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What Makes Them Masters of Survival? Unraveling the Intricacies of Adaptive Thrive of *Tetragonula iridipennis* Smith in the Dynamic Ecosystems of Tamil Nadu, India

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Abstract
Stingless bees (Hymenoptera: Apidae), commonly called 'Dammar bees in India,' inhabit concealed environments across various ecosystems. They possess significant ecological importance as 'Keystone Pollinators', contributing crucially to the pollination of diverse flowering plants. Increased attention is being given to the patterning aspects of nests in nature. Our investigation into this occurrence within human-inhabited environments is characterized by repeated spatio-temporal patterns. Various criteria, including habitat preference, nesting pattern, orientations, elevation, and nest architecture, were chosen to assess the preferences of an indigenous stingless bee, *Tetragonula iridipennis* Smith, at 30 different locations in Tamil Nadu, India. Most colonies inhabited walls (50.0%), followed by tree trunks (33.33%). The maximum height of nest entrances from ground level, a significant parameter for adaptive defense patterns, ranged from 1.3 m to 2.98 m. Additionally, the circumference of nest entrances varied from 1.29 cm to 2.45 cm. The length of the external tube ranged from 1.1 cm to 3.24 cm, playing a crucial role in defense by presenting a thin layer of mud capping to prevent the entry of intruders. Bees can effectively thwart threats and predators Through complex nesting architecture, including resin and mud coating, pillar-like batumen structures, waxy solid buildings, and multiple nest entrances. Thus, the population and their colony propagation could be sustained.

Introduction

Stingless bees are efficient pollinators of a wide variety of crops, including fruits, vegetables, and nuts. They exhibit behaviors such as buzz pollination, which enhances pollen release from flowers (Kishan Tej et al., 2017). The pollination services provided by stingless bees directly contribute to food security by increasing crop productivity and ensuring the availability of diverse and nutritious food sources. Their activities enhance fruit sets, seed production, and crop quality (Rader et al., 2020). Stingless bees may offer resilience to climate change-induced disruptions in pollination services. Stingless bee diversity exhibits regional variation influenced by climate, vegetation, and habitat types (Sahoo et al., 2023). Their adaptability to diverse environmental conditions and ability to forage in various habitats make them valuable allies in maintaining food security under changing climatic conditions (Rasmussen & Camargo, 2008). There are over 500 species of stingless bees globally, each with unique nesting behaviors and adaptations to their environments (Michener, 2000). India harbors a diverse array of stingless bee species, with an estimated 60-70 species recorded across different regions (Ascher & Pickering, 2017).

Exploring the intricate world of stingless bees unveils a fascinating journey into their nesting behavior. They construct intricate nests typically found in hollow trees, crevices, or underground cavities (Nunes et al., 2011). Within the confines of their carefully constructed abodes, these remarkable insects

exhibit a repertoire of behaviors aimed at ensuring the survival and prosperity of their colonies. Understanding the nesting behavior of stingless bees is crucial for conserving these essential pollinators and managing their populations effectively. Recent studies have delved into various aspects of their nesting habits, shedding light on their intricate social structures and nesting strategies. Dias et al. (2023) offered insights into the nest architectural diversity of stingless bees and the factors influencing their choice of nesting sites. The significance of nest architecture in regulating temperature and humidity levels is crucial for brood development and colony survival (Quezada-Euán et al., 2022). Moreover, pheromones are vital in mediating nestmate recognition and maintaining colony cohesion among stingless bees (Camargo et al., 2002).

The characteristics of nests, such as their location, complexity in design, and materials used, can vary depending on the species, offering valuable insights into the evolutionary behavior of different taxa and broader groups. For instance, the nest structure of stingless bees typically includes an entrance, a tunnel for access, storage compartments for nectar and pollen (food pots), and an area for brood development, often arranged in horizontal combs or clusters (Rasmussen & Camargo, 2008). Nests are constructed using wax secreted from the metasomal terga, which is then combined with resins and gums gathered by foragers. Some species incorporate mud, feces, or other materials into various parts of the nest. Across all species, there are variations in composition and texture among different sections of the nest. In certain species, brood combs are shielded by layers of wax involucrum, while storage pots are fashioned from cerumen (Michener, 2000). Several bee species construct erect and robust structures using a mixture of cerumen, mud, and resin, known as scutellum or batumen. In some instances, the supports and batumen plates are constructed from durable materials, while the external sheet of batumen encasing specific exposed nests tends to be harder and more brittle (Michener, 2000).

The stingless bee honey is characterized by its darker color, more acidic taste, and higher content of phenolic compounds, as documented by Garedew et al. (2003) and Kek et al. (2014). Propolis, another product of stingless bees, exhibits antibacterial, antimicrobial, and antifungal properties, as highlighted by Sforcin and Bankova (2010). Compared to honey bees, stingless bees are known to produce higher quantities of propolis, as noted by Fatoni (2008). However, natural enemies such as predators and parasites can diminish bee productivity, disrupt colonies, alter nest structure and composition, or even result in the death of individual bees. Some assailants, including wasps, ants, spiders, and centipedes, target stingless bee colonies. Centipedes, in particular, pose a significant threat due to their ability to swiftly inflict damage on bee colonies within 2-5 seconds, facilitated by their specialized morphological features for prey capture (Kumar et al., 2012). In defense, stingless bees resort to a "suicide bite" as a protective measure against predators (Shackleton et al., 2015). Honey bees commonly face parasitic infestations from mites, fleas, moths, and small beetles, which can compromise their health (Strauss et al., 2013). The prosperous survival of *T. iridipennis* was investigated by understanding their nesting behaviors and dynamic habitats. Therefore, the conservation of stingless bees could be effective. The study's objective was to investigate the nesting site preference, nesting pattern, predators, and unique aspects of nest structure associated with defense from the natural enemies of stingless bees.

Materials and Methods

Study area

The research was conducted across 30 distinct sites spanning eight different districts

(Fig 1) of Tamil Nadu, namely Coimbatore, Cuddalore, Dharmapuri, Dindigul, Kallakurichi, Madurai, Salem, and Thiruchirappalli. Exploration for colonies of *T. iridipennis* extended to all conceivable hidden spots of different listed sites of Tamil Nadu (Table 1). Observation-based data were recorded over ten months, from April 2023 to January 2024, between 08:00 and 16:00, employing an exhaustive search approach relying solely on visual observation. The primary objective involved locating feral colonies, which was facilitated by monitoring the movements of bees towards their nest entrances.

Nesting habitat

Systematic surveys were conducted within the study area, wherein nests of *T. iridipennis* were visually inspected in various locations, encompassing residential buildings, educational institutions, and roadsides, as well as natural vegetation, including trees and shrubs to determine the habitat preference (Fig 2). To evaluate unaltered nests, their orientation was categorized into eight levels based on cardinal directions (North, South, East, and West) and four intermediate directions (northeast, southeast, southwest and northwest) spanning specific degrees. The geo-coordinates and the nest orientations of stingless bee colonies were recorded and documented.

Nesting patterns and internal structures

The nesting substrate, shape, color, and material of the nest entrance structure were recorded along with measurements of the length and diameter of the entrance tube. Observations were recorded, encompassing details such as the nesting site, the number of colonies housed in each location, distance from the ground level, the dimensions of the entrance tube (length and width), and all the nesting patterns of *T. iridipennis*.

The internal configuration of the nest was investigated to measure brood cells, pollen and honey pots, cell shape and color, and cell positioning within the cavity. Additionally, observations were conducted to identify any natural enemies that had infiltrated the colony, and any adaptive defense behaviors exhibited to ward off the predators were also documented.

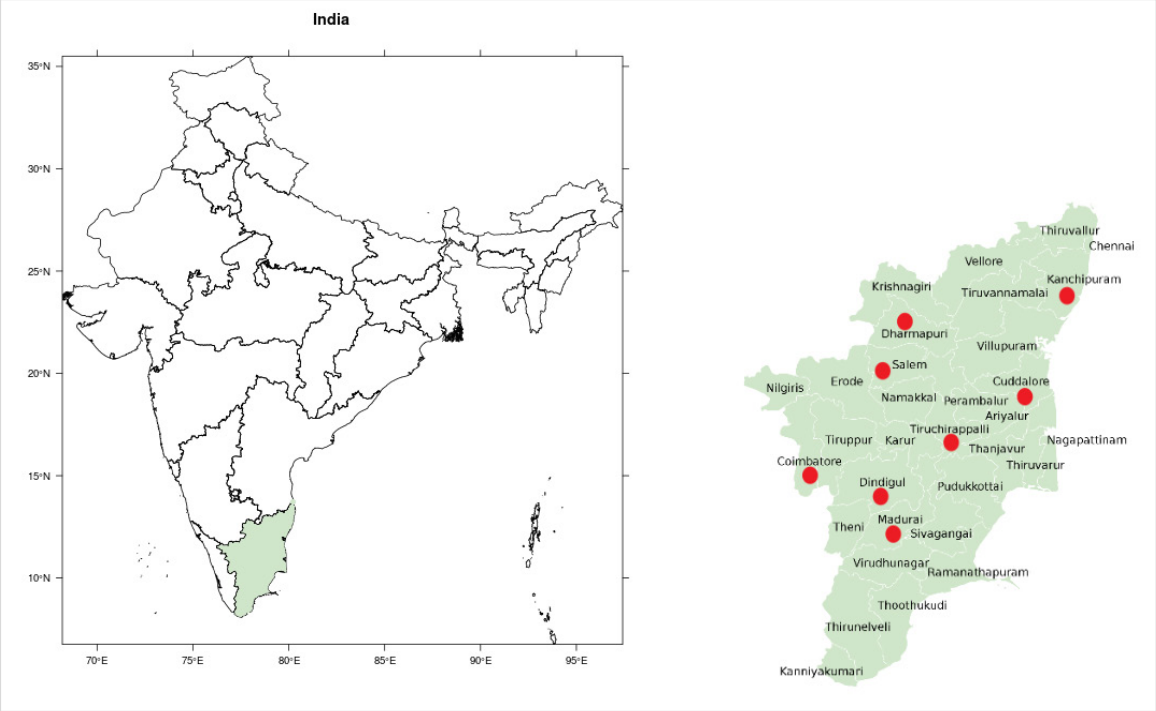
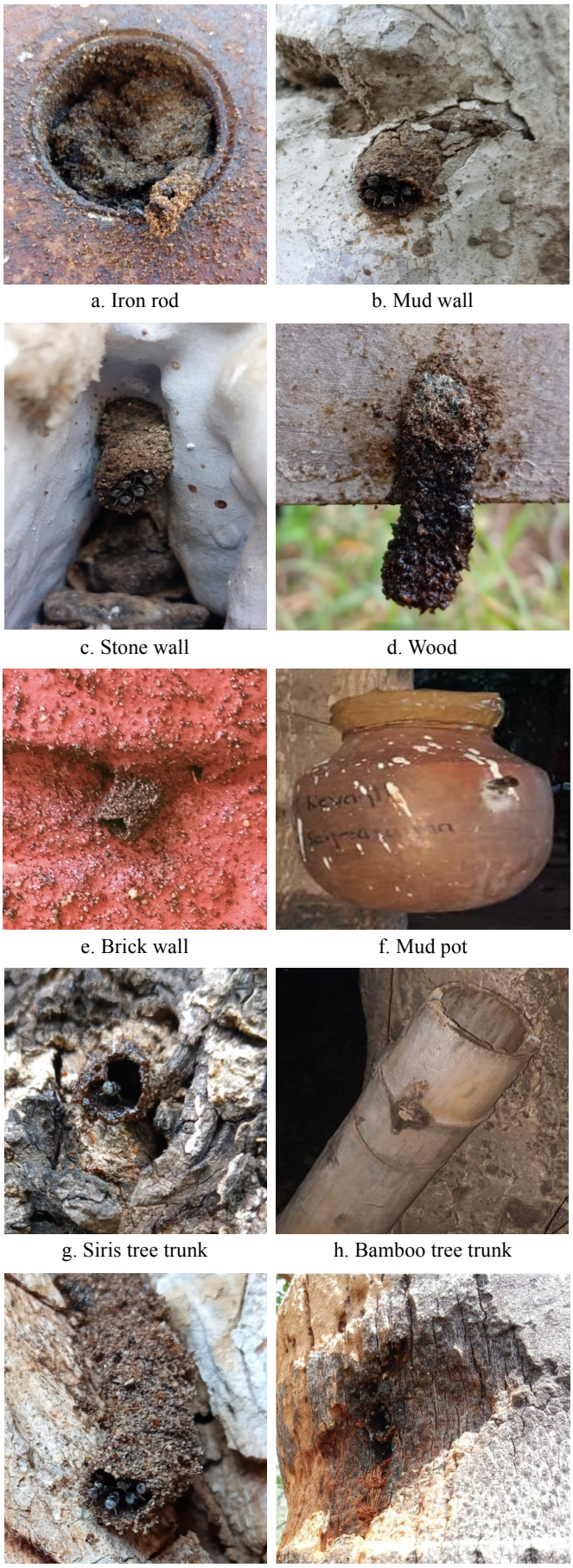


Fig 1. Selected nesting sites of stingless bee colonies, *Tetragonula iridipennis.*

Table 1. Nesting habitat of stingless bees, *Tetragonula iridipennis* in Tamil Nadu.

Places visited (30)	Geographical position		Substrate
	Latitude	Longitude	
Peerakankaranai	12° 52’ 46.24” N	80° 05’ 06.37” E	Tree trunk
Thiruvidanthai	12° 46’ 42.64” N	80° 14’ 53.74” E	Stone wall
Pennagaram	12° 08’ 05.40” N	77° 53’ 34.15” E	Stone wall
Mettur	11° 46’ 59.51” N	77° 47’ 48.12” E	Tree trunk
Virudhachalam	11° 34’ 08.48” N	79° 20’ 37.88” E	Mud wall
Sivapuri	11° 22’ 23.40” N	79° 42’ 48.64” E	Mud wall
Insectary, TNAU	11° 01’ 00.93” N	76° 55’ 44.62” E	Wood, Tree trunk
Botanical garden	11° 00’ 55.12” N	76° 55’ 55.64” E	Siris tree trunk
P. N. Pudhur	11° 00’ 32.41” N	76° 55’ 44.96” E	Brick wall
Veerakeralam	10° 59’ 43.91” N	76° 54’ 58.69” E	Mud wall
PBS, TNAU	10° 59’ 43.58” N	76° 54’ 59.45” E	Stone wall
Thondamuthur	10° 59’ 34.41” N	76° 50’ 36.74” E	Stone wall
Thenkarai	10° 58’ 23.55” N	76° 50’ 07.84” E	Iron rod, Tree trunk
Tanmanpuru	10° 58’ 23.32” N	76° 49’ 02.44” E	Banyan tree trunk
Thennamanallur	10° 58’ 22.42” N	76° 49’ 03.16” E	Mud wall
Perur	10° 57’ 56.44” N	76° 47’ 12.97” E	Banyan tree trunk
Alanthurai	10° 57’ 28.50” N	76° 46’ 46.31” E	Iron rod, Mud wall
Woraiyur	10° 49’ 43.89” N	78° 41’ 30.65” E	Mud wall
Muthukulam	10° 45’ 21.47” N	78° 36’ 06.68” E	Stone wall
Navalurkottapattu	10° 45’ 07.58” N	78° 36’ 08.83” E	Banyan tree trunk
Manapparai	10° 36’ 34.21” N	78° 25’ 25.58” E	Tree trunk
Arasappapillaipatty	10° 28’ 32.09” N	77° 44’ 00.45” E	Coconut tree trunk
Vakkampatty	10° 19’ 48.02” N	77° 53’ 31.55” E	Banyan tree trunk
Athoor	10° 19’ 12.04” N	77° 54’ 10.46” E	Stone wall
Thandikudi	10° 17’ 48.53” N	77° 42’ 31.63” E	Wood, tree trunk
Nilakottai	10° 14’ 03.58” N	77° 53’ 48.94” E	Mud wall
Narasingam	09° 58’ 30.78” N	78° 12’ 28.91” E	Brick wall
Melur	09° 58’ 13.10” N	78° 12’ 17.39” E	Mud wall
Vowal thottam	09° 57’ 52.13” N	78° 12’ 05.86” E	Bamboo tree trunk
Chittangulam	09° 55’ 30.72” N	78° 07’ 11.19” E	Mud pot, tree trunk



a. Iron rod b. Mud wall

c. Stone wall d. Wood

e. Brick wall f. Mud pot

g. Siris tree trunk h. Bamboo tree trunk

i. Banyan tree trunk j. Coconut tree trunk

Fig 2. Nesting habitat preference of stingless bees.

Statistical analysis

The study employed Descriptive Statistics, including means with standard deviations (SD), to analyze the significant differences among data pertaining to the nest characteristics of *T. iridipennis* under investigation. Also, Principal Component Analysis (PCA) was employed by using Google Colab notebook (Python program) to perform dimensional reduction in the data set, thus expressing the significant variation among the variables (nesting attributes).

Results

Habitat preference

Instances of habitat on walls (mud, stone, brick) exhibited a distinct preference, accounting for 50.0%. In comparison, other substrates, including tree trunk (33.33%), iron rod (6.66%), wood (6.66%), and mud pot (3.33%), served as intermediates for the specific nesting preferences of *T. iridipennis*. Data concerning the orientation of the nest indicated a notable trend: a significantly higher proportion (76.66%) of colonies displayed a preference for establishing a nest entrance facing east, followed by those opting for a west-facing (23.33%) entrance (Table 2 & 3).

Nesting pattern

The color of the nest entrance varied, encompassing hues of black, grey, and reddish-brown, influenced by the materials used for construction, such as sand, mud, grease, resin, and wax. Regarding the shape of the nest opening, round to oval shapes were consistently observed over irregular ones (Fig 3).

Nests positioned at the lowest height from ground level were predominantly found within wooden structures, with an average elevation of 1.3 m (ranging from 0.93 m to 1.67 m). Conversely, the highest nests, reaching up to 2.98 m (ranging from 0.52 m to 3.45 m), were located in walls, followed by those on iron rods at 2.03 m (1.92 m to 2.14 m). Colonies housed in tree trunks exhibited a mean height of 1.89 m (1.65 m to 2.05 m). Notably, the maximum population of worker guard bees was observed in walls, ranging from 3 to 10 individuals per colony, showcasing a defensive behavior against intruders (Table 3).

The nesting sites exhibited varying colony densities, with the highest observed on wall substrates, ranging from 2 to 10 colonies per site, followed by tree trunks, typically hosting up to 3 colonies per site. Other habitats generally supported only a single colony per site. Wall-inhabiting colonies exhibited denser vegetation in the surrounding microhabitat compared to other habitats, while sunlight exposure remained consistently low across all nesting substrates.

The diameter of nest entrances was largest among colonies residing in walls, measuring approximately 1cm (Fig 3h), followed by those in tree trunks at 0.86 cm.

Table 2. Quantitative nesting traits of stingless bee colonies, *Tetragonula iridipennis* in different agro climatic zones of Tamil Nadu.

Nesting sites	*Mean ± S.E.				
	NEH	NGB	NC/NS	CNE	ETL
Peerakankaranai	1.68 ± 0.01 (1.64)	5.00 ± 0.12 (2.44)	1.33 ± 0.11 (1.52)	1.53 ± 0.04 (1.59)	1.97 ± 0.02 (1.72)
Thiruvidanthai	0.61 ± 0.02 (1.27)	4.33 ± 0.19 (2.29)	2.33 ± 0.09 (1.82)	1.61 ± 0.08 (1.61)	0.63 ± 0.03 (1.28)
Pennagaram	0.65 ± 0.01 (1.28)	6.00 ± 0.11 (2.64)	2.00 ± 0.00 (1.73)	2.27 ± 0.06 (1.81)	0.76 ± 0.02 (1.33)
Mettur	1.87 ± 0.01 (1.69)	6.33 ± 0.23 (2.69)	1.33 ± 0.11 (1.52)	1.59 ± 0.03 (1.61)	2.84 ± 0.16 (1.95)
Virudhachalam	0.75 ± 0.03 (1.32)	6.67 ± 0.16 (2.76)	2.33 ± 0.09 (1.82)	2.58 ± 0.09 (1.89)	1.78 ± 0.02 (1.67)
Sivapuri	1.16 ± 0.04 (1.47)	6.33 ± 0.27 (2.68)	2.00 ± 0.00 (1.73)	2.49 ± 0.10 (1.86)	2.67 ± 0.10 (1.91)
Insectary, TNAU	1.52 ± 0.10 (1.58)	5.67 ± 0.16 (2.57)	1.33 ± 0.11 (1.52)	1.73 ± 0.01 (1.65)	1.82 ± 0.17 (1.66)
Botanical garden	1.93 ± 0.02 (1.71)	6.00 ± 0.22 (2.63)	1.33 ± 0.11 (1.52)	2.3 ± 0.07 (1.82)	3.67 ± 0.07 (2.16)
P.N. Pudhur	1.6 ± 0.14 (1.60)	6.00 ± 0.31 (2.61)	2.33 ± 0.09 (1.82)	2.38 ± 0.06 (1.84)	2.62 ± 0.03 (1.90)
Veerakeralam	2.18 ± 0.04 (1.78)	5.33 ± 0.26 (2.49)	2.67 ± 0.17 (1.90)	2.39 ± 0.09 (1.84)	2.21 ± 0.10 (1.79)
PBS, TNAU	3.01 ± 0.09 (2.00)	4.67 ± 0.19 (2.37)	8.33 ± 0.14 (3.05)	2.26 ± 0.09 (1.80)	1.53 ± 0.04 (1.59)
Thondamuthur	1.68 ± 0.23 (1.61)	8.67 ± 0.24 (3.09)	3.33 ± 0.21 (2.06)	2.38 ± 0.06 (1.84)	2.52 ± 0.12 (1.87)
Thenkarai	1.92 ± 0.01 (1.71)	5.00 ± 0.30 (2.41)	1.33 ± 0.11 (1.52)	2.33 ± 0.18 (1.81)	1.39 ± 0.17 (1.53)
Tanmanpuru	1.83 ± 0.03 (1.68)	5.00 ± 0.30 (2.41)	8.00 ± 0.10 (3.00)	1.96 ± 0.03 (1.72)	2.9 ± 0.12 (1.97)
Thennamanallur	2.58 ± 0.12 (1.88)	5.67 ± 0.07 (2.58)	2.33 ± 0.09 (1.82)	2.16 ± 0.07 (1.77)	2.55 ± 0.05 (1.88)
Perur	1.90 ± 0.01 (1.70)	6.00 ± 0.22 (2.63)	1.33 ± 0.11 (1.52)	1.82 ± 0.02 (1.68)	4.44 ± 0.06 (2.33)
Alanthurai	1.93 ± 0.03 (1.71)	4.67 ± 0.07 (2.38)	2.00 ± 0.17 (1.72)	1.93 ± 0.08 (1.71)	4.06 ± 0.11 (2.24)
Woraiyur	2.55 ± 0.09 (1.88)	7.33 ± 0.15 (2.88)	2.33 ± 0.09 (1.82)	2.56 ± 0.09 (1.88)	3.23 ± 0.07 (2.06)
Muthukulam	2.71 ± 0.11 (1.92)	6.67 ± 0.36 (2.72)	2.33 ± 0.09 (1.82)	2.54 ± 0.09 (1.88)	2.72 ± 0.04 (1.93)
Navalurkottapattu	1.82 ± 0.01 (1.68)	5.67 ± 0.18 (2.57)	1.33 ± 0.11 (1.52)	1.91 ± 0.03 (1.71)	2.49 ± 0.08 (1.86)
Manapparai	1.89 ± 0.03 (1.70)	6.33 ± 0.23 (2.69)	1.33 ± 0.11 (1.52)	1.79 ± 0.02 (1.67)	2.76 ± 0.02 (1.94)
Arasappapillaipatty	1.78 ± 0.02 (1.67)	6.00 ± 0.22 (2.63)	1.67 ± 0.11 (1.63)	1.82 ± 0.02 (1.68)	2.79 ± 0.05 (1.95)
Vakkampatty	1.94 ± 0.02 (1.71)	5.67 ± 0.18 (2.57)	1.00 ± 0.00 (1.41)	1.70 ± 0.00 (1.64)	3.28 ± 0.06 (2.07)

Table 2. Quantitative nesting traits of stingless bee colonies, *Tetragonula iridipennis* in different agro climatic zones of Tamil Nadu. (Continuation)

Nesting sites	*Mean ± S.E.				
	NEH	NGB	NC/NS	CNE	ETL
Athoor	2.90 ± 0.04 (1.98)	6.00 ± 0.28 (2.62)	2.00 ± 0.00 (1.73)	2.49 ± 0.10 (1.86)	2.12 ± 0.06 (1.76)
Thandikudi	1.84 ± 0.03 (1.69)	7.00 ± 0.10 (2.83)	1.33 ± 0.11 (1.52)	2.27 ± 0.03 (1.81)	2.04 ± 0.11 (1.74)
Nilakottai	2.50 ± 0.08 (1.87)	4.33 ± 0.19 (2.29)	1.00 ± 0.00 (1.41)	2.33 ± 0.13 (1.81)	2.05 ± 0.09 (1.74)
Narasingam	1.76 ± 0.02 (1.66)	6.00 ± 0.34 (2.60)	1.33 ± 0.11 (1.52)	2.29 ± 0.10 (1.81)	2.71 ± 0.03 (1.93)
Melur	2.40 ± 0.10 (1.84)	7.00 ± 0.21 (2.81)	1.33 ± 0.11 (1.52)	2.12 ± 0.09 (1.76)	2.74 ± 0.04 (1.93)
Vowal thottam	1.82 ± 0.02 (1.68)	6.00 ± 0.22 (2.63)	1.33 ± 0.11 (1.52)	2.07 ± 0.05 (1.75)	2.29 ± 0.07 (1.81)
Chittangulam	1.65 ± 0.02 (1.63)	5.33 ± 0.25 (2.49)	1.67 ± 0.11 (1.63)	1.63 ± 0.03 (1.62)	3.44 ± 0.10 (2.10)
CD @ 5%	0.20	0.19	0.29	0.20	0.21
SE (m)	0.07	0.21	0.10	0.07	0.08
SE (d)	0.10	0.29	0.15	0.10	0.11

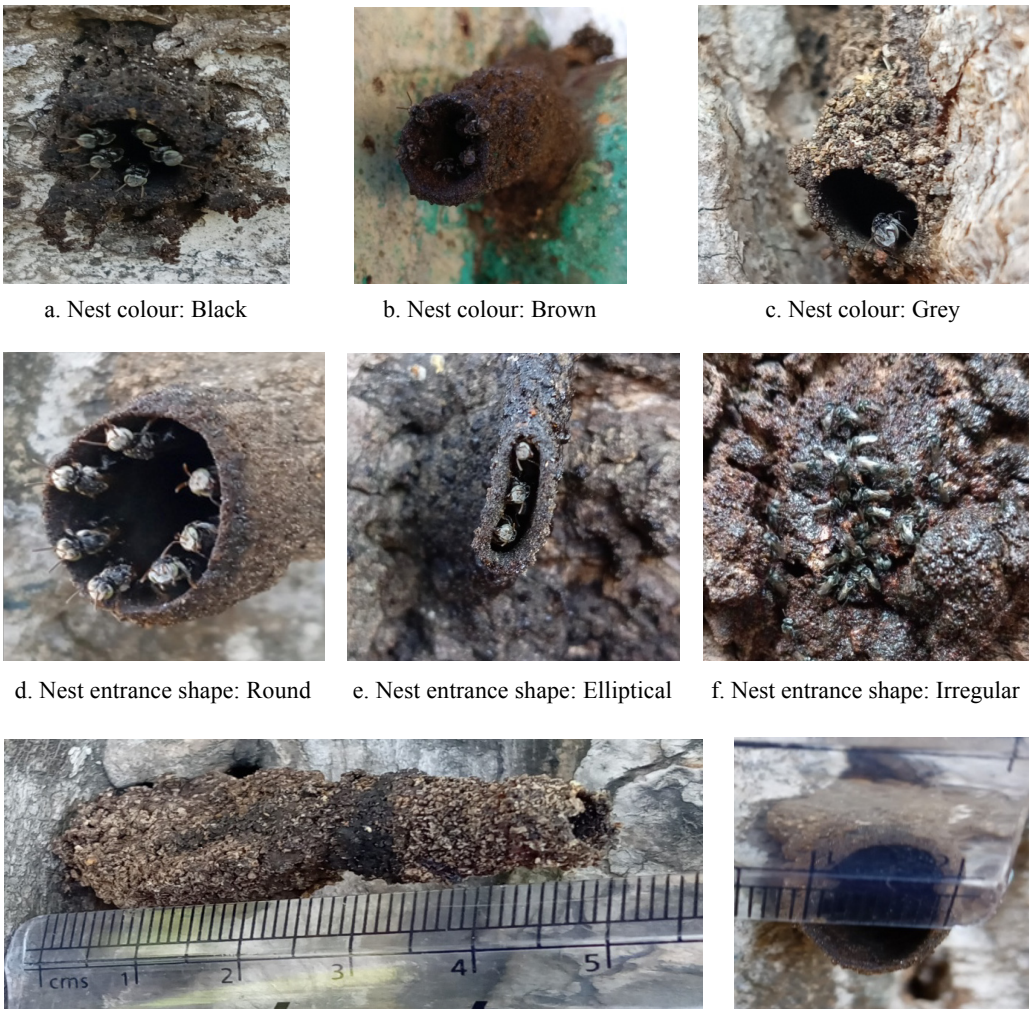
Note: *Mean of three replications. Figures in the parentheses are square root transformed values. SE: Standard Error; CD: Critical Difference ($p<0.05$); NEH: Nest Entrance Height from ground; NGB: No. of Guard Bees at nest entrance; NC/NS: No. of Colonies per Nesting Site; CNE: Circumference of Nest Entrance; ETL: External Tube Length.

The circumference of nest entrances across all habitats ranged from 1.29 to 2.45 cm. Colonies inhabiting tree trunks exhibited the longest external tube lengths, measuring 3.24 cm (ranging from 1.80 to 5.10 cm). In contrast, those in wall cavities had shorter external tubes, measuring 1.92 cm (ranging from 0.50 to 3.20 cm). Wooden structures harbored colonies with the shortest external tube lengths, ranging from 0.80 to 1.40 cm.

Table 3. Nesting characteristics of stingless bees, *Tetragonula iridipennis* in Tamil Nadu.

Attributes /Habitat	Wall (50.0%)	Tree trunk (33.33%)	Iron rod (6.66%)	Wood (6.66%)	Mud pot (3.33%)
Nest colour	Black, grey, brown	Black, brown	Brown	Black	Reddish brown
Nest orientation	South east, east and northwest	South east and south west	South and east	East and south east	North east
Shape of the nest opening	Round to oval	Round, Oval to irregular	Round	Round to elliptical	Round
Material deposited at nest entrance	Mud and resin	Resin, sand and wax	Wax, pollen and resin	Resin, mud and wax	Mud and resin
Micro habitat around the colony	VD - low; SI - low	VD - high; SI - low	VD - medium; SI - low	VD - medium; SI - low	VD - low; SI - low
Nest entrance height from ground (NEH) (m)*	2.98 ± 0.23 (0.52 - 3.45)	1.89 ± 0.11 (1.65 - 2.05)	2.03 ± 0.15 (1.92 - 2.14)	1.3 ± 0.52 (0.93 - 1.67)	1.53
No. of guard bees at nest entrance (NGB)	3 - >10	4 – 8	3 – 5	4 – 6	3
No. of colonies per nesting site (NC/NS)	2 - >10	1 – 3	1	1	1
Circumference of nest entrance (CNE) (cm)*	2.45 ± 0.53 (1.26 - 3.14)	2. 19 ± 0.15 (1.38 - 2.70)	1.29 ± 0.16 (1.13 - 1.45)	2.10 ± 0.63 (1.76 - 2.45)	1.45
External tube length (ETL) (cm) *	1.92 ± 0.13 (0.50 - 3.20)	3.24 ± 1.53 (1.80 - 5.10)	1.90 ± 1.50 (0.40 - 3.40)	1.1 ± 0.30 (0.80 - 1.40)	2.60

Note: *Mean values ± SD (Standard Deviation); VD: Vegetation Density; SI: Sunlight Incidence.



a. Nest colour: Black b. Nest colour: Brown c. Nest colour: Grey

d. Nest entrance shape: Round e. Nest entrance shape: Elliptical f. Nest entrance shape: Irregular

g. Measure of external tube h. Measure of nest opening

Fig 3. Nesting characteristics of stingless bees, *Tetragonula iridipennis.*

Principal Component Analysis (PCA) for nesting ecology of ***T. iridipennis***

The numerical data shows 30 distinct locations and their correlation with other data, indicating collinearity. Here, PC 1 exhibits a 21.07% variation, and PC 2 exhibits a 19.39% difference among all the sites regarding nesting traits. PCA exhibits that nesting habitat significantly influences the variation among nesting characteristics, with maximum diversity observed in various substrates.

Principal Component Analysis (PCA) (Table 4) was conducted to exhibit the significant variation among nesting characteristics utilized in studying the nesting ecology of stingless bee colonies observed during the survey. Typically, an analysis's primary principal component (PC) demonstrates the most significant axis of variation. Subsequently, the second PC illustrates the following largest uncorrelated axis of variation. The determination of the number of components to include was based on eigenvalue. The axial loads of PC1 (21.07%) and PC2 (19.39%) were plotted along the abscissa and ordinate, respectively.

Table 4. PCA – Eigenvalues of Covariance Matrix (Nesting attributes).

Particulars	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalues	3.553	2.943	1.428	0.946	0.648	0.503	0.403	0.243	0.056
Proportion	0.331	0.274	0.133	0.088	0.060	0.047	0.038	0.023	0.005
Cumulative Proportion	0.331	0.606	0.739	0.827	0.888	0.935	0.972	0.995	1.000

Natural enemies

Predators (Table 5) commonly observed in proximity to the nest entrance and within the colony included ants (*Oecophylla smaragdina* and *Tetraponera rufonigra)* (Fig 4a & c), wasps (*Vespa* sp. and *Leucospis* sp.), and spiders (*Phidippus* sp.).

Ants were noted for scavenging nourishment from honey, pollen cells, and brood compartments. Wasps were noted for their tendency to pilfer pupal broods, stalling their development. Additionally, spiders were observed constructing intricate webs (Fig 4b) within the colony, ensnaring foragers and impeding their movements. The nest entrance exhibits intricate architectural features that hinder intruders as they navigate through the nest.

a. *Oecophylla smaragdina*

b. Webbing by *Phidippus* sp.

c. *Tetraponera rufonigra*

Fig 4. Natural enemies of stingless bee colony.

Nest architecture

Each colony comprised internal compartments dedicated to food storage and brood development. The food storage section was further subdivided into pollen and honey zones, with separate pots for storing each. However, these pots were frequently found intermingled within the storage area.

The oval-shaped pollen containers were crafted from soft cerumen, exhibiting a dark brown hue, and typically situated at the periphery region of the colony. The dimensions of these pots varied, with lengths ranging from 5.10 to 6.70 mm and widths from 3.80 to 4.90 mm, averaging 5.90 ± 0.60 mm in length and 4.24 ± 0.45 mm in width (Table 6). The honey pots, which were slightly larger than the pollen pots, had an oval shape and were situated towards the inner edge of the colony. Some were often mixed among the pollen pots. Their dimensions varied, with lengths ranging from 5.60 to 6.90 mm and widths from 4.90 to 6.10 mm, averaging 6.36 ± 0.51 mm in length and 5.46 ± 0.54 mm in width. The cells housing eggs and larvae exhibited a brownish hue, contrasting with the creamy white color of pupal cells. Brood cells ranged from 1.80 to 2.90 mm in length and 1.80 to 2.70 mm in width, averaging 2.32 ± 0.44 mm and 1.92 ± 0.37 mm, respectively (Table 6).

Table 5. Defensive behavior of stingless bee colonies, *Tetragonula iridipennis*.

Predators of stingless bee colony noticed	Essence of impairment	Kind of defensive mechanism observed
Oecophylla smaragdina and *Tetraponera rufonigra* (Ants)	Acquiring nourishment from honey and pollen cells along with brood compartments	Attending of guard bees near the entrance orifice
	Suppression of adult emergence and devastation of storage pots	Formation of intricate nest structures by establishing root like batumen to ward off the intruders
	Predation on immature stages and newly emerged young ones	Deposition of wax and resin smear (propolis) surrounds the nest entrance
Vespa sp. and *Leucospis* sp. (Wasps)	Robbing of pupal broods and arrest their development	Complex nesting architecture with multiple entrance
		Fresh yellowish brown resin coating inside the nest to defend the colony
Phidippus sp. (Spiders)	Formation of complex web inside the colony	Using sharp mandibles or by fluttering its wings to escape from the web
	Entanglement of stingless bee foragers by its web	Thin layer of mud capping sealed with resin at nest entrance to trap the invaders

Table 6. Parameters of Nest storage structures of stingless bees, *Tetragonula iridipennis*.

Attributes/ Nest storage structures	Colour	Mean size (mm) ± SD	
		Length	width
Honey storage pots	Brown to black	6.36 ± 0.51 (5.60 – 6.90)	5.46 ± 0.54 (4.90 – 6.10)
Brood cells	Pale yellowish brown	2.32 ± 0.44 (1.80 – 2.90)	1.92 ± 0.37 (1.80 ± 2.70)
Pollen storage pots	Yellowish brown	5.90 ± 0.60 (5.10 – 6.70)	4.24 ± 0.45 (3.80 – 4.90)

Note: The values in parentheses are mean values (mm).

Discussion

Nesting habitat and habits

Nests are a significant aspect of the communal life of social insects, playing a crucial role in offering physical shelter from environmental disturbances. Additionally, in certain instances, the nest's microclimate contributes to maintaining a stable temperature. The primary materials used for constructing nests were mud, wax, resin, pollen, and sand, reinforcing the nest entrance. Nest-site preferences reflect an adaptive response to the fitness costs associated with variations in microclimate and other nesting attributes (Table 2). The selection of nesting sites across various habitats is contingent upon the availability of suitable substrates and food resources. Interior spaces such as residential and institutional buildings tend to accommodate more nests than open areas like roadsides. The variation in food supply around the nest emerges as a key factor contributing to differences in habitat attractiveness. However, forecasting the choice of nest sites remains challenging due to bees dedicating considerable effort to scouting potential locations. The primary materials used for constructing nests were mud, wax, resin, pollen, and sand, reinforcing the nest entrance. Correspondingly, a higher proportion of nests (50.0%) were observed in interior regions, including mud walls, stone walls, and brick walls, compared to other habitats, which are on par with the results of Danaraddi et al. (2009), Basavarajappa (2010) & Saaivignesh and Manickavasagam (2023) who noted that cracks or cavities in brick walls were favored sites for nest construction of *T. iridipennis*, with percentages of 70.59% and 78.79%, respectively. Similarly, Pavithra et al. (2013) also documented brick walls as the preferred substrate for nesting of *T. iridipennis*. Habitat preferences are viewed as adaptations influenced by enhanced fitness in favored environments, a process shaped by natural selection and genetic influences.

Hiding nests within tree trunks or wall crevices, clustering nests together, and elevating them to a suitable height above ground level can offer protection against potential threats and predators (Antoine & Forrest, 2021). The elevation of nests from ground level ranged from 1.30 to 2.98 m, consistent with the observations of Bhatta et al. (2019) and Saaivignesh et al. (2023), who reported that the maximum nest height from ground level was 3.0 m. A closer observation of the height of nests by climbing over trees using suitable accessories can provide a better picture of the

maximum height at which the stingless bees can build their nests. The strategic positioning of nests at varying heights within habitats offers defense against various environmental elements, including predators and abiotic factors (Antoine & Forrest, 2021). This adaptive tactic in colony construction, influenced by adjusting to different elevations, underscores height's significance in securing colony survival and environmental adaptation.

The external appearance of the nest displays a combination of colors, with black deposits being more frequently observed, likely serving as a defense mechanism against predators when compared to other colors. Entrances to the nests tend to have a higher concentration of mud and sand deposits, while others may exhibit accumulations of resin, pollen, and wax. Our findings align with those of Roubik (2006), who documented that typical nest entrances in the *Tetragonula* genus include flexible tubes, mud, sticky resin, fecal matter, pollen, wax, wood fibers, and various types of debris utilized as nest materials. Insulation provided by wood, resin, mud, sand, and other substrates prevents colonies from overheating.

The entry points of nests hold significant importance in shaping the overall traits of the nests. In stingless bees, the nest entrance usually manifests as a slight protrusion at the entrance hole's base. The studies revealed that the nest entrance measured their diameter upto 1 cm. However, Roubik (2006) documented that in some *Tetragonula* species, the nest entrance is notably larger. Moreover, they preferred circular to oval-shaped entrances rather than irregular ones.

Adaptive defense mechanisms

In our investigation, ants, wasps, and spiders were observed to harm pupal broods within cells, resulting in adverse effects on the emergence of adult bees. This behavior is associated with plundering honey and resin resources within the hive. These findings are consistent with Kumar et al. (2012), who highlighted ants as significant predators in stingless bee colonies and as primary robbers of sugar treasury in controlled bee colonies. The adaptive defense behavior prioritized consistent brood emergence and efficient task allocation for guarding and foraging, complemented by protective nest architecture. The presence of guards (Fig 5a) stationed at the hive entrance exemplified the common adaptive strategy employed by bees to protect the colony from intruders. Applying fresh resin at the nest entrance is crucial for shielding the nest against intruders such as ants (Leonhardt & Bluthgen, 2009). The intruders, especially spiders, were noticed to get ensnared within layers of a thin cap composed of mud coated with resin. Both resin and mud were utilized to obstruct spiders from entering the colony.

The study findings underscored that stingless bees exhibit adaptive defense behavior alongside an additional and intricate nest architecture to safeguard the colony during its initial stages of establishment (Fig 5). This defense mechanism involves trapping and eliminating accidental intruders within the colony. Batumen (Fig 5b) is a resinous material stingless bees collect for nest construction and maintenance. It is distinct from propolis and may have different chemical compositions depending on the plant sources available to the bees. Batumen serves similar functions to propolis in reinforcing the nest structure and defending against external threats (Alves et al., 2023). Worker bees deposited propolis at the nest entrance (Fig 5c) as a deterrent against intruders, illustrating their defensive activity. Additionally, colonies with multiple entrances (Fig 5d) can swiftly evade external attacks.

Resin (Fig 4e) acts as a protective measure against natural predators, as emphasized by Roubik (2006) and Leonhardt et al. (2010). Stingless bee workers also display defensive behavior by standing watch or hovering close to the hive entrance. The intruders, particularly spiders, were observed to become trapped between layers of a thin cap of mud coated with resin (Fig 5f) at the nest entrance. Resin and mud were employed to prevent invaders from entering the colony.

Nest architectures

The interior structure of stingless bee nests may be partitioned into chambers or sections, each serving specific functions such as brood rearing, food storage, and nest maintenance. These partitions contribute to the organization and efficiency of colony activities. Typically, stingless bee nests comprise elements such as the entrance, inner tunnel, storage pots for honey (Fig 6a) and pollen (Fig 6b), brood cells (Fig 6c), and layers of batumen. The nest texture becomes harder with a more significant proportion of wax compared to resin. Mud can further solidify the waxy nest to form batumen (Fig 6d-e). Additionally, cerumen (Fig 6f), involucrum, is positioned around the brood cells to shield the nest from predators and parasites (Michener, 2007).

Stingless bees construct intricate brood cells (Fig 6c) within their nests to accommodate eggs, larvae, and pupae. These cells are arranged in clusters and vary in size and shape depending on the species (Chinh et al., 2005). According to Leonhardt et al. (2010), the absence of plant resins results in transparent food storage pots and brood cells. Two varieties of brood cells exist: cluster and comb. Brood cells lacking eggs exhibit a darker hue, while those with eggs are transparent. According to Chinh et al. (2005), newly formed cells appear yellow-brown but become transparent as the wax erodes. Cells are interconnected via pillars (Fig 6d). The cells housing eggs and larvae exhibited a brownish hue, whereas pupal cells appeared creamy white.

The pollen pots (Fig 6b) were densely packed with pellets and sealed shut. Only a few pots were left open to store freshly collected pollen. The stored pollen was slightly moist with a sour taste. The area designated for pollen storage typically exceeded that for honey storage, suggesting an

a. Guard bees at nest entrance

b. Brood cells covered by root like batumen

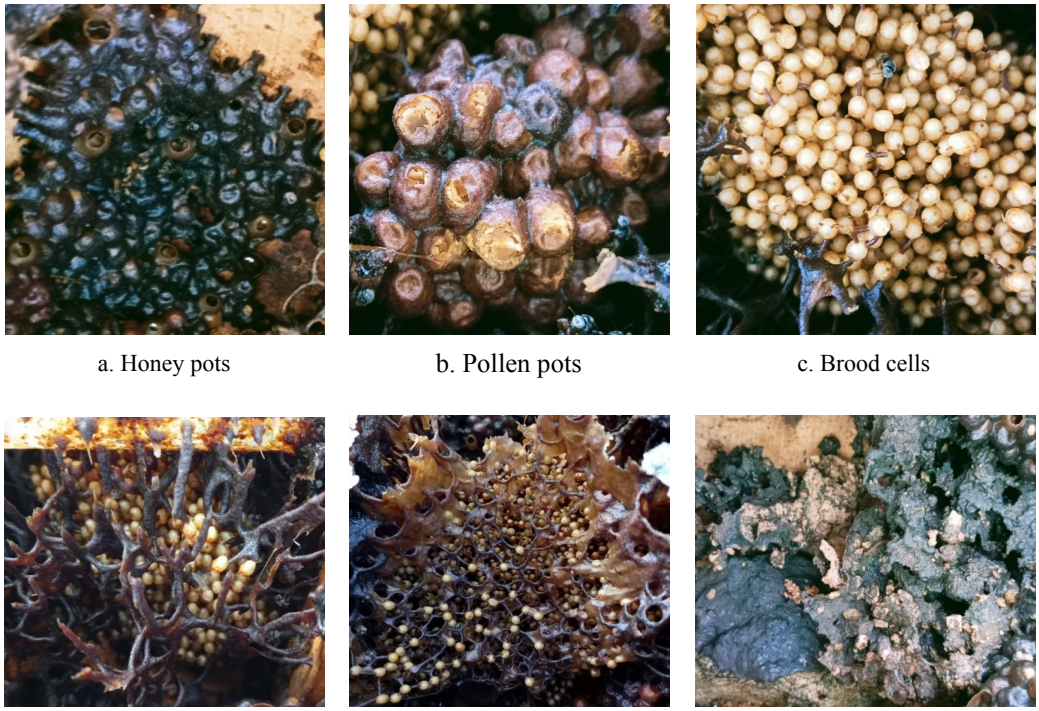
c. Deposition of propolis at the nest entrance

d. Multiple nest entrance

e. Sticky resin coating inside the colony

f. Mud capping with resin at nest entrance

Fig 5. Defensive behavior expressed by stingless bees *Tetragonula iridipennis*.



a. Honey pots b. Pollen pots c. Brood cells

d. Pillar like batumen e. batumen Connectives f. Cerumen

Fig 6. Nesting structures inside the stingless bees colony.

abundance of pollen in the study region. When the pollen pots reached full capacity, they were sealed shut. New pots were constructed adjacent to them, and occasionally, they were mixed.

Within their nest, stingless bees build honey pots (Fig 6a), varying in shape from oval to round and brown to black, serving as food reserves. Usually located close to the center of the nest, these pots are crucial resources during periods of scarcity.

PCA for nesting attributes of stingless bee colonies

Factor analysis techniques such as Principal Component Analysis (PCA) are employed to reduce the dimensionality of a large dataset by identifying a smaller set of uncorrelated variables. The goal is to maximize the explained variance using the minimal number of principal components. Sample plots (Fig 7a) and loading plots (Fig 7b) visually depict the relationships between variables and the first two principal components (Fig 7c). A score plot (Fig 7d) is utilized to determine the optimal number of components that capture the majority of the variance in the dataset. Cumulative proportion indicates the total variance these components explain, establishing a threshold for acceptable variance. In our investigation, the initial four principal components collectively represent 82.7% of the dataset's variance. Eigenvalues guide the retention of principal components, prioritizing those exceeding 1. In our study, ETL, NGB, SNE, and NO notably demonstrate significant positive loadings on the primary component (21.07%), highlighting their substantial contributions among the nine nesting attributes. Conversely, NS and NC/NS exhibit marked negative loadings on the second component (19.39%). Thus, the first principal component correlates positively with ETL, NGB, SNE, and NO, emphasizing their pronounced variability among their nesting sites.

Conclusion

Stingless bees' habitat preference and nesting behavior demonstrate their remarkable adaptability to diverse environments. By carefully selecting nesting sites and intricate construction of nests, these bees optimize their chances of survival and reproductive success. Our findings highlight the importance of suitable substrates and food availability in influencing nesting habitat choice, with interior regions such as housing and old buildings often hosting more nests. Additionally, the strategic placement of nests at varying elevations within habitats protects against environmental factors and potential predators. The defensive behaviors exhibited by stingless bees, such as propolis deposition and entrance guarding, further contribute to colony resilience. Understanding stingless bees' habitat preferences and nesting behaviors is crucial for conservation efforts and maintaining healthy ecosystems. Further research in this field will deepen our knowledge of these fascinating insects and their interactions with their environment.

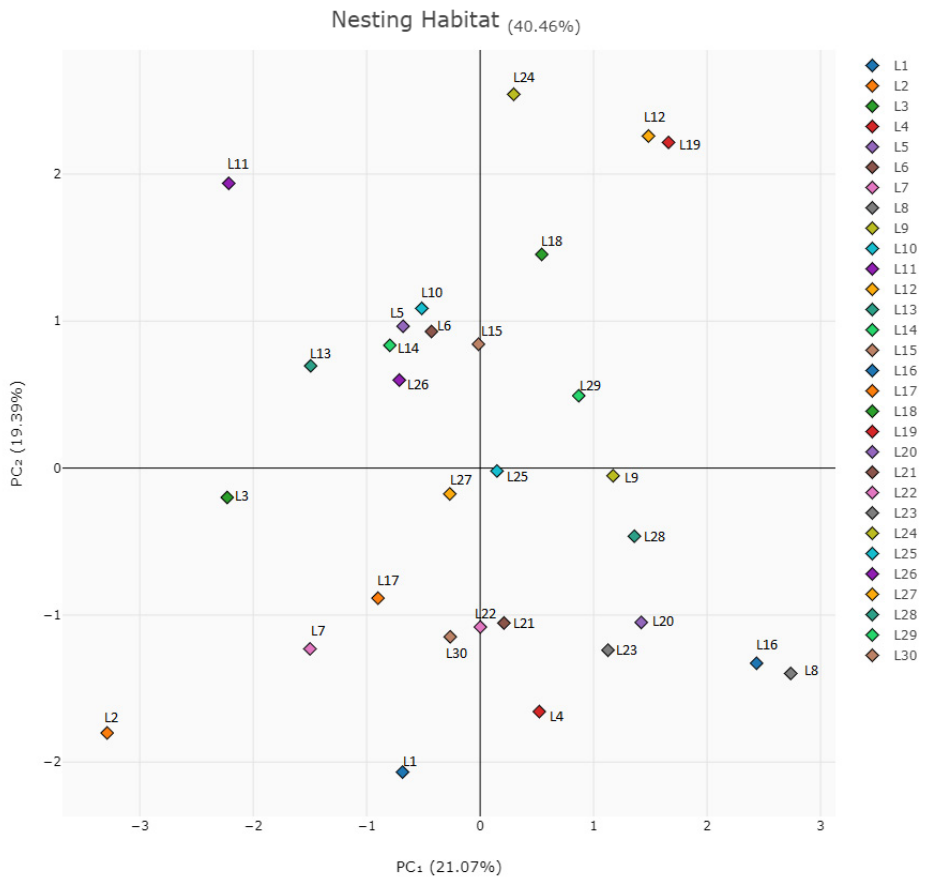


Fig 7a. Sample plot – Principal Component Analysis of nesting habitat from thirty different locations.

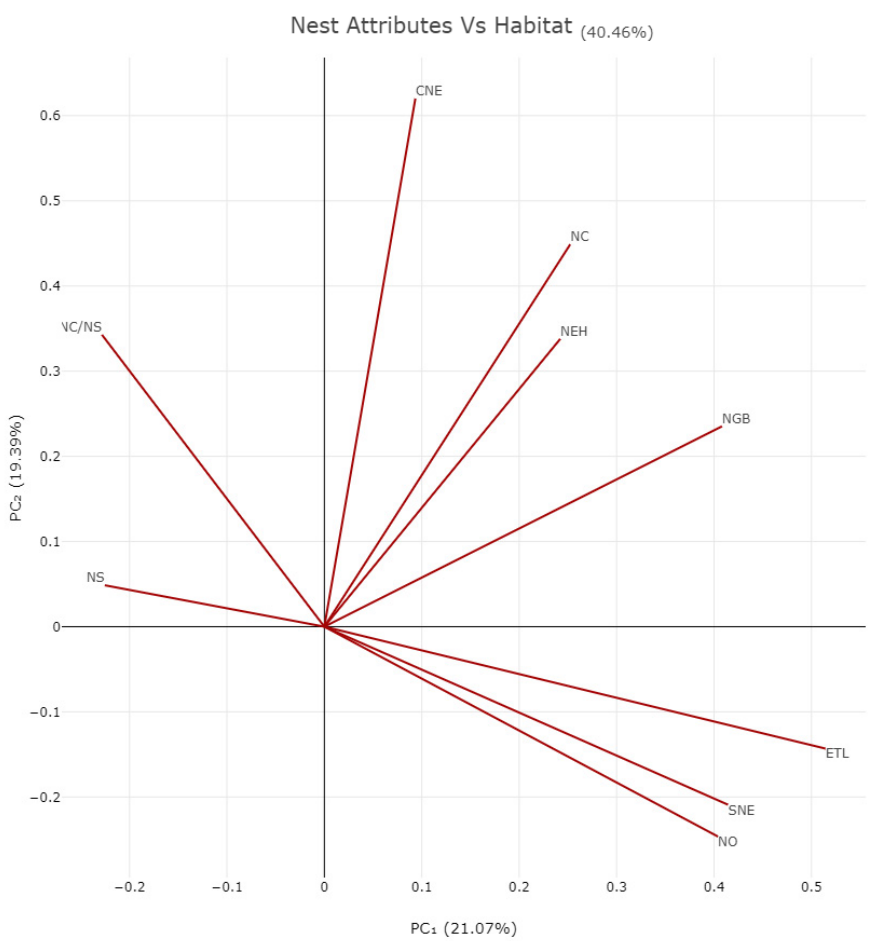


Fig 7b. Loading of variables (Nesting attributes).

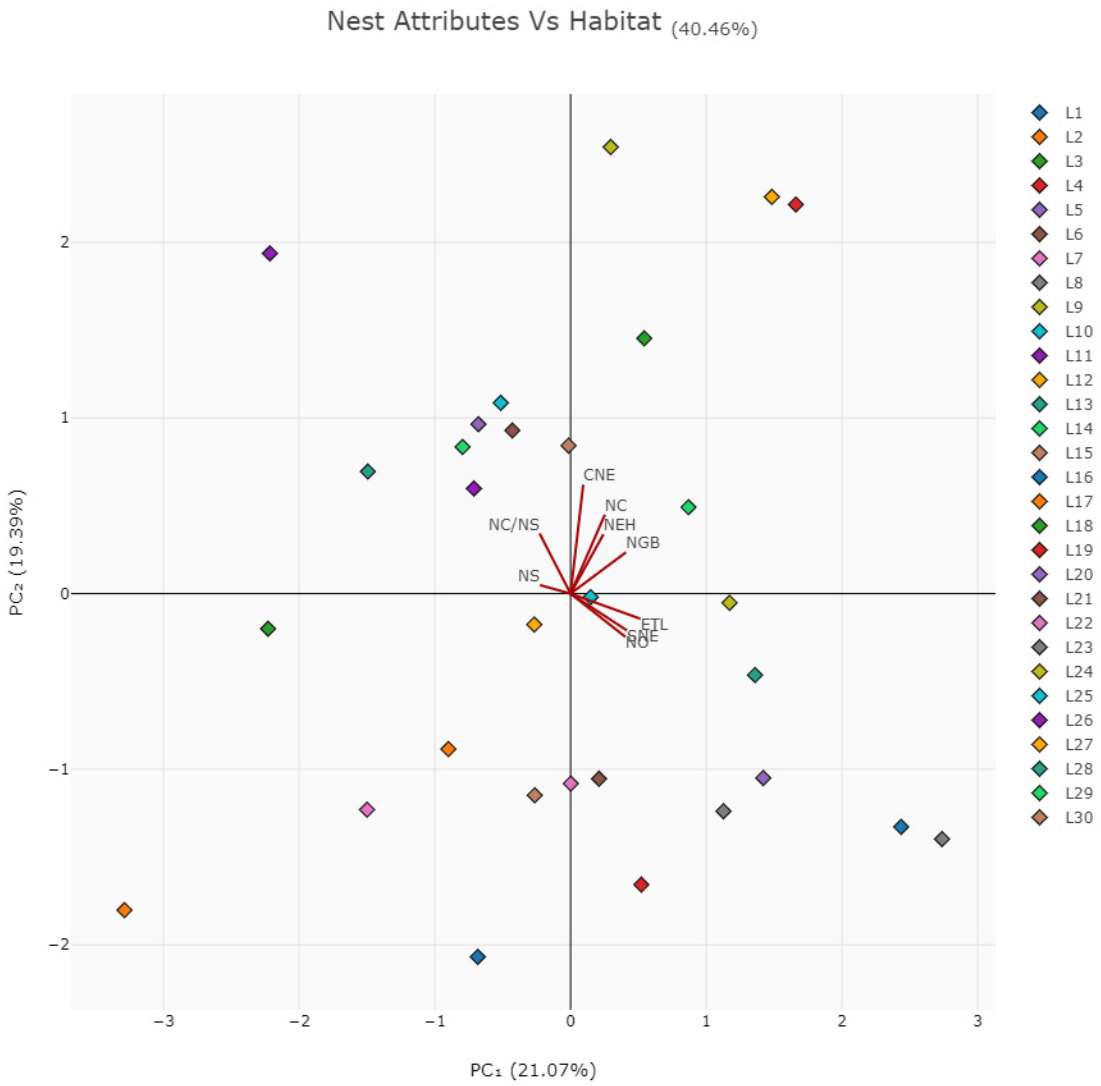


Fig 7c. Sample score and variable loads on Principal components.

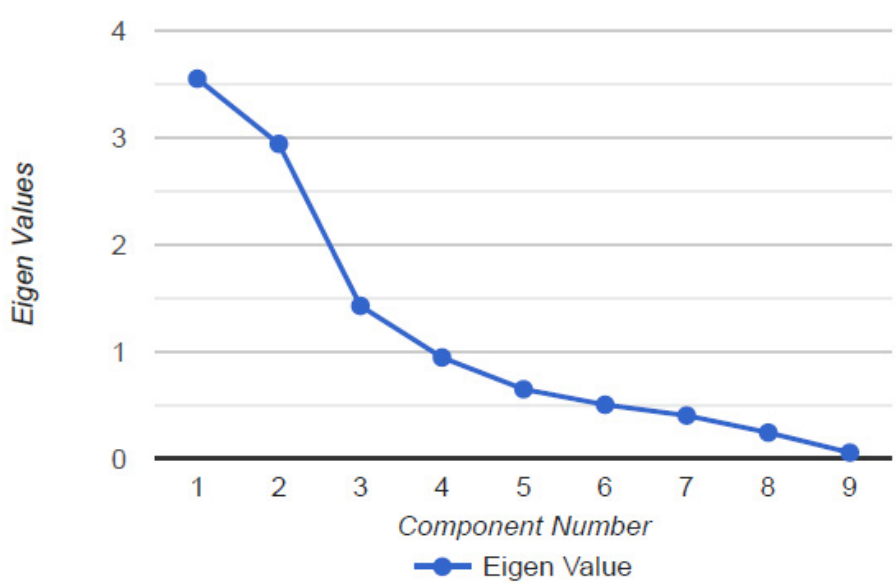


Fig 7d. Score plot of variables (Nesting attributes).

Fig 7. Principal Component Analysis.

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Authors' Contribution

SP: Conceptualization, methodology, and writing of original draft.
SMR: Supervision, review and editing, and data curation.
MM: Validation, review and editing, and supervision.
SVR: Formal analysis and supervision.
All authors have gone-through and approved the manuscript.

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