

## Natural History and Ecological Correlates of Fungus-Growing Ants (Formicidae: Attini) in the Neotropical Cerrado Savanna

INARA R. LEAL,<sup>1,2</sup> PAULO S. D. SILVA,<sup>3</sup> AND PAULO S. OLIVEIRA<sup>4</sup>

Ann. Entomol. Soc. Am. 104(5): 901–908 (2011); DOI: 10.1603/AN11067

**ABSTRACT** Fungus-growing ants (Formicidae: Attini) comprise a diverse and ecologically important group in Neotropical habitats. Compared with leaf-cutters, however, relatively little is known about the biology of less conspicuous attine species. Here, we compare nest size and structure, colony size and demographic composition, and worker size and polymorphism among the genera *Cyphomyrmex*, *Mycetarotes*, *Mycocarpus*, *Myrmicocrypta*, *Sericomyrmex*, and *Trachymyrmex*. In total, 25 ant colonies (one species per genus) were investigated at one site in the Brazilian savanna. Results indicate a consistent variation in nest size and structural complexity (architecture), colony and worker size, and a tendency to polymorphism among the studied genera. In addition, nest mound volume was found to be a good predictor of both worker number and volume of the fungus garden. Based on morphometric analyses, *Sericomyrmex* and *Trachymyrmex* together formed a distinct group from the other genera. The transition from the ancestral agricultural system toward the derived leaf-cutting habit also is followed by remarkable changes in nest size and architecture, colony size, and worker size and polymorphism. Our results support other recent studies that consider *Sericomyrmex* and *Trachymyrmex* as possessing transitional habits, distinct both from species that cultivate fungus by using mostly nonplant items (insect feces and corpses) as well as from typical leaf-cutters *Atta* and *Acromyrmex*. This is the first study to detect correlations of nest traits with worker number and size of fungus garden in the less conspicuous attines. Results highlight the importance of combining data on natural history and morphometry to understand the evolutionary history of fungus-growing ants.

**KEY WORDS** attine ants, colony size, demographic attributes, nest architecture, worker morphometry

The ant tribe Attini (Myrmicinae) includes 15 genera and >230 species occurring in the tropical and subtropical Americas (Mehdiabadi and Schultz 2010). These ants are known as fungus growers because they maintain an obligate mutualism with fungi cultured inside their nests, and which is the only food source for the larvae and an important resource for the adult ants as well (Weber 1972). A wide variety of material can be used as substrate for fungus culturing (Hölldobler and Wilson 1990, Rico-Gray and Oliveira 2007), and this variation can be used to categorize the Attini into two groups based on their agricultural habits. The so-called lower attines include species that collect mainly flowers, fruits, insect corpses, and feces fallen in the vicinity of their nests (Leal and Oliveira 2000, Mehdiabadi and Schultz 2010). The higher attines include nonleaf-cutting species (genera *Trachy-*

*myrmex* and *Sericomyrmex*) that collect mostly fallen leaflets, fruit, and flowers, as well as the typical leaf-cutters that collect fresh leaves from shrubs and trees (genera *Atta* and *Acromyrmex*) (see Schultz and Brady 2008, Mehdiabadi and Schultz 2010).

Leaf-cutting ants are very well known due to their remarkable effects on vegetation as herbivores. For example, they can 1) remove up to 15% of the standing leaf crop (Wirth et al. 2003, Urbas et al. 2007) and up to 50% of the plant species in their foraging territory each year (Vasconcelos and Fowler 1990); 2) reduce the vegetation cover by up to 18% and increase light availability within foraging areas (Wirth et al. 2003); and 3) secondarily disperse seeds of forest and savanna plants (Leal and Oliveira 1998, Silva et al. 2007, Christianini and Oliveira 2009). Moreover, leaf-cutters are considered important ecosystem engineers because their huge nests can significantly alter soil attributes (Sternberg et al. 2007) and light regimes (Farji-Brener and Illes 2000), which in turn may influence forest structure, composition, and dynamics (Corrêa et al. 2010), even after 15 yr of colony death or nest abandonment (Bieber et al. 2010).

Although nonleaf-cutters comprise a diverse and abundant group of fungus-growing ants, only more recently the general biology of these less conspicuous

<sup>1</sup> Programa de Pós-Graduação em Ecologia, Universidade Estadual de Campinas, C.P. 6109, 13083-862 Campinas SP, Brazil.

<sup>2</sup> Current affiliation: Departamento de Botânica, Universidade Federal de Pernambuco, 50670-901, Recife, PE, Brazil.

<sup>3</sup> Laboratório de Biossistemática Animal, Universidade Estadual do Sudoeste da Bahia (UESB), BR 415, Km 03, s/nº, 45700-000 Itapetinga BA, Brazil.

<sup>4</sup> Corresponding author: Departamento de Biologia Animal, Universidade Estadual de Campinas, C.P. 6109, 13083-862 Campinas SP, Brazil (e-mail: pso@unicamp.br).

attine species have been investigated in greater detail (Fernández-Marín et al. 2004, Pitts-Singer and Espelie 2007, Adams and Longino 2007, Diehl-Fleig and Diehl 2007, Klingenberg and Brandão 2009, Solomon et al. 2011). Lack of field data are probably due to their small colonies and the small size and discrete habit of foragers, which make them less noticeable in nature (Weber 1972, Hölldobler and Wilson 2011). Perhaps most importantly, because nonleaf-cutters use mostly fallen plant material and feces as substrate for fungus-culturing, their impact on vegetation and economic importance as crop pests is irrelevant compared with leaf-cutting *Atta* and *Acromyrmex* (Vander Meer et al. 1990, Wirth et al. 2003, and references therein).

Several biological traits such as colony size, nest structure, worker size, and polymorphism may influence the foraging ecology of nonleaf-cutting attines and their relationship with co-occurring plant and animal species (Hölldobler and Wilson 1990; Leal and Oliveira 1998, 2000). In addition, studying these traits may clarify understand the appearance of some relevant evolutionary steps within the Attini, such as the gradual increase in colony size, nest size and complexity, worker size and polymorphism, and the use of fresh material, especially leaves (Hölldobler and Wilson 1990, 2011). Indeed, Leal and Oliveira (2000) documented that the substrate used for fungus-culturing by nonleaf-cutting attines varies from mainly insect feces and corpses in genera such as *Cyphomyrmex*, *Mycetarotes*, and *Mycocepurus*, to mostly fallen soft leaflets in *Sericomyrmex* and *Trachymyrmex* (also see Schultz and Brady 2008, Mehdiabadi and Schultz 2010). Although the types of fungal substrate used by different genera suggest a trend in agricultural habits, other biological features of the Attini remain poorly documented.

In the current study, we compare several biological traits of six genera of nonleaf-cutting Attini (*Cyphomyrmex*, *Mycetarotes*, *Mycocepurus*, *Myrmicocrypta*, *Sericomyrmex*, and *Trachymyrmex*) in the cerrado savanna of Brazil. We used the most abundant species in each genus to assess nest size (volume of nest mound) and structure (chamber depth and volume), size of fungus garden, and colony size and demography (number of immatures, workers, queens, and alates). In addition, we investigated whether the external structure of the nests is a good predictor of worker number and volume of the fungus garden. Finally, we evaluated worker size and polymorphism in excavated colonies. We discuss the evolutionary syndromes within the Attini based on the documented biological traits.

#### Materials and Methods

The study was carried out in the cerrado reserve of the Estação Experimental de Mogi-Guaçu (22° 18' S, 47° 13' W), located in São Paulo state, southeastern Brazil. The Brazilian cerrados cover an area of 2 million km<sup>2</sup>, and within their distribution they present several intergrading physiognomic forms ranging from closed forest to open grassland with scattered shrubs,

and occasional forest strips along streams known as gallery forests (Oliveira-Filho and Ratter 2002). The climate in the study region is characterized by a cold-dry season occurring from April to September and a warm-rainy season from October to March. The maximum monthly rainfall is 235 mm in December and the mean temperature reaches a minimum of 8.7°C in July and a maximum of 30.4°C in February (De Vuono et al. 1986). The reserve presents floodplain soils and yellow-red latosols with medium texture to clay, and altitude varies from 585 to 635 m (Mantovani 1983).

Five trails (totaling 7 km) were selected in the cerrado sensu stricto (i.e., dense scrub of shrubs and trees) as well as in the transition between cerrado and gallery forest (Oliveira-Filho and Ratter 2002). Baits made of fruits (orange) and dry oats were used to attract attine ants and locate their nests. External and internal nest traits and demographic data were recorded for 25 ant colonies during March and April 1995 (end of warm-rainy season). We excavated at least three colonies of the most abundant species in each genus as follows: *Cyphomyrmex* gr. *rimosus* sp. 2, *Mycetarotes parallelus* (Emery), *Sericomyrmex* sp., *Trachymyrmex* sp. 4 (three colonies each), *Myrmicocrypta* sp. (five colonies), and *Mycocepurus goeldi* Forel (eight colonies). Except for *Mycetarotes* colonies, which were collected in gallery forest, all other ants were collected in cerrado sensu stricto. The material collected as fungal substrate by these colonies has previously been described by Leal and Oliveira (2000), and included leaves, flowers, fruit, seeds, wood, mosses, lichens, insect feces, and corpses (also see Fernández-Marín et al. 2004; Schultz and Brady 2008; Mehdiabadi and Schultz 2010; Hölldobler and Wilson 1990, 2011, and references therein). Species names and morphospecies designations used here follow Leal and Oliveira (2000); hereafter, they will be referred to by their generic names only. Ant voucher specimens are deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Nest size was evaluated based on the volume of the mound (i.e., width, length and height of soil deposition around the main nest entrance), number of chambers, as well as the depth and volume of each chamber. Each nest excavation began by digging a circle around the main nest entrance, so as to form a cylinder (1 m in diameter; 50 cm in depth) that included the whole nest. We then progressively removed thin soil layers from the outer surface of the cylinder toward its center, and described the nest chambers as they appeared. For each chamber, we used a caliper to measure its width, length, and height to calculate the volume to the nearest 1 cm<sup>3</sup>. Upon finding the chamber containing the fungus garden, we carefully removed the fungus with the ants and measured the width, length, and height of the mycelia mass to obtain its volume. Because nest mounds, chambers, and fungus gardens frequently presented an irregular form, their volumes were calculated assuming a rectangular pattern (i.e., width by length by height; nearest 1 cm<sup>3</sup>).

In the laboratory we estimated colony size and demographic composition by counting the number of

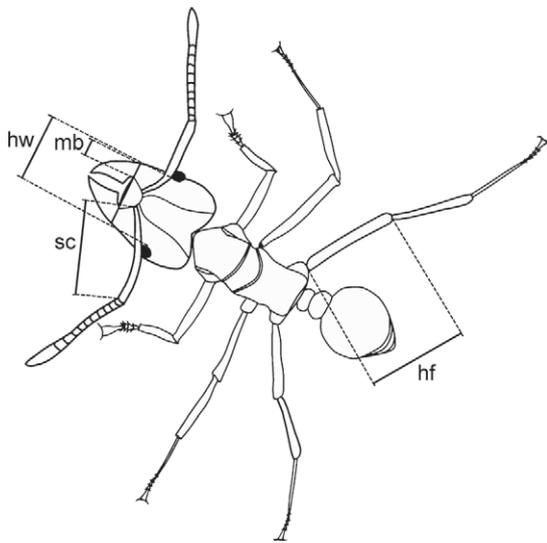


Fig. 1. Diagram showing the morphometric measurements taken from workers of *Cyphomyrmex*, *Mycetarotes*, *Mycocepurus*, *Myrmicocrypta*, *Sericomyrmex*, and *Trachymyrmex* in a cerrado savanna in southeastern Brazil. Data were taken from 120 ants of the largest excavated colony in each genus. hw, head width between eyes; hf, length of hind femur; mb, width of mandible basal margin; sc, length of antennal scape.

eggs, larvae, pupae, workers, queens and alates (young reproductive females and males). Worker size and polymorphism was assessed by morphometric analyses of each of the largest excavated colony in each genus. For each colony we randomly selected 120 ants to measure the body dry mass to the nearest 0.01 mg by using a CAHN H51 AR balance. The following measurements were taken of individual workers (to the nearest 0.01 mm): 1) head width between eyes, 2) width of mandible basal margin, 3) length of hind femur, and 4) length of antennal scape (Fig. 1).

One-way analyses of variance followed by Fisher post hoc comparisons were used to detect differences between genera regarding 1) volume of nest mound, 2) chamber depth and volume, 3) volume of fungus

garden, 4) number of workers, and 5) ant morphometry. Pearson correlations were used to evaluate the relationship between volume of nest mound and number of workers, and volume of nest mound and volume of fungus garden. We also performed a nonmetric multidimensional scaling (NMDS) ordination to investigate whether genera segregate from each other based on the morphometric measures. For this analysis, we used morphometric measures of 30 individual ants of each of the six studied genera (randomly selected from the largest excavated colony in each genus; see above). The ordination NMDS was developed using Bray-Curtis similarity coefficient. To examine the morphometric relationships among genera, measurements were considered as factors in an analysis of similarities via ANOSIM test (Clarke and Gorley 2001). In addition, polymorphism within genera was investigated by comparing the standard error of the head width measurements. This measurement was chosen as a polymorphism indicator because head width retains a constant proportion relative to body length, such that more polymorphic genera should present higher standard errors (Hölldobler and Wilson 1990, Wirth et al. 2003).

## Results

The studied genera differed significantly regarding the external and internal structure of the nests (Tables 1 and 2). The presence of a conspicuous nest mound was observed in almost all colonies, except those of *Cyphomyrmex*. In this genus, the only visible mound-like structure consisted of a pile of insect exoskeletons deposited around the nest entrance. The mean volume of true nest mounds was significantly higher in *Sericomyrmex* and *Trachymyrmex* than in *Mycetarotes*, *Mycocepurus*, and *Myrmicocrypta* ( $F = 23.93$ ,  $df = 4$ ,  $P < 0.0001$ ; Table 1). Chambers were not clearly defined in *Cyphomyrmex* nests; ants and yeast nodules were usually scattered among the superficial soil and leaf litter. *Mycetarotes* nests presented only one chamber, whereas those of *Myrmicocrypta* and *Sericomyrmex* had up to two chambers, and those of *Mycocepurus* and *Trachymyrmex* presented up to four nest chambers.

Table 1. Volume of nest mound and depth of nest chambers recorded in 22 colonies from five attine genera in a cerrado savanna in southeastern Brazil

	<i>Mycetarotes</i>	<i>Mycocepurus</i>	<i>Myrmicocrypta</i>	<i>Sericomyrmex</i>	<i>Trachymyrmex</i>
<i>Mycetarotes</i>	16 ± 8 14 ± 1	*	ns	*	*
<i>Mycocepurus</i>	ns	1,238 ± 270 35 ± 4	*	*	*
<i>Myrmicocrypta</i>	ns	ns	92 ± 33 41 ± 6	*	*
<i>Sericomyrmex</i>	*	*	*	3,165 ± 817 70 ± 9	ns
<i>Trachymyrmex</i>	*	*	ns	*	4,318 ± 321 44 ± 10

Values in the main diagonal of the matrix (mean ± 1 SE) refer to volume of nest mound (upper; cubic centimeters) and chamber depth (lower; centimeters). Differences between pairs of genera are based on Fisher post-test; significance levels are shown above (mound volume) and below (chamber depth) the main diagonal. ns, = not significant; \*,  $P < 0.05$ .

**Table 2.** Volume of nest chambers and fungus garden recorded in 22 colonies from five attine genera in a cerrado savanna in southeastern Brazil

	<i>Mycetarotes</i>	<i>Mycocepurus</i>	<i>Myrmicocrypta</i>	<i>Sericomyrmex</i>	<i>Trachymyrmex</i>
<i>Mycetarotes</i>	755 ± 248 318 ± 30	ns	ns	*	*
<i>Mycocepurus</i>	ns	629 ± 234 399 ± 148	ns	*	*
<i>Myrmicocrypta</i>	ns	ns	195 ± 46 113 ± 29	*	*
<i>Sericomyrmex</i>	*	*	*	2,918 ± 216 1,827 ± 159	ns
<i>Trachymyrmex</i>	*	*	*	ns	2,528 ± 330 1,764 ± 228

Values of volume (cubic centimeters) in the main diagonal of the matrix (mean ± 1 SE) refer to chambers (upper) and fungus garden (lower). Differences between pairs of genera are based on Fisher pos-test; significance levels are shown above (nest chamber) and below (fungus garden) the main diagonal. ns, not significant; \*,  $P < 0.05$ .

Chambers in *Trachymyrmex* nests were found >1 m deep in the ground, whereas in the other genera they were observed from 20 to 80 cm below the soil surface. Mean depth of nest chambers was significantly greater in *Sericomyrmex* and *Trachymyrmex* compared with *Mycetarotes*, *Mycocepurus*, and *Myrmicocrypta* ( $F = 5.1352$ ,  $df = 4$ ,  $P < 0.006$ ; Table 1). Comparisons of chamber volumes followed the same pattern ( $F = 25.8796$ ,  $df = 4$ ,  $P < 0.0001$ ; Table 2).

The structure of the fungus garden also differed among the six studied genera. In *Cyphomyrmex* nests the yeast garden was scattered among the leaf litter and was formed by small pieces of substrate covered by slender filaments of up to 8 mm in length (thus the garden volume could not be measured). In nests of *Mycetarotes*, *Mycocepurus*, and *Myrmicocrypta*, the fungus garden consisted of laminar sponges suspended from the ceiling of the chambers, or occasionally on plant roots. In nests of *Sericomyrmex* and *Trachymyrmex*, the fungus gardens were amorphous sponges located on the floor of the chambers. Mean volume of fungus garden was over four-fold higher in nests of *Trachymyrmex* and *Sericomyrmex* than in nests of *Mycetarotes*, *Mycocepurus*, and *Myrmicocrypta* ( $F = 40.6582$ ,  $df = 4$ ,  $P < 0.001$ ; Table 2).

The number of workers per colony differed significantly among the six genera ( $F = 3.37$ ;  $df = 5$ ;  $P = 0.023$ ), varying from 183 in *Myrmicocrypta* to up to 573 in *Sericomyrmex* (Table 3). Number of dealate queens per colony ranged as follows among genera: one (*Cyphomyrmex* and *Mycetarotes*), two (*Myrmicocrypta* and *Sericomyrmex*), three (*Mycocepurus*), and seven (*Trachymyrmex*). Overall, the volume of the nest

mound correlated strongly and significantly with both worker number (Pearson's  $r^2 = 0.75$ ,  $P < 0.05$ ) and volume of the fungus garden (Pearson's  $r^2 = 0.90$ ,  $P < 0.05$ ; Fig. 2A and B).

In general the six attine genera differed significantly with respect to the various morphometric parameters examined ( $P < 0.0001$ ; F-tests; Fig. 3A–E). Except for the antennal scape, *Trachymyrmex* and *Sericomyrmex* presented higher values than *Mycetarotes*, *Cyphomyrmex*, *Mycocepurus*, and *Myrmicocrypta* (Fig. 3A–E).

The NMDS ordination clearly revealed two distinct clusters: one formed by *Cyphomyrmex*, *Mycetarotes*, *Mycocepurus*, and *Myrmicocrypta*, and another comprised by *Sericomyrmex* and *Trachymyrmex* (Fig. 4). Moreover, ANOSIM test indicated a significant difference among genera ( $R = 0.914$ ,  $P = 0.001$ ).

The degree of polymorphism varied markedly among the attine genera investigated, as expressed by the standard error of the head width measurements. Workers of *Trachymyrmex* ( $SE = 63.29 \times 10^{-4}$ ) and *Myrmicocrypta* ( $58.97 \times 10^{-4}$ ) were the most polymorphic, followed by *Sericomyrmex* ( $29.35 \times 10^{-4}$ ), *Mycocepurus* ( $28.28 \times 10^{-4}$ ), *Mycetarotes* ( $28.17 \times 10^{-4}$ ), and *Cyphomyrmex* ( $17.23 \times 10^{-4}$ ).

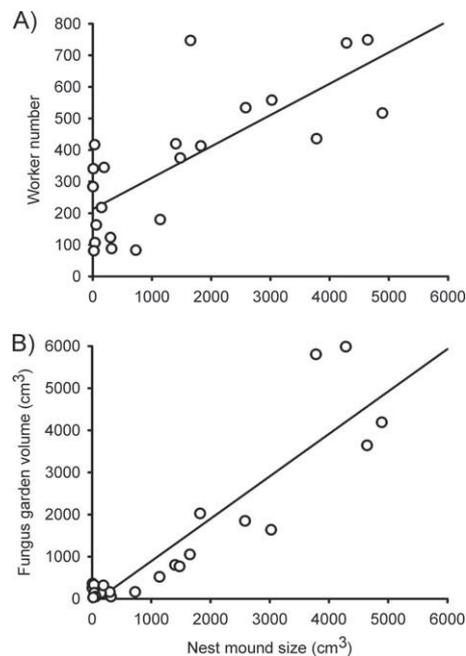
## Discussion

Our results indicate a nongradual variation of colony size, structural complexity of the nest (architecture), and worker size and polymorphism among the studied genera. In addition, nest mound volume was considered a good predictor of both worker number

**Table 3.** Number of workers recorded in 25 colonies from six attine genera in a cerrado savanna in southeastern Brazil

	<i>Cyphomyrmex</i>	<i>Mycetarotes</i>	<i>Mycocepurus</i>	<i>Myrmicocrypta</i>	<i>Sericomyrmex</i>	<i>Trachymyrmex</i>
<i>Cyphomyrmex</i>	187 ± 33	ns	ns	ns	*	*
<i>Mycetarotes</i>		347 ± 39	ns	ns	ns	ns
<i>Mycocepurus</i>			319 ± 86	ns	*	*
<i>Myrmicocrypta</i>				183 ± 47	*	*
<i>Sericomyrmex</i>					573 ± 97	ns
<i>Trachymyrmex</i>						564 ± 91

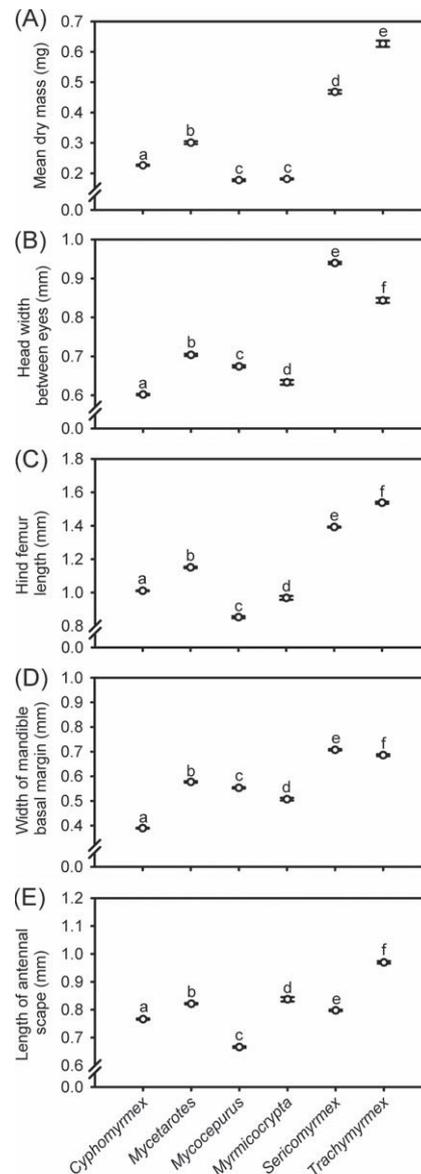
Values in the main diagonal of the matrix are means ± 1 SE. Differences between the pairs of genera are based on Fisher pos-test. ns, not significant; \*,  $P < 0.05$ .



**Fig. 2.** Correlation of nest mound volume with worker number (Pearson's  $r^2 = 0.75$ ,  $P < 0.05$ ) (A) and fungus garden volume (Pearson's  $r^2 = 0.90$ ,  $P < 0.05$ ) (B) in 25 attine colonies considering a minimum number of three colonies of the same species per studied genus (*Cyphomyrmex*, *Mycetarotes*, *Mycocepurus*, *Myrmicocrypta*, *Sericomyrmex*, and *Trachymyrmex*) located in Cerrado vegetation, state of São Paulo, southeastern Brazil.

and volume of the fungus garden. Morphometric analyses revealed that *Sericomyrmex* and *Trachymyrmex* together formed an isolated group, distinct from the other genera (*Cyphomyrmex*, *Mycocepurus*, *Myrmicocrypta*, and *Mycetarotes*). This suggests that the transition from the ancestral agricultural system toward the derived leaf-cutting habit is followed by remarkable changes in colony and worker traits, as well as nest architecture.

The external structure of *Cyphomyrmex* (*rimosus* group) nests in the cerrado savanna consisted mostly of insect exoskeletons deposited around the nest entrance, as also recorded by Fernández-Marín et al. (2004) in Central American forests. For the other genera, an external mound was always present, which in *Sericomyrmex* and *Trachymyrmex* were significantly greater than in *Mycetarotes*, *Mycocepurus*, and *Myrmicocrypta*. With respect to the internal nest structure, in *Cyphomyrmex* the chamber was not clearly well formed; the yeast garden and the ants were seen in the leaf litter down to only 5 cm below the ground in the cerrado. Similarly, Mueller and Wcislo (1998) observed only a single chamber in *C. longiscapus* (*costatus* group) in central Panama, and in most nests the fungus garden was partly exposed and could be seen from the nest entrance. According to Mayhé-Nunes (1995a) the same superficial nest pattern is found in the genus *Apterostigma*. However, nest depth in



**Fig. 3.** Mean morphometric measures of 120 ants per colony of the largest excavated colony of each studied genus (*Cyphomyrmex*, *Mycetarotes*, *Mycocepurus*, *Myrmicocrypta*, *Sericomyrmex*, and *Trachymyrmex*) located in Cerrado vegetation, state of São Paulo, southeastern Brazil. The evaluated morphometric measures were mean dry mass ( $F = 1082.36$ ,  $P < 0.0001$ ) (A), head width between eyes (hw;  $F = 1006.0$ ,  $P < 0.0001$ ) (B), hind femur length (hf;  $F = 2299.1$ ,  $P < 0.0001$ ) (C), width of mandible basal margin (mb;  $F = 1160.5$ ,  $P < 0.001$ ) (D), and length of antennal scape (sc;  $F = 560.3$ ,  $P < 0.0001$ ) (E). Different letters above the symbols indicate statistically significant differences between genera ( $P < 0.05$ ; Fisher post-test).

*Mycocepurus* and *Myrmicocrypta* were greater than in *Cyphomyrmex* and contained usually more than one chamber, supporting recent field reports on these

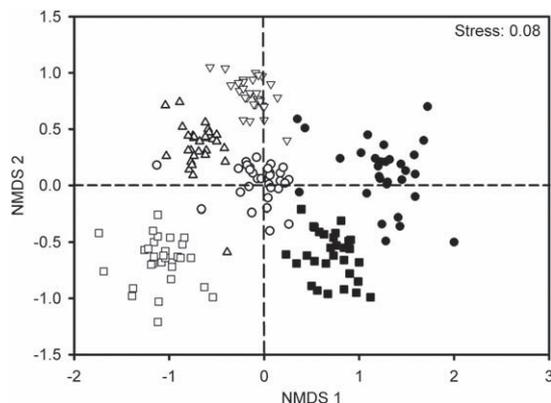


Fig. 4. NMDS as a basis for the ordination analysis based on worker morphometric attributes (i.e., dry mass, head width between eyes, hind femur length, width of mandible basal margin, and length of antennal scape) measured in 120 ants of the largest excavated colony of each studied genus located in Cerrado vegetation, state of São Paulo, southeastern Brazil. White inverted triangles, *Cyphomyrmex*; white circles, *Mycetarotes*; white squares, *Mycocephurus*; white triangles, *Myrmicocrypta*; black squares, *Sericomyrmex*; and black circles, *Trachymyrmex*.

genera (Murakami et al. 2000, Fernández-Marín et al. 2005, Rabeling et al. 2007). In general, our results on nest architecture and nest depth for *Mycetarotes parallelus* (10–16 cm) in cerrado match the data reported for this and other species in this genus at various regions in South America (Mayhé-Nunes 1995b; Solomon et al. 2004, and references therein).

Three basic types of fungus garden were detected: 1) pieces of substrate enveloped by yeast (*Cyphomyrmex*, *rimosus* group), 2) laminar fungus suspended in the chamber ceiling or plant roots (*Mycetarotes*, *Mycocephurus*, and *Myrmicocrypta*), and 3) amorphous fungus on the floor of the chamber (*Sericomyrmex* and *Trachymyrmex*). These patterns are consistent with previous reports (Wheeler 1907, Weber 1941, Kempf 1965, Solomon et al. 2004, Rabeling et al. 2007). The advantage of a suspended fungus garden is not clear, but this culturing behavior is frequently found among the lower attines and is rare in *Acromyrmex* and *Atta* that cultivate the fungus on the floor of the chambers (Weber 1937, Mayhé-Nunes 1995a, Wirth et al. 2003, Fernández-Marín et al. 2004). Garling (1979) suggested that the habit of culturing suspended fungus was set as the ants initiated fungus growth on plant roots.

Our results further showed strong and consistent differences among the attines for all evaluated demographic attributes. With the exception of *Mycetarotes*, the mean number of workers in *Sericomyrmex* and *Trachymyrmex* was significantly higher than in *Mycocephurus*, *Cyphomyrmex* and *Myrmicocrypta*. In addition, the total number of workers per colony ranged from 81 in *Myrmicocrypta* to 749 in *Sericomyrmex*, in accordance with Wetterer et al. (1998) that suggested that transitional attines tend to have small colonies of <3,000 workers. Indeed, similar results were found

in other demographic studies of *Cyphomyrmex* (20–300 workers), *Myrmicocrypta* (100–1,558), *Mycocephurus* (171–1,352), *Mycetarotes* (5–258), *Sericomyrmex* (200–1,621), *Trachymyrmex* (117–1,763) (Weber 1972, Fowler et al. 1986, Solomon et al. 2004, Rabeling et al. 2007). In our study, although the genera *Sericomyrmex*, *Trachymyrmex*, and *Mycetarotes* presented intermediate number of workers, they were numerically much closer to *Mycetarotes*, *Mycocephurus*, *Cyphomyrmex*, and *Myrmicocrypta* compared with the populous colonies of *Acromyrmex* (100,000–270,000) and *Atta* (2.5–8 million) (see Weber 1972, Fowler et al. 1986, Wirth et al. 2003).

Our data suggest that nest mound volume is a good parameter to estimate colony size and volume of fungus garden among attines. Although relationships between colony size and nest mound volume and/or basal area of the nest mound have already been reported for *Acromyrmex* and *Atta* (Autuori 1941, Fowler et al. 1986, Hölldobler and Wilson 1990), to our knowledge our study is the first to detect such correlations in the so-called lower attines.

The genera *Sericomyrmex* and *Trachymyrmex* presented higher values for most morphometric parameters compared with the other four genera, and as such formed a distinct isolated group. This is consistent with the findings of Leal and Oliveira (2000) that reported *Sericomyrmex* and *Trachymyrmex* collecting more vegetative plant parts compared with the other genera (*Cyphomyrmex*, *Mycetarotes*, *Mycocephurus*, and *Myrmicocrypta*), which tended to use mostly nonplant items (insect feces and corpses) for fungus culturing. Indeed, *Sericomyrmex* and *Trachymyrmex* were recently categorized as performing the culturing system labeled as “higher agriculture,” which also encompasses the leaf-cutters *Acromyrmex* and *Atta* (see Schultz and Brady 2008 for more information on attine agricultural systems).

Although polymorphism in nonleaf-cutting attines has been considered feeble or nonexistent (Weber 1972, Hölldobler and Wilson 1990), our morphometric analyses suggest a greater tendency toward polymorphism in *Trachymyrmex* and *Myrmicocrypta* compared with the other four genera. According to Hölldobler and Wilson (1990), the genera *Cyphomyrmex*, *Mycetophylax*, *Mycocephurus*, *Myrmicocrypta*, *Apterostigma*, *Mycetosoritis*, *Sericomyrmex*, and *Trachymyrmex* are considered monomorphic whereas *Acromyrmex* and *Atta* are polymorphic. A study by Beshers and Traniello (1994) with *Trachymyrmex septentrionalis* (McCook) suggests that population differences in colony life-history strategies may account for worker size variation in this weakly polymorphic species. Colony demography in *T. septentrionalis* seems to have been shaped by selection for colony growth rate and survival in its habitat, rather than an adaptation for efficient division of labor under specific ecological conditions (Beshers and Traniello 1994). The advantages of a more clear division of labor associated with a high polymorphism can be directly related to increased foraging efficiency (Helantera and Ratnieks 2008). For example, to maintain their large colonies, *Atta* and

*Acromyrmex* foragers must be large to be able to cut fresh leaves for fungus culturing, but small-sized workers are also necessary to care for the minute fungi in the nest (Hölldobler and Wilson 1990, 2011). The degree to which slight polymorphism is associated with foraging behavior in the studied genera has yet to be assessed.

Further investigation is needed on the internal complexity of attine nests among taxa with lower, transitional and advanced agricultural habits (Fernández-Marín et al. 2004, Adams and Longino 2007, Diehl-Fleig and Diehl 2007, Verza et al. 2007, Solomon et al. 2011). In addition, studies on ant morphometry should evaluate how the transition from monomorphism to polymorphism is associated with the increased input of vegetative items and consequently the development of larger colonies. Finally, a combination of phylogenetic with biological and ecological data should be critical to elucidate pathways in attine agricultural habits. This study highlights the importance of field accounts on natural history and biological features of attines to understand evolutionary trends within fungus-growing ants.

#### Acknowledgments

The attine genera were identified by A. J. Mayhé-Nunes and C.R.F. Brandão. We are grateful to the Instituto de Botânica de São Paulo for allowing us to work in the cerrado reserve in Mogi-Guaçu and for logistic support during fieldwork. We thank A. G. Bieber, F. Fernandez, A.V.L. Freitas, A. J. Mayhé-Nunes, and E. Sanhudo for helpful comments on the manuscript. The final version was considerably improved by comments from two anonymous referees. Financial support to P.S.O. was provided by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 301853/2009-6), Fundo de Apoio ao Ensino, à Pesquisa e Extensão, and Fundação de Amparo à Pesquisa do Estado de São Paulo (08/54058-1). I.R.L. was supported by a doctoral fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and a research grant from the CNPq.

#### References Cited

- Adams, R.M.M., and J. T. Longino. 2007. Nesting biology of the arboreal fungus-growing ant *Cyphomyrmex cornutus* and behavioral interactions with the social-parasitic ant *Megalomyrmex mondabora*. *Insectes Soc.* 54: 136–143.
- Autuori, M. 1941. Contribuição para o conhecimento da saúva (*Atta* spp.: Hymenoptera: Formicidae). I. Evolução do saúveiro (*Atta sexdens rubropilosa* Forel, 1908). Secretaria da Agricultura, São Paulo, Brazil.
- Beshers, S. N., and J.F.A. Traniello. 1994. The adaptiveness of worker demography in the attine ant *Trachymyrmex septentrionalis*. *Ecology* 75: 763–775.
- Bieber, A.G.D., M. A. Oliveira, R. Wirth, M. Tabarelli, and I. R. Leal. 2010. Do abandoned nests of leaf-cutting ants enhance plant recruitment in the Atlantic Forest? *Austral Ecol.* 36: 220–232.
- Christianini, A. V., and P. S. Oliveira. 2009. The relevance of ants as seed rescuers of a primarily bird-dispersed tree in the Neotropical cerrado savanna. *Oecologia* 160: 735–745.
- Clarke, K. R., and R. N. Gorley. 2001. PRIMER version 5: user manual/tutorial. PRIMER-E Ltd., Plymouth, United Kingdom.
- Corrêa, M. M., P.S.D. Silva, R. Wirth, M. Tabarelli, and I. R. Leal. 2010. How leaf-cutting ants impact forests: drastic nest effects on light environment and plant assemblages. *Oecologia* 162: 103–115.
- De Vuono, Y. S., E. A. Batista, and E.A.L. Funari. 1986. Balanço hídrico da reserva biológica de Mogi-Guaçu, São Paulo, Brasil. *Hoehnea* 13: 73–86.
- Diehl-Fleig, E., and E. Diehl. 2007. Nest architecture and colony size of the fungus-growing ant *Mycetophylax simplex* Emery, 1888 (Formicidae, Attini). *Insectes Soc.* 54: 242–247.
- Farji-Brener, A. G., and A. E. Illes. 2000. Do leaf-cutting ant nests make “bottom-up” gaps in Neotropical rain forests? A critical review of the evidence. *Ecol. Lett.* 3: 219–227.
- Fernández-Marín, H., J. K. Zimmerman, and W. T. Wcislo. 2004. Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). *Biol. J. Linn. Soc.* 81: 39–48.
- Fernández-Marín, H., J. K. Zimmerman, W. T. Wcislo, and S. A. Rehner. 2005. Colony foundation, nest architecture and demography of a basal fungus-growing ant, *Mycocetopus smithii* (Hymenoptera, Formicidae). *J. Nat. Hist.* 39: 1735–1743.
- Fowler, H., L. Da-Silva, C. Forti, and N. Saes. 1986. Population dynamics of leaf-cutting ants, pp. 123–145. *In* S. Logfren and R. Vander Meer [eds.], *Fire and leaf-cutting ants: biology and management*. Westview Press, Boulder, CO.
- Garling, L. 1979. Origin of ant-fungus mutualism—new hypothesis. *Biotropica* 11: 284–291.
- Helantera, H., and F.L.W. Ratnieks. 2008. Geometry explains the benefits of division of labour in a leafcutter ant. *Proc. R. Soc. Biol. Sci. Ser. B* 275: 1255–1260.
- Hölldobler, B., and E. Wilson. 1990. *The ants*. Harvard University Press, Cambridge, MA.
- Hölldobler, B., and E. Wilson. 2011. *The leafcutter ants: civilization by instinct*. W.W. Norton & Company, New York.
- Kempf, W. W. 1965. A revision of the Neotropical fungus-growing ants of the genus *Cyphomyrmex* Mayr. Part II: Group of *rimosus* (Spinola) (Hym., Formicidae). *Stud. Entomol.* 8: 161–200.
- Klingenberg, C., and C.R.F. Brandão. 2009. Revision of the fungus-growing ant genera *Mycetophylax* Emery and *Paramycetophylax* Kusnezov rev. stat., and description of *Kalathomyrmex* n. gen. (Formicidae: Myrmicinae: Attini). *Zootaxa* 2052: 1–31.
- Leal, I. R., and P. S. Oliveira. 1998. Interactions between fungus-growing ants (Attini), fruits and seeds in cerrado vegetation in southeast Brazil. *Biotropica* 30: 170–178.
- Leal, I. R., and P. S. Oliveira. 2000. Foraging ecology of attine ants in a Neotropical savanna: seasonal use of fungal substrate in the cerrado vegetation of Brazil. *Insectes Soc.* 47: 376–382.
- Mantovani, W. 1983. Composição e similaridade florística, fenologia e espectro biológico do cerrado da Reserva Biológica de Moji-Guaçu. Universidade Estadual de Campinas, Campinas, SP, Brazil.
- Mayhé-Nunes, A. J. 1995a. Filogenia de los Attini (Hym., Formicidae): un aporte al conocimiento de las hormigas fungívoras. Universidad Simón Bolívar, Caracas, Venezuela.
- Mayhé-Nunes, A. J. 1995b. Sinopsis do gênero *Mycetarotes* (Hym., Formicidae), com a descrição de duas espécies novas. *Bol. Entomol. Venez. N.S.* 10: 197–205.

- Mehdiabadi, N. J., and T. B. Schultz. 2010. Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecol. News* 13: 37–55.
- Mueller, U. G., and W. T. Wcislo. 1998. Nesting biology of the fungus-growing ant *Cyphomyrmex longiscapus* Weber (Attini, Formicidae). *Insectes Soc.* 45: 181–189.
- Murakami, T., S. Higashi, and D. Windsor. 2000. Mating frequency, colony size, polyethism and sex ratio in fungus-growing ants (Attini). *Behav. Ecol. Sociobiol.* 48: 276–284.
- Oliveira-Filho, A. T., and J. A. Ratter. 2002. Vegetation physiognomies and woody flora of the Cerrado biome, pp. 91–120. *In* P. S. Oliveira and R. J. Marquis [eds.], *The cerrados of Brazil: ecology and natural history of a Neotropical savanna*. Columbia University Press, New York.
- Pitts-Singer, T. L., and K. E. Espelie. 2007. Nest demographics and foraging behavior of *Apterostigma collare* emery (Hymenoptera, Formicidae) provide evidence of colony independence. *Insectes Soc.* 54: 310–318.
- Rabeling, C., M. Verhaagh, and W. Engels. 2007. Comparative study of nest architecture and colony structure of the fungus-growing ants, *Mycocepurus goeldii* and *M. smithii*. *J. Insect Sci.* 7: 40.
- Rico-Gray, V., and P. S. Oliveira. 2007. *The ecology and evolution of ant-plant interactions*. University of Chicago Press, Chicago, IL.
- Schultz, T. R., and S. G. Brady. 2008. Major evolutionary transitions in ant agriculture. *Proc. Natl. Acad. Sci. U.S.A.* 105: 5435–5440.
- Silva, P. D., I. R. Leal, R. Wirth, and M. Tabarelli. 2007. Harvesting of *Protium heptaphyllum* (Aubl.) March. seeds (Burseraceae) by the leaf-cutting ant *Atta sexdens* L. promotes seed aggregation and seedling mortality. *Rev. Bras. Bot.* 30: 553–560.
- Solomon, S. E., C. T. Lopes, U. G. Mueller, A. Rodrigues, J. Sosa-Calvo, T. R. Schultz, and H. L. Vasconcelos. 2011. Nesting biology and fungiculture of the fungus-growing ant, *Mycetagroicus cerradensis*: new light on the origin of higher-attine agriculture. *J. Insect Sci.* 11: 12.
- Solomon, S. E., U. G. Mueller, T. R. Schultz, C. R. Currie, S. L. Price, A.C.O. da Silva-Pinhati, M. Bacci, and H. L. Vasconcelos. 2004. Nesting biology of the fungus growing ants *Mycetarotes* Emery (Attini, Formicidae). *Insectes Soc.* 51: 333–338.
- Sternberg, L. D., M. C. Pinzon, M. Z. Moreira, P. Moutinho, E. I. Rojas, and E. A. Herre. 2007. Plants use macronutrients accumulated in leaf-cutting ant nests. *Proc. R. Soc. Biol. Sci. Ser. B* 274: 315–321.
- Urbas, P., M. V. Araújo, Jr., I. R. Leal, and R. Wirth. 2007. Cutting more from cut forests: edge effects on foraging and herbivory of leaf-cutting ants in Brazil. *Biotropica* 39: 489–495.
- Vander Meer, R. K., K. Jaffe, and A. Cedeno. 1990. *Applied myrmecology: a world perspective*. Westview Press, Boulder, CO.
- Vasconcelos, H. L., and H. G. Fowler. 1990. Foraging and fungal substrate selection by leaf-cutting ants, pp. 411–419. *In* R. K. Vander Meer, K. Jaffe, and A. Cedeno [eds.], *Applied myrmecology: a world perspective*. Westview Press, Boulder, CO.
- Verza, S. S., L. C. Forti, J.F.S. Lopes, and W.O.H. Hughes. 2007. Nest architecture of the leaf-cutting ant *Acromyrmex rugosus rugosus*. *Insectes Soc.* 54: 303–309.
- Weber, N. A. 1937. The biology of the fungus-growing ants. Part II. Nesting habits of the bachac (*Atta cephalotes*). *Trop. Agric.* 14: 223–226.
- Weber, N. A. 1941. The biology of fungus-growing ants, Part VIII: the Barro Colorado Island, Canal Zone, species. *Rev. Entomol.* 12: 93–130.
- Weber, N. A. 1972. *Gardening ants, the attines*. American Philosophical Society, Philadelphia, PA.
- Wetterer, J. K., T. R. Schultz, and R. Meier. 1998. Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. *Mol. Phylogenet. Evol.* 9: 42–47.
- Wheeler, W. M. 1907. The fungus-growing ants of North America. *Bull. Am. Mus. Nat. Hist.* 23: 669–U130.
- Wirth, R., W. Beyschlag, R. Ryel, H. Herz, and B. Hölldobler. 2003. The herbivory of leaf-cutting ants. A case study on *Atta colombica* in the tropical rainforest of Panama. *Ecological Studies*, Springer, Berlin, Heidelberg, New York.

Received 6 April 2011; accepted 22 May 2011.