



## SHORT NOTE

### Cohabitation Inquiline-host in Termite Nests: Does it Involve Distinct Mechanisms?

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#### Abstract

Nests of the termite *Constrictotermes* sp. can be cohabited by obligatory inquilines *Inquilinitermes* sp. Recent studies have shown that inquilines establish themselves in mature nests, possibly during the nidification transition phase of the colony (e.g., from the epigeal to the arboreal habit). It is believed that cohabitation is maintained through spatial segregation of the cohabitants since the inquilines aggregate in the central nest region. Here, we described the cohabitation between *I. microceus* (Silvestri) and a *Constrictotermes* species in Sergipe, Northeast Brazil. We compared the cohabitation observed in distinct regions considering hypotheses related to the entry and maintenance of the inquiline in the nests. All *Constrictotermes* sp. nests found in this region are epigeal, unlike already reported in other studies. Approximately 45% of the nests analyzed were associated with *I. microcerus*, and cohabitation seemed to be dependent on nest size. The entrance of the inquiline was not related to changes in the habit of nidification from soil to trees, as suggested in other studies. In addition, inquilines were not associated with and segregated into the dark walls in the central part of the nest. The cohabitation observed here could involve mechanisms which are distinct from the mechanisms reported in other regions.

The local coexistence of species intrinsically depends on their adaptations to abiotic conditions, as well as the result of interspecific interactions that can either restrict or facilitate the establishment and maintenance of the species (Valladares et al., 2015). Nests of eusocial insects – physical structures used to accommodate their own colonies – consist of small ecosystems with controlled conditions and/or a stock of resources that are attractive to invasive or opportunistic organisms (Costa-Leonardo, 2002; Jones & Oldroyd, 2006; Menzel & Blüthgen, 2010). On this restricted local scale, long-term close interspecific associations (e.g., inquilinism) typically require adaptations or specific strategies. Relationships of inquilinism in ant (Hymenoptera) nests are usually represented by social parasitism (Suhonen et al., 2019)

and involve the evolution of diverse strategies, such as mimicry (Turillazzi et al., 2000; Sledge et al., 2001), chemical insignificance (Lenoir et al., 2001), inhibition of aggressiveness (Ortolani et al., 2010), and confounding or avoidance (Akino, 2008). However, the evolution of inquilinism in termites (Blattodea) is still poorly understood (*but see* Cristaldo et al., 2012, 2014, 2016a, 2016b; Hugo et al., 2020).

Termite colonies exhibit different defense mechanisms, including the physical barrier of the nest, a morphologically differentiated caste with chemical and/or mechanical defense (Prestwich, 1984), and a worker caste capable of discriminating the chemical profile of individuals and exhibiting aggressiveness against intruders (Queller & Strassmann, 2002). Despite these adaptations, termite nests are frequently



cohabited by a series of other organisms (Cunha & Brandão, 2000). The most intriguing cases are reports of obligatory inquilinism by termites of the genus *Inquilinitermes* in nests of *Constrictotermes* (Mathews, 1977; Redford, 1984), the only ones frequently found in association with these hosts. Species of the genus *Constrictotermes* are restricted to South America (Constantino, 1999; Krishna et al., 2013), and in Brazil, they are found in the Cerrado, Amazon (Constantino, 1999), and Caatinga biomes (Vasconcellos et al., 2007). Colonies of *Inquilinitermes* spp. live strictly within these nests and, up to now, outside foraging has not been observed. According to Barbosa-Silva et al. (2016), *Inquilinitermes* species feed on material excreted by their hosts, which has a dark color composed of humus, mainly lignin and cellulose.

Some studies have attempted to evaluate the factors related to the entry of inquilines into host nests. Rodrigues et al. (2018) suggested that there is a trade-off between defence and reproduction, which makes mature nests less defended and more prone to invasion. In fact, Cristaldo et al. (2012) showed that the probability of finding inquilines increases in nests larger than 13.6 L that have already reached the reproductive phase. Godinho et al. (1989), also observed *Inquilinitermes* spp. only in nests with volume larger than 10 L. Oliveira et al. (2022) cited that these large nests could be associated with more feces and other resources that form the black walls of the nests.

In the Caatinga biome, Vasconcellos et al. (2007) reported that *C. cyphergaster* nests are usually built on trees but also on rock substrates. In the Brazilian Cerrado, the nests have two distinct nidification phases: they are initially constructed in the soil (epigeal phase), with later migration to tree trunks as they mature (arboreal phase) (Mathews, 1977). Some authors have hypothesized that the entry of inquilines may occur precisely during this migration, when nests are more vulnerable (Vasconcellos et al., 2007; Cristaldo et al., 2012).

Other studies have shown that inquiline permanence within nests seems to be related to spatial segregation between inquiline and host colonies. Cunha et al. (2003) showed that inquilines have smaller colonies than their hosts and are restricted to the dark walls, well delimited in the central region of the nest. Studies suggest that segregation could also occur because inquilines appear to repel their hosts or benefit from the perception of their chemical cues. Soldiers of *I. inquilinus* produce chemical substances that are repellent to *C. cavifrons* (Jirošová et al., 2016), whereas the feces of *I. microcerus* appear to be repellent to their host *C. cyphergaster* (Hugo et al., 2020). Cristaldo et al. (2014; 2016) showed that *I. microcerus* recognizes the trail and alarm signals of *C. cyphergaster*, using them for its own benefit to avoid spaces occupied by the host.

Although the host-inquiline system in termites has been considerably studied in recent years (Oliveira et al., 2022), such studies have been restricted to the Cerrado and Caatinga biomes. In the present study, we describe some characteristics

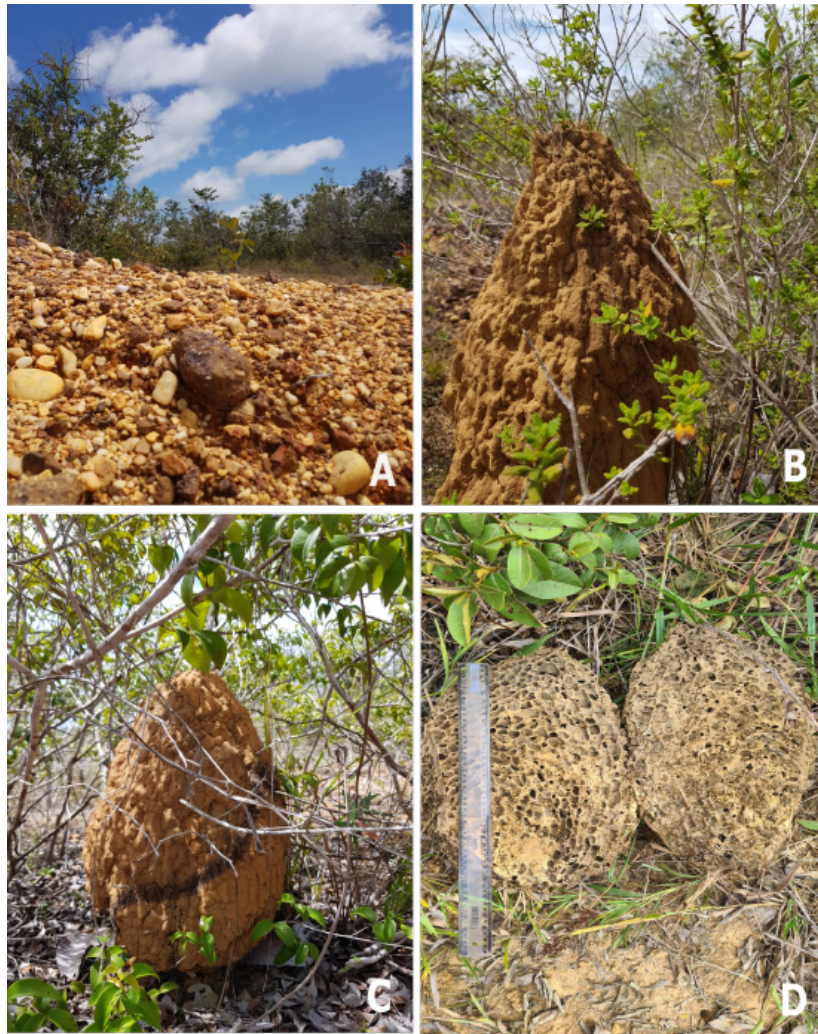
of the cohabitation between *Constrictotermes* sp. and the inquiline *I. microcerus* (Silvestri). Here, we provide a descriptive comparison of cohabitation between species of these genera considering some results and hypotheses already proposed in other studies. Specifically, the following questions were addressed: (i) What is the nesting habit of *Constrictotermes* sp. observed here? (ii) What is the minimum nest size with a greater probability of inquilinism? (iii) Is there evidence of inquiline segregation in the dark and central parts of nests?

The study was conducted in the municipal region of São Cristóvão (11°01'S and 37°12'W), Sergipe, Brazil, in transitional and open areas (Fig 1) surrounded by the Atlantic Forest biome. The altitude of the location is 200 m above sea level, the mean annual rainfall of the region is 1,200 mm, and the mean temperature is 25 °C. According to the Köppen classification, the climate is As' (tropical, with rainy winters and dry summers) (Pidwirny, 2011). The soil at the location of sample collection has a rocky appearance (Fig 1A), classified as *Dystrophic Haplic Plinthosol* (Nascimento & Pedrotti, 2004; Santos et al., 2018), with flat to slightly rolling topography. The local soil presented the following mean values: pH = 5.01, Mg = 0.48 cmolc.dm<sup>-3</sup>, Ca = 0.58 cmolc.dm<sup>-3</sup>, Al = 0.36 cmolc.dm<sup>-3</sup> e P = 0.56 mg.dm<sup>-3</sup>. The vegetation is sparse, with a predominance of herbaceous plants and small trees (Fig 1).

To respond to the above questions, we collected 45 active nests of *Constrictotermes* sp. of different sizes. The nest volume was measured according to the method described by Cristaldo et al. (2012). Subsequently, the nests were broken up to analyze the distribution of the dark walls and were scrutinized in the search for inquilines. The individuals found were collected, stored in 80% alcohol, and identified (Constantino, 1999). The data were analyzed using generalized linear models (GLM) to evaluate the relationship between the presence and absence of inquilines ( $y$  variable) with the nest volume ( $x$  variable) using the binomial error distribution on the R software (R Development Core Team 2015).

Our results showed that 51% of the nests of *Constrictotermes* sp. sampled were cohabited by *I. microcerus*. All nests sampled were on the soil surface, usually adhering to herbaceous plants of the *Lippia* genus (Fig 1B). Other studies have found that *C. cyphergaster* nests are associated with trees, especially larger or adult nests (Cunha, 2000; Lima-Ribeiro et al., 2006; Leite et al., 2011), which probably provides more food and stability to nests (Cunha, 2000; Gonçalves et al., 2006). On the other hand, the areas where we found *Constrictotermes* sp. in Sergipe were characterized by the predominance of herbaceous plants and shrubs with few and small trees (Fig 1). This environmental characteristic, combined with other local conditions (e.g., constancy of temperature and soil type), may be one of the determining factors of the nesting habits of *Constrictotermes* sp.

Interestingly, because the *Constrictotermes* sp. nests studied here occur only in the soil (Fig 1), we can discard

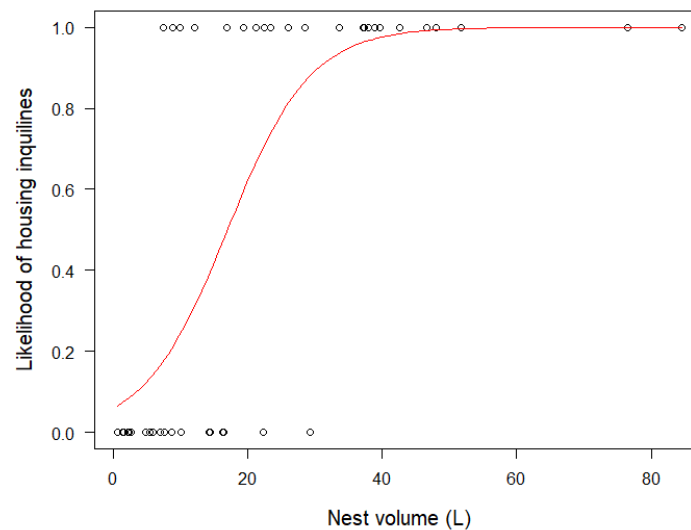


**Fig 1.** Nests of *Constrictotermes* sp. sampled in the present study. **A)** Aspect of the rocky soil and vegetation in the studied region; **B)** Epigeal nest adhered to herbaceous plant of the *Lippia* genus. **C)** Epigeal nest with the external wall of clear color; **D)** Internal aspect the nest without evidence of black and wetter mass concentration. Sergipe, Brazil.

the hypothesis that the entry of inquiline occurs during the transition of the epigeal-arboreal nidification habit, as suggested for the *C. cyphergaster* and *I. microcerus*. The probability of finding inquilines increased with the volume of nests ( $c^2 = 27.401$ ,  $df = 43$ ,  $p < 0.001$ ). Inquilines are more frequently found in nests larger than 20.07 L (Fig 2), a size slightly larger than that reported by Cristaldo et al. (2012) in *C. cyphergaster* nests (= 13.6 L) in the Cerrado biome.

In contrast with observations made in other studies (Mathews, 1977; Cunha et al., 2003) the nests studied here do not present any concentration of black and wetter mass (Fig 1D). Inquilines are usually found in the basal third of the nest. Thus, if there is some type of spatial segregation within the nests studied, this could occur with greater proximity between the galleries in which the inquiline and host are distributed, compared to the nests already observed in other regions. Furthermore, inquilines and hosts were seen moving freely throughout the nest immediately after it broke up. At no time was aggressiveness observed between the species.

Among the different attempts to clarify the cohabitation of nests between *Constrictotermes* and *Inquilinitermes*, some mechanisms have been effectively tested, whereas others have only been hypothesized. Here, we emphasize that even if such hypotheses are valid for explaining cohabitation in other regions, they do not appear to apply to the present system. On the one hand, the cohabitation studied here is also dependent on nest size, which could be related to the maturity of the host colony (Cristaldo et al., 2012), less patrolling of the host nest (DeSouza et al., 2016), or even the greater temporal appearance of the nests. Nevertheless, the entry and maintenance of inquilines are not related to nidification transition or spatial segregation between nest parts. In conclusion, our study emphasizes that the cohabitation observed here could involve mechanisms which are distinct from the mechanisms reported in other regions. Future studies may be directed to evaluate the variations in the intrinsic and environmental mechanisms involved in cohabitation between *Constrictotermes* sp. and *Inquilinitermes microcerus* in different regions.



**Fig. 2.** Effect of the volume of the *Constrictotermes* sp. nest on the presence of *Inquilinitermes microcerus*, São Cristóvão, SE, Brazil, 2019-2021. On the x-axis, 0 and 1 indicate the absence and presence of cohabitation with inquilines, respectively. The probability of finding inquilines follows the logistic equation  $\log(p/q) = -2.75759 + 0.16226v$ , where  $(p/q)$  is the inquiline habitation/non-habitation ratio, and  $v$  is the nest volume (L). The critical volume of the nest above which the probability of inquiline habitation exceeded 50% occurs at 20.07 L.

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### Declarations Ethics

Approval not applicable.

### Conflict of Interest

The authors declare that they have no conflicts of interest.

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