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Research

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Characterization of Nest Architecture of an Indian Ant *Diacamma indicum* (Hymenoptera: Formicidae)

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Abstract

Nests are physical entities that give shelter to the inhabitants from natural adversities, predators and act as a platform for organization of tasks particularly in social insects. Social insect nests can range from simple structures consisting of a single entrance leading to a chamber to complex nests containing hundreds of connected shafts and chambers. This study characterizes nest architecture of a tropical ponerine ant *Diacamma indicum* (Hymenoptera: Formicidae), which has small colony sizes and is known to be a scavenger. We also examined if these nests vary seasonally. By examining the microhabitat in the vicinity of the nest, the nest entrance characteristics and casting 77 natural nests of *D. indicum* across a year, we found that this species occupies relatively simple nests consisting of a single entrance that leads to a single chamber. This chamber progressed to a secondary tunnel that terminated at a greater depth than the chamber. The nest volume was not correlated to the number of adult members in the colony. Even though the microhabitat around the nest and the entrance itself change across seasons, principle component analysis showed that the nest architecture remained similar. Only one parameter, the entrance tunnel showed significant difference and was longer during postmonsoon. Nests of colonies living in the immediate vicinity of human habitation were comparable to other nests. We conclude that *D. indicum* found in the Gangetic plains live in relatively simple nests that do not vary across seasons.

Key words: Ponerinae, seasonal variation, nest microhabitat, nest chirality, nest volume

A nest is a place or a specially modified structure serving as an abode for animals and especially during their immature young. Animal nests have fascinated us over the centuries with their monumental building efforts as well as their enigmatic architectural features (Hansell and Ruxton 2008). Nests are important to organisms that occupy them as they give protection from predators, environmental adversities, and provide a storage space for resources (Hansell 1993). Nests are also important for the establishment of territories and providing group identity and for the organization of work, particularly in the case of social insects (Robson et al. 1995). Nesting sites of ants are diverse and can range from subterranean soil nests to cavities in living trees or leaves stitched together forming arboreal chambers and occasionally even consist of living workers orienting themselves to form a chamber (Tschinkel 2015). The generalized architecture of subterranean ant nests consists of a vertical shaft connecting the entrance to a horizontal chamber. This unit can be repeated many times over to form complex structures such as those found in leaf cutter ant *Atta leviagata* nests, containing up to 7,800 chambers that extend as deep as 7 m underground (Moreira et al. 2004).

The impact of seasonal variation has been examined on life history traits like alate formation, brood production, and colony size, but to the best of our knowledge, its impact on nest architecture in tropics is relatively unexplored (Tschinkel 1999a,b; Cristiano et al. 2019). What we do know is that as colony size increases the number of chambers as well as the volume of these chambers increase in case of *Pogonomyrmex badius*, *Phedole morrisi*, *Solenopsis invicta*, and some other species (Tschinkel 2015). Sometimes, the chamber edges become more lobed as the chamber grows (Tschinkel 2015). In the temperate ant *Odontomachus brunneus*, winter nests are twice as deep (up to 170 cm) as summer nests (up to 60 cm), even though colonies consisted of similar number of adults (Hart and Tschinkel 2012). When *Temnothorax rugatulus*, colonies were subjected to higher humidity and lower airflow, they built nests with thicker walls, indicating that colonies are potentially capable of adapting their nests to differences in their natural habitat (DiRienzo and Dornhaus 2017).

There are several methods to study the architecture of subterranean ant nests. Careful excavation of the nest, layer by layer, exposing one chamber at a time can give an idea about chamber size and depth, but fine structural details are lost. Casting of nests provides detailed information regarding the nest structure and is considered to be the best and most convenient option. Materials like

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paraffin wax, dental plaster, or metals with low melting point like Al or Zn are poured into the nest. The molten metal occupies the hollow structures within the nest and solidifies to produce a three-dimensional cast of the nest. While wax and dental plaster allow for the recovery of the dead ants from the nest cast, metal casts provide greater detail of the three-dimensional nest structure and is less prone to fragmentation during recovery and storage (Tschinkel 2010). Both of these methods lead to the destruction of the nest and its inmates. Two noninvasive methods namely X-ray and computed tomography can also give information on the three-dimensional structure of the nest without destroying the nest and its inmates(Halley et al. 2005).

Diacamma indicum (Ponerinae), is a black, 1-cm long ant, found in India, Sri Lanka, and perhaps Japan. *Diacamma indicum* colonies consist of adult monomorphic adult females, that is, all the individuals (reproductive caste and worker caste) are similar in size and morphology and they show solitary foraging to bring back parts of termites and other dead insects (personal observation) (Kushankur Bhattacharyya). The colony size ranges from 12 to 260 adults. They live mostly in subterranean nests and occasionally occupy other locations like crevices below rocks, bricks, walls, and tree branches (Viginier et al. 2004, Kaur and Sumana 2014, Kolay and Annagiri 2015). This species is known to be monodomous and occupy shallow nests or move to higher elevations during monsoon (Kaur and Sumana 2014, Kolay and Annagiri 2015). Across several relocations conducted in nature, *D. indicum* occupied pre-existing holes or cavities and they were not seen excavating a nest of their own (Kaur et al. 2012). In this study, we did field surveys across a year to characterize the living quarters or nests of *D. indicum* in its natural habitat, in eastern India along the Gangetic plains. The annual temperature ranges from 8 to 48°C in this region and rainfall occurs mostly in the months of June to September while the remainder of the year it is mostly dry. We hypothesized that the seasonal variation will impact nest architecture of this species. We characterized the area surrounding the nest or the microhabitat they were found in and the nest entrance. We cast nests using aluminium in order to characterize nest architecture across seasons. Furthermore, we expected colony size to correlate with nest volume as seen in other ant species. In order to check this, we also cast nests using paraffin wax.

Methods

Study Area

Nests of *D. indicum* were studied in their natural habitat in the Gangetic planes of India (Nadia district, West Bengal) (22° 56′ N, 88° 31′ E), from August, 2015 to July, 2016. The area lies in the tropical region and the climatic conditions are wet dry with an annual rainfall around 200 cm mainly occurring during 3–4 months (monsoon) (Kolay and Annagiri 2015). The area, in which natural nests were located measured approximately 0.5 square kilometers. The study was carried out in three periods; monsoon from June, 2015 to September, 2015, postmonsoon from October, 2015 to January, 2016 and premonsoon from February, 2016 to May, 2016. The maximum rainfall occurred in the months of July and September (total 87.98 cm). The temperature ranges from 10°C to 42°C. The weather data are from the Weather Station (Nadia District, West Bengal) (22° 57′ N, 88° 31′ E).

Nest Entrance Characterization

Information regarding nest entrance of natural *D. indicum* nests were collected by multiple field visits every month (number of visits ranged from 3 to 5 per month). Nests were located by following foragers that were returning to their nest. A total of 77 nests were sampled across the study period: monsoon (28), postmonsoon (25), and premonsoon (24). Upon discovery of a nest several entrance features like diameter, decoration, and presence of mound were recorded and building index (BI) scores were assigned. BI values ranged from 0 to 5, where 0 was allocated for a bare open nest and 5 for a nest entrance which had elaborate decorations and an elevated entrance with a consolidated mound and many decorations around the entrance (Kolay and Annagiri 2015). For further examination, a photograph of each nest entrance was taken with a scale in the background using a still camera (Canon Powershot SX15X). Additional analyses of entrance diameter and area were carried out using these pictures with ImageJ software. In order to evaluate the area surrounding the nest entrance a circle with a radius of three *D. indicum* body length (3 cm) from the center of the nest entrance was drawn. Within this focal microhabitat, the area covered by the four most common materials, that is, soil, vegetative material, caterpillar cuticle, and bird feathers were measured using ImageJ software. For each of these materials, scores from 0 to 4 were assigned. Scores were given according to the percentage area covered by each of these materials except for soil. When any given material was absent, it was scored as 0. When a given material covered 1 to 25% area within the focal area, a score of 1 was assigned; similarly coverage between 25–50%, 51–75%, and 76–100% were scored 2, 3, and 4, respectively. However, with soil, the calculation of percentage soil would underestimate the height of soil present and also did not account for the labor associated with consolidation of soil as compared to loose soil around the entrance. Thus, for soil, we scored small amount of loose soil as 1; medium amount of loose soil as 2; small but consolidated soil mound as 3, and consolidated mound with large amount of soil as 4. Sum of the scores from all these four materials was termed as 'nest entrance microhabitat or NEMH' and was used to get a comprehensive view of the nest entrance surroundings. A potential limitation of this index is that the values do not directly reflect the different materials or the quantity of a given material used by the ants. However, as the first attempt to quantify the microhabitat in which nests occur, it would be a useful tool.

Nest Casting

Nest architecture studies were performed by making metal casts of natural nests by the methods described in Tschinkel et al. (2010). Six nests were cast in each month from August, 2015 to July, 2016. In the current study, Aluminium (Al) was used as it has a relatively low melting point (around 650°C), and it can produce a cast of the nest structures in great detail. Upon solidification, it becomes very hard making it suitable for excavation from the soil with minimum damage and is easily stored for long durations. An earthen oven, hard coke coal and an air blower was used to melt Al bars inside a graphite crucible. On liquefication, any impurities in the metal formed a thin layer on top of the metal and this was removed using a spatula. A shallow mud bib was constructed around the entrance of the nest to facilitate pouring of molten Al. Molten Al was poured through the mud bib slowly until it overflowed. In about 15 min, the cast had solidified and was dug out of the soil using a spade (Fig. 1a). After digging out the nests partially, a picture was taken so as to ascertain the angle and the orientation. On bringing the cast to the lab, it was washed with water and debris were cleared.

A drawback of Al casting is that the ants living inside the nest die and get embedded inside the cast permanently. Thus, information on the residents could not be collected using this method. In order to obtain the number of ants that were inside the nest, we cast

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another set of nests using paraffin wax. Paraffin can produce some structural details of the natural nests; however, these casts easily break into fragments. As the melting point of paraffin is relatively low (around 60°C), these casts however preserve the adult ants and pupae within it. Paraffin was molten using the same oven inside a steel kettle. Before pouring molten wax through the nest entrance, the nest entrance and surrounding area was sprinkled with water. This induced the foragers to return to their nests within a few minutes, and returning foragers were also collected and embedded into the cast. Despite these precautions, it is possible that a very small percentage of foragers were missing from the cast and hence from our data. Nests were cast in a similar fashion as Al casts as described in previous section. The casts were dug out and cleaned. Each nest was then recast into multiple thin rectangular sheets of fixed size (21.5×30 cm^2), (the thickness of the wax sheets varied according to the amount of wax needed to cast the nest) in order to calculate the volume as well as quantify the number of adult ants that occupied the chamber. Volumes of both Al and wax casts were by dipping them totally into a container filled with water and the volumes of both Al and wax casts were calculated by dipping them totally.

Determining Different Parts of a Cast

From the Al casts, we differentiated various parts of a natural nest. Nests typically started with a single lobular entry space termed as the nest entrance (Ne). There was a single passage leading from the entrance to the first chamber, designated as the entrance tunnel (Te). The comparatively broader area was considered as a nest chamber (Nc). Presence of adults and pupal cases in this area in most of the cases confirmed this. The passage going away from the last chamber

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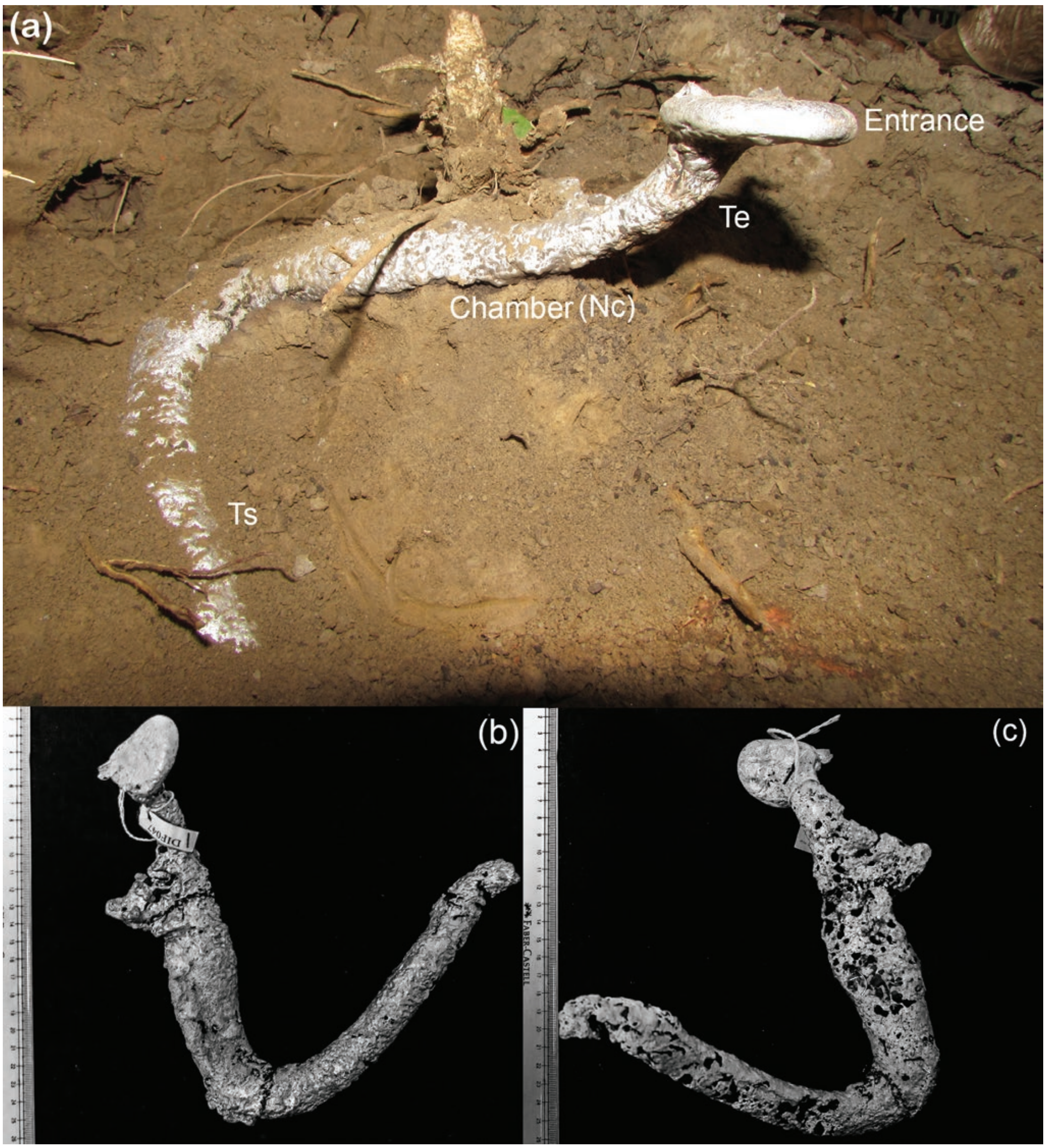

Fig. 1. Diacamma *indicum* nest cast: (a) Al cast in its original orientation half buried in soil within its natural habitat. The top disc represents the entrance, followed by entrance tunnel (Te), chamber (Nc), and secondary tunnel (Ts), ending at a depth of 15.9 cm vertically. Panels b and c show the same cast with a scale in two different orientations that differ from each other by 180 degrees. In the photographs remnants of ants in the chamber area are visible in panel c.

was termed the secondary tunnel (Ts). We call these passages as tunnels because qualitatively their angles were generally less than 90 degrees. As it was not clear how to quantify the start and termination points of the chamber, we made detailed measurements of tunnels across a cross section of nests. Ten random nests from different seasons were taken and the perimeter as well the diameter of the entrance tunnel (Te) and secondary tunnel (Ts) were measured. The tunnels' perimeters were measured with a cotton thread circumventing the tunnels followed by measuring the length of the thread. Ten measurements each was taken for both Te and Ts for every nest at approximately every centimeter or less. The average perimeter and diameter value of Te and Ts were calculated and used as the standard. Any part of the cast that had perimeter more than the standard value of both Te and Ts was considered as a chamber. In some of the nests, these parameters could not be distinguished clearly due to the presence of objects like brick and stones inside the structure or because of hollow structures produced presumably due to the presence of water or air pockets during casting. Casts ($N = 7$) having such indistinct structures or flaws were excluded from the dataset. Apart from tunnels and chambers mentioned earlier, any branch off structure, if any that was more than 0.5 cm in diameter was considered as an offshoot.

Quantification of the Internal Structure

To quantify internal structures, we measured several features of the Aluminium casts, such as number of entrance(s), entrance tunnel length (Te), number of chamber(s), total chamber volume, secondary tunnel length (Ts), and the diameter of Te and Ts. To understand the orientation of the nest inside the soil, three additional parameters were measured, which are vertical nest depth, that is, the perpendicular distance between nest termination point and the surface of the soil. Similarly, vertical chamber depth was measured by taking the average of the depths of the starting and termination point of the chamber. The nest angle was measured as the angle between cast entrance that is placed parallel to the horizontal plane and the tip of the secondary tunnel. The volume of chambers was measured by measuring the volume of displaced water. Nests were suspended upside down from a pulley and submerged to varying degrees into a large plastic tank filled with water to obtain the displacement by the nest chamber. Chirality of the nests is its rotational orientation either clockwise or anticlockwise. It was determined by hanging the nest vertically by its entrance and looking at the orientation of the secondary tunnel. All the parameters were compared across seasons.

Statistical Analysis

The statistical analyses were performed using statistical software StatistiXL (version 2.0) and ImageJ (version 1.51h). To compare different parameters across seasons Kruskal–Wallis tests were performed followed by post-hoc tests (Mann–Whitney *U* test) for pair wise comparisons. Spearmann Rank Correlation was conducted to correlate total nest volume with the colony size. Mean and standard deviation are presented in all the results unless mentioned otherwise. A Principal Component Analysis (PCA) was carried out taking into consideration all the parameters to create a consolidated nest architecture index. Six parameters were used as inputs for PCA: Te length, angle, Ts length, vertical nest depth, average chamber depth, and chamber volume. All PCAs were done using 'R' package version 3.4.2 and plotted using package Plot 3D. Unless mentioned otherwise the mean and standard deviation have been presented. In all cases, two-tailed *P* value equal to or lower than 0.05 was considered as the cut off for significance.

Results

A total of 107 natural nests were cast, of which 77 nests were cast using Al and 30 were cast using wax. Of these, seven and five nests, respectively were discarded as they either had materials like rocks or bricks incorporated in them or had air cavities. We found the nests in several different types of locations including human habitation. Eight such nests of the total 77 casts examined were located in close vicinity (nest entrances less than 2 cm away) to manmade structures like pavements, brick walls and those were considered as human-influenced nests. Thus, for 77 nests, we examined the microhabitat and compared nests across the seasons.

General Nest Architecture

The yearlong study on 77 natural *D. indicum* Al-cast nests conveys a great deal of information about the general nest architecture of this subterranean species. This species lives in relatively simple nests, which start with a single entrance. In a few cases ($N = 4$), we have encountered nests with two entrances. The nest entrance (Ne) leads to a single chamber through an entrance tunnel (Te) of variable angle which had an average length of 10.21 cm. In cases where there were multiple entrances, multiple entrance tunnels connected entrances to the chambers separately. In majority of the occasions, there was a single chamber, but on a few occasions several chambers were present and in all of them traces of ants were found. The highest number of chambers found was 3. The average chamber volume was 99.17 cc. After the chamber, there is another tunnel leading deeper down into the soil with an average length of 9.61 cm (Fig. 1) (Supp Figs. S1, S2, and S3 [online only]). In order to get a comprehensive view of nest architecture, we carried out a 'Principal component analysis' with length of Te, angle of the nest, chamber volume, vertical depth of the chamber, length of Ts, and vertical depth of the nest as input parameters. PCA1, 2 and 3 explained 73.41% of the variance in nest architecture, and the scatter plot with 70 nests showed that there was no clustering of nests across seasons, suggesting that, the generalized structure of the nest in *D. indicum* remains similar across the year (Fig. 2a).

We looked at chirality at two different levels: 1) chirality up to the chamber, that is, chirality of Te and 2) chirality from the chamber till the end of the nest, that is, chirality of Ts. The percentage of nests where Te was right handed or clockwise in premonsoon, monsoon, and postmonsoon were 42, 32, and 32%, respectively, whereas the percentage of nests where Te was anticlockwise were 20, 46, and 52%, respectively. Chirality of Ts was clockwise in 29, 21, and 32% of the premonsoon, monsoon, and postmonsoon nests, respectively, and 17, 39, and 44% of nests were oriented anticlockwise. No bias towards any of the chiral patterns were observed (Te – Chi square test: X^2 value = 0.286, df = 1, $P = 0.593$; Ts – Chi square test: X^2 value = 0.333, df = 1, $P = 0.564$). In total 28 cases of Te and 31 cases of Ts, the chirality could not be determined. This is because, the tunnel was very short or was placed directly below the entrance in the former and below the chamber in the latter case.

From the wax casts, we found that colonies contained an average of 81.6 ± 51.46 ants. There was no significant correlation between colony size and the total nest volume (Spearmann rank correlation test: $r_s = -0.08$, df = 25, $P = 0.69$; Fig. 2b). The average nest volume was 167.28 ± 89.94 cc. So, average nest volume per individual was 3.09 ± 2.49 cc (Supp Fig. S4 [online only]).

Though the general nest architecture was comparable throughout the year, the different parts might vary and show seasonality; thus, we examined this starting with the structures on the surface and moving deeper inside.

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Entrance

Nest entrance is an easily visible opening on the ground or in the crevices of soil mostly roundish in shape. It can be recognized by the presence of decorative materials and mounds in monsoon. The entrance is 2.5 ± 0.67 cm in diameter. BI was calculated for all the nests before they were cast using Al. The BI values were significantly different across seasons, (Kruskal–Wallis test: $X^2 = 24.625$, $P < 0.001$). Post-hoc tests also showed that BI during monsoon was significantly higher (average – 2.78 ± 1.26), than that of premonsoon (average – 1.21 ± 0.93) and postmonsoon (average – 1.2 ± 1.12) (Mann–Whitney *U* test: $U = 576.0$, df1 = 28, df2 = 25, $P < 0.001$, $U = 566.5$, df1 = 28, df2 = 24, $P < 0.001$; Fig. 3b). There was great heterogeneity

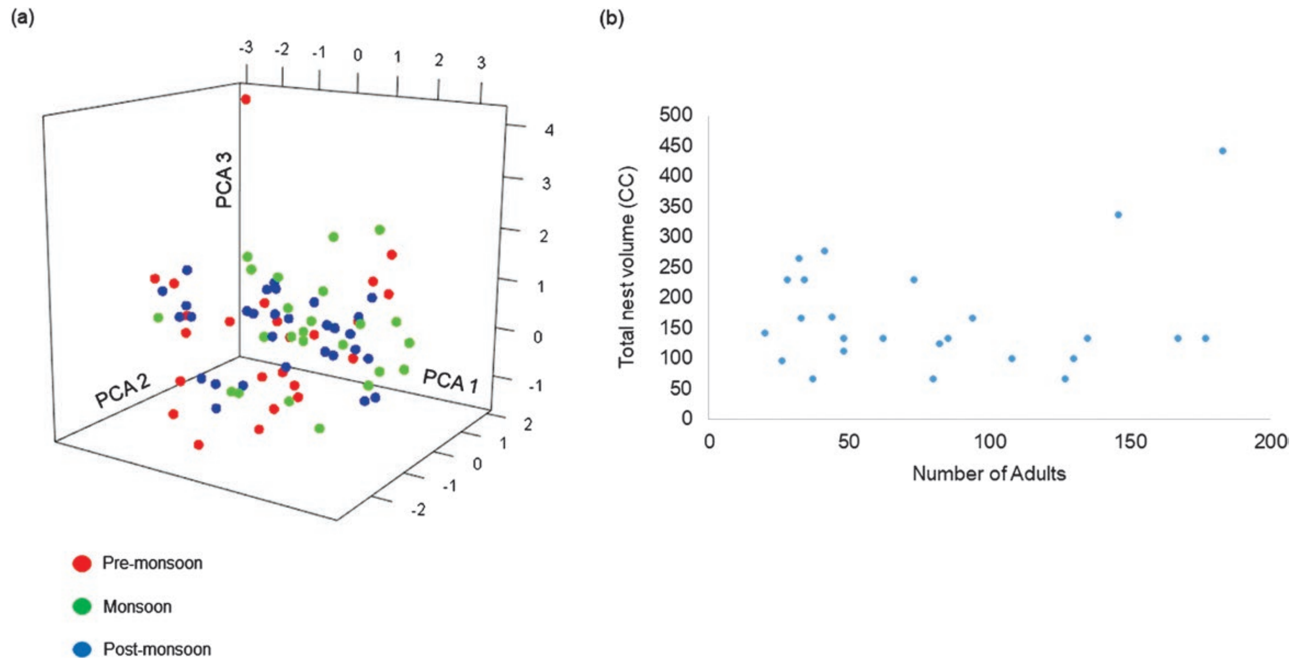

Fig. 2. *Diacamma indicum* nest architectural features: (a) Scatter plot of PCA1, 2 and 3 showing relative position of different nests across three different seasons ($N = 70$). (b) Scatter plot of number of adults in colonies and the nest volume across 25 colonies derived from wax casting.

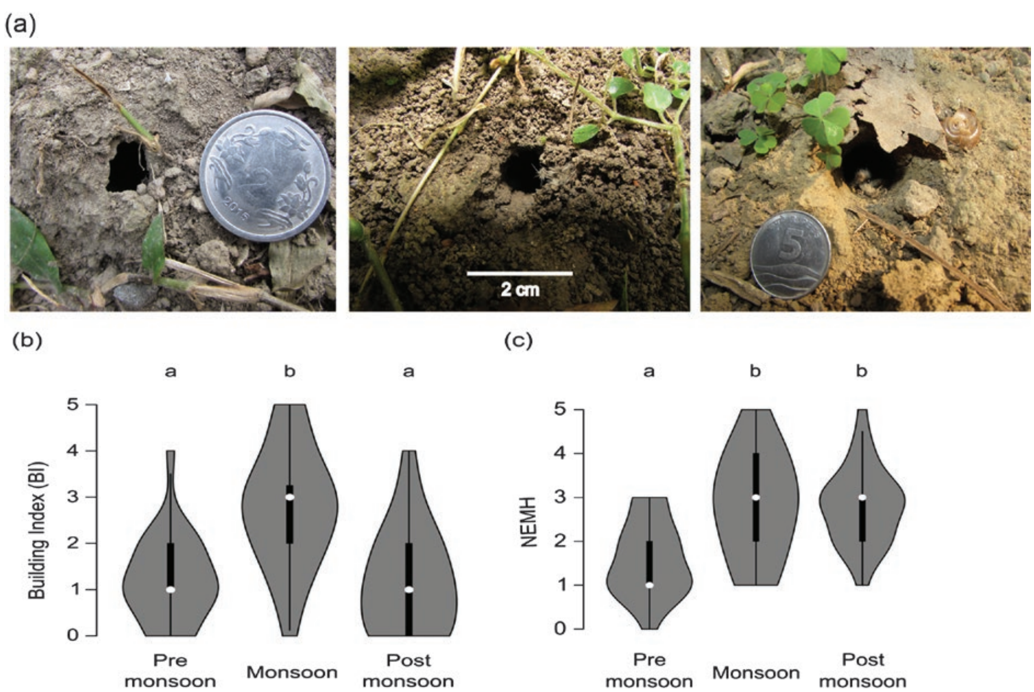

Fig. 3. *Diacamma indicum* nest entrance characteristics: (a) three natural nest entrances from three different seasons; premonsoon (left), monsoon (middle), and postmonsoon (right). The scale in the middle applies to all the three pictures. The diameter of the coins is 2.6 cm. (b) Comparison of BI values across three different seasons. (c) Comparison of Nest Entrance Microhabitat (NEMH) values across three different seasons. These violin plots display the median as a white dot and box on either side are the first and third quartiles with the lines representing the range of data. The gray region surrounding this represents the density distribution of the data. Significantly different bars carry different alphabets (Kruskal–Wallis test, $P < 0.05$).

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in the decoration and presence of vegetation around the nest entrance; dead and decaying vegetative matter was the most abundant material and feathers were scant. On quantifying the nest entrance microhabitat (NEMH), we found that the NEMH score also varied significantly across seasons (Kruskal–Wallis test: X^2 = 18.878, P < 0.001). Post-hoc tests showed that NEMH was comparable between monsoon (average – 2.85 ± 1.19) and postmonsoon (average – 2.8 ± 0.87), whereas NEMH in premonsoon was lower (average – 1.61 ± 0.84) (Mann–Whitney U test: U = 336, df1 = 28, df2 = 25, P = 0.845, U = 471, df1 = 28, df2 = 24, P < 0.001, U = 474.5, df1 = 25, df2 = 24, P < 0.001; Fig. 3c).

Entrance Tunnel (Te)

The entrance tunnel (Te) was situated at an angle varying from 20° to 90°. The average angle of Te is 55.34°. The average length of Te is 10.21 cm. The average width (diameter) of Te is 2.38 ± 0.4 cm. The vertical depth of Te is denoted by the perpendicular distance from the entrance to the end of Te and it is 5.89 ± 3.13 cm. Though the vertical depth remains comparable throughout the year, the length of Te varies. The length of Te was significantly greater in postmonsoon (average – 12.24 ± 3.72 cm) as compared to monsoon (average – 9.6 ± 6.13 cm) and premonsoon (8.78 ± 5.83 cm) (Kruskal–Wallis test: Chi square value: 8.385, P = 0.015) (post-hoc Mann–Whitney U test: U = 464, df1 = 25, df2 = 28, P = 0.042; U = 342.5, df1 = 25, df2 = 24, P = 0.003; Fig. 4a).

Chamber

The chamber is probably the most important part of the nest as it harbors the whole colony and provides space for rearing brood. The chamber is generally disc shaped and has rough edges. The majority of nests had a single chamber, and only six nests had multiple chambers and the maximum number of chambers observed was 3. The average height of chambers is 2.69 ± 1.14 cm and the average volume is 99.17 ± 56.2 cc. The average vertical depth of the chambers is 10.59 ± 4.48 cm.

Secondary Tunnel

The secondary tunnel is present in 50%, 67.86%, and 68% of the nests in premonsoon, monsoon, and postmonsoon nests, respectively. The average length of Ts is 9.69 ± 12.26 cm which is qualitatively similar to Te. The average vertical depth measured at the tip of the Ts (15.18 ± 6.44 cm) is significantly greater (Wilcoxon paired sample test: Z = 4.595, P < 0.001) than the average chamber depth (10.59 ± 4.48 cm) suggesting its functionality as a drainage system for water that may enter the nest chamber (Fig. 4b).

Human-Influenced Nests

A total of 8 nests among the dataset of 77 nests were influenced by humans. Qualitatively, these nests were similar to other nests. PCA confirmed that these nests had similar architectural features as other nests situated in nonhuman-influenced habitats.

Discussion

The generalized nest architecture of the tropical ponerine ant *D. indicum* has been investigated in this study in order to get basic information on their dwelling characteristics. This study reveals that *D. indicum* nests are relatively simple structures. They are comprised of a single chamber connected to a single entrance by a tunnel and has secondary tunnel starting from the chamber to the end at a greater depth. This information may be particularly useful in designing nests within the lab for this species as well as for asking questions in the context of relocation to a new nest. The presence of a single chamber suggests that *D. indicum* lacks the ability to store food in separate chamber or to have age-based spatial segregation as has been described in *Pogonomyrmex badius* (Tschinkel 2004). This species is a scavenger and has small colony size ranging from 12 to 260 with an average of

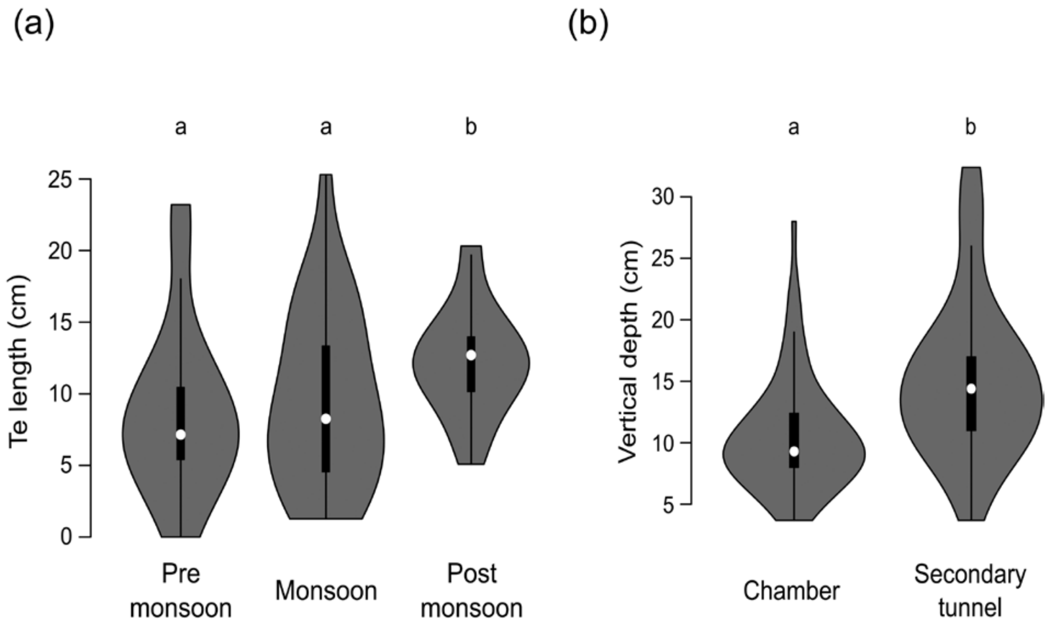

Fig. 4. *Diacamma indicum* entrance tunnel length and vertical depth: (a) Comparison among entrance tunnel lengths across three different seasons are presented as a violin plot. Significantly different bars carry different alphabets (Kruskal–Wallis test, $P < 0.05$). (b) Comparison of vertical depth of the chamber and the secondary tunnel is presented as a violin plot with significant different bars carrying different alphabets (Wilcoxon Paired Sample test, $P < 0.05$). These violin plots display the median as a white dot and box on either side are the first and third quartiles with the lines representing the range of data. The gray region surrounding this represents the density distribution of the data.

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90 adults, and perhaps they do not require multiple chambers, (Kaur et al. 2012). Compared to nests of species-like *Pogonomyrmex badius* (Tschinkel et al. 2004), *Dolichoderus brunneus* (Laskis and Tschinkel 2009), which contain multiple chambers that are connected together with a framework of tunnels, *D. indicum* occupy simple nests with a single entrance and a single chamber within. Further studies on the nest architecture of other *Diacamma* species as well as other ponerine species would be required to examine if these simple nests are a feature of this genus or subfamily.

The nest entrance is not particularly conspicuous and appears like any other hole in the ground with the diameter that is approximately twice the body length of *D. indicum*. It can be sometimes distinguished by the presence of decoration materials like plant materials, soil balls, caterpillar cuticle, and bird feathers. These materials are present on and around the nest entrance in all possible combinations and numbers. In a few cases, materials such as mollusc shells, insect wings, and even manmade objects like plastic were seen too. In order to get an idea about the microhabitat around the nest entrance, we devised a novel index 'nest entrance microhabitat (NEMH)' to characterize the abundance of these objects in the immediate vicinity across different seasons. These index values were higher in monsoon and postmonsoon as compared to premonsoon. Modification of the entrance itself was checked using BI. The current study found that BI is highest during monsoon as compared to pre- and postmonsoon, similar to what was reported previously (Kolay and Annagiri 2015). Given that mounds act as levees and reduce the amount of water that enters into the nest, it is not surprising that they are more common and better constructed during monsoon. But the function of the mounds may not be restricted to preventing inundation. Even in the absence of rainfall, damaged mounds were rebuilt (personal observation) indicating that they perform additional functions, like in the case of *Lasius flavus* ant which use their mounds to harvest sunlight (Kolay and Annagiri 2015, Véle and Holuša 2017). Variation in the mound and area in the immediate vicinity of the nest entrance has been seen in sand dune inhabiting ant species-like *Mycetophylax simplex*, *Trachymyrmex holmgreni* and these changes have been attributed to different environmental stress factors (Diehl-Fleig and Diehl 2007, Cristiano et al. 2019).

In *D. indicum*, the entrance tunnel length is significantly higher in postmonsoon period as compared to other seasons possibly to achieve optimal temperature during the winter. This hypothesis has to be examined with measurements of temperature inside the chambers across different seasons. The secondary tunnel was present in 61% of nests and was of varying length and it terminated at a higher depth as compared to the chamber in all cases. We hypothesize that this secondary tunnel is a probable effective drainage system for water that may enter the nest during nest inundation. It might also aid in achieving the proper microclimatic condition inside such as the regulation of CO_2 concentration (Cox and Blanchard 2000), or it may function as a dumping ground for organic and inorganic waste generated by the colony members. Typically, a single chamber is seen in the nest and thus it harbors the whole colony as well as brood. There was no correlation between number of adults in the colony and the nest volume with an average nest volume per individual at 3.09 cc. This is unlike what is known in other species-like *Pheidole morrisi* (Murdock and Tschinkel 2015) and may be caused due to the fact that they do not initiate their nest but occupy pre-existing holes in nature.

Further, PCA suggests that the overall nest architecture remains comparable across the year even though the abiotic characters show variation during this time. Multiple reasons that are not necessarily exclusive may bring about these generalized design of nest and lack of correlation between number of adults in a colony and nest volume. Some of these reasons could be that, *D. indicum* colonies do not initiate nest but occupy pre-existing holes in the ground when they relocate to a new nest. Colonies may occupy a given nest only for a short period of time and move to an alternate nest upon disturbance or other cause rather than make large-scale structural modification to their nest. It is also possible that this basic design provides optimal living conditions for these small sized colonies. Even colonies residing near human habitation were similar to other nests that were not directly impacted by humans, both qualitatively and quantitatively highlighting that this species lives in a relatively uniform, simple nest across a range of habitats throughout the year.

This study is important as it provides quantitative information from a large number of colonies from the tropics on a ponerine ant, a topic on which there is hardly any information. Further, it allows us to showcase the simplicity of these nests as compared to many other species and forms the basis for grounding experiments relating to relocation, colony maintenance, and nest construction to the natural parameter space of this species.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Author's Contribution

K.B. designed the experiments, carried out the experiments, did the data analysis, and wrote the manuscript. S.A. designed the experiments, revised the manuscript, and supervised the whole work.

Competing Interests

The authors declare no competing interests.

References

Cox, M. D., and G. B. Blanchard. 2000. Gaseous templates in ant nests. J. Theor. Biol. 204: 223–238.

Cristiano, M. P., D. C. Cardoso, M. V. Beirão, A. C. C. C. Reis, T. P. Pereira, and M. N. Moura. 2019. Nesting and distribution of *Trachymyrmex holmgreni* in *Brazilian restinga*. Insect. Soc. 66: 139–151.

Diehl-Fleig, E. D., and E. Diehl. 2007. Nest architecture and colony size of the fungus-growing ant *Mycetophylax simplex* Emery, 1888 (Formicidae, Attini). Insect. Soc. 54: 242–247.

DiRienzo, N., and A. Dornhaus. 2017. *Temnothorax rugatulus* ant colonies consistently vary in nest structure across time and context. Plos One. 12: e0177598.

Halley, J. D., M. Burd, and P. Wells. 2005. Excavation and architecture of Argentine ant nests. Insect. Soc. 52: 350–356.

Hansell, M. H. 1993. The ecological impact of animal nests and burrows. Funct. Ecol. 7: 5–12.

Hansell, M., and G. D. Ruxton. 2008. Setting tool use within the context of animal construction behaviour. Trends Ecol. Evol. 23: 73–78.

Hart, L. M., and W. R. Tschinkel. 2012. A seasonal natural history of the ant, *Odontomachus brunneus*. Insect. Soc. 59: 45–54.

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Kaur, R., and A. Sumana. 2014. Coupled adult-brood transport augments relocation in the Indian queenless ant *Diacamma indicum*. Insect. Soc. 61: 141–143.

Kaur, R., K. Anoop, and A. Sumana. 2012. Leaders follow leaders to reunite the colony: relocation dynamics of an Indian queenless ant in its natural habitat. Anim. Behav. 83: 1345–1353.

Kolay, S., and S. Annagiri. 2015. Dual response to nest flooding during monsoon in an Indian ant. Sci Rep. 5: 13716.

Laskis, K. O., and W. R. Tschinkel. 2009. The seasonal natural history of the ant, *Dolichoderus mariae*, in northern Florida. J Insect Sci. 9: 1–26.

Moreira, A., L. C. Forti, A. P. Andrade, M. A. Boaretto, and J. Lopes. 2004. Nest Architecture of *Atta laevigata* (F. Smith, 1858) (Hymenoptera: Formicidae). Stud. Neotrop. Fauna Environ. 39: 109–116.

Murdock, T. C., and W. R. Tschinkel. 2015. The life history and seasonal cycle of the ant, *Pheidole morrisi* Forel, as revealed by wax casting. Insect. Soc. 62: 265–280.

Robson, S. K., M. G. Lesniak, R. V. Kothandapani, J. F. A. Traniello, B. L. Thorne, and V. Fourcassié. 1995. Nonrandom search geometry in subterranean termites. Naturwissenschaften. 82: 526–528.

Tschinkel, W. R. 1999a. Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: distribution of workers, brood and seeds within the nest in relation to colony size and season. Ecol. Entomol. 24: 222–237.

Tschinkel, W. R. 1999b. Sociometry and sociogenesis of colony-level attributes of the Florida harvester ant (Hymenoptera: Formicidae). Ann Entomol Soc Am. 92: 80–89.

Tschinkel, W. R. 2004. The nest architecture of the Florida harvester ant, *Pogonomyrmex badius*. J Insect Sci. 4: 21.

Tschinkel, W. R. 2010. Methods for casting subterranean ant nests. J Insect Sci. 10: 88.

Tschinkel, W. R. 2015. The architecture of subterranean ant nests: beauty and mystery underfoot. J Bioecon. 17: 271–291.

Véle, A., and J. Holuša. 2017. Microclimatic conditions of *Lasius flavus* ant mounds. Int J Biometeorol. 61: 957–961.

Viginier, B., C. Peeters, L. Brazier, and C. Doums. 2004. Very low genetic variability in the Indian queenless ant *Diacamma indicum*. Mol. Ecol. 13: 2095–2100.

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