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## RESEARCH ARTICLE - ANTS

## The role of Senescent Stem-Galls over Arboreal Ant Communities Structure in *Eremanthus erythropappus* (DC.) MacLeish (Asteraceae) Trees

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### Abstract

The extensive occupation of canopy trees by ants can be attributed to many factors, such as the presence of structures that provide food and shelter. Structures induced by other insects in host plants, like senescent galls, can provide shelter and a nesting place for many species of ants. The main objectives of this work were: (1) to describe the ant communities found in canopies of candeia trees (*Eremanthus erythropappus*), including the species which use galls as nesting sites; (2) verify the role of galls in determining the structure and composition of the ant communities and (3) to evaluate whether the size and shape of galls are important to the choice of nesting sites by ants. Specifically, the following questions were investigated: 1 – Are larger galls more frequently occupied by ants than smaller galls? 2 – Does gall shape (globular and fusiform) influence occupation? 3 – Which species of ants are present in the canopies of candeias and which are occupying galls? Senescent galls were collected in locations in the southern portion of the Espinhaço Mountain Range, state of Minas Gerais, southeastern Brazil. In total, 3,195 galls were collected and 19 ant species were recorded. Only 176 galls (5.5%) had been occupied by ants, and these were represented by 11 species. The most frequent species found occupying galls were *Myrmelachista nodigera*, with 48 colonies; *Nesomyrmex spininodis*, with 37 colonies; and *Crematogaster* complex *crinosa* sp. 1, with 29 colonies. The ants occupied galls with greater volume and diameter. Even considering the low occupation frequency, senescent galls in *E. erythropappus* are used by ants, either as outstations or satellite nests of polydomic colonies, and may be important in determining ant species composition in canopy trees.

### Introduction

Ants (Hymenoptera: Formicidae) are an ecologically important group of arthropods because of their considerable species richness and biomass, and the important roles they play in the functioning of ecosystems (Hölldobler & Wilson, 1990; Lach et al., 2010). They are very conspicuous organisms in the arboreal environment due to the diversity of renewable food sources available to them, either produced by host plants, such as extrafloral nectaries, or by herbivorous insects

associated with plants, such as hemipteran exudates (Oliveira & Freitas, 2004; Del-Claro, 2004; Fagundes et al., 2012). Other important resources provided by plants to ants are nesting sites and shelter, such as domatias or cavities produced by the activity of endophytic insects (Oliveira & Freitas, 2004; Schoederer et al., 2010; Nascimento et al., 2012; Almeida et al., 2014). Therefore, the supply of food resources and places for shelter and nesting are both determining factors for the establishment and survival of arboreal ants on trees (Oliveira & Freitas, 2004).



Gall inducing insects are among the most sophisticated and specialized organisms that exist (Shorthouse et al., 2005). Galls are vegetable tumors induced by insects or other organisms; they are free of chemical compounds, rich in nutrients and provide a location for the larvae of gallers to develop (Mani 1964; Price et al., 1987; Dreger-Jauffret & Shorthouse, 1992; Raman et al., 2005; Fernandes & Santos 2014). Stem-galls persist on the plant and, after being abandoned by the inductor (= senescent galls), can be colonized by different organisms such as spiders, Coleoptera, Lepidoptera and, mainly, ants (Longino & Wheeler, 1987, 1988; Craig et al., 1991; Almeida et al., 2014). Due to the capacity of larvae to manipulate and modify their host plants, creating “new habitats” for other organisms, gall insects can be considered ecosystem engineers (Jones et al., 1994, 1997; Wright & Jones 2006). These “new habitats” are ecologically important for increasing both the species richness and abundance of local communities (Cornelissen et al., 2016).

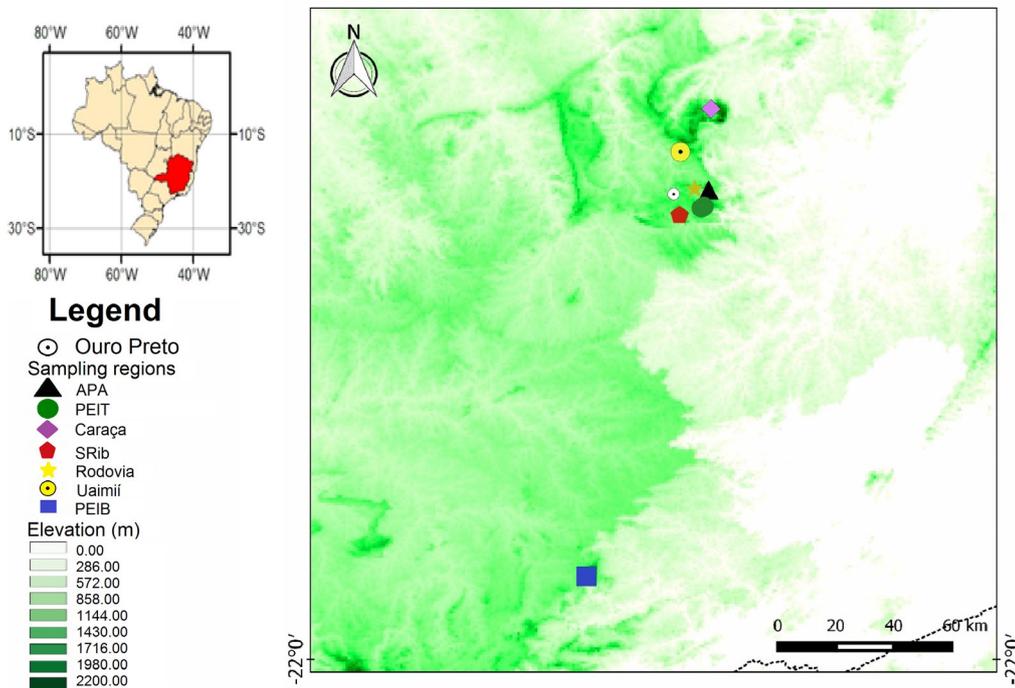
*Eremanthus erythropappus* (DC.) MacLeisch (Asteraceae), also known as “candeia”, is a pioneering tree species that forms dense, monodominant patches in areas in early stages of natural succession (Pedralli et al., 2000). It is native to South America, and is found mainly in Brazil, Argentina and Paraguay (Pereira et al., 2014). In Brazil, it is commonly found in the South and Southeast, as well as in the states of Bahia (Northeast) and Goiás (Central Brazil) (Pedralli et al., 1997; Lorenzi, 2009). On the Espinhaço Mountain Range, which extends throughout the states of Minas Gerais and Bahia, *E. erythropappus* is distributed across the entire altitudinal gradient, occupying high regions, rocky outcrops and riparian forests (Carneiro et al., 2009b). Due to its wide distribution in South America, and its pioneering character, candeia can be a key species in maintaining insect biodiversity. So far, six morphotypes of gall inducing insects have been described on candeia (Carneiro et al., 2009b).

Recently, Almeida et al. (2014) found eight ant species occupying senescent galls of candeia trees and suggested that the presence of senescent galls, an abundant resource, can increase the diversity of ant assemblages in the canopy of candeia trees. Therefore, this work aims at expanding the knowledge about ants associated with candeia galls on a regional scale, by describing the ant communities found in the canopies of candeia trees, including the species that use galls as nesting sites. Furthermore, this work also aims to verify the role of galls in determining the structure and composition of the ant communities and to evaluate whether the size and shape of galls are important to the choice of nesting sites by ants. Specifically, the following questions were investigated: 1 – Are larger galls more frequently occupied by ants than smaller galls? 2 – Does gall shape (globe and fusiform) influence occupation? 3 – Which species of ants are present in the canopy of candeias and which are occupying galls?

## Material and Methods

The study was conducted in seven regions of the state of Minas Gerais (Fig 1), which all possessed rupestrian field vegetation (vegetation in quartzite or ferruginous rocky outcrops) and a tropical montane climate. Six of the regions are near the municipality of Ouro Preto in the southern portion of the Espinhaço Range: 1) Itacolomi State Park (PEIT), 2) Environmental Protection Area of Andorinhas Waterfall (APA); 3) Uaimií State Forest (Uaimií), 4) populations along MG 356 Inconfidentes Highway (Rodovia); 5) Serra do Ribeiro (SRib); and 6) Private Natural Heritage Reserve - Caraça Sanctuary (Caraça). PEIT (20°22'30" S, 43°32'30" W) encompasses approximately 7,500 ha, with its highest point being the Itacolomi Peak at 1,772 m. Some mountain rainforest patches and Araucária trees complement the landscape (Araújo et al., 2003; IEF 2015). APA (20°22'34" S, 43°29'27" W) has an area of approximately 18,700 ha with an altitude of 1,300 m, and is located on the hills of Ouro Preto encompassing the headwaters of Rio das Velhas, a very important river in the state of Minas Gerais. Uaimií (20°14'43" S, 43°35'15.9" W) was established in 2003 in the area of APA, and has an area of 4,398 ha and elevations reaching to about 1,800 m (Rezende et al., 2011). The location called Rodovia (20°22'27" S, 43°32'22" W) is comprised of fire-damaged mesophilic vegetation, currently undergoing rehabilitation (Pedralli et al., 1997, 2000). SRib (20°27'55.4" S, 43°35'59" W) is a mountain formation located inside the Itatiaia State Natural Monument, a conservation unit with an area of 3,200 ha and an altitude of 1,568 m (IEF, 2015). Caraça (20°05'53.4" S, 43°29'17.7" W) is a private nature reserve with 11,233 ha of area at 1,240 m of altitude. In addition, samples were also collected in Ibitipoca State Park (PEIB) located in the Mantiqueira Mountain Range, in Lima Duarte, MG. PEIB (21°42'47.2" S, 43°54'07.7" W), with an area of 1,923 ha and elevations reaching 1,784 m. Seasonal montane forest of semi-deciduous trees and rupestrian fields cover most of the area of PEIB (Neto et al., 2007).

Ants and senescent galls were sampled during the period of January 2014 to March 2015. The study included seven regions throughout the host plant range to maximize sampling of ant species associated with *E. erythropappus*. Each plant and the population of trees were sampled once during the study period. In each region, two sites, separated by at least 1 km, were selected, and 15 individuals of candeia were randomly sampled from each, for a total of 30 individuals for each region and 210 for the entire study. Only plants no taller than three meters were sampled to facilitate canopy access. In addition to galls, foraging ants in the canopy were sampled with an entomological blow-type aspirator (Gibb & Oseto, 2006) during 15 minutes of inspection per plant. All senescent stem galls were collected and immediately deposited in a plastic bag specific for each plant. Once in the laboratory, the galls were measured using a digital caliper



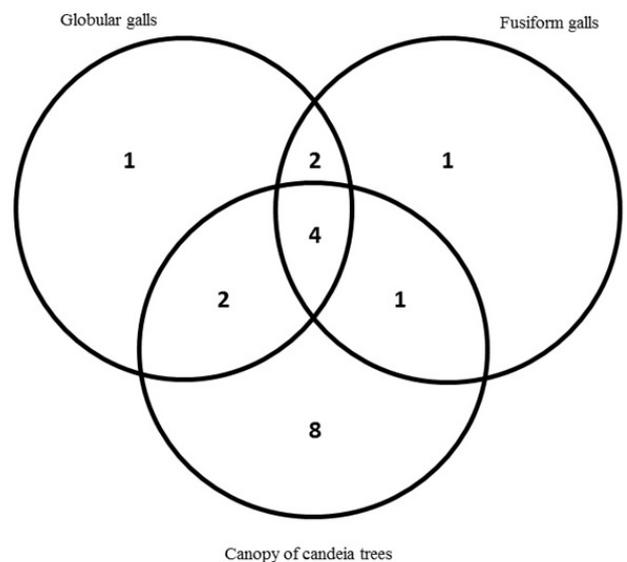
**Fig 1.** Map showing sampling regions in the state of Minas Gerais, Brazil.

(error:  $\pm 0.01\text{mm}$ ) to determine largest diameter, smallest diameter and height, and to calculate the volume of globular ( $V = 4/3 \pi r^3$ ) and fusiform ( $V = r^2 h \pi$ ) galls according to the method described by Almeida et al. (2014). In addition, all galls were opened to verify if ant colonies were present or not. Ants were identified as specifically as possible (down to genus or species) by RMF, who consulted as references Kempf (1959), Longino (2003) and Nakano et al. (2013). Specimens were subsequently deposited in the Entomological Collection Padre Jesus Santiago Moure (DZUP) at the Universidade Federal do Paraná, Curitiba, Brazil. Statistical analyses were performed using the statistical package R (R Development Core Team, 2012). To answer if the occupation of galls by ants increases with gall size, an adjusted a logistic regression model was used in which the presence / absence of ants was the response variable (binary) and gall size the predictor variable. However, residual analyses did not support the assumptions of the logistic regression model. Therefore, the non-parametric Wilcoxon test was used to determine preferences regarding size and shape, where the explanatory variables were occupation categories (unoccupied and occupied), and the responses variables were gall diameter and volume (Fernandes et al., 1988; Sprent & Smeeton, 2007). Non-parametric alternative methods for the analysis of two samples were used because the residual analyses did not support the parametric model assumptions (Sprent & Smeeton, 2007).

**Results**

In total, 3,195 senescent stem-galls were collected of two morphological types: 1,872 (58.6%) fusiform galls and 1,323 (41.4%) globular galls. Only 176 (5.5%) of the

senescent stem-galls had been occupied, of which 113 (64%) were fusiform and 63 (36%) globular. Occupancy rates were low (occupied galls/total galls), not exceeding 5.5% of available galls and in each of the populations: {PEIT [1= 5/238 (2,1%) and 2=17/182 (9,3%); APA [1= 17/367 (4,6%) and 2= 14/339 (4,1%); Caraca [1= 20/255 (7,8%) and 2= 7/192 (3,6%); PEIB [1= 4/110 (3,6%) and 2= 1/102 (0,98%); Uaimii [1= 14/231 (6,1%) and 2= 7/289 (2,4%); Rodovia [1= 17/197 (8,6%) and 2= 31/446 (6,9%); SRib [1= 11/78 (14,1%) and 2= 11/169 (6,5%)]. Nineteen ant species belonging to eight genera and three subfamilies were found (Fig 2). Myrmicinae was the richest subfamily, with nine species, followed by Formicinae, with seven species (Table 1). We found 11 ant

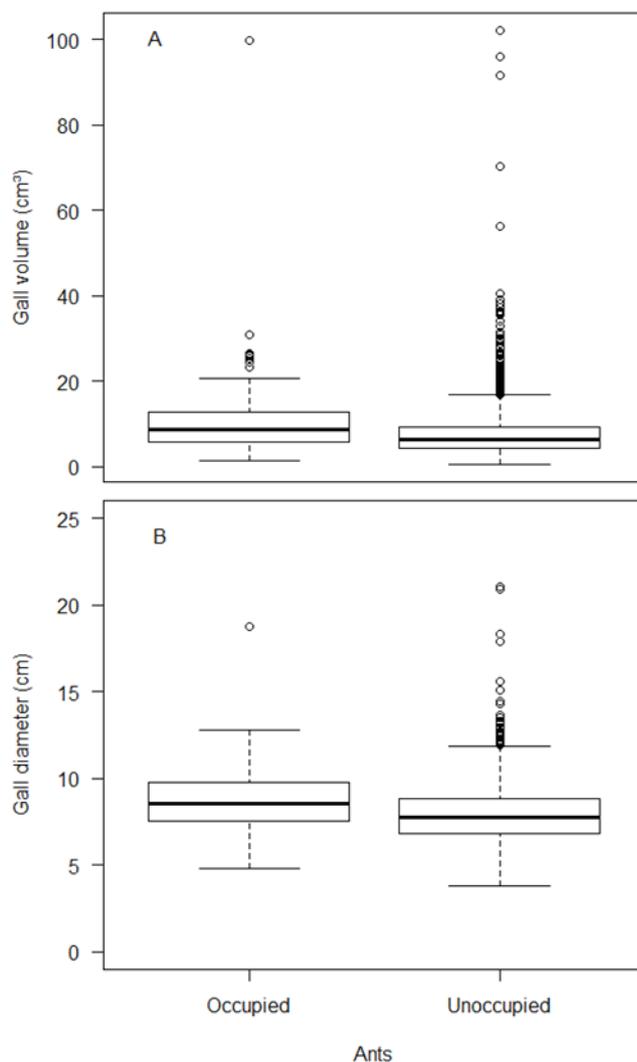


**Fig 2.** Venn diagram showing number of ant species found in galls of the two morphotypes and in canopies of candeia trees.

species occupying senescent stem-galls in candeia trees. Among these, two species were found only in fusiform galls, three only in globular galls and six in both morphological types (Table 1). Myrmicinae was the most common subfamily in galls (7), followed by Formicinae (3) and Pseudomyrmecinae (1) (Table 1). The species with the highest number of colonies present in galls was *Myrmelachista nodigera* Mayr, 1887 (Formicinae), with 43 colonies; *Nesomyrmex spininodis* (Mayr, 1887) (Myrmicinae), with 37; and *Creinatogaster complex crinosa* sp. 1 (Myrmicinae) with 29 colonies (Table 1).

**Table 1.** Ant species found in senescent stem-galls and canopy of *E. erythropappus* from the study sites. “X” means presence and “-” means absence.

Taxa	Number of colonies in galls	Galls morphology		Canopy
		Globular	Fusiform	
<b>Myrmicinae</b>				
<i>Creinatogaster complex crinosa</i> sp. 1	29	X	X	X
<i>Creinatogaster complex crinosa</i> sp. 2	2	X	-	-
<i>Nesomyrmex spininodis</i> (Mayr, 1886)	37	X	X	X
<i>Nesomyrmex</i> aff. <i>echinatinodis</i> Forel, 1886	15	X	X	X
<i>Solenopsis</i> sp.	4	-	X	-
<i>Cephalotes pusillus</i> (Klug, 1824)	-	-	-	X
<i>Cephalotes eduarduli</i> (Forel, 1921)	-	-	-	X
<i>Cephalotes maculatus</i> (Smith, 1876)	1	X	-	X
<i>Procryptocerus goeldii</i> Forel, 1899	1	X	-	X
<b>Formicinae</b>				
<i>Camponotus crassus</i> Mayr, 1862	-	-	-	X
<i>Camponotus rufipes</i> (Fabricius, 1775)	-	-	-	X
<i>Camponotus blandus</i> (Smith, 1858)	-	-	-	X
<i>Camponotus novogranadensis</i> Mayr, 1870	-	-	-	X
<i>Brachymyrmex</i> sp.	9	X	X	X
<i>Myrmelachista nodigera</i> Mayr, 1887	43	X	X	-
<i>Myrmelachista</i> sp.	18	X	X	X
<b>Pseudomyrmecinae</b>				
<i>Pseudomyrmex gracillis</i> (Fabricius, 1804)	-	-	-	X
<i>Pseudomyrmex</i> gr. <i>pallidus</i> sp.	-	-	-	X
<i>Pseudomyrmex</i> sp. 2	1	-	X	X



**Fig 3.** Size (volume and diameter) of senescent stem galls on candeia trees *Eremanthus erythropappus* (DC.) MacLeisch (Asteraceae). The volume ( $w = 342.370$ ;  $p = 0.001$ ; A) and the diameter ( $w = 336.890$ ,  $p < 0.001$ ; B) of occupied galls were higher than those of galls not occupied by ants.

In general, the occupied galls had greater volume ( $w = 342.370$ ;  $p = 0.001$ ;  $n = 3195$ ) (Fig 3A) and mean diameter ( $w = 336.890$ ;  $p < 0.001$ ;  $n = 3195$ ) (Fig 3B) than unoccupied galls. When both gall morphotypes were considered separately, the same pattern was found. Occupied fusiform galls had greater volume ( $w = 124.240$ ;  $p < 0.001$ ;  $n = 1872$ ) and diameter ( $w = 119.160$ ;  $p < 0.001$ ;  $n = 1872$ ). Likewise, the globular galls with higher volume ( $w = 57.604$ ;  $p < 0.001$ ;  $n = 1323$ ) and higher diameter ( $w = 57.236$ ;  $p = 0.001$ ;  $n = 1323$ ) were also more occupied by ants.

## Discussion

Except for the genus *Solenopsis* and some species of *Camponotus*, all ant species found in the present study represent typically arboreal genera (Brown, 2000). In fact, *Camponotus rufipes* and *C. crassus* are commonly reported as foraging on plants (Espírito Santo et al., 2011; Fagundes et al., 2013).

Four ant genera among those occupying galls, represented by four morphospecies (*Brachymyrmex* sp., *Cephalotes maculatus*, *Pseudomyrmex* gr. *pallidus* sp. and *Solenopsis* sp.), were not found in candeia galls by Almeida et al., (2014). So, the present work records, for the first time, these four morphospecies occupying galls on candeia trees, thus increasing the list presented in Almeida et al. (2014), from eight to twelve species. This increase is likely due to more sampling areas and to the greater sampling effort of the present work; n= 3195 galls in 210 plants in this study, as compared to n= 227 galls in 100 candeia trees in Almeida et al. (2014). However, *Crematogaster goeldii* (Forel, 1903), which was found by Almeida et al. (2014), was not recorded in the present study, and seems to be uncommon in candeia canopies (Almeida et al., 2014).

Despite the high number of species found in candeia galls in the present study, the occupancy rate was very low. In secondary vegetation, monodominant populations (as in the candeias studied here and in areas that have suffered some kind of environmental impact), ant diversity is usually low, as compared to primary and heterogeneous forests (Ribas et al., 2003; Klimes et al., 2012; Floren et al., 2014). Therefore, the low local richness of ants associated with candeia is likely a reflection of low structural complexity and environmental heterogeneity in the rupestrian fields of the study sites (Klimes et al., 2012; Stein et al., 2014).

This work supports the previous results of Almeida et al. (2014), which showed that larger galls have higher rates of occupation by ants. The preference for occupying larger galls has not only been related to ant body size and colony size, but also to the skill they have in modifying the habitat to accommodate the entire population of the colony as safely as possible (Fernandes et al., 1988; Araújo et al., 1995; Almeida et al., 2014). Moreover, the oldest and largest galls are naturally more lignified and, therefore, more resistant. They can offer greater protection to the colony from variation in the climate and other common stresses of the arboreal environment, such as strong winds and intense insolation, which can result in desiccation (Yanoviak & Kaspari, 2000).

During the opening of galls, we noticed that some galls contained only adults, or adults and immatures, or just the founding queen. This indicates the possible formation of new nests or the expansion of colonies by polydomy, a very common phenomenon among ants (Debout et al., 2007). Polydomy can be a strategy adopted by ants in the face of low environmental heterogeneity (Pfeiffer & Linsenmair, 1998) and low availability of nesting sites (Cereto et al., 2011). These characteristics are very common in monodominant candeia patches (Almeida et al., 2014), which are often found in highly impacted localities and areas affected by fire (Pedralli, 2000). Another similar strategy – outstations – consists of ants establishing nests on pre-existing structures in the environment (such as senescent galls or any physical structure) and using them as a rest place and/or shelter during

territory patrols (Anderson & McShea, 2001). Furthermore, these outstations enable ants to quickly respond to invasions, making them an important defense strategy to dominate their territories (Anderson & McShea, 2001; Lanan et al., 2011). Both polydomy and outstations are strategies that seem to be characteristic of the ant species found in this work, since we found multiple occurrences of the same species occupying different galls on the same plant, although no aggression tests were performed to determine the territoriality of the ants involved.

Even with a low frequency of gall occupation, not exceeding 5.5% of the total available, the galls sheltered more than half (11 of 19 species) of ant species collected in association with candeia. Thus, in general manner, the role of senescent stem galls is relevant for ant occurrence and coexistence, and for greater species diversity of ants associated with candeia trees.

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