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Morphometry of stingless bees (Hymenoptera: Apidae: Meliponini) of the genus *Lisotrigona* indicates presence of more than one species in India

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ABSTRACT: Morphometry of 53 stingless bees of the genus *Lisotrigona* collected from seven places in India by using 36 morphological parameters was studied. The data set also included morphometry data of primary types of *L. cacciae, L. chandrai* and *L. revanai* for comparison and was subjected to Factor and Canonical Discriminant analysis. All the bees collected from seven places formed two distinct clusters in the Factor analysis and five clusters in Canonical Discriminant analysis. In both the methods of analysis primary types of *L. cacciae, L. chandrai* and *L. revanai* were placed well separated from each other as well as from other bees. The bees from seven places also differed from the three known species in morphometry and ratios of length and width of parts of the body. Based on these results it is concluded that Indian stingless bees of the genus *Lisotrigona* consists of more than one species besides *L. cacciae*. The action of synonymizing *L. mohandasi, L. chandrai* and *L. revanai* with *L. cacciae* appears arbitrary; these three species should be considered valid until supported by male genital morphology or molecular characters.

KEYWORDS: Morphological parameters, *Lisotrigona* spp.*, factor analysis, canonical discriminant analysis*

INTRODUCTION

Indian stingless bees (Hymenoptera: Apidae: Meliponini) belong to three genera *Tetragonula* Moure, 1961*, Lepidotrigona* Schwarz, 1939 and *Lisotrigona* Moure, 1961 (Rasmussen, 2013; Viraktamath *et al*., 2020). The genus *Lisotrigona* includes tiny stingless bees (generally < 3 mm in length) and is quite rare. The genus is represented with four species in India *viz. L. cacciae* (Nurse, 1907), *L. mohandasi* Jobiraj and Narendran, 2004, *L. chandrai* Viraktamath and Sajan Jose, 2017 and *L. revanai* Viraktamath and Sajan Jose, 2017 distributed in Madhya Pradesh, Kerala and Maharashtra. Of the four species, three species (*L. cacciae, L. mohandasi* and *L. revanai*) are described based on the museum specimens while one species (*L. chandrai*) based on the detailed studies of males, females, queen and nest structure. No information is available on males, queens, nest structure and nesting habits about the other three species. Females of stingless bees are remarkably

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similar with very weak diagnostic characters. However, males and their genitalia have diagnostic characteristics which are species specific (Schwarz, 1939; Sakagami, 1978; Rasmussen, 2013; Attasopa *et al*., 2018). Hence Rasmussen (2013) and Attasopa *et al*. (2018) emphasized to make all out efforts to collect males and to include descriptions of both males and females while proposing new taxa.

As the diversity of Indian stingless bees is not fully known (Rasmussen, 2013), an intensive collection of stingless bees by surveying different parts of India was carried out. During our survey, we found feral colonies of *Lisotrigona* and collected series of samples of these bees from Maharashtra, Chhattisgarh and Mizoram. Recently Rasmussen *et al*., (2017) synonymized *L. mohandasi, L. revanai* and *L. chandrai* with *L. cacciae* without giving justification in spite of the fact that *L. chandrai* is described based on series of males and females. Since morphometry is one of the important tools to identify and delineate species in Meliponini which includes several cryptic and species complexes (Moure, 1961; Sakagami, 1978; Francoy *et al*., 2015; Halcroft *et al*., 2015), an exhaustive study on the morphometry of *Lisotrigona* bees was taken up to find - whether the genus *Lisotrigona* includes only one species (*L. cacciae*) or more in India?

MATERIALS AND METHODS

During our survey to collect stingless bees from different parts of India, we collected *Lisotrigona* bees in six places namely Darbha (18.85° N, 81.8689° E), Bhanupratappur (20.30^0 N, 81.07^0 E), Barnawapara (20.45^0 N, 81.97^0 E) (Chhattisgarh), Yavatmal (20.38° N, 78.13° E), Umred (20.84° N, 79.32° E) (Maharashtra) and Thenzawl (23.29^0 N, 92.75^0 E) (Mizoram) during 2019-2020 from their foraging sources. However, in Darbha and Barnawapara the samples were collected directly from their colonies. All the samples were collected in a specimen tube containing about 1 ml ethyl acetate absorbed in a cotton wad. Later the bees were transferred to a vial containing 95 per cent ethyl alcohol and labeled indicating the place and date of collection. All the samples were examined in the Systematic laboratory at the Department of Entomology, University of Agricultural Sciences, Bengaluru, under a Leica stereoscopic microscope (Model: M205C) to confirm the identity of the genus by using key characters enumerated by Rasmussen (2013). There were altogether 53 bees from seven places in the studies, including five bees of *Lisotrigona* found in the old collection of the Department of Entomology, UAS Bengaluru and Zoological Survey of India, Kolkata collected in Raipur (Chhattisgarh).

Thirty-six morphological parameters (modified from Sakagami, 1978; Rasmussen, 2013) were selected for morphometry studies (Table 1). The parameters that included various body parts of the head, thorax and abdomen were measured under the Jenco stereoscopic binocular microscope (Model: ZM-H-602) fitted with an ocular micrometer. The number of hamuli on the right-wing were counted. All the measurements were expressed in millimeters. Mean and standard deviation was calculated for each parameter. Ratios of measurement of different parts of the body as used by Sakagami (1978) were calculated. We also included morphometry data of primary type specimens of *L. chandrai, L. revanai* (Viraktamath and Sajan Jose, 2017) and *L. cacciae* (Rasmussen, 2013) in the analysis for comparison. All the data were subjected to $\log_{10}$ transformation before further analysis (Bookstein, 1985).

Two methods of statistical analysis by using SPSS software (version 16) were adopted to identify discrete morphological groups of bees from these seven places. The data were first subjected to Factor analysis which included analysis of variation, Principal Component analysis (PCA) on a correlation matrix of 30 measured variables. Six variables like DMO, FFl, SFL. TFL, TFW and HAM were not included as the variations among the samples were negligible. Sampling adequacy was verified by Kaiser-Meyer-Olkin measure and Bartlett's test of sphericity. Further, the variables were subjected to Varimax rotation with Kaiser normalization. Morphological groups were identified through a scatter plot by using regression factor score 1 and factor score 2. The second method of analysis was stepwise Canonical Discriminant

Table 1. Morphological parameters selected for morphometry and their abbreviations

Morphological Parameter	Abbreviation
Length of body	BL
Width of head including compound eyes	HW
Length of head	HL
Length of compound eye	EL
Width of compound eye	EW
Upper inter-orbital distance	UIOD
Diameter of median ocellus	DMO
Inter-ocellar distance	IOD
Ocello-ocular distance	OOD
Length of clypeus	CLL
Maximum width of clypeus	CLW
Length of malar space	MSL
Length of scape	SCL
Width of scape	SCW
Length of pedicel + flagellum	FL
Length of first flagellar segment	FFL
Length of second flagellar segment	SFL
Length of third flagellar segment	TFL
Width of third flagellar segment	TFW
Length of mandible	MNL
Width of mandible	MNW
Length of forewing	FWL
Width of forewing	FWW
Length of pterostigma	PTL
Length of marginal cell	MCL
Width of marginal cell	MCW
Diagonal length of forewing	FWD
Number of hamuli	HAM
Length of mesoscutum	MSCL
Maximum width of mesoscutum	MSCW
Length of mesoscutellum	SCTL
Maximum width of mesoscutellum	SCTW
Length of hind tibia	HTL
Width of hind tibia	HTW
Length of hind basitarsus	HBTL
Width of hind basitarsus	HBTW

analysis (CDA) by using all the 36 observed variables. A scatter plot was prepared by using the first two Discriminant Functions to study the clustering of samples.

RESULTS

Detailed morphometry of the stingless bees from seven places in comparison with three known species is presented in Table 2. *Lisotrigona* bees from Darbha and Barnawapara were larger with a body length of 3.10 mm as against the body length of 2.78, 2.95 and 2.58 mm in *L. chandrai, L. cacciae* and *L. revanai*, respectively. The body length of the bees varied from 2.71 to 3.02 mm in the remaining five places. The head width (including compound eyes) of the bees from Darbha was very close (1.18 mm) to that *L. cacciae* and *L. chandrai* (1.19 mm). While the head width of the bees from other places varied from 1.10 to 1.15 mm. The head length was greater in the bees from Darbha (0.94 mm) while it varied from 0.88 to 0.91 mm in the remaining six places. Head length was 0.77, 1.01, and 0.90 mm in *L. chandrai, L. cacciae* and *L. revanai,* respectively. Among the bees collected from seven places, longer forewings (2.65 mm) were recorded in the bees from Bhanupratappur while *L. chandrai* had the longest forewings (2.71 mm) among the three known species. In *L. cacciae* the forewings measured 2.65 mm. Similarly, the diagonal length of the forewing (FWD) was greater in the bees from Bhanupratappur (0.75 mm) while in the known species it was 0.70, 0.73 and 0.70 mm in *L. chandrai, L. cacciae* and *L. revanai*, respectively. The lowest diagonal length of the forewing (0.64 mm) was observed in the bees from Barnawapara. The longest hind tibia was recorded in the bees from Darbha (0.89) as compared to *L. chandrai* (0.84 mm), *L. cacciae* (0.86 mm) and *L. revanai* (0.86 mm).

The ratios of different parts of the body of the bees from seven places in comparison with the three known species are presented in Table 3. The ratio of length and width of head (HL/HW) was highest in *L. cacciae* (0.85) and lowest in *L. chandrai* (0.65). In the bees from seven places, the ratio varied from 0.77 to 0.82. On the other hand, the ratio of the length of scape and eye (SCL/EL) was the lowest in *L. cacciae* (0.45) and highest in the bees from Thenzawl (0.59). The ratio of width and length of the hind tibia (HTW/HTL) was higher in the bees from seven places (0.39 to 0.44) compared to the ratio observed in *L. chandrai* (0.39), *L. cacciae* (0.36) and *L. revanai* (0.34). Differences in the ratios of other body parts were not apparent between the freshly collected and the three known species.

Table 2. Morphometry of female stingless bees of the genus *Lisotrigona* from India (Mean in mm ± Standard deviation)

No	Parameter Place	DB N: 10	BPN: 01	BW N: 05	RP N: 04	YM N: 10	UR N: 10	TZ N: 10	*L chandrai**	*L cacciae***	*L. revanai**
1	BL	3.10 ±0.14	2.85	3.10 ±0.19	3.02 ±0.02	2.92 ±0.21	2.74 ±0.10	2.71 ±0.06	2.78	2.95	2.58
2	HW	1.18 ±0.02	1.15	1.14 ±0.01	1.10 ±0.00	1.11 ±0.03	1.10 ±0.01	1.15 ±0.04	1.19	1.19	1.14
3	HL	0.94 ±0.01	0.88	0.91 ±0.02	0.88 ±0.02	0.91 ±0.02	0.89 ±0.01	0.89 ±0.03	0.77	1.01	0.90
4	EL	0.84 ±0.02	0.85	0.82 ±0.02	0.81 ±0.01	0.81 ±0.04	0.77 ±0.02	0.81 ±0.03	0.83	0.83	0.88
5	EW	0.34 ±0.01	0.33	0.29 ±0.01	0.28 ±0.00	0.30 ±0.01	0.28 ±0.01	0.31 ±0.02	0.35	0.33	0.28
6	UIOD	0.75 ±0.02	0.75	0.73 ±0.02	0.74 ±0.03	0.72 ±0.02	0.73 ±0.02	0.75 ±0.02	0.76	0.75	0.78
7	DMO	0.11 ±0.01	0.12	0.11 ±0.01	0.10 ±0.01	0.10 ±0.00	0.12 ±0.00	0.10 ±0.01	0.09	0.11	0.11
8	IOD	0.28 ±0.00	0.28	0.27 ±0.01	0.27 ±0.01	0.26 ±0.01	0.25 ±0.01	0.27 ±0.01	0.26	0.27	0.29
9	OOD	0.14 ±0,01	0.14	0.14 ±0.01	0.14 ±0.01	0.15 ±0.01	0.14 ±0.01	0.16 ±0.01	0.18	0.18	0.18
10	CLL	0.26 ±0,01	0.25	0.24 ±0.01	0.25 ±0.00	0.25 ±0.01	0.25 ±0.01	0.24 ±0.01	0.25	0.24	0.28
11	CLW	0.55 ±0.01	0.55	0.57 ±0.01	0.52 ±0.01	0.49 ±0.01	0.51 ±0.02	0.56 ±0.02	0.58	0.42	0.39
12	MSL	0.01 ±0.00	0.01	0.02 ±0.00	0.05 ±0.00	0.01 ±0.00	0.02 ±0.00	0.04 ±0.01	0.03	0.02	0.03
13	SCL	0.42 ±0.01	0.45	0.41 ±0.01	0.42 ±0.02	0.41 ±0.01	0.39 ±0.01	0.41 ±0.01	0.49	0.37	0.47
14	SCW	0.08 ±0.00	0.08	0.08 ±0.00	0.10 ±0.01	0.07 ±0.01	0.07 ±0.00	0.07 ±0.01	0.08	0.07	0.07
15	FL	0.89 ±0.04	0.90	0.92 ±0.03	0.81 ±0.03	0.85 ±0.04	0.87 ±0.02	0.85 ±0.01	0.92	—	0.91
16	FFL	0.06 ±0.00	0.06	0.05 ±0.01	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01	0.06 ±0.01	0.08	0.06	0.08
17	SFL	0.07 ±0.00	0.07	0.06 ±0.01	0.09 ±0.01	0.06 ±0.00	0.06 ±0.01	0.05 ±0.00	0.07	0.06	0.08
18	TFL	0.08 ±0.01	0.08	0.06 ±0.01	0.08 ±0.01	0.06 ±0.00	0.06 ±0.01	0.05 ±0.00	0.07	0.07	0.09
19	TFW	0.10 ±0.00	0.10	0.26 ±0.03	0.09 ±0.01	0.10 ±0.00	0.10 ±0.00	0.09 ±0.01	0.10	0.11	0.10
20	MNL	0.43 ±0.02	0.50	0.42 ±0.02	0.43 ±0.01	0.43 ±0.02	0.44 ±0.01	0.47 ±0.00	0.48	0.45	0.45
21	MNW	0.18 ±0.00	0.22	0.18 ±0.01	0.18 ±0.01	0.18 ±0.00	0.16 ±0.01	0.18 ±0.00	0.16	0.12	0.16
22	FWL	2.59 ±0.05	2.65	2.48 ±0.08	2.52 ±0.02	2.50 ±0.06	2.44 ±0.04	2.63 ±0.07	2.71	2.65	2.69
23	FWW	0.97 ±0.03	0.95	0.83 ±0.03	0.91 ±0.02	0.87 ±0.03	0.90 ±0.04	0.94 ±0.05	1.00	0.95	1.04
24	PTL	0.45 ±0.01	0.45	0.41 ±0.01	0.44 ±0.01	0.40 ±0.01	0.44 ±0.01	0.45 ±0.01	0.41	0.36	0.40
25	MCL	0.77 ±0.02	0.73	0.75 ±0.00	0.75 ±0.01	0.73 ±0.02	0.75 ±0.01	0.79 ±0.01	0.78	0.89	0.77
26	MCW	0.19 ±0.01	0.22	0.21 ±0.01	0.18 ±0.01	0.18 ±0.01	0.20 ±0.01	0.20 ±0.01	0.18	0.18	0.17
27	FWD	0.74 ±0.01	0.75	0.64 ±0.01	0.69 ±0.01	0.65 ±0.02	0.66 ±0.02	0.72 ±0.02	0.70	0.73	0.70
28	HAM	5.00 ±0.00	5.00	5.00 ±0.00	5.00 ±0.00	5.00 ±0.00	5.00 ±0.00	5.10 ±0.03	5.00	6.00	5.00
29	MSCL	0.77 ±0.02	0.75	0.70 ±0.01	0.70 ±0.04	0.71 ±0.02	0.69 ±0.02	0.71 ±0.01	0.60	0.71	0.60
30	MSCW	0.92 ±0.02	0.95	0.88 ±0.02	0.87 ±0.02	0.88 ±0.02	0.88 ±0.03	0.91 ±0.02	0.90	0.93	0.90
31	SCTL	0.25 ±0.01	0.25	0.24 ±0.01	0.24 ±0.01	0.21 ±0.01	0.21 ±0.01	0.25 ±0.00	0.23	0.23	0.28
32	SCTW	0.75 ± 0.01	0.75	0.75 ±0.04	0.63 ±0.02	0.74 ±0.03	0.72 ±0.02	0.78 ±0.04	0.45	0.48	0.64
33	HTL	0.89 ±0.02	0.85	0.85 ±0.04	0.73 ±0.02	0.82 ±0.02	0.83 ±0.02	0.88 ±0.03	0.84	0.86	0.86
34	HTW	0.35 ±0.01	0.37	0.34 ±0.01	0.32 ±0.01	0.32 ±0.01	0.34 ±0.01	0.35 ±0.01	0.33	0.31	0.29
35	HBTL	0.44 ±0.01	0.45	0.46 ±0.02	0.42 ±0.03	0.45 ±0.01	0.45 ±0.00	0.45 ±0.01	0.42	0.35	0.38
36	HBTW	0.21 ±0.01	0.23	0.23 ±0.02	0.21 ±0.01	0.20 ±0.03	0.21 ±0.01	0.22 ±0.01	0.23	0.18	0.21

DB: Darbha; BH: Bhanupratappur; BW: Barnawapara; RP: Raipur; YM: Yavatmal; UR: Umred; TZ:Thenzawl

*L cacciae*** - Primary Type; *L chandrai** ; *L cacciae**; *L. revanai** (N: 10)

The validity of PCA of the 30 morphological parameters was justified as the KMO measure of sampling adequacy was 0.661 and Bartlett's test of sphericity were significant (<0.001). The PCA of the bees from seven places and three known species extracted seven components with Eigen value of more than 1.00 which altogether explained the variation to the extent of 75.17 percent. In the Principal Component 1, morphological characters like FWD, FWL, FWW, SCTL, UIOD, MCL, HW, OOD, MSCW, MNL, HTL and IOD had significantly higher loading factors that ranged from 0.494 to 0.877. All these parameters together accounted for 25.51 percent variation. In Principal Component 2, seven morphological parameters had significantly higher loading factors which ranged from 0.573 to 0.832 and influenced the variation to the extent of 14.26 percent. Both these two components together accounted for 39.77 percent variation.

The scatter plot drawn by using Regression Factor score 1 and 2 resulted in the formation of the following two major clusters (Fig. 1).

Cluster 1. Bees from Barnawapara + Raipur + Yavatmal + Umred

Cluster 2. Bees from Darbha + Bhanupratappur + Thenzawl

Three known species (*L. chandrai, L. cacciae* and *L. revanai*) were placed well separated indicating their distinctiveness from each other.

In the Canonical Discriminant analysis (CDA), eight functions were extracted with Eigen value more than 1.00 which together influenced 99.3 percent variation. Wilk's lambda values for all these eight functions were significant (<0.001). In the first function morphological parameters like MSCL, UIOD, FL, EW, HW, HBTL and MNW had higher

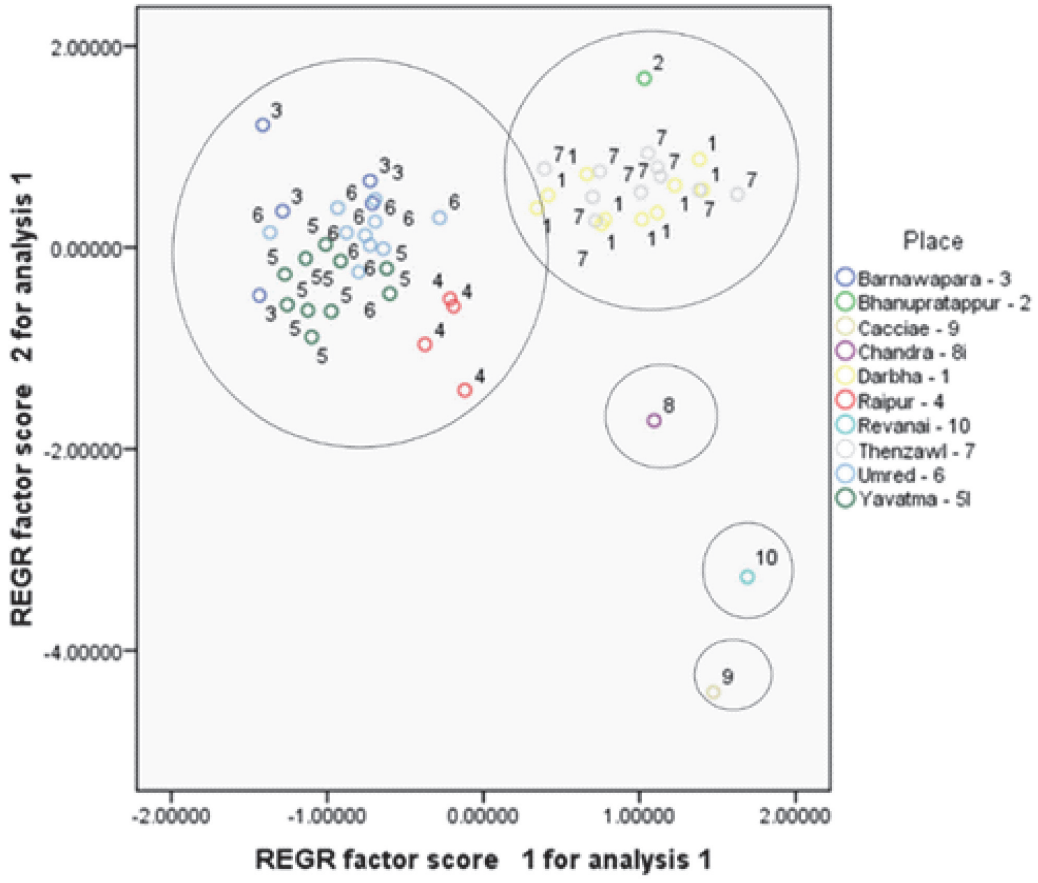

Fig. 1. Factor analysis scatter plot showing clusters of female stingless bees of the genus *Lisotrigona* from India

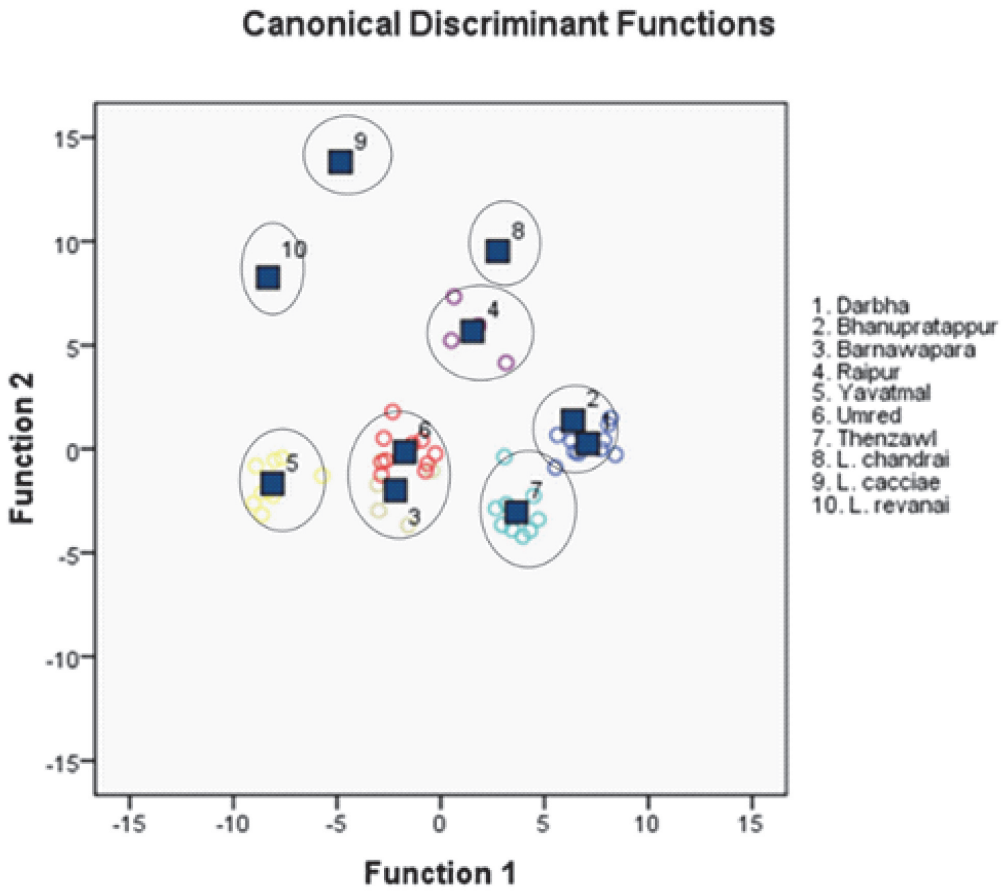

Fig 2. Discriminant analysis scatter plot showing clusters of female stingless bees of the genus *Lisotrigona* from India

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

SN	Parts of body	DB	BP	BW	RP	YM	UR	TZ	*L. chandrai*	*L. cacciae*	*L. revanai*
1	HL/HW	0.79	0.77	0.79	0.80	0.82	0.81	0.77	0.65	0.85	0.79
2	EL/UIOD	1.12	1.13	1.12	1.19	1.13	1.05	1.08	1.09	1.11	1.13
3	IOD/UIOD	0.37	0.37	0.37	0.36	0.36	0.34	0.36	0.34	0.36	0.37
4	SCL/EL	0.50	0.53	0.50	0.52	0.51	0.51	0.51	0.59	0.45	0.53
5	FWL/FWW	2.67	2.79	2.98	2.77	2.87	2.71	2.79	2.71	2.79	2.59
6	FWD/HW	0.63	0.65	0.56	0.63	0.59	0.60	0.63	0.59	0.61	0.61
7	HTL/HW	0.75	0.74	0.75	0.66	0.75	0.75	0.76	0.71	0.72	0.75
8	HTL/FWD	1.20	1.13	1.33	1.06	1.26	1.26	1.22	1.20	1.18	1.23
9	HTW/HTL	0.39	0.44	0.40	0.44	0.39	0.41	0.40	0.39	0.36	0.34
10	HBTW/HTW	0.48	0.51	0.50	0.50	0.44	0.47	0.49	0.55	0.51	0.55

DB: Darbha; BH: Bhanupratappur; BW: Barnawapara; RP: Raipur; YM: Yavatmal; UR: Umred; TZ:Thenzawl

Table 4. Comparison of morphometry of three recently described species of *Lisotrigona* from India with that of *Lisotrigona cacciae*

Parameter	*Lisotrigona mohandasi*[*]	*Lisotrigona revanai*[**]	*Lisotrigona chandrai*[**]	Lectotype - *Lisotrigona cacciae*[***] (India)	*Lisotrigona cacciae* - southeast Asia[****]
Length of body	3.00	2.58	2.78	2.95	3.06-3.64
Width of head including eyes	1,28	1,14	1.19	1,19	1.0-1.25
Ratio between length and width of head	2.34	2.26	2.34	2.48	--
Length of head (Clypeal apex-posterior margin of vertex)	--	0.99	0.77	1.01	0.98-1.13
Length of compound eye	--	0.88	0.83	0.83	0.80-0.90
Width of compound eye	--	0.28	0.35	0.33	--
Upper interorbital distance	--	0.78	0.76	0.75	0.73-0.80
Maximum interorbital distance	--	0.82	0.79	0.85	0.78-0.88
Lower interorbital distance	--	0.68	0.61	0.65	0.63-0.70
Diameter of median ocellus	--	0.11	0.09	0.11	--
Interocellar distance	--	0.29	0.26	0.27	0.21-0.28
Ocello-orbital distance	0.20	0.18	0.18	0.18	0.13-0.23
Length of clypeus	--	0.28	0.25	0.24	--
Maximum width of clypeus	--	0.39	0.38	0.42	--
Inter-tentorial distance	--	0.28	0.26	0.36	--
Clypeo-ocellar distance	--	0.74	0.68	0.06	--
Length of malar space	--	0.03	0,03	0.02	--
Length of antennae	--	1.38	1.41	--	--
Length of scape	0.20	0.47	0.49	0.37	0.33-0.40
Diameter of scape	--	0.07	0.08	0.07	--
Ration between length and diameter of scape	2.86	6.71	6.13	5.29	--
Diameter of third flagellomere	--	0.10	0.10	0.11	--
Length of first flagellomere	--	0.08	0.08	0.06	0.08
Length of second flagellomere	--	0,08	0.07	0.06	0.08
Length of third flagellomere	--	0.09	0.07	0,07	--
Length of mandible	--	0.45	0.48	0.45	--
Width of mandible	--	0.16	0.16	0.12	--
Ratio between length and width of mandible	--	2,81	3,00	3.75	--
Parameter	*Lisotrigona mohandasi*[*]	*Lisotrigona revanai*[**]	*Lisotrigona chandrai*[**]	Lectotype of *Lisotrigona cacciae*[***](India)	*Lisotrigona cacciae* from southeast Asia[****]
Length of proboscis	--	1.19	1.18	--	--
Length of forewing excluding tegula	--	2.49	2.51	2.37	--
Length of forewing including tegula	2,60	2.69	2.71	2.65	--
Width of forewing	--	1.04	1.00	0.95	--
Ratio of forewing length including tegula and width of forewing	--	2.59	2.71	2.79	--

Length of pterostigma	--	0.40	0.41	0.36	--
Width of pterostigma	--	0.10	0.10	0.12	--
Ratio between length and width of pterostigma	--	4.00	4.10	3.00	--
Length of marginal cell	--	0.77	0.78	0.89	--
Width of marginal cell	--	0.17	0.18	0.18	--
Ratio of length and width of marginal cell	--	4.53	4,33	4.94	--
Hamuli	--	5.40	5.00	6	--
Length of mesoscutum	--	0.60	0.60	0.71	--
Width of mesoscutum	--	0.90	0.90	0.93	--
Length of scutellum	--	0.28	0.23	0.23	--
Width of scutellum	--	0.64	0.45	0.48	--
Length of femur III	--	0.66	0.68	--	--
Length of tibia III	--	0.86	0.84	0.86	--
Width of tibia III	--	0.29	0.33	0.31	--
Ratio of length of tibia III and width	--	2.96	2.54	2.77	--
Length of basitarsus III	--	0.38	0.42	0.35	--
Width of basitarsus III	--	0.21	0.23	0.18	--
Ratio of length and width of basitarsus III	--	1.81	1.83	1.94	--
Ratio of length of basitarsus III and head width	--	0.33	0.35	0.29	--
Width of tergum III	--	--	--	1.05	--
Length of hairs on clypeus	--	0.02	0.02	<0.01	--
Length of hairs on frons	--	0.04	0.04	0.02	--
Length of hairs on the vertex	--	--	--	0.04	--
Length of hairs on scutellum apex	--	0.12	0.09	0.13	--

* Data from Jobiraj and Narendran (2004)
** Data from Viraktamath and Sajan Jose (2017)
*** Data from Lectotype (Rasmussen, 2013)
****Data from Engel (2000)

loading factors ranging from 0.211 to 0.353 which accounted for 43.8 percent variation. Results of CDA scatter plot drawn by using Canonical Discriminant function 1 and 2 segregated the bees from seven places into the following five distinct clusters.

Cluster 1. Bees from Darbha + Bhanupratappur

Cluster 2. Bees from Barnawapara + Umred

Cluster 3. Bees from Raipur

Cluster 4. Bees from Yavatmal

Cluster 5. Bees from Thenzawl

As observed in PCA, *L. chandrai, L. cacciae* and *L. revanai* were placed well separated from each other confirming their distinctiveness from other bees (Fig. 2). Classification of the bees into various clusters by the CDA indicated that 98.10 percent of original grouped cases and 90.60 percent of cross-validated grouped cases were correctly classified.

DISCUSSION

Stingless bees of the genus *Lisotrigona* are distributed in India, Sri Lanka and southeast Asia. The genus altogether includes six species namely

L. carpenteri Engel 2000, *L. furva* Engel 2000, *L. cacciae, L. mohandasi, L. chandrai* and *L. revanai.* Among these, *L. carpenteri* is the most distinctive species with yellow maculation on the face and a body length > 4.00 mm (Engel, 2000). However, *L. furva* and *L. cacciae* which are sympatric, were difficult to identify because of overlapping coloration, pilosity and morphometry (Michener, 2007). *L. cacciae* is the only species with a wide range of distribution occurring in India, Sri Lanka, Malaysia, Thailand, Cambodia and Vietnam (Engel, 2000; Michener, 2007; Karunaratne *et al*., 2017). Only two species (*L. furva* and *L. chandrai*) are known with both females and males and the male genitalia are quite distinct from each other (Michener, 2007; Viraktamath and Sajan Jose, 2017). *L. cacciae* is the first species of the genus described by Nurse in 1907 with Hoshangabad, India as the type locality (Rasmussen 2013). But the males of this species are unknown so far. The body length of the primary type of *L. cacciae* from India is 2.95 mm (Rasmussen, 2013) but the bees from southeast Asia measure from 3.06 to 3.64 mm (Engel 2000). Three species described from India have a body length of 3.00 mm (*L. mohandasi*), 2.58 mm (*L. revanai*) and 2.78 mm (*L. chandrai*) (Jobiraj and Narendran, 2004; Viraktamath and Sajan Jose, 2017). Similarly, the head width, head length, forewing length, forewing diagonal length and hind tibial length differ among these species. In spite of these and other differences (Table 4), Rasmussen *et al*. (2017) synonymized *L. mohandasi*, *L. chandrai* and *L. revanai* with *L. cacciae* without giving justification. The decision was probably based on similarities in coloration among these species. The variation among these by selecting 36 morphological parameters and compared them with the primary types of *L. cacciae, L. chandrai* and *L. revanai,* indicated that results of CDA were more robust than those of Factor analysis. In both the methods of analysis freshly collected bee samples and all the three known species segregated into distinct clusters. In Factor analysis bees from seven places formed two distinct clusters while in CDA they formed five clusters. All three known species (*L. cacciae, L. chandrai* and *L. revanai*) were placed well separated from each other as well as from other bees in both Factor and CDA. These results indicate that more than one species of *Lisotrigona* (other than *L. cacciae*) exist in India. There would have been a single compact cluster in both Factor and CDA if there was only one species. Hence, the action of Rasmussen *et al*. (2017) needs to be considered as arbitrary until supported by male genital morphology or molecular characters.

There is a need to collect males of *L. cacciae* from the areas of its currently known distribution and make comparative studies of male genitalia and sternal characters as they have clear diagnostic characters as reported by Schwarz (1939), Sakagami (1978), Rasmussen (2013) and Attasopa *et al.*(2018). In addition, molecular sequences of *L. cacciae* collected from different places need to be studied and compared. Until these results are available, it is proposed that all the described species of *Lisotrigona* from India (*L. mohandasi, L. chandrai* and *L.* revanai) should be treated as valid species.

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