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Fungi associated with nests of *Nasutitermes corniger* (Motschulsky) (Isoptera: Nasutitermitinae) in a semiarid region of Brazil

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Abstract

Mello AP, Corrêa EB, Barbosa-Silva AM, Bezerra-Gusmão MA. 2016. Fungi associated with nests of *Nasutitermes corniger* (Motschulsky) (Isoptera: Nasutitermitinae) in a semiarid region of Brazil. Entomotropica 31(37): 302-310.

There is a large diversity of fungi associated with termites. However, there are few studies on this subject, especially for South America. This work analyzed the community of fungi associated with nests of *Nasutitermes corniger* in urban environments of four cites (Pocinhos, Campina Grande, Areia, Bananeiras) with different climates (brejo de altitude and caatinga) in a semiarid region of northeastern Brazil. Successive dilutions, to 10^{-4} g/ml, were made with material from external and internal parts of 20 *N. corniger* nests. A solution was prepared with 1g of each nest, and 100 µl of each dilution (in five replicates) was placed in a Petri dish with potato dextrose agar medium (BDA). Twenty-three morphotypes distributed in eight genera were recorded, and the greatest richness, diversity and abundance of colonies were from the brejo de altitude nests. *Penicillium* sp.1, *Cladosporium* sp., *Aspergillus flavus* and *A. niger* were the most abundant in both regions studied.

Additional key words: Microbiology, semideciduous forest, termite, urban environments.

Resumen

Mello AP, Corrêa EB, Barbosa-Silva AM, Bezerra-Gusmão MA. 2016. Hongos asociados con nidos de *Nasutitermes corniger* (Motschulsky) (Isoptera: Nasutitermitinae) en una región semiárida de Brasil. Entomotropica 31(37): 302-310.

Hay una amplia variedad de hongos asociados con termitas. Sin embargo, hay pocos estudios sobre este tema, sobre todo en América del Sur. Hemos analizado la comunidad fúngica asociada con nidos de *Nasutitermes corniger* en entornos urbanos de cuatro ciudades (Pocinhos, Campina Grande, Areia, Bananeiras) con diferentes condiciones climáticas (bosque húmedo semideciduo de altura y caatinga) en una región semiárida del noreste de Brasil. Diluciones sucesivas hasta 10^{-4} g/ml, se hicieron a partir de porciones internas y externas de 20 nidos de *N. corniger*. Se preparó una solución con 1 g de cada nido y 100 µl de cada dilución (en cinco réplicas) y se colocaron en placas de Petri con un medio de agar de papa y dextrosa (PDA). Veintitrés morfotipos distribuidos en ocho géneros se registraron y se observó una mayor riqueza, diversidad y abundancia de colonias en los nidos de los pantanos de altitud. *Penicillium* sp.1, *Cladosporium* sp., *Aspergillus flavus* y *A. niger* fueron las especies más abundantes en ambas regiones.

Palabras clave adicionales: Bosques semideciduos, caatinga, entornos urbanos, microbiología, termitas.

Introduction

Termites are social insects and are among the most abundant arthropods in tropical ecosystems. These insects live in systems of nests constructed of soil, feces and saliva, which are humid environments rich in organic material (Sands 1969).

There is a large diversity of fungi associated with termites and their presence with these insects has often been classified as beneficial because these symbiotic associations provide termites metabolic pathways for processing carbon and nitrogen fixation from the breakdown of lignocellulosic components (Sands 1969, Lima and Costa-Leonardo 2007). However, some species of fungi are entomopathogens that severely impact the health of termite colonies, mainly because many of them prosper in hot, humid environments, such as termite nests (Batra and Batra 1979).

Most studies about termite/fungi associations have concentrated on symbiotic interactions of fungi cultivated in the nests of the termite subfamily Macrotermitinae (12 genera and 330 species) from Africa and Asia (Hendee 1933, 1934; Sands 1969, Aanen and Boomasm 2006, Aanen et al. 2007). Other studies have evaluated the ability to biologically control termites using entomopathogenic fungi, as well as the benefits provided by fungi that decompose lignocellulose to termites that colonize wood (Rouland-Lefevre 2000, Lopes et al. 2011, Passos et al. 2014). For the Semiarid Region of Brazil, there is a lack of studies related to these associations, where, in an area of caatinga, Barbosa-Silva et al. (2016), recorded 21 fungal isolates in the nests and food channels of *Constrictotermes cyphergaster* (Silvestri, 1901) and *Inquilinitermes fur* (Silvestri, 1901) (both Termitinae).

Nasutitermes corniger (Motschulsky, 1855), one of the main species in the genus, is a generalist feeder that does not discriminate between different species of wood (Traniello 1982). For this species, the search for food begins in tunnels that are mostly made of feces, where soldiers go on expeditions for food sources. When a source is located the soldiers return to the nest and recruit workers (Traniello 1982, Fontes 1995, Reinhard and Kaib 2001). This species is widely distributed in Brazil and is known for the economic damage it causes (Reinhard and Kaib 1995, Reinhard et al. 1997, Reinhard and Kaib 2001, Mello et al. 2014).

Within the Semiarid Region of northeastern Brazil there is approximately 735 000 km^2 of caatinga, which is a hot environment, with long dry periods, and is covered with a vegetation of spiny shrubs and seasonally dry forest (Leal et al. 2005). However, within the caatinga there are also areas of humid forest (seasonal semideciduous forest) that are locally called brejos de altitude (Andrade-Lima 1982, Rodal and Sales 2008). These areas, when compared to adjacent regions, have milder environments with higher precipitation, humidity (in the soil, air and vegetation) and fog (Andrade-Lima 1982, Rodal and Sales 2008).

This study analyzed the fungal community associated with the nests of *N. corniger* in urban environments with different climates, and verified the richness, diversity and similarity of fungi between populations of this termite species within the study areas.

Material and Methods

Study area

The nest samples analyzed were collected in urban areas of four cities, in a semiarid region of Paraíba, in northeastern Brazil. The cities of Pocinhos (lat 07° 04' 37" S, long 36° 03' 39" W) and Campina Grande (lat 07° 13' 50" S, long 35° 52' 52" W) are in regions that have a semiarid climate and were designated in the study as caatinga. These cities have arid climates, high temperatures, irregular patterns of precipitation, elevations between 500 and 640 m, temperatures between 21 °C and 30 °C, and an average annual rainfall of 382 and 802 mm, respectively (Atlas Geográfico da Paraíba 1985). In contrast, the cities of Areia (lat 06° 57´ 42” S, long 35° 41´ 43” W) and Bananeiras (lat 06° 45’ 00” S, long 35° 37’ 58” W) are found in parts of the semiarid region that have a humid climate with forest called brejo de altitude. These areas have elevations ranging from 650 to 1 000 m, average annual temperatures between 18 °C and 28 °C, and an average annual rainfall of 1 194 mm. The high humidity of these regions is due to an orographic effect, which reduces the thermal indices (Vivo 1997, Barbosa et al. 2004, Santos et al. 2007).

Isolation and identification of fungal colonies

The fungal composition of the nests was evaluated from 200 g samples from internal and external portions of 20 *N. corniger* nests. The material was individually homogenized, 1 g of each sample was transferred to test tubes containing 9 ml of sterilized, distilled water, and successive dilutions were made until 10^{-4}g/ml. Following this, 100 µl of each dilution was placed in sterilized Petri dishes, containing potato dextrose agar medium (BDA), and 40 µl of gentamicin sulfate was immediately added to prevent bacterial contamination. The test was carried out with five replicates for each nest sample. The plates, after solidification, were incubated for six days at room temperature (25 ± 2 °C). The fungi were identified to the generic level from stained mycelia, conidia and spores using Aman blue, and by consulting Barnett and Hunter (1999) and Singh et al. (1991). When possible, the isolates were identified to species.

Edaphic-climatic factors

The climate data was obtained from the Boletim Agroclimatológico Mensal of the National Institute of Meteorology (Inmet 2012, 2013). For each field site, the temperature and relative humidity of the air were obtained using a thermohygrometer. Mixed soil samples from around the trees that supported the nests were collected (ca. 200 g, to 20 cm deep) from three points equidistant from the host plant. The physical properties (granulometrics) of the soil were analyzed using the hydrometer method proposed by Bouyoucos (1951) and modified by Day (1965), as described by Forsyth (1975), and the chemical properties (organic material, pH, carbon, and electrical conductivity) and soil humidity were measured. The analyses were performed at the Soil Physics and Chemistry Laboratory at the Federal University of Paraíba, Campus II, Areia, PB.

Data analysis

The abundance of colonies of the morphotypes was determined by directly counting the number of isolated colonies. The Lilliefors normality test was performed in BioEstat 5.0, followed by an analysis of variance between the samples using the Kruskal-Wallis test in BioDiversity Pro 2.0. The similarity of the fungal communities among the study areas was evaluated with a non-metric multidimensional scaling (NMDS) diagram, based on Jaccard’s similarity. This was a qualitative analysis where the present/absent data were analyzed with Primer® version 6. The spatial difference between the areas was evaluated with a Principle Component Analysis (PCA), based on climatic factors (temperature, humidity and precipitation) and soil parameters (granulometrics, humidity, carbon percentage, organic material, pH and electrical conductivity). This analysis was made using Past 3.04.

Results and Discussion

Twenty-three fungal morphotypes, in eight genera of Ascomycota, were recorded from the *N. corniger* nests. The fungal community isolated in this study is frequently found in soils (Moreira and Siqueira 2006) and some of the fungi observed, such as *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp. and *Fusarium* spp., are anemophilous microorganisms (Lacaz

1977, Silva 1983). These fungi might be abundant in the nests of *N. corniger* (from urban areas) because their spores adhere to the cuticles of foraging termites, which transport them into the nests, and because soil (rich in fungi) is one of the materials used for constructing and repairing nests (Sands 1969, Cavalcanti and Maia 1994).

The highest richness, diversity and abundance of fungal colonies were from the brejo de altitude environment. Only two fungal isolates, one from Areia and another from Bananeiras, were not identified (Table 1). The higher fungal richness and diversity indices in the nests of *N. corniger* from brejo de altitude can probably be explained because areas of this environment are considered points of ecological disjunction of the Atlantic Forest that are reminiscent of regions with high biodiversity (Veloso et al. 1991). Compared to caatinga this environment is more humid, with milder temperatures, and has a higher amount of organic mater and humidity in the soil (Barbosa et al. 2004). This, in combination with the favorable microhabitat of the nests, provides optimal development conditions for colonies of fungi.

The Kruskal-Wallis analysis of variance showed significant differences of abundance between the isolates, mainly between the samples from Areia and Pocinhos (h=9.57; p=0.022). There were fungi exclusive to four nests from Bananeiras, seven from Areia and two from Campina Grande (Table 1). *Trichoderma* sp. was encountered in the nests from Areia and Campina Grande, and *Paecilomyces* sp.1. was found in the nests from Bananeiras, Areia and Campina Grande (Table 1).

The isolates *Penicillium* sp.1, *Cladosporium* sp., *Aspergillus flavus* and *A. niger* were present in both environments analyzed and in some cases with a high abundance of colonies isolated (Table 1) and which have also been reported to have high frequencies in nests of *Reticulitermes*, *Zootermopsis* and *Constrictotermes cyphergaster* (Hendee 1933, Zoberi and Grace 1990, Barbosa-Silva et al. 2016). This association might be related to the favorable conditions that the inside of nests provide to the fungi, such as partial temperature and humidity control (around 20 °C and 96 %, respectively), factors ideal for the sporulation and growth of colonies (Domsch et al. 1993, Alves 1998). In addition, it is known that termite nests are environments rich in organic and inorganic compounds, which is based mainly on the composition of the material used to construct the nests and the fact that food accumulates inside the nests (Bandeira 1983, Bezerra-Gusmão et al. 2010). *Paecilomyces* sp.3, *Cephalosporium* sp., *Fusarium* sp., *Aspergillus fumigatus*, *Aspergillus* sp.1, *Aspergillus* sp.2, *Aspergillus* sp.3 and *Aspergillus* sp.4 had punctual occurrences with low abundance in the colonies of the analyzed samples (Table 1). Previous studies conducted in urban environments found that *Aspergillus* spp. and *Penicillium* spp. are some of the main anemophilous fungi in the world (Richardson and Warnock 1993, Menezes et al. 2006). Perhaps their presence in these areas is associated with the continuous flux of people and animals on public roads, in addition to the influence of environmental factors such as ventilation, temperature and relative humidity that facilitate the dissemination of fungal spores inside the nests (Souza et al. 2012).

The non-metric multidimensional scaling (NMDS) diagram, based on Jaccard similarity of showed a separation between the areas in relation to species richness (Figure 1).

The difference observed in the ecological parameters of the isolated fungal communities between the study sites, with large geoclimatic differences, indicates that the heterogeneity of the environment can influence the fungal composition, richness and abundance present in termite nests. Possibly, the low richness of fungi isolated from nests from Pocinhos, as seen in the non-metric multidimensional scaling (NMDS) diagram (Figure 1), can be explained

Table 1. Richness and abundance [average ± standard deviation ($\overline{X}$ ± SD)] of fungal colonies in nests of *Nasutitermes corniger* (Isoptera: Nasutitermitinae) and percentage of infected nests (%) in urban environments in a semiarid region of northeastern Brazil.

Species	Brejo de Altitude				Caatinga			
	Areia		Bananeiras		Campina Grande		Pocinhos	
	($\overline{X}$ ± SD)	%	($\overline{X}$ ± SD)	%	($\overline{X}$ ± SD)	%	($\overline{X}$ ± SD)	%
Penicillium sp.1	23.20 ± 15.15	80	1.60 ± 3.58	40	13.60 ± 4.98	100	80.60 ±119.7	40
Penicillium sp.2	20.8 0± 30.15	40	-	0	-	0	23.60 ± 32.38	40
Penicillium sp.3	-	0	-	0	-	0	7.40 ± 16.55	20
Penicillium sp.4	-	0	10.60 ± 23.70	20	-	0	10.60 ± 23.70	20
Penicillium sp.5	12.60 ± 17.28	40	25.60 ± 28.08	60	27.60 ± 1.82	100	-	0
Verticillium sp.1	4.80 ± 8.67	40	-	0	-	0	5.20 ± 9.55	40
Verticillium sp.2	-	0	6.40 ± 9.02	40	-	0	-	0
Aspergillus niger	9.00 ± 8.6	60	8.60 ± 10.76	60	4.20 ± 2.94	80	7.40 ± 10.13	40
Aspergillus flavus	5.20 ± 8.67	40	1.60 ± 3.58	20	17.00 ± 18.91	60	2.60 ± 3.58	40
Aspergillus fumigatus	-	0	0.80 ± 1.79	20	-	0	-	0
Aspergillus sp.1	2.40 ± 5.36	20	-	0	-	0	-	0
Aspergillus sp.2	3.60 ± 8.05	20	-	0	-	0	-	0
Aspergillus sp.3	2.20 ± 4.91	20	-	0	-	0		0
Aspergillus sp.4	-	0	-	0	2.20 ± 4.92	20	-	0
Cladosporium sp.	3.40 ± 4.67	40	5.80 ± 7.36	60	1.80 ± 4.02	20	0.80 ± 1.79	20
Paecilomyces sp.1	-	0	11.60 ± 25.93	20	1.00 ± 2.23	20	-	0
Paecilomyces sp.2	1.20 ± 2.68	20	1.40 ± 3.13	20	-	0	-	0
Paecilomyces sp.3	0.80 ± 1.79	20	-	0	-	0	-	0
Trichoderma sp.	5.40 ± 7.60	40	-	0	1.20 ± 2.68	20	-	0
Cephalosporium sp.	0.80 ± 1.79	20	-	0	-	0	-	0
Fusarium sp.1	-	0	3.20 ± 7.15	20	-	0	-	0
S/1*	-	0	0.80 ± 1.79	20	-	0	-	0
S/2*	6.20 ± 8.50	40	0.60 ± 1.34	20	-	0	-	0

* =Isolates not identified.

by the fact that the region has long, dry, hot periods with low indices of precipitation and air humidity. In natural environments, these factors cause termites to forage less (Moura et al. 2006, Vasconcellos et al. 2007), which reduces the chances of transporting fungal spores. Thus, in this type of environment, the richness of fungi in the nests tends to be lower with only highly competitive species.

The granulometric analysis and the chemical components of the soils showed that the regions of caatinga had sandy soils with an alkaline pH and the regions of brejo de altitude had higher quantities of clay, organic material, carbon and

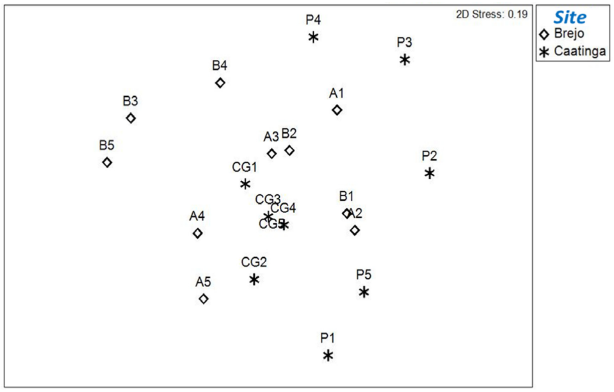

Figure 1. Ordenation of the non-metric multidimensional scaling (NMDS) analysis of the fungal similarity of nests of *Nasutitermes corniger* (Isoptera: Nasutitermitinae) from urban areas of brejo de altitude and caatinga in a semiarid region of northeastern Brazil. (Nests city Areia (A1, A2, A3, A4 and A5); Nests city Bananeiras (B1, B2, B3, B4, and B5); Nests city Campina Grande (CG1, CG2, CG3, CG4 and CG5); Nests city Pocinhos (P1, P2, P3, P4 and P5).

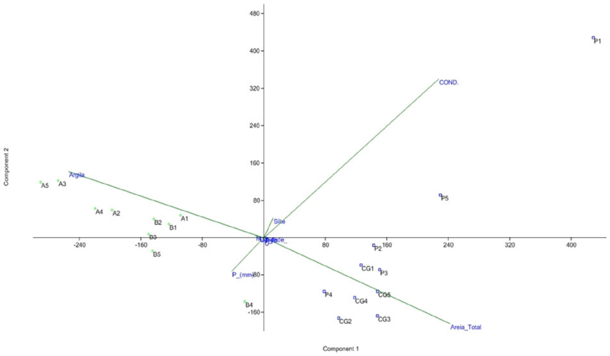

Figure 2. Principal component analysis (PCA) of the climatic parameters and soil composition for the edaphic differences between the brejo de altitude and caatinga environments in a semiarid region of northeastern Brazil.

humidity, which are characters of clay soils with an acidic to neutral pH.

The PCA showed that the areas studied have distinct environmental characteristics that influence the differences between them. The first two principle components of the PCA were responsible for 96 % of the total variation (Figure 2), and the most influential variables for this differentiation were percentage of sand and electrical conductivity of the soil, while the second principle components were clay and precipitation (Figure 2). It is also likely that the low amount of organic material and differences in soil pH of a region influence and determine the richness and abundance of fungi in nests. However, although there was a difference in the fungal composition associated with the *N. corniger* nests, the existence of fungal specificity in nests from a region cannot be affirmed because the species that occurred exclusively in one area were in low abundance of colonies and classified as punctual.

The isolated *Penicillium* spp., *Paecilomyces* spp., *Aspergillus* spp., *Cladosporium* sp. and *Trichoderma* sp. found in this study are potentially entomopathogenic (Laakso and Gloer 1993, Costa and Oliveira 1998, Rouland-Lefevre 2000, Moraes et al. 2001) and there was no colonization specificity of these in relation to the area of the nest, which can be a risk for termite colonies.

However, studies have shown that termites probably control the proliferation of entomopathogenic fungi in their nests with antifungal substances in defensive secretions produced by soldiers (Rosengaus et al. 2000, Fuller 2007, Mello et al. in preparation). Besides of this, it is known that situations that subject a colony to extreme risk can cause the colony to migrate and to form of a new nest in another location in order to reduce the chances of mortality. However, it is likely that the failure to produce chemical defenses by termites could contribute to mass infection by entomopathogenic fungi, such as those identified in this study, resulting in nest abandonment and or death of the colony.

Conclusion

The composition of the fungal microbiota associated with *N. corniger* nests from urban areas with brejo de altitude and caatinga had dissimilar richness and diversity patterns, and this is probably related to the environmental characteristics of each region. The climate and geomorphology of brejo de altitude, in addition to the microhabitat in the nests, likely offers ideal conditions for the development of a greater diversity of fungi compared to the nests from caatinga. Among the isolates, entomopathogenic fungi were found in the nests from both regions.

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