 ± 0.05	1.31 ± 0.08	1.38 ± 0.05	1.32 ± 0.07	1.44 ± 0.04	1.33 ± 0.09	1.32 ± 0.06	1.35
16	FFL	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0	0.12 ± 0.04	0.10 ± 0	0.1
17	SFL	0.12 ± 0.01	0.12 ± 0	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.1
18	TFL	0.12 ± 0.01	0.12 ± 0	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0	0.13 ± 0.01	0.11 ± 0.01	0.12
19	TFW	0.12 ± 0.01	0.13 ± 0	0.13 ± 0	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0	0.13 ± 0	0.12 ± 0.01	0.14
20	MNL	0.66 ± 0.05	0.64 ± 0.02	0.65 ± 0.02	0.67 ± 0.03	0.68 ± 0.03	0.69 ± 0.02	0.67 ± 0.03	0.63 ± 0.04	0.65
21	MNW	0.27 ± 0.03	0.28 ± 0	0.27 ± 0.02	0.27 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.27 ± 0.03	0.25 ± 0.01	0.21
22	FWL	4.49 ± 0.13	3.67 ± 1.6	4.15 ± 0.11	4.45 ± 0.1	4.37 ± 0.14	4.48 ± 0.08	4.48 ± 0.16	4.17 ± 0.13	4.6
23	FWW	1.53 ± 0.09	1.35 ± 0.08	1.47 ± 0.03	1.48 ± 0.04	1.48 ± 0.06	1.50 ± 0	1.55 ± 0.07	1.42 ± 0.06	1.65
24	PTL	0.72 ± 0.03	0.73 ± 0.03	0.72 ± 0.03	0.72 ± 0.04	0.72 ± 0.03	0.70 ± 0.06	0.73 ± 0.04	0.70 ± 0.04	0.71
25	MCL	1.37 ± 0.06	1.25 ± 0.02	1.26 ± 0.03	1.35 ± 0.04	1.34 ± 0.07	1.37 ± 0.06	1.39 ± 0.09	1.26 ± 0.05	1.4
26	MCW	0.32 ± 0.03	0.30 ± 0	0.30 ± 0	0.33 ± 0.02	0.33 ± 0.02	0.34 ± 0.01	0.34 ± 0.03	0.29 ± 0.01	0.32
27	FWD	1.28 ± 0.04	1.25 ± 0	1.25 ± 0	1.26 ± 0.04	1.25 ± 0.04	1.28 ± 0.03	1.23 ± 0.04	1.23 ± 0.02	1.33
28	HAM	6.09 ± 0.29	6.00 ± 0	6.00 ± 0	6.10 ± 0.31	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6
29	MSCL	0.97 ± 0.06	1.01 ± 0.04	0.98 ± 0.03	1.00 ± 0.03	0.99 ± 0.04	1.02 ± 0.04	1.05 ± 0.03	0.95 ± 0.03	1.08
30	MSCW	1.17 ± 0.07	1.26 ± 0.03	1.18 ± 0.03	1.26 ± 0.04	1.23 ± 0.06	1.28 ± 0.02	1.23 ± 0.03	1.19 ± 0.03	1.26
31	SCTL	0.30 ± 0.03	0.31 ± 0.03	0.32 ± 0.01	0.33 ± 0.01	0.32 ± 0.03	0.33 ± 0.01	0.37 ± 0.04	0.33 ± 0.02	0.28
32	SCTW	0.93 ± 0.05	1.00 ± 0.03	0.95 ± 0	0.96 ± 0.04	1.01 ± 0.08	0.96 ± 0.04	0.71 ± 0.04	0.95 ± 0.03	0.94
33	HTL	1.47 ± 0.05	1.46 ± 0.03	1.38 ± 0.03	1.45 ± 0.03	1.47 ± 0.05	1.50 ± 0	1.47 ± 0.03	1.38 ± 0.05	1.43
34	HTW	0.52 ± 0.06	0.54 ± 0.02	0.50 ± 0	0.56 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.51 ± 0.02	0.57
35	HBTL	0.60 ± 0.04	0.66 ± 0.03	0.63 ± 0.03	0.63 ± 0.04	0.67 ± 0.31	0.67 ± 0.06	0.64 ± 0.02	0.59 ± 0.03	0.62
36	HBTW	0.37 ± 0.03	0.40 ± 0.01	0.40 ± 0.04	0.39 ± 0.01	0.40 ± 0.02	0.40 ± 0.01	0.36 ± 0.02	0.38 ± 0.02	0.26

AP- Arunachal Pradesh, AS- Assam, MN- Manipur, MG- Meghalaya, MZ- Mizoram, NG- Nagaland, SK- Sikkim, TR- Tripura, PT- Primary type of *Lepidotrigona arcifera*

Table 3: Ratios of different parts of the body of stingless bees of the genus *Lisotrigona* from India

SN	Ratio between	AP	AS	MN	MG	MZ	NG	SK	TR	PT
1	HL/HW	0.71	0.72	0.77	0.72	0.72	0.73	0.7	0.72	0.8
2	EL/UIOD	0.9	0.92	0.96	0.92	0.95	0.92	0.93	0.97	0.92
3	IOD/UIOD	0.29	0.29	0.3	0.29	0.29	0.28	0.29	0.28	0.29
4	SCL/EL	0.62	0.6	0.53	0.59	0.59	0.59	0.59	0.57	0.53
5	FWL/FWW	2.93	2.72	2.82	3.01	2.95	2.99	2.89	2.93	2.79
6	FWD/HW	0.71	0.69	0.73	0.7	0.69	0.7	0.67	0.71	0.7
7	HTL/HW	0.82	0.81	0.81	0.8	0.82	0.82	0.81	0.8	0.76
8	HTL/FWD	1.15	1.17	1.1	1.15	1.18	1.17	1.19	1.12	1.07
9	HTW/HTL	0.35	0.37	0.36	0.39	0.37	0.37	0.38	0.37	0.4
10	HBTW/HTW	0.71	0.74	0.8	0.7	0.73	0.71	0.64	0.74	0.46

AP- Arunachal Pradesh, AS- Assam, MN- Manipur, MG- Meghalaya, MZ- Mizoram, NG- Nagaland, SK- Sikkim, TR- Tripura, PT- Primary type of *Lepidotrigona arcifera*

Table 4: Eigen values and percentage of variance in different Principal Components in the analysis of stingless bees of the genus *Lisotrigona* from India

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative	Total	% of Variance	Cumulative
1	7.239	20.109	20.109	7.239	20.109	20.109	3.931	10.92	10.92
2	3.524	9.788	29.897	3.524	9.788	29.897	3.494	9.706	20.626
3	2.522	7.006	36.903	2.522	7.006	36.903	3.224	8.956	29.582
4	2.18	6.057	42.959	2.18	6.057	42.959	2.674	7.429	37.011
5	1.907	5.296	48.256	1.907	5.296	48.256	2.09	5.806	42.817
6	1.713	4.757	53.013	1.713	4.757	53.013	1.966	5.462	48.278
7	1.428	3.968	56.98	1.428	3.968	56.98	1.916	5.322	53.6
8	1.322	3.674	60.654	1.322	3.674	60.654	1.707	4.742	58.342
9	1.201	3.336	63.99	1.201	3.336	63.99	1.548	4.3	62.642
10	1.109	3.08	67.07	1.109	3.08	67.07	1.351	3.753	66.395
11	1.067	2.963	70.034	1.067	2.963	70.034	1.31	3.638	70.034
12	0.955	2.652	72.685						
13	0.898	2.494	75.179						
14	0.812	2.255	77.434						
15	0.794	2.204	79.638						
16	0.693	1.924	81.562						
17	0.657	1.826	83.388						
18	0.611	1.698	85.086						
19	0.558	1.55	86.636						
20	0.521	1.447	88.084						
21	0.458	1.271	89.354						
22	0.443	1.23	90.584						
23	0.414	1.149	91.734						
24	0.36	1.001	92.734						
25	0.348	0.966	93.7						
26	0.315	0.876	94.576						
27	0.299	0.832	95.408						
28	0.278	0.772	96.179						
29	0.238	0.662	96.842						
30	0.226	0.629	97.47						
31	0.204	0.568	98.038						
32	0.195	0.541	98.579						
33	0.166	0.462	99.041						
34	0.161	0.448	99.489						
35	0.1	0.278	99.768						
36	0.084	0.232	100						

Extraction Method: Principal Component Analysis.

(0.15 to 0.16 mm in diameter) than the median ocellus of the primary type (0.08 mm). Mandibles were 1.20 to 1.33 times wider (0.25 to 0.28 mm wide) than the primary type (0.21 mm). Bees from Arunachal Pradesh, Nagaland and Sikkim had longer and wider forewings (4.48 to 4.49 and 1.50 to 1.55 mm, respectively) while the bees from Assam had the short and narrow wings (3.67 and 1.35 mm, respectively). The diagonal width of the forewings varied from 1.23 to 1.28

mm in the bees from eight states while it was 1.33 mm in the primary type. Longest hind tibia and hind basitarsus was found in the bees from Nagaland (1.50 and 0.67 mm, respectively). Bees from Mizoram also had equally long hind basitarsus (0.67 mm) closely followed by the bees from Assam (0.66 mm). In the primary type of *L. arcifera* the length of hind tibia and hind basitarsus were 1.43 and 0.62 mm, respectively.

The ratios of different parts of the body of the bees from eight

Table 5: Rotated component matrix in Principal Component analysis of female stingless bees of the genus *Lisotrigona* from India

	Rotated Component Matrix										
	Component 1	2	3	4	5	6	7	8	9	10	11
HTL	0.744										
FWD	0.642						0.386				
SCL	0.622										
FWW	0.596				0.51						
HW	0.556			0.384				0.34			
MCL	0.549		0.369				0.326				
UIOD	0.512			0.352						0.332	
BL	0.511	0.433									
MNL	0.491	0.368							-0.376		
HL	0.435			0.301					0.346		
HBTW		0.761									
SCTW		0.644									
EL		0.615									
CLW		0.591		0.456	0.335				0.381		0.322
MSCW		0.54		0.309							
TFL			0.853								
SFL	0.385		0.783								
SCTL			0.626								
TFW			0.59								
OOD				0.746							
FL				0.727							
MSL		0.366		0.562		0.313					
MCW					0.675			0.328			
FWL					0.618						
MSCL						0.757					
SCW						0.699					
FFL			0.461		0.485	0.505					
MNW				0.397		0.492					-0.303
CLL							0.71		-0.374		
DMO							0.708				
IOD								0.678			
HBTL		0.445						0.48			
HTW	0.309	0.387						0.422		0.302	
EW									0.766		
HAM										0.767	
PTL											0.863

Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization.,a. Rotation converged in 22 iterations.

Table 6: Eigen values and Canonical correlations of different functions in Discriminant analysis of female stingless bees of the genus *Lisotrigona*

Function	Eigenvalues				Canonical
	Eigenvalue	% of Var	Cumu	Correlation	
1	7.543a	38	38	0.94	
2	4.124a	20.8	58.8	0.897	
3	2.754a	13.9	72.6	0.857	
4	2.040a	10.3	82.9	0.819	
5	1.520a	7.7	90.6	0.777	
6	1.355a	6.8	97.4	0.759	
7	.515a	2.6	100	0.583	

a. First 7 canonical discriminant functions were used in the analysis.

northeastern states in comparison to the primary type of *L. arcifera* as envisaged by Sakagami (1978) are presented in table 3. The ratio between length and width of head was low in the bees from eight states that ranged from 0.70 to 0.77 as against the ratio observed in the primary type (0.80). Similar low ratio was observed between width and length of hind tibia (0.35 to 0.39) in the bees of eight states compared to the primary type (0.40). Contrarily higher ratios between length of

Table 7: Wilks' Lambda significance in Discriminant analysis of female stingless bees of the genus *Lisotrigona* from India

Test of Function(s)	Wilks' Lambda			
	Wilks' Lambda	Chi-square	df	Sig.
1 through 7	0	832.593	252	0
2 through 7	0.002	620.223	210	0
3 through 7	0.01	458.464	170	0
4 through 7	0.037	327.493	132	0
5 through 7	0.111	217.42	96	0
6 through 7	0.28	125.916	62	0
7	0.66	41.105	30	0.085

hind tibia and width of head (0.80 to 0.82), length of hind tibia and diagonal width of forewing (1.10 to 1.19) and width of hind basitarsus and hind tibia (0.64 to 0.80) were recorded in the bees of eight states. The corresponding ratios in the primary type were 0.76, 1.07 and 0.46, respectively.

Principal Component analysis (PCA) of 122 female bees resulted in 11 components with Eigen values more than 1.00 which explained the variation among the *Lepidotrigona* bees to the extent of 70.03 per cent (Table 4). In the Principal Component 1, morphological parameters viz. HTL, FWD, SCL,

Table 8: Standardized Canonical Discriminant Function Coefficients in different functions in the Discriminant analysis of female stingless bees of the genus *Lisotrigona* from India

Structure Matrix	Function						
	1	2	3	4	5	6	7
SCTW	.427*	-0.091	0.221	0.102	0.128	-0.13	-0.169
HBTW	.226*	0.084	0.165	0.207	0.147	0.019	-0.025
SFL	-.223*	-0.172	0.075	0.072	-0.118	0.049	0.182
FFL	-0.15	.223*	0.069	0.03	-0.053	0.115	-0.012
SCTL	-0.121	.218*	0.004	0.106	0.04	-0.015	0.084
FWD	-0.003	-.203*	0.07	0.02	0.063	-0.121	0.111
HBTL	0.095	0.168	.386*	0.261	-0.031	0.21	0.135
MCW	-0.125	0.064	.375*	0.126	0.133	-0.126	0.022
HTL	-0.065	-0.128	.341*	0.149	-0.151	-0.05	0.091
MNL	-0.029	0.03	.253*	0.053	0.083	-0.012	0.092
HW	-0.118	-0.059	.247*	0.236	-0.08	-0.159	-0.029
MCL	-0.155	-0.083	.226*	-0.014	0.053	-0.141	0.025
FWW	-0.192	-0.109	.207*	-0.19	0.175	-0.023	0.073
BL	0.023	-0.012	.137*	0.012	0.13	0.098	-0.019
MSCW	0.007	0.122	0.092	.384*	0.044	-0.127	0.12
MSL	0.1	0.05	-0.059	.328*	-0.035	-0.231	-0.322
HTW	-0.057	0.107	0.224	.296*	0.063	-0.151	-0.088
MSCL	-0.125	0.063	0.07	.257*	-0.023	0.081	0.152
IOD	-0.07	-0.166	0.207	.242*	0.162	0.131	0.035
HL	0.002	-0.049	0.141	.241*	0.171	0.091	0.215
UIOD	-0.088	-0.125	0.117	.231*	0.058	-0.215	0.192
TFW	-0.031	0.011	0.011	.168*	0.094	0.006	-0.011
SCW	0.086	-0.003	0.153	0.113	-.356*	0.26	-0.215
CLW	0.082	0.173	0.059	0.198	.257*	-0.058	-0.194
FWL	-0.104	-0.047	0.157	-0.119	.188*	-0.17	0.027
DMO	0.006	-0.103	0.017	-0.05	.119*	0.066	-0.091
SCL	-0.038	-0.117	0.215	0.014	-0.217	-.257*	-0.007
MNW	-0.028	-0.07	0.003	0.09	-0.012	.111*	0.002
TFL	-0.217	-0.058	-0.011	0.047	-0.139	-0.02	.323*
FL	-0.003	0.043	-0.033	0.312	0.059	-0.195	.315*
EW	0.137	-0.009	-0.009	-0.033	-0.096	-0.118	.297*
CLL	-0.024	-0.087	0.057	0.089	0.15	-0.058	-.294*
EL	0.076	0.207	0.102	0.031	0.075	0.029	.254*
PTL	-0.041	-0.023	0.004	0.082	0.003	0.089	-.213*
OOD	-0.058	-0.122	-0.185	0.129	-0.029	-0.004	.188*
HAM	-0.03	-0.087	-0.036	0.028	0.074	-0.1	-.122*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions; Variables ordered by absolute size of correlation within function;*. Largest absolute correlation between each variable and any discriminant function

FWW, HW, MCL, UIOD, BL, MNL and HL had higher component loading that ranged from 0.435 TO 0.744 (Table 5) and all these parameters together contributed for 20.11% variation (Table 4). Principal component 2 included five parameters (HBTW, SCTW, EL, CLW and MSCW) which together influenced 9.79% variation. Both these components explained the variation to the extent of 29.90% cumulatively (Table 4). Scatter plot drawn by using regression factor score 1 and 2 (Fig 1) revealed the following five clusters.

Cluster 1: Bees from Sikkim

Cluster 2: Bees Arunachal Pradesh

Cluster 3: Bees from Arunachal Pradesh and Tripura

Cluster 4: Bees from Sikkim and Tripura

Cluster 5: Bees from all eight states

In CDA, six functions were extracted with Eigen values more than 1.00 which explained the variation to the extent of 97.40% (Table 6). Wilk's Lambda values were significant for all the extracted functions (Table 7). In the first function morphological parameters viz. SCTW, HBTW and SFL had the higher influence with loading factor that ranged from 0.223

to 0.427 (Table 8). FFL, SCTL and FWD were the morphological features that had the next higher loading factor values (0.203 to 0.223) in the second function. Both first and second functions together influenced the variation to the extent of 58.80%.

Results of CDA scatter plot drawn by using function 1 and 2 showed the following four clusters (Fig 2).

Cluster 1: Bees from Arunachal Pradesh

Cluster 2: Bees from Sikkim

Cluster 3: Bees from Tripura

Cluster 4: Bees from Assam, Manipur, Meghalaya, Mizoram and Nagaland

Classification and cross validation of results of CDA indicated that 98.40% grouped samples were correctly classified. In cross validation, 82.00% of grouped samples of bees were correctly classified (Table 9).

All the bees from eight northeastern states of India included in the present study belonged to *L. ventralis* group as the length of body and forewing was less than 5 mm (Rasmussen, 2013). *L. arcifera* is the only species reported from India so far from

Table 9: Classification and cross validation of results in Discriminant analysis of female stingless bees of the genus *Lisotrigona* from India

Classification Results ^{b,c}		State Code	Predicted Group Membership								Total
Original	Count	1	2	3	4	5	6	7	8		
		1	22	0	0	0	0	0	0	22	
		2	0	6	0	0	0	0	0	6	
		3	0	0	4	0	0	0	0	4	
		4	0	0	0	19	0	0	0	19	
		5	0	0	0	0	44	1	0	46	
		6	0	0	0	0	0	6	0	6	
		7	0	0	0	0	0	0	9	9	
		8	0	0	0	0	0	0	10	10	
	%	1	100	0	0	0	0	0	0	100	
		2	0	100	0	0	0	0	0	100	
		3	0	0	100	0	0	0	0	100	
		4	0	0	0	100	0	0	0	100	
		5	0	0	0	0	95.7	2.2	0	100	
		6	0	0	0	0	0	100	0	100	
		7	0	0	0	0	0	0	100	100	
		8	0	0	0	0	0	0	100	100	
Cross-Count validated ^a		1	17	0	0	4	0	1	0	22	
		2	0	4	0	0	1	0	0	6	
		3	0	0	4	0	0	0	0	4	
		4	1	0	0	15	1	2	0	19	
		5	0	0	0	3	38	4	0	46	
		6	0	0	0	2	0	4	0	6	
		7	0	0	0	0	0	0	9	9	
		8	0	0	0	0	0	1	0	10	
	%	1	77.3	0	0	18.2	0	4.5	0	100	
		2	0	66.7	0	0	16.7	0	0	100	
		3	0	0	100	0	0	0	0	100	
		4	5.3	0	0	78.9	5.3	10.5	0	100	
		5	0	0	0	6.5	82.6	8.7	0	100	
		6	0	0	0	33.3	0	66.7	0	100	
		7	0	0	0	0	0	0	100	100	
		8	0	0	0	0	0	10	0	100	

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case; b. 98.4% of original grouped cases correctly classified; c. 82.0% of cross-validated grouped cases correctly classified.

this group though Schwarz (1939) synonymized it with *L. flavibasis* (Cockerell) and Moure (1961) suggested synonymizing with *L. hoozana* (Strand). According to Rasmussen (2013) two more species of ventralis group namely *L. ventralis* and *L. flavibasis* are expected in the Indian subcontinent. However, all these species are differentiated based on relative morphological features like colour of the hairs and other body parts which show intraspecific variation making it difficult to identify the species (Rasmussen, 2013). Hence, we studied morphometry of *Lepidotrigona* bees and subjected the data by two methods of statistical analysis. Our results showed that all the 122 bees formed five clusters in PCA while in CDA they formed four distinct clusters. Hence, we conclude that Indian fauna of *Lepidotrigona* bees consists of 4 to 5 species of ventralis group including *L. arcifera*. Further detailed studies need to be focused on inclusion of both females and associated males in the description of these species based on absolute morphological characters rather than relative characters like coloration.

ACKNOWLEDGEMENTS

This study was funded by the Indian Council of Agricultural Research, New Delhi. We gratefully acknowledge the faculty members of the Department of Entomology of different State

Agricultural Universities, ICAR Institutes and Central Agricultural University, Imphal, for providing facilities in collection of bee samples. We also acknowledge Mr. Ravi Umadi, Dr. Manha Bathari and Dr. Malar Bui for sparing some samples for our study.

REFERENCES

- Francoy T. M, Bonatti, V., Viraktamath, S. and Rajankar, B.R. 2015. Wing morphometrics indicates two distinct phenotypic clusters within populations of *Tetragonula iridipennis* (Apidae: Meliponini) from India. *Insectes Sociaux*. **63**: 109-115.
- Halcroft M. T., Dollin, A., Francoy, T.M., King, J.E., Riegler, M., Haigh, A.M., Spooner-Hart, R.N. 2015. Delimiting the species within the genus *Austrolebeia*, an Australian stingless bee, using multiple methodologies. *Apidologie*.
- Heard, T.A. 1999. The role of stingless bees in crop pollination. *Annual Review of Entomology*. **44**:183-206.
- Michener, C. D. 2000. The bees of the world. Baltimore: Johns Hopkins University Press, P.913 .
- Moure, J. S. 1961. A preliminary supra-specific classification of the old world Meliponini bees (Hymenoptera, Apoidea). *Studia Entomologica*. **4**:181-242
- Rasmussen, C. 2013. Stingless bees (Hymenoptera: Apidae: Meliponini) of the Indian subcontinent: Diversity, taxonomy and current status of

knowledge. *Zootaxa* .**3647**: 401-428

Roubik, D. W. 1989. Ecology and natural history of tropical bees. Cambridge University Press, New York.

Ruttner, F. 1988. Biogeography and Taxonomy of honey bees. Springer-Verlag Berlin Heidelberg, P.284 .

Sakagami ,S. F. 1978. Tetragonula Stingless Bees of the continental Asia and Sri Lanka (Hymenoptera: Apidae). *J. the Faculty of Science Hokkaido University Series VI Zoology*. **21**: 165–247.

Schwarz, H.F. 1939. The Indo-Malayan species of *Trigona*. Bulletin of the American Museum of Natural History, **76**: 83–141.

Viraktamath, S. and Rojeet, T. 2021. Two new species of *Tetragonula* (Hymenoptera: Apidae: Meliponini) from north-east India with notes on their nest structure. *Biologia*

Viraktamath, S., Tanuja, N. and Sishira, D. 2021. Morphometry of female and associated male stingless bees of the genus *Tetragonula* (Hymenoptera: Apidae: Meliponini) from India. *The Bioscan*. **16**: 9-21.