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Description of two new species of *Lisotrigona* (Hymenoptera, Apidae, Meliponini) from Central India and their nests

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ABSTRACT: *Lisotrigona darbhaensis* **sp. nov**. and *L. kosumtaraensis* **sp. nov** from Chhattisgarh and Maharashtra states, respectively, are described along with the additional description of the male of *L. chandrai*. *Lisotrigona darbhaensis* nested in the tree trunk of teak (*Tectona grandis*) while *L. kosumtaraensis* in the Indian frank incense (*Boswellia serrata*) and Indian boxwood (*Gardenia latifolia*). Brood cells of *L. kosumtaraensis* were arranged in clusters. The colony of *L. kosumtaraensis* consisted of 921 female and 40 male bees. The detailed studies on male genitalia, metasomal sterna, and morphometry with associated female bees collected from Maharashtra and Chhattisgarh provided conclusive evidence as these bees were found different from the known species of *Lisotrigona* and new to science. Both the new species are distinct and different from other known species of *Lisotrigona*. The diversity of *Lisotrigona* bees in India is rich with six valid species and the action of synonymizing all Indian species of *Lisotrigona* with *L. cacciae* is arbitrary.

KEY WORDS: *Lisotrigona darbhaensis*, *L. kosumtaraensi*s, stingless bees

INTRODUCTION

Stingless bees belonging to the tribe Meliponini of the family Apidae are doing yeoman service to the humans along with the honey bees by yielding honey of high medicinal value and pollinating several plant species including cultivated crops (Crane, 1999; Heard 1999; Cortopassi-Laurino *et al.,* 2006). Honey of stingless bees fetches a premium price in India ranging from rupees 1,500 to 10,000 per kilogram (Viraktamath *et al*., 2021a). The pollination services of honey bees, stingless bees, and other pollinators are worth US $ 577 billion per year (Lautenbach *et al*., 2012).

Among the three genera (*Tetragonula* Moure, 1961*, Lepidotrigona* Schwarz, 1939 and *Lisotrigona* Moure,1961) that occur in India, the genus *Lisotrigona* is characterized by smaller size (usually measuring 3.00 mm or less in body length), short linear malar space, converging inner eye margins, and reduced wing venation (Michener 2000). The first species of *Lisotrigona* from India (type locality: Hoshangabad: Madhya Pradesh) was described as *Melipona cacciae* by Nurse (1907), which was later transferred to the new genus *Lisotrigona* described by Moure (1961). After a gap of 97 years, Jobiraj and Narendran (2004) described *L. mohandasi,* and two species

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(*L. revanai* and *L. chandrai*) were added by Viraktamath and Sajan Jose (2017).

Females of most of the species of stingless bees are remarkably similar with very weak diagnostic characters resulting in difficulty in the identification of the species. Contrarily, the males have strong diagnostic characters that are species-specific and very useful in identifying the species (Schwarz, 1939; Sakagami, 1978; Rasmussen, 2013; Attasopa *et al*., 2018). Recently Engel *et al.* (2022) transferred *L. carpenteri* Engel to a new genus *Ebaiotrigona* when the males of the species were discovered after 22 years, and male genitalia were found unique and different from the typical *Lisotrigona* genus.

Bees from Kerala and Maharashtra were described as *L. chandrai* and *L. revanai* by Viraktamath and Sajan Jose (2017), respectively. However, Rasmussen *et al*. (2017) synonymized *L. mohandasi, L. revanai,* and *L. chandrai* with *L. cacciae* without giving justification. Recent studies by Viraktamath *et al*. (2021b) on morphometry of female *Lisotrigona* bees collected from seven places of India from Maharashtra, Chhattisgarh, and Mizoram in comparison with *L. mohandasi, L. chandrai, L. revanai* and *L. cacciae* (Primary type specimens) revealed that the dataset formed more than one cluster in Principal Component Analysis and Canonical Discriminant analysis indicating the occurrence of more than one species in India.

In this paper, *Lisotrigona darbhaensis* from Chhattisgarh and *L. kosumtaraensis* from Maharashtra are described as new species along with the additional descriptions of metasomal sterna of male *L. chandrai*. Brief notes on the nests of these two new species are also provided.

MATERIALS AND METHODS

In the endeavour to collect male with associated female stingless bees from various parts of India, four colonies of *Lisotrigona* in Kerala, six in Maharashtra, two in Chhattisgarh were found besides many foraging female bees in Maharashtra and Mizoram; and the authors were successful in collecting both males and females from three states (Kerala, Chhattisgarh, and Maharashtra). Male and female bees were collected from one colony of *Lisotrigona* nested in a teak tree (*Tectona grandis*) at Darbha, Chhattisgarh (18.85° N; 81.8689° E) (Figs. 6 A-1, A-2) by inserting one end of a narrow glass tube (15 cm long) in the colony and aspirating from the other end where a rubber tube (45 cm long) was connected. Glass and rubber tubes were separated by a thin muslin cloth to prevent the entry of bees in the mouth while aspirating. However, in Maharashtra adopted two methods for collecting male and female bees. All the bees of one *Lisotrigona* colony nested in an Indian frankincense tree (*Boswellia serrata*) at Kosumtara (21° 16’ 6’’N; 80° 32’ 37’’ E). were captured by using chloroform (Fig. 6 B-1). At Navatolla (21° 16’ 52’’N; 80° 33’ 36’’ E) a water trap (Viraktamath *et al.,* 2020) was used to collect male and female bees from one colony nested in an Indian boxwood tree (*Gardenia latifoila*). All the collected bees were transferred to vials containing ethyl alcohol (95%), labeled, and later sexed at the Systematic laboratory of the Department of Entomology, University of Agricultural Sciences Bengaluru (UASB) under a stereo binocular microscope. All the male bees and representative female bees were mounted on a triangular point and each bee was labeled with collection data (place, date, and collector’s name).

Followed the methods described by Viraktamath and Rojeet (2021) for the description of the species by studying the morphometry of 36 morphological traits, structure of male metasomal sterna and genitalia. Besides, subjected the morphometry dataset to the Principal Component Analysis (PCA) and mapped the species on a PCA plot to understand the relationships among the species. The terminology used in the description was that of Sakagami (1978) and Rasmussen (2013). The procedure to study the male genitalia, metasomal terga, and sterna were similar to that described by Viraktamath and Rojeet (2021). Images of the bees, genitalia, and metasomal sterna were taken under Leica microscopes (M205C and DM2000) fitted with a digital camera (DFC425) having a range of magnification from 7.8 to 160x and 40 to 400 x,

respectively. The images were later processed using Photoshop. Measured the length and width of the penis valve, gonostylus, and gonocoxite, under the microscope using Leica measurement software.

Holotypes and paratypes were deposited at the Department of Entomology, UASB. One paratype of each species will be deposited at the Zoological Survey of India, Kolkata, ZSIK.

The nests of both the species besides the internal structure of *L. kosumtaraensis* by breaking open the colony in Kosumtara were also studied. Since the entire colony was captured, total female and male bees were counted to estimate the strength of the colony excluding the brood. A sample of brood cells, honey, and pollen pots was measured (20 cells each) by using an ocular micrometer fitted in a stereo binocular microscope. Photographs were taken to depict the internal structure of the colony.

RESULTS

***Lisotrigona darbhaensis* Viraktamath sp. nov.**

LSIDurn:lsid:zoobank.org:act:E5D496A5-2B97-4EEF-B06B-444953E6297C

Diagnosis. Male bees measure a mean of 3.20 mm in body length and 1.14 mm in head width with forewings 2.65 mm long and 1.00 mm wide. Female bees measure a mean of 3.10 mm in body length, 1.18 mm head width with 2.59 mm long, and 0.97 mm wide forewings. This species differs from *L. kosumtaraensis* and males of other known species (*L. chandrai* and *L. furva*) in the following features. In the metasomal sternum 4, the gradulus briefly touches the antecosta medially as found in *L. kosumtaraensis* but does not touch in *L. chandrai;* the setae arising from the basal area of gradulus do not extend beyond the apical margin but in *L. kosumtaraensis* and *L. chandrai,* they extend beyond the apical margin (Figs. 3 A, B, C S4). The gradulus in sternum 5 touches the antecosta medially as in *L. kosumtaraensis* but in *L. chandrai* the gradulus runs very close to the antecosta without touching it; the apical margin is weakly bisinuate in *L. darbhaensis* but nearly straight in *L. kosumtaraensis,* and distinctly bisinuate in *L. chandrai* (Figs. 3A, B, C S5), distinctly inverted U-shaped in *L. furva* (see fig. 5 of Michener 2007). Antecosta nearly straight medially in sternum 6 in *L. darbhaensis* whereas it is distinctly convex in *L. kosumtaraensis*, distinctly concave in *L. chandrai,* and weakly concave in *L. furva* (see fig. 4 of Michener 2007)*;* apicomedian lobe of sternum 6 wider than long (as long as broad in *L. kosumtaraensis,* longer than wide in *L. chandrai* and *L. furva* (Figs. 3A, B, C S6). Gonocoxae elongate (0.81 mm), flipped J-shaped with a maximum width of 0.35 mm in *L. darbhaensis* and *L. kosumtaraensis* whereas they are shorter (0.79 mm), characteristically C-shaped, distinctly wider (0.41 mm) in *L. chandrai* (Figs. 4 A-1, B-1, C-1). The terminal part of penis valves distinctly curved and pointed in *L. darbhaensis, L. kosumtaraensis, L. chandrai* (Figs. 4 A-2, B-2, C-2) but straight and bluntly pointed in *L. furva* (See the fig. 2 of Michener, 2007). Gonostylus long (1.06 mm) arising from the basal part of gonocoxa in *L. darbhaensis* and *L. kosumtaraensis* but shorter, arising from basal half of gonocoxa in *L. chandrai* (0.87 mm) and *L. furva;* gonostylus broader with the tapered bluntly rounded, curved apex in *L. darbhaensis, L. kosumtaraensis, L. chandrai* (Figs. 4 A-3, B-3, C-3) but very slender in *L. furva* (see description and fig. 2 of Michener 2007).

Female bees are distinctly punctate on mesoscutum as also in *L. kosumtaraensis, L. revanai,* and *L. chandrai.* However, *L. cacciae* has exceedingly minute faint punctures while *L. furva* has strong punctures. The ratio of inter-ocellar to ocello-ocular distance is higher in *L. darbhaensis* (1.93) and *L. kosumtaraensis* (1.92) while lower in *L. chandrai* (1.44), *L. cacciae* (1.50), and *L. revanai* (1.61) (Table 2).

Description Males:

Coloration. Head, mesosoma and metasoma shiny, black (Fig. 1a, b). Labrum yellowish-brown; clypeus blackish brown; scape dark reddish-brown except the basal bulb and the socket yellowish-brown; pedicel, flagellar segments blackish-brown on the upper side but lighter on the lower side; ocelli transparent, shiny, light brown; compound eyes blackish brown (Fig. 1c). Wings hyaline; tegula,

Table 1. Morphometry* of two new species of *Lisotrigona* in comparison with type specimens of *L. chandrai* and *L. cacciae*

↓Parameter / Species→	*L. darbhaensis sp. nov.*		*L. kosumtaraensis sp. nov.*		*L. chandrai***		*L. cacciae****
	Male	Female	Male	Female	Male	Female	Holotype
	n-2	n-10	n-10	n-10	--	--	--
Length of body	3.20 ± 0.01	3.10 ± 0.14	3.32 ± 0.20	3.12 ± 0.25	3.01	2.78	2.95
Width of head including eyes	1.14 ± 0.02	1.18 ± 0.02	1.14 ± 0.03	1.19 ± 0.04	1.18	1.19	1.19
Length of head	0.88 ± 0.01	0.94 ± 0.01	0.89 ± 0.04	0,94 ± 0.04	0.88	0.86	1.01
Length of eye	0.84 ± 0.01	0.84 ± 0.02	0.88 ± 0.02	0,87 ± 0.04	0.82	0.83	0.83
Width of eye	0.38 ± 0.01	0.34 ± .01	0.36 ± 0.02	0.33 ± 0.02	0.35	0.35	0.33
Upper interocular distance	0.67 ± 0.03	0.75 ± 0.02	0.71 ± 0.02	0.79 ± 0.02	0.68	0.76	0.75
Diameter of median ocellus	0.13 ± 0.00	0.11 ± 0.01	0.14 ± 0.01	0.12 ± 0.00	0.13	0.09	0.11
Inter ocellar distance	0.29 ± 0.01	0.28 ± 0.00	0.29 ± 0.01	0.28 ± 0.00	0.26	0.26	0.27
Ocello-ocular distance	0.09 ± 0.00	0.14 ± 0,01	0.10 ± 0.01	0.15 ± 0.00	0.13	0.18	0.18
Length of clypeus	0.25 ± 0.00	0.26 ± 0,01	0.27 ± 0.02	0.26 ± 0.01	0.33	0.25	0.24
Maximum width of clypeus	0.45 ± 0.00	0.55 ± 0.01	0.49 ± 0.01	0.58 ± 0.05	0.49	0.38	0.42
Malar space length	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.01	0.03	0.02
Length of scape	0.33 ± 0.00	0.42 ± 0.01	0.33 ± 0.02	0.43 ± 0.02	0.38	0.49	0.37
Width of scape	0.09 ± 0.01	0.08 ± 0.00	0.10 ± 0.00	0.08 ± 0.00	0.09	0.08	0.07
Length of pedicel + flagellum	1.22 ± 0.00	0.89 ± 0.04	1.27 ± 0.06	0.93 ± 0.05	1.26	0.92	--
Length of flagellomere 1	0.05 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.06 ± 0.02	0.04	0.08	0.06
Length of flagellomere 2	0.11 ± 0.00	0.07 ± 0.00	0.11 ± 0.01	0.09 ± 0.01	0.11	0.07	0.06
Length of flagellomere 3	0.10 ± 0.00	0.08 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.11	0.07	0.07
Width of flagellomere 3	0.13 ± 0.01	0.10 ± 0.00	0.12 ± 0.00	0.10 ± 0.01	0.13	0.10	0.11
Length of mandible	0.30 ± 0.02	0.43 ± 0.02	0.31 ± 0.01	0.55 ± 0.00	0.30	0.48	0.45
Width of mandible	0.15 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.22 ± 0.00	0.14	0.16	0.12
Length of forewing + tegula	2.65 ± 0.00	2.59 ± 0.05	2.82 ± 0.13	2.64 ± 0.09	2.55	2.71	2.65
Width of forewing	1.00 ± 0.00	0.97 ± 0.03	0.99 ± 0.06	0.96 ± 0.03	0.95	1.00	0.95
Length of pterostigma	0.45 ± 0.00	0.45 ± 0.01	0.43 ± 0.02	0.45 ± 0.01	0.41	0.41	0.36
Length of marginal cell	0.88 ± 0.02	0.77 ± 0.02	0.85 ± 0.01	0.78 ± 0.03	0.90	0.78	0.89
Width of marginal cell	0.20 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.20	0.18	0.18
Wing diagonal length	0.71 ± 0.01	0.74 ± 0.01	0.79 ± 0.01	0.74 ± 0.01	0.70	0.66	0.73
Number of Hamuli	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00	5.00	6.00
Length of mesoscutum	0.75 ± 0.02	0.77 ± 0.02	0.79 ± 0.02	0.77 ± 0.02	0.77	0.60	0.71
Width of mesoscutum	0.88 ± 0.02	0.92 ± 0.02	0.94 ± 0.03	0.97 ± 0.05	0.91	0.90	0.93
Length of scutellum	0.27 ± 0.01	0.25 ± 0.01	0.26 ± 0.02	0.23 ± 0.02	0.25	0.23	0.23
Width of scutellum	0.77 ± 0.02	0.75 ± 0.01	0.84 ± 0.02	0.78 ± 0.03	0.76	0.45	0.48
Length of hind tibia	0.80 ± 0.00	0.89 ± 0.02	0.80 ± 0.01	0.91 ± 0.03	0.81	0.81	0.86
Width of hind tibia	0.26 ± 0.01	0.35 ± 0.01	0.26 ± 0.01	0.37 ± 0.02	0.23	0.33	0.31
Length of hind basitarsus	0.39 ± 0.00	0.44 ± 0.01	0.41 ± 0.01	0.48 ± 0.02	0.39	0.42	0.35
Width of hind basitarsus	0.14 ± 0.01	0.21 ± 0.01	0.15 ± 0.00	0.23 ± 0.02	0.13	0.23	0.18

* All measurements in mm ± SD, **: Data based on Viraktamath & Sajan Jose (2017); ***: Data based on Rasmussen (2013)

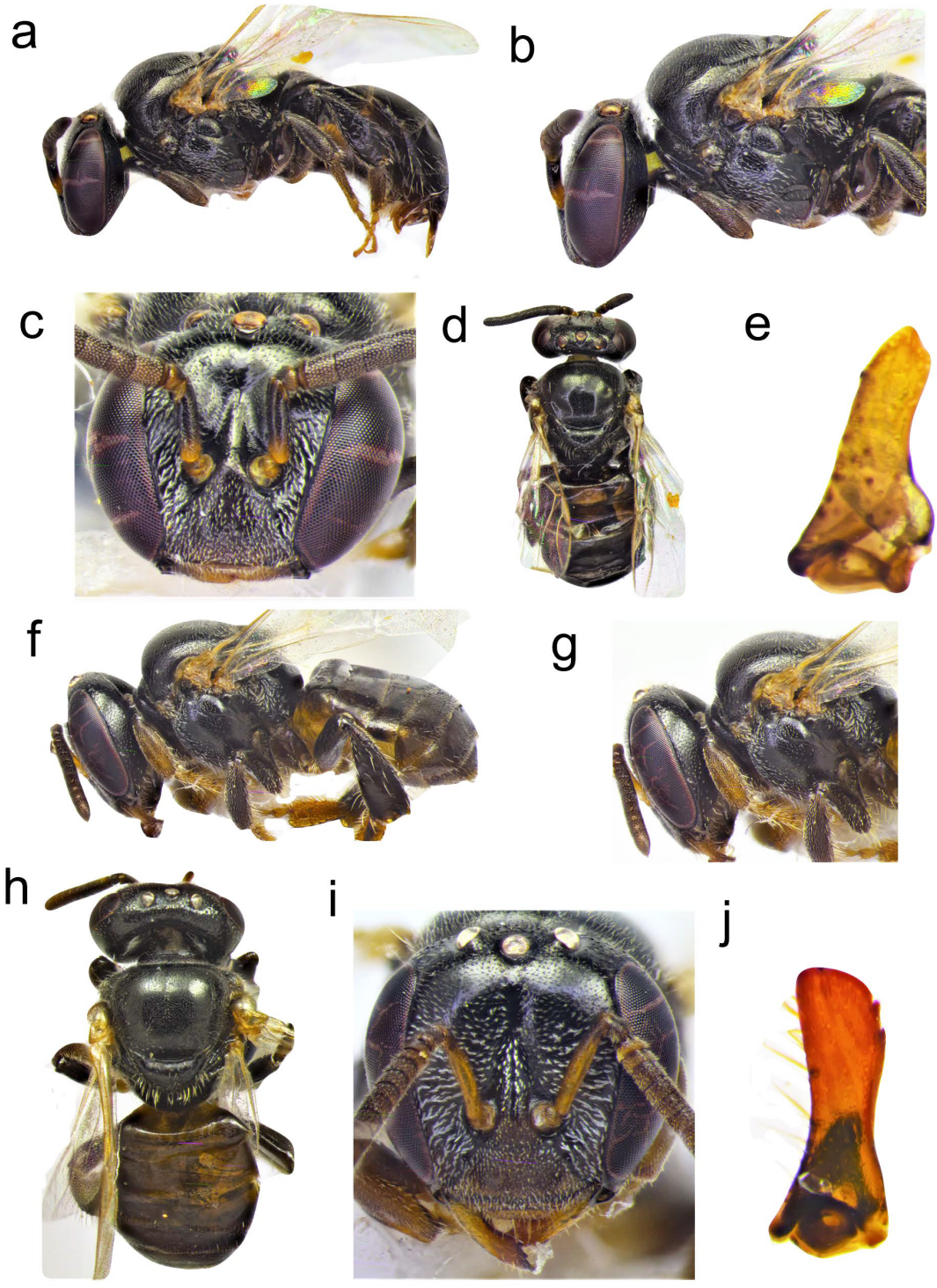

Fig. 1 *Lisotrigona darbhaensis* **sp. nov.** (a). Male lateral, (b). Head and thorax lateral (c). Head frontal, (d). Head, mesosoma and metasoma dorsal, (e). mandible (f). Female lateral, (g). Head and mesosoma lateral (h). Head, mesosoma, metasoma dorsal, (i). Head frontal (j). Mandible

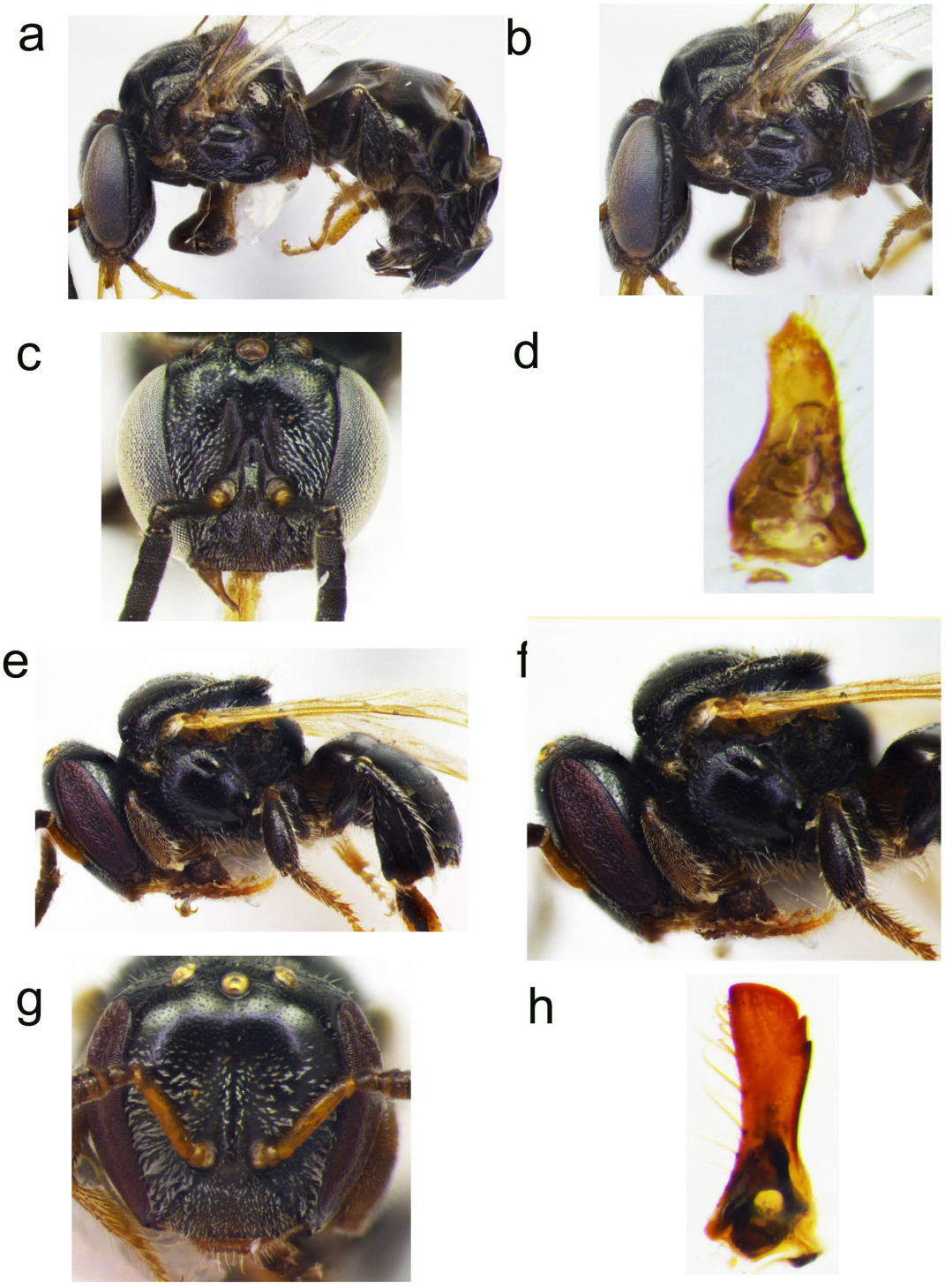

Fig. 2 *Lisotrigona kosumtaraensis* **sp. nov.** (a). Male lateral, (b). Head and thorax lateral (c). Head frontal (d). Mandible (e). Female. Lateral (f). Head and thorax lateral (g). Head frontal (h). Mandible

pterostigma, veins light brown. Mandibles ochraceous on the upper half with dark brown mottling on the basal half (Fig. 1e) All legs dark reddish-brown except the coxae, trochanters, and tarsi light brown. Metasomal terga black with light brown mottling on T2 to T4 (Fig. 1d); metasomal sterna dark reddish-brown.

Pilosity. Labrum fringed with long ochraceous hairs. Clypeus, the lower part of face covered with yellowish plumose tiny hairs without obscuring underlying integument; the upper and middle part of the face with fine yellowish hairs (Fig. 1c); vertex with brownish erect hairs. Mesoscutum, scutellum clothed with whitish fine hairs; mesoscutellar margin fringed with long whitish hairs (Fig. 1b, d). Mesepisternum clothed with white plumose hairs while metepisternum largely bare with a few long white hairs on the lower part (Fig. 1b). Propodeum with white, plumose short hairs on both lateral areas. Coxae, trochanters heavily fringed with yellowish hairs while femora, tibiae covered with short white hairs. The upper surface of the hind tibia with sparse white hairs while anterior and posterior margins fringed with white hairs. Metasomal terga T1 to T4 with sparse simple hairs along apical margins; apical terga with a transverse row of fine hairs near the apical margin; lateral and apical margins of terminal terga with a few long, stiff hairs.

Integument. Clypeus, entire face, vertex distinctly punctate. Integument surface in between antennal sockets distinctly depressed. Mesoscutum and pleural area punctate densely; mesoscutellum shiny, finely punctate. The middle part of propodeum imbricate; metasomal terga sparsely punctate otherwise glabrous.

Morphometry. Males measure a mean of 3.20 ± 0.01 mm in body length and 1.14 ± 0.02 mm in head width including eyes (Table 1). Head length 0.88 ± 0.01 mm; upper interocular distance 0.67 ± 0.03 mm; interocellar distance 0.29 ± 0.01 mm; diameter of median ocellus 0.13 mm; malar space length 0.02 mm; wing length 2.65 mm; wing width 1.00 mm; wing diagonal length 0.71 ± 0.01 mm; hind tibial length 0.80 mm and hind basitarsus length 0.39 mm. The ratio of head length to head width 0.77 (Table 2); eye length to upper interocular distance 1.25; interocellar distance to cello-ocular distance 3.22; scape length to eye length 0.39; forewing diagonal length to head width 0.63; hind tibial width to hind tibial length 0.33; hind basitarsus width to hind tibial width 0.54.

Metasomal sterna and genitalia. The following description is based on dissection of two male bees. Metasomal sternum 4, 0.25 mm long and 0.95 mm wide (Table 3); gradulus briefly touches the antecosta in the middle; lower 1/3rd area with setae that do not extend beyond the apical margin (Fig. 3 A-S4). Sternum 5 measures 0.13 mm long and 0.82 mm wide; gradulus touches the antecosta in the middle; apical margin weakly bisinuate with long setae arising from the margin (Fig. 3 A-S5). Antecosta in sternum 6 nearly straight in the middle region; the apicomedian lobe 0.20 mm long and 0.23 mm wide, broadly rounded at the apex; apodemal lobes moderately separated by a distance of 0.55 mm (Fig. 3 A-S6). Genitalia yellowish brown except the apical half of penis valves dark reddish-brown; if not exerted, lie within the abdomen cavity occupying from sternum 3 to 6. Each gonocoxa with elongate, slightly curved basal extension the apex of which curved inwards and then upwards resembling a flipped J-shape (Fig. 4 A-1); both gonocoxae with the widest region at the upper 1/3rd where both nearly touch each other, then separated and again coming close at the basal curved region. gonocoxae measure 0.81 mm long with a maximum width of 0.35 mm. Penis valves arise from the anterior margin of gonocoxae, 0.18 mm wide at the base, 0.50 mm long, gently curved with pointed curved apices (Fig. 4 A-1, A-2). Each gonostylus arises from basal 1/3rd length of gonocoxa, elongate (1.06 mm), tapering towards the terminal end with bluntly rounded, curved apex from where two long curved setae arise and short setae on the lateral side of terminal 1/3rd region (Fig. 4 A-3).

Females:

Coloration. Head and mesosoma black while metasoma dark reddish to black (Fig. 1f, g). Labrum, clypeus, compound eyes reddish brown to blackish brown. Antennal socket, scape, and its basal bulb yellowish-brown to dark reddish-brown; flagellar

Table 2. Ratios* of body parts of two new species of *Lepidotrigona* and holotypes of *L. chandrai, L. cacciae* and *L. revanai*

↓Ratio / Species→	*L. darbhaensis* **sp.nov.**		*L. kosumtaraensis* **sp.nov.**		*L. chandrai***		*L. cacciae****	*L. revanai***
	Male	Female	Male	Female	Male	Female	Holotype (Female)	Holotype (Female)
	n-2	n-05	n-05	n-05	--	--	--	--
Head length / Head width	0.77 ± 0.01	0.79 ± 0.01	0.78 ± 0.02	0.77 ± 0.06	0.75	0.72	0.85	0.82
Eye length / Upper interocular distance	1.25 ± 0.05	1.10 ± 0.05	1.24 ± 0.01	1.12 ± 0.02	1.21	1.09	1.11	1.13
Interocellar / Ocello-ocular distance	3.22 ± 0.11	1.93 ± 0.06	2.90 ± 0.19	1.92 ± 0.05	2.00	1.44	1.50	1.61
Scape length / Eye length	0.39 ± 0.01	0.50 ± 0.02	0.38 ± 0.02	0.48 ± 0.04	0.46	0.59	0.45	0.53
Forewing length / Forewing width	2.65 ± 0.00	2.72 ± 0.05	2.85 ± 0.24	2.75 ± 0.13	2.68	2.71	2.79	2.59
Forewing diagonal length / Head width	0.63 ± 0.01	0.62 ± 0.01	0.69 ± 0.01	0.61 ± 0.03	0.83	0.55	0.61	0.61
Hind tibial length / Head width	0.70 ± 0.01	0.77 ± 0.01	0.70 ± 0.01	0.76 ± 0.06	0.69	0.68	0.72	0.75
Hind tibial length / Forewing diagonal length	1.13 ± 0.01	1.24 ± 0.02	1.01 ± 0.02	1.21 ± 0.06	1.12	1.23	1.18	1.23
Hind tibial width / Hindi tibial length	0.33 ± 0.01	0.39 ± 0.01	0.33 ± 0.01	0.41 ± 0.02	0.28	0.41	0.36	0.34
Hind basitarsus width / Hind tibial width	0.54 ± 0.01	0.57 ± 0.00	0.58 ± 0.01	0.56 ± 0.05	0.57	0.69	0.58	0.72

* All measurements in mm ± SD, **: Data based on Viraktamath & Sajan Jose (2017); ***: Data based on Rasmussen (2013)

segments blackish-brown on the upper side but lighter on the lower side. Ocelli shiny, yellowish-brown (Fig. 1i). Mandibles reddish-brown except for the lower 1/3rd mottled with dark reddish-brown (Fig. 1j). Tegula yellowish-brown; wings hyaline with pale brown pterostigma and veins. Legs dark reddish-brown except for the coxae, trochanters, and tarsi pale yellowish-brown; hind basitarsus with a reddish-brown broad longitudinal stripe in the middle. Metasomal T1 to T4 light brown anteriorly, dark brown posteriorly while the T5-T6 darker (Fig. 1h). In some all metasomal terga dark brown to black. Metasomal sterna yellowish-brown anteriorly and dark brown posteriorly.

Pilosity. Labrum fringed with long yellowish hairs. Clypeus, lateral areas of face clothed with white plumose short hairs without obscuring the underlying integument. Middle and upper part of the face with fine white hairs (Fig. 1i); vertex with brownish erect hairs. Mesoscutum, mesoscutellum with fine white hairs while the mesoscutellum fringed with long yellowish hairs. Anterior half of mesepisternum with short, plumose white hairs density and length of which increases on the lower quarter; metepisternum largely bare on the upper half while the lower half with fine short white plumose hairs (Fig. 1g). Coxae, trochanters heavily fringed with yellow hairs. The upper part of the hind tibia with sparse white hairs; anterior and posterior margins fringed with plumose white hairs. Metasomal T1-T4 with few short white hairs arranged in the transverse band, the density and length increasing on the terminal terga; sterna with a dense transverse band of short hairs on each with density increasing on the terminal segments.

Integument. Similar to the males.

Morphometry. Females measure 3.10 ± 0.14 mm in body length and 1.18 ± 0.02 mm in head width (Table 1); head 0.94 ± 0.01 mm long; upper interocular distance 0.75 ± 0.02 mm while interocellar distance 0.28 ± 0.00 mm; malar space length 0.01 mm; forewing 2.59 ± 0.05 mm long, 0.97 ± 0.03 mm wide; forewing diagonal length 0.74 mm ± 0.01 mm; hind tibial and hind basitarsus 0.89 ± 0.02 and 0.44 ± 0.01 mm long and 0.35 ± 0.01

Fig. 3 Metasomal sterna: (A). *Lisotrigona darbhaensis* **sp. nov.** (B). *L. kosumtaraensis* **sp. nov.** and (C). *L. chandrai* (S4). Sternum 4, (S5). Sternum 5, (S6). Sternum 6

Table 3. Morphometry of male metasomal sterna and genitalia structures of two new species of *Lisotrigona* in comparison with male paratypes of *L. chandrai*

↓Parameter / Species→	*L. darbhaensis* **sp. nov.**	*L. kosumtaraensis* **sp. nov.**	*L. chandrai*
	n-05	n-05	n-03
Length of sternum 4	0.25	0.28 ± 0.02	0.27 ± 0.04
Width of sternum 4	0.95	1.03 ± 0.05	1.04 ± 0.02
Length of sternum 5	0.13	0.15 ± 0.00	13.5 ± 0.01
Width of sternum 5	0.82	0.93 ± 0.09	0.89 ± 0.04
Width of sternum 6	0.55	0.60 ± 0.02	0.58 ± 0.00
Length of the median lobe of sternum 6	0.20	0.19 ± 0.01	0.18 ± 0.00
Width of the median lobe of sternum 6	0.23	0.19 ± 0.01	0.15 ± 0.00
Length of gonocoxa	0.81	0.83 ± 0.04	0.79 ± 0.06
Width of gonocoxa at the mid-region	0.35	0.35 ± 0.03	0.41 ± 0.02
Length of penis valve	0.50	0.57 ± 0.04	0.54 ± 0.04
Width of penis valve at the base	0.18	0.18 ± 0.02	0.17 ± 0.02
Length of gonostylus	1.06	1.06 ± 0.22	0.87 ± 0.04

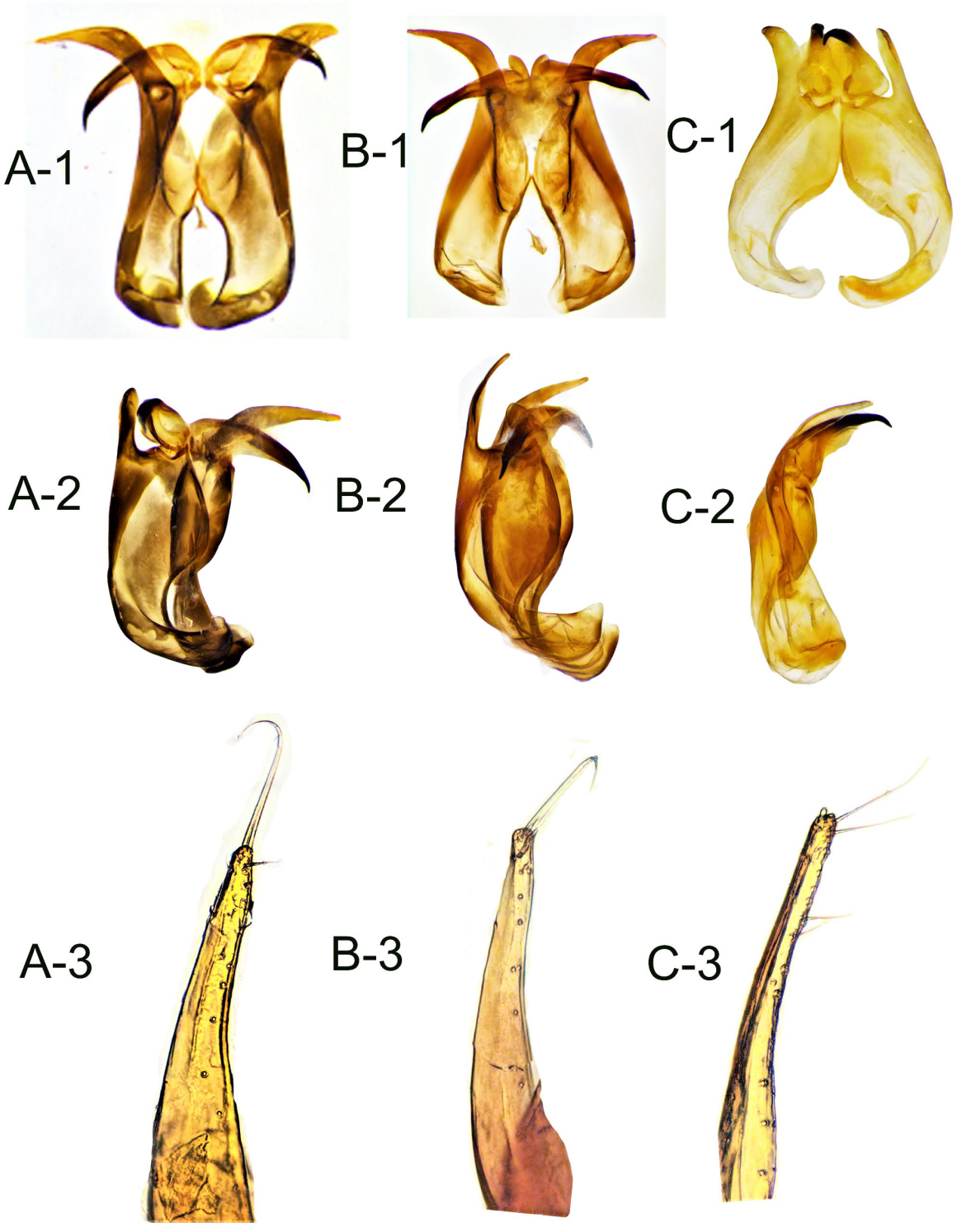

Fig. 4 Male genitalia: (A). *Lisotrigona darbhaensis* **sp. nov.** (B). *L. kosumtaraensis* **sp. nov.** (C). *L. chandrai*, (1). Genitalia ventral view, (2). Lateral view (3). Terminal part of gonostylus

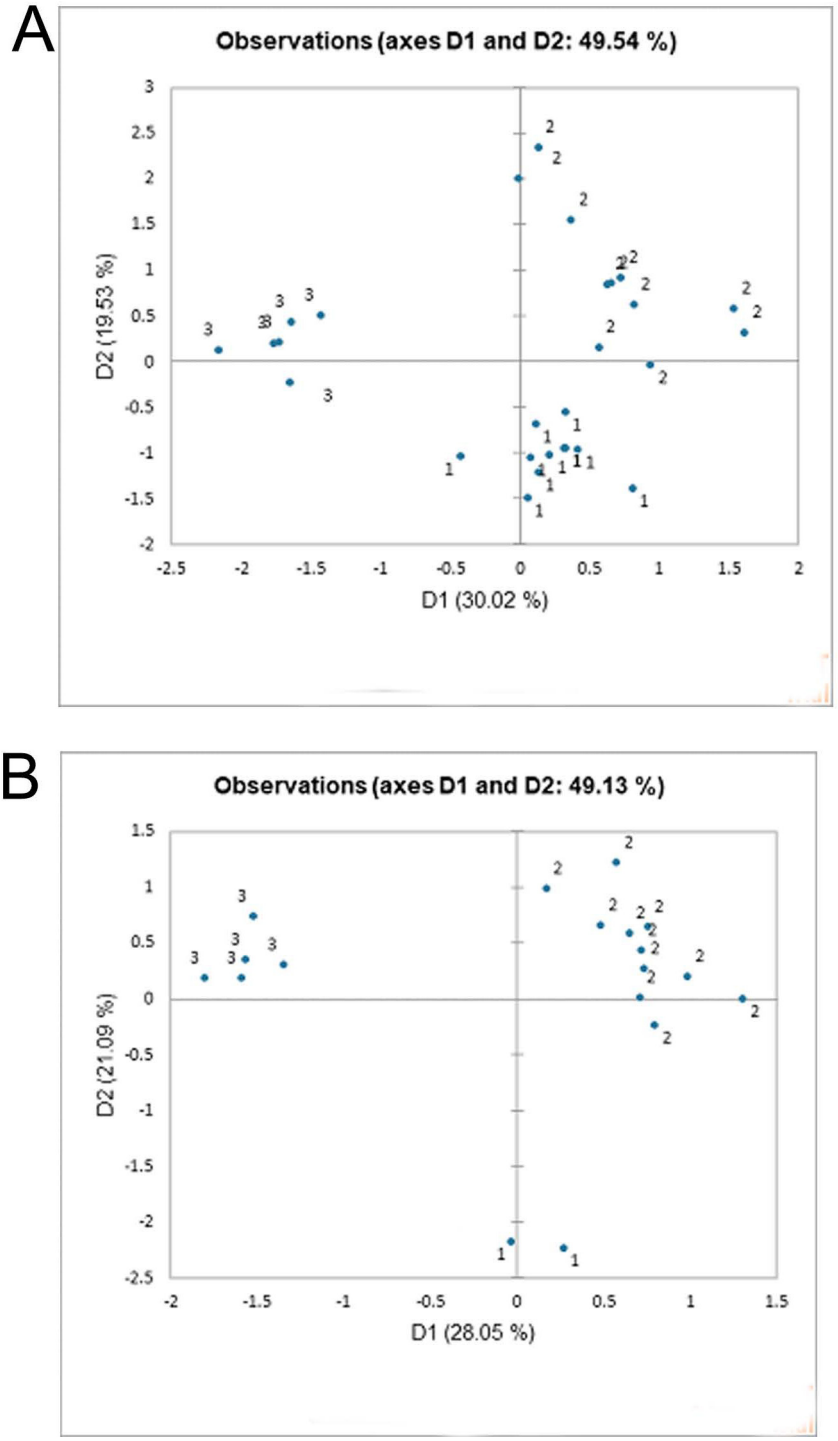

Fig. 5 Clusters of (1) *Lisotrigona darbhaensis* **sp. nov.** (2) *L. kosumtaraensis* **sp. nov.** and (3) *L. chandrai* on PCA plots formed by using 36 morphological traits. (A) Females, (B) Males

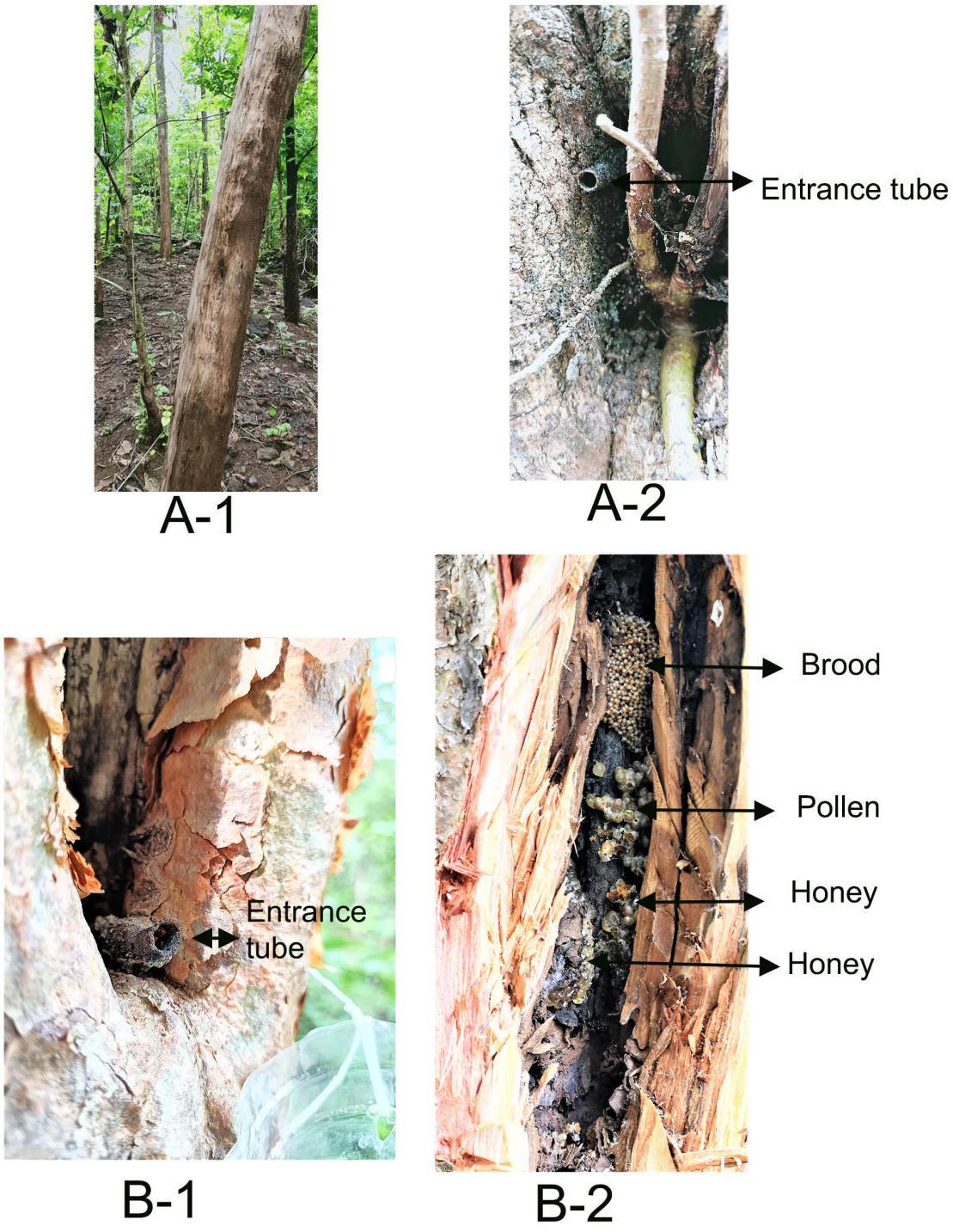

Fig. 6 Nests of (A) *Lisotrigona darbhaensis* **sp. nov.** and (B) *L. kosumtaraensis* **sp. nov.** (A-1) Nest in teakwood tree (*Tectona grandis*), (A-2) Close view of the colony showing entrance tube, (B-1) Nest in Indian frankincense tree (*Boswellia serrata*), (B-2) Inner structure of the nest showing brood, pollen and honey areas

and 0.21 ± 0.01 mm wide, respectively. Ratio of head length to width 0.79 ± 0.01 (Table 2); interocellar to ocello-ocular distance 1.93 ± 0.06; forewing diagonal to head width 0.62 ± 0.01; hind tibial length to forewing diagonal length 1.24 ± 0.02; hind basitarsus width to hind tibial width 0.57.

Nest. A single colony of *L. darbhaensis* was found in a trunk of a young teak wood tree (*Tectona grandis*) at Darbha, Chhattisgarh (Fig. 6 A-1). The colony had an entrance tube of 2 cm in length with a round opening of 1 cm in diameter (Fig. 6 A-2). The bees were very shy and stopped foraging with a slight disturbance.

Material examined. Holotype 1♂ adult. Chhattisgarh: Darbha ((18.85° N; 81.8689° E, Altitude 557 m a.s.l), 17. xi. 2020, leg. Shubham Rao with genitalia stored in genitalia vial pinned to the same pin, deposited at UASB. *Paratypes*: 1♂, 13♀ with the same collection data deposited at UASB; 1♀ paratype to be deposited at ZSIK.

Etymology. This species is named after the type locality Darbha.

***Lisotrigona kosumtaraensis* Viraktamath and Jagruti sp. nov.**

LSIDurn:lsid:zoobank.org:act:1A5D7F3B-048C-49E1-B588-67433B0B7682

Diagnosis. This species is larger than *L. darbhaensis* with males measuring a mean of 3.32 mm in body length and 1.14 mm in head width; forewings 2.82 mm long and 0.99 mm wide. The species is distinct in the following aspects: setae arising from basal area of gradulus of the metasomal sternum 4 extend beyond the apical margin (Fig. 3 B-S4); apical margin of metasomal sternum 5 nearly straight (Fig. 3 B-S5); antecosta in metasomal sternum 6 distinctly convex medially (Fig. 3 B-S6); apicomedian lobe of the metasomal sternum 6 as long as broad with broadly rounded apex; gonocoxae elongate and narrower, flipped J-shaped as in *L. darbhaensis* (Figs. 4. B-1, B-2); penis valves with distinctly curved pointed apices as in *L. darbhaensis,* and *L. chandrai* (Figs. 4 B-2); gonostyli long and wider. Female bees with distinct punctures on mesoscutum as in *L. darbhaensis, L. chandrai* and *L. revanai*.

Description. Males:

Coloration. Head, mesosoma and metasoma black (Fig. 2 a,b). Labrum yellowish-brown to dark reddish-brown; clypeus dark reddish-brown; scape, pedicel, flagellar segments dark reddish-brown approaching to black except for the basal bulb of scape and antennal sockets light reddish-brown. Pedicel and flagellar segments brownish-black. Ocelli shiny, light reddish-brown; compound eyes brown to dark reddish-brown approaching black, but in some appearing greyish black in frontal view (Fig. 2c). Mandibles ochraceous with light brown mottling in the basal and middle region (Fig. 2d). Pronotal lobe, tegulae light reddish-brown. Wings hyaline with brownish pterostigma and veins. Mesoscutellum light reddish-brown to black. Legs dark reddish-brown except for coxae, trochanter, and tarsi ochraceous. Metasomal terga with light brownish irregular mottling; sterna ochraceous medially

Pilosity. Labrum with sparse white hairs. Clypeus clothed with fine white plumose hairs without obscuring underlying integument. The lower part of the face with white plumose prominent hairs while the upper part with fine white hairs (Fig. 2c). Vertex with white erect hairs. Mesoscutum covered with fine white hairs. Mesoscutellum fringed with long white hairs. Coxae, trochanters, tarsi heavily fringed with long yellowish hairs while femora, tibiae are clothed with short white hairs; Upper surface of the hind tibia with sparse white hairs while anterior margin fringed with white hairs and posterior margin with dense plumose white hairs. The anterior and lower part of mesepisternum, the lower part of metepisternum with white, plumose long hairs; the lateral surface of propodeum with dense short white plumose hairs (Fig. 2b). Posterior margin of metasomal terga and sterna with a transverse band of very fine white hairs the density and length of which increasing towards terminal metasoma.

Integument: Clypeus, entire face, vertex distinctly punctate. Integument surface in between antennal sockets distinctly depressed. Mesoscutum, mesoscutellum, pleural area punctate densely;

mesoscutellum shiny, finely punctate. The middle part of propodeum imbricate; metasomal terga sparsely punctate otherwise glabrous.

Morphometry. Male bees measure a mean of 3.32 ± 0.20 mm long with 1.14 ± 0.03 mm wide head; head length 0.89 ± 0.04 mm; upper interocular distance 0.71 ± 0.02 mm; interocellar distance 0.29 ± 0.01 mm; diameter of median ocellus 0.14 ± 0.01 mm; forewings 2.82 ± 0.13 mm long, 0.99 ± 0.06 mm wide; forewing diagonal length 0.79 ± 0.01 mm; hind tibiae 0.80 ± 0.01 mm long, 0.26 ± 0.01 mm wide while the hind basitarsus 0.41 ± 0.01 mm long, 0.15 mm wide (Table 1). Mean ratio of head length to width 0.78; eye length to upper interocular distance 1.24; interocellar to ocello-ocular distance 2.90; scape length to eye length 0.38; forewing diagonal length to head width 0.69; hind tibial width to length 0.33 and hind basitarsus width to hind tibial width 0.58 (Table 2).

Metasomal sterna and genitalia. The following description is based on the dissection of five male paratypes. Sternum 4 measures 0.28 mm long, 1.03 mm wide (Table 3); gradulus touching the antecosta briefly at the medial region; lower 1/3rd area of the sternum with long setae extending beyond the apical margin (Fig. 3 B-S4); Sternum 5 densely pigmented, 0.13 mm long with widely separated apodemal lobes (0.93 mm); gradulus in touch with antecosta medially; apical margin nearly straight with long setae arising submarginally (Fig. 3 S-5); in sternum 6, the antecostal margin distinctly convex medially; apicomedian lobe as long as broad (0.19 mm) with broadly rounded apex (Fig. 3 S-6). Genitalia yellowish brown except the terminal half of penis valves dark reddish-brown; each gonocoxa with an incurved basal extension (0.83 mm long) the terminal part of which curved inwards and then upwards resembling a flipped J-shape (Figs. 4 B1, B2); broadest (0.35 mm) at the apical half where both gonocoxae come in close contact; penis valves arise from the anterior margin of gonocoxae, 0.18 mm wide at the base, tapering towards apical region terminating in curved pointed apex (Figs. 4 B-2); gonostylus arises from the lateral side at basal 1/3rd of each gonocoxa, wider at the midlength and tapering terminally with broad rounded, curved apex; two long and curved setae arise from the apical margin; a circlet of short spines submarginally and a longitudinal row of short spines along the terminal half (Fig. 4 B3); each gonostylus 1.06 mm in length.

Females:

Coloration. Head, mesosoma, metasoma black (Fig. 2 e,f.) Labrum reddish brown; clypeus dark reddish-brown; compound eyes blackish brown; ocelli shiny with brownish tinge; scape reddish-brown to black except for basal bulb and antennal socket light reddish-brown; flagellar segments blackish-brown on the upper side but light brown on the lower side (Fig. 2g); mandibles reddish-brown with dark reddish-brown mottling on basal 1/3rd (Fig. 2h) Pronotal lobe, tegula yellowish-brown; wings hyaline with reddish-brown tinge; pterostigma, veins light brown. All legs black except coxae, trochanters, and tarsal segments reddish-brown; hind basitarsus with the broad reddish-brown longitudinal band.

Pilosity. Labrum fringed with reddish-brown long hairs. Clypeus with fine white hairs; lower part of the face with distinct plumose white hairs; (Fig. 2g) vertex with short brownish erect hairs; gena, post gena fringed with sparse long white hairs; occipital area with fine white hairs. Mesoscutum and mesoscutellum with fine white hairs; mesoscutellar margin fringed with long ochraceous hairs. Anterior half of mesepisternum with distinct, long plumose white hairs; metepisternum with sparse white hairs; the lateral surface of propodeum with thick short plumose white hairs (Fig. 2f). Metasomal tergum with a transverse band of white hairs near basal margin the density and length of which increases towards the caudal end; sterna with a very dense transverse band of yellowish hairs with density increasing towards caudal end.

Integument. Head, mesosoma and metasoma shiny. Clypeus with dense fine punctures; entire face, vertex with distinct punctures; gena glabrous. Mesoscutum, pleural area, mesoscutellum with dense minute punctures. Middle area of propodeum imbricate. Metasomal terga with very sparse punctures otherwise glabrous.

Table 4. Differentiating characters among the males of known species of *Lisotrigona* from the world

↓Parameter / Species→	*Lisotrigona darbhaensis* **sp. nov.**	*Lisotrigona kosumtaraensis* **sp. nov.**	*Lisotrigona chandrai*	*Lisotrigona furva* ----
Metasomal sternum 4	Gradulus briefly touches antecosta medially	Gradulus briefly touches antecosta Medially	Gradulus does not touch antecosta	
	Setae from gradulus do not extend beyond the apical margin	Setae from gradulus extend beyond the apical margin	Setae from gradulus extend beyond the apical margin	
Metasomal sternum 5	Gradus briefly touches antecosta medially	Gradus briefly touches antecosta medially	Gradulus does not touch but is very close to the antecosta.	Apical margin distinctly inverted U-shaped
	Apical margin weakly bisinuate	Apical margin weakly bisinuate	Apical margin distinctly bisinuate	
Metasomal sternum 6	Antecosta straight in the middle	Antecosta distinctly convex in the middle	Antecosta distinctly concave in the middle	Antecosta weakly concave
	Apicomedian lobe wider than long	Apicomedian lobe as long as broad	Apicomedian lobe longer than wide	Apicomedian lobe longer than wide
Gonocoxa	Elongate, narrower, flipped J-shaped	Elongate, narrower, flipped J- shaped	Shorter, wider, C-shaped	Shorter, wider, C-shaped
Penis valve	Elongate with curved pointed terminally	Elongate with curved pointed terminally	Elongate with curved pointed terminally	Broader with straight, slender apically
Gonostylus	Long, broader with bluntly rounded apex	Long, broader with bluntly rounded apex	Long, broader with bluntly rounded apex	Shorter, very slender

Morphometry. Female paratypes measure 3.12 ± 0.25 mm, 1.19 ± 0.04 and 0.94 ± 0.04 mm in body length, head width and head length, respectively (Table 1); upper interocular distance 0.79 ± 0.02 mm; interocellar distance 0.28 ± 0.00 mm; diameter of median ocellus 0.12 ± 0.00 mm; forewings 2.64 ± 0.09 mm long, 0.96 ± 0.03 mm wide; wing diagonal length 0.74 ± 0.01 mm; hind tibia and hind basitarsus 0.91 ± 0.03 and 0.48 ± 0.02 mm long, respectively. Ratio of head length to width 0.77; interocellar to cello-ocular distance 1.92; forewing diagonal length to head width 0.61; hind tibial length to forewing diagonal length 1.21; hind basitarsus width to hind tibial width 0.56 (Table 2).

Nest. One colony of *L. kosumtaraensis* was found in a vertical cavity (54.40 x 5.6 cm) of an Indian frankincense tree at a height of 124 cm from the ground (Fig. 6 B-1). The colony had a long, dark brown entrance tube of 6.9 cm in length with an oval opening of 0.6 x 0.8 cm. The inner surface of the entrance tube was thin, but the exterior surface was rough made of propolis. Brood was a mix of spheroid and ellipsoid cells, arranged in a cluster in the middle of the cavity (Fig. 6 B-2). The brood cell was 2.24 ± 0.09 mm wide and 2.72 ± 0.36 mm in height. All the brood cells were connected by thin cerumen connectives and to the cavity by the pillars. Honey cells were located above the brood area as well as at the bottom of the cavity. Pollen cells were found just below the brood (Fig. 6 B-2). Each honey and pollen cell measured 0.84 ± 0.25 and 0.87 ± 0.23 mm in width and 1.10 ± 0.18 and 1.11 ± 0.16 mm in length, respectively. The colony consisted of 621 female and 40 male bees which formed 4.16 percent of the total population of the bees.

Material examined. Holotype: 1♂ adult. Maharashtra: Kosumtara, (21°16' 6"N; 80° 32' 37" E, Altitude 355 m.s.l), 25. vii. 2021, leg. Jagruti Roy deposited at UASB. *Paratypes*: 13♂, 13♀ with same collection data; 7♂, 2♀ with same data but collected on 16. vii. 2021, 10♂, 9♀ collected at Maharashtra: Navatolla (21° 16' 52"N; 80° 33' 36" E), 16. vii. 2021, leg. Jagruti Roy, deposited at UASB; 1♀ paratype to be deposited at ZSIK.

Etymology. This species is named after the type locality Kosumtara.

***Lisotrigona chandrai* Viraktamath and Sajan Jose, 2017**

Lisotrigona chandrai was described and illustrated by Viraktamath and Sajan Jose (2017) from the type locality Kanhangad, Kerala. Detailed morphometry of male and female type specimens is presented in table 1 (Viraktamath and Sajan Jose 2017) and the ratios of body parts in table 2. Male metasomal sterna provide important diagnostic characters along with genitalia structures to identify the species (Attasopa *et al*., 2018). However, Viraktamath and Sajan Jose (2017) did not describe the male metasomal sterna of *L. chandrai* in their publication. Hence, we describe these sterna to compare with all the known species of *Lisotrigona* after re-examining the type specimens deposited at the UASB.

Description of metasomal sterna. Sternum 4 is lightly pigmented with gradulus not touching the antecosta; setae arising from gradulus extend beyond the apical margin (Fig. 3 C-S4); sternum 5 lightly pigmented, 0.15 mm in length, 0.93 mm in width; gradulus very close to antecosta without touching it medially; short setae arise from most of gradulus (Fig. 3 C-S5); the apical margin distinctly bisinuate; sternum 6 lightly pigmented except lateral apodemal lobes and apicomedian lobe; antecosta distinctly concave medially; the apicomedian lobe longer (0.18 mm) than wide (0.15 mm) with broadly pointed apex (Fig. 3 C-S6).

DISCUSSION

Stingless bees that belong to the genus *Lisotrigona* are rare (Engel 2000; Viraktamath *et al*., 2021b) and occur in India and Southeast Asia. *Lisotrigona cacciae* the type species of the genus described from India is also reported from Thailand, Borneo, and Sri Lanka (Michener 2007; Karunaratne *et al*. 2017). Engel (2000) described *L. carpenteri* from Vietnam, Cambodia, and *L. furva* from Thailand. However after 22 years males of *L. carpenteri* were discovered and the male genitalia were found unique and different from those of other known species of *Lisotrigona.* Hence, Engel et al (2022) transferred *L. carpenteri* to a new genus *Ebaiotrigona* with *L. carpenteri* as type species of the new genus. So far five species of *Lisotrigona* are known in the world. Michener (2007) compared the populations of *L. cacciae* and *L. furva* from Thailand and reported that both species are extremely similar and all the characters (except the head width) which seemed to differentiate both species failed. Engel (2000) reviewed the genus *Lisotrigona* from Indo-Malayan region and compared *L. cacciae* and *L. scintillans* (Cockerell). Since he did not find differences in size, integument sculpturing and major differences in coloration, he synonymized *L. scintillans* with *L. cacciae.* Interestingly no efforts have been made to collect and describe males, queens, and nest structure of *L. cacciae* which is reported to be very common in Thailand and Southeast Asia.

In India, besides *L. cacciae,* three species of *Lisotrigona* namely, *L. mohandasi, L. revanai,* and *L. chandrai* were described (Jobiraj and Narendran 2004; Viraktamath and Sajan Jose 2017). However, Rasmussen *et al.* (2017) synonymized these species with *L. cacciae* though Viraktamath and Sajan Jose (2017) provided critical differences in morphometry, male genitalia structures among known species of *Lisotrigona* besides describing queen and nest structure for *L. chandrai*.

Recent efforts in the collection of large samples of *Lisotrigona* female bees from feral colonies and analysis of morphometric data of these samples indicated the occurrence of more than one species in India (Viraktamath *et al.* 2021b). The authors success in collecting male bees in association with females from two states (Chhattisgarh and

Maharashtra) and the study of morphometry of 36 parameters, structure of male metasomal sterna, male genitalia along with mapping of the species on a PCA plot, revealed the distinctiveness of these bees which led to the description of *L. darbhaensis* and *L. kosumtaraensis* as new species. These two new species are different from each other as well as other known species of *Lisotrigona* in morphometry, the structure of male metasomal sterna and genitalia as enumerated in (Tables 1 to 4 and Figs. 3, 4; also see the diagnostic characters under *L. darbhaensis*).

Female bees are distinctly punctate on mesoscutum of *L. darbhaensis, L. kosumtaraensis, L. revanai,* and *L. chandrai.* However, *L. cacciae* has exceedingly minute faint punctures while *L. furva* has strong punctures. Both the new species differ from *L. scintillans* in having the face, vertex, mesoscutum, abdomen clothed with short white hairs while in *L. scintillans,* these body parts are not hairy except little pale hairs at the sides of face (Cockerell, 1920). The ratio of interocellar to ocello-ocular distance is higher in *L. darbhaensis* (1.93) and *L. kosumtaraensis* (1.92) while lower in *L. chandrai* (1.44), *L. cacciae* (1.50), and *L. revanai* (1.61). Analysis of morphometry data of both male and female bees of *L. darbhaensis, L. kosumtaraensis* and *L. chandrai* also confirmed the distinctiveness of these new species as all the three species formed distinct clusters on the PCA plots (Fig. 5A, B).

The colony strength of 961 bees (921 females + 40 males) in *L. kosumtaraensis* is an approximate estimation as it was not sure whether all the foraging bees had returned at the time of capturing the colony and hence not considered the brood cells. Interestingly male bees formed 4.16 percent of the total bee population. However, Viraktamath (unpublished) obtained only 6 males (2.68%) and 224 females when an entire colony of *L. chandrai* was captured.

The discovery of these two new species increases the number of *Lisotrigona* species to six from India. However, a critical study of male metasomal sterna, genitalia with associated female bees may reveal the underlying rich diversity of India which remains largely unexplored. The results strongly indicate the necessity of revision of the genus from the Indian subcontinent.

Based on the evidence of the present study, it is proposed that all the three species namely, *L. mohandasi, L. chandrai,* and *L. revanai* synonymized with *L. cacciae* by Rasmussen *et al.* (2017) need to be considered as valid and distinct from *L. cacciae*. The authors are also of the opinion that the occurrence of *L. cacciae* outside India needs to be verified by collecting males with correctly associated females and a critical study of male genitalia along with metasomal sternal structures.

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