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Short communication

Characterization of fungi associated with the nasal hairs of Molossid bats

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ABSTRACT

Fungal pathogens have become a serious threat to wildlife, threatening populations of even once common, abundant species. We describe the mycobiota associated with the nasal hairs of three Molossid bat species, *Cynomops planirostris*, *Molossus molossus*, and *Molossus rufus*, in southwest Brazil. Bats were captured in the Cerrado and Pantanal biomes. We cultured 22 fungal isolates from twelve individual bats. Sixteen sequences of the ITS region were obtained, yielding 11 unique sequence types from the genera *Aspergillus*, *Cladosporium*, *Paecilomyces*, and *Penicillium*. No obvious detrimental effects on the bats from the fungi were observed, although some species or genera that we identified are known pathogens in other species. This is the first report of such fungi associated with the nasal hairs of Molossid bats. Our results indicate the need for further research on the biodiversity, ecological role, and potential effects of this mycobiota on Molossid bats.

1. Introduction

Mycoses are rapidly becoming one of the leading threats to wildlife (Daszak et al., 2001; Fisher et al., 2012). A number of emerging fungal pathogens have reduced populations of diverse taxa including sea corals (Geiser et al., 1998), reptiles (Thomas et al., 2002; Bowman et al., 2007; Allender et al., 2011), and amphibians (Daszak et al., 1999; Briggs et al., 2010). In 2006, bats in the northeastern United States were reported with white fungus on their nose and wings (Blehert et al., 2009). The pathogen, which invades the dermal tissue, has devastated the bat populations of the region (Blehert et al., 2009; Frick et al., 2010). The novel fungus, *Pseudogymnoascus desctructans* (Minnis and Lindner, 2013), has also been reported in bats in Europe, although it does not appear pathogenic there (Wibbelt et al., 2010; Puechmaille et al., 2011).

The mycobiota of bats in Brazil is largely unknown. We observed the presence of filamentous fungi among the hairs between the snout and upper lip of three species of Molossid bats, *Cynomops planirostris*, *Molossus molossus*, and *Molossus rufus* (Fig. 1). Worldwide, there are approximately 90 species of Molossid bats, which can be found on every continent (Wilson and Reeder, 2005). These bats are small to medium size insectivores characterized by a free tail extending beyond the uropatagium, narrow wings, dark, velvety fur, and an internal keel (Gregorin and Taddei, 2002). Brazil is home to 29 species of Molossid bats (Nogueira et al., 2014), 14 of which can be found in Mato Grosso do Sul state (Cáceres et al., 2008; Bordignon et al., 2011; Santos and Bordignon, 2011).

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Fig. 1. *Cynomops planirostris* presenting visible fungal filaments around the nasal hairs.

These fungi on the nasal hairs of Molossid bats have not been previously reported or described. The objective of this study was to identify these fungi. We also compared the species richness and composition of fungi isolated from the different bat species and from the two different habitat types.

2. Materials and methods

2.1. Study site

We collected bats from around Mato Grosso do Sul, southwest Brazil between April 2012 and January 2013 in both the Cerrado and Pantanal. The Cerrado is a large seasonal xeromorphic plant formation located on the plateau of Central Brazil, commonly called "Brazilian Savanna" (Eiten, 1972). The Pantanal is an extensive, unpredictable wetland, influenced by the vegetation classes that border it, such as the Cerrado, Amazonian forest, and Chaco, favoring a great variety of vegetation types (Pott et al., 2011). In the Cerrado, bats were collected on rural properties outside of Campo Grande and within the city of Campo Grande. Trapping in the Pantanal occurred around the Negro River and Miranda River (Table 1).

2.2. Bat capture and fungus isolation

We captured bats of three species for this study: *Cynomops planirostris*, *Molossus molossus*, and *Molossus rufus*. In the study area, only bats of the genera *Cynomops* and *Molossus* have the nasal hairs which appear to host fungi. Other Molossid bats have wrinkled upper lips and therefore no hairs on the muzzle. To capture bats, mist nets were placed near roosts, small water bodies, and possible flyways. Bats were removed from mist-nets using latex gloves and placed in sterilized cloth bags. The bats were identified to species level according to Gregorin and Taddei (2002). Forceps were sterilized in a flame and then dipped in alcohol. Visible fungus filaments were removed with forceps and placed in Petri dishes on sterile potato dextrose supplemented with chloramphenicol (100 μg ml^{-1}) and gentamicin (50 μg ml^{-1}) to prevent bacterial contamination. Each Petri dish was then capped and sealed with plastic tape immediately after placing the filament on the agar. When filaments were not visible, the sterilized forceps were passed over the nasal hairs and pressed onto the agar. These plates were closed and then sealed with plastic film.

Cultures were left to grow at room temperature in sealed Petri dishes. There was no significant variation in room temperature during the incubation period, which lasted approximately 15 d. When there was more than one morphotype on a dish, each was placed onto a different plate. This was done in a sterilized laminar flow hood using sterile forceps to prevent any contamination.

2.3. Molecular methods

To more accurately identify fungal cultures, the rRNA internal transcribed spacer region (ITS1, 5.8S, and ITS2; ca. 600 pb) was sequenced. DNA was extracted from circular fragments of mycelium with a diameter of approximately 0.5 cm following a modified CTAB protocol (Doyle and Doyle, 1987). The isolated material was quantified using NanoDrop 2000 (Thermo Scientific) and its quality was assessed using 1% agarose gel electrophoresis. Amplifications were performed using primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). Each PCR reaction contained 0.2 mM of dinucleotide triphosphate (dNTPs), 0.2 μM of each primer (ITS1F and ITS4), 1X GoTaq buffer (Promega), 1 U of GoTaq DNA polymerase (Promega), and 1 μl of genomic DNA. The final volume of amplification was 25 μl. PCR reactions were amplified in an Applied Biosystems Veriti 96-Well Thermal Cycler using the following parameters: 5 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 45 s at 55 °C, and 1 min at 72 °C, and then a final elongation of 10 min at 72 °C. After PCR, an aliquot of 2 μl from each sample was checked by horizontal electrophoresis in 1% agarose gel. PCR products were cleaned with PEG 20% (Dunn and Blattner, 1987) and sequenced according to the protocols of Macrogen Inc. (Seoul, South Korea).

Sequencing reads were assembled and edited using Geneious® 7.1.7 (http://www.geneious.com; Kearse et al., 2012). High-quality consensus sequences of each isolate were compared to the NCBI GenBank database with BLAST (Altschul et al., 1990). Each sequence was compared to the first 30 matches in GenBank. If at least 20 of the first 30 sequences matched the same genus or species, the isolate was identified as belonging to that genus or species.

All sequences were submitted to GenBank under accession numbers **KR610358 – KR610373**.

3. Results and discussion

A total of 22 ascomycete colonies were cultured from 12 individual bats of three different bat species: *Cynomops planirostris* (n = 6), *Molossus molossus* (n = 3), and *Molossus rufus* (n = 3) (Table 1, Fig. 2). Five bats were captured near the Negro River and Miranda River, in the Pantanal. Within the Cerrado, six bats were collected on rural properties and one was collected within the city of Campo Grande. Of the 22 isolates cultured, adequate sequences for identification were obtained from 16 of them, and they were identified to the level of genus or species (Table 1). From the 16 isolates, eleven unique ITS sequence types were obtained from different species in four genera. At the species level, *Aspergillus terreus* (three isolates with identical sequences) was identified from three cultures from two different bats. Two additional sequence types of *Aspergillus* were also identified, one sequence type of *Cladosporium*, one sequence type of *Paecilomyces*, and eight sequence types of *Penicillium*. The divergence among the different sequence types of *Aspergillus* was about 10% while in *Penicillium*, divergence ranged from 1 to 6.5%. *Penicillium* sp. isolate 6.1 was the most different from the other isolates, diverging 6.5% from other *Penicillium* spp. sequence types. The other seven *Penicillium* spp. isolates diverged <2% from each other.

Some fungal isolates appeared to be common across bat species and the two biomes, Cerrado and Pantanal. Seven similar *Penicillium* isolates (divergence <2%) were cultured from seven

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