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SHORT NOTE

Nest Biology and Demography of the Fungus-Growing Ant *Cyphomyrmex lectus* Forel (Myrmicinae: Attini) at a Disturbed Area Located in Rio Claro-SP, Brazil

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Abstract

Cyphomyrmex ants are a basal group of small fungus-growers (Myrmicinae:Attini), which differ profoundly from their most studied relatives Atta and Acromyrmex. Our objective was to investigate the nest biology and demography of the fungus-growing ant Cyphomyrmex lectus in a transitional area (savanna-forest) disturbed by an annual fire regime, in Southeast Brazil. The colonies of C. lectus were located close to each other (mean distance between nests, 3.38 ± 2.75 m). Its external nest structure consisted of a single circular nest-entrance hole but without a conspicuous nest mound. Nests were relatively simple consisting of a single well-formed chamber and a single gallery connecting to the nest entrance. No relationships were found between ant number per colony and gallery length, as well as chamber volume. Nonetheless, we detected an effect of the ant number per colony on chamber depth. In this sense, a deeper chamber could warrant a better protection against fire, natural enemies and offer favorable micro-conditions to yeast culture. The colonies of C. lectus are small (a mean of 70 ± 49.4 individuals/colony). Alates, larvae and pupae were found only in one out of eight colonies. A single dealate queen per colony was observed strongly suggesting that this species is monogynous. Further studies could evaluate whether (1) nests density differ before and after fire periods, (2) fire occurrence affects the survival and establishment rate of new colonies, as well as (3) the nest microclimatic conditions necessary to yeast culture.

Among the fungus-growing ant genera included in the tribe Attini (Myrmicinae), *Cyphomyrmex* presents peculiar traits (*e.g.* nest structure and distribution, colony demography, two distinct forms of fungus cultivation: mycelium and "yeast" cultivation) and behavior that have called the attention of researchers (e.g., Murakami & Higashi, 1997; Mueller & Wcislo, 1998; Schultz et al., 2002; Klingenberg et al., 2007; Leal et al., 2011; Mehdiabadi et al., 2012). For example, along the Atlantic seashore (called "restinga"), state of Bahia, Brazil, colonies of *Cyphomyrmex transversus* were observed nesting under and inside dry coconuts (*Cocos nucifera L.*) on the ground (Ramos-Lacau et al., 2012). Coconuts not only may function as a physical barrier against natural predators of *C. transversus*, but also provide thermal insulation. According Ramos-Lacau *et al.* (2012), this behavior associated to the

simultaneous occurrence of multiple dealate queens per colony, conferring to the population a greater capacity for population expansion and maintenance of competitive nest densities to *C. transversus* in harsher environmental conditions, such as those seen in the Restinga of Brazilian coastal zones.

However, little was published in particular on nest ecology and demography of *Cyphomyrmex lectus* (e.g. Kempf, 1964), as well as other aspects on its biology. Our study provides new information about its nesting distribution, its internal and external nest architecture and the population structure of *C. lectus* colonies in a transitional zone between the Brazilian Cerrado and the Atlantic forest affected by annual fire regime. Fire is considered a major but normal disturbance in the Brazilian savanna (Cerrado) (Miranda et al., 2002), and may influence ant ecology (Frizzo et al., 2012)



and ant colony establishment (Araújo et al., 2003). In details, we investigated (1) the occurrence of other Cyphomyrmex species around the nests of C. lectus and (2) described the respective nest internal and external architecture of C. lectus colonies. Secondly, we evaluated whether colony size of C. lectus affects positively its nest internal architecture. This was done correlating ant number per colony with gallery length, nest chamber depth and chamber volume. Therefore, we discuss about what factors can be favoring colony survival of C. lectus in an area on fire annual regimes based on nest structure. Our study was carried out in an area influenced by an annual fire regime and located at the Floresta Estadual Edmundo Navarro de Andrade (FEENA), Campus of UNESP (Universidade Estadual Paulista), Rio Claro, SP, Brazil (22°23'44"S 47°32'03"W). As cited above, FEENA is considered a transitional zone between the Brazilian savanna (cerrado) and the Atlantic forest (Pirani et al., 2005). The vegetation is composed by several grassland species with scattered shrubs, typical vegetation of Brazilian savanna (see Oliveira-Filho & Ratter, 2002; formore information about vegetation physiognomies and woody flora of the Cerrado Bioma). Mean annual precipitation is 1534 mm (dry season extends from April to September and rainy season from October to March) and mean annual temperature is 20.6°C (Pirani et al., 2005).

In June 2005, immediately after a bushfire, Cyphomyrmex colonies were located and two workers per colony were collected to confirm the identification of the species. Vouchers were deposited in the Centro de Estudos de Insetos Sociais (CEIS), UNESP, Rio Claro - SP, and also in the CPDC collection of the Laboratório de Mirmecologia (CEPLAC / CEPEC / SECEN, Ilhéus-BA), Brazil. Eight nests of Cyphomyrmex lectus were thus excavated (five in June and three in October) aiming to study the internal nest architecture in seven of them and all the ants were collected for the demography survey. In each colony studied for nest structure, five biological traits were measured, such as (1) nest distance between colonies, (2) diameter of nest role entrance, (3) principal gallery length (i.e., length from nest role entrance to next superficial gallery), (4) chamber depth, (5) chamber dimensions, *i.e.* maximum width and height in order to calculate chamber volume.

Single t tests were used to verify the differences between colonies regarding diameter of nest-entrance hole, gallery length, chamber depth, chamber volume and number of individuals. Single regressions were performed to evaluate the effect of ant number per colony on gallery length, nest chamber volume and chamber depth. The analyses were developed using the software BioEstat (Ayres et al., 2007). All the average values are informed followed by Standard Deviation (SD).

In the sampled locality, the colonies of *C. lectus* were aggregated and located relatively close to each other, with a mean distance between them of 3.38 ± 2.75 m. The external nest structure of the ant consisted of a single circular nest-

entrance hole with mean diameter of 0.21 ± 0.06 cm but without a well-formed nest mound on the ground surface. The diameter of nest-entrance hole varied significantly between colonies (*t* test = 8.22; p < 0.001). The internal structure was formed by a single oval chamber connected to the outside via a single gallery. A yeast-like fungi forming a spongy mass was observed and disposed at the bottom of the chamber. No dedicated garbage chamber was observed in all studied colonies.

Gallery length varied from 8.5 to 17 cm with mean length of 11 ± 3 cm. Mean chamber depth varied from 2 to 3.8 cm reaching a mean of 3.05 ± 0.6 cm. Chamber volume varied from 8.2 to 54.3 cm³ with average value of 27.5 ± 15.1 cm³. These measured nest attributes varied significantly between colonies (i.e. gallery length: T test = 9.7 and p < 0.001; chamber depth: T test = 13.4 and p < 0.001; and chamber volume: T test = 4.8 and p = 0.003). Two other species of the Cyphomyrmex genus (i.e. C. strigatus and C. rimosus) were simultaneously observed in the area with similar patterns of external nest architecture of C. lectus. For C. lectus colonies, the mean number of workers was of 70 ± 49.4 individuals per colony. No significant effect of total number of ant individuals (*i.e.*, colony size) on both gallery length (F=0.059, g.l. = 1; p = 0.83; Fig 1A) and nest chamber volume (F= 0.22, g.l. = 1; p = 0.65; Fig 1B) was observed but the nest chamber depth increased significantly with increasing the number of ants per colony (F= 7.88, g.l. = 1; P = 0.04; Fig 1C). Ant number per colony explained 54% of the observed variation regarding the chamber depth. As observed to all studied nest attributes, the number of ant individuals also varied significantly between colonies (T test = 4.83 and p = 0.003).

Alates (young reproductive females and males), larvae and pupae were found only in one colony (sampled in October). This colony contained eight females, two males, three larvae and nine pupae. None of the eight investigated colonies contained eggs. A single dealate queen was observed in each eight studied colonies strongly suggesting that this species is monogynous.

The nest external and internal architecture in *Cyphomyrmex* genus is considered geometrically simple (e.g. Leal et al., 2011; Ramos-Lacau et al., 2012) when compared to Atta and Acromyrmex genera (Wirth et al., 2003; Silva Junior et al., 2013). Here, one single circular nest-entrance hole without a well-formed nest mound was observed in C. lectus. Similar structure was also reported to Cyphomyrmex morschi (Klingenberg et al., 2007) and in other Attine genera such as Mycocepurus Forel, Mycetarotes Emery, Trachymyrmex Forel and Sericomyrmex Mayr (Mayhé-Nunes, 1995; Leal et al., 2011). In our study area, two other species of the Cyphomyrmex genus (C. strigatus and C. rimosus) were also observed with similar patterns of external nest architecture as C. lectus. However, other Cyphomyrmex species (e.g. C. transversus and C. cornutus) present a highly distinctive nesting biology compared to C. lectus, C. strigatus and C. rimosus (Adams & Longino, 2007; Ramos-Lacau et al., 2012). For example, in Costa Rica, on Atlantic slopes, Adams

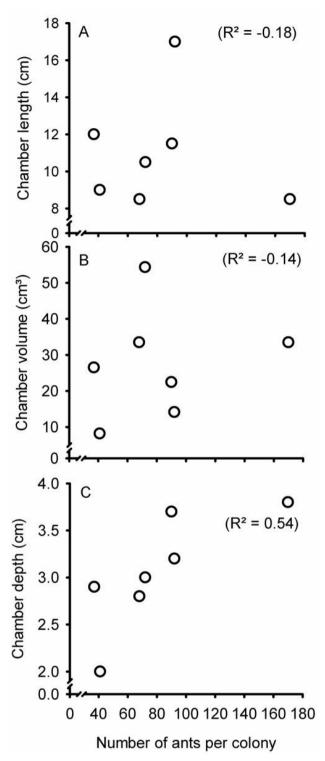


Fig 1. Ant number effect on (A) gallery length, (B) chamber volume and (C) chamber depth in colonies of *C. lectus* observed in Rio Claro-SP, Brazil.

and Longino (2007) described nest of *Cyphomyrmex cornutus* nests formed by large masses of accreted soil suspended in the low arboreal zones. In fact, as referred by Schultz *et al.* (2002) to *Cyphomyrmex longiscapus*, *Cyphomyrmex* genus comprises species that may be considered as a model of organism for the study of behavior, ecology, mating frequency, cultivar specificity, etc.

Contrasting with another Cyphomyrmex species (rimosus group) described by Leal et al. (2011), the fungus garden in C. lectus was well-defined (yeast-like fungi formed a sponge mass) and arranged at the bottom of the chambers. This pattern has been also illustrated for C. transversus (Ramos-Lacau et al., 2012). According to Leal et al. (2011), the fungus garden in Cyphomyrmex colonies was usually characterized by yeast nodules scattered among the superficial soil and leaf litter, in Brazilian savanna (Cerrado). In this biome, the same authors detected three basic types of fungus garden in six Attines genera: (1) pieces of substrate enveloped by yeast (Cyphomyrmex, rimosus group), (2) laminar fungus suspended in the chamber ceiling or plant roots (Mycetarotes, Mycocepurus, and Myrmicocrypta), and (3) amorphous fungus on the floor of the chamber (Sericomyrmex and Trachymyrmex; see also Solomon et al., 2004; Rabeling et al., 2007; Mehdiabadi et al., 2012).

With respect to the demographic observations, the colonies of C. lectus had an average worker number per colony smaller compared to two other Cyphomyrmex species studied, C. cornutus (Adams & Longino, 2007) and C. transversus (Ramos-Lacau et al., 2012). Colonies of C. lectus presented a single dealate queen. The reproductive and alates forms were not common in these colonies during our two sampling periods, since juvenile mates were observed only in a single colony sampled in October. The occurrence of colonies with multiple dealate queens in habitats on fire regimes could provide a significant competitive advantage and facilitates the persistence of colonies. In Attines, studies have demonstrated that colonies with multiple dealate queens may have a higher number of workers, a better developed fungus garden and higher survival rates (e.g., Mintzer, 1987; for Atta texana). Colonies with multiple dealate queens are frequently observed among Attines (Delabie, 1989; Leal et al., 2011; Ramos-Lacau et al., 2012), where the colonies can be both haplometrotic and pleometrotic (Fernández-Marín et al., 2004). Regarding the absence of alates in our study, it is possible that this occurred simply because the majority of colonies (five) were sampled in June, during the middle of the dry and cold season. This month may be unfavorable to the alate production.

In our study, although there is no significant relationship between colony size with some nest attributes, such as gallery length and chamber volume, we detected a positive effect of the colony size over chamber depth Possibly, colonies with deeper chambers could be better protected against fire and natural enemies, and offer more favorable microclimatic conditions to yeast culture.

Further studies on *Cyphomyrmex* species, especially in Brazilian savanna, could evaluate whether (1) the nests density differ before and after fire periods, (2) fire affect survival and establishment rate of new colonies and, finally, (3) fire affect the microclimatic conditions necessary to yeast culture. Indeed, the vegetation cover reduction by fire can promote ant species composition by altering the species dominance status (see Vasconcelos et al., 2008), given that the soil surface becomes exposed to the wind and solar radiation incidence (Miranda et al., 2002).

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