



Two new species of *Tetragonula* (Hymenoptera: Apidae: Meliponini) from North-East India with notes on their nest structure

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Abstract

We describe *Tetragonula kyrdemkulaiensis* Viraktamath and Rojeet sp. n. with associated females and males from Kyrdemkulai, Meghalaya. This appears to be one of the largest species of the genus with male holotype measuring 5.20 mm in body length and 1.85 mm in head width, while female paratypes measuring 4.93 and 1.82 mm in body length and head width, respectively. Male gonostylus arises laterally and is gently clavate apically. Penis valve is robust with a characteristic shape. One nest was found in the soil at 90 cm depth with cells arranged in a cluster. We also describe *Tetragonula srikantanathi* Viraktamath sp. n. based on females from Salema, Tripura. Female holotype measures 3.50 mm in body length and 1.60 mm in head width, while the paratypes measure 3.58 and 1.59 mm in body length and head width, respectively, with the whole orange metasoma. One nest of this species was found in mud wall cavity with unique single-cell thick layer of brood combs which has not been reported in any other Indian species of *Tetragonula*. Both species were compared with the images and descriptions of primary type specimens of *Tetragonula* species occurring in the Indo-Malayan region and were found to be distinct in morphological characters, male genitalia and nest structure.

Keywords *Tetragonula kyrdemkulaiensis* · *Tetragonula srikantanathi* · Stingless bees · North-East India

Introduction

Stingless bees are one of the important groups of beneficial insects. Honey of stingless bees fetches a premium price because of its high medicinal value and taste (Crane 1999; Choudhari et al. 2012; Surendra et al. 2012; Rasmussen 2013). Heard (1999) reviewed the importance of stingless bees as pollinators and concluded that they play an important role in pollinating more than 60 cultivated crops besides many more other plant species.

More than 600 species of stingless bees are described in over 60 genera (Wille 1983; Michener 2000; Rasmussen and Cameron 2010). Recently, Rasmussen et al. (2017) listed 35 species of the genus *Tetragonula* from the Indomalayan-

Australasian regions. Indian stingless bees belong to three genera, namely *Tetragonula* Moure, 1961, *Lisotrigona* Moure, 1961 and *Lepidotrigona* Schwarz, 1939 of which *Tetragonula* is the most dominant (Rasmussen 2013). Among the 14 species of stingless bees reported from India, nine belong to *Tetragonula*, viz. *T. iridipennis* (Smith, 1854), *T. ruficornis* (Smith, 1870), *T. bengalensis* (Cameron, 1897), *T. laeviceps* (Smith, 1857), *T. gressitti* (Sakagami, 1978), *T. fuscobalteata* (Cameron, 1908), *T. calophyllas* Shanas and Faseeh, 2019, *T. perlucipinnae* Faseeh and Shanas, 2019 and *T. travancorica* Shanas and Faseeh, 2019 (Sakagami 1978; Rasmussen 2013; Viraktamath and Sajan Jose 2017; Shanas and Faseeh 2019), four species belong to *Lisotrigona* and one to *Lepidotrigona*.

Northeast India, located at the confluence of Indo-Malayan, Indo-China, Indo-Myanmar and Indo-Bangladesh borders and joining the foothills of Himalaya, is considered as hot spot of biodiversity (Chatterjee et al. 2006; Tripathi et al. 2016). It is composed of eight states, Assam, Arunachal Pradesh, Nagaland, Meghalaya, Manipur, Mizoram, Tripura and Sikkim. This region with its wide range of diverse climate, terrain and ecological habitats forms a gateway for rich variation in the flora and fauna of India

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(Myers 1988). Consequently, it is identified as the Indo-Burma hotspot that forms one of the 35 Global biodiversity hotspots (Myers 1988). The region harbours several endemic flora and fauna.

Of the five species of stingless bees reported from this area, four belong to the genus *Tetragonula* (Akum et al. 2012; Rahman et al. 2013; Rathor et al. 2013; Das et al. 2019). In this paper we describe two new species along with their nest characteristics from this region.

Materials and methods

During a survey to collect stingless bees in northeast India, we came across two feral colonies, one in a mud wall cavity at Salema, Tripura (24.01° N and 91.83° E) and the other in the soil at Kyrdemkulai, Meghalaya (25.44° N and 91.47° E). Samples of outgoing foraging bees from these two colonies were collected separately in specimen tubes containing ethyl acetate absorbed in a cotton pad. Later, we transferred these bees to vials containing 95% ethyl alcohol for preservation. Each vial was labeled with data containing the name of place, date, and collector's name. These vials were brought to the laboratory at the Department of Entomology, University of Agricultural Sciences, Bengaluru, Karnataka for detailed studies. Bees were sexed under a binocular stereoscopic microscope. Males were identified by the protruded gonostylus and penis valve at the posterior end of metasoma. We mounted a few bees on card points and the rest were preserved in vials containing 95% ethyl alcohol.

All bees were identified to the generic level by using the key of Rasmussen (2013). Morphological parameters selected for detailed studies were those used by Sakagami (1978), Ruttner (1988), Rasmussen (2013) and Viraktamath and Sajan Jose (2017). Morphometry of 34 and 38 morphological parameters in male and female bees, respectively, were studied for description of the species. Landmarks used for measuring various parameters in morphometry studies are presented in Table 1. Ten ratios of different morphological parameters (Sakagami 1978) were used in the description of the species. We followed the terminology used by Rasmussen (2013). Morphometry was studied with an Olympus (SZ 61) stereoscopic binocular microscope with 6.7 to 45× magnification fitted with ocular micrometer. Male genitalia, metasomal terga and sterna were studied by carefully detaching the metasoma from a card mounted male and gently heating it to the boiling point for a minute in a coning test tube containing 10% KOH solution in a water bath. Later, the metasoma containing genitalia was taken out from the tube, washed in distilled water, and dissected under the microscope in a cavity block to study the detailed structure. After the studies, all parts of the genitalia were placed in the metasoma using it as a storage bag and then transferred to a genitalia vial containing

glycerin. This vial was pinned back to the same pin with the card mounted male from which the metasoma was removed. Photographs were taken under Leica microscopes (M205C and DM2000) fitted with digital camera (DFC425) having a range of magnification from 7.8 to 160× and 40 to 400×, respectively. The images were later improved by using Photoshop.

The nest of *T. kyrdemkulaiensis* was excavated on 20.02.2019 and that of *T. srikantanathi* on 8.11.2017 separately. Both the nests were photographed separately by using a digital camera. Size of the entrance, length of the entrance tube, size of the nest cavity honey and pollen cells was recorded. The arrangement of the brood area, food stores and waste dumping area were also recorded.

Holotypes and paratypes are deposited at the Department of Entomology, University of Agricultural Sciences, Bengaluru (UASB). One paratype of each species is deposited at the Department of Entomology, Indian Agriculture Research Institute, New Delhi (IARID) and Zoological Survey of India, Kolkata (ZSIK).

Tetragonula kyrdemkulaiensis Viraktamath and Rojeet sp. n.

Diagnostic characters

One of the largest species with male holotype 5.20 mm in body length and 1.85 mm in head width; brownish black; clypeus very distinct in male with dense, dark brown closely appressed plumose hairs; flagellum uniformly dark brown both on upper and lower side; gonostylus arise from lateral side of gonocoxa, gently clavate at apex; penis valve robust with characteristic shape resembling upside-down sea horse. This species resembles *Tetragonula gressitti* in body coloration and in having a clavate gonostylus. But distinctly differs from it in having closely appressed plumose hairs on the clypeus and frons opposed to simple hairs in *T. gressitti*; mandibles normal (receded in *T. gressitti* see Fig. 107 of Sakagami 1978); shorter, weakly sinuate, clavate gonostylus (Fig. 3a) (very long, distinctly sinuate, clavate in *T. gressitti* see Fig. 82a of Sakagami 1978); penis valve robust, distinctly curved inwards near base, outwards near mid-length and then terminating into a sickle shaped structure apically (Fig. 3a, b) (penis valve rod like in *T. gressitti*); sternum 5 with distinct, inverted U shaped medio apical emargination (very narrow small emargination in *T. gressitti*) and medio apical process shorter and broader in sternum 6 (Fig. 3c) as against narrow and very long in *T. gressitti*.

Females measure 4.93 mm (4.60 to 5.20 mm) in body length and 1.82 mm (1.80 to 1.90 mm) in head width. They differ in morphometry from all primary type specimens of *Tetragonula* occurring in the Indian subcontinent with greater

Table 1 Landmarks used for measuring various parameters in morphometry studies of *Tetragonula kyrdemkulaiensis* sp. n. and *T. srikantanathi* sp. n

Parameters	Landmarks for measuring parameters
Length of body	Anterior margin of face to posterior margin of metasoma measured from lateral longitudinal axis of the body
Width of head including eyes	Outer margin of left compound eye to outer margin of right compound eye measured from dorsal side of head on transverse axis
Length of head	Apical margin of clypeus to anterior margin of median ocellus measured from frontal side
Length of eye	Distance between dorsal and ventral margin of eye measured on the mid-vertical axis of the eye
Width of eye	Distance between two anterior and posterior margins on the mid-longitudinal axis of the eye
Upper inter-ocular distance	Distance between inner margins of both compound eyes on the dorsal side
Diameter of median ocellus	Distance between outer margins of median ocellus on transverse axis
Inter-ocellar distance	Distance between inner margins of two dorsal ocelli on transverse axis
Ocello-ocular distance	Distance between outer margin of right dorsal ocellus and inner margin of right compound eye
Length of clypeus	Distance between apical and basal margin of clypeus
Maximum width of clypeus	Maximum distance between two lateral margins
Malar space length	Distance between ventral margin of left compound eye and basal margin of mandible
Length of scape	Distance between basal and apical margin of scape excluding basal bulb
Width of scape	Maximum distance between lateral margins
Length of pedicel+flagellum	Basal margin of pedicel to apical margin of terminal segment of flagellum
Length of flagellomere 1	Maximum length between basal and apical margin measured from lateral longitudinal axis
Length of flagellomere 2	Maximum length between basal and apical margin measured from lateral longitudinal axis
Length of flagellomere 3	Maximum length between basal and apical margin measured from lateral longitudinal axis
Width of flagellomere 3	Maximum diameter
Length of mandible	Distance between basal to apical margin of mandibular tooth
Width of mandible	Maximum distance between two lateral margins near the basal margin
Length of forewing+tegula	Basal margin of tegula to outermost margin of forewing along its longitudinal axis
Width of forewing	Maximum width between costal and anal margin measured along its transverse axis
Length of pterostigma	Distance between basal margin to apical margin of pterostigma
Length of marginal cell	Distance between basal margin to apical margin along with longitudinal axis
Width of marginal cell	Maximum distance between anterior and posterior margin along with transverse axis
Wing diagonal	Distance between bifurcation of M-Cu bifurcation and basal tip of marginal cell
Length of mesoscutum	Distance between anterior and posterior on mid-dorsal longitudinal axis
Maximum width of mesoscutum	Maximum distance between two lateral margins along with transverse axis
Length of hind tibia	Maximum distance between basal and apical margins along with longitudinal axis
Width of hind tibia	Maximum distance between two lateral margins along with transverse axis
Length of hind basitarsus	Maximum distance between basal and apical margins along with longitudinal axis
Width of hind basitarsus	Maximum distance between lateral margins along with transverse axis

values in length of body, head, eye, scape, antenna, mandible forewing, pterostigma, marginal cell, wing diagonal, hind tibia and hind basitarsus (Table 2). Similarly, width of head, clypeus, mandible, forewing, hind tibia and hind basitarsus have greater values.

Holotype: Male

Integument coloration. Head, thorax and metasoma dark brown to brownish black (Fig. 1a, b); labrum, clypeus reddish brown; scape, pedicel, first flagellar segment dark reddish

brown except basal bulb of scape light brown; flagellar segments dark brown on both upper and lower side; ocelli reddish brown with transparent areas within each ocellus; eyes light brown (Fig. 1a, b); mandibles dark brown basal 2/3rd, light brown apically (Fig. 2b). Thorax brownish black except pronotal lobe light brown and anterior half of scutellum ochraceous (Fig. 1b). Mesoscutum without distinct longitudinal bands of hairs. All legs reddish brown except trochanter and terminal four tarsal segments light brown. Wings with light brown tinge, anterior veins and pterostigma dark brown. Lateral areas of metasomal sterna light brown (Fig. 1a).

Table 2 Comparison of morphometry between primary type specimens* of female *Tetragonula* bees from Indian subcontinent with the *T. kyrdemkulaiensis* sp. n. and *T. srikantanathi* sp. n

Parameter	<i>T. iridipennis</i> (n = 1)	<i>T. praeterita</i> (n = 1)	<i>T. ruficornis</i> (n = 1)	<i>T. bengalensis</i> (n = 1)	<i>T. kyrdemkulaiensis</i> (n = 12) Mean $\pm$ SD	<i>T. srikantanathi</i> (n = 10) Mean $\pm$ SD
Length of body	3.55	3.33	3.45	3.55	4.93 $\pm$ 0.37	3.58 $\pm$ 0.06
Width of head including eyes	1.60	1.52	1.66	1.70	1.82 $\pm$ 0.03	1.59 $\pm$ 0.02
Length of head	1.10	1.04	1.13	1.13	1.37 $\pm$ 0.06	1.15 $\pm$ 0.01
Length of eye	1.10	1.04	1.13	1.13	1.27 $\pm$ 0.04	1.04 $\pm$ 0.01
Width of eye	0.40	0.40	0.42	0.43	0.45 $\pm$ 0.01	0.36 $\pm$ 0.01
Upper inter-ocular distance	1.00	1.04	1.02	1.02	1.11 $\pm$ 0.05	1.05 $\pm$ 0.00
Diameter of median ocellus	0.15	0.14	0.14	0.15	0.19 $\pm$ 0.01	0.14 $\pm$ 0.01
Inter-ocellar distance	0.33	0.35	0.38	0.38	0.38 $\pm$ 0.02	0.35 $\pm$ 0.00
Ocello-ocular distance	0.23	0.24	0.24	0.23	0.21 $\pm$ 0.01	0.22 $\pm$ 0.01
Length of clypeus	0.33	NA	0.39	0.33	0.41 $\pm$ 0.03	0.31 $\pm$ 0.02
Maximum width of clypeus	0.70	NA	0.71	0.65	0.87 $\pm$ 0.06	0.67 $\pm$ 0.03
Malar space length	0.03	NA	0.05	0.06	0.04 $\pm$ 0.01	0.03 $\pm$ 0.00
Length of scape	0.57	0.59	0.57	0.57	0.71 $\pm$ 0.02	0.56 $\pm$ 0.01
Width of scape	0.10	0.08	0.10	0.10	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00
Length of pedicel + flagellum	1.24	NA	1.25	1.30	1.53 $\pm$ 0.06	1.25 $\pm$ 0.04
Length of flagellomere 1	0.09	0.05	0.07	0.11	0.09 $\pm$ 0.00	0.07 $\pm$ 0.01
Length of flagellomere 2	0.07	0.12	0.11	0.11	0.14 $\pm$ 0.01	0.12 $\pm$ 0.01
Length of flagellomere 3	0.10	0.10	0.11	0.11	0.14 $\pm$ 0.01	0.12 $\pm$ 0.01
Width of flagellomere 3	NA	NA	NA	NA	0.14 $\pm$ 0.01	0.13 $\pm$ 0.00
Length of mandible	0.62	NA	0.62	0.60	0.79 $\pm$ 0.01	0.56 $\pm$ 0.02
Width of mandible	0.19	NA	0.21	0.18	0.29 $\pm$ 0.01	0.29 $\pm$ 0.02
Length of forewing + tegula	3.44	3.44	3.20	3.50	4.74 $\pm$ 0.10	3.76 $\pm$ 0.07
Width of forewing	1.32	NA	1.28	1.25	1.62 $\pm$ 0.08	1.27 $\pm$ 0.04
Length of pterostigma	0.48	0.48	0.51	0.48	0.69 $\pm$ 0.01	0.57 $\pm$ 0.03
Length of marginal cell	1.21	1.21	1.23	1.21	1.50 $\pm$ 0.04	1.27 $\pm$ 0.03
Width of marginal cell	0.30	NA	0.3	0.29	0.39 $\pm$ 0.01	0.29 $\pm$ 0.01
Wing diagonal	1.01	0.95	0.95	0.95	1.32 $\pm$ 0.04	1.00 $\pm$ 0.00
Hamuli	5	5	5	5	6.00 $\pm$ 0.00	5.00 $\pm$ 0.00
Length of mesoscutum	0.87	0.85	0.83	1.00	1.18 $\pm$ 0.03	0.91 $\pm$ 0.04
Maximum width of mesoscutum	1.01	1.00	1.05	1.13	1.25 $\pm$ 0.02	1.05 $\pm$ 0.01
Length of hind tibia	1.55	1.47	1.43	1.51	1.74 $\pm$ 0.03	1.32 $\pm$ 0.04
Width of hind tibia	0.54	0.54	0.52	0.54	0.61 $\pm$ 0.02	0.50 $\pm$ 0.01
Length of hind basitarsus	0.50	0.48	0.48	0.50	0.71 $\pm$ 0.07	0.57 $\pm$ 0.03
Width of hind basitarsus	0.29	0.27	0.27	0.29	0.37 $\pm$ 0.03	0.26 $\pm$ 0.02
Head length/head width	2.22	2.19	2.08	2.09	2.71 $\pm$ 0.02	2.25 $\pm$ 0.01
Eye length/ocular distance	1.10	1.04	1.11	1.11	1.14 $\pm$ 0.02	0.99 $\pm$ 0.01
Inter-ocellar distance/ocular distance	0.33	0.34	0.37	0.37	0.34 $\pm$ 0.03	0.33 $\pm$ 0.02
Scape length/eye length	0.52	0.57	0.50	0.50	0.56 $\pm$ 0.03	0.54 $\pm$ 0.02
Forewing length/Forewing width	2.61	--	2.50	2.80	2.93 $\pm$ 0.13	2.96 $\pm$ 0.07
Wing diagonal/head width	0.63	0.63	0.57	0.56	0.73 $\pm$ 0.02	0.63 $\pm$ 0.01
Hind tibial length/head width	0.97	0.97	0.86	0.88	0.96 $\pm$ 0.02	0.83 $\pm$ 0.03
Hind tibial length/Wing diagonal	1.53	1.55	1.51	1.59	1.32 $\pm$ 0.03	1.32 $\pm$ 0.04
Hind tibial width/hind tibial length	0.35	0.37	0.36	0.36	0.35 $\pm$ 0.02	0.38 $\pm$ 0.02
Hind basitarsus width/hind tibial width	0.54	0.50	0.52	0.54	0.61 $\pm$ 0.03	0.52 $\pm$ 0.04

*Based on Rasmussen (2013). All measurements are in millimeters except for hamuli

Pilosity. Labrum and lateral margins of mandibles fringed with long golden yellow hairs; clypeus very distinct, with dense, dark brown closely appressed plumose long brown hairs (Fig. 2c); lower part of face with white short plumose hairs while upper part with dark brown hairs; vertex, occiput, lower part of occiput with erect yellow intermixed with dark brown long hairs. Pronotal lobes densely covered with long white plumose hairs; mesoscutal bands of hairs indistinct; lateral margin of mesoscutum fringed with short yellow hairs; scutellum densely fringed with long yellow hairs (Fig. 2a); mesepisternum clothed

with long plumose white hairs, the density increasing towards lower side; metepisternum and lateral part of propodeum densely clothed with short plumose hairs entirely obscuring underlying integument (Fig. 1b). Metasomal terga with sparse yellow hairs, the density increasing towards terminal terga; sterna with dense long yellow hairs.

Integument and other structures. Head, thorax, metasoma, legs shiny; clypeus, face, vertex, mesoscutum, mesoscutellum finely punctate, denser on head; antennae densely punctate with dark

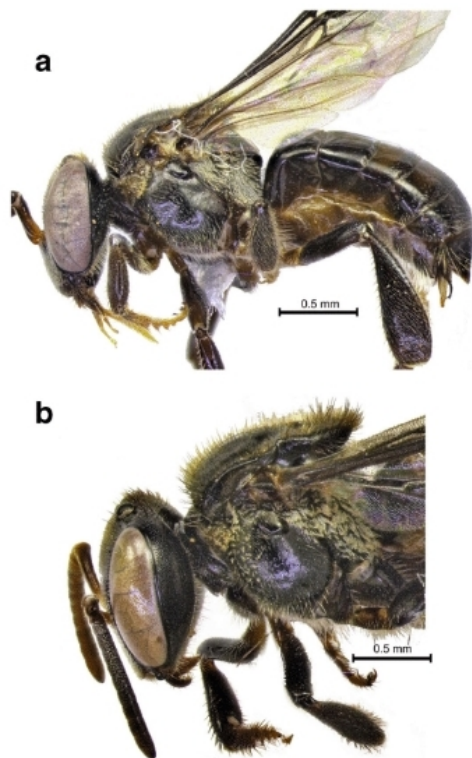


Fig. 1 *Tetragonula kyrdemkulaiensis* sp. n. Male holotype: **a** lateral habitus; **b** head, thorax closer lateral view

brown pits both dorsally and ventrally; anterior metasomal terga T1 to T3 sparsely punctate, density of which increasing on posterior terga. Legs densely punctate. Gradulus in touch with antecosta medially on sternum 5 (Fig. 3c); postgradular area with distinct dark brown punctures from which long golden yellow setae arise; apical margin fringed with long golden yellow hairs. Antecosta on sternum 6 sinuate medially, with medio apical process longer than wide with rounded apex (Fig. 3c).

Morphometry. Body 5.20 mm in length and 1.85 mm in width (Table 3). Head length 1.32 mm; upper interocular distance 1.05 mm; inter-ocular distance 0.38 mm; ocello-ocular distance 0.15 mm; clypeus length 0.5 mm, width 0.75 mm; mandible twice as long as wide (0.25 mm); forewing length 4.8 mm, width 1.45 mm; hamuli 6; hind tibia 1.6 mm long and 0.60 mm wide; hind basitarsus length 0.65 mm, width 0.35 mm.

Ratios of head length and width 0.71; eye length and upper inter-ocular distance 1.28; interocellar and ocello-ocular distance 2.53; scape length and eye length 0.41; forewing length and width 3.31; wing diagonal and head width 0.68; hind tibial length and head width 0.86; hind tibial length and wing diagonal 1.28; hind tibial width and length 0.38; hind basitarsus width and length 0.54.

Genitalia. Genitalia structures light brown to dark brown; gonocoxa wider than long (Fig. 3a); gonostylus not sclerotized,

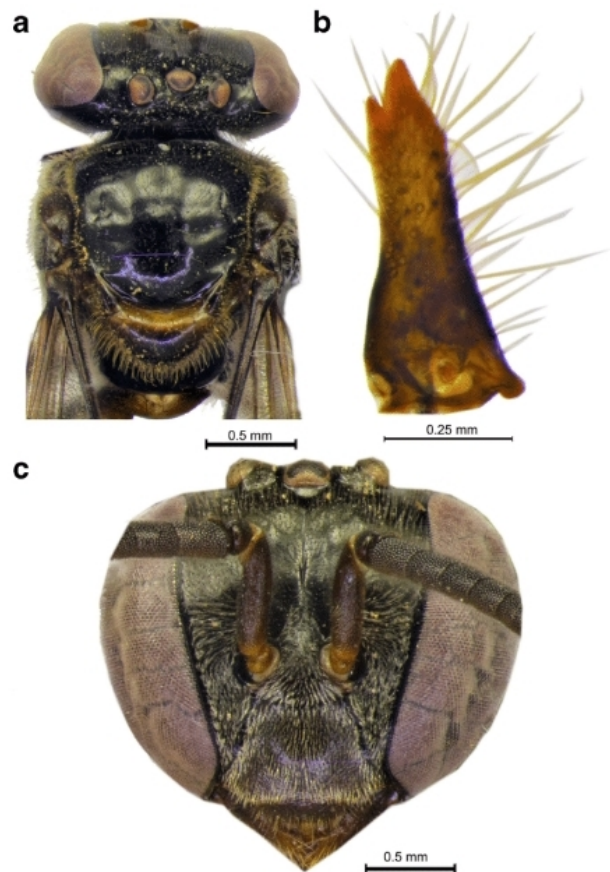


Fig. 2 *Tetragonula kyrdemkulaiensis* sp. n. Male holotype: **a** head, thorax dorsal view; **b** mandible; **c** head frontal view

arising from lateral side of gonocoxa, 0.86 mm long, gently sinuate at 2/3rd of its length, slightly clavate apically (Fig. 3a, b); each gonostylus 104 μ m wide at the base narrowing to 64 μ m after a short distance, then slightly widening to 77 μ m before narrowing again to 43 μ m; terminal clavate end 59 μ m wide. Distal 1/4th of the inner surface of gonostylus with a row of small slender setae; outer surface with subapical stout setae (Fig. 3b). Penis valve sclerotized resembling to the shape of an upside down seahorse; 0.75 mm long, robust, with characteristic bisinuate shape (Fig. 3a), 178 μ m wide at the base, gently curved inwards after a short distance, then widened to 199 μ m at the middle, curving outwards and then strongly curving inwards and gradually narrowing to 46 μ m before attaining a strongly curved sickle shaped structure terminally at 3/4th length of the valve (Fig. 3a, b).

Variation in male paratype. The male paratype is identical to the holotype in coloration, pilosity and integument sculpture but differs from the holotype in the following morphometry.

Body 5.00 mm in length and 1.80 mm in width. Width of clypeus 0.70 mm; length of mesoscutum 1.20 mm, width

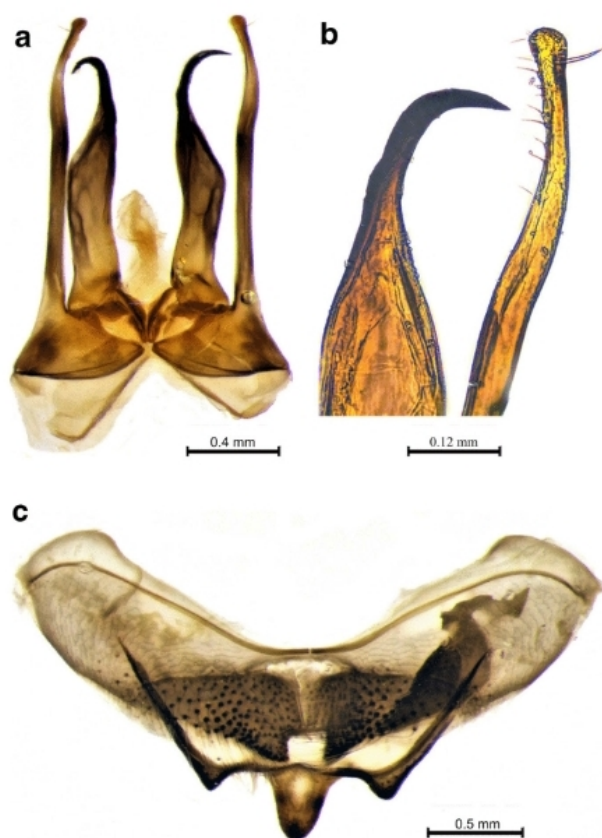


Fig. 3 *Tetragonula kyrdemkulaiensis* sp. n. Male holotype: **a** genitalia with gonocoxae, gonostyli, penis valves; **b** terminal parts of gonostylus, penis valve; **c** sterna 5 and 6

1.30 mm; forewing length 4.6 mm; hind tibiae 0.55 mm wide; length of marginal cell 1.55 mm, width 0.35 mm; hamuli 5; width of hind tibiae 0.55 mm; hind basitarsus length 0.60 mm.

Ratio of head length and width 0.73; eye length and upper inter-ocular distance 1.24; scape length and eye length 0.42; forewing length and width 3.17; wing diagonal and head width 0.69; hind tibial length and head width 0.89; hind basitarsus width and length 0.58.

Paratypes: Females

Coloration. Head, thorax, and metasoma brownish black to black (Fig. 4a, b); labrum chestnut brown; clypeus brownish black with yellowish apical margin (Fig. 5d); ocelli pale ochraceous; scape chestnut brown, apical 2/3rd with blackish tinge; pedicel, flagellum fuscous dorsally, pale ochraceous ventrally; mandibles bidentate subapically, chestnut brown except basal 1/3rd lateral and apical margins dark brown (Fig. 5c). Wings with light brown tinge, anterior veins and pterostigma dark brown. All legs brownish black except trochanters and terminal four tarsal segments ochraceous. Anterior half of metasomal terga in

some bees ochraceous and basal half dark brown; in some, this pattern reduced to a narrow transverse band, thus giving a banded appearance to metasoma (Fig. 5b); sterna black basally, blackish green apically.

Pilosity. Lower part of face including clypeus with dense short plumose white hairs (Fig. 5d); labrum fringed with golden yellow short mixed with long hairs; upper part of face, vertex with brownish plumose hairs; basal margin of vertex fringed with long pale brown mixed with golden yellow hairs; occipital area, lower part of occiput with long pale yellow hairs often mixed with brown hairs. Pronotum including pronotal lobes with white plumose hairs; pale yellow hairs on anterior and lateral margins of mesoscutum; mesoscutellum fringed with long, yellow dense hairs mixed with dark brown hairs; mesoscutum without distinct longitudinal band of hairs; anterior part of mesepisternum with long white hairs at lower side (above coxae) while upper side (below wing bases) with plumose white short hairs; posterior part of mesepisternum with brown sparse hairs; metepisternum, lateral area of propodeum clothed with dense, plumose white hairs (Fig. 4b). All coxae, trochanters heavily fringed with golden yellow hairs; anterior margins of femora and tibiae lined with golden yellow hairs while posterior margins, first tarsal segments with dense dark brown hairs. Metasomal terga with yellow short thin band of hairs at the basal margin; sterna 1 to 5 with broad band of long, white silky hairs becoming dense medially.

Integument surface. Face, vertex, thorax shiny, punctate; scape, pedicel, and flagellar segments heavily punctate; Metasomal terga shiny, sparsely punctate progressively increasing in density towards terminal terga; metasomal sterna heavily punctate. Legs shiny and punctate.

Morphometry. Detailed morphometry is presented in Table 2. Mean body length 4.93 mm; head wider (1.82 mm) than long (1.37 mm); eye length 1.27 mm, width 0.45 mm; upper inter-ocular distance 1.1 mm; inter-ocular distance 0.38 mm; ocello-ocular distance 0.21 mm; median ocellus diameter 0.19 mm; clypeus length 0.41 mm, width 0.87 mm; malar space length 0.04 mm; scape length 0.71 mm, width 0.10 mm; first and second flagellar segments as long as wide (0.14 mm); mandible 2.7 x longer (0.79 mm) than its width (0.29 mm); forewing 4.74 mm long, 1.62 mm wide; wing diagonal 1.32 mm; hind tibia 1.74 mm long, 0.61 mm wide; hind basitarsus 0.71 mm long and 0.37 mm wide.

Ratio of head length/head width 0.74; eye length/ocular distance 1.16; interocular distance/ocular distance 1.77; scape length/eye length 0.58; forewing length/width 3.02; wing diagonal/head width 0.73; hind tibial length/head width 0.95; hind tibial length/wing diagonal 1.31; hind tibial width/length 0.34; hind basitarsus width/hind tibial width 0.64.

Table 3 Morphometry of type specimens of *Tetragonula kyrdemkulaiensis* sp. n. and *T. srikantanathi* sp. n

Parameters	<i>T. kyrdemkulaiensis</i> sp. n.		<i>T. srikantanathi</i> sp. n.	
	Holotype: Male (<i>n</i> =1)	Paratypes: Females (<i>n</i> =12) Mean±SD	Holotype: Female (<i>n</i> =1)	Paratypes: Females (<i>n</i> =10) Mean±SD
Length of body	5.20	4.93±0.37	3.50	3.58±0.06
Width of head including eyes	1.85	1.82±0.03	1.60	1.59±0.02
Length of head	1.32	1.37±0.06	1.15	1.15±0.01
Length of eye	1.35	1.27±0.04	1.05	1.04±0.01
Width of eye	0.57	0.45±0.01	0.40	0.36±0.01
Upper inter-ocular distance	1.05	1.11±0.05	1.05	1.05±0.00
Diameter of median ocellus	0.20	0.19±0.01	0.15	0.14±0.01
Inter-ocellar distance	0.38	0.38±0.02	0.37	0.35±0.00
Ocello-ocular distance	0.15	0.21±0.01	0.20	0.22±0.01
Length of clypeus	0.50	0.41±0.03	0.30	0.31±0.02
Maximum width of clypeus	0.75	0.87±0.06	0.65	0.67±0.03
Malar space length	0.01	0.04±0.01	0.02	0.03±0.00
Length of scape	0.55	0.71±0.02	0.55	0.56±0.01
Width of scape	0.15	0.10±0.00	0.10	0.10±0.00
Length of pedicel+flagellum	2.20	1.53±0.06	1.30	1.25±0.04
Length of flagellomere 1	0.08	0.09±0.00	0.08	0.07±0.01
Length of flagellomere 2	0.20	0.14±0.01	0.12	0.12±0.01
Length of flagellomere 3	0.18	0.14±0.01	0.12	0.12±0.01
Width of flagellomere 3	0.15	0.14±0.01	0.12	0.13±0.00
Length of mandible	0.50	0.79±0.01	0.57	0.56±0.02
Width of mandible	0.25	0.29±0.01	0.28	0.29±0.02
Length of forewing+tegula	4.80	4.74±0.01	3.75	3.76±0.07
Width of forewing	1.45	1.62±0.08	1.25	1.27±0.04
Length of pterostigma	0.65	0.69±0.01	0.55	0.57±0.03
Length of marginal cell	1.60	1.50±0.04	1.25	1.27±0.03
Width of marginal cell	0.33	0.39±0.01	0.30	0.29±0.01
Wing diagonal	1.25	1.32±0.04	1.00	1.00±0.00
Hamuli	6.00	6.00±0.00	0.05	5.00±0.00
Length of mesoscutum	1.25	1.18±0.03	0.95	0.91±0.04
Maximum width of mesoscutum	1.40	1.25±0.02	1.05	1.05±0.01
Length of hind tibia	1.60	1.74±0.03	1.40	1.32±0.04
Width of hind tibia	0.60	0.61±0.02	0.50	0.50±0.01
Length of hind basitarsus	0.65	0.71±0.07	0.55	0.57±0.03
Width of hind basitarsus	0.35	0.37±0.03	0.25	0.26±0.02
Length of tergum 3	--	0.86±0.00	--	0.44±0.01
Width of tergum 3	--	2.54±0.01	--	1.88±0.03
Length of sternum 3	--	0.69±0.01	--	0.40±0.01
Width of sternum 3	--	1.81±0.01	--	1.64±0.01

All measurements are in millimeters except for hamuli

Morphometry of 34 parameters of female bees of *T. kyrdemkulaiensis* sp. n. was compared with that of primary type specimens of *Tetragonula* (Rasmussen, 2013) from the Indian subcontinent (Table 4). Values for 28 morphological parameters considerably larger in *T. kyrdemkulaiensis*

compared to the values of morphometry of primary type specimens. Some of the notable morphological parameters with greater values in *T. kyrdemkulaiensis* were length of body, head, eye, scape, antennae, mandible forewing, pterostigma, marginal cell, wing diagonal, hind tibia and hind basitarsus.

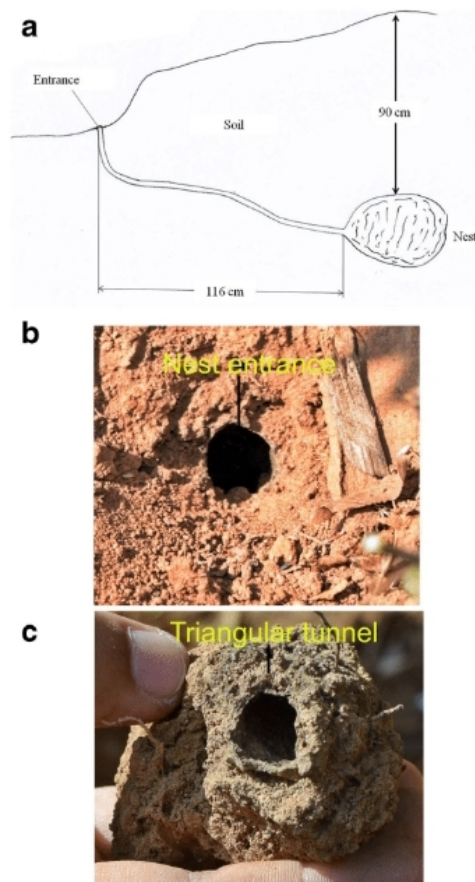


Fig. 6 *Tetragonula kyrdemkulaiensis* sp. n. Nest: **a** position of nest; **b** entrance; **c** triangular tunnel

Advance fronts (new brood cells) were clearly distinguishable by their dark brown colour from the older cells which were light brown to golden yellow. Multiple advance fronts were found on two sides of the brood area (Fig. 8a). Thin layers of cerumen separated part of brood cell in the peripheral region. There were vertical small spaces distributed irregularly in the midst of brood area (Fig. 8a). Diameter of the brood area was 7 cm.

Tetragonula srikantanathi Viraktamath sp. n.

Diagnosis

This species is known by female which can be identified by its morphometry, presence of dense plumose hairs on the face; distinct longitudinal bands of hairs on the mesoscutum; entire metasoma uniformly orange. The nest is very characteristic with single cell thick, horizontally suspended brood combs. The species differs from all other *Tetragonula iridipennis* group of species (*T. iridipennis*, *T. praeterita* (Walker, 1860), *T. ruficornis* and *T. bengalensis*) from Indian subcontinent in having entire metasoma uniformly orange (dark brown or black or jet black in other *Tetragonula* species); shorter (0.56) but wider mandibles



Fig. 7 *Tetragonula kyrdemkulaiensis* sp. n. Nest: **a** nest covered by involucrum; **b** brood area

(0.29 mm) as opposed to longer (0.6 to 0.62 mm) and narrower mandibles (0.18 to 0.21 mm) in other *Tetragonula* species of *iridipennis* group; longer brownish forewings, pterostigma and marginal cell (3.76, 0.57 and 1.27 mm, respectively) compared to other *Tetragonula* species (3.20 to 3.50, 0.48 to 0.51 mm, 1.21 to 1.23 mm, respectively); shorter hind tibia (1.32 mm) as opposed to longer (1.43 to 1.55 mm) in other *Tetragonula* species (Table 4). Similarly, this species differs from other known species of *Tetragonula* (*T. laeviceps*, *T. gressitti* and *T. kyrdemkulaiensis*) in metasomal coloration (uniformly orange as against dark brown to jet black metasoma) and morphometry. Body length varies from 3.4 to 4.90, 6.09 to 6.12 and 4.60 to 5.20 mm in *T. laeviceps*, *T. gressitti* and *T. kyrdemkulaiensis*, respectively. The most striking difference, however, is building very characteristic single cell thick, horizontally suspended brood combs as opposed to irregular cluster of brood cells in all other *Tetragonula* species reported so far from Indian subcontinent.

Holotype: Female

Integument coloration. Head, thorax black but metasoma uniform orange (Fig. 9a, b). Clypeus black except middle part brown; labrum, scape, pedicel reddish brown; flagellar segments dark brown on the upper side, ochraceous on the lower side;

Table 4 Nest characteristics of *Tetragonula* species

Parameter	<i>T. kyrdemkulaiensis</i> sp. n. (n=1) Dimensions (cm)	<i>T. srikantanathi</i> sp. n. (n=1)
Entrance diameter	1.6	2.25
Entrance length	116.0	1.5
Nest length	20.0	45
Nest height	14.0	--
Nest width	13.5	24
Nest depth	90	35
Diameter of the brood chamber	7.0	--
Brood cell height*	0.50±0.07	0.32±0.03
Brood cell width*	0.30±0.05	0.23±0.05
Pollen pot height*	1.23±0.12	0.75±0.09
Pollen pot width*	1.11±0.07	0.46±0.05
Honey pot height*	1.14±0.08	0.79±0.06
Honey pot width*	0.67±0.08	0.54±0.07

* n = 10

intersegmental area of flagellar segments light brown (Fig. 10a); mandibles dark brown with two subapical teeth and black irregular markings at the basal margin (Fig. 10b). Thorax black; tegulae dark brown; wings with light brown tinge appearing brownish; anterior veins, pterostigma light brown (Fig. 10c); all legs dark brown except terminal four tarsal segments light brown.

Pilosity. Face including clypeus clothed with white plumose hairs density being more on the lower part of face; labrum, lateral margins of mandible fringed with long yellowish brown hairs (Fig. 10a); vertex, occipital area, with dark brown erect simple hairs; lower part of occiput fringed with yellow long hairs. Pronotal lobes densely clothed with white, short plumose hairs; mesoscutum with five longitudinal bands of white hairs; lateral margins of mesoscutum fringed with yellow short hairs; scutellum heavily fringed with long, yellow intermixed with dark

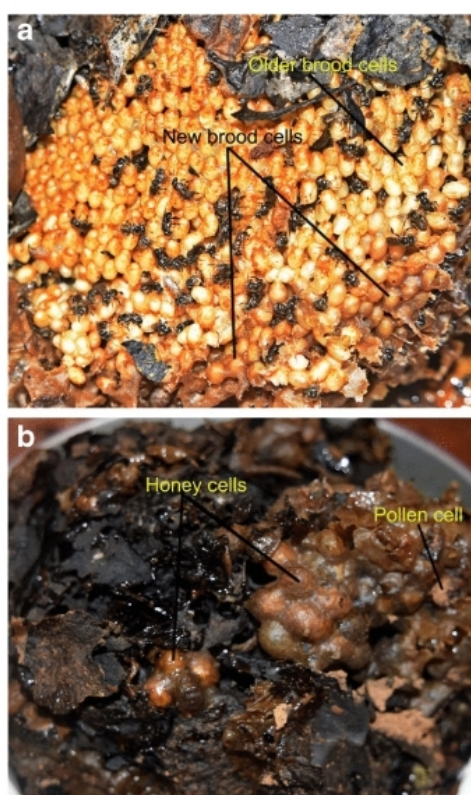


Fig. 8 *Tetragonula kyrdemkulaiensis* sp. n. Nest: **a** brood with advance fronts; **b** pollen and honey cells

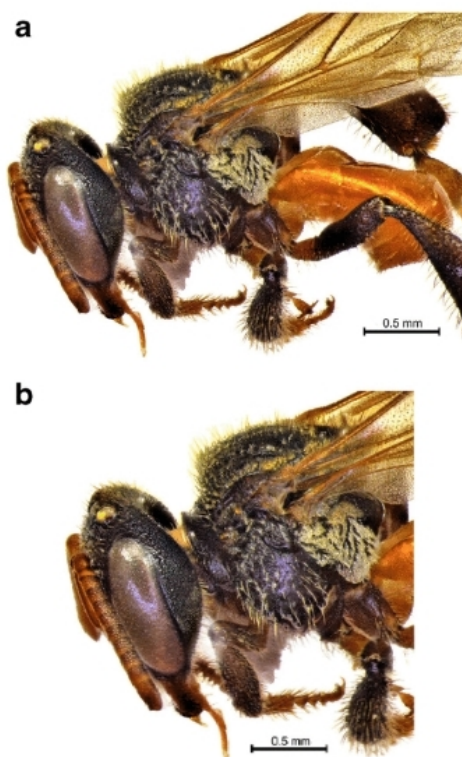


Fig. 9 *Tetragonula srikantanathi* sp. n. Female holotype: **a** lateral habitus; **b** head, thorax lateral closer view

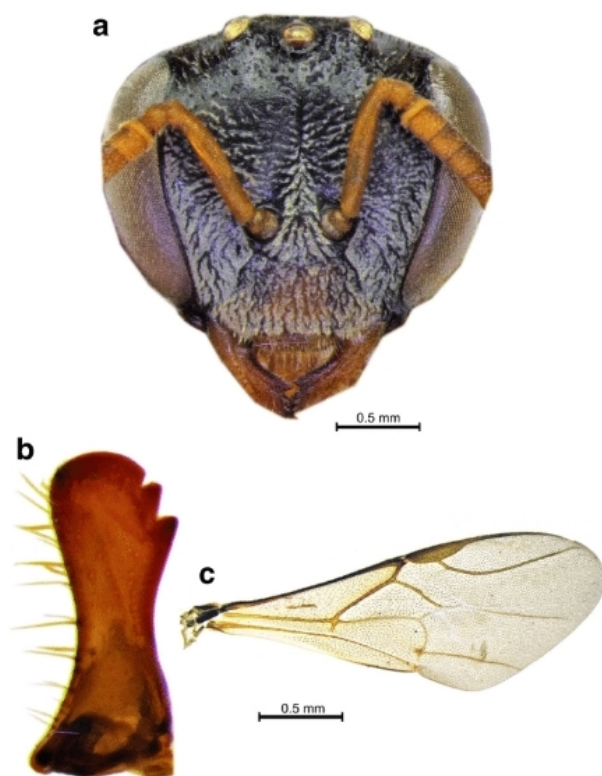


Fig. 10 *Tetragonula srikantanathi* sp. n. Female holotype: **a** head frontal view; **b** mandible; **c** forewing

brown hairs; anterior part of mesepisternum, whole of metepisternum, lateral part of propodeum densely clothed with white short plumose hairs while posterior part of mesepisternum with long plumose hairs (Fig. 9b). Metasomal terga with sparse yellow hairs the density increasing towards terminal end; sterna heavily fringed with short white hairs on both lateral sides, long white hairs medially. All legs clothed with pale yellow hairs.

Morphometry. Detailed morphometry is presented in Table 2. Body length 3.5 mm; head wider (1.60 mm) than long (1.15 mm); eye length 1.05 mm, width 0.40 mm; inter ocular distance 1.05 mm; inter-ocular distance 0.37 mm; diameter of median ocellus 0.15 mm; ocello-ocular distance 0.20 mm; clypeus wider (0.65 mm) than long (0.30 mm); malar space length 0.02 mm; scape length excluding basal bulb 0.55 mm; first flagellar segment very short (0.08 mm); second and third flagellar segments more or less equal in length and diameter (0.12 mm); mandibles 2.04× longer than its width (0.28 mm); forewing length 3.75 mm, width 1.25 mm; wing diagonal 1.00 mm; hind tibia 2.80× longer (1.40 mm) than wide (0.50 mm); hind basitarsus 2.20× longer (0.5 than its width (0.25 mm).

Ratio of head length and width 0.72; eye length and upper inter-ocular distance 1.00; interocellar and ocello-ocular distance 1.85; scape length and eye length 0.52; forewing length

and width 3.00; wing diagonal and head width 0.63; hind tibial length and head width 0.88; hind tibial length and wing diagonal 1.40; hind tibial width and length 0.36; hind basitarsus width and length 0.45.

Integument surface. Mandibles, labrum, upper part of face, vertex, occiput, punctate; integument of clypeus, lower part of face, mesepisternum, metepisternum, lateral of propodeum obscured by dense plumose hairs; thorax and metasoma dull, finely punctate.

Male: Unknown

Variation. Paratypes are identical to the holotype but the metasoma in some appears uniform brown than orange. Morphometry data of paratypes is presented in Table 3.

Material examined. *Holotype:* Female: Tripura: Salema (24.01° N 91.83° E, Altitude 89 m a.s.l.), 8. xi. 2017, Coll. S. Viraktamath, deposited at UASB. *Paratypes:* 164 workers (25 mounted on card point, 139 preserved in 95% ethyl alcohol) with same collection data deposited at UASB; one paratype deposited each at IARID and ZSIK.

Name derivation. This species is named in honor of Mr. Srikanta Nath, Assistant Director of Agriculture of the Tripura State Agriculture Department who searched the nest and helped in studying its structure.

Nest structure

This species was found nesting in mud wall vertically elongate cavity (Fig. 11b). The nest cavity measured 24 cm wide, 45 cm long and 35 cm deep. Entrance tube was 1.5 cm long with an oval opening with maximum width of 2.25 cm (Fig. 11a). There was no tunnel and the short entrance tube led to the nest cavity directly. The nest was divided into five areas arranged vertically from bottom to top were, resin dump, food storage, brood, food storage and resin dump. Brood area was in the middle of the nest cavity with honey and pollen storage both above and below the brood area. Bees stored honey behind the brood area also deep in the nest cavity. Resin dump was on topmost and at the bottom of the cavity, divided by hard, black walls of batumen.

Brood cells were in a single cell thick horizontal layer of combs arranged one above the other and attached to a hard, black batumen wall on one to three sides and suspended horizontally (Fig. 11c) However, one comb was suspended obliquely. Older brood cells were bright yellow, vertically elongate, and measured 0.32 cm in height and 0.23 cm in width (Table 3). Newly formed brood cells were brown. All brood cells were closely adhered together without any space in between. Interestingly, freshly built brood cell combs



Fig. 11 *Tetragonula srikantanathi* sp. n. Nest: **a** colony entrance; **b** colony inner view; **c** brood combs

(advance fronts) were found in the middle of the cavity behind the matured cells containing pupae. Pollen and honey cells were dark brown to black, soft, irregular in shape and larger measuring 0.75×0.46 and 0.79×0.54 cm, respectively. Adjacent cells shared the common walls.

Discussion

Northeast India with its remarkably diverse terrain, climate and ecological habitats is one of the global hotspots of diversity (Tripathi et al. 2016). Among 14 species of stingless bees reported from India, five species namely *Lepidotrigona arcifera* (Cockerell, 1929), *T. iridipennis*, *T. laeviceps*, *T. bengalensis*, *T. ruficornis* occur in the northeast India. The species belonging to the genus *Lepidotrigona* appear to be endemic to this region as the species belonging to this genus are not reported from anywhere else from India. The genus *Tetragonula* includes several species complexes which are morphologically similar (Rasmussen 2013). Unfortunately, all the species reported from northeast India by Akum et al. (2012), Rahman et al. (2013), Rathor et al. (2013), Das et al. (2019) are based on morphometry and colour of worker bees. Until now no information is available on male bees of *Tetragonula* associated with females from this region. Hence, a systematic and exhaustive study on diversity of stingless bees based on both male and female bees may result in discovery of many more species from this hot spot region.

In an endeavour towards this goal, two new species of *Tetragonula* have been reported here from this region thus elevating the number of species to seven. We compared both the new species with the images and descriptions of the primary type specimens of *Tetragonula* occurring in Indian

subcontinent published by Rasmussen (2013). We also compared the new species with the identified *Tetragonula* species from Asia deposited in the national collections like ZSI Kolkata, IARI New Delhi and UAS Bengaluru in addition to the rich collection of more than 30,000 stingless bees made by the senior author from different parts of India. Similarly, we have confirmed the distinctiveness of these two species from those described in the published literature from Indomalayan and Australasian regions (Cockerell 1929; Schwarz 1939; Moure 1961; Sakagami 1975, 1978; Dollin et al. 1997; Rasmussen 2013; Rasmussen et al. 2017).

Sakagami (1978) divides *Tetragonula* species into two groups based on whether they have distinct mesoscutal hair bands or not. The former group (*iridipennis* group) includes *T. hirashimai* (Sakagami, 1978), *T. pagdeni* (Schwarz, 1939), *T. pagdeniformis* (Sakagami, 1978), *T. iridipennis*, *T. minor* (Sakagami, 1978) and *T. fuscobalteata* (Cameron, 1908). The latter group (*laeviceps* group) includes *T. latigenalis* (Sakagami, 1978), *T. geissleri* (Cockerell, 1918), *T. melina* (Gribodo, 1893), *T. zucchini* (Sakagami, 1978), *T. sarawakensis* (Schwarz, 1937), *T. laeviceps* and *T. gressitti*. Though this character cannot be used with precision, Sakagami (1978) opines that this is one of the few reliable diagnostic character for species groups. Accordingly, *T. kyrdemkulaiensis* without distinct mesoscutellar bands belongs to the *laeviceps* group while *T. srikantanathi* with distinct bands to the *iridipennis* group.

Stingless bees in India nest in cavities of tree trunks, mud and stone walls, crevices in rocks, old electric meter boxes, pipes and other such concealed places Danaraddy et al. 2009; Nayak et al. 2013; Singh 2016; Layek and Karmakar 2018). However, none of the stingless bee species belonging to the genus *Tetragonula* is reported nesting in soil from India. Singh (2016) reported *Homotrigona* (*Lophotrigona*) *canifrons* (Smith, 1857) nesting in soil at a depth varying from few centimetres to over 150 centimetres in Nagaland. *T. kyrdemkulaiensis* sp. n. known from both male and female bees is very distinct, living 90 cm deep in the soil from ground level and has unique body colouration and pilosity in both male and female bees. It appears to be one of the largest stingless bee species occurring in India differing in body coloration, pilosity, morphometry and male genitalia from its close relative *T. gressitti* (Table 5).

Tetragonula srikantanathi sp. n. is described only by female as the males are yet to be collected. Females of different species of *Tetragonula* look similar and have weak diagnostic characters (Rasmussen 2013). Hence, identification of these species is mainly based on morphometry, pilosity and nest structure (Schwarz 1939; Sakagami 1978; Dollin et al. 1997; Rasmussen 2013). Hence, we compared the morphometry of this new species with that of all the primary type specimens of *Tetragonula* from the Indian subcontinent. Sakagami (1978), Rasmussen (2013) and more recently Attasopa et al. (2018) emphasized the importance of inclusion of both males and

Table 5 Diagnostic characteristics of *Tetragonula kyrdemkulaiensis* sp. n. and *T. gressitti*

Parameter	<i>T. gressitti</i> (Sakagami 1978)		<i>T. kyrdemkulaiensis</i> sp. n.	
	Male (<i>n</i> =2)	Female (<i>n</i> = 9 to 53)	Male (<i>n</i> =2) Mean±SD	Female (<i>n</i> =12) Mean±SD
Length of body	--	4.10	5.10±0.10	4.93±0.37
Width of head including eyes	1.77	1.79	1.83±0.02	1.82±0.03
Length of forewing+ tegula	5.00	4.8	4.70±0.14	4.74±0.10
Wing diagonal	1.40	1.32	1.25±0.00	1.32±0.04
Length of hind tibia	1.75	1.60	1.58±0.04	1.74±0.03
Eye length/ocular distance	1.10	1.05	1.27±0.04	1.14 ± 0.02
Inter-ocellar distance/ocello ocular distance	--	1.70	2.53±0.00	1.81±0.08
Scape length/eye length	0.45	0.60	0.41±0.00	0.56±0.03
Ratio of wing diagonal/head width	0.85	0.8	0.68±0.10	0.73±0.02
Hind tibial length/head width	1.00	0.95	0.86±0.01	0.96±0.02
Hind tibial length/Wing diagonal	1.35	1.30	1.28±0.00	1.32±0.03
Hind tibial width/hind tibial length	0.30	0.36	0.36±0.03	0.35±0.02
Hind basitarsus width/hind tibial width	0.60	0.65	0.60±0.04	0.61±0.03
Frontal hairs	Not plumose	Not plumose	Plumose	Plumose
Mandibles	Receded	Normal	Normal	Normal
Mesoscutal hairs	Testaceous	Dark		Yellow intermixed with dark brown hairs
Apical margin of sternum 5	Deeply but narrowly invaginated		Distinct inverted U-shaped invagination	--
Apico-medial process of sternum 6	Very long and slender		Shorter and broader	--
Gonostylus	Very long, and slender 65 µ wide at base, conspicuously sinuate at 1/3rd, apex clavate with 75 µ width		Shorter, broader 104 µ wide at base, gently sinuate terminal clavate end 59 µ wide	--
Penis valve	Rod like, slender, basally 85 µ and medially 80 µ wide, apical half forming a broad curve, apex not much pointed		Robust, bisinuate, 178 µ wide at the base, a strongly curved sickle shaped structure terminally	--

All measurements are in millimeters

females in describing new taxa in stingless bees. However, uniform coloration of metasoma, morphometry of this species (shorter and wider mandibles; longer brownish forewings, pterostigma and marginal cell; shorter hind tibia compared to primary types of *Tetragonula*) and unique nest structure (building parallel combs of single layer thick brood cells, suspended horizontally). justify treating *T. srikantanathi* as a new species.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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