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Characterization of External and Internal Nesting Ecology of Stingless Bee (*Tetragonula iridipennis*) within Managed Habitats (Meliponaries) of Central Western Ghats, Karnataka

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ABSTRACT

The study investigated the nesting habits and colony structures of the stingless bee, *Tetragonula iridipennis*, across four districts in Karnataka: Dakshina kannada (Panaje), Kodagu (Ponnampet), Chikkamagaluru (Kalasa) and Hassan (Arakalagudu). Results showed that while all bees follow a similar patterns in building round, black entrance tubes and oval brood cells, the size and strength of the colonies changed significantly based on their location. Colonies in the coastal and hilly regions of Panaje and Ponnampet were featuring longer entrance tubes (up to 41.52 mm), more guard bees(14 and 11) and higher numbers of honey (105 and 80) and pollen pots (85and 60, respectively). In contrast, colonies in the drier plains of Arakalagudu were significantly smaller in size, with shorter entrance tubes (11 mm length) and nearly 70 per cent fewer brood cells. These findings suggest that the environments of the central Western Ghats provide better resources and climate for stingless bees, allowing them to build larger nests and store more food compared to the drier interior regions.

Keywords : Stingless bees, Pollen pots, Honey pots, Brood cells, Guard bees

NESTING ecology is the key factor while characterizing species however, only nest architecture and nest site location documentation has been inconsistently done for few stingless bee species in southeast Asian countries as well as in Indian subcontinent (Layek *et al*., 2025 and Gupta, 2014). Stingless bees face several threats to their colonies during the day time as well as in the night (Bennett *et al*., 2021). In order to overcome these threats multiple defensive strategies has been adopted by these bees includes both behavioural defence (Shackleton *et al*., 2015). During day time honey bee guards stand at the entrance of the colony and touch incoming bees to identify nest mates. The most vulnerable structure of the nest in stingless bees and other eusocial insects is nest entrance (Roubik, 2021). This place must act as balancing bridge between colony defence and traffic flow for other colony functions such as foraging, waste removal and construction (Roubik *et al*., 2018).

Roubik 2021, reported nest entrance manipulation in order to balance between foraging and guarding in several species across genera *Friesella, Meliponula, Pariotrigona, Scaptotrigona, Plebeia, Nannotrigona, Schwarziana* and *Trigona*. Charanakumar *et al*., 2022, reported pig mouth shaped (locally called *Pannimookam* or *Pandimogam*) entrance shape in *Tetragonula* from the Western Ghats region of India. For the first time, (Bennett *et al*., 2021) examined behaviours employed by stingless bees, *Tetragonisca angustula* at night and reported two aspects of defence including nest closing and guarding behaviour at the nest entrance during night time. Ethnic communities, forest tribes and other traditional forest dwellers in the Western Ghats region of Karnataka state have been practicing meliponi culture but, it is not well documented (Charanakumar *et al*., 2022). One of the key benefits of sustainable meliponiculture is the ability to transfer stingless bee colonies into artificial hives, enabling regular inspections and improved management practices (Qu *et al*., 2022). Notably, ethnic communities in the central Western Ghats region of India have developed innovative artificial hives alongside traditional ones, assessing their effectiveness through direct comparisons with conventional hive structures (Charanakumar *et al*., 2022). Many tribal families rely on honey hunting as a primary source of livelihood in India. *Tetragonula iridipennis* Smith is the most widely distributed and well-known stingless bee species in India, including Karnataka (Moulya *et al*., 2023 and Padmini *et al*., 2024). In Karnataka, stingless bee products are valued for their uses in food, medicine, crafts and cultural practices. Information regarding the nesting ecology of the stingless bee, *Tetragonula iridipennis* in the central Western Ghats-a vital biodiversity hotspot-remains fragmented and poorly documented. To address this gap, the present study was made to provide a detailed account of both the external and internal nesting characteristics of this species within the Karnataka region of the central Western Ghats. Knowledge on these ecological parameters, helps to enhance our understanding of the nesting requirements and habitat preferences of *T. iridipennis* in this unique landscape.

Material and Methods

The ethno-biological investigations were conducted across four districts in the state of Karnataka: Dakshina Kannada (Panaje), Chikkamagaluru (Kalasa), Hassan (Arakalagudu) and Kodagu (Ponnampete). This study area is characterized by a diverse landscape of tropical evergreen forests, moist deciduous formations and extensive plantations of coffee, areca nut and spices. The region's unique agro-climatic conditions-ranging from the coastal plains of Dakshina Kannada to the high-altitude Malnad hilly terrains of Kodagu and Chikkamagaluru-provide an ideal environment for the traditional practice of meliponi culture and the natural nesting of *Tetragonula iridipennis*. The field study was conducted in May 2024 to June 2025.

To document the nesting ecology of *Tetragonula iridipennis*, progressive stingless beekeepers were identified acrosseach study location (Table 1). A detailed nest characters analysis was carried out on five rectangular wooden hives per location. To ensure uniformity in colony vigor and resource availability, only colonies with equal population strength that had remained unharvested for the previous year were selected for the sampling.

The external nesting characteristics were recorded by observing the presence or absence, shape, colour and construction materials of the nest entrance tube. Internally, the study focused on the morphometrics and morphology of the colony structures. Brood cell parameters included length, width, total number, shape and colour. Similarly, honey and pollen pots were evaluated based on their dimensions (length and width), total count (specifically for honey pots), shape and coloration. All physical measurements were conducted to assess the structural variations of the nests within the meliponiculture practices of the central Western Ghats. The collected data were analyzed using the Randomized Block Design (RBD). We used the GRAPES 1.1.0 statistical software, developed by the Kerala Agricultural University (KAU), to find out if there were any

Table 1

Geographical coordinates and altitude of the study locations

Location	Lattitude (N)	Longitude (E)	Altitude (E)
Panaje	12.7649	75.1740	131
Ponnampet	12.1403	75.9448	892
Kalasa	13.2296	75.3663	833
Arakalagudu	12.7649	76.0570	947

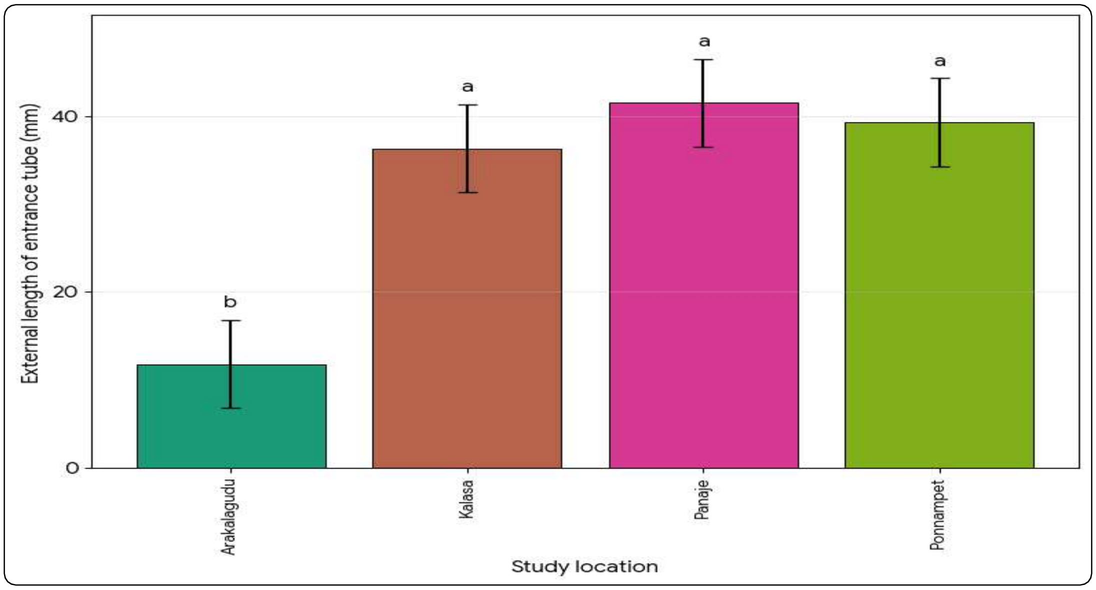

Fig. 1 : Effect of different locations on external length of entrance tube (mm)

significant differences between the four study locations.

Results and Discussion

The external nest architecture and defense mechanisms of *Tetragonula iridipennis* across four locations in the central Western Ghats reveals a significant geographic influence reflecting the colony's adaptation to local ecosystem and available resources. The length of the entrance tube was significantly higher and statistically at par in Panaje (41.52 mm), Ponnampet (39.30 mm) and Kalasa (36.30 mm) locations, than Arakalagudu, which recorded a drastic shorter tube length of only 11.80 mm (Fig. 1). Almost a similar trend was observed in the width of the entrance tube too, Panaje (11.5 mm), Ponnampet (10.7 mm), Kalasa (9.2 mm) and Arakalagudu (8.0 mm) (Fig. 2). Furthermore, the number of guard bees stationed at the entrance followed this pattern, peaking at 14.0 in Panaje and

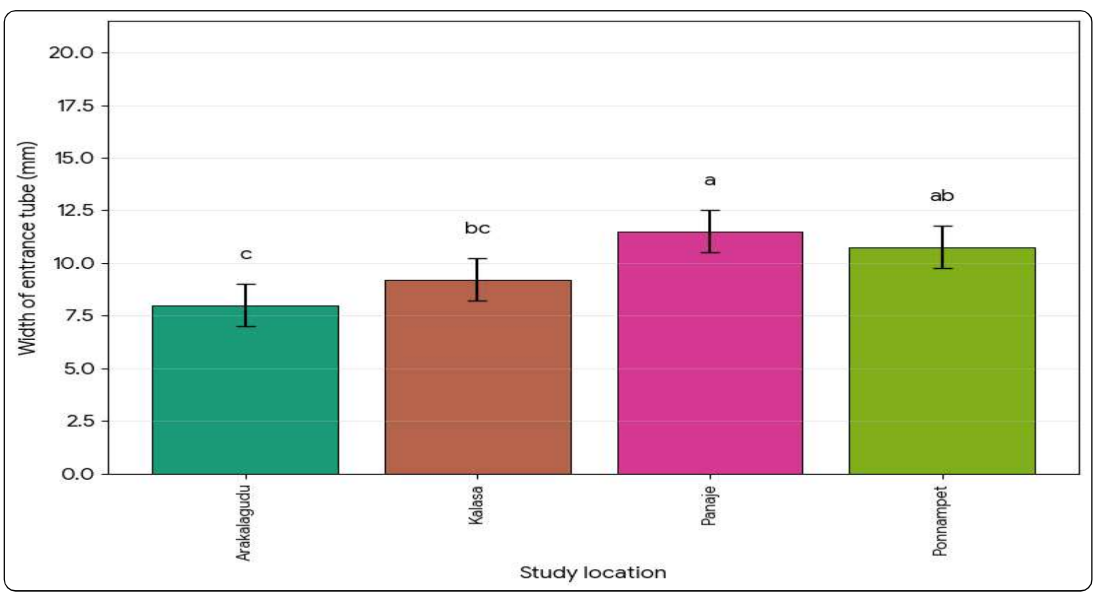

Fig. 2 : Effect of different locations on width of entrance tube (mm)

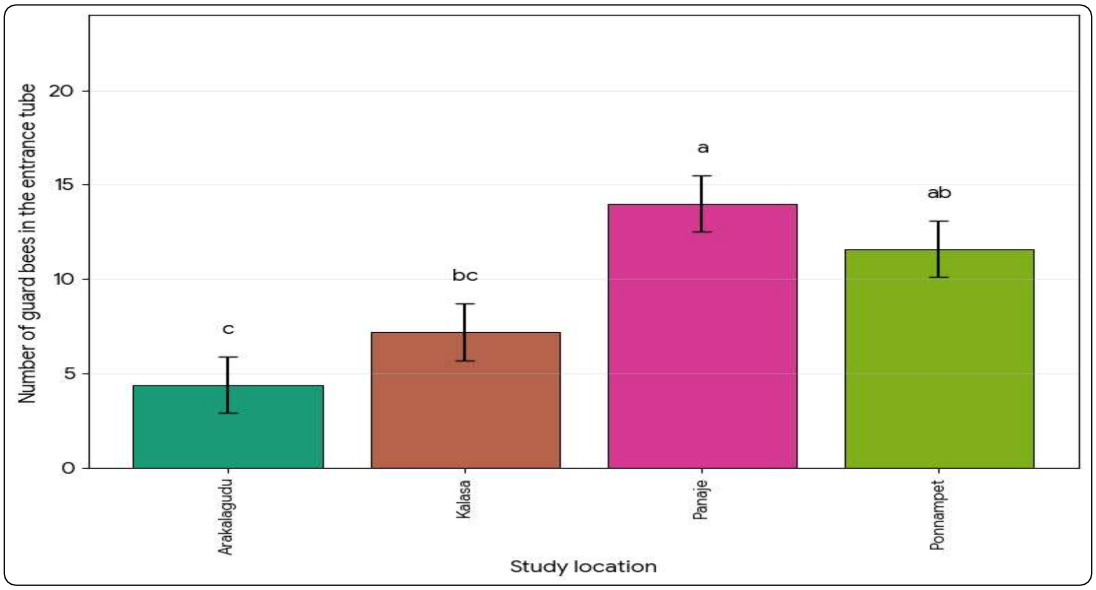

Fig. 3 : Effect of different locations on number of guard bees in the entrance tube

dropping to a minimum of 4.4 in Arakalagudu (Fig. 3). The moderate to high Coefficient of Variation (CV) for guard bees (36.265%) indicates that location and habitat being the primary drivers besides the individual colony strength which plays a vital role in determining defensive investment.

Results illustrates significant variations in the morphometrics of brood cells of stingless bees across four locations in central western Ghats of Karnataka, indicating that geographical and environmental factors influencing nest architecture. Panaje recorded the highest dimensions for both brood cell length (4.0144 mm) and width (3.2068 mm), being statistically superior to all other study locations. In contrast, Ponnampet and Kalasa showed intermediate values that were statistically at par with each other. The significantly smallest brood cell dimensions were observed in Arakalagudu (Table 2). The CV values indicated moderate levels of variability across the sampled colonies indicating that *T. iridipennis* adapts its brood cell size based on local environmental conditions or the availability of resources at specific altitudes (Table 1) and habitats.

The (Table 2) shows significant variations in the total brood cell number across the four study locations. Panaje recorded the highest mean brood cell count (929.6) and this was followed closely by Ponnampet (913.4), which is almost three folds of that recorded in Arakalgudu (296.4 cells). There was a moderate level of variability (CV=20.547) which is quite obvious die to micro climate and habitat variations in the study locations likely characterized by higher floral availability and more favourable climatic thermal refuges in Panaje and Ponnampet which have supported significantly larger and more robust colonies compared to the drier or more seasonally extreme environment of Arakalagudu.

Table 2

Effect of Locations on Brood Cell morphometries and Number

Location	Length (mm)	Width (mm)	Number
Panaje	4.0144 [a]	3.2068 [a]	929.6 [a]
Ponnampet	3.2880 [b]	2.4360 [b]	913.4 [ab]
Kalasa	3.0416 [b]	2.2820 [b]	714.0 [b]
Arakalagudu	2.2944 [c]	1.6196 [c]	296.4 [c]
CD	0.606	0.520	201.98
CV (%)	13.929	15.809	20.547

The data indicates a significant regional variation in the physical dimensions of honey pots (Table 3).

TABLE 3

Effect of Locations on Honey Pot morphometrics and Number

Location	Length (mm)	Width (mm)	Number
Panaje	13.33 [a]	9.90 [a]	105.6
Ponnampet	8.34 [b]	6.71 [b]	80.0
Kalasa	7.87 [bc]	6.67 [b]	78.4
Arakalagudu	6.43 [c]	5.49 [c]	35.8 [c]
CD	1.58	0.66	9.205
CV (%)	12.742	6.612	8.913

TABLE 4

Effect of Locations on Pollen Pot morphometrics and Number

Location	Length (mm)	Width (mm)	Number
Panaje	11.32 [a]	8.74 [a]	85.6 [a]
Ponnampet	7.18 [b]	5.71 [b]	60.0 [b]
Kalasa	6.87 [b]	5.67 [b]	58.4 [b]
Arakalagudu	5.43 [c]	4.49 [c]	17.2 [c]
CD	1.03	0.641	9.265
CV (%)	9.71	7.56	12.16

Panaje recorded the largest honey pots, with a mean length of 13.33 mm and width of 9.90mm, significantly outperforming all other locations. Ponnampet and Kalasa showed intermediate dimensions; whose widths were statistically at par (6.712 mm and 6.672 mm, respectively).

Parallel to the size of the pots, the total number of honey pots per colony varied significantly across locations. Panaje exhibited the highest storage capacity with a mean of 105.6 honey pots, significantly higher than the other regions. Ponnampet (80.0) and Kalasa (78.4) showing non-significant difference between each other. Arakalagudu showed the lowest storage capacity with only 35.8 pots, matching the trend observed in the brood cell data. The moderate CV of 8.913 per cent indicates that the number of honey pots is relatively stable trait within the study populations across study area (Table 3).

There are significant statistical differences in the physical dimensions of pollen pots between the four study sites. Panaje recorded the largest pollen pots, with a mean length of 11.32 mm and a width of 8.74 mm, placing it in the superior statistical category. In contrast, Ponnampet and Kalasa formed an intermediate groupshowing no significant difference between each other. The smallest dimensions were observed in Arakalagudu, which was statistically inferior (Table 4).

The total number of pollen pots followed a nearly identical trend, reflecting the overall resource-gathering efficiency of the colonies. Panaje exhibited the highest number of pots (85.6), indicating a robust surplus of protein storage. Ponnampet and Kalasa showed moderate storage levels with 60.0 and 58.4 pots, respectively and were statistically at par with each other. A drastic reduction was seen in Arakalagudu, which recorded only 17.2 pots, suggesting significantly lower pollen availability or a smaller foraging force in this region. The CV for the number of pots (12.159%) moderate indicating some variability across the region (Table 4).

The qualitative assessment of nesting parameters revealeda high degree of structural and chromatic consistency in the nesting habits of *T.iridipennis* across all four locations. The presence of a nest entrance tube was a universal feature in all sampled colonies, predominantly appearing in black or blackish-brown colour. While the tube shape was consistently round across all sites, colonies in Panaje and Kalasa also exhibited a distinct bell-shaped morphology. The primary construction material used for these tubes was cerumen (a mixture of bees wax and plant resins), though colonies in Ponnampet and Kalasa were observed incorporating debris into the structure.

Brood cells in all locations were oval in shape, with a clear color transition from darker shades

TABLE 5
Other Nesting Parameters Studied in the Study Area

Parameters/Locations	Panaje	Ponnampet	Kalasa	Arakalagudu
Altitude	131 M	892 M	833 M	947 M
Mean annual rainfall	3789 M	2800 M	4000 M	815 M
Mean temp	21-330 C	16-340 C	17-290 C	24-330 C
Presence or absence nest entrance tube	Present	Present	Present	Present
Shape of nest entrance tube	Round, bell shape	Round	Round, bell shape	Round
Colour of entrance tube	Black, Blackish brown	Black, Blackish brown	Black, Blackish brown	Black
Material used to construct nest entrance tube	Ceruman	Ceruman, debris	Ceruman, debris	Ceruman
Young Brood cell colour	Young brood cell are dark brown, light pinkish, light blakish in colour	Young brood cell are dark brown, in colour	Young brood cell are dark brown, in colour	Young brood cell are light brown in colour
Mature Brood cell colour	Matured brood cells are light brown, creamish in colour	Matured brood cells are light brown, creamish in colour	Matured brood cells are light brown, creamish in colour	Matured brood cells are light brown, creamish in colour
Honey pots colour	Dark brown	Dark brown	Dark brown	Dark brown
Pollen pots colour	Light brown	Light brown	Light brown	Light brown
Brood cell shape	Oval	Oval	Oval	Oval
Honey pots shape	Round, uneven shape	Round	uneven shape	Round
Pollen pots shape	Round, uneven shape	Round	uneven shape	Round

(dark brown, light pinkish or blackish) in young cells to lighter tones (light brown or creamish) in mature cells. Honey and pollen pots were generally round or uneven in shape; notably, honey pots consistently maintained a dark brown color, while pollen pots were consistently light brown across the study area.

Nesting Ecology of Stingless Bees

Results clearly suggests that the elaborate entrance tubes in the Central Western Ghats and coastal regions (Panaje and Ponnampet) are a strategic response to higher colony productivity and predator pressure (Couvillon *et al.,* 2008). Larger colonies with abundant honey and pollen stores, as seen in these high-performing locations, require a more sophisticated bottleneck defence provided by a longer tube to prevent the entry of pests like phorid flies or ants ((Roubik, 2006). This infrastructure is supported by the rich availability of resins and plant gums in the humid-tropical flora of these zones. Conversely, the minimalist entrance architecture in Arakalagudu likely reflects a resource-conservation strategy; in the drier plains, the energy cost of secreting resin for a long tube is high and a smaller entrance may be an adaptation to regulate internal nest humidity and temperature. The positive correlation between entrance dimensions and guard numbers confirms that external nest architecture serves as a visible indicator of internal colony vigour and the ecological suitability of the habitat reported/documented by Hora *et al*. (2023) lends support to present findings too.

The significant variations in brood cell dimensions and colony strength across the four locations suggest that *T. iridipennis* exhibits high architectural plasticity in response to local environmental quality. The higher brood cell length and numbers recorded in Panaje (Table or Fig.) indicate an optimal ecological niche where high resource availability and climatic stability allow for maximum biological investment. In contrast, the drastic reduction in all parameters at Arakalagudu suggests that environmental stressors, likely related to higher temperature, seasonality and limited floral diversity in the plains, force colonies to adopt a resource-conservation strategy characterized by smaller cell sizes and lower population counts (Ali *et al*., 2025).

The statistical parity between Ponnampet and Kalasa further highlights the influence of the Western Ghats' humid-tropical climate, which acts as a 'thermal refuge' supporting significantly more robust colonies than the drier interior regions. Ultimately, these results demonstrate a strong positive correlation between architectural investment and colony productivity, proving that the geographic location is the primary determinant of both the physical size of the offspring's developmental environment and the overall reproductive success of the colony (Bhatta *et al*., 2019).

Honey Pots, Pollen Pots Morphometrics and Numbers

The significant regional variations in honey storage architecture and capacity reflect the diverse resource availability and foraging efficiencies of *T.iridipennis* across Central Western Ghats of Karnataka. The superior performance in Panaje, characterized by both the largest honey pot dimensions (13.33 mm length) and the highest count (105.6), suggests a 'resource surplus' environment. In these coastal-influenced regions, high floral density and favourable humidity levels likely allow colonies to invest heavily in large-scale storage vessels to capitalize on consistent nectar flows. This creates a compounding advantage: larger pots provide a higher volume-to-surface-area ratio for honey maturation, while a higher number of pots ensures a larger energy reserve for colony maintenance and defence (Bhatta *et al*., 2019). Conversely, the significantly smaller and fewer honey pots in Arakalagudu (6.43 mm length; 35.8 pots) point toward environmental constraints typical of the plains, where erratic nectar availability and higher temperature fluctuations may limit a colony's ability to produce wax and store food. The statistical parity between Ponnampet and Kalasa suggests that the mid-elevation Western Ghats ecosystem provides a moderately stable environment, allowing for substantial but not peak storage development. Ultimately, the strong correlation between pot size and pot number across all sites indicates that honey storage is a highly plastic trait, with the physical architecture of the nest serving as a direct reflection of the habitat's carrying capacity and the colony's foraging success (Charanakumar *et al*., 2022).

The data for pollen storage confirms a scaling effect in *T.iridipennis* colonies across Karnataka. In high-density regions like Panaje, colonies do not simply build more pots; they build larger pots, thereby exponentially increasing their total storage volume for protein resources. The significant reduction in all three parameters (length, width and number) in Arakalagudu suggests that this location may represent a sub-optimal habitat where environmental constraints limit both the physical size of the storage architecture and the colony's ability to accumulate pollen reserves. These results correlate strongly with the brood data, as higher pollen storage in Panaje likely supports the significantly higher brood cell numbers observed earlier (Charanakumar *et al*., 2022 and Ali *et al*., 2025).

The observed colour transition in brood cells from dark to light brown/creamish is a critical developmental indicator; as the larvae pupate and the cell walls thin out or are modified by the bees, the lightening of the color serves as a reliable marker for maturity (Charanakumar and Venkatesha, 2024) a phenomenon consistently noted in all four study areas. Colonies in the humid Western Ghats regions (Panaje and Ponnampet) are significantly more robust, featuring larger nests and greater storage capacities compared to the smaller, resource-conservative colonies found in the drier plains of Arakalagudu. Geographical location acts as the primary determinant for these variations, with environmental quality driving both the physical investment in nest architecture and overall colony productivity.

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