# Ectoparasitic Skin Diseases of Two-toed and Three-toed Sloths in Costa Rica

## Stephanie Dewell

# Faculty Sponsor: Dr. Clay Britton

Department of Biology

#### Abstract

A total of 16 sloths in the wild and in captivity (eight B. variegatus and eight C. hoffmanni) were evaluated for dermatitis over a two-week period in Manuel Antonio, Costa Rica. Fungal culture, skin impression, and skin scrapings were conducted on each individual case in order to follow the differential diagnostic minimum database of small animal dermatology. Captive population samples were taken from a wildlife sanctuary and The Sloth Institute Costa Rica rehabilitation facility. A definitive diagnosis was not possible in five of the cases. In the captive population (six B. variegatus and five C. hoffmanni), dermatophytosis by Microsporum gypseum was detected in three C. hoffmanni adults (18.7%), sarcoptic mange in three pruritic B. variegatus infants (18.7%), and pyotraumatic dermatitis in one adult C. hoffmanni (6.2%). Zoonotic ectoparasites were found exclusively in the captive sloth populations at the wildlife sanctuary. Five sloths from the wild presented with skin lesions: electric shock trauma from telephone wires was the primary cause of 80% of these lesions, and secondary Malassezia dermatitis (6.3%) and pyotraumatic dermatitis (12.5%) were confirmed with skin impression. The general trends of the trial indicate that the most prevalent cause of dermatitis was zoonotic ectoparasites (37.5%). Proper management and increased biological security in captive populations may contribute to the prevention of disease transmission.

#### Introduction

Bradypus variegatus and Choloepus hoffmanni, respectively known as the three-toed and two-toed sloths, are inhabitants of tropical rainforests of the Caribbean and Pacific regions of Central and South America (McCarthy et al. 1999). Sloths are primarily arboreal mammals and belong to the taxonomic order Pilosa. According to the Red List of Threatened Species published by the International Union for Conservation of Nature and Natural Resources (IUNC), nearly half of the genera belonging to the family Bradypodidae are recognized as critically endangered, with decreasing population trends. C. hoffmanni shares the phylogenic family Megalonychidae with one other sloth species, Choloepus didactylus. Though C. hoffmanni and B. variegatus are currently listed as two species of least concern (IUNC 2016), their future survival is threatened by trends of rapid deforestation and human encroachment on their natural habitat (Laurence et al. 1999, Sibaja-Morales et al. 2009).

Serving as a pilot study for development of a record of ectoparasitic infections among Costa Rican sloths, the current research uses the dermatologic testing minimum database to enable identification of specific ectoparasitic organisms in the subject population of 16 sloths, some captive and some wild. The results support the proposition that zoonotic ectoparasites—parasites that can be transmitted from animals to humans are the most common cause of dermatitis in captive sloths and thus that proper management and increased biological security in captive populations are desirable to prevent disease transmission in both human and animal populations.

#### Habitat Loss & Fragmentation

During the 1960s and 1970s, Costa Rica experienced some of the highest rates of population growth and of deforestation in the world (Bixby and Palloni 1998, Sader and Joyce 1988). Habitat loss and fragmentation threaten sloth survival as they not only displace the sloths from their appropriate niche but also subject the species to a vast array of physiological stressors they would not experience in the wild. The Costa Rican government has promoted ecotourism to preserve the deteriorating tropical landscape of Costa Rica, but its policy has several limitations (Langholz et al. 1999). By definition, ecotourism enables travelers from around the world to experience the natural forest and wildlife while inflicting minimal human impact on the native ecosystems. Yet studies analyzing the mechanisms by which tourism of the tropics is developed, operated, and organized indicate that current practices allow for negative impact on the maintenance of overall healthy ecosystems (Isaacs 2000).

Over the last two decades, sloths have become an increasingly popular attraction to travelers in Central America. Young sloths are often captured and taken from their mothers in the wild, and held captive in improvised shelters; here they are sold to international travelers (Moreno and Plese 2006). Both buyers and sellers of the sloths are often unaware of the specific dietary needs of the animal, resulting in the surrender of extremely malnourished individuals to rehabilitation facilities. Of the 102 sloths intercepted by officials from traffickers in 2004, only 23 sloths were able to survive rehabilitation—13 of the 81 B. variegatus (16%) species and 10 of the 21 C. hoffmanni species (48%) (Moreno and Plese 2006).

#### **Clinical Disorders**

Sloths are becoming increasingly vulnerable to disease as urbanization and ecotourism introduce direct pathways for infectious organisms that are uncommon in the wild. As wild habitats shrink and wild sloths are captured, not only are sloths exposed to unfamiliar parasites and other organisms carried by domestic animals, but also their immunocompetency is compromised by the stresses of urban hazards and inappropriate diets. Prevalent clinical disorders recorded among both species are nutritional disorders (45.7%), digestive maladies (12.3%), respiratory complications (12.3%), and injuries (6.1%) (Diniz and Oliviera 1999). One case of habitat destruction resulted in the displacement of nearly 600 sloths to grasslands and beaches, where they suffered from starvation, dehydration, and parasite infection (Moreno and Plese 2006). Common physical injuries reported after the habitat disturbance included vehicle-induced

trauma, stoning by children, and electrical shock from telephone wires (Moreno and Plese 2006). A health survey of 277 sloths conducted at the Unau Foundation in Central America indicated that human-induced traumas, such as nail mutilation, filed down teeth, contusions, burns and other wounds, accounted for 2% of the sloth injuries treated, all of which increased the sloths' susceptibility to secondary bacterial infection (Moreno and Plese 2006).

Sloth pelage, or fur, shares a symbiotic relationship with diverse algal communities and plays an important role as a vector for several non-pathogenic arthropods (Gilmore 2001, Suutari et al. 2010). It has been suggested that these symbiotic relationships not only play an important role in camouflaging sloths, but also serve as source of nutrition via cutaneous absorption and diffusion along the hair shafts (Gilmore et al. 2001). Skin diseases that occur as a result of injury and uncommon parasitism disrupt these mutualistic relationships and are associated with 35% of nutritional and digestive illnesses (Diniz and Oliviera 1997). Hindrances to the symbiotic relationships between algal communities and pelage, as a result of dermatitis, can have hazardous effects on important physiological processes of both sloth species.

#### **Dermatitis: Transmission and Manifestations**

Dermatitis is a general term used to describe inflammation of the skin caused by allergens, disease, or infection. Lesions caused by physical injuries are the primary cause of secondary bacterial skin diseases in mammals; these diseases include pyotraumatic dermatitis, superficial pyoderma, and deep pyoderma (Diniz and Oliviera 1999, Hnilica, 2006). Since individuals with differing secondary bacterial skin diseases experience similar symptoms, each case must be investigated thoroughly to determine the appropriate therapy. Pyotraumatic dermatitis is characterized by an acutely pruritic, rapidly enlarging area of alopecia and erosive skin with moist exudate (Hnilica 2006). In contrast, both superficial and deep pyoderma involve hair follicles and adjacent epidermis, resulting in individuals presenting with multifocal or generalized areas of papules, epidermal collarettes, localized alopecia, and erythema (Hnilica, 2006, Wilson and Chen 2004).

Secondary fungal infections can also result from skin damage caused by chronic trauma and moisture (Hnilica 2006). Malasseziasis, more commonly known as yeast dermatitis, presents as nonhealing, erythematous, crusted skin or nail bed lesions (Black 2012, Hnilica 2006). Wounds must be treated immediately to avoid these bacterial and fungal skin diseases that ultimately hinder the sloth's mobility and ability to feed (Diniz 1999). Pyotraumatic dermatitis and Malassezia dermatitis are considered to be non-zoonotic infections, which indicates these diseases cannot be spread from animals to humans.

In contrast, sarcoptic mange is a highly contagious skin disease caused by the zoonotic arthropod itch mite, Sarcoptes scabiei. A study analyzing infectious diseases in the human population of international travelers stated that skin disorders are the third most common health ailment globally at 8% (Wilson and Chen 2004). Of the skin lesions present in travelers, arthropod-related dermatitis constituted 9.7% of disease found in persons seen at tropical disease medical units over a three-year period (Wilson and Chen 2004). The mite is highly transmissible due to its utilization of both animals and humans as carriers and its ability to infect by skin-to-skin contact between individuals (Black 2012, Diaz 2006). Infestation of humans from the animal mange is generally self-limiting

because the species of mite that infects animals is only able to reproduce on its specific host (Bandi and Saikumar 2013, Walton 2007). S. scabiei infestation on both the animal host and human reservoir presents as lesions, including papules, alopecia, erythema, crusts, and excoriations (Black 2012, Diaz 2006, Hnilica 2006). Lesions formed by hypersensitivities to the by-products of the animal mange mite lead to secondary bacterial infection; therefore, differential diagnosis is crucial to the overall health of the animal host.

Dermatophytosis (ringworm) is a zoonotic fungal infection of the hair shafts caused by keratinophilic fungi from the genera Microsporum, Trichophyton, and Epidermophyton (Xavier et al. 2006). Unlike yeast dermatitis, dermatophyte organisms are transmitted through direct contact with fur and dandruff that contain fungal particles, originating from an infected animal or environment. Skin involvement in both humans and animals may be localized or generalized with areas of circular, irregular, or diffuse alopecia with scaling. Other symptoms include erythema, papules, crusts, seborrhea, and onychodystrophy of the digits (Hnilica 2006).

#### **Differential Diagnosis**

The dermatologic diagnostic minimum database includes skin scrapes, cutaneous skin cytology/impression, and fungal cultures (Hnilica 2006). Differential diagnosis is crucial to determine the primary cause of a skin disease since lesions appear similar; however, these diseases can also co-occur and require different methods of treatment. As discussed in detail below, published research has not applied these differential diagnostic standards, possibly resulting in the publication of false-negative findings.

Over a one-year period, 65 Costa Rican sloth species from Sau Paulo Zoo were examined for gastrointestinal parasites and ectoparasites. The study concluded that 27 sloths presented with pruritic lesions, erythema, and hair loss, and were positive for S. scabiei itch; no evidence of fungal infection was found (Sibaja-Morales et al. 2009). Though Sibaja-Morales and colleagues' study was the first to discover that captive sloths can be infected with sarcoptic mange, it is of concern that no diagnostic test other than skin scraping was documented. The diagnosis was further supported when four researchers developed rashes after handling infected sloths (Sibaja-Morales et al. 2009). Nonetheless, this method raises several questions for concern, as the accuracy of skin scrapings is less than 50 percent when no other diagnostic test is performed (Hnilica 2006, Walter 2011).

An additional study in South America discovered that 100% of fungal cultures taken from sloths that presented with skin lesions tested positive for dermatophytosis from the genera Microsporum (Xavier et al. 2006). The researchers in this study did not perform additional skin impression and skin scraping tests, which fails the standard of differential diagnosis in small animal dermatology. Such incomplete protocols can lead to ineffective treatments with hazardous effects not just on the animal's health but also on the validity of statistics for future research.

Research pertaining to B. variegatus and C. hoffmanni ectoparasitic diseases is a relatively new field. While a few studies have investigated the presence of clinical diseases in sloths—and incorporate a brief synopsis of dermatological illnesses—the researchers have not conducted differential diagnosis of skin diseases, whether in wild or captive

animals (Diniz and Oliviera 1999, Moreno and Plese 2006, Sibaja-Morales et al. 2009, Xavier et al. 2007).

The objective of the present research is to serve as a pilot study to aid in establishing a recorded foundation of ectoparasitic infection incidence in sloths across various environments in Costa Rica by utilizing the dermatologic testing minimum database. The hypothesis of this study is that zoonotic ectoparasites are the primary cause of dermatitis in both captive and wild sloth populations in Costa Rica. The data collected throughout this experiment can be utilized in future long-term studies investigating the primary cause of skin diseases. Furthermore, an increased frequency of zoonotic parasite infection would indicate the need for establishing preventive measures to avoid further transmission in both the human and animal populations.

### Materials and Methods

#### Location

Differential diagnostics were conducted on sloth species C. hoffmanni and B. variegatus residing in the wild and in captivity over a two-week period in Manuel Antonio, Costa Rica. Samples from captive specimens were obtained from two separate facilities. The first facility, a wildlife sanctuary, is located 7 km outside Quepos Central. The sanctuary is open three hours daily (0900-1200) to the general public for wildlife tours. Sloths are separated by age and placed in either the nursery or exhibition enclosure. The nursery is a solid studio structure that serves as housing for infant and juvenile sloths of both species. The adult C. hoffmanni sloths are held in a steel wire exhibition enclosure (6 x 6 x 6 m), as there are no adult B. variegatus individuals. The second sampling site, the Sloth Institute Costa Rica (TSI), located in Manuel Antonio, is a research facility that rehabilitates and reintroduces sloths into the wild. TSI houses B. variegatus and C. hoffmanni adult individuals that are held in enclosures (6 x 6 x 6 m) divided by steel fencing for separation of the species. Sloths are in direct contact with trained research professionals for approximately one to two hours daily, and the enclosures are not accessible to the general public.

#### Skin Scraping: Technique and Interpretation

Superficial skin scrapes of the sloth lesions were taken by applying moderate pressure with a dulled No. 10 sterile scalpel blade in the direction of hair growth on the affected area. Hair and debris collected on the blade were then transferred to a standard microscope slide prepared with three drops of veterinary grade mineral oil at the surface. Slides were examined microscopically under 400x total magnification to visualize superficial ectoparasitic mites. S. scabiei can be identified based on the presence of a long, unjointed pedicel with a sucker, on the end of the extremities (Hendrix 2007).

The ideal depths for skin scrapes depend on the organism in question (Hendrix 2007, Hnilica 2006); therefore, secondary skin scrapes were required. Secondary samples were taken in order to expel mites from deep within the hair follicles. Mineral oil was applied to the affected area, which was then scraped repeatedly in the direction of hair growth until the skin appeared pink, with the capillaries visible and oozing blood (Hendrix 2007, Walton 2007). False-negative findings can result if the deep skin scraping fails to provide a small amount of blood due to an increased potential for mites to be left

within the follicle. The deep skin scrape samples were examined under 400x total magnification for hair follicle mites (Demodex spp.).

#### Skin Impression: Technique and Interpretation

Secondary bacterial infection and Malassezia dermatitis prevalence or absence were evaluated utilizing acetate tape preparations of superficial samples. The acetate tape preparation technique involves the use of transparent adhesive tape to collect a sample of hair or superficial skin debris. The adhesive side of the tape was applied repeatedly to the lichenified lesions and processed with VetOne Rapid Differential Stain #3 (methylene blue CAS 1120-79-3, phosphate salts CAS 7778-770) for 30 seconds. The tape was rinsed for five seconds with deionized water, placed on a microscope slide, and examined under 1000x total magnification to visualize the presence or absence of bacteria and yeast (Malassezia) outgrowth.

The cytology of skin taken from sloth individuals with pyotraumatic dermatitis revealed the presence of mixed bacteria, whereas Malasseziasis was confirmed by the finding of several round to budding yeasts (Hnilica 2006). The bacteria in this technique are described based on morphology and not identified to species level.

#### Fungal Culture: Inoculation, Incubation, and Examination

Hardy Diagnostics Derm-Duet<sup>TM</sup> II\*, RSM<sup>TM</sup>/DTM, 15x100 Biplate, 15ml/15ml, individually wrapped Bacti-Lab Culture System<sup>TM</sup> was used to isolate and identify dermatophyte organisms. This culture plate has two sections: DTM and RSM. Dermatophyte fungal contaminant growing on DTM plates forms alkaline products that change pH indicators in the medium from yellow to deep red. The RSM produces a blue color change in response to a dermatophyte organism and also expedites the sporulation of macroconidia. The two-section plate is equipped with an antibacterial and antifungal compound that prohibits the growth of saprophytic fungi and bacteria contaminants; however, bacteria are not completely suppressed and some non-dermatophytic fungi can cause a false-positive red (DTM) or false-positive blue-green (RSM) color change.

Lesions were scraped with a No. 10 sterile scalpel blade and transferred by rubbing the broad side of the scalpel blade directly onto the agar surface of the plate. Plucks of hair were taken from the diseased area with a hemostat and the resulting specimen was tapped gently into the surface of the media. Cultures were stored away from light, lid-side down, at 25° C for 10 days. The media were examined every 48 hours during the incubation period for characteristic color change and colony appearance. Cultures that exhibited color change on the RSM or DTM on the 10th day of incubation were examined microscopically.

A microscope slide was prepared with one drop of VetOne Rapid Differential Stain #3 (methylene blue CAS 1120-79-3, phosphate salts CAS 7778-770). Acetate tape preparation was then used to collect conidia from the RSM segment and placed adhesive side down on the prepared slide. The slide was examined for spores under 400x total magnification. Dermatophytes and saprophytes were identified down to species level with identification charts provided by Bacti-Lab Skin Culture Systems<sup>TM</sup>. Microscopic examination of fungal contaminants eliminated false-negative/false-positive results based solely on color change and colony morphology.

# Results

# **General Overview**

A total of 16 sloths were evaluated for dermatitis over a two-week period. Eleven sloths in captivity presented with dermal illness, 37.5% of which was attributed to zoonotic ectoparasite infection (Table 1). Zoonotic ectoparasites were found exclusively in the nursery and adult exhibition populations at the wildlife sanctuary. Five sloths from the wild presented with skin lesions: 80% of these lesions were attributed to electric shock trauma; three of the lesions had evidence of secondary bacterial infection (18.7%) and Malassezia overgrowth (6.3%).

Dermatitis	n (%) *	Cause (n)
Bacterial	4 (25.0)	Bite wound from non-pathogenic ectoparasite (1), Electric shock traumas (3)
Yeast	1 (6.3)	Malassezia overgrowth from electric shock trauma (1)
Zoonotic	6 (37.5)	Sarcoptes scabiei mange (3),
		Microsporum gypseum dermatophytosis (3)

\*Five cases with inconclusive test results constitute 31.2% of the lesions not formally diagnosed.



**Figure 1.** S. scabiei infection. **A,** Pruritic B. Variegatus with mange. **B,** Presence of S. scabiei mite under 400x total magnification after superficial skin scraping. Photographs by author.

# **Captive Juveniles**

Three B. variegatus juveniles from the wildlife sanctuary presented with pruritic lesions, and S. scabiei mites were confirmed post skin scraping (Figure 1). Skin impressions of the three individuals were negative for secondary bacterial infection and Malassezia overgrowth. Lack of blue color change on RSM agar indicated the absence of dermatophyte growth, which was confirmed with microscopic examination of fungal conidia on the 10th day of incubation.

# **Captive Exhibition Adults**

Three C. hoffmanni exhibition adults presented with lesions at the wildlife sanctuary. The first case involved a male sloth, estimated to be one year old, with lesions present on four limbs, measuring 4 x 2 cm on left lateral metacarpal, 2 x 3 cm on the ventral aspect of the metatarsal, and 4.5 x 2 cm the right rear carpus (Figure 2). A 10month-old female presented secondarily with bisymmetrical alopecia measuring 4 x 2 cm on the right and left metacarpals. The third and most severe of the cases was an approximately one-year-old male with lesions on stifle joint, elbow, lateral aspect of the antebrachium, and both carpi. Measurements of lesions were not obtained in this case due to the individual's aggressive behavior. Lesions were flaky, erythematous, and nonexudative, and had no callouses formed.



Figure 2. Lesions caused by dermatophyte infection. Note the multifocal scaling, erythema, and onychodystrophy of the digits. Photographs by author.

Blue color change on the RSM plates (Figure 3A, left half) and red color change on the DTM plates (Figure 3A, right half) indicated dermatophytosis. Colony top view appearance was cinnamon/mauve in color and reverse colony coloration was darkly pigmented. Microscopic examination of conidia revealed ovoid-shaped macroconidia with three to six cells, which confirmed Microsporum gypseum dermatophytosis (Figure 3B). Skin impressions for secondary bacterial infection of the skin and skin scrapings for superficial and deep mites were negative for all three exhibition cases.



**Figure 3.** Dermatophytosis in three captive C. hoffmanni individuals. **A**, Top view of fungal contaminant growing on DTM fungal culture agar at day 10 of incubation. **B**, Microscopic examination of dermatophyte Microsporum gypseum macroconidia under 400x total magnification. Note the macroconidia ovoid shape with six or fewer cell divisions. Photographs by author.

# **Captive Non-Exhibition Adults**

Two B. variegatus and one C. hoffmanni were evaluated for skin illness. A threeyear-old male C. hoffmanni presented for ectoparasite removal. An engorged nonpathogenic tick was found on the ventral aspect of the individual's neck near the trachea (Figure 4A) and a 2 x 2 cm lesion was observed at the attachment site post removal



**Figure 4.** Secondary bacterial infection. **A,** Tick attachment site on ventral neck of C. Hoffmanni. **B,** Residual wound from tick attachment. **C,** Non-pathogenic tick. Photographs by author.

(Figure 4B). Microscopic interpretation of skin impression taken of tick attachment site on the individual's neck wound was consistent for pyotraumatic dermatitis due to high levels (3+) of cocci bacteria (C. Tamamoto, NCSU College of Veterinary Medicine-Dermatology Telephone Consultation, personal communication Oct. 27, 2016).

Skin impression, skin scraping, and fungal culture results were negative for a two-and-a-half-year-old female B. variegatus and a one-and-a-half-year-old male B. variegatus that both presented with flaky skin. Causation for the skin conditions in these two cases was inconclusive.

# **Free-Living Population**

Five sloths from the wild presented with severe dermal lesions. Fungal cultures and skin scrapings from all five individuals were negative for mite infection and dermatophytosis. Pyotraumatic dermatitis was diagnosed in three of the five cases from the wild that presented with skin lesions. In the first case, a female B. variegatus was found with nails that were abnormally dark, hair thinning on the left forearm, and skin on the carpus that was easily removed and moist. The second and third cases were C. hoffmanni individuals that presented with burns on forearms that extended to the abdomen. Skin impression results from the three individuals were positive for cocci (3+) bacterial infection.

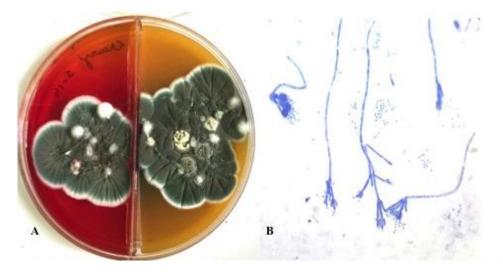


Figure 5. Yeast dermatitis. A, Ventral neck second-degree burn from electric shock trauma on an adult *C. hoffmanni* sloth found in the wild. B, Microscopic image of *Malassezia* yeast organism overgrowth under 1000x total magnification from acetate tape preparation. Photographs by author.

A single C. hoffmanni adult male presented with multiple areas of hair loss and second-degree burns after accidental electric shock trauma from telephone wires (Figure 5A). Skin impression revealed more than 10 Malassezia organisms per field of view (Figure 5B). The combination of clinical signs and high levels of organisms present on skin impression confirmed yeast dermatitis in this case (C. Tamamoto, NCSU College of Veterinary Medicine-Dermatology Telephone Consultation, personal communication Oct. 27, 2016).

#### **Atypical Findings**

Fungal culture results for the captive non-exhibition sloths had red color change on the RSM side (Figure 6A). Microscopic examination of the fungal contaminant revealed the presence of conidiophores that resembled paintbrushes, with conidia emanating from the top and conidiospores in finger-like chains on phialides projecting from vesicles (Figure 6B), which confirmed the presence of saprophyte Penicillium spp.



**Figure 6.** Saprophytic fungi growth. **A,** Red color change on RSM plate with absence of blue color change on DTM plate. **B,** Microscopic examination of fungal contaminant. Note the conidiophores that resemble paintbrushes with conidia emanating from the top. Photographs by author.

One adult C. hoffmanni female from the wild presented with extremely flaky skin and no apparent lesions or ectoparasites. On the 10th day of incubation, the fungal culture had both blue and red color change. From the top view, multiple fungal colonies appeared buff/powdery with reverse dark brown colony coloration on both RSM and DTM segments. Microscopic examination of fungal contaminant revealed curled hyphae consistent with Trichophyton mentagrophtes, but results were inconclusive due to the absence of macroconidia.

#### Discussion

The purpose of this study was to differentiate among skin diseases of Costa Rican sloth populations by utilizing the dermatologic diagnostic minimum database. Disease frequencies were then compared in order to establish which was the most prevalent. Due to pseudo-replications in the sampling procedure, chi-square analysis was not conducted on the data in this experiment; therefore, there is no significant statistical evidence to suggest that zoonotic ectoparasites are the primary cause of dermatitis in Coast Rican sloth populations. Nevertheless, the general trends of the trial indicate that the highest frequency of dermatitis was induced by zoonotic ectoparasites (37.5%). The presence of S. scabiei mites on the sloths in this study confirmed the previous report by Sibaja-Morales et al. (2009), which recorded the first case of this parasite affecting both C. hoffmanni and B. variegatus individuals in Costa Rica. It is noteworthy that during this study two of the volunteer staff at the wildlife sanctuary developed pruritic rashes that resolved without any medical therapy. These rashes can possibly be attributed to zoonosis caused by the animal species S. scabiei, as human infestation is generally self-limiting.

Additional evidence of zoonotic disease was found in the exhibition population of C. hoffmanni individuals. The clinical presentations of the dermatophyte lesions and the microbiological findings observed in the present study are supported by previous reports from Xavier et al. (2008), which described dermatophytes in free-living B. variegatus individuals of Brazil. This is the first study to document dermatophytosis caused by the keratinophilic fungi Microsporum gypseum in captive Costa Rican C. hoffmanni populations. All of the sloths that presented with zoonotic ectoparasite infection were residents at the wildlife sanctuary.

Although it is not possible to confirm the origin of the infections at the wildlife sanctuary, several husbandry factors might have contributed to the increased rate of zoonotic parasitism observed in its populations. The nursery not only functioned as housing for infant sloths but also as a living space for volunteer staff and quarantined animals. Though quarantined animals were separated to prevent direct contact with the nursery sloth population, the personnel involved in the care of the nursery wildlife were a possible source for transmission of parasites, as they come in direct prolonged contact with both the quarantined individuals and the nursery sloths throughout the day. In addition, inspection of the exhibition cage revealed a suspended kennel containing stagnant water and numerous piles of moldy excrement on the cage floor. Sloths must travel to the ground to urinate and defecate, making them more at risk of fungal contraction, since M. gypseum is considered to be a geophilic dermatophyte found in the soil. However, transmission from direct contact with fur and dandruff that contain fungal particles must also be considered.

Secondary pyotraumatic dermatitis (18.8%) and Malassezia dermatitis (6.3%) from electric shock traumas were the most common skin ailments of sloths that presented from the wild. The four cases with shock wounds were found on the ground after heavy storms. Manuel Antonio experiences increased rates of rainfall from May to November; therefore, the frequency of shock traumas should be statistically analyzed in future studies to correlate seasonal variations with disease patterns. Common wounds of sloths in captivity, such as wire-induced injuries and fight wounds reported by Diniz and Oliviera (1999), were not observed in this study. Adequate enclosure size for the sloth populations at TSI and the exhibition population at the wildlife sanctuary enabled each sloth to establish its own territory; therefore, the risk for aggression between individuals was decreased.

Eleven fungal cultures in this study were positive for the saprophytic fungi Penicillium spp. Saprophytes live primarily on decaying vegetation and are commonly observed in human and animal clinical practices. Though the presence of the fungus does not generally indicate disease, penicilliosis can be pathogenic and fatal in immunocompromised individuals (Mok et al. 1997). As previously reported by Sibaja-Morales et al.(2009), sloths are at a higher risk of contracting disease due to the stress factors associated with captivity. Immunosuppression from stress and constant exposure to materials harboring the Penicillium spp. fungus in sloth enclosures puts captive sloth individuals in danger of contracting nephrotoxic and carcinogenic mycotoxins produced by Penicillium spp. During this study, two B. variegatus individuals that were not evaluated for skin disease presented with sudden onset of upper respiratory distress. Pneumonia was confirmed in both cases at necropsy. This is important to note, as Penicillium spp. has been isolated from patients with pneumonia and respiratory disorders are the second most common organic disorder of sloths in captivity, as reported by Diniz and Oliviera (1999). Future studies investigating the pathogenicity of Penicillium spp. in captive sloths should be conducted to aid wildlife rehabilitators in maintaining the upper respiratory health of the species.

# Conclusion

Increased biological security must be considered in order to eliminate further transmission of zoonotic diseases to wildlife staff, the general public, and other captive species. The wildlife sanctuary should establish preventive measures, such as volunteer dormitories and a separate isolation ward for quarantined animals. Cage-specific clothing, such as coveralls and disposable shoe covers, should be worn by staff at the wildlife sanctuary and at TSI whenever they are handling wildlife and entering enclosures, in order to avoid cross-contamination in their wildlife populations. The practice of responsible ecotourism by persons traveling to Costa Rica is also essential for the success of both species in captivity and in the wild. Practices such as touching, holding, and taking photographs that physically disrupt the sloth should be eliminated as this contributes to increased stress levels, disruption of the symbiotic algal communities on the sloth fur, and risk of zoonosis.

Acknowledgements: I would like first to thank both the Center for Research and Creativity and the Biology Department at Methodist University for making this project financially possible. Secondly, to Sam Trull, the director of the Sloth Institute of Costa Rica, who trusted me to work with her sloth population, and to Dr. Marie Burris, for her continuous support and technical assistance, I extend my deep appreciation.

#### References

- Bandi K, Saikumar C. 2013. Sarcoptic mange: A zoonotic ectoparasitic skin disease. Journal of Clinical and Diagnostic Research 7(1):m156-157.
- Bixby L, Palloni A. 1998. Population and deforestation in Costa Rica. Population and Environment: A Journal of Interdisciplinary Studies 20(2):149-185. Available from http://ccp.ucr.ac.cr/bvp/pdf/bosques/rosero48.pdf
- Black JG. 2012. Microbiology: Principles and Explorations. 8th ed. MA: John Wiley & Sons Inc: 599-600.
- Diaz J. 2006. The epidemiology, diagnosis, management, and prevention of ectoparasitic diseases in travelers. Journal of Travel Medicine 13(2):1195-1982. Available from http://www.ncbi.nlm.nih.gov/pubmed/16553596

- Diniz L, Oliveira P. 1999. Clinical problems of sloths (Bradypus sp. and Choloepus sp.) in captivity. Journal of Zoo and Wildlife Medicine 30(1):76-80. Available from http://www.jstor.org/stable/20095824?seq=1#page\_scan\_tab\_contents
- Gilmore DP, Da Costa CP, Duarte DPF. 2001. Sloth Biology: An update on their physiological ecology, behavior and role as vectors of arthropods and arboviruses. Brazilian Journal of Medical and Biological Research 34:9-25. Available from http://www.scielo.br/scielo.php?script=sci\_arttext&pid=S0100-879X2001000100002&lng=en&tlng=en
- Hendrix CM. 2007. Laboratory Procedures for Veterinary Technicians. 5th ed. MI: Mosby Inc: 231-263.
- Hnilica K. 2011. Small Animal Dermatology: A Color Atlas and Therapeutic Guide. 3<sup>rd</sup> ed. CA: Elsevier Inc: 22-38.
- Isaacs J. 2000. The limited potential of ecotourism to contribute to wildlife conservation. Wildlife Society Bulletin 28(1):61-69. Available from http://www. globalforestcoalition.org/wp-content/uploads/2010/12/Ecotourism-Isaacs.pdf
- IUCN, 2016. The IUCN Red List of Threatened Species. Version 2016-2. Available from http://www.iucnredlist.org
- Langholz J, Lassoie J, Schelhas J. 1999. Incentives for biological conservation: Costa Rica's private wildlife refuge program. Conservation Biology 14(6):1735-1743. Available from https://www.jstor.org/stable/2641525?seq=1#fndtnpage\_scan\_tab\_contents
- Laurance W, Vasconcelos H, Lovejoy T. 1999. Forest loss and fragmentation in the Amazon: Implications for wildlife conservation. Oryx 34(1):39-45. Available from https://www.sciencebase.gov/catalog/item/50540553e4b097cd4fcfb09f
- McCarthy T, Anderson D, Gustavo A, Cruz D. 1999. Tree sloths (Mammalia Xenarthra) in Nicaragua and Honduras, Central America. The Southwestern Naturalist 44(3):410-414. Available from

http://www.jstor.org/stable/30055246?seq=1#page\_scan\_tab\_contents

- Mok T, Koehler A, Yu M, Ellis D, Johnson P, Wickham N. (1997) Fatal Penicillium citrinum pneumonia with pericarditis in a patient with acute leukemia. Journal of Clinical Microbiology 35(10): 2654–2656. Available from http://jcm.asm.org/content/35/ 10/2654.full.pdf+html
- Moreno S, Plese T. 2006. The illegal traffic in sloths and threats to their survival in Colombia. BioOne 7:10-67. Available from http://www.bioone.org/doi/pdf/ 10.1896/1413-4411.7.1.64
- Sader S, Joyce A. 1988. Deforestation rates and trends in Costa Rica, 1940 to 1983. Biotropica 20(1):11-19. Available from

http://www.jstor.org/stable/2388421?seq =1#page\_scan\_tab\_contents Sibaja-Morales K, de Oliveira J, Rocha A, Gamoa JH, Gamboa JP, Murillo F, Sandi J, Nunez Y, Baldi M. 2009. Gastrointestinal parasites and ectoparasites of Bradypus variegates and Choloepus hoffmanni sloths in captivity from Costa Rica. Journal of Zoo and Wildlife Medicine 40(1):86-90. Available from http://www.ncbi.nlm.nih.gov/ pubmed/19368244

Suutari M, Majaneva M, Fewer D, Voirin D, Aiello A, Friedl T, Chiarello GA, Blomster J. 2010. Molecular evidence for a diverse green algal community growing in the hair of sloths and a specific association with Trichophilus welckeri (Chlorophyta, Ulvophyceae). BMC Evolutionary Biology 10:86. Available from http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-10-86

- Walter B. 2011. Comparison of dermoscopy, skin scraping, and the adhesive tape test for the diagnosis of scabies in a resource-poor setting. Arch Dermatology 147(4):468-73. Available from https://www.ncbi.nlm.nih.gov/pubmed/21482897
- Walton S. 2007. Problems in diagnosing scabies, a global disease in human and animal populations. Clinical Microbiology Reviews 20(2):268-279. Available from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865595/
- Wilson M, Chen L. 2004. Dermatologic infectious diseases in international travelers. Current Infectious Disease Reports 6:54-62. Available from http://www.ncbi.nlm.nih.gov/ pubmed/14733850
- Xavier G, Silva LB, Silva DR, Peixoto R, Lino G, Mota R. 2008. Dermatophytosis caused by Microsporum canis and Microsporum gypseum in free-living Bradypus variegates in the State of Pernambuco Brazil. Brazilian Journal of Microbiology. 39:508-510. Available from http://www.ncbi.nlm.nih.gov/pmc/PMC3768448