

ORIGINAL ARTICLE

## Primitive nest architecture and small monogynous colonies in basal Attini inhabiting sandy beaches of southern Brazil

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(Received 14 February 2006; accepted 12 October 2006)

### Abstract

The species *Mycetophylax conformis*, *M. simplex* and *Cyphomyrmex morschi*, all belonging to the basal Attini, occur exclusively on beaches along the Atlantic shores of South America. In Brazil *C. morschi* colonies were found sympatrically with the two *Mycetophylax* species which, however, had no overlap in their geographic distribution. We recorded species-specific nesting site preferences resulting in a zonal colonization of dune and restinga areas, with distinct preferences in microhabitat choice. After excavating subterranean nests, we were able to describe details of nest architecture, especially with regard to the number and position of the fungus chambers. The nest structure is similar in all species, but the colony size differed with the highest number of workers in *M. simplex*. All nests were headed by a single mated queen. First attempts were undertaken to maintain colonies under laboratory conditions.

### Resumo

As espécies *Mycetophylax conformis*, *M. simplex* e *Cyphomyrmex morschi* fazem parte do grupo dos Attini basais e ocorrem exclusivamente em praias do Atlântico na América do Sul. No Brasil *C. morschi* ocorre simpatricamente com as espécies de *Mycetophylax*, mas as duas espécies de *Mycetophylax* não tem uma sobreposição na sua distribuição geográfica. Também detectamos preferências específicas na escolha da área da nidificação o que resulta em uma colonização zonal das dunas e restingas com preferências distintas na escolha do microhabitat. Com escavações cuidadosas de um número representativo de ninhos obtivemos detalhes sobre a arquitetura, em especial o número e posição das câmaras de fungo. A estrutura subterrânea dos ninhos é similar nas espécies estudadas mas o tamanho da população difere entre as espécies com o maior número de operárias em *M. simplex*. Todas as colônias tem uma rainha que copulou apenas uma única vez. Primeiras tentativas de manutenção de colônias no laboratório foram feitas.

**Keywords:** Brazil, coastal sand habitats, colony structure, *Cyphomyrmex*, *Mycetophylax*, nest architecture, basal Attini

### Introduction

The fungus-growing ants (Formicidae; tribe Attini) are distributed mainly in the Neotropics (Weber, 1972; Fowler, 1983; Hölldobler & Wilson, 1990; Wirth et al., 2003). Most species inhabit areas with dense plant cover, like rain forests or other inland vegetation (Schultz, 1999). The highly derived Attini of the genera *Atta* and *Acromyrmex*, the leaf cutter ant species, form colonies populated by millions of polymorphic workers. Their nests can comprise more than a thousand fungus chambers

(Moreira et al., 2004). These ants cut green leaves which are prepared and used as substrate for culture of their fungus symbionts.

An intermediate status is represented by ants of the genera *Trachymyrmex* and *Sericomyrmex* (Fernández-Marín et al., 2004). They are characterized by a monomorphic worker caste (Hölldobler & Wilson, 1990), facultative leaf and flower cutting (Weber, 1972; Leal & Oliveira, 2000) and medium to small colonies (Hölldobler & Wilson, 1990). The symbiotic fungi of these genera are closely related to those of the leaf cutter ants (Chapela et al., 1994).

The numerous species of the more original Attini are considered to represent several ancient characters (Mueller, 2002). They all have a monomorphic worker caste (Hölldobler & Wilson, 1990). These ants do not cut fresh leaves but instead collect dry plant matter, seeds, flowers, feces and insect carcasses as substrate for their fungus gardens (Leal & Oliveira, 1998, 2000). Their colonies are small (Hölldobler & Wilson, 1990), varying from 30 to more than 1000 individuals (Mueller & Wcislo, 1998; C. Rabeling, personal communication). Their nests have been little studied, and only in a few species. Often merely one fungus chamber was found (Mueller & Wcislo, 1998; Schultz et al., 2002), but other records documented several chambers (Hölldobler & Wilson, 1990; Solomon et al., 2004; Fernández-Marín et al., 2005). Their symbiotic fungi are closely related to some free-living forms (Mueller et al., 1998). Various nesting habitats were described (Green et al., 2002; Solomon et al., 2004), amongst them sandy beaches (Albuquerque et al., 2005). For a project on taxonomy of basal Attini in Brazil, we collected ants of the species *Mycetophylax conformis*, *M. simplex* and *Cyphomyrmex morschi* at such sites in southern Brazil. Since more information about these taxa will certainly contribute to our understanding of the evolution of agriculture by insects, we decided to study their occurrence, nest architecture and colony composition in more detail. We addressed the following questions: (1) Are there differences in the preference for particular nesting microhabitats between the three species studied? (2) How are the subterranean nests structured? (3) Are there species-specific peculiarities in the nest population?

## Materials and methods

Field studies were carried out in the dunes at Cabo Frio, Marica and Araruama, Rio de Janeiro state (22°S, 42°W), Joaquina beach and Pântano do Sul beach, Florianópolis, Santa Catarina Island, Santa Catarina state (27°S, 48°W). Colonies of *M. conformis* were collected at Cabo Frio, Marica and Araruama in February and July 2002, nests of *M. simplex* at Joaquina in September 2003, and those of *C. morschi* at Joaquina and Pântano do Sul in April 2003. Additional data on the geographic distribution of the three species in the states of São Paulo and Rio Grande do Sul were obtained from material in the ant collection of the Zoological Museum of the University of São Paulo.

Nest entrance tunnels and the subterranean constructions were excavated according to the following protocol. The entrance was marked with a stick. About 0.5 m apart, a hole of about 1 m depth

was dug out. Then the sand towards the nest tunnel was carefully removed until parts of the nest structure were exposed. We tried to find the complete entrance tunnel ending in a chamber containing the fungus garden. The exact position of the tunnel was best seen when ants were just passing during excavation. The whole content of the chamber was transferred with a spoon into a plastic container (diameter 10 cm, 8 cm high). We also tried to collect all the ants disturbed during our digging. The details of the nest architecture were documented and measured.

At the Museu de Zooloiga da Universidade de São Paulo the colonies were maintained at 25°C in containers with a plaster layer on the bottom that was moistened regularly. In the laboratory, the numbers of workers, queens, gynes, males and brood per nest were recorded. The artificial nests were provided with triturated corn, dry grass and other plant matter taken from the nest collection sites. During the first days in the laboratory the material carried in by the foragers was determined by examination of the remnants in the waste heap.

## Results

### Occurrence and nesting site

Nests of the three species were found in sandy habitats of the dunes at Atlantic beaches (Figure 1). *Cyphomyrmex morschi* occurred sympatrically with *M. conformis* at the beaches of Rio de Janeiro and São Paulo states and with *M. simplex* at the beaches of Santa Catarina Island and Rio Grande do Sul state. *Mycetophylax conformis* and *M. simplex* did not overlap in their geographic distribution along the Atlantic coast. Their nests were positioned near the shore side where vegetation cover was sparse. The distance to the seawater was just enough to prevent flooding of the colonies. The exact position differed in relation to the slope. Nests of *C. morschi* were found on the lee side of the dunes, in the so-called restinga area. They were located under or close to bushes in areas where vegetation cover was denser and not likely to be covered by sand of

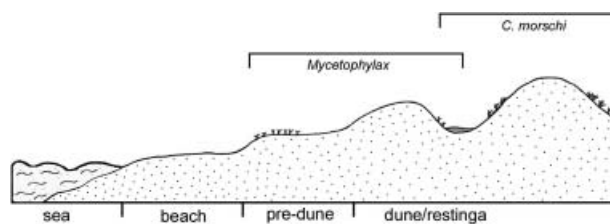
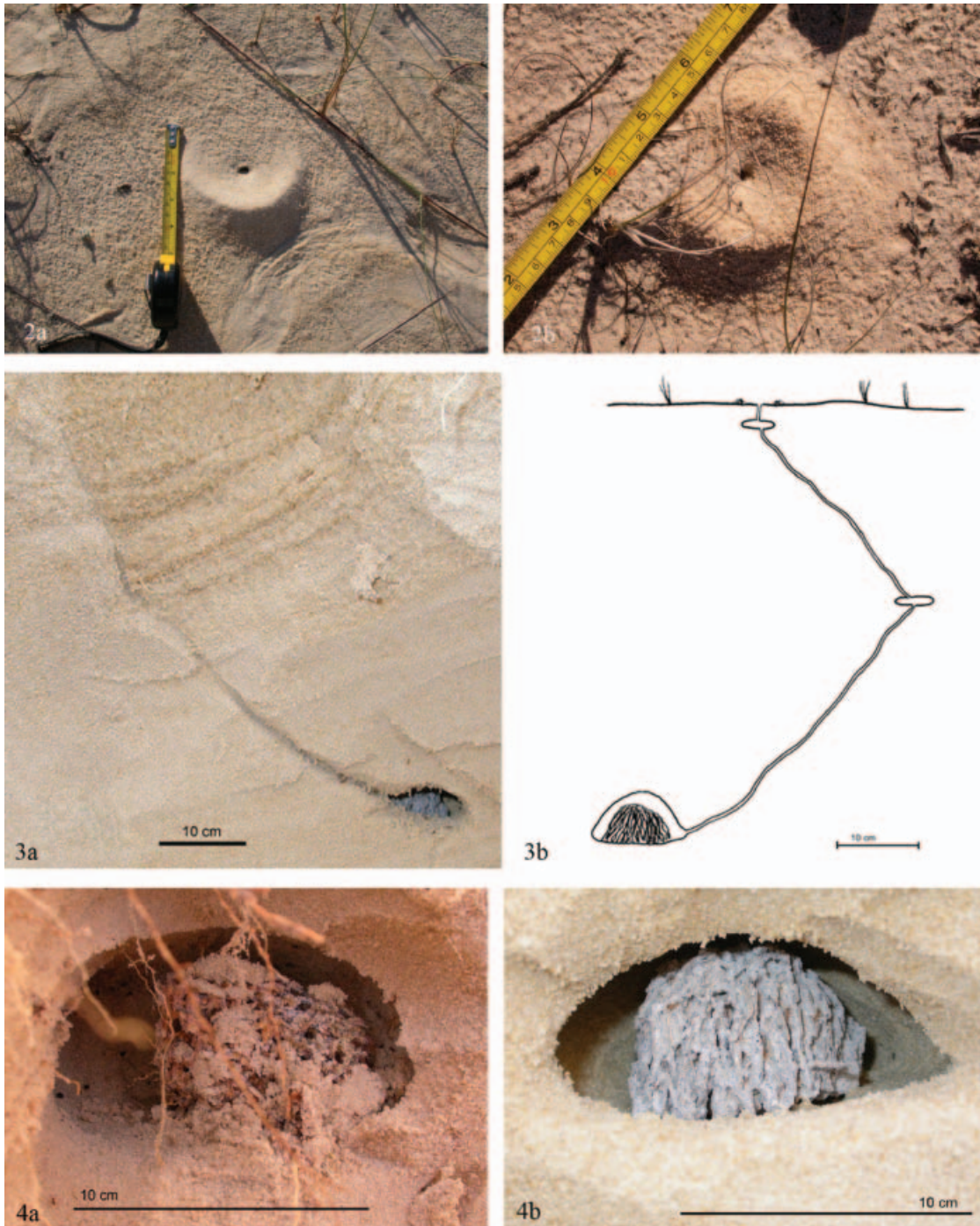


Figure 1. Nesting microhabitats of the ants *Mycetophylax conformis*, *M. simplex* and *Cyphomyrmex morschi* in the dunes at shores of the Atlantic ocean in southern Brazil.



Figures 2–4. (2) Entrance structure of nests in the sandy soil: (a) *Cyphomyrmex morschi*; (b) *Mycetophylax simplex*. (3) Subterranean nests of *M. simplex*: (a) tunnel ending in the fungus chamber; (b) system of tunnels with intermediate empty chambers and terminal chamber with fungus garden. (4) Fungus chamber in the nests of: (a) *C. morschi*; (b) *M. simplex*. There was some variation in the color of the fungus mycel, but no species-specific difference.

moving dunes. Only one *C. morschi* nest was situated directly aside a *M. simplex* colony. Two nests of *M. conformis* were located near small freshwater pools in the dunes which occasionally caused flooding of the fungus chamber floors. However, in these cases we observed that the fungus mycelium was suspended on grass roots at the roof of the chamber, preventing direct contact with the water.

#### Nest architecture

The entrance consisted of a small opening measuring about 0.5 cm, surrounded by a circular or semi-circular crater of sand (Figure 2a, b). Only at Araruama beach in some *M. conformis* nests was the opening on a little tumulus ca. 1 cm high. The entrance may be closed when the weather is windy or

on days without foraging activity. In *M. conformis* and *M. simplex*, we observed that after rainfall new entrances were built up to 15 cm aside the original ones.

The subterranean tunnels had species-specific properties. In *M. conformis* and *C. morschi* nests they had diameters of only 3 mm. In *M. simplex* the tunnel diameter measured 5 mm (Figure 3). Usually the tunnels in nests of *M. conformis* and *C. morschi* lead straight towards the fungus chamber located 20–40 cm laterally. In nests of *M. simplex* the tunnel was directed slightly downwards, making some curves and passing through two or three empty chambers (2 cm high, 5 cm wide), finally ending in the fungus chamber. The first chamber was always located only 3–5 cm under the nest entrance in a horizontal distance of up to 10 cm (Figure 3b) and mostly contained some workers, rarely also males.

#### Fungus gardens

Position and size of the fungus chamber varied with site, season and colony size (Table I), but no species-specific properties in the appearance of the fungus gardens could be identified. In nests of *M. conformis* the fungus chamber was found up to 50 cm under the surface in summer but only 20 cm down in winter. Then the previous summer chamber contained only waste and roots. In chambers with an actively cultivated fungus garden this always occupied almost the whole volume and waste was absent.

Only one nest had three fungus chambers, the largest of which (392 ml volume) contained the queen and fresh fungus while the smaller two (45 ml volume) held some workers and males together with old fungus gardens.

In nests of *M. simplex* and *C. morschi*, the fungus garden was relatively small, filling about two-thirds of the chamber volume (Figure 4; Table I). Waste was present in the fungus chamber in most of the nests of *M. simplex*. Three *C. morschi* nests had a second chamber of the same size near the fungus chamber containing merely accumulated waste. In one nest the waste was deposited beneath the fungus garden.

#### Composition of the waste

In all nests the waste consisted mainly of plant remnants. Only in nests of *M. conformis* were parts of insect bodies detected in addition, especially of *Notoxus* spp. (Coleoptera, Anthicidae), but also of dead conspecifics. In all cases numerous live mites and nematodes colonized the waste heaps.

#### Colony population

Colony size varied with species (Table II) and never exceeded 600 individuals. *Mycetophylax simplex* nests had the largest population. All colonies were headed by one egg-laying queen. When no queen was observed after colony removal, we assumed that

Table I. Nest structure in three species of basal Attini at sandy beaches of southeastern and southern Brazil.

Description	<i>Mycetophylax conformis</i>	<i>Mycetophylax simplex</i>	<i>Cyphomyrmex morschi</i>
No. of excavated nests	18	8	15
No. of fungus chambers	1–3	1	1–2
Position of fungus chambers underground (cm)	43 (35–50) (summer) 18 (15–20) (winter)	54 (35–80)	27 (7–40)
Horizontal distance of fungus chambers to entrance (cm)	20–40	60	20–40
Fungus chamber volume (ml)	250 (80–392)	1300 (300–2464)	950 (120–2550)

Table II. Detailed colony analysis in three Attini species.

Colony no.	<i>Mycetophylax conformis</i>				<i>Mycetophylax simplex</i>				<i>Cyphomyrmex morschi</i>			
	Workers	Queens	Males	Gynes	Workers	Queens	Males	Gynes	Workers	Queens	Males	Gynes
1	31	1	0	0	252	1	37	0	39	1	0	0
2	337	1	0	0	365	1	21	2	148	1	29	30
3	9	0	0	0	63	0	5	0	58	1	1	0
4	71	0	0	11	245	0	3	2	167	1	4	103
5	231	1	0	15	129	1	5	0	252	1	5	12
6	46	1	0	8	535	1	14	59	41	1	0	0
7	104	0	1	5	33	0	0	0	38	0	0	0
8	–	–	–	–	335	1	3	3	150	1	0	0
9	–	–	–	–	–	–	–	–	106	1	1	0
10	–	–	–	–	–	–	–	–	227	1	24	12

she had been lost during excavation. The number of males in nests of *M. conformis* and *C. morschi* was smaller than in *M. simplex*. The number of gynes also varied between species and colonies. The number of alates was not correlated with the worker population of a nest.

## Discussion

### Occurrence and nesting sites

Our study confirms the few previous biogeographic records on basal Attini inhabiting coastal environments (Kempf, 1964; Diehl-Fleig et al., 2003; Albuquerque et al., 2005). For the first time information on preferred microhabitats at sandy beach sites is provided. Our data indicate that *C. morschi* coexists sympatrically with *M. conformis* and *M. simplex*, but occupies clearly different microhabitats that do not overlap within the coastal dune zone. The geographic distribution of the two *Mycetophylax* species, however, is allopatric. While the southern limit of the geographic range of *M. conformis* is located in São Paulo state, the northernmost occurrence of *M. simplex* is at the beaches of Santa Catarina Island, Santa Catarina state. This distribution pattern results in a partitioning of the resource nesting space by the three ant species and probably reduces interspecific competition for material foraged as fungus substrate.

### Structure of the subterranean nests

The general design of the excavated nests of the three species was rather similar. This is in particular true for the nest entrance. The tunnels were simple and just wide enough to allow the nest owners to pass. These features are probably due to the unstable dry sandy soil (Fernández-Márin et al., 2005).

We can assume that one fungus chamber per nest is the minimal requirement for a colony of fungus-growing ants, and this original design is still represented in *Mycocrepus*, *Myrmicocrypta*, *Cyphomyrmex*, *Apterostigma* and *Mycetophylax* (Hölldobler & Wilson, 1990; Mueller & Wcislo, 1998; Schultz et al., 2002; Villesen et al., 2004). It remains open whether this simple nest construction is a conserved ancient character or a derived one, applied under restrictive ambient conditions. In fact, more than one chamber was sometimes observed in our study and has also been reported for nests of *Mycetophylax simplex* (Diehl-Fleig et al., 2003) and *Mycocrepus smithii* (Fernández-Márin et al., 2005). Such constructions may be correlated with more favorable forage environments.

The function of small empty chambers is not quite clear, this was also found in some *Mycocrepus*

species (Fernández-Márin et al., 2005; C. Rabeling, personal communication). The occurrence of different summer and winter fungus chambers may ensure optimal microclimatic conditions. We conclude that a small nest with merely one fungus chamber can be hypothesized as the original design in ancient fungus-growing ants with only small colonies.

### Colony populations

All colonies excavated by us were monogynous. Thus, this status has to be regarded normal for basal Attini (Villesen et al., 1999; Murakami et al., 2000). We assume the same social organization for all monomorphic Attini (Villesen et al., 2002). Another common trait is the small number of workers per nest, as already used by Hölldobler & Wilson (1990) for their classification of the original Attini. Because our sample of excavated nests consisted of colonies of unknown age, no final population size can be stated as typical for the three species. Small numbers of ants per nest were recorded for *Cyphomyrmex longiscapus* and *C. muelleri* with averages of 23 and 44 workers, respectively (Schultz et al., 2002). In colonies of *C. rimosus* about 70 workers and in nests of *Myrmicocrypta ednaella* 71 workers were counted (Murakami & Higashi, 1997). In habitats rich in plant biomass more populous colonies and a higher diversity of fungus-growing ants have been described (Majer et al., 1997; Silva & Silvestre, 2004).

## Acknowledgments

We would like to thank Dinair Couto Lima, Benedito C. Lopes, Rogerio Rosa da Silva, Bodo Hasso Dietz and Marisa Vianna Ferraz for help with the field work. The authors received support in Brazil from CAPES, CNPq, FAPESP, the Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, the Museu de Zoologia da Universidade de São Paulo, and in Germany from GRAFÖG, University of Tübingen, DAAD, and the Natural History Museum Karlsruhe.

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