

TWO NEW SPECIES OF *LISOTRIGONA* MOURE (HYMENOPTERA: APIDAE: MELIPONINI) FROM INDIA WITH NOTES ON NEST STRUCTURE

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ABSTRACT

Lisotrigona rewanai and *L. chandrai* have been described as new species along with notes on nest structure of *L. chandrai*. *L. rewanai* measures 2.58 mm in length and 1.14 mm in head width while *L. chandrai* 2.78 and 1.19 mm, respectively. Forewing is smaller in *L. rewanai* (2.49 mm) than *L. chandrai* (2.51 mm). Ocelli are transparent white but reddish brown to dark brown in *L. chandrai*. Apical margin of clypeus is flat in *L. rewanai* while gently concave in *L. chandrai*. Male and queen of *L. rewanai* are unknown. Male of *L. chandrai* measures 3.01 mm in length and 1.18 mm in head width. Male gonocoxites are strongly curved medially. The queen measures 4.24 mm in length and 1.18 mm in head width. *L. chandrai* nests in hard laterite rocks, foundation walls, and tree cavities in Kerala with short entrance tube. The brood is in cluster form surrounded by pollen and honey storage pots on either side. A key for identification of known species of *Lisotrigona* is presented. These two new species are the smallest among the known species of *Lisotrigona* from India.

INTRODUCTION

Stingless bees are closely related to the honey bees, bumble bees and orchid bees and are the oldest highly eusocial bees. Fossils of bees preserved in amber are 80 million years old (Grimaldi *et al.*, 1995; Michener and Grimaldi, 1988). They are distributed in all the tropical and subtropical areas of Afrotropical, Australasian, Indo-Malayan and Neotropical regions of the world (Ruttner, 1988; Roubik, 1989; Michener, 2000). Like honey bees, they are highly social, with morphologically and physiologically distinct queens and workers. They build nests in a variety of tree hollows and ground cavities (Ruttner, 1988).

Rearing of stingless bees (meliponiculture) has an ancient history in Mayan culture. Natives of Mayans have practiced meliponiculture for thousands of years. The traditional Mayan name for this bee is *Xunan kab*, literally meaning "lady bee". The bees were once the subject of religious ceremonies and were a symbol of the bee god *Ah-Muzen-Cab*, who is known from the *Madrid Codex* (Cortopassi-Laurino *et al.*, 2006). Meliponiculture is traditionally practiced in India for centuries (Kumar *et al.*, 2012; Rasmussen, 2013).

Stingless bees yield from a few to 5000 grams of honey annually, depending on the species (Michener, 2000). However, their honey is claimed to have very high medicinal value (Cortopassi-Laurino *et al.*, 2006). These bees also play an important role in pollination of several species of cultivated crops, wild and forest plants. Heard (1999) reviewed the

importance of these bees as pollinators of tropical crops. Nine species of crops are confirmed as effectively pollinated by stingless bees while they contribute to the pollination of nearly 60 other crops.

More than 600 species of stingless bees are described in over 60 genera (Wille, 1983; Rasmussen and Cameron, 2010). In India, the most common and widely distributed species of stingless bee is reported to be *Trigona iridipennis* (Sakagami, 1978; Ramanujam *et al.*, 1993; Raakhee and Devanesan 2000; Swaminathan, 2000; Roopa, 2002; Roubik, (personal communication). Rasmussen and Cameron (2007) based on the molecular phylogenetic studies recommended that the generic name *Trigona* be applied only to the Neotropical taxa while the name *Tetragonula* be applied for the taxa of Indo-Malayan/Australasian region. Recently Rasmussen (2013) summarized the information of eight described species of stingless bees from Indian subcontinent along with morphometric data of the type specimens as well as photographic documentation of their habitus.

Among more than 60 genera of stingless bees, the genus *Lisotrigona* includes minute stingless bees with body length varying from 2.50 to 3.00 mm; short linear malar space; converging inner eye margins; greatly reduced wing venation (Michener, 2000). This genus was described by Moure (1961) with *Melipona cacciae* Nurse as the type species. The genus is distributed in Sri Lanka, India, Vietnam, Borneo, Sumatra, and China (D. W. Roubik, personal communication). Engel (2000) added two species, *L. carpenteri* Engel and *L. furva*

Engel and revised the genus from Indo-Malayan region. Jobiraj and Narendran (2004) described *L. mohandasi* Jobiraj and Narendran and provided revised key to the world species of *Lisotrigona*. Chinh *et al.* (2005) gave nest and colony characteristics of *L. carpenteri* from Vietnam. Michener (2007) described the male genitalia and provided justification for the generic rank to *Lisotrigona*. Rasmussen and Cameron (2007) have discussed the phylogenetic relationships among stingless bees including *Lisotrigona* based on molecular studies. Rasmussen (2008) included four species of *Lisotrigona* in the catalogue of Indo Malayan and Australasian stingless bees. Peter *et al.* (2014) reported molecular characteristics of one unknown species of *Lisotrigona* from Kerala.

Rasmussen (2013) reported that full diversity of stingless bees in Indian subcontinent remains unknown because of the lack of morphological structure of male genitalia and molecular data. Hence a survey was undertaken to study the diversity of stingless bees from different parts of India. During this survey, we collected two species of *Lisotrigona* which were found to be new to science and the same are described in this paper along with male genitalia and nest structure for one species.

MATERIALS AND METHODS

The standard parameters adopted to study morphometry were as per the methods used by Sakagami (1978), Ruttner (1988), Rasmussen (2013) and Viraktamath (2015) with slight modifications. Forty-eight morphological parameters and nine ratios of different body parts (Table 1) were used to study the detailed morphometry of worker bees. However, 28 parameters and 13 ratios of body parts were used for males and queen (Table 3). All the measurements were taken by Leica stereoscopic microscope (model M-165 C) with Leica Application Suit (version 3.01) measurement software. Morphometry data pertaining to the lectotype of *L. cacciae* provided by Rasmussen (pers. Communication and Rasmussen, 2013) was used for comparison. Similarly, morphometry data of *L. mohandasi* (Jobiraj and Narendran, 2004) in respect of five parameters was also used.

Male genitalia were studied by dissecting two male specimens under the Leica stereoscopic microscope and photographed with the digital Leica camera attached to the microscope.

Behavior of bees (*L. chandrai*) was observed in four wild colonies and detailed nest structure was observed by breaking open two feral colonies nested in crevice of a laterite rock.

The type depositories of the new species are abbreviated as follows

- IARID : Department of Entomology, Indian Agricultural Research Institute, New Delhi
 UASB : Department of Entomology, University of Agricultural Sciences, Bengaluru
 ZSIK : Zoological Survey of India, Kolkata

RESULTS AND DISCUSSION

Lisotrigona revanai sp. nov. (Fig. 1 a-c)

Coloration

Clypeus brown with subapical black transverse border; frons,

vertex, gena, postgena black; ocelli distinctly transparent white; transverse indistinct groove just anterior to median ocellus followed by a distinct Y shaped furrow, the divergent arms surrounding antennal bases and extending up to clypeus; eyes reddish brown to fuscous; scape ochraceous with apical quarter dark brown; pedicel dark brown, flagellum black mottled with ochraceous round minute spots throughout. Mandibles ochraceous except basal 1/3 black; proboscis brown at basal half and ochraceous at apical half; labial and maxillary palps ochraceous. Thorax black and shiny; tegulae reddish brown. wings hyaline, iridescent, with light brownish veins; pterostigma lightly pigmented ochraceous. Coxa, trochanter ochraceous; femur brown; fore tibia ochraceous while middle and hind tibia brown; tarsi ochraceous. Abdomen reddish brown dorsally with blackish apical border; ochraceous with light brown anterior border ventrally. A comparative account in coloration of different body parts in known species of *Lisotrigona* from India is presented in table 2.

Pubescence

Clypeus with apical margin flat, fringed with reddish hair; face covered with numerous plumose white hair; dense ochraceous short hair on antennae; gena, post gena sparsely covered with fuscous hair. Vertex finely punctate, shiny with minute fuscous hair. Mesoscutum finely punctate, shiny with few white hairs medially; scutellum with margin fringed with white hair. Metanotum finely punctate with reddish brown hair. Episternum shiny with white hair at the anterior margin; propodeum reticulated with few hairs laterally. Legs clothed with reddish brown hair. Metasomal tergites and sternites with few sparse hairs except at the basal margins fringed with hair.

Morphometry

Detailed Morphometry is presented in Table 1. Body length ranging from 2.39 to 2.71 with a mean of 2.58 mm; head width 1.06 to 1.24 mm (mean 1.14 mm); scape 0.45 to 0.49 (0.47 mm) in length; third flagellar segment 0.07 mm in length and 0.10 mm in width; antennal length 1.33 to 1.47 mm (mean 1.38 mm) forewing length excluding tegula 2.46 to 2.54 mm in length (mean 2.49 mm), width 1.02 to 1.06 mm (mean 1.04 mm). Hind tibial length 0.83 to 0.89 mm (mean 0.86 mm), width 0.28 to 0.30 mm (mean 0.29 mm); hind basitarsus length 0.36 to 0.41 mm (mean 0.38 mm) and width 0.21 mm.

Material examined

Holotype: Worker: India: Maharashtra: Sawantawadi, 13.iii.2009, Coll. S.I. Rami deposited at UASB. *Paratypes*: 22 workers with same collection data at UASB, IARID and ZSIK.

Remarks

Males are unknown. This species resembles *Lisotrigona chandrai* in general coloration but is distinctly smaller in body length (2.58 mm) and head width (1.14 mm) than the remaining known species from India namely *L. cacciae* which measures 2.95 and 1.19 mm and *L. mohandasi* that measures 3.00 and 1.28 mm in length and head width as reported by Rasmussen (2013) and Jobiraj and Narendran (2004), respectively. *L. chandrai* measures 2.78 and 1.19 mm, respectively. Forewing excluding tegula distinctly longer (2.49 mm) than *L. cacciae* (2.37 mm) but smaller than *L. chandrai* (2.51 mm). Similarly,

Table 1: Detailed morphometry of known species of *Lisotrigona* from India

Parameter	<i>Lisotrigona cacciae</i> *	<i>Lisotrigona mohandasi</i>	<i>Lisotrigona revanai</i> sp.nov	<i>Lisotrigona chandrai</i> sp, nov
Length of body	2.95	3,00	2.58	2.78
Width of head including eyes	1,19	1,28	1,14	1.19
Ratio between length and width of head	2.48	2.34	2.26	2.34
Length of head (Clypeal apex- posterior margin of vertex)	1.01	-	0.99	0.77
Length of compound eye	0.83	-	0.88	0.83
Width of compound eye	0.33	-	0.28	0.35
Upper interorbital distance	0.75	-	0.78	0.76
Maximum interorbital distance	0.85	-	0.82	0.79
Lower interorbital distance	0.65	-	0.68	0.61
Diameter of median ocellus	0.11	-	0.11	0.09
Interocellar distance	0.27	-	0.29	0.26
Ocello-orbital distance	0.18	0.20	0.18	0.18
Length of clypeus	0.24	-	0.28	0.25
Maximum width of clypeus	0.42	-	0.39	0.38
Inter-tentorial distance	0.36	-	0.28	0.26
Clypeo-ocellar distance	0.06	-	0.74	0.68
Length of malar space	0.02	-	0.03	0,03
Length of antennae	-	-	1.38	1.41
Length of scape	0.37	0.20	0.47	0.49
Diameter of scape	0.07	-	0.07	0.08
Ration between length and diameter of scape	5.29	2.86	6.71	6.13
Diameter of third flaegellomere	0.11	-	0.10	0.10
Length of first flagellomere	0.06	-	0.08	0.08
Length of second flagellomere	0.06	-	0,08	0.07
Length of third flagellomere	0,07	-	0.09	0.07
Length of mandible	0.45	-	0.45	0.48
Width of mandible	0.12	-	0.16	0.16
Ratio between length and width of mandible	3.75	-	2,81	3,00
Length of proboscis	-	-	1.19	1.18
Length of forewing excluding tegula	2.37	-	2.49	2.51
Length of forewing including tegula	2.65	2,60	2.69	2.71
Width of forewing	0.95	-	1.04	1.00
Ratio of forewing length including tegula and width of forewing	2.79	-	2.59	2.71
Length of pterostigma	0.36	-	0.40	0.41
Width of pterostigma	0.12	-	0.10	0.10
Ratio between length and width of pterostigma	3.00	-	4.00	4.10
Length of marginal cell	0.89	-	0.77	0.78
Width of marginal cell	0.18	-	0.17	0.18
Ratio of length and width of marginal cell	4.94	-	4.53	4,33
Hamuli	6	-	5.40	5.00
Length of mesoscutum	0.71	-	0.60	0.60
Width of mesoscutum	0.93	-	0.90	0.90
Length of scutellum	0.23	-	0.28	0.23
Width of scutellum	0.48	-	0.64	0.45
Length of femur III	-	-	0.66	0.68
Length of tibia III	0.86	-	0.86	0.84
Width of tibia III	0.31	-	0.29	0.33
Ratio of length of tibia III and width	2.77	-	2.96	2.54
Length of basitarsus III	0.35	-	0.38	0.42
Width of basitarsus III	0.18	-	0.21	0.23
Ratio of length and width of basitarsus III	1.94	-	1.81	1.83
Ratio of length of basitarsus III and head width	0.29	-	0.33	0.35
Width of tergum III	1.05	-	-	-
Length of hairs on clypeus	<0.01	-	0.02	0.02
Length of hairs on frons	0.02	-	0.04	0.04
Length of hairs on the vertex	0.04	-	-	-
Length of hairs on scutellum apex	0.13	-	0.12	0.09

Data from lectotype (Rasmussen, 2013)

length of pterostigma (0.40 mm) is more than in *L. cacciae* (0.36 mm) but marginal cell distinctly smaller (0.77 mm) than in *L. cacciae* (0.89 mm) and nearly equal to *L. chandrai* (0.78 mm). Ocelli are transparent white compared to yellow in *L.*

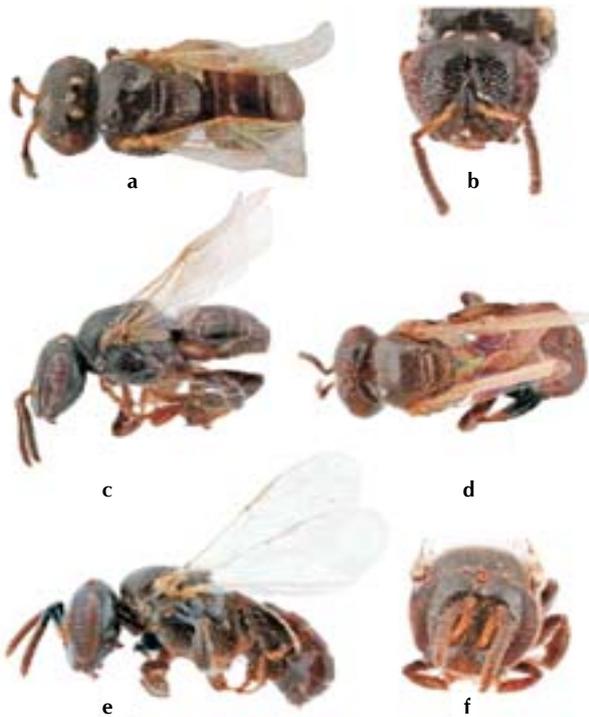
cacciae, reddish brown to dark brown in *L. mohandasi* (Jobiraj and Narendran, 2004) and *L. chandrai*. Apical margin of clypeus flat.

***Lisotrigona chandrai* sp. nov.**

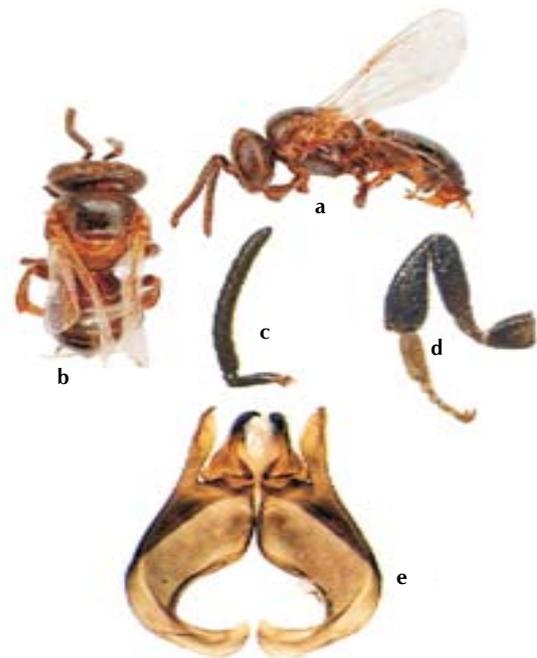
Table 2: Comparative coloration in known Indian species of *Lisotrigona*

Part of the body	<i>Lisotrigona cacciae</i> *	<i>Lisotrigona mohandasi</i>	<i>Lisotrigona revanai</i> sp.nov	<i>Lisotrigona chandrai</i> sp.nov
Clypeus	Reddish brown	Dark brown	Brown with subapical black transverse border	Black with reddish brown apical border
Frons	Reddish brown	black	black	Black
Vertex	Reddish brown	black	black	Black
Gena	Reddish brown	black	black	Black
Post gena	Reddish brown	black	black	Black
Ocelli	Yellow	Dark brown tint	Transparent white	Reddish brown to dark brown
Eyes	Reddish brown	Brown	Reddish brown to dark brown	Black
Scape	Yellow with apical quarter sometimes brown	Yellowish brown with apical quarter dark brown	Ochraceous with apical quarter dark brown	Ochraceous with apical half grayish black
Pedicel	Light brown	Light brown	Dark brown	Greyish black
Flagellum	Light brown	Light brown	Black	Black
Pronotum	Dark brown	Black	Black	Black
Mesonotum	Dark brown	Black	Black	Black
Metanotum	Dark brown	Black	Black	Black
Scutellum	Dark brown	Black	Black	Black
Coxa	Brown	Dark brown	Ochraceous	Fuscous
Trochanter	Yellow	Yellow	Ochraceous	Reddish brown to fuscous
Femur	Brown	Dark brown	Brown	Fuscous
Tibia	Brown	Dark brown	Fore tibia ochraceous; middle and hind tibia brown	Fuscous
Meta-tarsus	Brown with yellow border	Dark brown	Ochraceous	Fuscous
Tarsal segments	Yellow	Yellow	Ochraceous	Ochraceous
Abdomen dorsum	Light to dark brown	Brown to dark brown	Reddish brown with blackish apical border	Black
Abdomen ventrum	Light to dark brown	Brown to dark brown	Ochraceous with light brown anterior border	Black

Data from lectotype (Rasmussen, 2013)

**Figure 1: *Lisotrigona revanai* (a-c) and *L. chandrai* (d-f)****Workers (Fig. 1 d-f)****Coloration**

General body colour similar to *L. revanai* except the following (Table 2). Clypeus black with reddish brown apical border. Lateral ocelli fuscous, median ocellus reddish brown. "Y" shaped furrow anterior to median ocellus similar to *L. revanai*.

**Figure 2: Male *Lisotrigona chandrai* sp. nov.**

Eyes black. Mandibles black only at the basal ¼ and distal 3/4 dull yellow; proboscis, maxillary and labial palps ochraceous; scape ochraceous with apical ½ greyish black; pedicel greyish black; Coxae fuscous, trochanter reddish brown to fuscous. Femora, tibiae, metatarsi fuscous while tarsal segments ochraceous. In some, base of femur ochraceous.

Table 3: Detailed Morphometry of male and queen of *Lisotrigona chandrai* sp. nov.

Morphometric parameters	Male	Queen
Body Length	3.01	4.24
Vertex Length	0.23	0.34
Head Width including eyes	1.18	1.18
Ratio of Vertex Length and Width	0.19	0.28
Proboscis Length	1.02	1.00
Distance between ocelli	0.26	0.24
Ocello-Ocular distance	0.13	0.20
Ratio of Distance between Ocelli & Ocello-Ocular distance	2.00	1.2
Antennal Length	1.64	1.75
Scape	0.38	0.47
Length of IV flagellar segment	0.11	0.07
Width of IV flagellar segment	0.13	0.11
Ratio of IV Flagellar segment length and width	0.84	0.63
Mandible length	0.30	0.39
Mandible width	0.14	0.14
Ratio of Mandible Length and width	2.14	2.78
Hind Femur Length	0.65	0.81
Hind tibial length	0.81	1.01
Hind tibial width	0.23	0.33
Ratio of hind tibial length and width	3.52	3.06
Hind basitarsus Length	0.39	0.51
Hind basitarsus width	0.13	0.12
Ratio of Tarsus Length and Head Width	3.00	4.25
Fore wing Length	2.55	2.52
Fore wing Width	0.95	0.93
Ratio of Fore Wing Length and Width	2.68	2.70
Pterostigma length	0.41	0.38
Pterostigma width	0.10	0.11
Ratio of Pterostigma Length and Width	4.10	3.45
Hind wing length	1.73	1.81
Hind wing width	0.55	0.52
Ratio of Hind wing length and width	3.14	3.48
Hamuli	5	4
3 rd Tergite Length	0.47	0.82
3 rd sternite length	0.35	0.36
3 rd sternite width	1.24	1.30
Ratio of Sternite Length and Width	0.28	0.27
Distance between M-Cu bifurcation and basal tip of marginal cell.	0.70	0.70
Ratio of Distance between M-Cu bifurcation and basal tip of marginal cell and Head width	0.59	0.59
Ratio of Hind Tibial Length and distance between M-Cu bifurcation and basal tip of marginal cell	1.15	1.44
Ratio of width of hind basitarsus and width of hind Tibia	0.56	0.36

Table 4: Morphometry of Nest and developmental stages of *Lisotrigona chandrai* sp.nov.

	Length (mm)	Width (mm)
Brood cell	3.09	2.22
Grown larva	4.96	1.28
Pollen pot	7.28	6.37
Honey pot	7.28	6.25

Abdomen black both dorsally as well as ventrally.

Pubescence

Clypeus with dense ochraceous hair and with fringed, gently concave apical margin; face with pale ochraceous several plumose hairs; vertex shiny, finely punctate, covered with minute fuscous hair; flagellar segments with ochraceous short hair; gena and post gena with sparse fuscous hair. Mesonotum, metanotum, scutellum finely punctate, sparsely hairy; scutellum basally and laterally fringed with whitish hair; metanotum with reddish brown hair. Propodeum finely punctate, fringed

laterally with white hair. Legs densely clothed with reddish brown hair. Metasomal tergites and sternites sparsely hairy but apical margins of tergites fringed with hair.

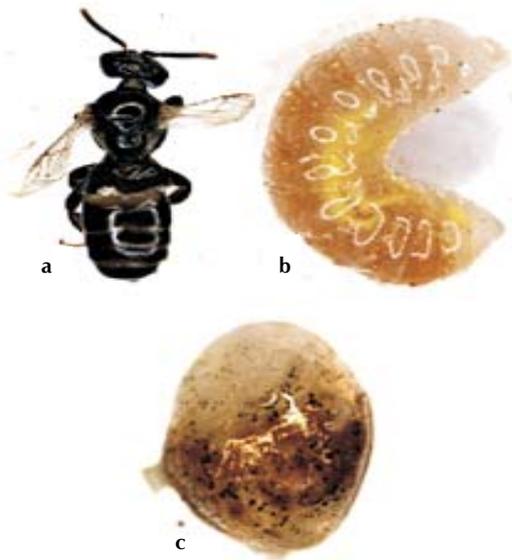
Morphometry

Detailed Morphometry is presented in Table 1. Body length varies from 2.77 to 2.90 with a mean of 2.78 mm. Head width 1.17 to 1.26 mm (mean 1.19 mm); scape 0.48 to 0.51 (0.49 mm) in length; fourth flagellar segment 0.07 mm in length and 0.11 mm in width; antennal length 1.32 to 1.47 mm (mean 1.41 mm). Forewing length 2.38 to 2.60 mm in length (mean 2.51 mm), width 0.92 to 1.04 mm (mean 1.00). Hind tibial length 0.81 to 0.92 mm (mean 0.84 mm), width 0.30 to 0.37 mm (mean 0.33 mm); hind basitarsus length 0.40 to 0.49 mm (mean 0.42 mm).

Male (Fig. 2 a-d)

Coloration

Head dark brown to black; thorax and abdomen dark reddish brown. Clypeus dark brown to black, gently convex dorsally.



a. Queen, b. Full grown larva, c. Brood cell

Figure 3: *Lisotrigona chandrai* sp. nov.

Mandibles reddish brown with a blunt projection at apical margin. Proboscis, maxillary and labial palps ochraceous. Vertex and frons black. Antennae dark brownish to black with antennal bases and basal part of scape ochraceous; flagellar segments with yellowish punctures. Eyes fuscous; ocelli reddish brown, arranged more or less in a transverse straight line. Tegulae ochraceous, wings with very faint reduced venation. Pterostigma faintly pigmented. All coxae and trochanters light reddish brown; femora and tibiae fuscous and tarsi ochraceous. Mesonotum, metanotum, scutellum and thoracic sterna shiny, reddish brown to fuscous. Abdominal terga and sterna shiny and fuscous.

Pubescence

Clypeus with short white hair with apical margin fringed with longer white hair. Frons, vertex, gena and post gena punctate with very fine minute indistinctly plumose white pubescence with a few longer white setae near ocelli. Flagellar segments with fuscous short but dense setae. Mandibles with apical margin fringed with long brownish setae. Meso and metanota punctate, sparsely covered with white minute setae. Lateral and basal margins of nota fringed with longer white setae. Scutellum basally and laterally fringed with long white hair. Episternum, thoracic sterna and propodeum with white fine pubescence. Legs with dense reddish brown setae. Metasoma and metasternum with sparse white hair.

Morphometry

Body length varies from 2.98 to 3.03 with a mean of 3.01 mm (Table 3). Head width 1.11 to 1.25 mm (mean 1.18 mm); vertex length 0.21 to 0.24 mm (mean 0.23 mm); scape 0.38 mm in length; fourth flagellar segment 0.11 mm in length and 0.13 mm in width; antennal length 1.64 mm; forewing 2.55 mm in length and 0.95 mm in width while hind wing 1.73 mm in length and 0.55 mm in width. Hind tibial length 0.81 mm, width 0.23 mm; hind basitarsus length 0.39 mm and width



a. Nest entrance, b. Brood, pollen and honey, c. Queen, d. Harvested honey pots

Figure 4: Nest characteristics of *Lisotrigona chandrai* sp. nov.

0.13 mm.

Male genitalia (Fig 2 e)

Both gonocoxites strongly curved medially, extending basally reaching very close to each other and again recurved inwardly as in figure 2 e. Gonostylus with maximum width of 0.63 mm ante apically and about 5.25 times longer than its maximum width, with incurved setiferous apices. Valves elongate triangular, 0.50 to 0.54 mm in length with maximum width of 0.20 to 0.27 mm basally. Apical 1/3rd dark brown and apices strongly curved inwards.

Queen (Figs. 3 a and 4 c)

Coloration

Antennae, head and thorax dark brown; abdomen ochraceous with reddish brown to fuscous transverse band basally on each tergum while sterna pale brown when alive (Fig. 4 c). terminal abdominal segment dark brown; legs dark brown but tarsi ochraceous. Head, thorax and abdomen turning to dark brown after preservation in 85 per cent alcohol (Fig. 3 a)

Morphometry

Detailed morphometry is presented in Table 3. Body longer (4.24 mm) than the male and worker with head including eyes as wide as the males and workers (1.18 mm); proboscis short 1.00 mm in length; antennae longer (1.75 mm) than male (1.64 mm) and the worker (1.41 mm); scape, fourth flagellar segment measures 0.47 and 0.07 mm; femur, tibia and metatarsus of hind leg 0.81, 1.01, 0.51 mm, respectively; forewing 2.52 mm long and 0.93 mm wide; hindwing 1.81 mm long 0.52 mm wide; pterostigma 0.38 mm long and 0.11 mm wide; third tergite and third sternite length 0.82 and 0.36 mm.

Material examined

Holotype male, INDIA: Kerala: Kanhangad: 20. ii. 2012: S.

Viraktamath (genitalia in micro vial) deposited at UASB. Five paratypes with same collection data, but two collected by S. Viraktamath and three by Bhaktibhavana R. deposited at UASB. Five workers, INDIA: Kerala: Thaliparamba, 03.iii.2011; Sajan Jose K.; 10 workers: INDIA: Kerala: Kanhangad: 20.ii.2012: S. Viraktamath; 14 workers, INDIA: Kerala: Kanhangad: 20.ii.2012: Bhaktibhavana R, deposited at UASB, IARID and ZSIK.

Colony structure (Fig. 4)

L. chandrai bees were observed nesting in Thaliparamba and Kanhangad in Kerala in hard laterite rocks, foundation walls, and tree cavities and the size of the colonies was small. The entrance tube was short, cylindrical and slightly projecting outside (Fig. 4 a). The entrance was surrounded by black resinous material generally. Two to three workers were guarding the entrance of the nest. The workers were very calm, shy and withdrew with slight disturbance. Workers flew away when the colony was disturbed leaving the queen and young workers. The entrance tube opened into the nest cavity the walls of which were lined with blackish cerumen as also noticed in *Tetragonula iridipennis* (Sajan Jose and Thomas 2014). The brood area was in the center of the colony surrounded by cluster of pollen and honey storage pots on either side (Fig. 4 b) as well as in a separate chamber above the brood. The brood cells were arranged in clusters which were attached to the floor of the nest cavity with very thin connectives. Both freshly constructed (dull yellow) as well as mature brood cells (bright yellow) were observed. Hard, porous dark grey compartments made up of batumen separated these areas. However, such compartment walls were noticed only when the nest cavity was large in *Tetragonula iridipennis* (Sajan Jose and Thomas, 2014). The cerumen (resin and wax mixture) was very soft and white. Resin deposit was observed near the entrance while waste dump at the extreme left and right side of the nest cavity.

The brood cells were dull yellow to bright yellow (Fig. 3 c); 2.84 to 3.32 (mean 3.09) mm in length and 1.88 to 2.62 (mean 2.22) mm in width (Table 3) (Fig. 3 c and 4 b). The full-grown larva was white to creamy yellow and curved (Fig. 3 b) measuring 3.96 to 5.37 (mean 4.96) in length and 1.11 to 1.48 (mean 1.28 mm) mm in width.

Pollen pots were light yellow and measured 6.98 to 7.63 (mean 7.28) mm in length and 5.88 to 6.79 (mean 6.37) mm in width. Honey pots were translucent, snowy white to amber colored (Fig. 4 b, d) and measured 6.97 to 7.46 (mean 7.28) mm in length and 5.66 to 6.73 (mean 6.25) mm in width. The honey was light yellow and transparent.

Remarks

Male genitalia of this species are unique and quite different from the genitalia of *L. furva* Engel from Thailand the only other species wherein male genitalia are described by Michener (2007). Males are not known in other Indian species of the genus. The workers resemble *L. revanai* in general coloration and appearance but are distinctly longer (2.78 mm) compared to *L. revanai* (2.58 mm) and the apical margin of the clypeus is gently concave (flat in *L. revanai*).

Both *L. revanai* and *L. chandrai* appear to be the smallest stingless bees from India, comparable to the Neotropical

Trigonisca Moure and *Leurotrigona* Moure. The head width of these two new species is distinctly narrower (1.14 and 1.19 mm, respectively) than that of *L. mohandasi* (1.28 mm) but is similar to *L. cacciae* reported from India. But *L. cacciae* is distinctly longer (> 2.95 mm) (Engel, 2000; Rasmussen 2013). Both the species also distinctly differ in the ratios of length and breadth of scape, mandible, forewing, pterostigma, marginal cell of the forewing and basitarsus III (Table 1 and Fig. 5).

The partial sequence of the COI gene of *L. chandrai* (referred as Kannur sample by Peter et al 2014) has shown 77 per cent similarity with *Tetragonula iridipennis* and 82.78 per cent similarity with *L. cacciae* and 82.07 per cent similarity with *L. furva* (Peter et al., 2014) which clearly establishes distinctiveness of this species from *L. cacciae*.

Key for identification of species of *Lisotrigona* Moure (Workers)

1. Clypeus entirely yellow; face with yellow markings; mesoscutum with thin yellow margins bordering tegulae.....*L. carpenteri* Engel
- Clypeus dark brown to black; face without yellow markings; mesoscutum without yellow margins bordering tegulae.....2
2. Punctures of mesoscutum small and strong.....*L. furva* Engel
- Punctures of mesoscutum extremely small and faint.....3
3. Body length > 2.90 mm.....4
- Body length < 2.90 mm.....5
4. Body length 2.95 mm; clypeus with minute hairs; metanotum sculpture finely imbricate*L. cacciae* (Nurse)
- Body length 3.00 mm; clypeus with plumose hairs; metanotum sculpture reticulate anteriorly..... *L. mohandasi* (Jobiraj and Narendran)
5. Body length 2.77 to 2.90 mm; head width including eyes 1.14 mm; clypeus brown with black subapical transverse border; apical margin straight; ocelli transparent white.....*L. revanai* sp.nov.
- Body length 2.39 to 2.71 mm; head width including eyes 1.14 mm; clypeus black with reddish brown apically and faint concave apical margin; ocelli reddish brown to fuscous..... *L. chandrai* sp.nov.

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REFERENCES

- Chinh, T. X., Sommeijer, M. J., Boot, W. J. and Michener, C. D. 2005.** Nest and colony characteristics of three stingless bee species in Vietnam with the first description of the nest of *Lisotrigona carpenter*. *J. Kans. Entomol. Soc.* **78**: 363-372.
- Cortopassi-Laurino, M., Imperatriz-Fonseca, V. L., Roubik, D. W., Dollin, A., Heard, T., Aguilar, I., Venturiei, G. C., Eardley, C. and Nogueira-Neto, P. 2006.** Global meliponiculture: challenges and opportunities. *Apidologie*. **37(2)**: 275-292.
- Engel, M. S. 2000.** A review of the Indo-Malayan Meliponine genus *Lisotrigona*, with two new species (Hymenoptera: Apidae), *Orient. Insects*. **34**: 229-237.
- Jobiraj, T. and Narendran, T. C. 2004.** A revised key to the world species of *Lisotrigona* Moure (Hymenoptera: Apoidea: Apidae) along with description of a new species from India. *Entomon*. **29**: 39-43.
- Grimaldi, D. A., Shedrinsky, A., Ross, A. J. and Baer, N. S. 1995.** Forgeries of fossils in amber ; history, identification and case studies. *Curator (New York)* **37**: 251-274.
- Heard, T. A. 1999.** The role of stingless bees in crop pollination. *Annu. Rev. Etnomol.* **44**: 183-206.
- Kumar, M. S., Singh, A. J. A. R. and Alagumuthu, G. 2012.** Traditional beekeeping of stingless bee (*Trigona* sp) by Kani tribes of Western Ghats, Tamil Nadu, India. *Indian J. Trad. Know.* **11(2)**: 342-345.
- Michener, C. D. 2000.** *The Bees of the World*, John Hopkins University Press, Baltimore, USA.
- Michener, C. D. 2007.** *Lisotrigona* in Thailand, and the male of the genus (Hymenoptera: Apidae: Meliponini). *J. Kans. Entomol. Soc.* **80**: 130-135.
- Michener, C. D. and Grimaldi, D. A. 1988.** The oldest fossil bee: Apoid history, evolutionary stasis and antiquity of social behavior. *Proc. National Acad. Sci. USA.* **85**: 6424-6426.
- Moure, J. S. 1961.** A preliminary supra-specific classification of the Old World meliponine bees (Hym. Apoidea). *Studia Entomology.* **4**: 181-242
- Peter, R., Jose, S. K., Subin, C. S. and Thomas, S. 2014.** Taxonomy of stingless bees (Hymenoptera: Apidae) of Kerala, India. In. Thomas, S. and Joseph, G.K. (Eds.) *Invertebrates: Systematics and Biology*, Nirmala and Academic Pub. pp. 102-108.
- Raakhee, M. and Devanesan, S. 2000.** Studies on behavior of stingless bee, *Trigona iridipennis* Smith (Apidae: Meliponinae). *Indian Bee. J.* **62(4)**:59-62.
- Ramanujam, C. G. K., Fatima, K. and Kalpana, T. P. 1993.** Nectar and pollen sources for dammer bee (*Trigona iridipennis* Smith) in Hyderabad (India). *Indian Bee J.* **55(1/2)**: 25-28.
- Rasmussen, C. 2008.** Catalog of the Indo Malayan/Australasian stingless bees (Hymenoptera: Apidae: Meliponini). *Zootaxa.* **1935**: 1-80.
- Rasmussen, C. 2013.** Stingless bees (Hymenoptera: Apidae: Meliponini) of the Indian subcontinent: Diversity, taxonomy and current status of knowledge. *Zootaxa.* **3647(3)**: 401.
- Rasmussen, C. and Cameron, S. A. 2007.** Molecular phylogeny of the Old World stingless bees (Hymenoptera: Apidae: Meliponini) and the non-monophyly of the large genus *Trigona*. *Syst. Entomology.* **32**: 26-39.
- Rasmussen, C. and Cameron, S. A. 2010.** Global stingless bee phylogeny supports ancient divergence, vicariance and long distance dispersal. *Biol. J. Linn. Soc.* **99**: 206-232.
- Roopa, C. A. 2002.** Bioecology of stingless bees, *Trigona iridipennis* Smith. M.Sc. (Agri) Thesis, UAS, Bangalore.
- Roubik, D. W. 1989.** *Ecology and natural history of tropical bees*. Cambridge University Press, New York. M.Sc. (Agri)
- Ruttner, F. 1988.** *Biogeography and Taxonomy of Honey bees*. Springer-Verlag Berlin. p. 284.
- Sajan Jose, K. and Thomas, S. 2014.** Nest architecture of stingless bee, *Trigona iridipennis* (Smith). In. Thomas, S. and Joseph, G.K. (Eds.) *Invertebrates: Systematics and Biology*, Nirmala and Academic Pub. pp. 82-86.
- Sakagami, S. F. 1978.** *Tetragonula* stingless bees of the continental Asia and Sri Lanka (Hymenoptera: Apidae). *J. Fac. Sci. Hokk. Univ. Ser. VI. Zoology.* **21**: 165-248.
- Swaminathan, S. 2000.** *Bioecology of stingless bee (T. iridipennis)*. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Viraktamath, S. 2015.** Morphometry and genitalia of drones of *Apis* honey bee species from India. *The Bioscan.* **10**: 1057-1067.
- Wille, A. 1983.** Biology of the stingless bees. *Annu. Rev. Entomol.*, **28**: 41-64.