An Acad Bras Cienc (2024) 96(Suppl. 1): e20231272 DOI 10.1590/0001-3765202420231272
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Identification of insect body fragments found in fecal samples of *Sapajus nigritus* (Primates: Cebidae) from five Conservation Units in Rio de Janeiro state, Brazil

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Abstract: *Sapajus nigritus* (Primates: Cebidae) is endemic of the Atlantic Forest, occurring from the Southeastern and Southern regions of Brazil to Northeastern Argentina. This species plays a role in two ecosystem services: seed dispersal through endozoochory and insect population control in agricultural plantations and forest environments. Fruits and invertebrates represent approximately 90% of their diet, and there is a large number of insects in the diet of *S. nigritus* in urban and conserved areas. However, it is known that insect diversity decreases in anthropized environments. Our objective was to identify the insects present in the fecal samples of *S. nigritus* from five Conservation Units in Rio de Janeiro state, Brazil. We aim to estimate the percentage of each taxon of insects found in feces either, hypothesizing that there are a greater variety of insect species in the diet of *S. nigritus* that inhabit preserved forested areas. A fecal screening was conducted using a light microscopy and the insects were identified based on their external morphology. Insect fragments were found in eight out of ten fecal samples of *S. nigritus*, revealing that they belonged to insects from five orders: Hymenoptera, Hemiptera, Orthoptera, Coleoptera and Blattodea, suggesting a good conservation status of the sampling areas.

Key words: ecosystem services, invertebrates, neotropical primates, primate diet.

INTRODUCTION

The capuchin monkey, *Sapajus* spp. (Primates: Cebidae), inhabits the north region of South America, Southern Brazil and Northeastern Argentina (Vilanova et al. 2005, Lynch Alfaro et al. 2012). Currently, eight species are recognized: *Sapajus apella*, *S. macrocephalus*, *S. libidinosus*, *S. cay*, *S. robustus*, *S. xanthosternos* and *S. nigritus* (Lynch Alfaro et al. 2012). The latter, *Sapajus nigritus* (Goldfuss 1989), is endemic to the Atlantic Forest, with a distribution mostly in the Southeastern and Southern regions of Brazil, as well as Northeastern Argentina (Rylands et al. 2005, Lynch Alfaro et al. 2012). As an opportunistic feeding species, *S. nigritus* also occupies forest fragments near agricultural plantations and urban areas (Cowlishaw & Dunbar 2000, Corrêa et al. 2018, Gonçalves 2019, Mikich et al. 2015) and according to its trophic plasticity, its diet tends to be adapted to the available resources (Brown & Zunino 1990, Ludwig et al. 2005). Due to the variety of their diet, these primates play an important role in ecosystem services, such as seed dispersal through endozoochory and insect population control in agricultural plantations and forest environments (Mikich et al. 2015, Liebsch et al. 2018).

Sapajus nigritus (Primates: Cebidae) is omnivorous with a diet based on leaves, sap, seeds, nectar, eggs, ripe, small vertebrates, fruits, and invertebrates (Ludwig et al. 2005, Mikich 2001). The last two items represent approximately 90% of their diet (Mikich 2001, Ludwig et al. 2005, Gonçalves 2019). This species has wide canine teeth capable of tearing food (Mikich & Liebsch 2014) and has specialized abilities for food capture and manipulation, which allow them to dig surfaces by removing tree bark to search for small invertebrates (Mannu & Ottoni 2009). In addition, the protein intake is directly related to the consumption of invertebrates (Rothman et al. 2014, Santos 2015), and this essential nutrient is a limiting component for wild animals because, for vertebrates, they are only provided by feeding (Santos 2015, Barboza et al. 2009). Its varied diet ensures nutritional and energy sources that contribute for survival and individual reproduction (Garber 1987, Jesus et al. 2022). A wide insect variety has been found in the *S. nigritus* diet, including the orders Orthoptera, Hymenoptera, Hemiptera, Coleoptera, Blattodea, Diptera and Lepidoptera (Mikich et al. 2015, Ludwig et al. 2005).

In areas with a high degree of conservation, invertebrates are food items often described in the primate diet (Pickett et al. 2012, Jesus et al. 2022). Similarly, in forest fragments even when close to urban centers and agricultural plantations, it was found that *Sapajus* sp. does not abandon wild foods, including the consumption of invertebrates (Galetti & Pedroni 1994, Gonçalves 2019). In a forest fragment in the urban matrix in Foz do Iguaçu in Paraná state, Southern Brazil, despite the abundant supply of anthropogenic food sources, it was noticed a high consumption of invertebrates (45.73%), followed by fruits (40.41%) among the items identified in the diet of *Sapajus* spp. (Gonçalves 2019).

Within invertebrates, insects are highly occurring organisms in tropical forests, accounting for 89% of terrestrial animals (Buzzi & Miyazaki 1993), but a reduction in insect diversity due to natural disturbances such as environmental fragmentation and anthropization is observed (Thomazini & Thomazini 2002). Thus, insects are bioindicators of environmental impacts and have varied roles in ecosystem functioning (Thomazini & Thomazini 2002). Considering the importance of assessing the actual need for conservation, since the S. nigritus is significantly impacted by natural disturbances, particularly forest resource exploitation (Chapman et al. 2013), and understanding the dietary intake of primates, according to their diet flexibility and the location they inhabit, can provide us information on the impact of primates and their ecological functions for species conservation planning (Chapman et al. 2012).

There are several methodologies to evaluate the diet of primates, among them, the analysis of fecal samples (Moreno-Black 1978, Mikich et al. 2015, Ludwig et al. 2005), direct observation of individuals foraging behavior (Gonçalves 2019, Moreno-Black 1978, Bione 2011), and metagenomic studies (Pickett et al. 2012). The first type of analysis can be done from samples obtained during captures for different research purposes and even in the case of samples found in resting places of primate groups (Pickett et al. 2012).

Our objective was to identify by light microscopy the taxonomic order of insects found in the diet of *S. nigritus* through fecal samples collected during the capture of individuals in five Conservation Units of the Atlantic Forest in the state of Rio de Janeiro. We will seek to advance to the maximum in identification. We hypothesized that there are a variety of insect species in the diet of *Sapajus nigritus* that

inhabit preserved forested areas. Additionally, we aim to estimate the percentage of each taxon of insects found in feces.

MATERIALS AND METHODS

Sampling areas

Sampling was conducted in the following five Conservation Units in Rio de Janeiro state: Itatiaia National Park (IT), Marambaia Island (MI), Tijuca National Park (TI) Poço das Antas Biological Reserve (PA) and Serra dos Órgãos National Park (SO) (Figure 1). IT is located in Serra da Mantiqueira (22°27'S/44°36'W) with 280 km^2 (Morim & Barroso 2007) covering the municipalities of Resende and Itatiaia in the Southwest of Rio de Janeiro state and South of Minas Gerais state. The area has mountainous relief with the Pico das Agulhas Negras (the fifth highest point in the country), and it is the first National Park in Brazil by state decree 1.173/37 (Brasil 1937). MI is in the municipality of Mangaratiba in the South of the state of Rio de Janeiro (23°04'S/43°53'W), has 42 km^2 of area with mountainous relief and is included in the Environmental Protection Area of Mangaratiba by state decree 9.802/87 (Brasil 1987). TI is part of Serra da Carioca (22°57'S/43°14'W) located in Rio de Janeiro city, comprising an area of 32 km^2 (Freitas et al. 2006) and was categorized as a National Park by federal decree 50.923/61 (Brasil 1961). PA in Casimiro de Abreu and Silva Jardim municipalities (22°30'S/42°15'W), has 50 km^2 and was the first Biological Reserve in Brazil by decree 73.791/74 (Brasil 1974). SO (22°23'S/43°10'W) covers four municipalities (Petrópolis, Magé, Guapimirim and Teresópolis) in the state of Rio de Janeiro with 200 km^2 of area

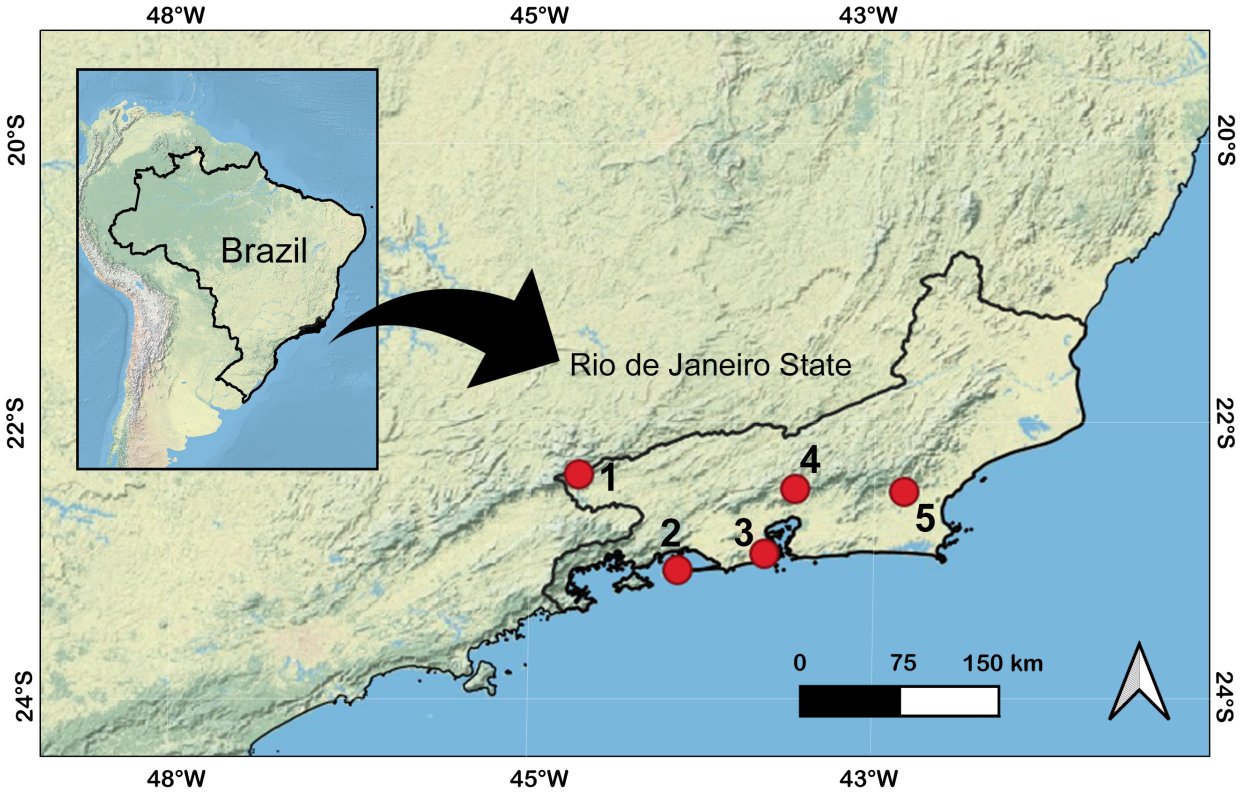

Figure 1. ***Sapajus nigritus*** **fecal samples collection sites in five Conservation Unities in the state of Rio de Janeiro, Brazil. (1) Itatiaia National Park; (2) Marambaia Island; (3) Tijuca National Park; (4) Serra dos Órgãos National Park; (5) Poço das Antas Biological Reserve.**

(Guimarães et al. 2009, Aximoff et al. 2015) and was created by decree 1.822/39 (Brasil 1939).

Sample collection and fecal analysis

Ten individuals of *Sapajus nigritus* were captured at different times in five Conservation Unities in Rio de Janeiro state, with some degree of environmental protection: Marambaia Island (MI), Tijuca National Park (TI), Itatiaia National Park (IT), Poço das Antas Biological Reserve (PA) and Serra dos Órgãos National Park (SO) in 2015, 2017 and 2019. The fecal samples were collected from five adult males (three from MI and two from TI), one sub-adult male (SO), that has intermediate characteristics between the adult and young (Izawa 1980), one young male (MI), two adult females (from MI and PA), and one young female (IT), that did not have marked tufted hair on the crown (Izawa 1980).

The animals were captured with Tomahawk traps (70 x 40 x 40 cm and 115 x 45 x 45 cm, being CxLxA) set up on top of bamboo platforms. The individuals were sedated with intramuscular injection of Ketamine hydrochloride (15 mg/kg) and Midazolam (0.05/kg), in the thigh for collection of blood and morphological measurements for population genetic analysis. The feces were collected during these procedures and each one was stored in 70% ethanol solution in Falcon tubes of 15 mL, kept refrigerated. After recovering from sedation, the individuals were released at the same place of capture. All procedures were approved by the Ethic Committee of Animal Use from the Instituto de Ciências Biológicas e da Saúde of the Universidade Federal Rural do Rio de Janeiro (013/2018) authorized by SISBIO/ICMBio (license 57417-6).

The fecal screening was performed with a NOVAXTX-5C stereomicroscopic with a 20x magnification. The samples were placed in a Petri dish and with the aid of fine-tipped tweezers, fruit remains, seeds and invertebrates body fragments were separated and stored under refrigeration. The invertebrates found were photographed at different angles with a Leica M205C stereomicroscope, obtaining photographs for staking performed in LAS Suite V2.0 software aiming to achieve the most accurate taxonomic identification.

RESULTS

Insect fragments were found in the eight fecal samples of ten different individuals of *Sapajus nigritus* captured from five Conservation Unities in the state of Rio de Janeiro, Brazil. The insect body parts found were legs, heads, antenna, and abdomen.

Insect fragments of Hymenoptera order were evidenced by ambulatory legs with basitarsus represented by the first article of tarsus enlarged (Figure 2a). An intact prey composed of a head with geniculate antenna represented by an elongated scape and pedicel with flagellum forming a 90-degree angle characteristic of ants of the genus *Solenopsis* from the Formicidae family (Figure 2b). Beyond this, two pointy tubular abdomens with absence of appendages named as gaster, observed in insects of suborder Apocrita of the Hymenoptera family (Figure 2c and 2d), was observed. This order was found in five fecal samples, two from MI, two from TI and one from PA (Table I).

A leg with the presence of sensory structures of locusts which characterize an arolium (Figure 2e), many tibias with the presence of thorns, the salter leg of the third pair of legs, with thorns facing back (Figure 2f and 2g) were found indicating the presence of insects of the order Orthoptera. Insect fragments of this order were found in four samples, two samples from MI, one from TI and one from PA, among the food items of *Sapajus nigritus* (Table I).

Figure 2. Classification of insects (CI) found in fecal samples of *Sapajus nigritus* that inhabit Conservation Units in the state of Rio de Janeiro. (a) Hymenoptera leg; (b) Ant of the genus *Solenopsis* (Formicidae: Hymenoptera); (c) and (d) Two gasters of suborder Apocrita (Hymenoptera); (e) Leg with arolium of order Orthoptera; (f) and (g) Tibias with the presence of thorns of order Hymenoptera; (h) A beetle leg of the Scarabaeidae family, Coleoptera; (i) Beetle leg of order Coleoptera; (j) Beetle body (Coleoptera); (k) A beetle leg of the family Curculionidae, Coleoptera; (l) Antenna of suborder Heteroptera, Hemiptera; (m) Blattodea antenna.

From the order Coleoptera it was possible to identify two families based on the fragments of insects found among the diet of *S. nigritus*. One leg of beetle with dilated tibias and jagged or wavy margin (Figure 2h) of the family Scarabaeidae was found in a sample of MI (Table I). A leg with the bilobate tarsomere, probably being of the Curculionidae family (Figure 2k), a dorsal part of a beetle body with a rigid wing named elytra (Figure 2j) and abeetle leg (Figure 2i) were found in TI samples (Table I).

A part of a setaceous antenna with an escape, pedicel and the first flagellomere, judgingby the length of this last segment is characteristic, probably, of bedbugs, order Hemiptera and suborder Heteroptera (Figure 2l). This fragment was found in a fecal sample of MI (Table I).

From the order Blattodea just one beaded antenna with rounded antennomeres (Krishna et al. 2013) (Figure 2m) was found in one sample of MI (Table I).

In 50% of the fecal samples analyzed from *Sapajus nigritus*, the order Hymenoptera was found and identified, including the suborder Apocrita, family Formicidae, down to the genus *Solenopsis*. In 40%, the order Orthoptera was identified. The order Coleoptera, including the families Scarabaeidae and Curculionidae, was identified in 37.5% of the samples. The orders Hemiptera, including the suborder Heteroptera, and the order Blattodea were found in 12.5%. In 10% of the samples, it was not possible to identify any taxonomic level. In 20% of the samples, no insect fragments were found (Figure 3).

Table I. Number of insect fragments (NIF) from fecal samples of *Sapajus nigritus* (Primates), collected in five Conservation Units (CU) in the state of Rio de Janeiro, Brazil, taxonomic orders identified and date of collection.

CU/ID	Date	NIF	CI			
			O	S	F	G
MI01/A	23/04/2015	2	HYM	NI	NI	NI
MI02/Y	23/04/2015	-	-	NI	NI	NI
MI03/A	23/04/2015	3	ORT	NI	NI	NI
MI04/A	27/05/2017	19	HYM, ORT, HEM	APOC, HETE	NI	NI
MI05/A	15/11/2019	5	COL, BLA	NI	SCAR	NI
IT01/Y	22/11/2018	2	NI	NI	NI	NI
TI01/A	07/09/2019	7	HYM, ORT	NI	NI	SOLE
TI02/A	08/09/2019	7	HYM, COL	NI	NI	NI
PA01/A	27/09/2018	12	HYM, ORT, COL	APOC	CURC	NI
SO01/SA	01/11/2018	-	-	NI	NI	NI

CU: Conservation Units; ID: Identification of individuals; NIF: Number of insect fragments; CI: Classification of insects; MI: Marambaia Island. IT: Itatiaia National Park. TI: Tijuca National Park. PA: Poço das Antas Biological Reserve. SO: Serra dos Órgãos Biological Reserve. A: Adult; Y: Young; SA: Sub-adult; O: Order; S: Suborder; F: Family; G: Genus; HYM: Hymenoptera; ORT: Orthoptera; HEM: Hemiptera; COL: Coleoptera; BLA: Blattodea; APOC: Apocrita; HETE: Heteroptera; SCAR: Scarabaeidae; CURC: Curculionidae; SOLE: *Solenopsis*; -: Not found; NI: No identified.

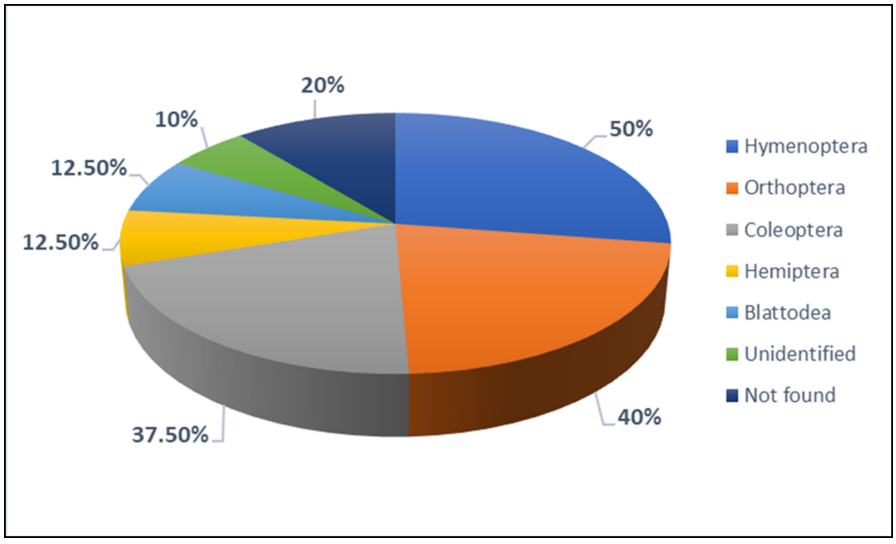

Figure 3. Percentage of fecal samples from Sapajus nigritus in which insect orders were found and identified, samples where insects were found but remained unidentified, and samples where no insects were found.

DISCUSSION

In 80% of the fecal samples at least one insect order was found. Among these, it was possible to identify insects in 90%. In the only sample of IT, two insect fragments were found, but they could not be identified. Despite being insect body parts, confirmed by their stiffness and appearance, they were broken, making it difficult to identify them. In one sample of MI and in the only one of SO, the presence of insects in the diet was not detected. Out of the total of 57 insect fragments, the highest number was detected

in MI (19), followed by PA (12), from three orders in both cases: Hymenoptera and Orthoptera in both, and Hemiptera and Coleoptera in each, respectively.

The fecal sample with the highest quantity of insect fragments was collected in May from Marambaia (MI04), which corresponds to a dry season. This finding supports the hypothesis that insect consumption is expected to increase during this season (Gonçalves 2019, Galetti & Pedroni 1994, Fragaszy et al. 2004) as capuchin monkeys tend to adopt a more generalist diet (Gonçalves 2019).

All the orders found in this study have already been described in the diet of primates, including *Sapajus* spp. (Gonçalves 2019, Ludwig et al. 2005, Jesus et al. 2022), with the predominance of the orders Orthoptera, Hymenoptera and Coleoptera (Mikich et al. 2015, Moreno-Black 1978, Ludwig et al. 2005). Furthermore, the consumption of insects from the order Blattodea has also been described predominantly in the diet of *Sapajus* sp. (Jesus et al. 2022).

The diversity of consumed insects tends to vary according to the location and primate species. Regarding studies with *Sapajus* spp., investigating the diet of 60 individuals of *Sapajus nigritus* from an urban forest remnant by identifying insect fragments through direct observation and fecal sample analysis, five orders of insects were found, including Coleoptera, Hemiptera, Hymenoptera, Diptera and Lepidoptera (Ludwig et al. 2005). Whereas in a preserved area, examining 29 stomach samples from *Sapajus* spp., nine insect orders were identified, including Hymenoptera with the Formicidae family, as well Coleoptera, Orthoptera, Hemiptera, Blattodea, Diptera, Lepidoptera, Arachnida and Myriapod orders (Jesus et al. 2022). The identification of five insect orders in ten fecal samples from *S. nigritus*, is suggestive of a good state of conservation in the areas where our samples were collected. It is important to highlight that in areas with high degrees of conservation, primates tend to frequently consume insects (Pickett et al. 2012, Jesus et al. 2022).

In forest fragments surrounded by plantations, wild species need to adapt their diet to survive (Lowry et al. 2013). *Sapajus nigritus*, a resident of these locations, is considered an agricultural pest by rural producers, as they invade the plantations while foraging (Ludwig et al. 2006). However, it has been demonstrated that they provide an environmental service by controlling insects, which are also considered pests to the crops (Mikich et al. 2015). So, despite the decrease in insect diversity in fragmented areas due to natural disturbances (Thomazini & Thomazini 2002), capuchin monkeys still consume this food item in these areas to supplementtheir diet.

Finally, the identification of insects found in fecal samples of *S. nigritus* through light microscopy was an efficient method, allowing for identification at the genus level, Solenopsis sp. (Formicidae: Hymenoptera). Typically, when using this method, the identification of insects is limited to the order, suborder, and family (Ludwig et al. 2005, Mikich et al. 2015, Gonçalves 2019).

Acknowledgments

We thank the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the research support. We thank to Vinícius da Rocha Miranda and Gustavo Henrique Soares Guedes, Ph.D. students from the Universidade Federal Rural do Rio de Janeiro/UFRRJ for the assistance with the insect photographs, to the graduates Carolina Penha da Silva Branco and Fernanda Soares Nogueira, as well as to José Francisco Santos da Silva, laboratory technician, for their support at various stages of the analysis, and to Vinícius Siqueira Gazal e Silva, agronomist and professor in the Department of Entomology and Plant Pathology at UFRRJ, for the assistance in identifying the insect fragments.

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How to cite

MEDEIROS JM, PENEDO DM, RESENDE ALS & NOGUEIRA DM. 2024. Identification of insect body fragments found in fecal samples of *Sapajus nigritus* (Primates: Cebidae) from five Conservation Units in Rio de Janeiro state, Brazil. An Acad Bras Cienc 96: e20231272. DOI 10.1590/0001-3765202420231272.

Manuscript received on December 1, 2023;
accepted for publication on May 31, 2024

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Author contributions

JMM and DMN designated the study. DMP conducted the field work with the collection of fecal samples. JMM conducted laboratory experiments analyzing the insects by a light microscopy and photographing the fragments. ALSR identified the insects through the photographs. JMM, DMN, ALSR and DMP wrote the manuscript, read, and approved the final version.