Large-scale evaluation of water traps for their efficiency in collecting male stingless bees (Apidae: Meliponini)

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A large-scale evaluation of water traps for their efficiency in trapping male stingless bees was done, which involved 41 locations from 9 states and 88, 7 and 5 stingless bee colonies of *Tetragonula*, *Lepidotrigona* and *Lisotrigona* species respectively. We succeeded in collecting male bees in 82 colonies of *Tetragonula* spp., five of *Lepidotrigona* spp. and two of *Lisotrigona* spp. The male bees were trapped throughout the year with more than 10 males/colony in March, April, July, September, October and December. Males of *Tetragonula*, *Lepidotrigona* and *Lisotrigona* formed 0.17–14.29%, 0.54–21.53% and 12.35–14.21% of the total bees trapped per colony respectively. We conclude that a water trap is the best technique to collect male bees of all three genera (*Tetragonula*, *Lepidotrigona* and *Lisotrigona*) occurring in India within a brief period during any time of the year.

Keywords: Collection efficiency, flight pattern, male stingless bees, water trap.

STINGLESS bees are one of the economically important groups of hymenopterans yielding medicinally highly valued honey and pollinating several plant species, including cultivated crops[1–3]. Despite their wide distribution in India, their diversity is not fully known, according to Rasmussen[1], who listed only seven species from the country (eight species from the Indian subcontinent). Remarkable similarity among different species of female bees, the prevalence of several sibling and cryptic species besides species complexes, the lack of systematic explorations and critical studies of males with associated female bees, and no representation of males in museum collections are the major reasons for this poor knowledge of Indian fauna. The importance of male bees in the identification of species has been emphasized by Schwarz[4] and Sakagami[5]. Rasmussen[1] as well as Attasopa *et al.*[6] stressed that all efforts should be made to collect and study male bees as they have clear diagnostic features for the identification of species. Rasmussen[1] also emphasized that no additional species of *Tetragonula* should be proposed from India until males are discovered. Obviously, this is also valid for the species of *Lepidotrigona* and *Lisotrigona.* Though *T. iridipennis* was described in 1854 by Smith[7] with Kandy, Sri Lanka, as the type locality, no male bees of this species have been described from India so far. Vijayakumar and Jeyaraaj[8] have described males of *Tetragonula iridipennis*; however, species identity remains to be confirmed. Similarly, males of *Lepidotrigona arcifera* and *Lisotrigona cacciae* are unknown, though these species were described from India in 1929 and 1907 by Cockerell[9] and Nurse[10] respectively. Viraktamath *et al.*[11] developed a simple technique using a water trap to collect male bees of *Tetragonula* spp. and evaluated it successfully in seven diverse locations. Using this technique, Viraktamath and Rojeet[12], and Viraktamath and Roy[13] described six new species of *Tetragonula* based on males with associated female bees, thus increasing the total number of stingless bees to 22 from 7 species in 2013. Recently, Engel *et al.*[14] removed *Lisotrigona carpenteri* Engel previously considered a species of *Lisotrigona* to a new genus *Ebaiotrigona* Engel & Nguyen after discovering its males, which were found to have unique genitalia. Considering the importance of male bees, a large-scale evaluation is needed for the robustness of this technique. Hence, the present study was conducted with two objectives, namely (i) to check whether the technique developed by Viraktamath *et al.*[11] is robust enough in collecting males across wide varying ecological conditions in different states of India, and (ii) whether males of *Lepidotrigona* and *Lisotrigona* can also be collected using this technique. The study was conducted in 41 locations belonging to 9 states of India, across the 3 genera of stingless bees, namely *Tetragonula*, *Lepidotrigona* and *Lisotrigona*. To the best of our knowledge, evaluation of this technique to collect males of *Lisotrigona* has not been carried out before. The results of this large-scale evaluation are presented here.

We conducted an intensive survey for the collection of male and associated female stingless bees in nine states from August 2019 to March 2022. During this endeavour, we evaluated the water traps for their efficiency following the method described by Viraktamath *et al.*[11] in 36 locations in the colonies of *Tetragonula* spp., three and two locations each in *Lepidotrigona* spp. and *Lisotrigona* spp. respectively (Table 1). In each location, we selected 1–7 natural colonies for trapping the bees depending on the availability of the colonies. Thus, we evaluated water traps in 88, 7 and 5 colonies of *Tetragonula*, *Lepidotrigona* and *Lisotrigona* species respectively. The trapping duration was 1–15 days, which varied in different locations. As this technique was developed to collect male bees, the trapping duration was irrelevant. Hence, we did not consider this parameter while interpreting the data. It is obvious that once the male bees are collected just a day after the installation of the trap, there is no need to continue trapping further, according to Viraktamath *et al.*[11]. Even a single male can help the taxonomist in the correct identification of the species.

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Table 1. Evaluation of water traps for their efficiency in the collection of male stingless bees

State: place	Coordinates	Month of trapping	Duration of the trap used (days)	Mean number of female bees trapped/ colony	Mean number of male bees trapped/ colony	Mean total number of bees trapped/ colony	Percentage of male bees trapped/ colony
Tetragonula spp.							
Karnataka: Bagalkot	16.16°N, 75.66°E	July 2019	15	1424	15	1439	1.04
Andhra Pradesh: Visakhapatnam	17.68°N, 83.21°E	August 2019	3	795	6	801	0.75
Madhya Pradesh: Jabalpur	23.20°N, 79.95°E	January 2019	3	387	4	391	1.02
Madhya Pradesh: Hoshangabad	22.75°N, 77.71°E	January 2019	1	325	5	330	1.52
Madhya Pradesh: Gwalior	26.21°N, 78.17°E	June 2019	1	63	2	65	3.08
Madhya Pradesh: Bagaditola	21.23°N, 80.25°E	July 2021	3	767	8	775	1.03
Madhya Pradesh: Paraswada	21.22°N, 80.26°E	July 2021	3	601	7	608	1.15
Chhattisgarh: Ambikapur	23.14°N, 83.14°E	March 2019	2	509	5	514	0.97
Chhattisgarh: Kondagaon	19.59°N, 81.66°E	December 2019	3	85	5	90	5.56
Chhattisgarh: Bastar	19.20°N, 81.93°E	December 2019	3	559	1	560	0.18
Chhattisgarh: Dantewada, Godre	18.84°N, 81.86°E	September 2019	1	6	1	7	14.29
Chhattisgarh: Kerlapal	18.31°N, 81.59°E	December 2019	3	814	16	830	1.93
Chhattisgarh: Bhardevbhata	20.26°N, 81.49°E	November 2019	1	69	1	70	1.43
Chhattisgarh: Kopra	20.87°N, 81.92°E	October 2019	3	599	1	600	0.17
New Delhi: Indian Agricultural Research Institute	28.63°N, 77.15°E	March 2019	3	1240	16	1256	1.27
Rajasthan: Jaipur	26.91°N, 75.78°E	March 2019	3	207	15	222	6.76
Rajasthan: Udaipur	24.58°N, 73.71°E	March 2019	3	180	2	182	1.09
Maharashtra: Nagpur	21.09°N, 79.04°E	September 2019	15	661	11	672	1.64
Maharashtra: Sawali (Sawli (Bu))	21.16°N, 78.55°E	December 2019	6	568	7	575	1.22
Maharashtra: Wardha	20.44°N, 78.36°E	January 2020	10	589	1	590	0.17
Maharashtra: Durgapur	19.49°N, 79.48°E	January 2020	5	607	5	612	0.82
Maharashtra: Pauni	20.45°N, 79.37°E	February 2020	1	343	4	347	1.15
Maharashtra: Manewada	21.06°N, 79.06°E	March 2020	3	768	57	825	6.91
Maharashtra: Ajni Colony	21.07°N, 79.05°E	January 2021	5	372	1	373	0.27
Maharashtra: Adyal	20.55°N, 79.43°E	February 2021	3	228	4	232	1.72
Maharashtra: Zaliya	21.22°N, 80.23°E	May 2021	4	1025	3	1028	0.29
Maharashtra: Hazr fall	21.16°N, 80.33°E	May 2021	5	432	3	435	0.69
Maharashtra: Navatolla	21.16°N, 80.33°E	May 2021	3	645	3	648	0.46
Maharashtra: Sonartola	21.16°N, 80.33°E	May 2021	5	806	4	810	0.49
Maharashtra: Dhiwrintola	21.07°N, 80.24°E	July 2021	5	347	1	348	0.29
Maharashtra: Kosatola	21.03°N, 80.24°E	July 2021	5	204	7	211	3.32
Maharashtra: Dadapur	20.35°N, 80.20°E	March 2022	3	706	2	708	0.28
Maharashtra: Warvi	20.34°N, 80.20°E	March 2022	3	210	4	214	1.87
Mizoram: Thenzawl	23.17°N, 92.45°E	September 2020	15	91	8	99	8.08
Mizoram: Thenzawl	23.17°N, 92.45°E	March 2021	15	329	01	330	0.30
Mizoram: Thenzawl	23.17°N, 92.45°E	April 2022	15	1185	03	1188	0.25
Lepidotrigona spp.							
Mizoram: Thenzawl	23.17°N, 92.45°E	October 2020	15	113	31	144	21.53
Mizoram: Thenzawl	23.17°N, 92.45°E	April 2021	15	414	10	424	2.36
Arunachal Pradesh: Kamakhyapur	27.56°N, 96.18°E	March 2020	3	370	2	372	0.54
Lisotrigona spp.							
Maharashtra: Navatolla	21.16°N, 80.33°E	July 2021	7	163	27	190	14.21
Maharashtra: Kosumtara	21.16°N, 80.32°E	July 2021	7	71	10	81	12.35

The bees in each trap were collected daily, counted and stored separately in 5 ml plastic vials containing 95% ethyl alcohol and labelled. The mean number of male, female and total bees trapped per colony for each place, irrespective of the trapping duration, was recorded after examining each sample under a binocular microscope and identifying to the generic level in the laboratory at the Department of Entomology, University of Agricultural Sciences, Bengaluru (UASB). The percentage of male bees was calculated for each location separately based on the total trap catches.

Both female and male bees were collected in the water traps. Females always outnumbered males in all the locations (Table 1). Interestingly, some males were in the late pupal stage, and some were in the pupal stage. Among 88 colonies of *Tetragonula*, we successfully trapped males in 82 colonies, with a success rate of 93.18%. Though the trapping duration varied from 1 to 15 days, males were trapped within one day in five locations (13.89%), three days in 17 locations (47.22%), 10 days in nine locations (25.00%), and beyond 10 days in five locations (13.89%) (Table 1). The number of males trapped varied from 1 to 57/colony, while the percentage of males among the total bees trapped varied from 0.17 to 14.29. Interestingly, males were trapped in all the months during the period of evaluation (Table 2), but more than 10 males/colony were trapped in March, July, September and December.

Our results strongly endorse the earlier results of Viraktamath *et al.*[11], who collected male bees within a day in 2 locations and within 15 days in the other 5 locations in *Tetragonula* colonies. Further, they reported trapping of males throughout the year, with a peak trapping in September.

The water traps were evaluated in seven colonies of *Lepidotrigona* spp. in three locations for 3 to 15 days (Table 1). We collected males from five colonies resulting in a success rate of 71.43%. The number and percentage of males trapped were more in Thenzawl (10–31 males/colony and 2.36–21.53%) than in Kamakhyapur (two males/colony and 0.54%).

In the five *Lisotrigona* bee colonies evaluated for seven days in two locations, we succeeded in collecting males in two colonies, with a success rate of 40%. Maximum males were trapped in Navatolla (27 males/colony) than in Kosumtara (10 males/colony), forming 14.21% and 12.35% of the total trap catches respectively.

Prior to the development of the technique to collect male bees by Viraktamath *et al.*[11], all the known species of stingless bees from India were described based on females alone using relative characteristics like body colouration, pilosity, hair colour and body measurements. Holotypes of these species were also female bees. As female bees of different species are remarkably similar, it is nearly impossible to identify the species. Hence, Rasmussen[1] categorically stated that 'species of the "iridipennis" species group are extremely similar in external morphology of the workers, and a taxonomic revision of the species of India should include morphological characters of the male genitalia and molecular data'. Further, he mentioned that 'until their males are discovered, further collections are made, and already described species from southeast Asia are compared with Indian specimens, it remains premature to describe and propose additional species of *Tetragonula* from India'.

Hitherto methods for collecting males included continuous monitoring of the colonies for the emergence of mating flights, laborious searching of male congregations (leks), the complete killing of bees of a colony, collecting outgoing bees and obtaining males as a chance factor[6,11]. However, the technique proposed in this study is effective in overcoming all these problems and providing males within a brief period, irrespective of the season and the genera occurring in India. Our large-scale evaluation undoubtedly demonstrates the robustness of this technique to collect males of *Tetragonula*, *Lepidotrigona* and *Lisotrigona* stingless bees occurring in India within a brief period. This technique may also be useful across other genera occurring elsewhere.

Though Viraktamath *et al.*[11] reported that the flight pattern of outgoing bees is the major reason why the males and females are trapped in water, many unanswered questions remain: Why late pupal stage, as well as teneral males, are trapped in water? Do all the species of Meliponini produce males throughout the year? Further detailed species-wise studies may provide answers to these questions.

Table 2. Pattern of trapping of males of stingless bees in different months (August 2019–April 2022)

↓ Month/Year →	2019	2020	2021	2022
January	+	+	+	ND
February	ND	ND	+	+
March	+	+	+	ND
April	ND	ND	+	+
May	ND	ND	+	
June	+	ND	ND	
July	+	ND	+	
August	+	ND	ND	
September	+	+	ND	
October	+	+	ND	
November	+	ND	ND	
December	+	ND	ND	

+, Males trapped; ND, No data.

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Hot springs of Demchok, Ladakh, India

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In this study, two thermal springs are reported from the Demchok area in Ladakh, India. These are characterized by water having low total dissolved solids (TDS) content (~250 mg/l) as well as high pH (9.5) and surface temperature (75°C). Although these hot springs and their medicinal properties are known to locals, they have not been scientifically studied. Relatively low TDS despite high temperature could be due to sluggish ion-exchange processes in the geothermal reservoir. Such a situation might have developed because of the high water-to-rock ratio and/or smaller residence time of the geothermal fluid in the reaction zone.

Keywords: Geothermal zone, hot springs, ion-exchange process, medicinal properties, water–rock ratio.

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HOT springs with conspicuously high solute concentrations compared to local groundwater occur in geothermal anomalous zones[1–3]. Prominent geothermal zones are present in seven major provinces of India, characterized by different litho-tectonic settings. According to the Geological Survey of India (GSI), there are about 350 hot springs in these geothermal provinces[4–6]. The Himalayan Geothermal Province (HGP), stretching for over 1500 km at altitudes between 1500 and 4500 m amsl, consists of more than half of all the thermal resources in India[4,7]. Within the HGP, geothermal zones are particularly predominant in the Union Territory of Ladakh, India, the most important geothermal areas being in the Puga Valley, Chumathang and Nubra valley[2] (Figure 1). Puga and Chumathang thermal springs have been the subject of detailed geothermal exploration by the Geological Survey of India (GSI) since 1973 when the Puga multipurpose project was carried out[8,9]. Here, hot springs discharge water at 84–87°C (boiling point of water at an elevation of 4000–4400 m amsl), and some of the thermal zones, including shallow wells, provide evidence of subsurface boiling[9]. Only preliminary exploratory studies have been taken up to date in the Nubra Valley. Subduction tectonism and shallow crustal melting are the primary reasons for high geothermal gradient anomalous heat flow (>180 mW/m^2) in the geothermal province of Ladakh[7,10].

Located about 300 km southeast of Leh, the district headquarters and the capital of Ladakh, Demchok is well known for the occurrence of hot springs and their medicinal properties. These hot springs are roughly the same altitude as those at Puga, i.e. 4400 m amsl. The Demchok hot springs can be reached after about 10–12 h of a strenuous drive along the left bank of the River Indus or via the Loma–Hanle–Umling La road (the highest motorable La or pass at an altitude of 5790 m amsl).

Ladakh Granitoid Complex of Cretaceous to Tertiary age overlain by Quaternary deposits occurs in the Demchok thermal spring area[11,12] (Figure 1). These hot springs occur in two clusters. One cluster consists of two hot springs, viz. HS-DEM-01 and HS-DEM-02 with surface discharge temperatures of 74°C and 75°C respectively (Table 1), just 10°C less than the local boiling point of water (84°C) at the given altitude (Figure 2). HS-DEM-02 shows minor leakage of thermal waters from a small stagnant pond (Figure 2), while discharge from HS-DEM-01 is estimated to be around 5 litre/min. The other cluster is located at Amchi's village (residents of Demchok) and consists of numerous hot springs with medicinal properties. These hot springs are controlled by Amchis (local healers) and are accessible only on some specific occasions. These springs are used to treat skin diseases, digestive issues, pain healing, etc. The low ionic content in these hot springs accounts for their balneological importance. During this study, the second cluster could not be visited despite our best efforts.

Table 1 shows the results of the chemical analysis of water from these hot springs and non-thermal waters. Water