Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 47 Issue 1 January 2026 pp. 142-152
DOI: http://doi.org/10.22438/jeb/47/1/MRN-5747

J. Environ. Biol., 2026

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Unveiling the stingless bee: Biological and morphometric insights into *Tetragonula iridipennis* (Smith) from Assam, North-east India

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Received: 17 April 2025 **Revised:** 27 July 2025 **Accepted:** 29 September 2025

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Abstract

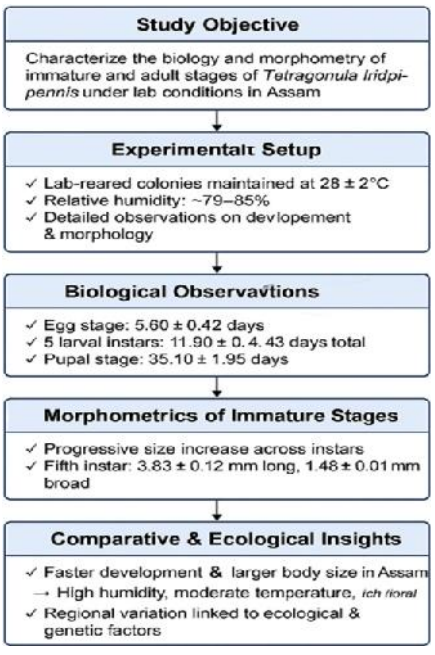
Aim: To investigate the biology and morphometric characteristics of stingless bee, *Tetragonula iridipennis*.

Methodology: The study on biology involved the records on developmental durations which were carried out by designing a simple patentable model under laboratory condition. This model was exploited to record the developmental duration of individual stages in the life cycle of *T. iridepennis*. Morphometrics of adult worker bees were collected from the apiary and killed using chloroform and preserved in 70% ethyl alcohol.

Results: The developmental durations of individual life stages of *T. iridipennis* were recorded as egg period (5-6 days), larval period (11-12 days) and pupal period (35-36 days). The present study also revealed the mean length and breadth of the egg, second instar, third instar, fourth instar and fifth instars larvae of *T. iridipennis*. The mean length and breadth of pupae, and adult body length range were also measured.

Interpretation: The findings of this study will pave a way towards popularization, domestication and management of these stingless bees in this region.

Key words: Biology, Developmental stages, Morphometrics, Stingless bee, Tetragonula iridipennis



How to cite: Saranya, P.S., S. Borkataki, A. Rahman, M.K. Deka, A. Devee, B. Bhattacharyya, K. Das, S.P. Nanda, R. Boruah, K.S. Bhairavi, S. Sen, R.R. Taye and D. Sorahia: Unveiling the stingless bee: Biological and morphometric insights into *Tetragonula iridipennis* (Smith) from Assam, North-east India. *J. Environ. Biol.*, **47,** 142-152 (2026).

Introduction

Beekeeping of *Apis cerana* Fab. and *Apis mellifera* L. has played a pivotal role contributing to more sustainable development initiatives, owing to the large net gains from labour in conventional honey production compared to other agricultural operations (Rasmussen and Gonzalez, 2013). Additionally, meliponiculture has also been practised in the Old World for millennia, alongside the popular practice of raising honey bees. Stingless bees, constitute a large monophyletic group of eusocial bees including many distinct tropical and subtropical species (Chuttong *et al.,* 2016). The term 'meliponine' is a synonym for stingless bee. Stingless bees are classified under the order Hymenoptera, family Apidae, subfamily Meliponinae and tribe Meliponini (Chuttong *et al.,* 2016). They are closely related to common honey bees (Tuksitha *et al.,* 2018) with a notable exception in their vestigial stingers that are remarkably rudimentary and cannot be utilised as a defensive tool. However, presence of stout mandibles can be seen in certain species that are sufficiently strong to inflict a mild bite to intruders and also irritates them by pulling their hairs or crawling into their ears or nostrils.

Taxonomically, stingless bees are broadly classified into two major genera, *Trigona* and *Melipona,* which are among the approximately 50 recognized genera under the subfamily Meliponinae known to be domesticated globally (Michener, 2007; Grüter, 2020). Although scientists continue to discover and describe new species, over 600 stingless bee species are currently recognized worldwide (Grüter, 2020; Melo, 2020). Their greatest diversity occurs in the Neotropics, with around 450 species in the Central and South America. Africa (including Madagascar) harbors about 36 species, while the Indo Malayan and Australasian regions (Asia plus Australia) are home to approximately 90 species. Notably, the genus *Trigona* is exclusively Neotropical, despite earlier reports suggesting an Old-World distribution. Contemporary surveys across the Indian subcontinent indicate that stingless bee communities are predominantly composed of the genera *Tetragonula* and *Lisotrigona*, with *Lepidotrigona* appearing mostly in North-eastern regions.

According to recent taxonomic and ecological studies, *Tetragonula* now comprises more than 30 described species in India, whereas *Lisotrigona* includes about four species and *Lepidotrigona* remains comparatively rare. The most common stingless bee species found in India are *Tetragonula iridipennis*, popularly known as the Indian stingless bee or dammer bee as they collect dammer (kind of resin) from dipterocarp trees, which is used in the construction of the comb structures (Saranya *et al.,* 2024). This species was originally described from Sri Lanka. Stingless bees are also considered as promising pollinators of specific crops belonging to families Compositae, Cruciferae, Malvaceae, Nuciferae *etc.,* where honey bees fail to pollinate due to large size as compared to stingless bees. The pollination services provided by stingless bees are considerably efficient due to various factors such as polylecty and floral constancy where they can pollinate numerous plant species and the worker visits only single species of plant on one trip (Ramalho *et al.,* 1994). As stingless bees provide short range pollination services, they can be successfully utilized in polyhouses and shade net houses (Rasmussen, 2013). Also, they are appropriate for pollination in closed enclosures like glass houses (Slaa *et al.,* 2000). Furthermore, stingless bees are highly distinguishable from other corbiculate Apinae by the presence of jugal lobe in the hind wing and reduced forewing venation. However, despite its importance, exclusive studies on stingless bee *T. iridepennis* including biology, morphometrics and its multiplication in different parts of North-east India are lacking for which it has failed to attract the attention of beekeepers. Thus, a maiden attempt was made to study the biology and morphometrics of *T. iridipennis* under laboratory conditions as meagre information are available on this aspect from this region in particular.

Materials and Methods

Biology of *Tetragonula iridipennis*: The study on the biology of *Tetragonula iridipennis* was conducted at the Insect Rearing Laboratory of Department of Entomology, Assam Agricultural University, Jorhat, Assam under controlled laboratory conditions (temperature = 28.00 ± 2.00°C, relative humidity = 79%), utilizing a specially designed, patentable observation model. This model was developed with the objective of enabling clear and continuous monitoring of brood pots by ensuring proper aeration and addressing the challenges associated with observing brood within sealed pots. The customized setup facilitated precise documentation of the developmental duration of each life stage of *T. iridipennis.* Daily observations were recorded to accurately track the instar progression and stadium duration from egg to adult emergence. The biological observations on developmental stages were based on five replications (n = 5), with daily monitoring of individual brood cells. For adult morphometric assessments, measurements were taken from ten bees (n = 10) to ensure statistical robustness.

Morphometrics of *Tetragonula iridepennis*: Adult worker bees were collected from the apiary and killed using chloroform and preserved in 70% ethyl alcohol. The different body segments were carefully dissected after boiling in 10 % KOH solution for 2-3 min, followed by rinsing of the parts in water and placing in glacial acetic acid to neutralize KOH. Body parts *viz.,* head, wings and hind legs were dissected for studying the morphometrics of adults. For immature stages, healthy brood cells were selected. The measurements were recorded under a Zeiss Stemi 2000-C stereozoom microscope at 4 x 10x magnification. The measurements were recorded in millimeter (mm). Twenty morphometric characteristics were examined in the study (Ruttner, 1988; Sakagami, 1978).

Statistical analysis: The data were subjected to statistical analysis to determine the treatment effects. The mean was computed to represent the central tendency of the observations. Standard deviation was calculated to measure the extent of variability among the observations. To assess the accuracy of

mean, standard error was estimated. Critical difference (CD) at a 5% significance level was used to evaluate the statistical significance between the treatment means Fisher's t-test values.

Results and Discussion

The study comprehensively documented the biology and morphometrics of *Tetragonula iridipennis* under controlled laboratory conditions. The developmental period from egg to adult spanned approximately 52 to 54 days, with the egg, larval, and pupal stages lasting on average 5.60, 11.90, and 35.10 days, respectively. Significant differences in larval instar durations and morphometrics were recorded, with progressive increase in body size across instars. Adult morphometric analysis revealed an average body length of 4.65 mm. Detailed measurements of key body parts, including the head, thorax, abdomen, wings and legs, were recorded. Regional variations observed in developmental durations and body size compared to earlier studies likely reflected the influence of unique climatic conditions and floristic diversity of Assam, highlighting the importance of localized ecological factors in shaping bee biology. The findings are presented elaborately (Table 2,3; Fig. 1,2) under different sub headings in this section.

Biology and morphometrics of immature stages

Egg: The egg was transparent to white coloured, cylindrical in shape with broader end at one side. A single egg was laid in the center of each brood cell filled with brood food. The average length and breadth of the eggs ranged between 0.80 to 0.26 mm. The egg hatched in 5.50 to 6.00 days with mean of 5.60 days.

Larva: The larva was transparent white to light yellowish colour, C shaped and apodous. Five larval instars were recorded in its complete life cycle with significant differences. The total larval period occupied 11-12 days with mean of 11.90 days. The measurements of individual instars were also recorded. The first instar larva was transparent white colour, about 0.92 mm long and 0.40 mm wide. The duration ranged from 1.50 to 2.50 days with mean duration of 2.01 days. The body of the second instar larva was slender and white to pale yellowish in colour with yellowish mass visible inside the body. The larva was about 2.08 mm long

Table 1: Morphometric characteristics of adult stages of *Tetragonula iridipennis*

Characteristics	Sl. No.	Characteristics	
I. Body		**iii. Wings**	
a.	Head length (*Hdl*)	l.	Forewing length (*Fwl*)
b.	Head width (*Hdw*)	m.	Forewing width (*Fww*)
c.	Thorax length (*Thl*)	n.	Hindwing length (*Hwl*)
d.	Thorax width (*Thw*)	o.	Hindwing width (*Hww*)
e.	Abdomen length (*Abdl*)	p.	Number of hamuli in hind wing (*Nh*)
f.	Abdomen width (*Abdw*)	**iv. Legs**	
g.	Body length (*Bl*)	q.	Femur length (*Fl*)
ii. Head		r.	Femur width (*Fw*)
h.	Antennal length (*Anl*)	s.	Tibial length (*Tbl*)
l.	Compound eye length (*Cmpl*)	t.	Tibial width (*Tbw*)
j.	Compound eye width (*Cmpw*)		
k.	Ocello-ocular distance (*Ocd*)		

Table 2: Biology of developmental stages of *Tetragonula iridipennis*

Developmental stages	Duration (in days) Mean (mm)* ± SD
Egg	5.60 ± 0.42
First Instar	2.01 ± 0.35
Second Instar	2.50 ± 0.50
Third Instar	2.61 ± 0.42
Fourth Instar	2.30 ± 0.67
Fifth Instar	2.50 ± 0.50
Total larval period	**11.90 ± 0.43**
Pupa	35.10 ± 1.95
Adult	42.60 ± 2.30
Total life cycle	95.20 ± 5.08

*Data represents mean of five observations

Table 3: Morphometric study of developmental stages of *Tetragonula iridipennis*

Developmental stages	Mean (mm)* ± SD	
	Length	Breadth
Egg	0.81 ± 0.01	0.26 ± 0.01
Larva		
First Instar	0.92 ± 0.02	0.40 ± 0.01
Second Instar	2.08 ± 0.01	0.67 ± 0.04
Third Instar	2.41 ± 0.01	0.97 ± 0.01
Fourth Instar	2.73 ± 0.02	1.15±0.01
Fifth Instar	3.83 ± 0.12	1.48 ± 0.01
Pupa	3.99 ± 0.04	1.53 ± 0.04
Adult	4.74 ± 0.26	1.58 ± 0.04

*Data represents mean of five observations

Table 4: Morphometrics of *Tetragonula iridepennis* adult

	Characteristics	Mean (mm)* ± SD
Body		
	Head length	1.29 ± 0.11
	Head width	1.72 ± 0.07
	Thorax length	1.60 ± 0.06
	Thorax width	1.50 ± 0.15
	Abdomen length	1.84 ± 0.20
	Abdomen width	0.78 ± 0.11
	Body length	4.65 ± 0.29
Head		
	Antennal length	1.58 ± 0.04
	Compound eye length	0.85 ± 0.03
	Compound eye width	0.23 ± 0.01
	Ocello-ocular distance	0.16 ± 0.01
	Wings	
	Fore wing length	3.81 ± 0.10
	Fore wing width	1.34 ± 0.17
	Hind wing length	2.55 ± 0.21
	Hind wing width	0.64 ± 0.14
	Number of hamuli in hind wing	5
Legs		
	Femur length	1.10 ± 0.04
	Femur width	0.25 ± 0.02
	Tibial length	1.53 ± 0.07
	Tibial width	0.45 ± 0.08

*Data represents mean of ten observations

and 0.67 mm wide. The duration ranged between 2 to 3 days with mean duration of 2.50 days. The third instar larva was pale yellowish in colour with a yellowish abdominal extension on posterior side of the body. The larva was about 2.41 mm long and 0.97 mm wide. The duration ranged from 2 to 3 days with mean duration of 2.61 days. The fourth instar larva was cream coloured, about 2.73 mm long and 1.15 mm wide. A significant increase in size of the larva was observed as the instar advanced. The duration ranged between 2 to 3 days with mean duration of 2.30 days. The fifth instar larva was pure white and the brood pot was completely devoid of larval food. The length and breadth of larva were 3.83 mm and 1.48 mm, respectively. The duration ranged between 2 to 3 days with mean duration of 2.50 days.

Pupa: When the last larval instar approached pupation, the brood pot completely dried up. The pupa was pure white in colour and exarate type where all appendages were well developed. In later stages, there was a prominent change in eye colour from transparent to red due to sclerotization. The average length of the pupa was 3.99 mm and the average breadth was 1.53 mm. The pupal period lasted for 32 to 36 days with mean ranging between 35.10 days. These findings are in agreement with the reports of Roopa *et al.* (2015) who reported that egg period ranged between 5.50 to 6.00 days, total larval period ranged 11.00 to 13.50 days with a mean of 12.70 days and pupal period ranged from 33.00 to 38.00 days with a mean of 35.80 days from Regional Research Station, UAS, GKVK, Bangalore. Limited studies have been conducted on the biology of *T. iridepennis* and morphometrics of individual instars/stadium. Therefore, the present study can be considered as a pioneer work done from Assam, North-east India.

The observed regional differences in the biology and morphometrics of *T. iridipennis* are likely the result of complex interactions between local ecological conditions, climatic factors, genetic variation and methodological approaches. Recognizing these factors is essential to understand intraspecific variability and to develop region-specific management practices for successful domestication and conservation of stingless bees in diverse agroecological landscapes. High relative humidity (~79-85%) and moderate temperatures (28±2°C) of Assam may contribute to faster development (egg period: 5.60 days) and slightly larger body size (mean body length: 4.65 mm). Rich floristic diversity of Assam within the Indo-Burma biodiversity hotspot likely ensured better brood nutrition, influencing both development and adult morphometry Altitudinal effects also suggested lower elevations typically correlate with slightly larger body sizes due to reduced metabolic constraints under Assam condition. Khambhu et al. (2021) documented the biology and developmental time in stingless bees *T. laeviceps.* The researchers reported the egg, larval and pupal period of *T. laeviceps* to be 5.37 ± 0.81, 11.20 ± 0.81 and 31.83 ± 1.35 days, and the total period of development from egg to adult was 48.77 ± 1.74 days. Further, the researchers revealed that the colour of newly emerged adult was light yellowing brown.

Morphometrics of *Tetragonula iridepennis* adult

Adult: The head and thoracic segments were dark brown to blackish colour whereas the abdomen was amber coloured. The results pertaining to this study are presented in Table 4 and illustrated in Fig. 3 to 9.

Body

Head length (*Hdl*): It was measured from the clypeo-labral ridge to the dorsal end of the head. The length ranged between 1.13 to 1.45 mm with a mean length measuring 1.29 mm.

Head width (*Hdw*): The longest distance from left side to right side of head was measured, including protruding eyes which varied from 1.59 to 1.82 mm with a mean head width of 1.72 mm.

Thorax length (*Thl*): The measurement was taken from the anterior to posterior base of the thorax, where the mean *Thl* was observed to be 1.60 mm. The range was 1.49 to 1.70 mm.

Thorax width (*Thw*): The measurement was nearer to the posterior angles where the thorax was broadest. The mean *Thw* was 1.50 mm with a range between 1.29 to 1.74 mm.

Abdomen length (*Anl*): The measurement was taken from second to last abdominal segments which varied 1.61 to 2.20 mm with a mean length range of 1.84 mm.

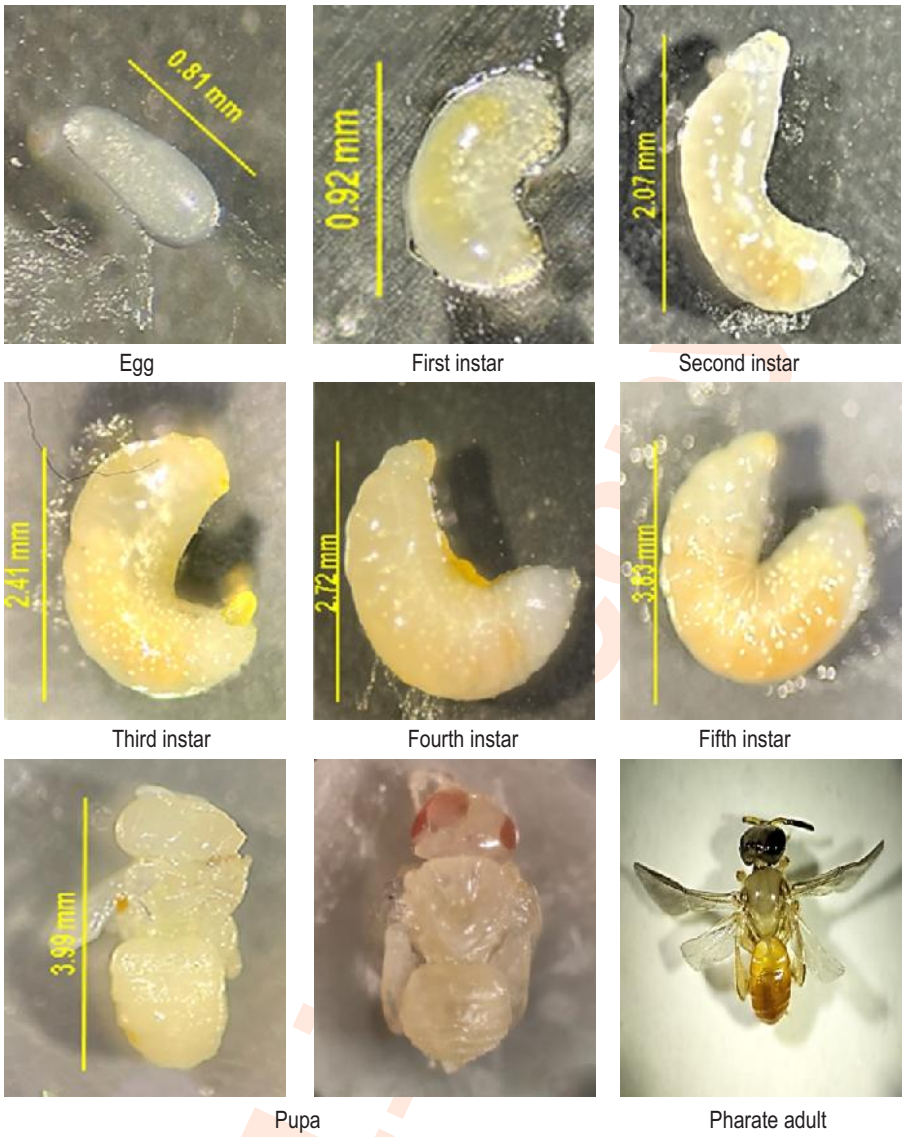


Fig 1: Morphometrics of developmental stages of *Tetragonula Iridipennis*.

Fig. 2: Developmental stages of *Tetragonula iridipennis*: (a) Egg, (b) First instar, (c) Second instar, (d) Third instar, (e) Fourth instar, (f) Fifth instar, (g) Pupa, and (h) Adult.

Abdomen width (*Anw*): The measurement was taken nearer to the posterior angles where the abdomen was broadest and varied from 0.64 to 0.97 mm, with a mean width range of 0.78 mm.

Body length (*Bl*): This measurement was taken between the anterior most point on head to the tip of abdomen. The average measure of the body length was 4.65 mm. The length varied from 4.00 to 4.96 mm.

The results are in close proximity with the findings of Patel and Pastagia (2016), who recorded the thoracic length 1.35 to 1.78 mm in *Tetragonula laeviceps* and thorax breadth varying between 1.30 to 1.41 mm from Navsari Agricultural University, Gujarat. Their studies have shown minor differences in thoracic breadth. The abdominal length and width were nearly similar with the studies of Patel and Pastagia (2016), who reported the abdomen length varying between 1.30 mm to 1.70 mm. Adult morphometric analysis revealed a mean body length of 4.65 mm, closely matching the measurements reported for *T. iridipennis* (Makkar *et al.* (2018). Makkar and his co-workers, through their detailed morphological investigations, reported that *Tetragonula iridipennis* exhibited an average body length of 3.653 mm, with the head measuring 1.232 mm in length and 1.605 mm in width. The thorax was 1.519 mm in length and 1.144 mm in width, while the abdomen measured 1.478 mm in length and 1.162 mm in width. These morphometric values provide a useful baseline for comparative studies and further emphasize the relatively small size and compact body structure characteristic of stingless bees. Tej *et al.* (2017) investigated the morphological diversity of *Tetragonula iridipennis* populations across Coimbatore, Erode and Tiruppur districts of Tamil Nadu. Morphometric analysis revealed that stingless bees from Coimbatore were comparatively larger, with a head length measuring 1.53 mm and head width 1.76 mm, followed by those from Tiruppur (head length: 1.35 mm; head width: 1.63 mm) and Erode (head length: 1.23 mm; head width: 1.62 mm) districts. Sharma *et al.* (2023) carried out detailed morphometric investigations on stingless bee Tetragonula iridipennis from the Saurashtra region of Gujarat. Their study documented considerable variation in body measurements, with the overall body length ranging between 4.10 and 4.66 mm. The head length was recorded in the range of 1.47 to 1.55 mm, while the thorax length varied 1.42 to 1.48 mm and thorax breadth 1.55 to 1.61 mm. Similarly, the abdomen length was found to range between 1.41 and 1.61 mm. These observations not only provide insights into the regional morphometric variability of *T. iridipennis* but also highlight subtle structural differences that could be attributed to ecological and environmental factors prevailing in the Saurashtra region. Sakagami (1978) elucidated that *Tetragonula iridipennis* possesses a head width of approximately 1.69 mm, providing one of the earliest morphometric accounts of this stingless bee species. Later, Rasmussen (2013) further contributed to the morphometric characterization of the species, documenting a total body length of about 3.55 mm and a head width of 1.60 mm. These findings, though reported from different studies and periods, collectively enrich the baseline information on the size attributes of *T. iridipennis,* thereby serving as valuable references for comparative morphometric evaluations across regions and populations. In a similar study, Vijayakumar and Jeyarraj (2017) conducted standard morphometric analyses to assess the population-level variation in *Tetragonula iridipennis* collected from seven different localities within the Coimbatore district.

Their findings revealed notable differences in body size among the sampled populations. Stingless bees collected from Nellithurai were comparatively larger, with a mean body length of 3.84 mm, head length of 1.35 mm and head width of 1.79 mm. In contrast, smaller morphometric values were observed in populations from Sambaravalli (body length: 3.25 mm; head length: 1.35 mm; head width: 1.79 mm) and Sirumugai (body length: 3.45 mm; head length: 1.29 mm; head width: 1.53 mm). Intermediate measurements were recorded in populations from Chickadasam Palayam (body length: 3.52 mm; head length: 1.31 mm; head width: 1.69 mm), Pungam Palayam (body length: 3.65 mm; head length: 1.30 mm; head width: 1.63 mm), Velliankadu (body length: 3.55 mm; head length: 1.27 mm; head width: 1.53 mm), and Vellakinaru (body length: 3.65 mm; head length: 1.30 mm; head width: 1.61 mm). These results highlight the existence of intra-population morphometric variability within *T. iridipennis* across different microhabitats of Coimbatore, suggesting that ecological and environmental conditions might play a role in shaping regional morphometric traits.

Head

Antennal length (*Anl*): The measurement was taken between the base of the scape on the head to the end of the flagellum. The length ranged between 1.52 to 1.64 mm with a mean *Anl* of 1.58 mm.

Compound eye length (*Cmpl*): The measurement was taken on the antero-posterior axis of the compound eyes. The length ranged between 0.81 to 0.89 mm. The average *Cmpl* was 0.85 mm.

Compound eye width (*Cmpw*): The measurement was taken along broadest distance on the dorso-ventral axis of compound eyes. The width ranged between 0.22 to 0.24 mm. The mean *Cmpw* was 0.23 mm.

Ocello-ocular distance (*Ocd*): The measurement was recorded between the simple eyes with mean distance of 0.16 mm. The range was 0.14 to 0.17 mm.

Makkar *et al.* (2018) further documented the morphometric features of visual organs in *Tetragonula iridipennis,* reporting a compound eye length of 1.052 mm and width of 0.249 mm, along with an ocello-ocular distance of 0.218 mm. Such precise measurements of ocular structures are critical, as they provide insights into the visual capacity and ecological adaptations of stingless bees. The relatively small ocello-ocular distance, in particular, may be indicative of their adaptation to foraging under low-light or shaded conditions, a feature

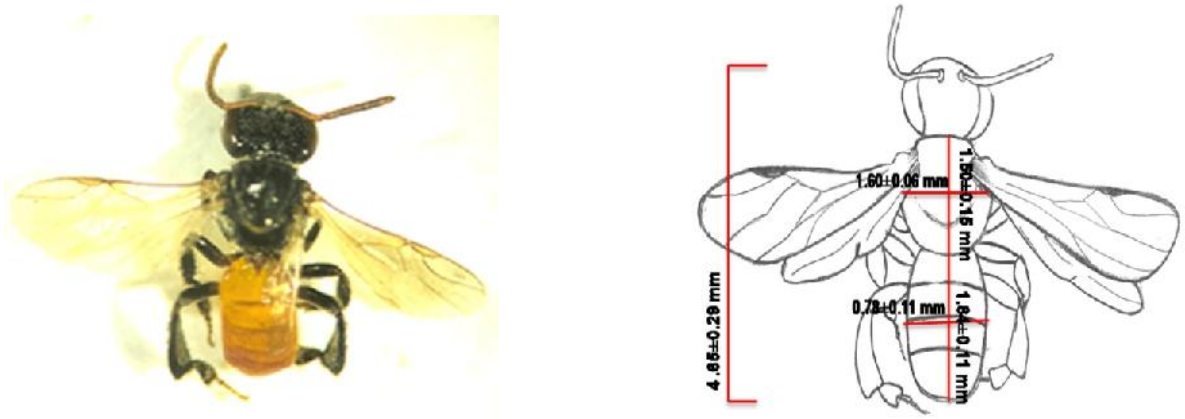


Fig. 3: Measurements of *Tetragonula iridipennis* adult (Tol=1.60 ± 0.06 mm; Tow = 1.50 ± 0.15 mm; Anl = 1.84 ± 0.11 mm; Anw = 0.78 ± 0.11 mm; Bl = 4.65 ± 0.29 mm).

Fig. 4: Measurements of *Tetragonula iridipennis* head (Anl = 1.58 ± 0.04 mm; Hdl = 1.29 ± 0.11 mm and Hdw = 1.72 ± 0.07 mm)

Fig. 5: Measurements of length and width of compound eyes (Cmpl = 0.85 ± 0.03 mm; Cmpw = 0.23 ± 0.01 mm) and ocello-ocular distance (Ocd = 0.16 ± 0.01 mm).

commonly associated with eusocial bees inhabiting tropical environments. Tej *et al.* (2017) examined antennal and ocello-ocular morphometrics of *T. iridipennis* collected from Coimbatore, Tiruppur and Erode. The results exhibited comparatively greater measurements, with antennal length of 1.87 mm and an ocello-ocular distance of 0.21 mm in stingless bees collected from Coimbatore, followed by populations collected from Tiruppur, which recorded antennal length of 1.78 mm and an ocello-ocular distance of 0.22 mm.

The smallest values were observed in stingless bees from Erode, with an antennal length of 1.67 mm and an ocello-ocular distance of 0.21 mm. Vijayakumar and Jeyarraj (2017) documented considerable variation in antennal length and ocello-ocular distance of *Tetragonula iridipennis* populations collected from different localities of Coimbatore district. Stingless bees from Nellithurai exhibited the highest values, with antennal length of 1.31 mm and ocello-ocular distance of 0.39 mm, indicating comparatively larger cranial dimensions. These were followed by populations from Sirumugai (antennal length: 1.26 mm; ocello-ocular distance: 0.33 mm) and Velliankadu (antennal length: 1.27 mm; ocello-ocular distance: 0.25 mm). Lower values were recorded in populations from Sambaravalli (antennal length: 1.19 mm; ocello-ocular distance: 0.30 mm), Chickadasam Palayam (antennal length: 1.24 mm; ocello-ocular distance: 0.29 mm), Pungam Palayam (antennal length: 1.22 mm; ocello-ocular distance: 0.28 mm) and Vellakinaru (antennal length: 1.21 mm; ocello-ocular distance: 0.31 mm). In a recent study, Sharma *et al.* (2023) reported that the antennal length of *Tetragonula iridipennis* varied within the range of 1.71 to 2.01 mm, indicating noticeable intra-population morphometric variation. Furthermore, Singh and Khan (2019) documented the compound eye dimensions of *Tetragonula iridipennis,* reporting a length of 0.975 mm and a width of 0.389 mm. These precise measurements contribute to the baseline morphometric characterization of species and serve as valuable reference points for comparative studies on visual morphology and its ecological significance.

Wings

Forewing length (*Fwl*): Measurements were taken between the articulatory point of forewing and its apical tip which varied from 3.69 to 3.99 mm with a mean *Fwl* of 3.81 mm.

Forewing width (*Fww*): The measurement was taken at the middle of forewing where it was maximum & varied from 1.00 to 1.56 mm with a mean *Fww* of 1.34 mm.

Hindwing length (*Hwl*): Measurements were taken between the articulatory point of hind wing and its apical tip. The mean *Hwl* was 2.55 mm and the range was 2.04 to 2.86 mm.

Hindwing width (*Hww*): Measurements were taken at the middle of hind wing where width was maximum. The mean *Hww* was 0.64 mm and range was 0.48 to 0.91 mm.

Number of hamuli (*Nh*): The number of hooks present on the costal margin of hind wings were counted and recorded to be five in all specimens examined.

Makkar *et al.* (2018) documented the morphometric attributes of the wings in *Tetragonula iridipennis,* recording an average forewing length of 3.739 mm and width of 1.382 mm, while the hindwing measured 2.476 mm in length and 0.671 mm in width. In addition, the number of hamuli was consistently observed to be five. These morphometric parameters highlight the relatively small yet proportionally well-developed wings of *T. iridipennis,* which are crucial for maintaining efficient flight despite their miniature body size. The forewings, being comparatively larger and broader than the hindwings, likely contribute to greater lift and stability during foraging flights whereas the reduced size of the hindwings correspond with their role in coupling with the forewings via hamuli during flight. The presence of five hamuli, as reported, is in agreement with previous observations in stingless bees, underscoring the structural adaptations of this group for coordinated wing movement. Such morphometric details are significant not only for taxonomic differentiation but also for understanding functional adaptations that support the ecological success of stingless bees in diverse habitats. In a similar investigation, Sharma *et al.* (2023) provided detailed morphometric measurements of *Tetragonula iridipennis* wings. The study revealed that the forewing length ranged from 3.30 to 3.60 mm, with a corresponding breadth of 1.33 to 1.55 mm. The hindwing measurements were comparatively smaller, with length varying between 2.46 and 2.75 mm and breadth ranging from 0.55 to 0.65 mm. Additionally, the number of hamuli on the hindwing was consistently recorded as five. Vijayakumar and Jeyarraj (2017) reported significant variation in wing morphometrics of *Tetragonula iridipennis* populations collected from different localities of Coimbatore district. Stingless bees from Nellithurai recorded comparatively larger wing dimensions, with a forewing length of 3.41 mm and a forewing width of 1.36 mm. In contrast, smaller measurements were observed in populations from Sambaravalli (forewing length: 3.28 mm; width: 1.18 mm), Chickadasam Palayam (forewing length: 3.32 mm; width: 1.22 mm), Pungam Palayam (forewing length: 3.31 mm; width: 1.27 mm), and Vellakinaru (forewing length: 3.29 mm; width: 1.27 mm). Intermediate values were recorded in bees from Sirumugai (forewing length: 3.37 mm; width: 1.28 mm) and Velliankadu (forewing length: 3.35 mm; width: 1.29 mm). These findings highlight the existence of micro-geographical variation in wing morphology among *T. iridipennis* populations, which could be influenced by local environmental conditions and genetic factors. Further, the number of hamuli on the hindwing was consistently observed to be five by the researchers (Vijayakumar and Jeyarraj, 2017).

Legs

Femur Length (*Fl*): The measurement was taken between the anterior tip and posterior tip of the femur. The *Fl* ranged 1.05 to 1.17 with a mean length of 1.10 mm.

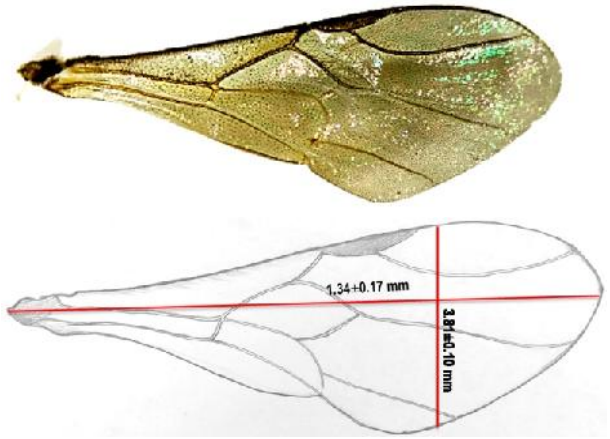


Fig. 6: Measurements of fore wing (Fwl = 3.81 ± 0.10 mm and Fww = 1.34 ± 0.17 mm).

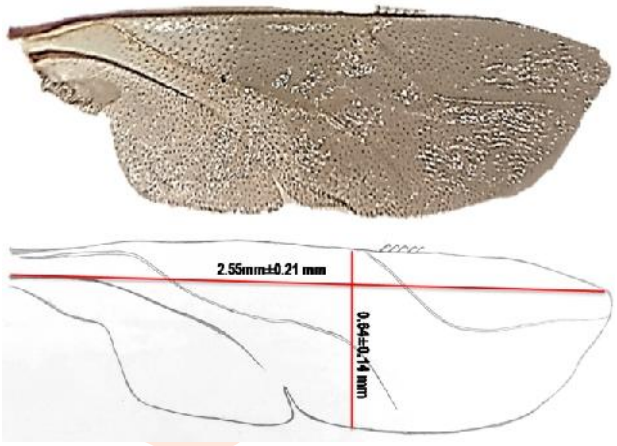


Fig. 7: Measurements of hind wing (Hwl = 2.55 ± 0.21 mm and Hww = 0.64 ± 0.14 mm)

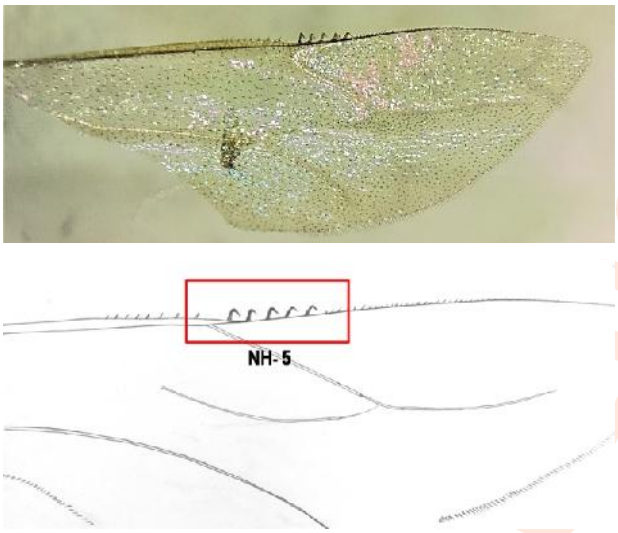


Fig. 8: Number of hamuli in hind wing (Nh = 5)

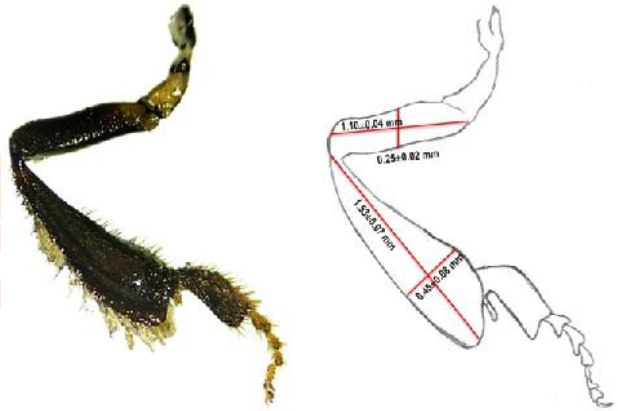


Fig. 9: Measurements of femur and tibia of hind leg (Fl = 1.10 ± 0.04 mm; Fw = 0.25 ± 0.02; Tbl = 1.53 ± 0.07 and Tbw = 0.45 ± 0.08).

Femur Width (*Fw*): The measurement was taken between the sides of the concave surface of the hind femur. The *Fw* ranged 0.23 to 0.30 mm with a mean width of 0.25 mm.

Tibial Length (*Tbl*): The measurement was taken between the anterior tip and posterior tip of tibia. The mean *Tbl* measured 1.53 mm and ranged from 1.42 to 1.63 mm.

Tibial Width (*Tbw*): Measurements were taken between the sides of the concave surface of the hind tibia. The mean *Tbw* measured was 0.45 mm which ranged from 0.25 to 0.52 mm.

Makkar *et al.* (2018) documented the morphometric attribute of the hind tibia in *Tetragonula iridipennis,* reporting an average length of 1.477 mm. This measurement holds functional significance, as the hind tibia in stingless bees is a critical structure associated with pollen collection and transport. Tej *et al.* (2017) reported distinct regional variation in the hind tibial morphometrics of *Tetragonula iridipennis.* In stingless bees collected from Coimbatore, the hind tibial length and width were recorded as 1.62 mm and 0.49 mm, respectively. Populations from Tiruppur exhibited comparatively smaller dimensions, with a hind tibial length of 1.34 mm and width of 0.43 mm, while those from Erode showed values of 1.31 mm for length and 0.49 mm for width. These differences highlight subtle morphometric variability among the populations from adjoining districts, potentially influenced by local ecological conditions. Sharma *et*

al. (2023) provided detailed morphometric observations on the hind legs of *Tetragonula iridipennis* where they revealed that the hind leg length ranged between 4.45 and 4.59 mm, while the hind leg width varied 0.44 to 0.51 mm. Vijayakumar and Jeyarraj (2017) documented notable variation in hind tibial morphometrics of *Tetragonula iridipennis* populations where stingless bees collected from Nellithurai exhibited the largest dimensions, with a hind tibial length of 1.53 mm and a width of 0.55 mm.

In comparison, smaller values were recorded in populations from Sambaravalli (length: 1.31 mm; width: 0.41 mm), Vellakinaru (length: 1.40 mm; width: 0.45 mm), and Chickadasam Palayam (length: 1.41 mm; width: 0.48 mm). Intermediate measurements were observed in bees from Sirumugai (length: 1.41 mm; width: 0.49 mm), Pungam Palayam (length: 1.42 mm; width: 0.43 mm), and Velliankadu (length: 1.46 mm; width: 0.47 mm). Furthermore, Singh and Khan (2019) provided morphometric details of the femur in *Tetragonula iridipennis,* recording a length of 0.906 mm and a width of 0.232 mm. Also, the researchers documented the length of hind tibia as 1.359 mm and width of hind tibia as 0.510 mm. These findings highlight intra-population variability in hind tibial traits of *T. iridipennis,* suggesting that subtle morphological differences may be shaped by local environmental conditions and microhabitat influences. The relatively small size, when compared to larger eusocial bees such as *Apis mellifera,* reflects the overall miniature body dimensions of *T. iridipennis*. Nonetheless, the structural adaptation of hind tibia enables efficient pollen storage and carriage to the nest, ensuring effective foraging even within their size constraints. Such morphometric information contributes to the growing body of knowledge required for species-level identification and offers a foundation for comparative studies across meliponine bees.

This study presents the first comprehensive account of the biology and morphometrics of *Tetragonula iridipennis* in Assam. While most traits aligned with reports from other regions of India, minor variations particularly in thoracic and pupal dimensions were observed, likely due to local environmental or genetic factors. Biological observations, including developmental durations, brood patterns, and colony dynamics, offer key insights into the life cycle of *T. iridipennis* under Assam's climatic conditions, aiding in the identification of optimal periods for colony division and propagation, an essential step for advancing meliponiculture in the region. Given that stingless bee domestication in Assam is still nascent, these standardized benchmarks have significant practical relevance. They contribute to taxonomic clarity, informed colony management, and the promotion of sustainable stingless beekeeping. Beyond meliponiculture, the findings support pollinator conservation, rural livelihood enhancement, and ecological agriculture in North-east India. Future work on genetic diversity, behavioral ecology, and agro-climatic influences will further strengthen conservation and optimize stingless bee management across diverse landscapes.

Acknowledgment

The authors take the privilege to express their deepest sense of gratitude to AICRP on Honey bees and Pollinators, AAU, Jorhat for providing necessary aid and help during the present study.

Authors' contribution: A. Rahman, S. Borkataki and P.S. Saranya: Conceptualization of work; Conducted experiments; **A. Devee, S. Borkataki, P.S. Saranya, S. Sen and R. Boruah:** Analysis of data and drafting manuscript; **B. Bhattacharyya, M.K. Deka, K. Sindhura Bhairavi, K. Das and D. Sorahia:** Preparation of manuscript; **S.P. Nanda, R.R. Taye and K. Das:** Revision and Proof reading.

Funding: Not applicable.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

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

Data availability: Data are presented in tabular manner within the research article.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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