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# Biologia Geral e Experimental

Universidade Federal de Sergipe

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## NUMBER OF INDIVIDUALS OF THE HUNTING AND WEB-BUILDING SPIDER GUILDS OF THE DRY GRASSLAND AND GALLERY FOREST OF CHAPADA DIAMANTINA, BAHIA (ARACHNIDA: ARANEAE)

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*Marcelo Cesar Lima Peres<sup>1</sup>*

### ABSTRACT

This study compares the number of individuals of the hunting and web-building guilds of spiders collected at the dry grassland and gallery forest of the Chapada Diamantina, State of Bahia. There was no difference between the number of individuals collected in the dry grassland (n = 39) and the gallery forest (n = 40), but the number of individuals of each guild was significantly different between the sampled areas. Individuals of the spider web-building guild prevailed in the gallery forest (n = 27), while those from the hunting guild prevailed in the dry grassland (n = 24). The importance of the microhabitats to the distribution and composition of spiders' guild are discussed.

**Keywords:** guilds, spiders, cerrado, Chapada Diamantina, Bahia.

### RESUMO

O estudo compara o número de indivíduos das guildas de aranhas caçadoras e construtoras de teias, coletadas no campo sujo e na mata ciliar na Chapada Diamantina, Estado da Bahia. Não houve diferença entre o número de indivíduos coletados no campo sujo (n = 39) e na mata ciliar (n = 40), mas o número de indivíduos de cada guilda foi significativamente diferente entre as áreas amostradas. Indivíduos da guilda das aranhas construtoras de teias prevaleceram na mata ciliar (n = 27), enquanto aqueles da guilda de aranhas caçadoras prevaleceram no campo sujo (n = 24). Discute-se brevemente a importância dos microhabitats na distribuição e composição de guildas de aranhas.

**Palavras-chave:** guildas, aranhas, cerrado, Chapada Diamantina, Bahia.

### INTRODUCTION

Several studies discuss the importance of the ecological factors in order to understand the relationships between groups of spiders that share the same resources, such as tolerance to the sunlight and shade, humidity and climate conditions, strategies

of prey capture, types of vegetation, shelters and reproductive behavior (Uetz, 1977; Sunderland & Greenstone, 1999; Höfer & Brescovit, 2001; Brescovit *et al.*, 2004; Peres *et al.*, 2007; Silva. & Coddington, 1996). These ecological groups of spiders are generically called guilds, a term that can be defined as a group of syntopic organisms that use resources in a

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similar way, utilizing the same trophic levels (Jaksic', 1981), or a cluster of species separated from all other such clusters by a distance greater than the largest distance between the two most disparate members of the guild concerned (Pianka, 1994). In spite of their differences in morphology, physiology and behavior, distinct spider guilds may be associated to explore microhabitats (Halaj *et al.*, 1998), but studies comparing guild habitat specificity are few (Höfer & Brescovit, 2001). In order to contribute to the understanding of how habitat and foraging behavior can determine spider guilds, we studied the hunting and web-building spider guilds of the dry grassland and gallery forest in the cerrado vegetation of Brazilian State of Bahia.

#### MATERIALS AND METHODS

The study was carried out in March, September and October of 2005 in the cerrado area of the Reserva Particular de Patrimônio Natural Adília Paraguassu (12°59'S, 41°23'W), located in the Chapada Diamantina, Bahia. Three dry grassland and three gallery forest areas were sampled utilizing two methods: i) sweeping net during 30 minutes in a quadrant (5 x 5 meters) inserted randomly in each area, ii) 60 pitfall-traps (9 centimeters of diameter) disposed in 6 lines separated by 1 meter, each one with 10 traps filled with 200ml of a solution containing 70% ethanol, water, formaldehyde 4% and detergent. The traps remained open for seven days. Most of the spiders were identified until genera. A chi-square test for homogeneity (Ayres *et al.*, 2000) was utilized in order to verify the differences between the number of individuals in the hunting/web-building species guilds presented in the dry grassland and gallery forest of the studied areas. The two categories of spider guilds adopted in the study followed Höfer & Brescovit (2001).

#### RESULTS AND DISCUSSION

Among the 79 collected spiders (around 37 species), 40 individuals occurred in the dry grassland and 39 in the gallery forest, heterogeneously distributed in relation to the two guild categories. Most of the web-building guild individuals ( $n = 27$ ) occurred in the gallery forest; for the hunting guild most individuals ( $n = 24$ ) occurred in the dry grassland ( $\chi^2 = 6.8$ ,  $p < 0.01$ , d.f. = 1, Table 1). The species composition of both guilds was different between and inside the sampled areas, except for the web-building *Dubiaranea* sp. (Linyphiidae) and two hunting lycosids (Lycosidae) that occurred together in the dry grassland and gallery forest (Table 1).

We assume that these differences were due to the habitat and microhabitat structures of the sampled areas, mainly the vegetation. Souza-Alves & Tinôco (2005) reported that the dry grassland areas are constituted by shrub vegetation and does not have a thick layer of litter, whereas the gallery forest areas have a more accentuated herbaceous and litter coverage. The presence of a high herbaceous covering and shrub layers can favor the web-building species, offering a greater diversity of microhabitats than the open areas do (Wise, 1993; Elton, 1973).

As for density, in the areas with low litter and diversity of microhabitats, like the dry grassland, the individuals are more exposed to predation (Uetz, 1979), such as the web-building spiders, and we expected to encounter more hunting guild spiders in these open areas. However, in habitats with a deep leaf litter layer, as the gallery forests, we expected a higher number of individuals of the web-building guilds, especially those that fix their webs among the leaves on the ground. Both expectancies were encountered in our study, suggesting that together with the morphological, physiological and behavioral features, we also have to consider the structure of the habitats and microhabitats for a better understanding of the regional distribution and composition of spider guilds.

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Table 1. Frequency of the number of individuals per species between the web-building and hunting spider guilds in the dry grassland (G) and gallery forest (F) of Chapada Diamantina, Bahia.

	Web-building		Hunting	
	G	F	G	F
TETRAGNATHIDAE				
<i>Tetragnatha</i> sp.	2	-		
<i>Glenognatha</i> sp.	1	-		
ULOBORIDAE				
<i>Zosis</i> sp.	-	1		
ARANEIDAE				
<i>Micrathena</i> sp.	-	1		
<i>Mangora</i> sp.	-	1		
DICTYNIDAE				
<i>Dictyna</i> sp.	1	-		
THERIDIIDAE				
<i>Dipoena</i> sp.	-	2		
<i>Coleosoma floridanum</i> Banks, 1900	1	-		
<i>Guaraniella</i> sp.	1	-		
<i>Euryopis</i> sp.	1	-		
<i>Euryopis</i> sp. 2	1	-		
<i>Steatoda</i> sp.	1	-		
LINYPHIIDAE				
<i>Dubiaranea</i> sp.	2	3		
Linyphiidae sp.	2	-		
OXYOPIDAE				
<i>Oxyopes salticus</i> Hentz, 1845	3	-		
<i>Peucetia</i> sp.	-	1		
HAHNIIDAE				
Hahniidae sp. 1	-	6		
Ochyroceratidae				
<i>Ochyrocera</i> sp.	-	4		
SICARIIDAE				
<i>Sicarius tropicus</i> Mello-Leitão, 1936	-	1		
PHOLCIDAE				
<i>Mesabolivar</i> sp.	-	5		
<i>Metagonia</i> sp.	-	2		
<b>Total</b>	<b>16</b>	<b>27</b>	<b>24</b>	<b>12</b>



# Biologia Geral e Experimental

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## CARDIOVASCULAR EFFECTS AND ACUTE TOXICITY OF THE AQUEOUS EXTRACT OF *COSTUS SPICATUS* LEAVES (ZINGIBERACEAE)

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Márcio Roberto Viana Santos<sup>1</sup>

### ABSTRACT

Cardiovascular effects and acute toxicity of the aqueous extract of *Costus spicatus* leaves (AECS) were studied in rats and mice. In non anaesthetized normotensive rats (n = 6), AECS (0.5, 1.0, 5.0 and 10.0 mg/kg; i.v., randomly) induced hypotension associated with tachycardia. In intact or endothelium-denuded rings of rat superior mesenteric artery, AECS (0.1 - 100.0 µg/mL, n = 4) provoked relaxation of the tonus induced by 10 µM phenylephrine. The extract showed low acute toxicity in mice.

**Keywords:** *Costus spicatus*, aqueous extract, cardiovascular effects, acute toxicity.

### RESUMO

Os efeitos cardiovasculares e a toxicidade aguda do extrato aquoso das folhas de *Costus spicatus* (AECS) foram estudados em ratos e camundongos. Em ratos normotensos não-anestesiados, (n = 6), AECS (0.5, 1.0, 5 e 10.0 mg/kg; i.v., aleatoriamente) induziu hipotensão associado com taquicardia. Em anéis intactos ou sem endotélio da artéria mesentérica superior de rato, AECS (0.1 - 100.0 µg/mL, n = 4) provocou relaxamento do tônus induzido por 10 µM de fenilefrina. O extrato demonstrou baixa toxicidade aguda em camundongos.

**Palavras-chave:** *Costus spicatus*, extrato aquoso, efeitos cardiovasculares, toxicidade aguda.

### INTRODUCTION

The plant *Costus spicatus* Swartz (Zingiberaceae) is a bush that grows up to 2 meters and can be found in the coastal Brazilian forest. Popularly known as “cana-do-brejo” or “cana-de-macaco”, this plant is used in folk medicine as diuretic (Martinez, 1984), for treatment of colds, sore throats, dysentery, diarrhea (Cruz, 1965), bladder, urethra and

kidney complaints (Manfred, 1947).

Phytochemical analysis of *C. spicatus* rhizomes and leaves showed the presence of flavonoids (Williams & Harbone, 1977), flavonol glycosides (Silva *et al.*, 2000), saponins and saponinins (Silva *et al.*, 1999a, 1999b). Other studies on this plant revealed antimicrobial (Misas *et al.*, 1979), haemolytic (Silva *et al.*, 1999a, 1999b) and diuretic activities (Caceres *et al.*, 1987; Souza *et al.*, 2004). The objective of this study

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is to evaluate the cardiovascular effects of AECS in rats, through the combined use of the *in vivo* and *in vitro* approaches.

#### MATERIAL AND METHODS

**Plant:** The plant *Costus spicatus* was collected around the city of Aracaju (10°54'S, 37°04'W). The voucher specimen was identified and deposited in the Herbarium of the Biology Department at the Universidade Federal de Sergipe.

**Aqueous extract:** The AECS was obtained from dried leaves (40g of powder) by an infusion with distilled water (1 L/100° C) followed by filtration. The filtrate was lyophilized (aqueous extract) and stored at 4° C. When required, extract was dissolved in a saline solution, for *in vivo* experiments, or nutritive solution, for *in vitro* experiments, at the desired concentrations.

**Animals:** Male Wistar rats (200 - 300 g) were used for the cardiovascular experiments; male Swiss mice (20-35g) were used in the toxicity experiments. Animals were housed under conditions of controlled temperature and lighting (lights on: 06:00 - 18:00 hours) and had free access to food and tap water.

**Drugs:** The drugs used were: acetylcholine chloride (Ach), L-phenylephrine chloride (Phe), (all from SIGMA), sodium nitroprusside (SNP), heparin sodium salt (ARISTON) and sodium thiopental (CRISTÁLIA). All compounds were freely dissolved in distilled water (for *in vitro* experiments) or saline (for *in vivo* experiments).

**Acute toxicity:** Experimental groups of 6 mice received orally doses of 1, 3 and 5 g/kg of AECS, while the control group received only vehicle (distilled water). All groups were observed for 48 hours; at the end of this period the mortality was recorded for each

group (Dietrich, 1983).

**Blood pressure measurements:** Under sodium thiopental anesthesia (45 mg/kg, i.v.), the lower abdominal aorta and inferior vena cava were cannulated via the left femoral artery and vein using polyethylene catheters. The catheters were then filled with heparinized saline solution and slid under the skin to emerge between the scapulae. Arterial pressure was measured after 24 h by connecting the arterial catheter to a pre-calibrated pressure transducer (Edwards Lifescience, Irvine, CA, USA) and pressure outputs were recorded by an amplifier-recorder (BioData, Model BD-01, PB, Brasil) connected to a personal computer equipped with an analog-to-digital converter board (BioData, PB, Brasil). Data were collected at a frequency of 200 Hz. For each cardiac cycle, the computer calculated mean arterial pressure (MAP) and pulse interval, referred to here as the heart rate (HR). The venous catheter was inserted for drug administration. Sodium nitroprusside (10 µg/kg) was injected to check the efficacy of catheter insertion.

**Rat superior mesenteric artery rings:** Rats were killed by stunning and exsanguination. The superior mesenteric artery was removed, cleaned from connective tissue and fat, and sectioned in rings (1 - 2 mm), which were suspended by cotton threads in organ baths containing 10 ml of Tyrode's solution (Composition mM: NaC 158.3, KCl 4.0, CaCl<sub>2</sub>·2H<sub>2</sub>O 2.0, NaHCO<sub>3</sub> 10.0, C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> 5.6, MgCl<sub>2</sub>·6H<sub>2</sub>O 1.05 and NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O 0.42), gassed with carbogenic mixture (95 % O<sub>2</sub> and 5 % CO<sub>2</sub>) and maintained at 37° C for isometric tension recordings. The stabilization period was of 1 h under a resting tension of 0.75 g. During this time the solution was changed every 15 min, to prevent the accumulation of metabolites. The isometric tension was recorded through a force transducer (Gould, Model GM2, USA) coupled to an amplifier-recorder (Gould, USA). Endothelium was removed by gently rubbing the intimal surface of the vessels. The

presence of functional endothelium was assessed by the ability of acetylcholine (ACh) (10  $\mu$ M) to induce more than 70 % relaxation of pre-contracted vessels with phenylephrine (10  $\mu$ M). The absence of the relaxation to ACh was taken as evidence that the vessel segments were functionally denuded of endothelium.

**In vivo experiments:** After cardiovascular parameters had been stabilized, MAP and HR were recorded before (baseline values) and after i.v. administration of randomized doses of AECS (0.5, 1.0, 5.0 and 10.0 mg/kg). Dose-response curves were then obtained. These administrations were separated by a time interval sufficient to allow full recovery of cardiovascular parameters. A control dose-response curve was then obtained.

**In vitro experiments:** After the stabilization period, two successive contractions of similar magnitude were induced with 10  $\mu$ M Phe in rings with or without endothelium. During the tonic phase of the third contraction, different concentrations of AECS (0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0  $\mu$ g/ml) were added cumulatively to the organ bath. The relaxations were measured by comparing the developed tension before and after the addition of AECS and expressed as percentage of relaxation from induced tonus.

**Statistics:** Values are expressed as means  $\pm$  SEM. When appropriate, Student's t test was conducted in order to evaluate the significance of the differences between two means. All procedures were carried out on Graph Pad Prism™ version 3.02 software.

## RESULTS

In non-anaesthetized normotensive rats, baseline MAP and HR values were  $117 \pm 1$  mmHg and  $369 \pm 10$  bpm, respectively. In these animals, the intravenous *in bolus* injections of AECS (0.5, 1, 5 and

10 mg/kg) induced a light and transitory hypotension ( $-7.4 \pm 0.5$ ;  $-14.9 \pm 0.6$ ;  $-11.5 \pm 0.4$  and  $-10.7 \pm 0.4$  %) associated with tachycardia ( $2.2 \pm 0.3$ ;  $8.9 \pm 0.4$ ;  $5.7 \pm 0.3$  and  $9.4 \pm 0.4$  %). The effects to each dose were fully recovered after 30 seconds (Figure 1). In rat isolated rings of the superior mesenteric artery with intact endothelium, AECS (0.1, 0.3, 1.0, 3.0, 10.0, 30.0 and 100.0  $\mu$ g/mL, n = 4, cumulatively) induced relaxations of tonus induced by 10  $\mu$ M phenylephrine, which was not significantly affected after the endothelium remotion (Figure 2). The AECS did not present toxicity up to the oral dose of 5g/kg, so the LD50 could not be determined.

## DISCUSSION

We chose to evaluate the effects of AECS on the cardiovascular parameters in non-anaesthetized rats, in order to avoid anesthesia and surgical stress influences (Smith & Hutchins, 1980; Fluckiger *et al.*, 1985). Baseline MAP and HR values were analogous to those previously reported in other studies (Lahlou *et al.*, 2002; Cunha *et al.*, 2004). In these animals, acute administration of AECS induced light hypotension associated with tachycardia.

Peripheral vascular resistance mainly maintains the blood control pressure and the major contributor is the vascular tone of several arterial beds (White *et al.*, 1996), as the mesenteric one (Mulvany & Aalkjaer, 1990). In order to verify if hypotensive response could be induced by the decrease of the peripheral vascular resistance due to a possible vasorelaxation, we performed experiments using rings from the rat superior mesenteric artery, an *in vitro* approach. In these preparations, AECS induced vasorelaxation suggesting that the hypotensive response appears to be due to a direct action on the peripheral vascular resistance.

It is well known that the endothelium is an important regulator of the vascular tone by releasing

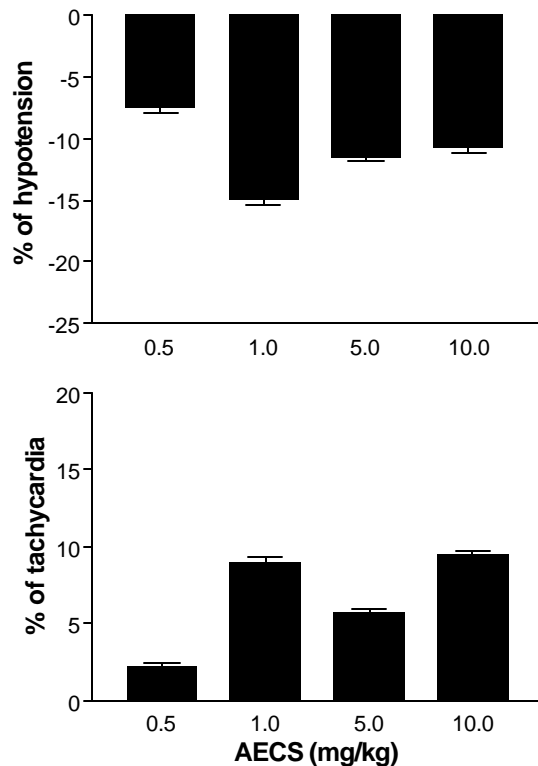


Figure 1. Hypotensive and tachycardic response induced by AECS (0.5, 1.0, 5.0, 10.0 mg/Kg; i.v.) in non-anaesthetized normotensive rats. Values are expressed as mean ± SEM.

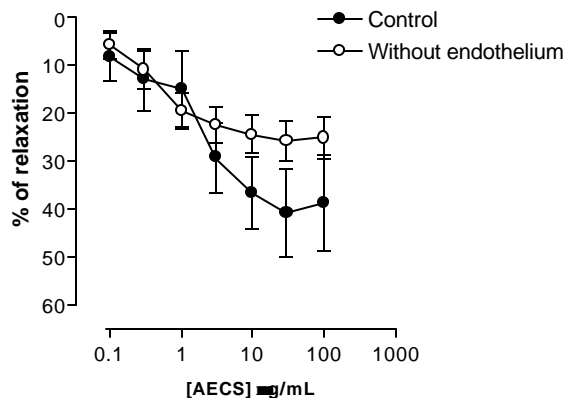


Figure 2 – Concentration-response curves to AECS (0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0 µg/ml) in rat superior mesenteric artery rings pre-contracted with 10.0 µM. Values are expressed as mean ± SEM. \*  $p < 0.05$  vs Control.

endothelium-derived relaxing factors (Moncada *et al.*, 1991), mainly NO and COX-derived products, such as PGI<sub>2</sub> (Furchgott & Zawadzki, 1980; Moncada *et al.*, 1991). In order to investigate the participation of the endothelium in the vasorelaxant response induced by AECS, we performed experiments in the absence of functional endothelium. In these conditions, the vasorelaxant response induced by AECS was not significantly changed. This suggests that the presence of endothelium is not essential for relaxant responses expressions and that an endothelium-independent pathway is probably implicated in this effect.

The cardiovascular effects of AECS may be due to the presence of flavonoids (Williams & Harbone, 1977), such as, quercetin, kaempferide, tamarixetin and isorhamnetin 3-O-neohesperidoside (Silva *et al.*, 2000), which have vasodilator properties (Perez-Viscaino *et al.*, 2002) possibly involving opening potassium channels (Calderone *et al.*, 2004). These cardiovascular effects associated with the recent discovery of *Costus spicatus* diuretic effect (Souza *et al.*, 2004) showed that this medicinal plant has a great therapeutic potential, because the association between diuretic and vasorelaxant effects are frequently used in treatment of hypertension and other cardiovascular diseases (World Health Organization, 1999; Boydak *et al.*, 2004). However, further pharmacological studies are necessary to confirm this assumption.

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# Biologia Geral e Experimental

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## ENTEROBACTERIACEAE E *PSEUDOMONAS* SP. MULTIRESISTENTES ISOLADAS DE EFLUENTES URBANOS EM ARACAJU, SERGIPE

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### RESUMO

O estudo avaliou a ocorrência de bactérias Gram-negativas multiresistentes a antimicrobianos presentes em efluentes urbanos na cidade de Aracaju, Sergipe. As bactérias isoladas foram *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas* sp. e *Pantoea agglomerans*. As colônias presuntivas (222) apresentaram resistência a ampicilina, tetraciclina, kanamicina e cloranfenicol ou a mais de um desses antimicrobianos.

**Palavras-chave:** Enterobacteriaceae, *Pseudomonas* sp., multiresistência, Sergipe.

### ABSTRACT

This study evaluated the occurrence of Gram-negative bacteria multiresistant to antimicrobians present in urban effluents in the city of Aracaju, Sergipe. The isolated bacteria were *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas* sp. and *Pantoea agglomerans*. Presumptive colonies (222) showed resistance to ampicilin, tetracyclin, kanamycin and chloramphenicol or to more than one of these antimicrobians.

**Keywords:** Enterobacteriaceae, *Pseudomonas* sp., multiresistance, Sergipe

### INTRODUÇÃO

A resistência bacteriana é um fenômeno biológico natural que se tornou problema de saúde pública, devido à utilização indiscriminada e em larga escala de antimicrobianos (ATMs). De origens diversas, os antimicrobianos despejados nos esgotos urbanos interagem com agentes patogênicos,

promovendo nestes a transferência e seleção de genes de resistência (Davison, 1999; Kümmerer *et al.*, 2000; Guardabassi *et al.*, 2002).

Dentre as bactérias Gram-negativas que apresentam elevada resistência aos ATMs destacam-se as espécies da família Enterobacteriaceae e do gênero *Pseudomonas*. Sabemos que as enterobacteriáceas estão presentes nos meios urbanos

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em geral (Farmer III *et al.*, 1985; Stover *et al.*, 2000; Meirelles-Pereira *et al.*, 2002), mas ao nível regional faltam informações sobre a frequência de ocorrência e potencial de resistência aos vários agentes antimicrobianos, objetivos deste estudo.

## MATERIAL E MÉTODOS

**Amostras:** Água coletada em efluentes urbanos dos bairros Jardins e Industrial da cidade de Aracaju, Sergipe. Foram isoladas presuntivamente enterobactérias e *Pseudomonas* sp., de acordo com método recomendado pela Associação Americana de Saúde Pública (Apha, 2000).

**Seleção de multiresistentes:** Os isolados foram selecionados por pressão auxotrófica (Sambrook *et al.*, 1989), em caldo Luria-Bertani - LB (0,1 % Tripton, 0,05 % extrato de levedura, 0,1 % NaCl). e suplementados separadamente com ampicilina (100 µg/mL), tetraciclina (50 µg/mL), cloranfenicol (170 µg/mL) e kanamicina (50 µg/mL) (Sigma-Aldrich). Os inóculos foram incubados por 24 horas a 37° C sob agitação de 150 rpm.

**Identificação das amostras multiresistentes:** As amostras que apresentaram resistência a dois ou mais dos quatro antimicrobianos testados foram identificadas por provas bioquímicas (Holt *et al.*, 1994), no Laboratório de Virologia Comparada da Universidade Federal de Sergipe, e confirmadas por sorotipagem no Laboratório de Enteropatógenos do Instituto Oswaldo Cruz, Rio de Janeiro.

## RESULTADOS

A Tabela 1 mostra os resultados. Foram isoladas amostras multiresistentes de *Escherichia coli* (9), *Enterobacter cloacae* (5), *Klebsiella pneumoniae* (6),

*Pantoea agglomerans* (3) e do gênero *Pseudomonas* sp. (3); entre parênteses o número de amostras. No bairro Jardins foram isoladas *Escherichia coli* (2), *Pseudomonas* sp. (2) e *Klebsiella pneumoniae* (1). No bairro Industrial foram isoladas *Escherichia coli* (7), *Enterobacter cloacae* (5), *Klebsiella pneumoniae* (5), *Pseudomonas* sp. (1) e *Pantoea agglomerans* (3); entre parênteses o número de amostras. Com relação aos antimicrobianos, foram isoladas 222 colônias presuntivas de *Pseudomonas* sp. e enterobactérias que apresentaram resistência à ampicilina (24), tetraciclina (25), kanamicina (24) e cloranfenicol (14) ou resistência a mais de um antimicrobiano (26); entre parênteses o número de isolados.

## DISCUSSÃO

A ocorrência da enterobactéria endofítica *Pantoea agglomerans* nas amostras pode estar relacionada à proximidade do mangue, vegetação que compõe os bairros estudados. A presença de *Escherichia coli* pode ser explicada pelos dejetos sanitários residenciais. Coliformes não-fecais comumente também ocorrem em áreas de despejo, como *Enterobacter* e *Klebsiella*, as últimas associadas a dejetos hospitalares que também explicaria a presença de *Pseudomonas* na área de estudo (Meirelles-Pereira *et al.*, 2002; Kümmerer & Henninger, 2004; Goñi-Urriza *et al.*, 2000).

Os resultados concordam com outros estudos que relataram resistência à ampicilina, tetraciclina e kanamicina (Al-Jebouri, 1985; Al-Ghazali *et al.*, 1988). A resistência ao cloranfenicol é rara, possivelmente como resultado do uso restrito dessa droga (Goñi-Urriza *et al.*, 2000). As amostras de Aracaju apresentaram resistência a alguns dos marcadores de resistência mais comuns de ATMs, empregados em antibioticoterapia humana e veterinária (Al-Jebouri, 1985; Al-Ghazali *et al.*, 1988; Goñi-Urriza *et al.*, 2000; Meirelles-Pereira *et al.*, 2002).



A resistência de bactérias a antimicrobianos pode ser devido ao uso indiscriminado desses ATMs, o que limitaria a eficiência dessas drogas no tratamento de infecções humanas e veterinárias. Neste estudo nós utilizamos provas bioquímicas para avaliar a resistência dos microorganismos aos ATMs, outros métodos poderão contribuir para uma melhor avaliação, como detecção de DNA plasmidial de baixo peso molecular.

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Tabela 1. Resistência a antimicrobianos (ATMs) em Enterobacteriaceae e *Pseudomonas* sp., Aracaju, Sergipe.

Amostra	Espécie	Bairro	Determinantes de Resistência			
			Amp	Tet	Clo	Kan
1	<i>Escherichia coli</i>	Industrial	+	+	+	+
2	<i>Escherichia coli</i>	Industrial	+	+	+	+
3	<i>Escherichia coli</i>	Industrial	+	+	+	+
4	<i>Escherichia coli</i>	Industrial	+	+	+	+
5	<i>Escherichia coli</i>	Industrial	+	+	+	+
6	<i>Klebsiella pneumoniae</i>	Industrial	+	+	+	+
7	<i>Klebsiella pneumoniae</i>	Industrial	+	+	+	+
8	<i>Enterobacter cloacae</i>	Industrial	+	+	+	+
9	<i>Enterobacter cloacae</i>	Industrial	+	+	+	+
10	<i>Enterobacter cloacae</i>	Industrial	+	+	+	+
11	<i>Pseudomonas</i> sp.	Industrial	+	+	+	+
12	<i>Pseudomonas</i> sp.	Jardins	+	+	+	+
13	<i>Pseudomonas</i> sp.	Jardins	+	+	+	+
14	<i>Escherichia coli</i>	Jardins	+	+	-	+
15	<i>Escherichia coli</i>	Industrial	+	+	-	+
16	<i>Klebsiella pneumoniae</i>	Jardins	+	+	-	+
17	<i>Klebsiella pneumoniae</i>	Industrial	+	+	-	+
18	<i>Klebsiella pneumoniae</i>	Industrial	+	+	-	+
19	<i>Klebsiella pneumoniae</i>	Industrial	+	+	-	+
20	<i>Enterobacter cloacae</i>	Industrial	+	+	-	+
21	<i>Pantoea agglomerans</i>	Industrial	+	+	-	+
22	<i>Pantoea agglomerans</i>	Industrial	+	+	-	+
23	<i>Pantoea agglomerans</i>	Industrial	+	+	-	+
24	<i>Enterobacter cloacae</i>	Industrial	+	+	+	-
25	<i>Escherichia coli</i>	Jardins	-	+	-	+
26	<i>Escherichia coli</i>	Industrial	-	+	-	+

+ presente, - ausente, amp - ampicilina, tet- tetraciclina, clo - cloranfenicol, kan - kanamicina

# Biologia Geral e Experimental

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## NOTES ON THE REPRODUCTION, BEHAVIOUR AND DIET OF *SAGUINUS NIGER* (PRIMATES: CALLITRICHIDAE) IN A FOREST REMNANT AT THE NATIONAL PRIMATE CENTRE, ANANINDEUA, PARÁ

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### ABSTRACT

This study presents data on the reproduction, behaviour and diet of a free-ranging group of black-handed tamarins, *Saguinus niger*, in an urbanised forest at the National Primate Centre in Ananindeua, eastern Brazilian Amazonia. The study group contained between five and seven members, changing due to the disappearance of an adult and the births of three infants. Quantitative behavioural data were collected in scan samples during five days each month between May and September 2000, covering the transition between wet and dry seasons. The general activity budget (n = 935 records) was 58.7% locomotion, 16.2% feed, 10.1% rest, 9.7% forage, and 5.3% social behaviour/others. During foraging, subjects spent 83.9% of their time scanning visually for signs of prey, and only 16.1% manipulating substrates. The diet was frugivorous-insectivorous, with 94.2% of feeding records involving fruit, and 5.8% insects. Nine of the plant species were identified, four of which were previously unrecorded for *S. niger*. All the identified insect prey were orthopterans. The group spent most of its time in the middle to lower forest strata moving on substrates of small diameter. Despite the overall limitations of the data collected, then, the study group exhibited typical tamarin patterns of behaviour, and was clearly well adapted to conditions at the study site, despite the degree of habitat disturbance.

**Key words:** *Saguinus niger*, behaviour, ecology, diet, habitat fragmentation, Amazonia.

### RESUMO

Este estudo apresenta dados sobre a reprodução, o comportamento e a dieta de um grupo silvestre de *Saguinus niger*, em um fragmento florestal urbano no Centro Nacional de Primatas em Ananindeua, Pará (Amazônia oriental). O grupo de estudo variou entre cinco e sete membros, mudando pelo desaparecimento de um adulto e o nascimento de três infantes. Dados quantitativos de comportamento foram coletados através de amostragem de varredura durante cinco dias por mês, entre maio e setembro de 2000, abrangendo a transição entre as estações chuvosa e seca. O orçamento geral de atividades (n = 935 registros) foi de 58,7% de locomoção, 16,2% de alimentação, 10,1% de descanso, 9,7% de forrageio e 5,3% comportamento social/outras. Durante o forrageio, os indivíduos gastaram 83,9% de seu tempo procurando visualmente por presas, e somente 16,1% manipulando substratos. A dieta foi frugívora-insetívora, com 94,2% de registros alimentares envolvendo frutos e 5,8% insetos. Nove espécies de plantas foram identificadas, das quais quatro não tinham sido registradas para *S. niger*. Todos os insetos consumidos foram da ordem Orthoptera. O grupo passou a maior parte de seu tempo nos estratos florestais medianos, utilizando substratos finos. Apesar de todas as limitações dos dados coletados, o grupo de estudo exibiu padrões comportamentais típicos do gênero, e está claramente bem adaptado às condições da área de estudo, apesar do grau de perturbação do habitat.

**Palavras-chave:** *Saguinus niger*, comportamento, ecologia, dieta, fragmentação de habitat, Amazônia.

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## INTRODUCTION

Remnant populations of primates isolated in fragments of habitat are relatively vulnerable to catastrophic events such as fires or epidemics, and may suffer inbreeding depression, all of which increase considerably the risks of local extinction (Lande & Barrowclough, 1987; Lacy, 1997). Such populations nevertheless play an increasingly important role in the conservation of many species, especially in the Atlantic Forest (Ferrari & Diego, 1995; Ruiz-Miranda et al., 2002; Jerusalinsky et al., 2006), but also in Amazonia (Silva, 2003; Gordo, 2005). However, the development of effective management strategies depends on the availability of reliable data on the ecology of the species, primarily in the context of habitat fragmentation.

The callitrichids (marmosets and tamarins) are among the most ecologically tolerant platyrrhines, and many species exhibit a clear preference for disturbed habitat in comparison with primary forest. Some species, such as the common marmoset (*Callithrix jacchus*) may even be abundant in urban environments (Cunha et al., 2006). Morphological specialisations for this “colonising” niche include small body size (Martin, 1992; Ferrari, 1993), which contributes to reduced spatial needs in comparison with larger-bodied primates. The success of callitrichids in marginal habitats may also be reinforced by the lack or absence of competition from these larger, ecologically less tolerant platyrrhines.

Despite being distributed throughout much of southeastern Amazonia (Hirsch *et al.*, 2002), the ecology of *Saguinus niger* is still poorly known, although the available evidence (Oliveira, 1996; Oliveira & Ferrari, 2000; Veracini, 2000) indicates that the behavioural ecology of this tamarin is typical of the genus, and that the species is essentially frugivorous-insectivorous. Data on both *S. niger* (Ferrari & Lopes, 1990, 1996; Veracini, 2000) and the

closely-related *Saguinus midas* (Egler, 1993) indicate that these species may be ecologically more successful than sympatric callitrichids, *Mico argentatus* and *Saguinus bicolor*, respectively. This implies that they may also be more tolerant of habitat disturbance, and in fact Egler (1993) concluded that habitat fragmentation east of Manaus has favoured expansion of the geographic range of *S. midas* into areas previously occupied by *S. bicolor*. Obviously, this process has deleterious implications for the conservation of *S. bicolor* – one of the most endangered of Amazonian primates – and there is an urgent need for more data on all aspects of the ecology of the two species (Gordo, 2005).

With this in mind, this study presents preliminary data on the behavior and diet of a free-ranging group of black-handed tamarins (*S. niger*) in an isolated forest fragment located within an urban environment in eastern Pará. In addition to providing new data on this poorly-known platyrrhine, it is hoped that the study will contribute to the development of effective management strategies for fragmented populations of tamarins throughout the Amazon.

## METHODS

**Study site:** The present study took place in an isolated fragment of approximately 25 hectares of Amazonian *terra firme* forest on the grounds of the National Primate Centre (CENP) in Ananindeua, Pará (01°21' S, 48°22' W). The forest has been logged selectively, but can be characterised as disturbed primary habitat (O. Cardoso, pers. comm.), with trees of up to 30 m and clearings dominated by palms and epiphytes. The area is criss-crossed by a system of trails, some of which are paved. The site is inhabited by one other primate (*Saimiri sciureus*), and a number of small- to medium-sized mammals, such as pacas (*Agouti paca*), sloths (*Bradypus tridactylus*), and squirrels (*Sciurus* sp.).

**Data collection:** A preliminary study took place between January and April, 2000, period that coincides with the first half of the wet season in the study area (monthly precipitation peaked at 481 mm in April). During this period, study group members were identified and habituated to the presence of human observers, and data collection procedures were tested. As the group was small in size, it was possible to distinguish members on the basis of body size and sex.

Between May and September (the dry season normally starts in July-August, although precipitation was never less 100 mm in any given month), quantitative behavioural data were collected in instantaneous scan samples (Martin & Bateson, 1993), using the sampling schedule recommended for callitrichids by Ferrari & Rylands (1994), and also used by Oliveira (1996) and Veracini (2000) in their field studies of *S. niger*. This schedule involves the collection of a one-minute sample at intervals of five minutes. Data were collected on five days each month, with observation sessions lasting between four and seven hours.

At the beginning of each scan sample, the time and location was recorded and, for each subject observed during the scan, the following information was collected: its activity state (categories: travel, forage, feed, rest, social behaviour, and miscellaneous); identity or age-sex class; its height above the ground (m) and the diameter (cm) of the supporting substrate; any other information considered relevant. The foraging category was divided into two subcategories: visual scanning for signs of prey, and manipulation of substrates. The set of information collected for a given subject during a scan is referred to here as a record. Complementary behavioural data were collected throughout the study period, in *ad libitum* (Altmann, 1974) fashion.

The group's activity budget was estimated according to the relative proportions of records of different behavioural categories. The composition of the

diet was based on the relative proportions of feeding records attributed to different food items. Whenever subjects were observed ingesting fruit, the source was marked, and samples were collected for taxonomic identification. Defecated seeds were also collected for identification and measurement. Insects were identified, whenever possible, through the collection of prey parts discarded by the subjects. For the analysis of vertical stratification, records of subject height were grouped in classes of 5 m, following Oliveira (1996).

## RESULTS

**Group composition:** At the beginning of the preliminary study, the group had five members, including three adults (two males and one female), a subadult female and a juvenile male. This increased to six with the birth of a single female infant in early January (no later than the 13th). In June, one of the adult males was captured by a resident of the housing estate located next to the CENP. This animal was released back into the CENP on request of the first author, but it subsequently disappeared, leaving the study group with a total of five members (Table 1).

By the end of July (no later than the 24th), a pair of twins were born, increasing group size to seven members. The infants were first observed on the back of the mother, which was having some difficulty moving and, at one point, was left hanging by her hands and feet, with her back – and the infants – facing the ground. Subsequently, other group members – in particular the adult male – carried infants, a typical behaviour pattern (Digby *et al.*, 2007), although normally only one at a time. During approximately two-thirds of the time, at least one of the infants remained with the mother. Transfers of infants were invariably stimulated by the prospective carrier, by approaching the infant.

By September, these twins were already exploring the environment independently of adult caregivers, principally during feeding bouts. Typically, during feeding bouts, the infants would explore the tree crown on their own, but would be retrieved by one of the adults when the group moved on. During the daily “siesta” period, around midday, the infants engaged in play behaviour, including the attempted capture of butterflies, and chasing small birds.

These infants begged food items from the adults and subadult in typical callitrichid fashion (Tardif et al., 1993), with shrill and insistent vocalisations, and invariably succeeded in obtaining the transfer of the desired food item. While this behaviour patterns is typical of all callitrichids, Oliveira (1996) only recorded food transfer between the mother and her infant in a group of *S. niger*, although this may have been because the infant had only just started to move independently at the time of monitoring.

**Activity budget** Monitoring of the study group was affected by a number of problems, including the capture of the adult male in June, which resulted in group members being more wary of observers during the following days. In subsequent months, which also coincided with major building work within the compound, the group spent increasingly more time – often more than four hours – in a sector of the fragment covered in extremely dense vegetation which had no access trails, as well as visiting the back gardens of houses that adjoin the 5-metre high perimeter wall of the CENP compound.

If monitoring was impaired in the first case, it became impossible in the second, given that there was no way of accompanying the animals beyond the perimeter wall. It wasn’t clear what resources were exploited in the gardens, but occasional shouts heard from neighbours indicate that they may even have entered some of the houses in search of food, a behaviour observed in some other urban callitrichids (SFF, pers. obs.). These visits lasted from 30 to 75

minutes, and were often continuous with time spent in the denser forest. In addition to these problems, administrative restrictions at the site limited data collection to periods of no more than seven hours, rather than continuous monitoring throughout the daily activity period, as originally planned.

Despite all these problems, quantitative data were recorded in a total of 424 scan samples, during which 935 records were collected. While the average number of records collected per scan (2.2) may be considered adequate, considering the size of the study group (Ferrari & Rylands, 1994), the small overall number of records limits conclusions on behaviour patterns. Despite these obvious limitations, the activity budget was typical of tamarin species, with the vast majority of activity time being devoted to locomotion, foraging and feeding (Figure 1). However, it seems likely that locomotion is overestimated relative to the other categories, considering both the observation conditions, as described above, and the intrinsic bias of the scan sampling procedure (Ferrari & Rylands, 1994).

During foraging (n = 150 records), 83.9% of time was spent scanning visually for signs of prey, the remainder being dedicated to the manipulation of substrates. Generally, live leaves and twigs were handled superficially, whereas dead leaves were opened destructively. Holes in the trunks of trees and the forks of branches were also investigated intensively.

**Social behaviour:** Four categories of social behaviour were recorded during the present study: allogrooming, agonism, play, and scent marking. Scent marking was the most frequently observed category, and almost all observations were of sternal and suprapubic marking. Typically, two or three individuals – normally adults – would mark the same place on the substrate consecutively. Circumgenital marking was rare (n = 3 records, two of which by juveniles), and did not involve urination.

Play was also a common behaviour, and invariably involved the immature individuals, which engaged in tag-chasing. On three occasions, birds (two small passeriformes and an immature toucan, *Ramphastos* sp.) were involved in the play, with the tamarins hiding behind branches and then jumping towards the birds. The passeriformes responded by hiding behind branches, whereas the toucan flew away immediately.

Allogrooming was observed primarily during rest periods, during the middle of the day, but were normally very discrete, reducing even further the visibility of the subjects. Juveniles generally groomed adults more than vice versa. In one case, the subadult female groomed the group's oldest adult male, who then inspected and sniffed her genitalia, while she adopted a solicit-grooming posture. At this moment, the male bit the female lightly on the ear and moved away. Agonism was restricted to occasional exchanges of excited vocalisations.

**Diet:** Once again, the relatively small number of feeding records collected ( $n = 85$ ) limits the analysis of the study group's diet. Despite this, the subjects were clearly frugivorous-insectivorous, dedicating 94.2% of feeding time to the ingestion of fruit, and 5.8% to the predation of insects. While plant exudates (gum) are a dietary staple for many callitrichids, the lack of consumption in the present study may be due to the period of the present study, given that in the previous study, gum was only exploited at end of the dry season (November and December), when fruit was scarce.

A total of nine tree species were exploited for their fruit (Table 2), only four of which had been recorded previously (Oliveira & Ferrari, 2000; Veracini, 2000). In four cases, the fruit pulp was ingested together with the seeds, which were defecated intact. It is interesting to note here that the largest seed ingested by *S. niger* in the study of Oliveira & Ferrari (2000) were 18 mm in length, whereas many seeds of larger size (more than 20 mm in length) were not

ingested. Only five insect prey were identified, and all belonged to the order Orthoptera, the archetypal callitrichid prey. Of these, three were grasshoppers (Acrididae), and two were crickets (Grillidae).

**Vertical Spacing:** Study group members were observed at all different levels within in the forest, but clearly preferred the middle to lower strata (Figure 2). Similar preferences have been recorded at other sites (Lopes, 1993; Oliveira, 1996; Bobadilla & Ferrari, 2000; Veracini, 2000). Despite being active at the lower levels in the forest, the subjects came to the ground only very rarely, and only to cross gaps in the forest.

This is consistent with typical tamarin behaviour, and also the preference of larger-bodied sympatric primates, such as *Alouatta* and *Chiropotes* for the higher strata (Bobadilla & Ferrari, 2000). Veracini (2000) recorded a very similar pattern, including the relative predominance of foraging behaviour in the 5-15 m stratum. Substrate sizes (Figure 3) reflect the preference for relatively small branches in the middle strata, and the relatively infrequent practice of the vertical clinging and leaping mode of locomotion on large, vertical trunks.

## DISCUSSION

Behaviour patterns are a fundamental component of the ecology of any primate species, and vary systematically in accordance with local conditions and seasonal fluctuations in resources. Understanding basic patterns and the response to shifts in resource availability provides important insights into evolutionary processes and ecological specialisations. The tamarins (*Saguinus* spp.), essentially Amazonian in their distribution (Hirsch *et al.*, 2002), are highly active frugivore-insectivores, and spend a large proportion of their time foraging and moving rapidly through the forest.

Comparisons among studies are hampered by differences in variables such as sampling procedures, study duration and timing, and group composition (Ferrari & Rylands, 1994). In the case of *S. niger*, all three studies conducted to date have followed the same sampling schedule recommended by these authors, and in this respect, are directly comparable (Table 3). While there are broad overall similarities, there are also notable differences, such as relatively high rates of locomotion in the CENP group, and low levels of social behaviour in Veracini's ECFPn group. Increased locomotion in the CENP group almost certainly reflects observation conditions, as indicated by differences in the number of records collected (for similar periods of data collection). By contrast, the time spent feeding was remarkably consistent across studies, which may reflect the relative importance of this activity.

While diets were also broadly similar, with regard to the predominance of fruit and the relatively minor contribution of arthropods (Table 4), Veracini's (2000) data are divergent in terms of the variety of plant resources exploited. A number of different factors may have contributed to this difference, including forest type, season, and in particular, sympatry with *M. argentatus*. The latter species occurs only at ECFPn, where it feeds intensively on exudates by gouging the bark of gum-producing species such as *Parkia ulei* (Mimosaceae). The behaviour of the marmosets thus provides an alternative resource for *S. niger*, which may be especially important during the dry season. The site at ECFPn was also within an area of continuous forest (rather than a fragment as at the other sites), with extensive river edge habitat and high concentrations of *Symphonia globulifera* (Guttiferae), a major source of nectar for many callitrichids. Most tamarins will consume exudates when or where they are available (Digby *et al.*, 2007), and sympatry with marmosets may be a significant factor in the ecology of a tamarin species (Lopes & Ferrari, 1994).

Despite its methodological limitations, then, the present study has shown that *S. niger* is not only able to survive and reproduce in a highly-disturbed forest matrix, it may do this without major alterations in behaviour patterns. This is promising for the long-term survival of the species, and eventual management of remnant populations in a critically impacted region.

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Table 1. Composition of the study group during the monitoring period.

Month	Adult males	Adult females	Subadult (M/F)	Juvenile (M/F)	Infant
December 1999	2	1	1F	1M	0
January 2000	2	1	1F	1M	1F
June	1	1	1F	1M	1F
July	1	2	1M	1F	2
September	2	2	0	1F	2

M: male; F: female.

Table 2. Tree species exploited for their fruit by the *S. niger* study group at CENP.

Taxon		Seed ingested	Maximum length x width of seed (mm)	Recorded for <i>S.m. niger</i> by
Boraginaceae	<i>Cordia</i> sp.	No		Veracini (2000)
Elaeocarpaceae	<i>Sloanea</i> sp.	Yes	12 x 10	-
Apocynaceae	<i>Lacmellea</i> sp.	No		Veracini (2000)
Chrysobalanaceae	<i>Licania alba</i>	No		-
Ochnaceae	<i>Ouratea</i> sp.	Yes	11 x 8	-
Mimosaceae	<i>Parkia nitida</i>	Yes	23 x 10	-
Moraceae	<i>Pouroma guianensis</i>	No		Oliveira & Ferrari (2000)
Sapotaceae	<i>Pouteria</i> sp.	Yes	14 x 7	Oliveira & Ferrari (2000)
Anacardiaceae	<i>Tapirira guianensis</i>	No		Veracini (2000) <sup>1</sup>

<sup>1</sup>Exudate exploited.Table 3. Activity budgets recorded for *S. niger* (all studies followed the same scan sampling schedule).

Study site	% of time spent:					N records	Source
	Travel	Forage	Feed	Rest	Misc.		
CENP	58.70	9.70	16.21	10.06	5.33	935	Present study
Fazenda Vitória	46.08	15.19	16.73	17.46	4.54	3659	Oliveira (1996)
ECFPn	42.38	18.86	17.64	14.50	0.53	1904	Veracini (2000)

Table 4. Composition of the diet of *S. niger* at different study sites.

Study site	% of feeding records:				Source
	RPPs <sup>1</sup>	Exudate	Animal prey	Nectar	
CENP	94.2	-	5.8	-	Present study
Fazenda Vitória	87.5	3.1	9.4	-	Oliveira & Ferrari (2000)
ECFPn	48.8	23.8	4.5	22.9	Veracini (2000)

<sup>1</sup>Reproductive Plant Parts (fruit, flowers).

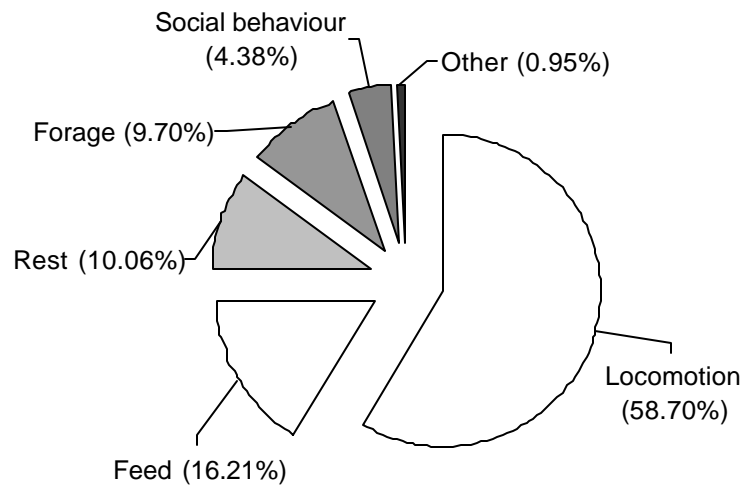


Figure 1. General activity budget of the *S. niger* study group (n = 935 records). The category “other” refers to defecation and urination.

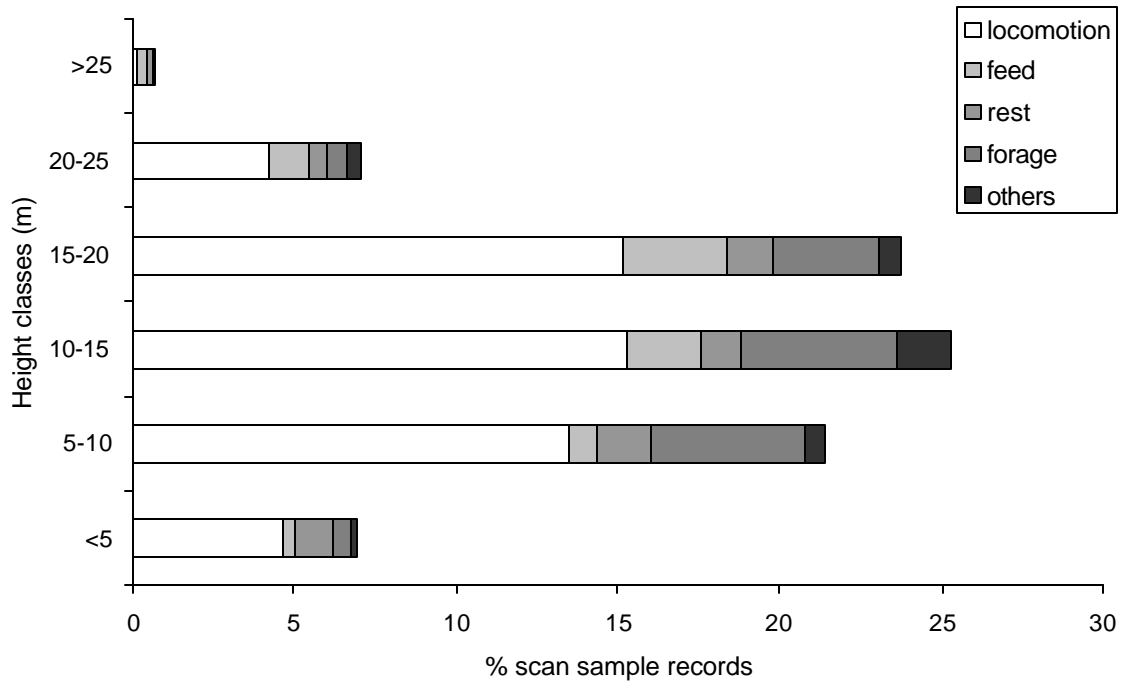


Figure 2. Use of different forest strata by the *S. niger* study group, according to scan sample records (n = 935). “Others” includes social behaviour and defecation/urination.

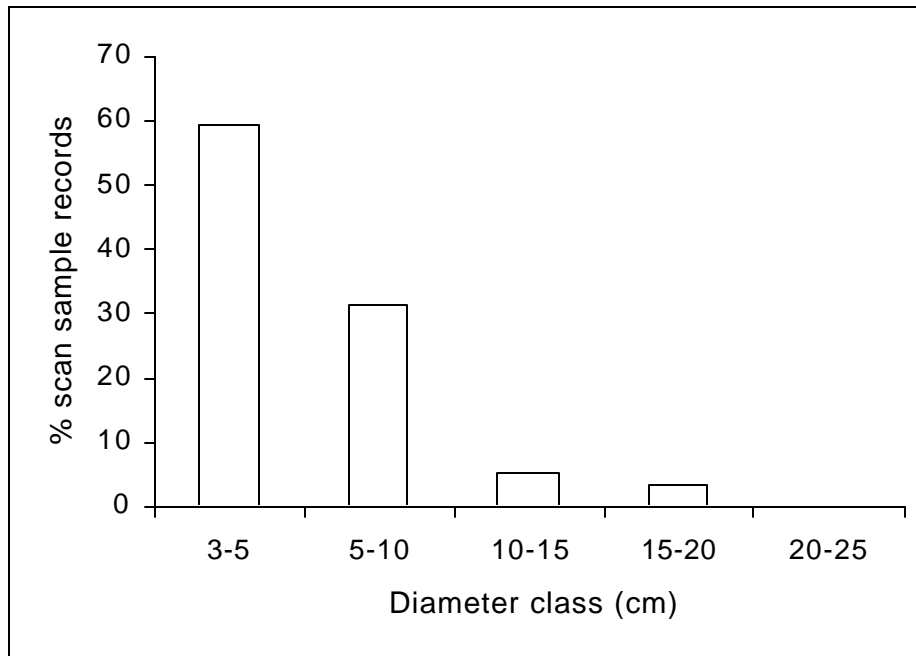


Figura 3. Use of substrates of different sizes by the *S. niger* study group, according to scan sample records (n=935).

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