https://doi.org/10.21708/avb.2025.19.2.12780

ORIGINAL ARTICLE

ACTA VETERINARIA BRASILICA

ISSN: 1981-5484

Identification of agents potentially causing dermatological problems in sloths *Bradypus variegatus* (Schinz, 1825) from brazilian northeastern states

Identificação de agentes potencialmente causadores de agravos dermatológicos em bichos-preguiça *Bradypus variegatus* (Schinz, 1825) provenientes de estados do nordeste brasileiro

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ABSTRACT: This study identified ectoparasites and dermatophyte fungi in the fur of sloths of the *Bradypus variegatus* species from different states in the Brazilian Northeast. For this purpose, the animals were divided into two groups: group 1 without lesions and group 2 with lesions. In the presence of macroscopic ectoparasites, specimens were removed and classified. A Wood's lamp was directed toward the furs of the sloths. Trichograms were performed, in which hairs were chosen randomly from the animals in group 1; while, in group 2, the hairs were extracted from the periphery of the lesion. In sloths with lesions, impressions were also made using adhesive tape, and the injured areas were scraped to prepare slides to identify dermatophyte fungi and microscopic ectoparasites. Part of the hair obtained in both groups was inoculated into VetCheck culture medium, and in case of fungal growth, a subculture was performed followed by a microculture. Based on the data obtained, the macroscopic ectoparasite *Amblyomma* sp. was observed in an uninjured pup. In an adult female, in one of her lesions, the microscopic ectoparasite *Lobalges trouessarti* was found. The Wood's lamp demonstrated fluorescence in all animals in group 2 and a large part of group 1, with the presence of the dermatophyte *Nannizzia gypsea*, confirmed through microscopic analysis of the macroconidia.

Keywords: Xenarthra; *Bradypus variegatus*; ectoparasites; dermatophytes.

RESUMO: Propôs-se identificar ectoparasitas e fungos dermatófitos no pelame de preguiças da espécie *Bradypus variegatus*, oriundas de diferentes estados do Nordeste brasileiro. Para isso, os animais foram distribuídos em dois grupos: o grupo 1, sem lesões cutâneas e o grupo 2, animais que apresentavam lesões. Mediante a presença de ectoparasita macroscópico, o espécime foi removido e classificado. A lâmpada de Wood foi aplicada sobre o pelame das preguiças. Realizou-se tricogramas, onde nos animais do grupo 1 os pelos foram escolhidos aleatoriamente; enquanto que, no grupo 2, os pelos foram extraídos da periferia da lesão. Nas preguiças com lesões, foram feitos ainda, a impressão com fita adesiva e o raspado das áreas lesionadas para preparação de lâminas, a fim de se identificar fungos dermatófitos e ectoparasitas microscópicos. Parte dos pelos obtidos em ambos os grupos foram inoculados em meio de cultura VetCheck e em caso de crescimento fúngico, foi realizado o repique seguido do microcultivo. Com base nos dados obtidos, foi observado o ectoparasita macroscópico *Amblyomma* sp., em um filhote sem lesão. Enquanto que, em uma fêmea adulta, numa de suas lesões, foi encontrado o ectoparasita microscópico *Lobalges trouessarti.* A lâmpada de Wood demonstrou fluorescência em todos os animais do grupo 2 e grande parte do grupo 1, sendo a presença do dermatófito *Nannizzia gypsea* confirmada a partir da análise microscópica dos macroconídeos.

Palavras-chave: Xenarthra; *Bradypus variegatus*; ectoparasitas; dermatófitos.

INTRODUCTION

Sloths, anteaters, and armadillos are included in the superorder Xenarthra. Its origin dates back to before the separation of Gondwana, and this group experienced its geographic isolation mainly during the Cretaceous Period, in the Mesozoic Era (Grassé, 1955; Engelmann, 1985; Hedges *et al*., 1996; Larrazábal, 2004). In the Pleistocene, many species, especially the large-sized ones, became extinct (Cartelle, 2000; Pough *et al.,* 1993). Xenarthrans are distributed into two orders: *Cingulata*, whose representatives have bodies covered by bony deposits in the form of plates, the osteoderms, as in armadillos, and the order *Pilosa*, in which the specimens have bodies covered by fur, as in the case of sloths and anteaters (Gardner, 2005).

Sloths are specifically distributed into two families, Bradypodidae (three-toed sloths) and Megalonychidae (two-toed sloths), with the genera *Bradypus* and *Choloepus*, respectively (Britton, 1941).

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Received: 01/16/2025.
Accepted: 05/27/2025.

They are found in forest regions of Tropical America, from Honduras and Nicaragua in the north, through Panama and further south, in Brazil and Bolivia. They occur on both sides of the Andes, a fact that early observers did not recognize (Gardner, 2005). Animals of the *Bradypus* genus are present in low-altitude neotropical locations and are especially prevalent in regions where the temperature does not fluctuate greatly, that is, in the depths of the forests (Britton, 1941). The *Bradypus variegatus* species (Schinz, 1825) is found mainly in the Amazon and in the Northeast of Brazil (Amorim *et al*., 2003).

Bradypodids are highly specialized in terms of anatomical and physiological characteristics, and in dietary and behavioral habits, in addition to peculiar ecological interactions (Britton, 1941). One of these particular morphological characteristics refers to the anatomy of their coat, which consists of long and thick hairs with irregular transverse cracks that increase in number and size with age (Aiello, 1985). In these cracks in their fur, occurrences of green algae identified as *Trichophilus* spp. have been reported, with which they are believed to maintain a symbiotic relationship (Pauli *et al*., 2014). Many researchers state that this is a valid assumption and that the association between the three-toed sloth and the algae encrusted in its fur is advantageous for both, with the algae obtaining shelter in the cracks of the hairs, while providing green camouflage, protection against ultraviolet rays, and dietary supplementation for the sloth (Aiello, 1985; Karsten *et al*., 2005; Suutari *et al.*, 2010; Pauli *et al*., 2014). A commensal relationship has also been attributed between these animals and pyralid moths (*Cryptoses* spp.), in which the moths require the association, but as they do not feed on the sloth's tissues, they impose no consequences on their host (Waage; Montgomery, 1976). When a sloth descends from a tree and defecates, pregnant female moths leave the animal's fur and oviposit in the feces. The larvae are coprophagous, developing entirely within the dung, and the adults emerge and fly to the canopy to find their mating ground in the sloth's fur and continue their life cycle (Pauli *et al*., 2014).

Another peculiar characteristic of the hairs of bradypodids is their unique transverse cracks, which retain water and allow the hair shaft to become saturated with rainwater, which could enable the absorption of the liquid like a sponge, possibly making it an even more ideal environment for algae, and leading to speculations that sloths receive nutrients from them by diffusion along the spongy outer portion of the hairs, followed by absorption through the skin (Aiello, 1985), although this process is not yet fully understood. However, it is known that the accumulation of water favors the development of algae that produce exopolymeric substances, which may give the hair the desired texture or allow the growth of beneficial bacteria (Karsten *et al*., 2005).

On the other hand, humidity may contribute to the development of opportunistic fungi that cause alopecia and dermatomycoses, potentially leading to secondary bacterial infections with itching and pustules (Xavier *et al*., 2008). In addition, such a condition may also encourage the occurrence of other organisms such as moths, cockroaches, beetles (Britton, 1941; Wujek; Cocuzza, 1986), and ectoparasites such as mites, ticks, and lice (Waage; Best, 1985; Diniz *et al*., 1997). However, information on fungal species that cause dermatophytoses and other agents associated with dermatological conditions in sloths is scarce, with few reports, and these are related to pathogens that are more common in domestic animals such as dogs and cats (Silva *et al.*, 2002; Chermette *et al*., 2008).

Due to the scarcity of data on skin problems that may affect sloths and the need to understand the pathology in order to guide the treatment of the animal, this study aimed to identify ectoparasites (macro and microscopic) and filamentous and dermatophyte fungi in the fur of sloths from different northeastern states, in order to contribute information that supports clinical decision-making to aid in the conservation of the species *B. variegatus* and the development of new research.

MATERIAL AND METHODS

This research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SinGen), under number AC9035E, and was conducted with authorization from the Ethics Committee on the Use of Animals of the Federal Rural University of Pernambuco (CEUA/UFRPE), number 2425250621. It also obtained approval from the Biodiversity Authorization and Information System of the Chico Mendes Institute (SisBio/ICMBio), number 46665/11. Sloths of the *Bradypus variegatus* species were used. Semiological evaluations of the fur were performed through visual inspection with the aid of a magnifying glass, searching for possible alterations in hair and skin such as alopecia, abscesses, crusts, and pustules, as well as the collection of macroscopic ectoparasites, when present. Once this was done, the animals were divided into two groups: group 1, composed of animals without skin lesions, and group 2, composed of animals presenting some type of lesion. Sloth analyses were carried out at two locations: the Recife Zoo, Dois Irmãos State Park, Recife, Pernambuco, through the Instituto Preguiça-de-Garganta-Marrom (IPGM), and at the Wildlife Screening Center (CETAS/Tangara), Recife, Pernambuco. Macroscopic organisms found on the fur were removed by using tweezers and stored in properly identified vials containing 70% ethanol, for subsequent classification at the Parasitology Laboratory of the Federal Rural University of Pernambuco (LAPAR/UFRPE).

Group 1 consisted of 10 animals: one adult female from the city of Cabo de Santo Agostinho, Pernambuco; one female and one young male from João Pessoa, Paraíba; one young male from Maceió, Alagoas; and another from Recife, PE, all rescued and sent to the IPGM at the Recife Zoo; one adult male, one young male, and one young female from Camaragibe, Pernambuco; and two juveniles of undetermined sex, one from Camaragibe, Pernambuco, and another from the municipality of Garanhuns, Pernambuco, both sent to CETAS/Tangara. For sample collection, the animals were combed by using a stainless-steel comb, and some of the hairs that fell out were randomly selected for

trichogram analysis. These hairs were placed on slides identified with the animal's name. The hairs were aligned in the same orientation, from root to tip, stabilized with mineral oil, and covered with a coverslip for fixation. The slides were examined under a light microscope to evaluate the presence of morphological abnormalities in the hairs.

Some of the hairs collected after combing were stored in sealed containers wrapped with plastic film, properly labeled, and sent to the Agricultural and Environmental Microbiology Laboratory of the Federal Rural University of Pernambuco (LAMAA/UFRPE). These hairs were inoculated in VetCheck culture medium (Dermatophytes – Tecsa Laboratories) and incubated at room temperature, monitored over a 14-day period. In cases of medium color change and fungal growth, microbial subcultures were performed by transferring material to Petri dishes containing Sabouraud Dextrose Agar supplemented with chloramphenicol. Once fungal growth was observed on the plates, microculture was performed, and slides were stained with Amman's Lactophenol or Cotton Blue for fungal sample identification. Additionally, animals were inspected with a Wood's lamp, with a wavelength of 360 nm, in a dark room, to also evaluate the presence of such fungi.

Group 2 consisted of five specimens: one adult female from Recife-Pernambuco; one young male from João Pessoa-Paraíba, both rescued and sent to IPGM at the Recife Zoo; and three adult females, one from Camaragibe, Pernambuco; another from Escada, Pernambuco; and another from Jaboatão dos Guararapes, Pernambuco, rescued by CETAS/Tangara. A detailed mapping of lesion occurrence in anatomical regions was made based on thorough observation of the fur. To determine abnormalities in hair morphology, and the presence of microscopic ectoparasites, hyphae, and arthroconidia, a trichogram and adhesive tape impression method on the lesion site were performed. Hairs were removed from the lesion periphery, since alopecias on the lesion were total. Extraction of hairs was performed by using hemostatic forceps with tips wrapped in pieces of cuff to epilate them completely in the direction of hair growth to avoid trauma. Hairs were placed on slides and analyzed following the same methodology as applied to group 1.

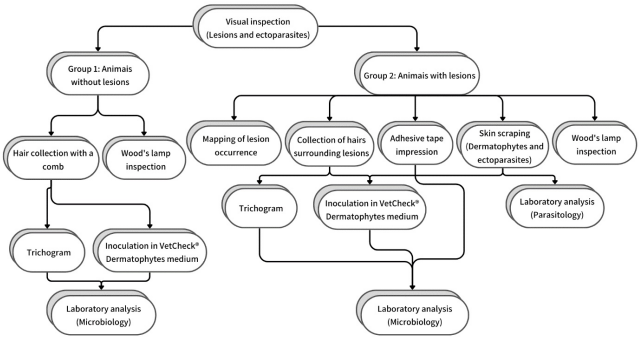
For verification of hyphae and arthroconidia, some hairs were placed in sealed containers, labeled, and sent to LAMAA/UFRPE, where the same incubation method used for group 1 samples was followed. The adhesive tape impressions from the lesions were also intended to confirm fungal presence. The tapes were adhered to microscope slides, identified, and sent to LAMAA/UFRPE. At LAMAA, slides were stained by using the Amman's Lactophenol method: the tape edge was lifted, a drop of stain applied to the slide, the tape replaced, and the slide observed under a light microscope.

To identify dermatophyte agents and assess microscopic ectoparasites, skin scrapings were performed at the lesion sites. Wood's lamp inspections were also performed on the animals. Skin scrapings for dermatophyte detection were performed with a microscope slide and used when hair analysis was inconclusive. Scrapings for ectoparasite detection were performed by using a scalpel blade, and the collected material was aligned on histological slides, covered with another slide, with edges sealed with adhesive tape to prevent ectoparasite escape and material loss. Slides were duly identified. At LAPAR/UFRPE, ectoparasite scrapings were examined and specimens classified by using a light microscope. At LAMAA/UFRPE, when necessary, scrape samples for dermatophyte evaluation were inoculated in VetCheck culture medium following the same protocols used for group 1. The methodologies applied to both groups are summarized in the flowchart shown in Figure 1.

RESULTS AND DISCUSSIONS

In animals from group 1, characterized by specimens without skin lesions, a macroscopic ectoparasite of the *Amblyomma* sp. species was observed in the juvenile from Camaragibe, Pernambuco (Figure 2). Ticks on *B. variegatus* have also been reported by other authors, such as Souza *et al.* (2016), in their records of occurrence of *Amblyomma geayi* in a sloth specimen from the northern region of Brazil, as well as Martins *et al.* (2015) and Martins *et al*. (2017), with their observations of *Amblyomma varium* in bradypodids from southeastern Brazil.

Figure 1 – Flowchart indicating the experimental design of groups 1 and 2 of *Bradypus variegatus* sloths, without and with skin lesions, respectively.



Source: Personal archive.

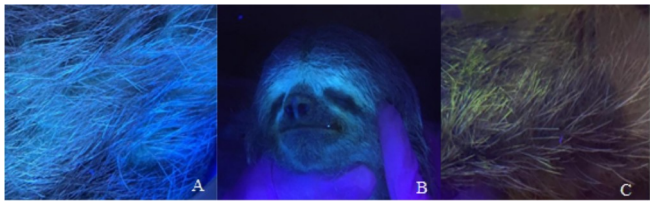
Figure 2 – *Amblyomma* sp. tick collected from the pelage of the *Bradypus variegatus* sloth.



Source: Personal archive.

The use of a Wood's lamp demonstrated the presence of fluorescence in different areas of the evaluated sloths' bodies, except in the young female from Camaragibe, Pernambuco, and in the young males from João Pessoa, Paraíba, Maceió, Alagoas, Camaragibe, Pernambuco, and Recife, Pernambuco. In the illuminated regions, scaling was observed in the adult female, young female, adult male, and the juveniles, as shown in Table 1 (Figure 3).

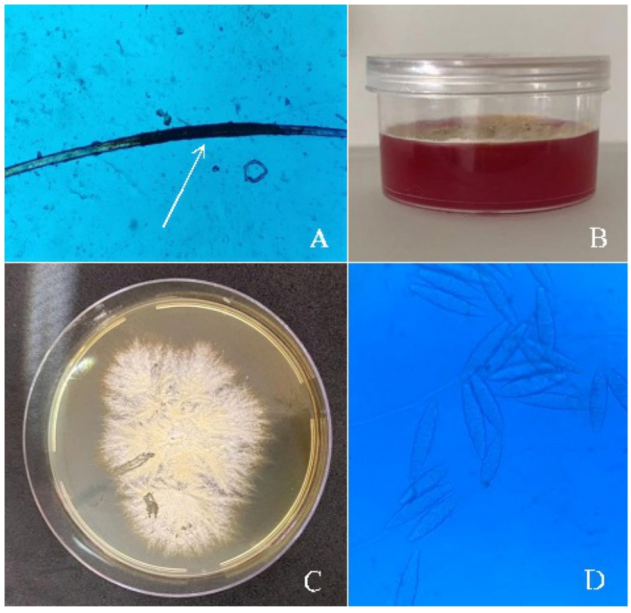
Figure 3 – Photomacrographs of body areas with fluorescence under a Wood's lamp in *Bradypus variegatus* sloths without skin lesions. A - Bluish fluorescence. B - Strong blue fluorescence. C - Green fluorescence.



Source: Personal archive.

Based on the microscopic evaluation of hair from the group 1 animals, no morphological abnormalities were reported, nor the occurrence of ectoparasites. However, in animals with positive fluorescence, the presence of fungal spores and hyphae was described. In the juvenile and adult male from Camaragibe, Pernambuco, the dermatophyte *Nannizzia gypsea* (synonym *Microsporum gypseum*) was identified through positive VetCheck culture medium, macroscopic fungal growth on the plate, and microscopic analysis of macroconidia (Figure 4).

Figure 4 – Identification of the dermatophyte fungus *Nannizzia gypsea* on the pelage of *Bradypus variegatus* sloths without lesions. A - Photomicrograph showing the dermatophyte fungus adhered to the hair (10x). B - VetCheck culture medium with reddish coloration, indicative of dermatophyte presence. C- Fungal growth on the agar plate after subculture. D - Photomicrograph of fungal macroconidia (40x).

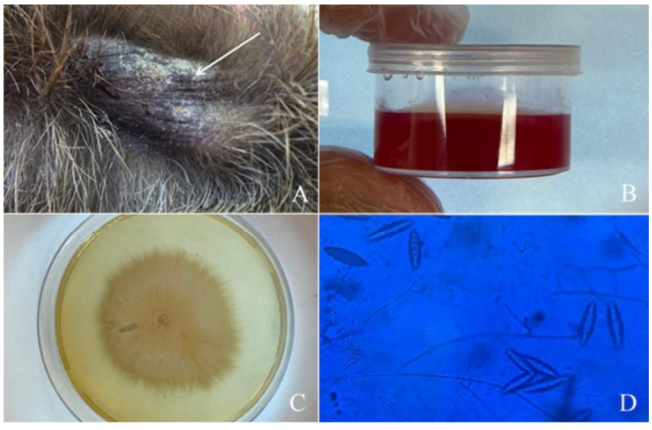


Source: Personal archive.

The young female from Camaragibe, Pernambuco, and the young males belonging to group 1 did not demonstrate potential causative agents of dermatological afflictions. The presence of an infectious agent, although potential, is not always associated with disease occurrence, since environmental factors and host-related characteristics, such as genetic traits, are closely linked to the individual's susceptibility and response capacity to the agent (Pan American Health Organization, 2010).

In animals from group 2, which presented skin lesions, no macroscopic ectoparasites were observed. Skin lesions were found in adult females from Camaragibe, Pernambuco, and Escada, Pernambuco, as well as in a young male from João Pessoa, Paraíba. The adult female rescued in Jaboatão dos Guararapes, Pernambuco, exhibited two lesions, while the adult female from Recife, Pernambuco, had only one lesion. The sloths did not show pruritus or pustules in the lesioned skin areas, which were characterized as dry areas with total alopecia, epidermal collars, and hyperkeratosis (Figure 5A). From the data collected, a higher frequency of lesions was noted on the animals' limbs, as detailed in Table 2. Similar findings were also observed by Cleff *et al.* (2008) in their case report of a tufted capuchin (*Cebus apella*) diagnosed with *Candida albicans* infection.

Figure 5 – Identification of the dermatophyte fungus *Nannizzia gypsea* in the pelage of sloths (*Bradypus variegatus*) with skin lesions. A – Skin lesion with epidermal collarettes and hyperkeratosis. B - VetChek culture medium with reddish coloration, indicative of the presence of a dermatophyte fungus. C- Fungal growth in Petri dish after subculture. D - Photomicrograph of fungal macroconidia (40x).



Source: Personal archive

With regard to Wood's lamp examination, fluorescence was detected in all animals in various body regions, including both lesioned and non-lesioned areas. In the latter, skin desquamation was observed (Figure 6).

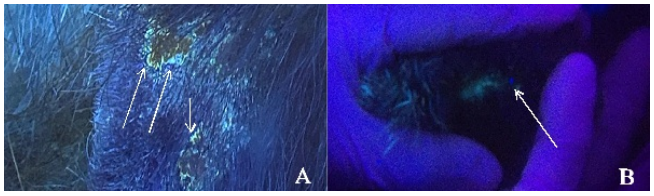
Exclusively in the adult female from Camaragibe, Pernambuco, three of her lesions showed no fluorescence. Likewise, none of the lesions in the adult female from Jaboatão dos Guararapes, Pernambuco, exhibited fluorescence. In the remaining animals, all lesions fluoresced (Table 2). The absence of fluorescence in some lesions, despite the broad

Table 1 – Indication of body areas with fluorescence detected by Wood's lamp in *Bradypus variegatus* sloths without skin lesions.

Sex	Age	Rescue Location	Body Areas with Wood's Lamp Fluorescence and Fluorescence Color
Female	Adult	Cabo de Santo Agostinho, PE	Thorax and dorsum, bluish fluorescence
Female	Juvenile	João Pessoa, PB	Mouth, strong blue fluorescence; dorsum, bluish fluorescence
Male	Adult	Camaragibe, PE	Abdomen, green fluorescence; distal region of the right thoracic limb, green fluorescence
Undetermined	Infant	Camaragibe, PE	Distal region of the left thoracic limb, bluish fluorescence; distal region of the right thoracic limb, strong blue fluorescence
Undetermined	Infant	Garanhus, PE	Dorsal region of the head, strong blue fluorescence; thorax, strong blue fluorescence; dorsum, strong blue fluorescence; distal region of the right thoracic limb, strong blue fluorescence

association of Wood's lamp findings with dermatological infections—especially fungal and bacterial (Veasey *et al.*, 2017)—may be related to trauma that had not fully healed, resulting from falls prior to rescue. Falls during the traversal of fragmented habitats have been reported by Silva *et al*. (2017) as one of the most common issues faced by sloths.

Figure 6 – Photomacrographs of body areas with fluorescence under Wood's lamp in sloths (*Bradypus variegatus*) with skin lesions. A- Bluish fluorescence in a lesioned area. B- Strong blue fluorescence in a lesioned area.



Source: Personal archive.

Microscopic examination of hair samples obtained through adhesive tape impressions and scrapings from lesion areas revealed the presence of fungal spores and hyphae in all tested specimens. The hairs did not exhibit morphological changes, aside from the fungal structures adhered to them. The presence of the dermatophyte *Nannizzia gypsea* was confirmed through a positive VetChek result, followed by macroscopic observation of fungal growth and microscopic identification of macroconidia, in all individuals except for the adult females from Jaboatão dos Guararapes, Pernambuco, and Escada, Pernambuco (Figure 5). Dermatophytoses caused by the *Microsporum* genus in sloths have previously been reported in Pernambuco by Xavier *et al.* (2008), which supports the findings of this study and highlights the need for the development of an effective treatment protocol.

Additionally, the mite *Lobalges trouessarti* was identified in hair samples collected from the median dorsal lesion of the adult female rescued in Camaragibe, Pernambuco. This microscopic ectoparasite may or may not be associated with dermatological conditions. The animal presented no signs of pruritus or discomfort in the affected skin areas (Figure 7), suggesting that this mite may be part of the diverse eukaryotic microbiota inhabiting the sloth's fur (Tipton; Machado-Allison, 1972; Suutari *et al*., 2010).

Figure 7 – Lobalges trouessarti mite observed in a skin scraping from a lesion in a sloth (*Bradypus variegatus*).



Source: Personal archive.

Based on the dermatological findings in the sloths examined in this study, a high frequency of the dermatophyte *N. gypsea* can be inferred in the fur of these animals, potentially being the main causative agent of skin lesions in comparison to other macro- or microscopic eukaryotes, particularly in the states of Pernambuco and Paraíba. The fact that animals positive for the dermatophyte did not exhibit green fluorescence under Wood's lamp underscores the importance of using high-specificity methods such as VetChek, since Wood's lamp fluorescence may result in false negatives when the fungus is not *Microsporum canis* (Kefalidou *et al*., 1997). Furthermore, the detection of *N. gypsea* in asymptomatic animals highlights the need for fur screening in rescued sloths, even when no macroscopic skin alterations are present. This is crucial to prevent immunosuppressed individuals from developing dermatophytosis after contact with healthy fungal carriers or to avoid releasing infected animals into the wild, where they could transmit the pathogen to free-living individuals. Such interactions commonly occur during reproductive periods and in maternal care (Moreno & Plese, 2006; Xavier, 2006).

Table 2 – Indication of body regions with skin lesions and areas with fluorescence detected by Wood's lamp in sloths (*Bradypus variegatus*).

Sex	Age	Rescue Location	Skin Lesion Regions	Fluorescent Areas (Wood's Lamp)
Female	Adult	Camaragibe, PE	Four dorsal lesions (median cranial, middle and caudal lesion and right caudolateral lesion); Lesion in the region of the left shoulder girdle; Lesion in the proximal region of the left pelvic limb	All areas of lesion, bluish fluorescence
Female	Adult	Recife, PE	Ventral lesion in the pelvic region	Lesion area and dorsum, bluish fluorescence
Female	Adult	Jaboatão dos Guararapes, PE	Ventral lesion in the thoracic region; Lesion in the distal region of the right thoracic limb	Proximal region of the left thoracic limb, bluish fluorescence
Female	Adult	Escada, PE	Middle median lesion on the back; Lesion in the distal region of the right thoracic limb; Lesion in the distal region of the right pelvic limb; Lesion in the distal region of the left pelvic limb	Lesion area in the distal region of the right thoracic limb, bluish fluorescence
Male	Youg	João Pessoa, PB	Lesions to the dorsal region of the head; Lesions to the distal region of the left thoracic limb; Lesions to the region of the right radiocarpal joint; Ventral lesion in the thoracic region; Two lesions to the left pelvic limb (proximal and distal lesion	Lesion area in the dorsal region of the head, strong blue fluorescence; Lesion areas in the left pelvic limb (proximal and distal lesion), bluish fluorescence; Left radiocarpal joint region, strong blue fluorescence; Back, bluish light; Right pelvic limb, bluish fluorescence

CONCLUSIONS

Among the *Bradypus variegatus* individuals evaluated, when present, skin lesions were primarily located on the limbs. Dermatological conditions in these animals are likely to be more commonly caused by the dermatophyte *N. gypsea* than by ectoparasites or other pathogenic eukaryotes.

ACKNOWLEDGEMENTS

We thank the Brown-Throated Sloth Institute (IPGM) and the Center for the Screening of Wild Animals (CETAS/Tangara) for providing and handling the sloths. We also thank Professor Dr. Jaqueline Bianque from the Biology Department of the Federal Rural University of Pernambuco (UFRPE) for the identification of the ectoparasites.

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