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A 3D model to illustrate the nest architecture of *Acromyrmex balzani* (Hymenoptera; Formicidae)

Nathan Rodrigues Batista[1,3]*, Vinicius Edson Soares de Oliveira[3], William Fernando Antonialli-Junior [1,2,3]

[1]*Universidade Federal da Grande Dourados, Programa de Pós-Graduação em Entomologia e Conservação da Biodiversidade, Dourados, MS, Brasil.*
[2]*Universidade Estadual de Mato Grosso do Sul, Programa de Pós-Graduação em Recursos Naturais, Dourados, MS, Brasil.*
[3]*Universidade Estadual de Mato Grosso do Sul, Centro de Estudos em Recursos Naturais, Laboratório de Ecologia Comportamental, Dourados, MS, Brasil.*

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ABSTRACT

For eusocial insects, the nest is a place where the main social interactions occur. The nest architecture ensures protection from predators and the environment, as well as suitable conditions for brood rearing, food storage, and in some cases the cultivation of fungus farms. Variations in nest architecture can occur, according to the environmental conditions. In order to elucidate the internal organization of nests, most studies use 2D schemes and photographs to illustrate the nest architecture models. However, 3D models can provide a different and more realistic view of the nest architecture. The aim of this study was to describe the nest architecture and colony size of the grass-cutting ant *Acromyrmex balzani* (Emery), using 3D models to illustrate these features. The structures of eight colonies were measured and the data were used to create a 3D model of each nest. Externally, the nests had one or more piles of loose soil and waste, with a single straw turret over the entrance. Underground, the nests had from 2 to 6 chambers, at a maximum depth of 122 cm. It could be concluded that the observed nest architecture of *Acromyrmex balzani* followed, at least in part, the pattern already reported in the literature. However, this is the first report of connection between two chambers made by two shafts, as well as the presence of the turret at the nest entrance/exit, regardless of the season of the year. These differences evidence that the nest structures may vary, depending on intrinsic or local environmental conditions.

Introduction

The nests of social insects provide protection and shelter for adults and brood, a safe place for food storage, a microenvironment suitable for the development of the brood, and are the places where most intracolonial interactions occur (Starr, 1991; Sudd, 1982). The nest architecture is the result of the energetically costly cooperative work of nestmates, while the nest also influences and modulates the flow of information necessary for social cohesion and efficiency in execution of the colony's tasks (Buhl et al., 2004b, 2004a; Perna and Theraulaz, 2017).

Most ants build their nests on the ground, digging shafts that interconnect chambers where the entire colony is housed (Tschinkel, 2015). Their subterranean nests are characterized by one or more external openings, with internal chambers connected by shafts and tunnels. Although studies indicate a species-typical pattern of organization of subterranean nests (Vieira et al., 2007; Cerquera and Tschinkel, 2010; Tschinkel, 2011), there may be variations caused by the conditions faced by the colonies in each environment (Toffin et al., 2010; Tschinkel, 2015).

In fact, ant workers can use environmental cues to guide the excavation of new structures, in search of more suitable conditions for the performance of colony functions (Bollazzi et al., 2008; Fröhle and Roces, 2009; Bollazzi and Roces, 2010a, 2010b; Römer and Roces, 2015). These environmental cues can modify the digging behavior or mediate the interactions between the workers, consequently resulting in intraspecific morphological variations in the nest architecture (Bollazzi et al., 2008).

Most studies investigating ant nest architectures (Vieira and Antonialli-Junior, 2006; Diehl-Fleig and Diehl, 2007; Forti et al., 2007; Rabeling et al., 2007; Vieira et al., 2007; Jesovnik et al., 2013) have used 2D schemes or photographs to illustrate them, which do not allow understanding of the real morphology of nest structures. In addition to the 2D models, another method widely used in the reconstruction of underground nests is to fill the nests with materials such as plaster,

* Corresponding author.
E-mail: nathan.bio.rb@gmail.com (N.R. Batista).

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molten metal, or cement (reviewed by Tschinkel, 2010). However, this method makes it harder to acquire information about other aspects of the colonies, such as their demographics.

On the other hand, the use of 3D models based on morphometric nest data, in addition to allowing the study of colony demographics, can illustrate the structures of the nests in their natural forms. However, there have been few studies describing the architecture of ant colonies using 3D models (Pinter-Wollman, 2015; Khuong et al., 2016; Guimarães et al., 2018).

Among the underground nests, those of leaf-cutter ants (*Atta* and *Acromyrmex*) present a great diversity of architectural models (Gonçalves, 1961; Moreira et al., 2004a, 2004b). These ants cut plant material and transport it into the nest, where they use it as a substrate upon which they cultivate symbiotic fungus, their main food source (Quinlan and Cherrett, 1979; Bass and Cherrett, 1995).

In leaf-cutter ants, nests of species of the genus *Acromyrmex* have a less complex architecture, compared to nests of the genus *Atta* (Moreira et al., 2004a, 2004b), and their colonies are generally smaller (Mehdiabadi and Schultz, 2009). The species *Acromyrmex balzani* (Emery) is a leaf-cutter ant species distributed in the center-west, southeast, and south regions of Brazil, as well as in Paraguay and Argentina (Gonçalves, 1961). The nest architecture of this species varies according to the biogeographical region, reaching a depth of 210 cm (Silva et al., 2010). The nests can have 5-14 chambers (Silva et al., 2010; Caldato et al., 2016) that are arranged vertically and connected by a single shaft (Ichinose et al., 2007; Pimenta et al., 2007; Poderoso et al., 2009; Silva et al., 2010; Camargo et al., 2016).

The present study was carried out with the objective of studying the external and internal architectures of the nests of *A. balzani* and presenting them in the form of a 3D model.

Materials and methods

Nest architecture

Eight nests of *Acromyrmex balzani* were excavated during the period from February to September 2018, three of them in the hot and rainy season, and five of them in the cold and dry season. The climate of the region is type Cwa (humid mesothermal), according to the classification of Kottek et al. (2006). The collection of nests in different seasons enables assessment of whether external structures, such as the turret and soil mounds, can vary under different climatic conditions (Pimenta et al., 2007). Individuals were taken to the laboratory and species identification was performed using the dichotomous key of Forti et al. (2006). The species was confirmed by Dr. Jacques H. C. Delabie of the Executive Committee of the Cocoa Plantation Plan (CEPLAC), Cocoa Research Center (CEPEC), Myrmecology Laboratory of the Universidade Estadual de Santa Cruz (UESC).

All the colonies were located near the Center of Studies in Natural Resources (CERNA), on the campus of the Universidade Estadual de Mato Grosso do Sul, in Dourados, Mato Grosso do Sul State (22°13'16" S, 54°48'20" W). The nest sites were found by active searching and following workers returning to the colony, and the distances between them were measured using a GPS. In order to ensure that the excavated nests were from different colonies, the shortest distance between one nest and another was 7 m and the longest distance was 111 m (Table S1). Caldato et al. (2016) reported that a distance of 5 m provided separation of the polydomic nests of this species.

Recording was made of the presence and height of the turret at the nest entrance, the presence of a loose soil mound with waste near the entrance, and the distance between this mound and the entrance of the nest (Silva et al., 2010).

The nests were excavated according to the methodology proposed by Antonialli-Junior and Giannotti (1997). Firstly, a trench 100 cm deep was dug 15 cm from the nest entrance. After digging the trench, the soil was slowly sliced away, in order to reach each chamber and/or shaft. When the deepest chamber in each nest was reached, the chamber walls were carefully inspected for another shaft, after which excavation was performed to about 50 cm below the chamber, to ensure that no nest structures or parts of the colony were lost. All the immature and adult individuals within these structures were collected, stored in 500 mL plastic containers, killed by freezing, and counted.

For each collected colony, the following data were recorded: (i) number of workers of each subcaste, (ii) presence or absence of queen in a chamber, (iii) maximum depth of the nest, (iv) number of chambers, (v) chamber depth (measured from the ground surface to the chamber roof), (vi) chamber length (the longer two edges), (vii) chamber width (the shorter two edges), and (viii) chamber height (measured from the floor to the roof of the chamber). These data were used to calculate the total and mean volumes of the chambers, using the following equation: volume = $3/4\pi LWH$ (ellipsoid volume), where L, W, and H are the chamber length, width, and height, respectively (Cardoso et al., 2014). Drawings of all the nests were made during the excavations, with these drawings and the size data subsequently being used to model, animate, and present the nests in 3D format, employing Promob v. 2016 software (serial number: 40693944; product code: 9CWWK2lM76).

Statistical analyses.

Pearson correlation tests were performed to determine whether there were any significant relationships between the number of workers and the number of chambers, between the number of workers and the nest volume, and between the number of chambers and the nest volume. These correlations could indicate the way in which the nest increased in size, whether by increasing the volume of preexisting chambers, or by adding more standard-sized chambers, as well as the influence of the colony population density on this increase (Tschinkel, 1999; Mikheyev and Tschinkel, 2004; Guimarães et al., 2018). These analyses were performed using R software (R Core Development Team, 2019).

Results

All the excavated nests had a single entrance covered by a turret composed of dry plant material, with a mean height of 2.5 ± 1.0 cm and mean diameter of 0.87 ± 0.20 cm (Table 1), regardless of the season. This structure was usually surrounded by a semi-circular mound of loose soil, with mean radius of 5.9 ± 1.8 cm. An exception was nest 5, which had two mounds arranged on opposite sides around the nest entrance. These mounds were composed mostly of soil, although in the part most distant from the entrance orifice, they also contained deposited waste, consisting of dead workers and portions of exhausted fungus.

The nests had between 2 and 6 chambers, and reached a mean depth of 61.9 ± 43.9 cm. The shallowest nest (nest 1) was 18 cm deep and had 2 chambers, while the deepest nest (nest 7) reached the maximum depth of 122 cm and had 6 chambers.

The chambers were ellipsoid, arranged vertically one under the other, and variable in size (2-16 cm width, 3-9 cm height, and 3-13 cm length, as shown in Table 2 and Figures 1 and 2). The nest chambers were interconnected by a single shaft, except for nest 8, where the first and second chambers were connected by 2 parallel shafts (Figure 2d). The first and second chambers of nests 1 and 2 had an additional

Table 1
Dimensions of external structures (centimeters) and allocation of fungal cultivar and brood of eight nests of *Acromyrmex balzani*.

Nest	Turret (cm)	Entrance diameter (cm)	Distance from entrance to soil mound (cm)	Chambers with fungus garden	Chambers with brood	Chamber with queen
1	1.0	0.8	5.0	2nd	2nd	2nd
2	2.3	1.0	4.8	1st, 2nd, 3rd	1st, 2nd, 3rd	1st
3	3.4	0.7	5.0	1st, 2nd	1st, 2nd	1st
4	3.0	1.2	10.0	2nd,3rd,4th	-	-
5	3.6	1.5	5.0	1st, 2nd	1st	1st
6	2.0	0.6	6.0	3rd,4th,5th	-	-
7	3.3	0.8	4.4	3rd	-	-
8	2.7	1.0	7.0	1st, 2nd, 3rd, 4th	3rd	3rd

Table 2
Size and depth (centimeters) of the chambers of the nests of *Acromyrmex balzani*.

Nest	Dimensions	Chambers					
		1	2	3	4	5	6
1	Depth	3	10.9				
	Width	16	6				
	Height	7.9	7				
	Length	10.5	10				
2	Depth	4	11.6	17.6			
	Width	6	5	11			
	Height	3.5	4	5			
	Length	5	6	10			
3	Depth	4	15.5				
	Width	6	11				
	Height	4.5	6				
	Length	7	8				
4	Depth	6	14.5	32	62		
	Width	6	9.5	12	10		
	Height	4.5	7	9	6.5		
	Length	6	13	12.5	10		
5	Depth	6	14.5				
	Width	4	6				
	Height	3	4.5				
	Length	6	7.5				
6	Depth	5	11.5	32.5	62	100	
	Width	2	8	12	13	12	
	Height	3	6	7	7	5	
	Length	3	8	11	10	10	
7	Depth	4	15	32	62	79.5	116
	Width	13	13	14.5	16	10	6
	Height	6	7	9	7	5.5	9
	Length	6	10	11	9	10	8
8	Depth	2.5	8	24	46.5	68.5	
	Width	7	11	10	6.5	10	
	Height	3	6.5	7.5	5	7.5	
	Length	7	11	10	6	10	

small ovaloid appendix-shaped structure (AP), directly connected to the chamber, whose mean volume was 8.6 ± 1.1 cm^3 (Figures 1a, b).

Nests 6 and 7 had an enlarged shaft between the first chamber and the ground surface, different from the patterns for the other chambers. The shafts had a mean volume of 7.9 ± 2.2 cm^3 (Figures 2b, c). All the nests had at least one chamber containing a fungus garden, where the brood was also found (Table 1). The fungus was suspended in the chamber, adhered to roots or in direct contact with the soil. Chambers without fungus gardens had only workers inside them. Rotating animations and computational models of the nests are provided for

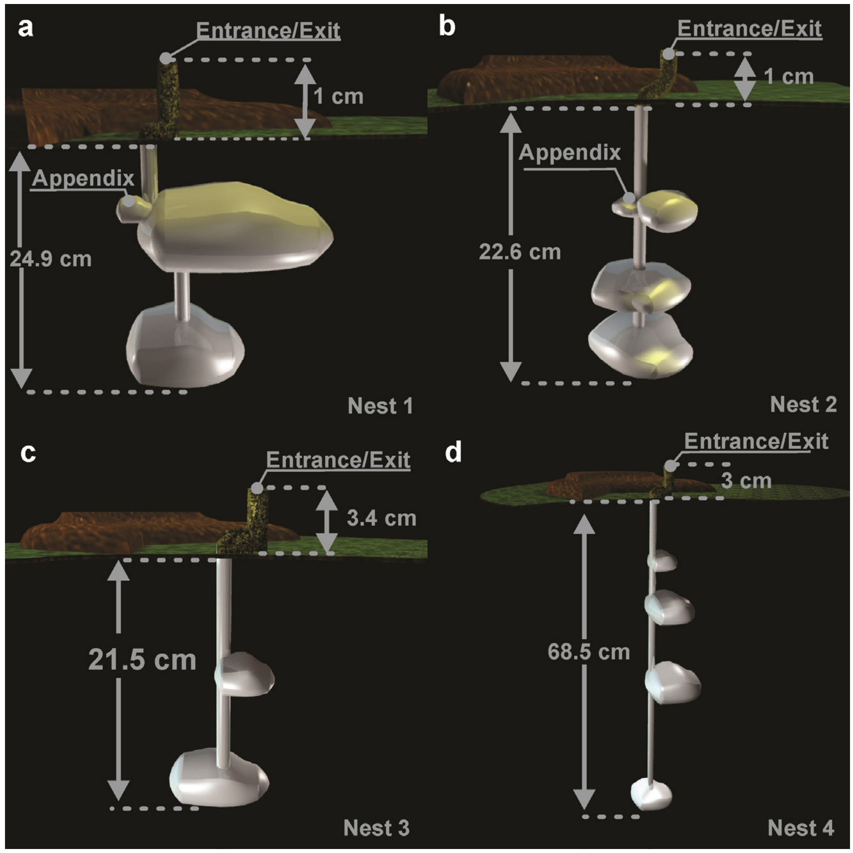

Figure 1 3D profile of nests 1 to 4, showing turret height, appendix location and maximum depth. a: nest 1; b: nest 2; c: nest 3 and d: nest 4.

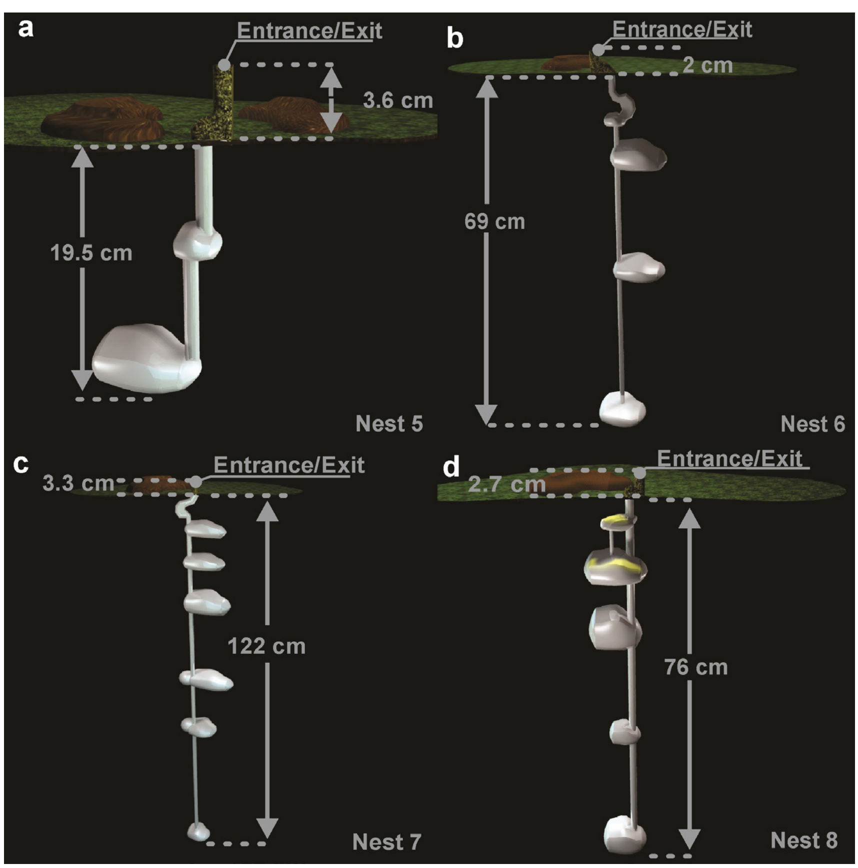

Figure 2 3D profile of nests 5 to 8, showing turret height and maximum depth. a: nest 5; b: nest 6; c: nest 7 and d: nest 8.

better visualization of the architectural features (Supplementary Materials 2 and 3, respectively).

The colony populations ranged from 621 to 1662 workers (mean: 986 ± 343 workers), with on average 287 ± 64 minor workers, 210 ± 92 medium workers, and 488 ± 216 major workers (Table 3). Queens and immature ants were not found in colonies 4, 6, and 7 (Table 1). When present, there was only one queen per nest, always in the chamber with the brood and the fungus garden.

The results revealed positive correlations between the number of workers and the number of chambers (r = 0.911, p = 0.001), between the number of workers and the nest volume (r = 0.796, p = 0.017), and between the number of chambers and the nest volume (r = 0.782, p = 0.021), as shown in Figure 3.

Discussion

This study elucidates the architecture of *A. balzani* nests, assisted by the creation of 3D models, and also describes the demography of the colonies. The external structures of the nests described here have been observed for nests in other populations of this species. The turrets are built by ants from different subfamilies, and in three species of the *Acromyrmex* genus (*A. balzani*, *A. landolti*, and *A. fracticornis*), these structures have been shown to be important to prevent flooding of the nests (Espina and Timaure, 1977; Navarro and Jaffe, 1985; LeBrun et al., 2011). Moreira et al. (2019) found evidence that this turret could also act as a visual clue used by foragers of *A. balzani* as a spatial reference during the return to the colony. This might explain the existence of these turrets regardless of the season, besides the fact that in the places where the nests were found, there was no transit of people or cattle that could destroy these structures.

The mounds of loose soil and waste found around the nest entrance were present outside all the nests. These mounds consisted of soil from excavation, dead workers, and deposition of fragments of leaves used to grow the fungal symbiont. The deposition of these mounds may vary, depending on nest activity. In dry and cold conditions, colonies of *A. balzani* can reduce their activities to save energy, due to resource shortages, consequently decreasing the number of trips to the surface (Verza et al., 2007; Caldato et al., 2016). Hence, it could be inferred that in the environment where the colonies were nested, the conditions were favorable for excavation activity, regardless of the period of the year.

In leaf-cutter ants, efficient waste disposal is essential for the health of the colonies, because although the symbiont fungus is used as food, it can contain microorganisms aggressive to the colony (Bot et al., 2001; Hart, 2002). One such microorganism is the *Escovopsis* fungus, a specialized genus of parasitic fungus that is only found in colonies of fungus-growing ants, which is not a danger to the ants themselves, but to their symbiont fungus, reducing its growth and sometimes causing death of the colony (Currie et al., 1999). Although no internal chambers with garbage deposits were found in the present work, the existence of such garbage chambers, as well as waste disposal outside the nests, was reported for *A. balzani* by Caldato (2010).

Table 3
Total and relative number of workers from each of the 3 subcastes of *Acromyrmex balzani* found in the 8 colonies.

Nest	Number of workers			Proportion of subcastes			Total
	Major	Medium	Minor	Major	Medium	Minor	
1	404	68	237	57%	10%	33%	709
2	525	216	284	51%	21%	28%	1025
3	228	184	209	37%	30%	34%	621
4	452	176	283	50%	19%	31%	911
5	322	146	217	47%	21%	32%	685
6	576	297	351	47%	24%	29%	1224
7	948	369	345	57%	22%	21%	1662
8	448	230	373	43%	22%	35%	1051

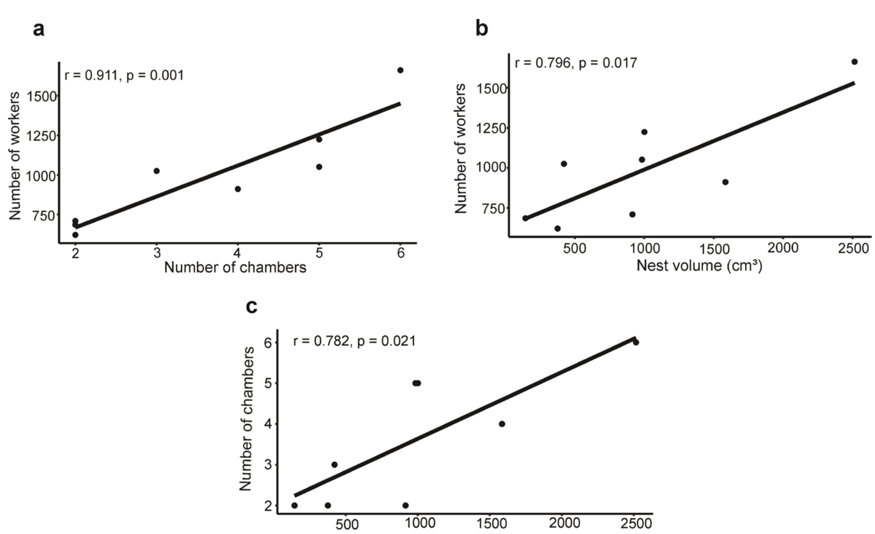

Figure 3 Pearson's Correlation between the number of workers and the number of chambers (a); between the number of workers and the nest volume (b) and between the number of chambers and the nest volume (c), with their respective r and p values.

The main difference in the building pattern of the excavated nests was the presence of two shafts connecting chambers (Figure 2d), unlike the features reported previously for *A. balzani*, where the nests had only one shaft connecting chambers (Pimenta et al., 2007; Verza et al., 2007; Silva et al., 2010; Caldato et al., 2016). The way that these shafts occur can vary intraspecifically (Tschinkel, 2015), but this variation has not been observed previously for nests of *A. balzani*. The increase in connectivity generated by the presence of more than one shaft leading to the entrance chamber could result in an increased flow of information within the colony, consequently increasing the rate of worker recruitment to exploit resources (Pinter-Wollman, 2015).

The number of chambers in the excavated nests ranged from two to six. The maximum number of chambers in colonies of *A. balzani* described previously ranged from five (Pimenta et al., 2007; Verza et al., 2017; Poderoso et al., 2009; Caldato et al., 2016) to fourteen chambers (Silva et al., 2010). This variation in the number of chambers observed here and in the previous studies could be related to specific conditions found in each nesting environment, such as soil physical characteristics (Toffin et al., 2010), climate (Bollazzi et al., 2008), and age of the colony (Van Gils and Vanderwoude, 2012).

Figures 1 and 2 show the aspects of the colonies, in comparison with the descriptions provided in previous studies (Caldato et al., 2016; Ichinose et al., 2007; Pimenta et al., 2007; Poderoso et al., 2009; Silva et al., 2010; Verza et al., 2017). It can be seen that the chamber surfaces were irregularly shaped, and it is possible to assess the volume of each chamber, as well as that of the nest as a whole. The 3D models also show how the chamber appendices were structured and connected. These structures were described in previous studies of the ant species *Ectatomma brunneum* and *Ectatomma vizottoi* (Vieira et al., 2007; Vieira and Antonialli-Junior, 2006), but only descriptions and 2D schemes were provided, which do not allow for such realistic illustration. It is also relatively easy to observe and understand how connections occurred through 2 shafts between chambers 1 and 2 of nest 8 (Figure 2d). Previously, in the work of Guimarães et al. (2018), it was shown that 3D schemes are very useful for detailed elucidation of the ways that the internal and external structures of the nest are organized.

The results obtained here showed that as the number of individuals in the colony increased, the workers increased the number of chambers and, consequently, the nest volume (Figure 3). In addition, the volume of the chambers was closely related to the number of chambers, indicating that nest growth was mainly due to the digging of more standard-sized chambers. Hence, the volume seemed to depend on the total number of ants in the colony, as similarly described for the nests of *Odontomachus brunneus* (Patton) (Cerquera and Tschinkel, 2010), *Formica pallidefulva* (Latreille) (Mikheyev and Tschinkel, 2004), and *Odontomachus chelifer* (Latreille) (Guimarães et al., 2018).

It could be concluded that the nest architecture of *Acromyrmex balzani*, illustrated in this study by means of 3D models, partly followed the pattern already reported in the literature. However, this is the first report of connection between two chambers involving two shafts, as well as the presence of the turret at the entrance to the nest throughout the year.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

All authors contributed to the study conception and design. The nests excavation, data collection and analysis were performed by NRB and VESO. The first draft of the manuscript was written by NRB and WFAntonialli-Junior and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Supplementary material

The following online material is available for this article:
Table S1 - Distance between nests of Acromyrmex balzani in meters.
Supplementary material 2 - Acromyrmex balzani nests in rotation illustrated in a 3D model.
Supplementary material 3 - Computational 3D models of Acromyrmex balzani nests.