Association of Fungi with Molossid Bats (Gervais 1856) in Mato Grosso do Sul, Southwest Brazil

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Abstract

We describe the mycobiota associated with the nasal hairs of three species of Molossid bats, *Cynomops planirostris*, *Molossus molossus*, and *Molossus rufus*, in Mato Grosso do Sul, southwest Brazil. Bats were captured in the Cerrado and Pantanal biomes; some had visible filaments and others did not. One to four morphospecies were cultured from each bat and we identified a total of 13 fungal morphospecies. Only four fungal species were cultured from more than one species; none were common to all three. In addition, only one species was common to bats from both the Cerrado and Pantanal. We did not observe any negative effects on the bats from the fungus, although some species or genera are known pathogens. Our results indicate the need for further research into biodiversity and role of the mycobiota of Molossid bats.

Keywords: Molossidae, fungi, nasal hair, Pantanal, Cerrado

1. Introduction

There are approximately 90 species of Molossid bats, which can be found on every continent (Mickleburgh *et al*. 2002). These bats are insectivores of small to medium size characterized by a free tail, narrow wings, dark, velvety fur, and an internal keel (Gregorin & Taddei 2002). Brazil is home to 26 species of Molossid bats (Reis *et al*. 2007) and 14 can be found in the state of Mato Grosso do Sul (Cáceres *et al*. 2008; Santos & Bordignon 2011).

There are several known associations of fungus with bat species. In the Western Hemisphere, bats are known to excrete *Histoplasma capsulatum* in their feces (Emmons 1958; McMurray & Russel 1982) and *Leptospira* in their urine (Bunnel *et al*. 2000; Bharti *et al*. 2003); both are pathogenic to humans. Studies have detected a variety of fungal species, including pathogens, superficially (Gandra *et al*. 2008; Jaya Seelan *et al*. 2008; Voyron *et al*. 2011) and within the internal organs of bats (Reis & Mok 1979; Mok *et al*. 1982; Dias *et al*. 2011). In 2006, bats in the northeastern United States were reported with white fungus on their nose and wings. The pathogen, which invades the dermal tissue, has devastated the bat populations of the region (Blehert *et al*. 2008). The novel fungus, *Geomyces destructans*, has also been reported in bats in Europe (Puechmaille *et al*. 2010; Wibbelt *et al*. 2010).

We observed the presence of filamentous fungi among the hairs between the snout and upper lip of Molossid bats of three species, *Cynomops planirostris*, *Molossus molossus*, and *Molossus rufus*, captured in the Cerrado and Pantanal biomes of Mato Grosso do Sul, southwest Brazil. These fungi have not been previously reported or described. This study had the objective to

identify and characterize these fungi. We also compared the species richness and composition of fungi isolated from the different bat species and from the different biomes.

2. Materials and Methods

2.1 Study site

The bats were collected from around Mato Grosso do Sul, Southwest Brazil between April 2012 and January 2013 from both the Pantanal and Cerrado biomes. Trapping occurred Rio Negro or Rio Miranda in the Pantanal (wetland) region. In the Cerrado, bats were collected on rural properties outside of Campo Grande in a region of Cerrado (savannas) and within the city of Campo Grande, which is also located within the Cerrado (Fig. 1, Table 1).

2.2 Capture of Bats and Removal of Fungi

Mist nets were placed near roosts, small water bodies, and possible flyways. Bats were taken out of mist-nets using latex gloves and placed in sterilized cloth bags. Individuals were identified by species according to Gregorin & Taddei (2002). Forceps were sterilized in a flame and with alcohol. Visible fungus filaments were removed with the forceps and placed in petri dishes on sterile potato dextrose treated with chloramphenicol (100µg/mL) and gentimicin (50µg/mL). Each petri dish was capped and sealed with plastic tape immediately after the filament was

placed on the agar. When filaments were not visible, sterile forceps were passed over the nasal hairs and pressed onto the agar. These plates were closed and then sealed with plastic film.

2.3 Fungal Cultures

Cultures were left to grow at room temperature in sealed petri dishes. Different morphologies growing in the same culture were separated onto different plates in a hood using sterile forceps. Fungal cultures were examined macroscopically and microscopically and identified by morphology according to Barnett and Hunter (1972).

3. Results and Discussion

We identified 13 Ascomycete morphospecies from 12 individual bats of three different species: *Cynomops planirostris*, *Molossus molossus*, and *Molossus rufus*. Five bats were captured near the Rio Negro or Rio Miranda in the Pantanal (wetland) region. Six bats were collected on rural properties outside of Campo Grande in a region of Cerrado (savannas). One bat was collected by the Centro de Controle de Zoonosis in the Prefeitura Municipal within the city of Campo Grande, which is also located within the Cerrado

We were able to identify four fungal specimens to the species level: *Aspergillus fumigatus, Aspergillus niger*, *Aspergillus terreus*, and *Emericella nidulans*. In addition, we were able to identify two other *Aspergillus* morphospecies, as well as *Cladosporium* sp., *Chrysosporium* sp.,

Exophiala sp., *Fonsecaea* sp., *Penicillium* sp., *Phaeoacremonium* sp., and *Scopulariopsis* sp. An additional two morphospecies, which we were unable to identify, were cultured and have been designated Species 3.1 and 5.1, originating from Bat 3 and Bat 5 respectively. Both were hyphomycetes with no asexual reproductive structures.

One to four fungal species were isolated from each bat. At least one fungal species was isolated from each bat, including those with no visible filaments. One to two fungal species were isolated from bats with no visible filaments, while up to four were isolated from those with visible filaments. Bats of all three species from both the Cerrado and Pantanal were collected with and without visible filaments. The bat with the most fungal species isolated (4) was collected within the city limits of Campo Grande.

The number of morphospecies that we found associated with the Molossid bats is high, especially the four species associated with one bat. Studies in Malaysia (Jaya Seelan *et al*. 2008) and Italy (Voyron *et al*. 2011) have found three and two different species, respectively, per bat species.

No fungal species was common to all three bat species. *A. terreus* and *Fonseceae* sp. were common to *M. molossus* and *M. rufu*s. *A. niger* and *Penicillium* sp. was cultured from both *C. planirostris*. *A. terreus* was the only fungal species cultured from specimens from the Cerrado and Pantanal. *A. niger* and *Chrysosporium* sp. were cultured from bats both with and without visible filaments. Our results point to the possibility that these fungi are highly diverse and may be largely specific to certain species or certain regions. However, it is also possible that some

fungi may be commonly found associated with bats around the world. *A. fumigatus* for example has been found on bats in Malaysia (Jaya Seelan *et al*. 2008) and Italy (Voyron *et al*. 2011). *A. niger* and *Cladosporium* sp. have also been found in Malaysia and Italy respectively (Jaya Seelan *et al*. 2008; Voyron *et al*. 2011).

The fungi did not appear to detrimentally affect the bats. All the bats were of normal size and weight. Fungal filaments were observed only in the hairs between the nose and upper lip. Many of the genera from which we cultured are common and widespread in the environment. There is no indication that these fungi are pathogenic, although many of them, such as *A. fumigatus, A. niger, A. terreus,* as well as species from the genera *Cladosporium*, *Chrysosporium*, *Emericella*, *Exophiala*, *Fonsecaea*, *Phaeoacremonium*, and *Scopulariopsis* are known pathogens or cause opportunistic infection. *Chrysosporium* in particular has been identified as an emerging pathogenic in reptiles (Thomas *et al*. 2002; Bowman *et al*. 2007; Allender *et al*. 2011). A variant of *A. fumigatus* has been identified as a pathogen of the bat species *Hipposideros cervinus* (Seelan Jaya & Anwarali Khan 2009). Given the growing number of fungi infecting various wildlife species across the world (Geiser *et al*. 1998; Daszak *et al*. 2001; Drew *et al*. 2002; Fisher *et al*. 2012), our results indicate the need for further research into the biodiversity of these fungi and the potential effects they may have on Molossid bats.

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