Isolation of *Microascus* sp. and *Cephalotheca* sp. from Bats in Argentina

Iara Figini,[1,2,3,6] **Alejandro N. Etchecopaz,**[1,4] **Paula Blanco,**[1,2,3] **Diana Arnica,**[1,5] **María L. Cuestas,**[1,4] **and M. Marcela Orozco**[1,2,3] [1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Godoy Cruz 2290, C1425QUB, Argentina; [2]Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA-CONICET), Ciudad Autónoma de Buenos Aires, Intendente Güiraldes 2160, C1428EGA, Argentina; [3]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Intendente Güiraldes 2160, C1428EGA, Argentina; [4]Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM-UBA CONICET). Ciudad Autónoma de Buenos Aires, Paraguay 2155, C1121ABG, Argentina; [5]Parque Nacional Ciervo de los Pantanos, Administración de Parques Nacionales (APN), Campana, Provincia de Buenos Aires, Rómulo Otamendi 1127, C2804, Argentina; [6]Corresponding author (email: iarafgn@gmail.com)

ABSTRACT: Bats worldwide play significant roles in ecosystem functions, encompassing pollination, seed dispersal, and pest control while concurrently serving as diseases reservoirs. As part of a comprehensive wildlife health surveillance effort, bats were systematically sampled within two national protected areas in Argentina. During this study 67 bats were examined and samples were collected from eight *Molossus* spp. individuals exhibiting conspicuous yellowish or white lesions on their noses. All samples were cultured on Sabouraud dextrose agar and lactrimel agar for fungal growth evaluation. Fungal isolates were identified using morphologic and molecular taxonomic techniques, leading to the detection of *Microascus* sp. in three *Molossus rufus* from Ibera National Park and *Cephalotheca* sp. in five *Molossus molossus* from Marsh Deer National Park. No fungal growth was identified in samples collected from the healthy hairs of the bats displaying lesions on their noses. The two fungi, which have not previously been isolated from bats, should be considered potentially pathogenic, evidenced by diseased hairs in the affected individuals.

Key words: Chiropteran, emerging fungi, Latin America, *Molossus* spp., hair lesions.

Bats (chiropterans) play essential roles in ecosystems, including pollination, seed dispersal, predation, and biological pest control (Kunz et al. 2011). Worldwide, 1,384 bat species have been described (Taylor and Tuttle 2019), including 69 in Argentina (Barquez and Díaz 2020). Chiropterans are also known for hosting several pathogenic species of bacteria, viruses, protozoa, and fungi (Joffrin et al. 2018). Several pathogenic fungi have been identified in bats, including *Histoplasma capsulatum* and *Pseudogymnoascus destructans*, and other potentially harmful species such as *Fusarium* spp. and *Aspergillus* spp. (Ludwig et al. 2023). Certain diseases, such as white-nose syndrome (WNS) caused by *P. destructans*, have led to significant declines in bat populations (Blehert et al. 2009). Within the context of wildlife health surveillance and through systematic bat sampling, we report the isolation of *Microascus* sp. and *Cephalotheca* sp. from Argentine bats of the genus *Molossus*.

Our study was conducted in two national protected areas from Argentina: Ibera National Park (INP) and Marsh Deer National Park (MDNP). The INP is a 183,500-ha protected area in Corrientes Province, located within the Ibera Wetlands, one of the largest freshwater systems in the world (Neiff and Poi de Neiff 2006). The MDNP, in Buenos Aires Province, covers 5,200 ha within the delta and islands of the Parana River ecoregion, a vast assemblage of floodable areas in the terminal segment of the Plata Basin (Burkart et al. 1999).

We conducted four field campaigns in 2022, two in each park, spanning autumn–winter (June and July) and spring–summer (October to December) seasons. The study adhered to the ethical and biosafety standards of the Faculty of Exact and Natural Sciences, University of Buenos Aires, with approval from the Institutional Animal Care and Use Committee (protocol no. 2021/164). Capture and transit permits were obtained from the National Parks Administration (NEA 513 Rnv 1). Capture sites were selected on the basis of the presence of bats, including places where they use human structures as refuge. These sites comprised the public camping and the park ranger station in INP, and the dock near the Parana River and the ranger station in MDNP (Fig. 1). Each campaign included one to three capture evenings using one to four black mesh mist nets (6 m wide, 2.6 m high,
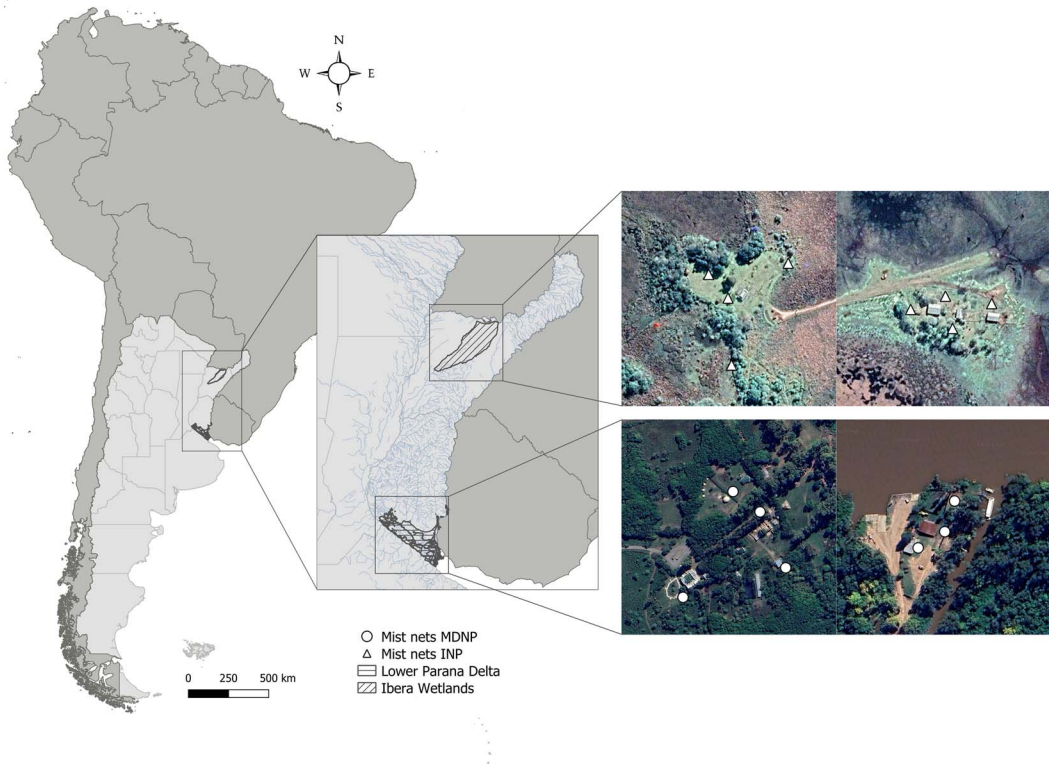
FIGURE 1. Location of the selected sampling sites of bats in Ibera National Park (INP), Corrientes (27°52.122′S, 56°52.810′W) and Marsh Deer National Park (MDNP), Buenos Aires (34°15′09″S, 58°52′04″W), Argentina.

38-mm mesh; Avinet Research Supplies, Portland, Maine, USA) placed in vegetation-free areas or near the bat shelter. Captured individuals were placed in cloth bags, weighed, examined for injuries, and identified on the basis of morphologic characteristics (Barquez and Díaz 2020). Individuals with a visible lesion affecting the hairs between the snout and upper lip were sampled (Fig. 2). The lesions were characterized by a yellow or white area of matted hairs that were easily removed, leaving an alopecic area below. We collected hairs using sterile dissecting forceps and stored them in sterile microtubes (Henso Medical, Hangzhou, Zhejiang, China) at room temperature. We then released bats at the capture site.

Samples were cultured on Sabouraud dextrose agar (Laboratorio Britania S.A, Ciudad Autónoma de Buenos Aires, Argentina) and lactrimel agar plates supplemented with 500 mg/L chloramphenicol (Laboratorio Britania S.A.) and incubated at 28 C and 10 C for up to 3 mo to evaluate fungal growth. All these procedures were conducted under strict biosecurity and sterile conditions.

Isolates were molecularly identified by amplification of the internal transcribed spacer (ITS)1–5.8S–ITS2 region of ribosomal DNA using universal fungal primers ITS1 and ITS4 (White et al. 1990) followed by sequencing of the amplicon. Species identification was further confirmed by bidirectional sequencing of the DNA fragments amplified from the β-tubulin gene with primers BT2a and BT2b (Glass and Donaldson 1995) using ABI BigDye Terminator v.3.1 (Macrogen Inc., Seoul, South Korea). The resulting nucleotide sequences were edited using Codon Code Aligner version 7.1.2 (Codon Code Corporation, Centerville, Massachusetts, USA) and compared with sequences published in the GenBank database (Benson et al. 2002). We used phylogenetic analysis by the neighbor-joining method to determine genetic relationships. The best model of evolution was estimated using JModelTest software (Posada lab, University of Vigo, Galicia, Spain)

FIGURE 2.   *Molossus rufus* bat (on the left) sampled in Ibera National Park, Argentina in June 2022 and *Mo. molossus* (on the right) sampled in July 2022 in Marsh Deer National Park, Argentina, both showing visible opaque yellow or white lesion around their muzzles.

considering both the Bayesian and the Akaike information criteria; the phylogenetic tree was constructed using PhyML 3.0 software (Montpellier bioinformatic, Montpellier, France). Clade stability was determined in a bootstrap resampling procedure with 1,000 replicates. Gaps were treated as missing data. The nucleotide sequences of the corresponding genes of the fungi *Cephalotrichum stemonitis* and *Wardomycopsis inopita* were used as outgroup for the β-tubulin phylogenetic tree and the nucleotide sequences of the corresponding genes of the fungus *Aurobasidium pullulans* were used as outgroup for the ITS1–5.8S–ITS2 phylogenetic tree.

We captured 67 individuals, 48 in INP and 19 in MDNP, representing five genera: *Myotis*, *Molossus*, *Tadarida*, *Eumops*, and *Eptesicus*. The genus *Molossus* was dominant, comprising 73% of the captured individuals (n=49), with two species identified: *Molossus rufus* in INP and *Molossus molossus* in MDNP. The sampling effort involved 1,117 m^2 net-hours yielding a capture success rate of 5.99 individuals per hour per net. Of the 67 bats, eight individuals (11.95%), consisting of three female *Mo. rufus* and five *Mo. molossus* (one female and four males), presented conspicuous superficial lesions affecting the hairs and skin in their muzzles, with invasion of the hair cortex by intracapillary

hyphae, resulting in the destruction of the hair structure (Fig. 3). This invasion had led to the loss of the medulla, a consistent reduction in hair diameter, and the deterioration of wall spicules along with a decline in hair vitality.

Fungal development was observed after 2 wk at 28 C and after 6 wk at 10 C in all tubes. The sampled *Mo. rufus* individuals were positive for *Microascus* sp., whereas *Mo. molossus* were positive for *Cephalotheca* sp. All positive samples were obtained during the autumn–winter campaigns. In tubes where healthy hair from the same bats was cultured, no fungal development was observed.

The similarity of the nucleotide sequences obtained herein to those obtained from the GenBank database suggested, with high bootstrap support, that all isolates recovered from lesions from *Mo. rufus* and *Mo. molossus* bats were *Microascus intricatus* and *Cephalotheca foveolata*, respectively (Figs. 4, 5). The newly generated sequences from our study were deposited in the GenBank database under accessions PP073988 to PP073990, PO028554, and PP037987 through PP037990.

A strong association between bats and fungi has consistently been demonstrated, attributed to their feeding behavior and habitat preferences that foster fungal proliferation and transmission (Karunarathna et al. 2023). Migrations
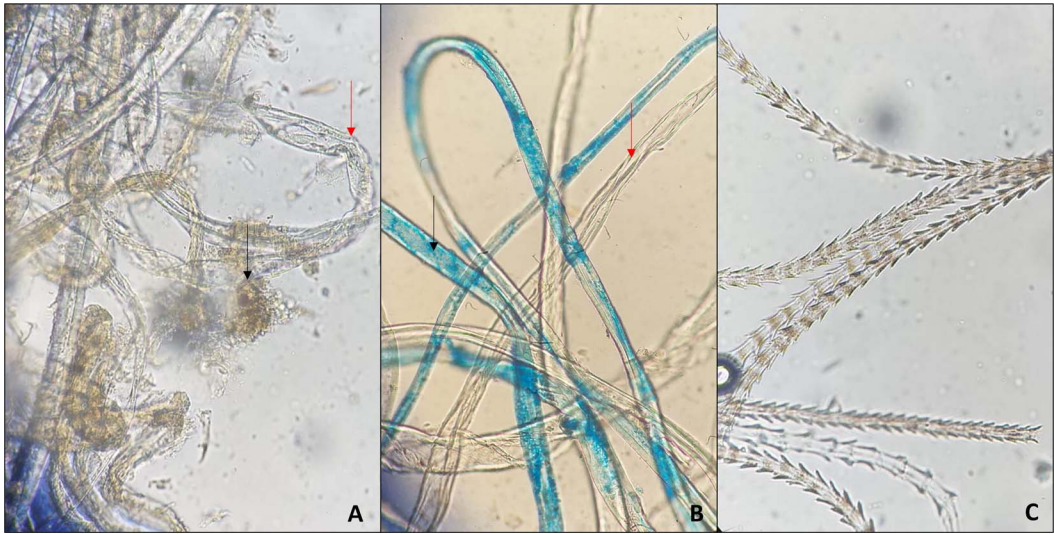
FIGURE 3.   (A, B) Hair from a *Molossus* sp. bat from Argentina, showing invasion of the hair cortex by intra-capillary hyphae, with the presence of microspores (black arrows) more notable with the addition of cotton blue stain, resulting in the destruction of the hair structure. This invasion has led to the loss of the medulla, a consistent change in hair diameter (arrows), and the deterioration of wall spicules along with a decline in hair vitality. (C) Healthy hair from the same region of a *Molossus* sp. bat without lesion. Note that the diameter of the hairs is constant and the hair structure is conserved, with medulla, cortex, and spicules in the cortex.

of bats between environments expose them to numerous fungi, aiding fungal spread among colonies via contact or contaminated surfaces, contributing to environmental dissemination (Karunarathna et al. 2023). Fungal infections causing population declines are emerging as cross-taxa pathogens (Fisher et al. 2012), with potential interspecies transmission, including to humans. *Molossidae* bats host a wide variety of fungi including *Scopulariopsis*, *Fusarium*, *Penicillium*, *Aspergillus*, and *Candida* spp., *H. capsulatum*, and *Paracoccidioides brasiliensis* (Shapiro et al. 2015; da Paz et al. 2018; Ludwig et al. 2023), demonstrating their role as fungal reservoirs and potential sources of infections.

There are no previous published reports of *Microascus* or *Cephalotheca* spp. isolated from bats with lesions on their nose hairs. Although the phylogenetic trees generated from the genes that we amplified do not permit a complete definition of the species, the sequences of at least the β-tubulin gene and ITS region of ribosomal DNA were similar to those of *Mi. intricatus* and *C. foveolata*, respectively.

Superficial growth of the fungus was only evident in the hair of the snouts of *Mo. molossus* and *Mo. rufus*. These bat species were frequent in their respective areas, with *Mo. rufus*

accounting for 79.2% of captures in INP and *Mo. molossus* 57.9% in MDNP, often using human structures or dwellings as their shelters. Remarkably, the genus *Molossus* is commonly associated with human-inhabited areas (da Paz et al. 2018). We cannot specify or assume that the presence of these fungi is causing illness in bats; however, some species from the detected genera are potentially pathogenic, such as *C. foveolata* and *Mi. intricatus*. *Cephalotheca foveolata* is an emerging human pathogen causing superficial infections in humans, subcutaneous hyalohyphomycoses, and bloodstream infections (Suh et al. 2006; Lu et al. 2015). It has also been found in respiratory secretions of cystic fibrosis patients, but the clinical implications remain unknown (Devoto et al. 2018). *Microascus* spp. are common in soils and decaying plant material; some species have been reported in bats from caves in Canada affected by WNS (Vanderwolf et al. 2016). Other species, including *Mi. intricatus*, have been identified as opportunistic pathogens affecting animals, particularly humans (Brasch et al. 2019), with reported cases of sinus, cutaneous, and disseminated infections mainly in immunocompromised patients (Sandoval-Denis et al. 2016).
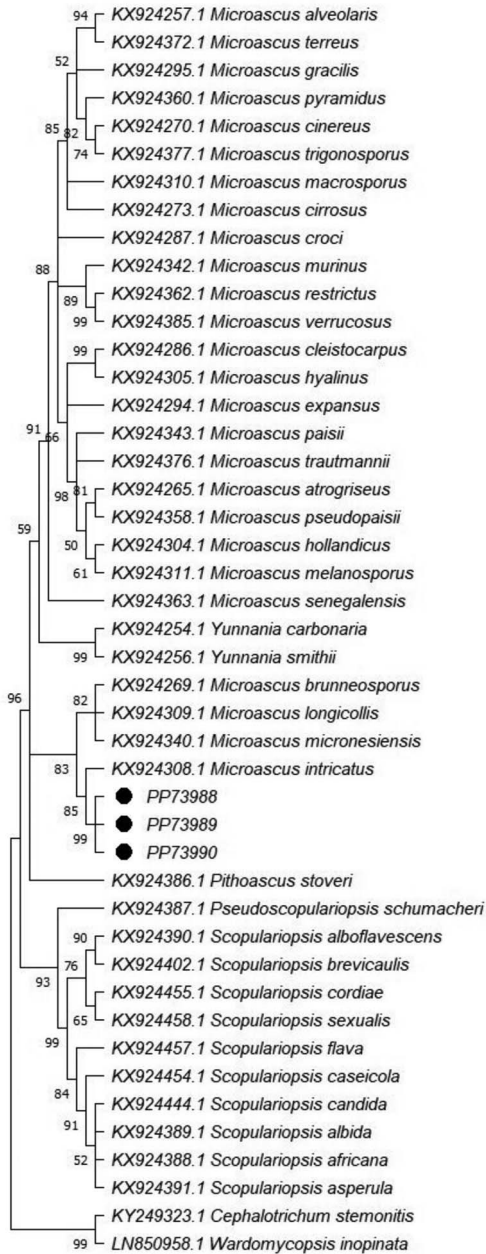
FIGURE 4.   Phylogenetic tree generated from a neighbor-joining analysis of a partial β-tubulin sequence alignment of the *Microascus intricatus* strains (● 2780312) isolated from *Molossus rufus* bats from Argentina.
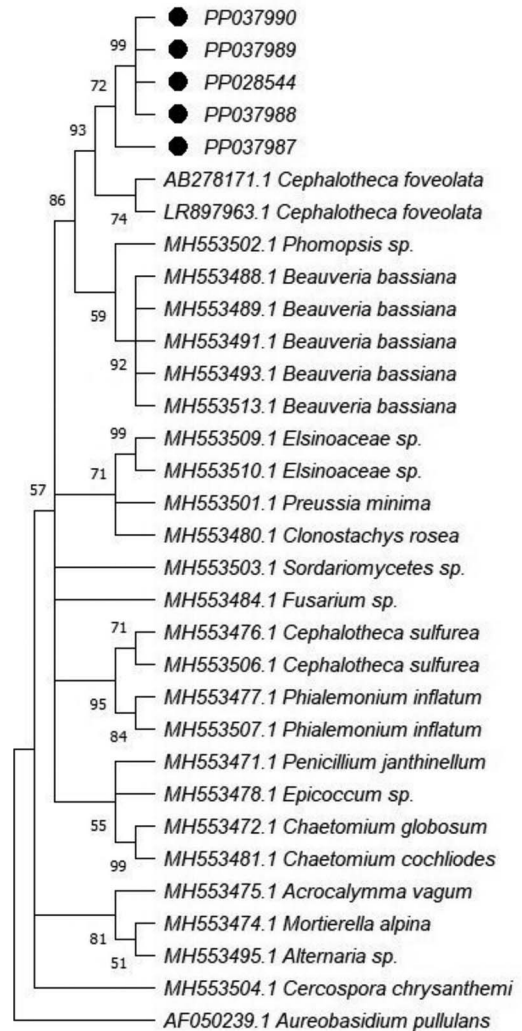

FIGURE 5.   Phylogenetic tree generated from a neighbor-joining analysis of the internal transcribed spacer (ITS)1–5.8S–ITS2 region of ribosomal DNA sequence alignment of the *Cephalotheca foveolata* strains (●) isolated from *Molossus molossus* bats from Argentina.

Notably, our findings of fungal species associated with muzzle hair invasion in bats were reported during the winter seasons, a period of higher energy demands and scarce food sources, potentially weakening the immune system of bats (Karunarathna et al. 2023). Further research is needed to fully understand the impact of these fungi on bat populations. Additionally, studying the epidemiology of bat-associated fungal diseases is important because of their potential role as cross-species reservoirs of fungal pathogens, helping us grasp the complexities of fungal environmental dynamics. Embracing a One Health approach in these studies, focusing on comprehensive wildlife health surveillance (FAO et al. 2022), allows a holistic understanding of disease

risks to animals, humans, and the environment, paving the way for more effective and all-encompassing public health interventions.

LITERATURE CITED

Barquez RM, Díaz MM. 2020. *Nueva guía de los murciélagos de Argentina.* Programa de Conservación de los Murciélagos de Argentina, Tucumán, Argentina, 183 pp.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL. 2002. GenBank. *Nucleic Acids Res* 30:17–20.

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, et al. 2009. Bat white-nose syndrome: An emerging fungal pathogen? *Science* 323:227.

Brasch J, Beck-Jendroschek V, Iturrieta-González I, Voss K, Gené J. 2019. A human subcutaneous infection by *Microascus ennothomasiorum* sp. nov. *Mycoses* 62:157–164.

Burkart R, Bárbaro NO, Sánchez RO, Gómez DA. 1999. *Eco-regiones de la Argentina.* Programa de Desarrollo Institucional Ambiental, Argentina, 42 pp.

da Paz GS, Adorno BMV, Richini-Pereira VB, Bosco SMG, Langoni H. 2018. Infection by *Histoplasma capsulatum*, *Cryptococcus* spp. and *Paracoccidioides brasiliensis* in bats collected in urban areas. *Transbound Emerg Dis* 65:1797–1805.

Devoto TB, Pola S, Rubeglio E, Finquelievich J, Gamarra S, García-Effron G, Cuestas M. 2018. Prevalence of fungal colonization in cystic fibrosis: A retrospective study over 3 years in Buenos Aires, Argentina. *Int J Infect Dis* 73:279–280.

FAO, UNEP, WHO, WOAH. 2022. *One Health Joint Plan of Action (2022–2026): Working together for the health of humans, animals, plants and the environment.* FAO, Rome, Italy. https://doi.org/10.4060/cc2289en. Accessed August 2023.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484:186–194.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 61:1323–1330.

Joffrin L, Dietrich M, Mavingui P, Lebarbenchon C. 2018. Bat pathogens hit the road: But which one? *PLoS Pathog* 14:e1007134.

Karunarathna SC, Haelewaters D, Lionakis MS, Tibpromma S, Jianchu X, Hughes AC, Mortimer PE. 2023. Assessing the threat of bat-associated fungal pathogens. *One Health* 16:100553.

Kunz TH, Braun de Torrez E, Bauer D, Lobova T, Fleming TH. 2011. Ecosystem services provided by bats. *Ann N Y Acad Sci* 1223:1–38.

Lu LL, Weil AA, Wiederhold N, Sutton DA, Chesnut L, Lindner J, Fan H, Tingpej B, El-Khoury J, Kwon DS. 2015. Probable case of *Cephalotheca foveolata* bloodstream infection. *JMM Case Rep* 2:1–5.

Ludwig L, Muraoka JY, Bonacorsi C, Donofrio FC. 2023. Diversity of fungi obtained from bats captured in urban forest fragments in Sinop, Mato Grosso, Brazil. *Braz J Biol* 83:e247993.

Neiff J, Poi de Neiff A. 2006. Situación ambiental en la ecorregión Iberá. In: *La Situación Ambiental Argentina 2005*, Brown A, Martinez Ortiz U, Acerbi M, Corcuera J, editors. Fundación Vida Silvestre Argentina, Buenos Aires, Argentina, pp. 177–184.

Sandoval-Denis M, Gené J, Sutton DA, Cano-Lira JF, de Hoog GS, Decock CA, Wiederhold NP, Guarro J. 2016. Redefining *Microascus, Scopulariopsis* and allied genera. *Persoonia* 36:1–36.

Shapiro JT, dos Santos TMR, Marchetti CR, Lorenz-Lemke AP, Delarmelina E, Bordignon MO. 2015. Characterization of fungi associated with the nasal hairs of Molossid bats. *Fungal Ecol* 18:126–129.

Suh MK, Lim JW, Lee YH, Ha GY, Kim H, Kim JR, Yaguchi T, Nishimura K. 2006. Subcutaneous hyalohyphomycosis due to *Cephalotheca foveolata* in an immunocompetent host. *Br J Dermatol* 154:1184–1189.

Taylor M, Tuttle M. 2019. *Bats: An illustrated guide to all species.* Smithsonian Books, Washington, DC, 400 pp.

Vanderwolf KJ, Malloch D, McAlpine DF. 2016. Fungi on white-nose infected bats (*Myotis* spp.) in Eastern Canada show no decline in diversity associated with *Pseudogymnoascus destructans* (Ascomycota: Pseudeurotiaceae) presence. *Int J Speleol* 45:43–50.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: A guide to methods and applications*, Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. Academic Press, London, UK, pp. 315–322.