PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07581, 2025
DOI: 10.1590/1678-5150-PVB-7581

Original Article
Small Animal Diseases

Phylogenetic analysis of *Sporothrix brasiliensis* isolated from feline sporotrichosis on São Luís Island, Maranhão, Brazil[1]

Allana F. Barros[2], Juliana S. Vitor[3], Yanara R.C.M. Esposito[3], Nayara S. Oliveira[2], Ellainy M.C. Silva[2], Ilka M.R. de Souza[4], Thiago A. de Melo[4], Elmary C. Fraga[5], Alcina V.C. Neta[6], Fábio H.E. de Andrade[6], Fernando Almeida-Souza[2,7], Larissa S.S. Ribeiro[6], Ana Lucia Abreu-Silva[6]*

ABSTRACT.- Barros AF, Vitor JS, Esposito YRCM, Oliveira NS, Silva EMC, De Souza IMR, De Melo TA, Fraga EC, Neta AVC, De Andrade FHE, Almeida-Souza F, Ribeiro LSS, Abreu-Silva AL. **Phylogenetic analysis of *Sporothrix brasiliensis* isolated from feline sporotrichosis on São Luís Island, Maranhão, Brazil**. *Pesquisa Veterinária Brasileira 45:e07581, 2025*. Departamento de Patologia Veterinária, Universidade Estadual do Maranhão, Campus Paulo VI, São Luís, MA, Brazil. E-mail: abreusilva.ana@gmail.com

Sporotrichosis is a zoonotic cutaneous mycosis caused by saprophytic fungi of the *Sporothrix*, affecting cats, horses, dogs, and humans. This study aimed to evaluate sporotrichosis in cats clinically and to phenotypically characterize and molecularly characterize *Sporothrix* species on São Luís Island, Maranhão, Brazil. From October 2022 to July 2023, clinical assessments and cytological examinations were performed on suspected feline sporotrichosis cases at the Francisco Edilberto Uchôa Lopes Veterinary Hospital, State University of Maranhão. Lesion exudates were collected via exfoliation or imprinting for fungal culture and species identification. Fungal cultures underwent species-specific polymerase chain reaction (PCR), genetic sequencing, and phylogenetic analysis. A total of 46 cats (33 males and 13 females) were assessed. Disseminated cutaneous sporotrichosis was observed in 70% of cases, with lesions predominantly on the face, ears, thoracic regions, and limbs. Initially, white fungal cultures gradually turned blackish with a coriaceous texture characteristic of *Sporothrix* spp. PCR amplification of the calmodulin (CAL) gene using *Sporothrix brasiliensis*-specific primers confirmed all 46 samples as *S. brasiliensis*. Phylogenetic analysis revealed genetic identity rates ranging from 90% to 100% with *S. brasiliensis* sequences. This seems to be the first molecular confirmation of *S. brasiliensis* causing feline sporotrichosis on São Luís Island.

INDEX TERMS: Cats, epidemiology, outbreak, phylogeny, sporotrichosis, *Sporothrix* spp., zoonosis.

[1] Received on January 3, 2025.
Accepted for publication on February 19, 2025.

[2] Graduate Program in Animal Science, Universidade Estadual do Maranhão (UEMA), São Luís, MA 65055-310, Brazil. *Corresponding author: abreusilva.ana@gmail.com

[3] Veterinary Medicine Course, Universidade Estadual do Maranhão (UEMA), São Luís, MA 65055-310, Brazil.

[4] Departamento de Química e Biologia, Laboratório de Fitopatologia, Universidade Estadual do Maranhão (UEMA), São Luís, MA 65055-310, Brazil.

[5] Departamento de Química e Biologia, Universidade Estadual do Maranhão (UEMA), Caxias 65604-380, Brazil.

[6] Departamento de Patologia Veterinária, Universidade Estadual do Maranhão (UEMA), São Luís, MA 65055-310, Brazil.

[7] Laboratório de Protozoologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ 21041-250, Brazil.

RESUMO.- [**Análise filogenética de *Sporothrix brasiliensis* isolada de felinos com esporotricose na Ilha de São Luís, Maranhão.**] A esporotricose é uma micose cutânea zoonótica causada por fungos saprófitas pertencentes ao gênero *Sporothrix* que acomete gatos, cavalos, cães e humanos. Este estudo teve como objetivo avaliar clinicamente a esporotricose em gatos e caracterizar fenotípica e molecularmente as espécies de *Sporothrix* sp. na ilha de São Luís, estado do Maranhão, Brasil. De outubro de 2022 a julho de 2023, avaliações clínicas e exames citológicos foram realizados em casos suspeitos de esporotricose felina no Hospital Veterinário Francisco Edilberto Uchôa Lopes, Universidade Estadual do Maranhão. Exsudados de lesões foram coletados por esfoliação ou *imprint*

para cultura fúngica e identificação de espécies. As culturas fúngicas foram submetidas à reação em cadeia em polimerase (RCP) espécie-específica, sequenciamento genético e analise filogenética. Um total de 46 gatos (33 machos e 13 fêmeas) foram avaliados. Esporotricose cutânea disseminada foi observada em 70% dos casos, com lesões predominantemente na face, orelhas, regiões torácicas e membros. Inicialmente, culturas fúngicas brancas gradualmente tornaram-se enegrecidas com uma textura coriácea característica de *Sporothrix* spp. A amplificação por PCR do gene calmodulina (CAL) usando *primers* específicos de *Sporothrix brasiliensis* confirmou todas as 46 amostras como *S. brasiliensis*. A análise filogenética revelou taxas de identidade genética variando de 90% a 100% com sequências de *S. brasiliensis*. Esta parece ser a primeira confirmação molecular de *S. brasiliensis* causando esporotricose felina na Ilha de São Luís.

TERMOS DE INDEXAÇÃO: Gatos, epidemiologia, surto, filogenia, esporotricose, *Sporothrix* spp., zoonose.

INTRODUCTION

Fungi of the genus *Sporothrix* are saprophytic, dimorphic organisms transitioning between filamentous forms and yeast-like phases during infection (Rossow et al. 2020). Historically, *Sporothrix schenckii* was considered the sole causative agent of sporotrichosis. However, it demonstrates a remarkable ability to infect various hosts and adapt to diverse geographical and environmental conditions, prompting extensive phylogenetic studies across global regions (Marimon et al. 2007, De Carolis et al. 2022).

The genus *Sporothrix* is classified into two clades: (1) the pathogenic clade, which includes the *S. schenckii* complex (*S. brasiliensis*, *S. schenckii*, *Sporothrix globosa*, and *Sporothrix luriei* – formerly *S. schenckii* var. *luriei*), responsible for infections in humans and animals, and (2) the environmental clade, comprising the *Sporothrix pallida* (*Sporothrix chilensis*, *Sporothrix mexicana*, *Sporothrix humicola*, and *S. pallida* formerly *Sporothrix albicans*) and the *Sporothrix stenoceras* complexes (Beer et al. 2016).

In urban environments, felines serve as primary hosts for fungi of the *S. schenckii* complex. Infection occurs through contact with contaminated soil or via bites or scratches from infected cats (Gremião et al. 2017).

Sporotrichosis presents in four clinical forms: fixed cutaneous, disseminated cutaneous, lymphocutaneous, and extracutaneous. Felines predominantly exhibit the disseminated cutaneous form due to the fungus' adaptation to the species (Nakasu et al. 2021). Conversely, humans most frequently develop the lymphocutaneous form (Orofino-Costa et al. 2022). In the fixed cutaneous form, lesions remain localized to the site of fungal inoculation and present as ulcerated or erythematous papules, plaques, or ulcers (Boechat et al. 2018). The disseminated cutaneous form involves multiple, widespread lesions, potentially associated with multiple trauma events (Orofino-Costa et al. 2017).

The diagnosis of sporotrichosis relies on clinical evaluation, epidemiological context, and laboratory testing (Lopes-Bezerra et al. 2018). Laboratory confirmation includes cytology, fungal culture, histopathology, and molecular techniques applied to lesion exudates or scrapings (Silva et al. 2015, Almeida et al. 2018).

Within the *S. schenckii* complex, *S. brasiliensis* is the primary species associated with epidemic outbreaks in South America. This species exhibits higher virulence, resistance to azole antifungals, and immune evasion capacity (Gómez-Gaviria et al. 2023).

Southern and southeastern Brazil report the highest sporotrichosis incidence due to hot, humid climates and abandoned animal populations (Alves et al. 2013, Silva et al. 2018a.) Cases are also documented in northeastern states, including Rio Grande do Norte (Figueira & Nunes 2010, Bento et al. 2021), Pernambuco (Spinelli et al. 2021), Paraíba (Nunes et al. 2011), Alagoas (Marques-Melo et al. 2014), Bahia (SES 2019), and Ceará (Aguiar et al. 2023).

The island of São Luís, Maranhão, is located in northeastern Brazil and comprises the municipalities of São Luís, Paço do Lumiar, and São José de Ribamar. The region experiences a hot and humid climate, with an average annual temperature of 26.8 °C (Araujo 2001, Climate-Data Org 2024). Tropical and subtropical areas like this are prone to neglected diseases due to low income, inadequate housing, large populations of infectious vectors, proximity to domestic animals, environmental degradation, and limited access to healthcare (WHO 2021).

Sporotrichosis, a neglected zoonotic disease, is prevalent in densely populated areas with poor sanitation and vulnerable social conditions (Rodrigues et al. 2013). As a public health concern, this study aimed to identify the *Sporothrix* species responsible for feline sporotrichosis and investigate the disease cycle's clinical, phenotypic, and molecular characteristics on São Luís Island.

MATERIALS AND METHODS

Ethical approval. The study was approved by the Ethics Committee on Animal Experimentation of the "Universidade Estadual do Maranhão" (CEEA/UEMA) under protocol number 60/2021, and adhered to the guidelines set by the National Council for the Control of Animal Experimentation (CONCEA).

Study site. The study was conducted in the São Luís Island metropolitan region, comprising the cities of São Luís, São José de Ribamar, Paço do Lumiar and Raposa. This area is situated in Maranhão State's coastal region, with geographic coordinates of Latitude 02°22′23″ and 02°51′00″ South and Longitude 44°26′41″ and 43°59′41″ West (Rocha et al. 2023).

Animals. The study included 46 felines (33 males and 13 females) with no restrictions on age, breed, or sex, all of which presented with skin lesions suggestive of sporotrichosis. These animals were treated at the Francisco Edilberto Uchôa Lopes Veterinary Hospital of the UEMA. Cats undergoing treatment with itraconazole or other antifungal drugs were excluded. Following initial screening, the animals underwent dermatological examination, anamnesis, clinical history assessment, and sample collection for cytological and fungal culture tests.

Cytology. Exudates from the lesions were collected using exfoliation or imprinting techniques to prepare cytology slides, which were stained with Romanowsky-type stain (Rapid Panothic-Laborclin) for visualizing cellular and fungal structures, as per the method described by Silva et al. (2018b).

Fungal culture and phenotypic evaluation. Lesions were disinfected with 2% chlorhexidine, and exudate samples were collected using sterile swabs and transported in a STUART medium. Samples were cultured on Sabouraud Dextrose Agar with chloramphenicol (S.D.A.; BD, Franklin Lakes, New Jersey) and incubated at 25 °C for up to 21 days. Colony growth was monitored daily. Colonies suggestive

of *Sporothrix* sp. were evaluated for macroscopic features, including surface topography (e.g., flat and irregular), texture (e.g., yeasty, granular, powdery, and cottony), and pigmentation (on the colony surface, colony reverse, and diffusion in the medium). Colony slides were stained with lactophenol cotton blue for phenotypic evaluation to assess the size, shape, and organization of conidiophores (Marimon et al. 2007). Confirmed *Sporothrix* genus colonies were further processed for DNA extraction and polymerase chain reaction (PCR) analysis.

DNA extraction and polymerase chain reaction (PCR) applied to *Sporothrix brasiliensis* based on the cal gene. DNA extraction and purification were performed using the Phenol-Chloroform-Isoamyl Alcohol protocol (Sambrook et al. 1989), with modifications such as overnight incubation and proteinase K (400 µg/mL) addition. Species-specific primers were used to amplify the calmodulin (cal) gene, as described by Rodrigues et al. (2015). PCR was conducted in a 25-µL reaction volume containing 250 ng of extracted DNA, 12.5 µL of PCR master mix (PROMEGA), and 0.2 µM of each primer. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 15 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 90 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. PCR products were visualized on gels stained with SYBR® Safe DNA Gel Stain under ultraviolet light after electrophoresis at 60 V for 40 min.

Amplified product purification and sequencing. Polymerase chain reaction products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega®) per the manufacturer's instructions. Purified DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, San Jose, California). Sequencing was performed using an ABI PRISM 3700 Analyzer (Applied Biosystem, Foster City, California) following the dideoxynucleotide chain termination method (Sanger et al. 1977). The sequences obtained in this research were submitted to genbank with accession number: PQ785783, PQ785784, PQ785786 and PQ801656.

Phylogenetic analysis. Electropherograms of the sequences were assessed using FinchTV 1.4 software (Geospiza). A consensus sequence combining forward and reverse DNA strands was generated using the CAP3 Sequence Assembly Program (PRABI-Doua). Nucleotide sequences were saved in "FASTA" format and aligned with homologous sequences from the GenBank database. The alignment of calmodulin (cal) gene sequences was performed using the Clustal/W tool in Bioedit 7.2 (Hall 1999) and manually refined. The alignment was set to 460 bp and included gaps. Outgroup sequences from Histoplasma capsulatum (U12505.1) were used. A phylogenetic tree was constructed using the maximum likelihood method based on the Kimura 2-parameter evolutionary model (Kimura 1980). Clade support was evaluated using bootstrap analysis (Felsenstein 1985) with 1,000 replicates in MEGA10 software (Kumar et al. 1994).

RESULTS

Clinical assessment. Of the 46 cats assessed, 14 (30%) exhibited the localized form of sporotrichosis, whereas 32 (70%) presented the disseminated form (Table S1). Localized lesions were observed on the snout, ear, right thoracic limb, scrotal sac, right pelvic limb, and near the caudal abdominal mammary glands (Fig.1-4). Respiratory symptoms, such as sneezing or nasal discharge, were detected in 26% (12/46) of the cats. In disseminated cases, lesions were most commonly distributed on the face, ears, dorsal thoracic region, and thoracic and pelvic limbs (Fig.5-8). Severe lesions were frequently observed on the digits and snout.

Cytology. Microscopic examination revealed a chronic inflammatory process characterized by hypersegmented and degenerated macrophages and neutrophils. Cigar-shaped yeasts, typical of *Sporothrix* spp., were also observed (Fig.9).

Fungal culture and phenotypic assessment. Colonies initially appeared white to cream-colored within five to seven days and later darkened to brownish or blackish hues (Fig.10-11). The cultures exhibited hyaline, septate, and branched hyphae with oval-to-rounded microconidia organized in a daisy bouquet-like arrangement (Fig.10-11), characteristic of the *Sporothrix* genus.

Molecular detection of *Sporothrix brasiliensis* using the cal gene. All samples yielded a 469-bp amplified product specific to the cal gene of *S. brasiliensis* (Fig.12).

BLASTn analysis of sequenced products. Fifteen positive samples, representing various collection intervals (five samples from localized clinical cases and 10 samples from animals presenting the disseminated cutaneous clinical form of the disease), were selected for genetic sequencing based on DNA concentration. Five (5/15) of them were quality sequenced. BLASTn analysis revealed 90% to 100% genetic identity with *S. brasiliensis* sequences previously deposited in the GenBank database, derived from hosts including humans, cats, and dogs. The location of isolates, identity rates, and parameters such as Query cover and E-value for the analyzed samples are summarized in Table 1. Consequently, only the remaining five samples were included in the phylogenetic analysis.

Fig.1-4. Skin lesions in a cat with localized cutaneous sporotrichosis. (**1**) Ulcerated lesion on a palmar pad of the right thoracic limb. (**2**) Ulcerated plaque-like lesions with erythema and crusts near the left ear. (**3** and **4**) Ulcerated lesions on the nasal plane.

Phylogenetic analysis of *S. brasiliensis* based on the cal gene. A total of 36 homologous sequences were selected and aligned with four consensus sequences obtained in this study to analyze the phylogenetic evolution of the isolates. DNA sequences from *S. brasiliensis*, *S. schenckii*, *S. globosa*, *S. mexicana*, *S. albicans*, and *S. pallida* were evaluated. The tree shown in Figure 13 reveals four clades in its topology, formed by *S. brasiliensis*, *S. schenckii*, *S. globosa*, and *S. mexicana*, all of which exhibited a high degree of support. The five isolates from cats assessed in this study were grouped within a clade with a high bootstrap value (98%), confirming their phylogenetic association with *S. brasiliensis*. This clade included sequences previously identified in *Homo sapiens* (KC693868, JX077113, and KF048980), *Canis lupus familiaris* (OR037312), and *Felis catus* (MZ261816) in Brazil, as well as sequences from Portugal (MT783949) and Spain (AM116886 and AM116877).

DISCUSSION

To the best of our knowledge, this is the first study to perform phylogenetic analysis on isolates from São Luís Island (MA) and confirm the presence of *Sporothrix brasiliensis* in cats from this region. The samples collected were grouped with Brazilian sequences from isolates obtained from humans, felines, and canines across various states, including Paraíba, Rio de Janeiro, São Paulo, the Distrito Federal, Pará, Pernambuco, and Minas Gerais. Internationally, sequences from Portugal and Spain were also included. The *S. brasiliensis* clade primarily encompasses isolates associated with clinical infections in humans and animals (Mora-Montes et al. 2015), particularly in Brazil (Xavier et al. 2023).

The *Sporothrix* fungus thrives in decaying organic matter, soil, and tree bark, predominantly in tropical and subtropical climates (Hernández-Castro et al. 2022). Similar environmental conditions, such as high humidity, significant rainfall, and extensive vegetation, are observed on São Luís Island and throughout Maranhão state, facilitating the development of this species. The molecular data collected in this study significantly advance the diagnosis and treatment of sporotrichosis. Enhanced diagnostic capabilities directly improve patient outcomes and play a critical role in controlling the spread of the fungus (Rossow et al. 2020, Stefaniszen et al. 2023). This improvement impacts clinical progression in patients and helps prevent fungal transmission in both feline and human populations (Arrillaga-Moncrieff et al. 2009, Zhang et al. 2015).

Sporotrichosis caused by *S. brasiliensis* is associated with severe clinical presentations and is characterized by abundant fungal cells in lesions. This, coupled with cats' social behavior, facilitates the transmission of the infection to human and non-human hosts. Disseminated sporotrichosis was the most common clinical manifestation observed in the felines studied, with over half presenting lesions on the face, ears, back, and limbs. According to Macêdo-Sales et al. (2018), lesions are more frequent on the head and body extremities, such as ear tips and paws, due to increased exposure during fights or contact with soil or plants.

Extracutaneous symptoms of feline sporotrichosis, including nasal or ocular mucosa lesions, sneezing, and dyspnea, are less common than skin manifestations and may precede or occur without them (Silva et al. 2022). Factors favoring extracutaneous forms include immunosuppressive diseases such as FIV and FeLV, high fungal loads in existing lesions that promote hematogenous spread, and the high virulence of *S. brasiliensis* (Miranda et al. 2018). Bastos et al.

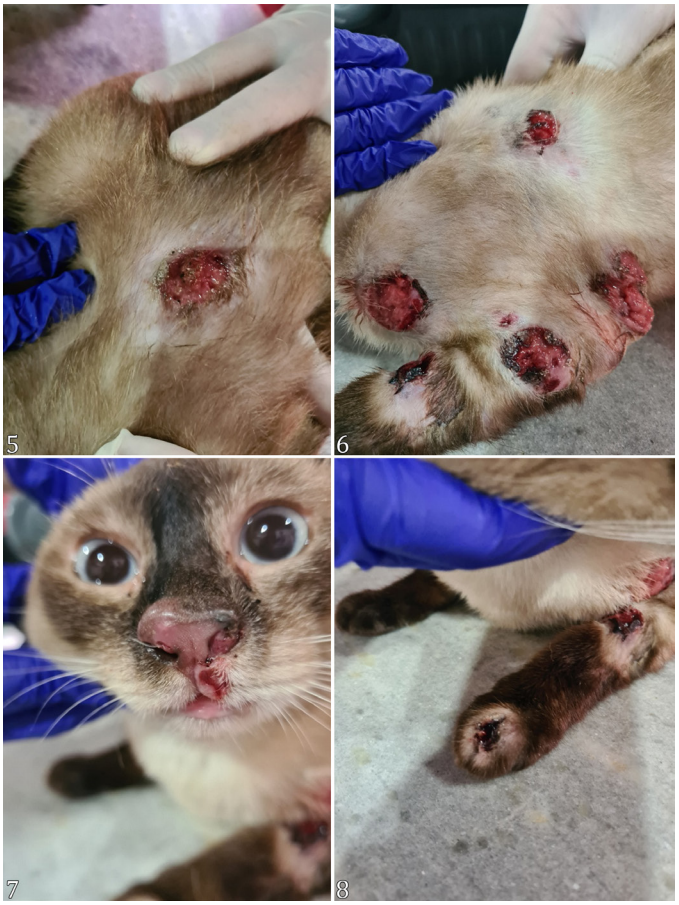

Fig.5-8. Cat affected by disseminated sporotrichosis. (**5**) Circular, ulcerated lesion on the back of the pelvic region. (**6**) Multiple ulcerated lesions with a gummy appearance on the left thoracic limb. (**7**) Nodular lesion on the snout and a small ulcer on the left upper lip. (**8**) Ulcerated lesion on a digit of the right thoracic limb.

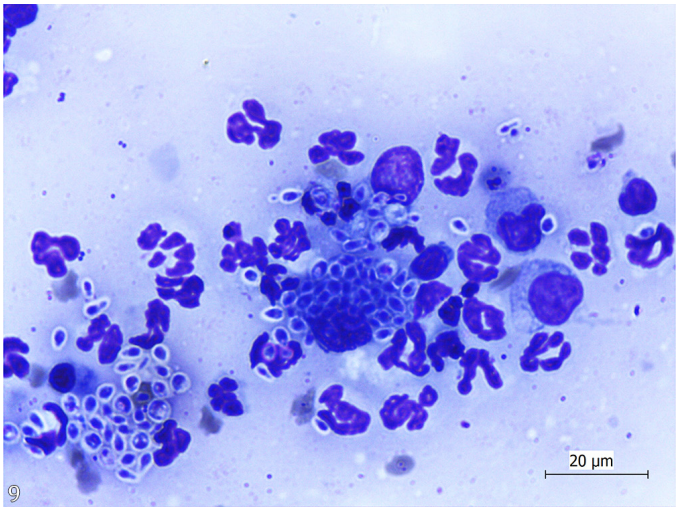

Fig.9. A swab from a feline lesion showing free and intracytoplasmic yeast forms of *Sporothrix* sp. in macrophages. Rapid panoptic staining, bar = 20 µm.

(2022) emphasized that respiratory symptoms, such as nasal discharge and sneezing, facilitate the zoonotic transmission of *Sporothrix* spp. through infectious secretions. Personal protective equipment is essential to reduce the spread of *Sporothrix* spp. (Gremião et al. 2021).

The virulence of *S. brasiliensis* is attributed to factors such as thermal dimorphism, biofilm formation, adhesins, and melanin production, contributing to its aggressive behavior (Barros et al. 2011, Brilhante et al. 2021). Progressive darkening of fungal cultures, indicative of melanin production, was a primary feature observed in *Sporothrix* cultures. Melanin protects the fungus from host defenses by resisting reactive oxygen species and nitric oxide (NO) and shielding against ultraviolet radiation (Smith & Casadevall 2019, García-Carnero & Martínez-Álvarez 2022).

While fungal culture and molecular techniques are excellent diagnostic tools, cytological assessment provides a rapid (Gonsales et al. 2020), cost-effective, and accessible method for diagnosing sporotrichosis in cats, especially in socioeconomically

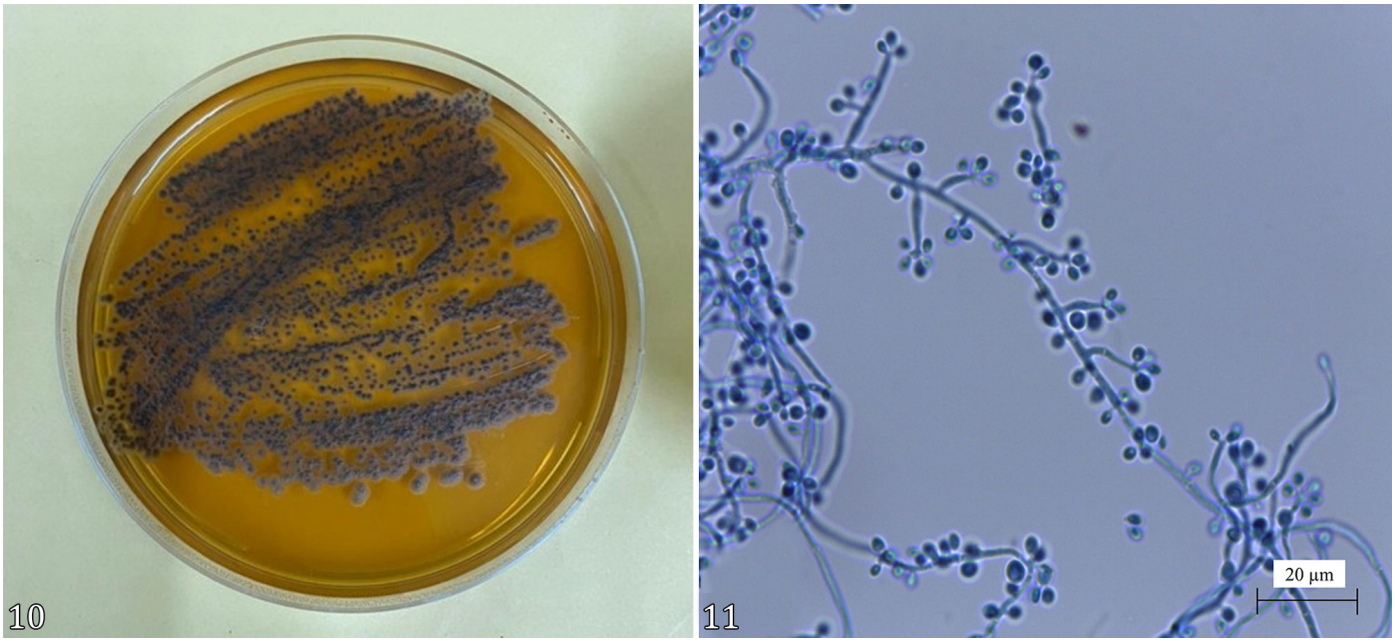

Fig.10-11. Macroscopic and microscopic analysis of *Sporothrix brasiliensis* culture. (**10**) Dark-colored colony indicative of melanin production after 14 days on Sabouraud Dextrose Agar with chloramphenicol, incubated at 25 °C. (**11**) Optical microscopy (100×) showing hyaline and septate hyphae with conidiophores presenting daisy bouquet-like microconidia. Bar = 20 µm.

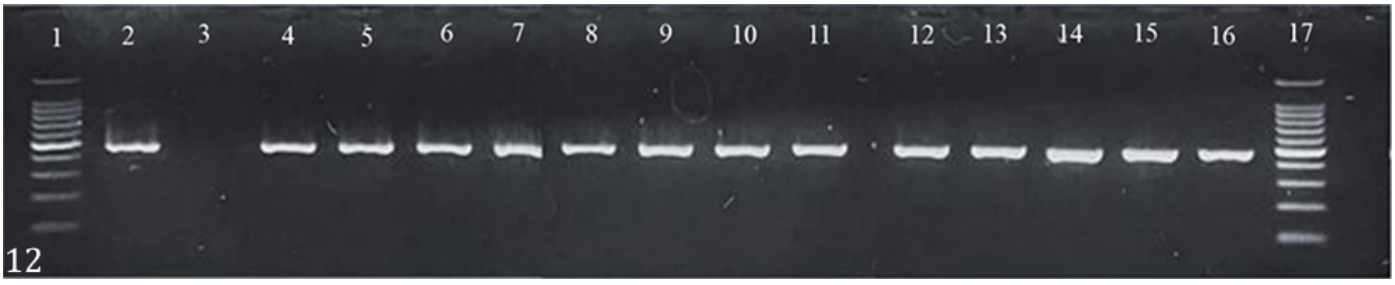

Fig.12. Agarose gel electrophoresis (1.5%) of PCR amplification for the cal gene of *Sporothrix brasiliensis* from feline samples. 1 and 17 = molecular weight marker; 2 = positive control; 3 = negative control; 4–16 = sporotrichosis-positive samples.

Table 1. Results of BLASTn analysis for Sporothrix sequences based on the cal gene

Sample ID	Scientific name	Query cover	E-value	Identity rate	Local	Host	Access number
Felis catus 66	*Sporothrix brasiliensis*	91%	0.0	97.39%	Brazil	*Felis catus*	MH453930Q865519OL953013
Felis catus 86	*Sporothrix brasiliensis*	100%	0.0	98.89%	Brazil	*Canis lupus familiaris, Homo sapiens* and *Felis catus*	OR037321ON397993OL888138
Felis catus 97	*Sporothrix brasiliensis*	99%	0.0	99.54%	Portugal and Brazil	*Felis catus* and *Homo sapiens*	MT783949MH453938KJ769111
Felis catus 108	*Sporothrix brasiliensis*	100%	0.0	98.89%	Portugal and Brazil	*Homo sapiens* and *Felis catus*	MT783938OP811261MH453942
Felis catus 110	*Sporothrix brasiliensis*	100%	0.0	100.00%	Brazil	*Homo sapiens, Canis lupus familiaris*	ON398001OR037312

vulnerable regions. This approach enables the visualization of numerous *S. brasiliensis* specimens in smears from skin lesion exudates (Silva et al. 2015, Gonsales et al. 2019).

The spread of sporotrichosis in Brazil is influenced by various factors, including the absence of comprehensive epidemiological surveillance systems targeting small animals. The limited use of sensitive and accessible diagnostic methods delays the identification (Silva et al. 2024), notification, and treatment of infected animals. Furthermore, the lack of national registries for disease notification in animals and humans highlights the need for improved surveillance programs and the development of novel diagnostic tools for field use. These improvements would enhance diagnostic protocols and promote preventive measures such as responsible pet ownership and early veterinary intervention (Rossow et al. 2020, Moreira et al. 2021). Establishing nationwide reporting systems for animal and human cases is critical for effectively monitoring and managing sporotrichosis outbreaks.

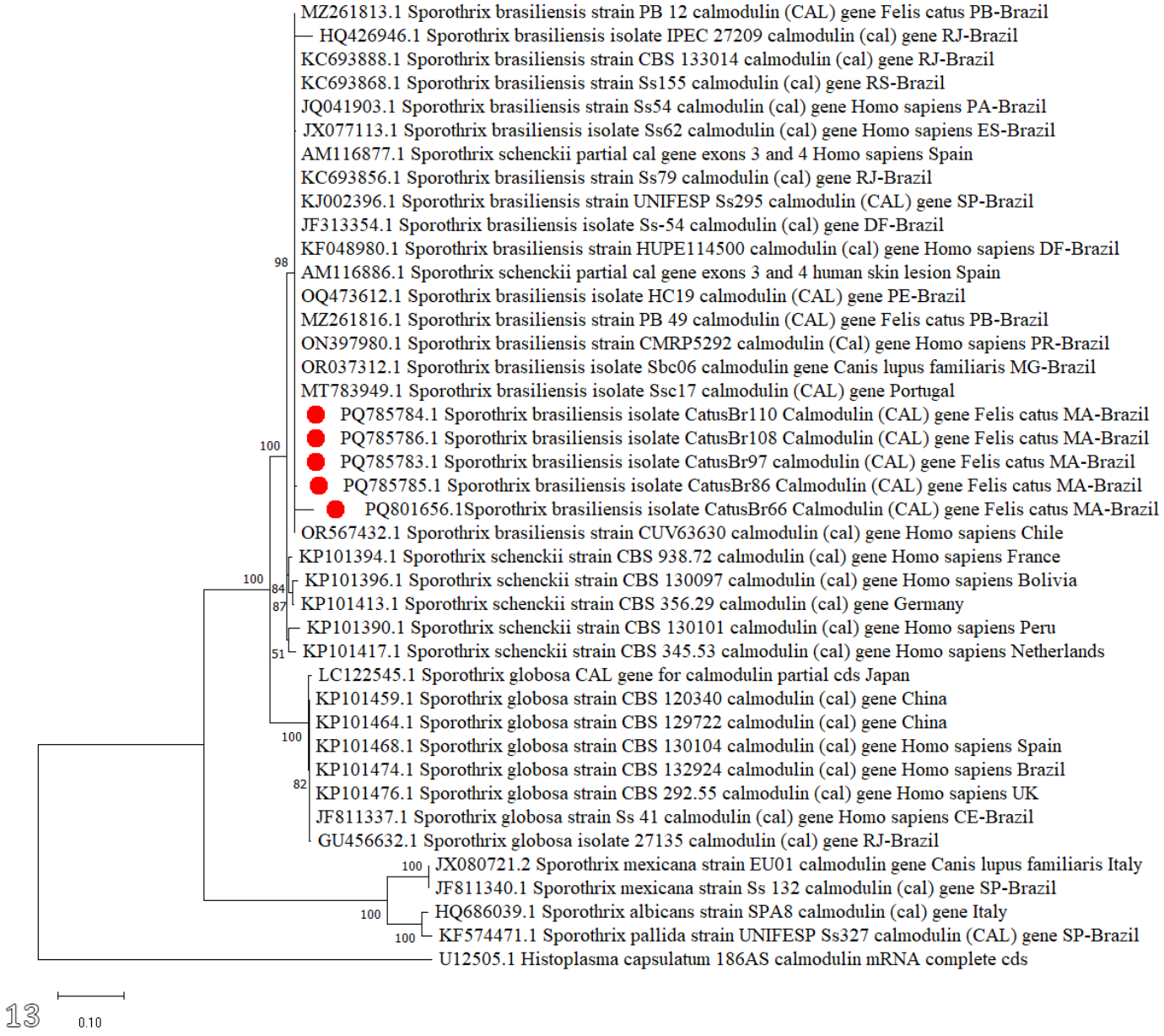

Fig.13. Phylogenetic classification of *Sporothrix brasiliensis* isolates from cats in São Luís Island, Maranhão. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. This analysis included 28 nucleotide sequences based on the cal gene. Evolutionary analyses were performed using MEGA X software.

CONCLUSION

Based on the data, *Sporothrix brasiliensis* was identified as the infected agent in felines from São Luís Island. Most felines presented disseminated cutaneous sporotrichosis, while localized forms were restricted to facial lesions. The fungal isolates exhibited a high degree of support compared to sequences from other Brazilian regions, confirming the disease's expansion. Public policies and health education initiatives are essential to guide pet owners and mitigate the spread of sporotrichosis to other areas of Maranhão.

Acknowledgments.- This study was supported by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (Coordination for the Improvement of Higher Education Personnel - CAPES), Brazil, Finance Code 001). Dra. Allana Freitas Barros was a doctoral scholarship holder funded by the "Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão" (Foundation to Support Research and Scientific and Technological Development of Maranhão - FAPEMA), Grant BD-01171/20. Dr. Fernando Almeida-Souza is a postdoctoral research fellow supported by "Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro" (FAPERJ) (Grant E-26/203.513/2023). Dra. Ana Lucia Abreu-Silva is a researcher productivity fellow funded by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (National Scientific and Technological Development Council - CNPq), Grant 313348/2021-9. We thank Dr. Dayvison Francis Saraiva Freitas ("Fundacão Oswaldo Cruz", RJ, Brazil) for kindly supplying us with some standard isolates of *Sporothrix* spp.

Conflict of interest statement.- The authors declare no conflicts of interest.

Credit author statement.- Allana F. Barros, Juliana S. Vitor, Yanara R.C.M. Esposito, Thiago A. de Melo, Ilka M.R. de Souza, and Larissa S. S. Ribeiro performed the experimental procedures and mycology laboratory analyses. Fábio H.E. Andrade and Ana Lucia Abreu-Silva conducted the histopathological analyses and contributed to data interpretation. Nayara S. Oliveira is responsible for the study design and dermatological clinical assessments. Elmary C. Fraga, Ellainy M. C. Silva, Alcina V.C. Neta, and Fernando Almeida-Souza contributed to the study design, execution, and PCR and phylogenetic analyses as well as data interpretation. Ana L. Abreu-Silva and Larissa S.S. Ribeiro participated in the study design, execution, and data interpretation and provided final approval of the manuscript.

Data availability statement.- The datasets analyzed during the present study are provided in the supplementary material.

REFERENCES

Aguiar BA, Borges IL, Silva BWL, Rodrigues FRN, Gonçalves LD, Casseb AR, Brito JS, Pinheiro AQ, Rocha MFG, Viana DA. First case report of feline sporotrichosis caused by *Sporothrix brasiliensis* in the state of Ceará – Brazil. Med Mycol Case Rep 2023; https://doi.org/10.1016/j.mmcr.2023.02.005

Almeida, AJ, Reis NF, Lourenço CS, Costa NQ, Bernardino MLA, Vieira-da-Motta O. Esporotricose em felinos domésticos (*Felis catus domesticus*) em Campos Dos Goytacazes, RJ. Pesq Vet Bras 2018; https://doi.org/10.1590/1678-5150-PVB-5559

Alves AJSE, Guilloux AGA, Zetun CB, Polo G, Braga GB, Panachão LI, Santos O, Dias RA. Abandono de cães na América Latina: revisão de literatura. Rev Educ Cont Med Vet Zootec 2013; https://doi.org/10.36440/recmvz.v11i2.16221

Araujo RR. O processo de urbanização na produção do clima urbano de São Luís-MA. Dissertação de Mestrado, Universidade Estadual Paulista Júlio de Mesquita Filho, Presidente Prudente, SP, 2001.

Arrillaga-Moncrieff I, Capilla J, Mayayo E, Marimon R, Marine M, Genis J, Cano J, Guarro J. Different virulence levels of the species of *Sporothrix* in a murine model. Clin Microbiol Infect 2009; https://doi.org/10.1111/j.1469-0691.2009.02824.x

Barros MBL, Paes RA, Schubach AO. *Sporothrix Schenckii* and sporotrichosis. Clin Microbiol Rev 2011; https://doi.org/10.1128/cmr.00007-11

Bastos FAGD, Cognialli RCR, Farias MR, Monti FS, Wu K, Queiroz-Telles F. Spread of *Sporothrix* spp. through respiratory droplets from infected cats: a potential route of transmission. Med Mycol 2022; https://doi.org/10.1093/mmy/myac079

Beer ZW, Duong TA, Wingfield MJ. The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship. Studies Mycology 2016; https://doi.org/10.1016/j.simyco.2016.07.001

Bento AO, Costa ASS, Lima SL, Alves MM, Melo ASA, Rodrigues AM, Silva-Rocha WP, Milan EP, Chaves GM. The spread of cat-transmitted sporotrichosis due to *Sporothrix brasiliensis* in Brazil towards the Northeast region. PLoS Negl Trop Dis 2021; https://doi.org/10.1371/journal.pntd.0009693

Boechat JS, Oliveira MME, Almeida-Paes R, Gremião IDF, Machado ACS, Oliveira RVC, Figueiredo ABF, Rabello VBS, Silva KBL, Zancopé-Oliveira RM, Schubach TMP, Pereira SA. Feline sporotrichosis: associations between clinical-epidemiological profiles and phenotypic-genotypic characteristics of the etiological agents in the Rio de Janeiro epizootic area. Mem Inst Oswaldo Cruz 2018; https://doi.org/10.1590/0074-02760170407

Brilhante RSN, Fernandes MR, Pereira VS, Costa AC, Oliveira JS, Aguiar L, Rodrigues AM, Camargo ZP, Pereira-Neto WA, Sidrim JJC, Rocha MFG. Biofilm formation on cat claws by *Sporothrix* species: an ex vivo model. Microbial Pathogenesis 2021; https://doi.org/10.1016/j.micpath.2020.104670

Climate-Data Org. Dados climáticos para cidades mundiais. 2024. Accessed March 10, 2024. https://pt.climate-data.org/america-do-sul/brasil/maranhao/sao-luis-1671/

De Carolis E, Posteraro B, Sanguinetti M. Old and new insights into *Sporothrix schenckii* complex biology and identification. Pathogens 2022; https://doi.org/10.3390/pathogens11030297

Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; https://doi.org/10.2307/2408678

Figueira KD, Nunes GDL. Esporotricose felina: primo relato na cidade de Mossoró, Rio Grande do Norte, Brasil. MEDVEP. Rev Cient Med Vet 2010;8(27):715-718.

García-Carnero LC, Martínez-Álvarez JA. Virulence factors of *Sporothrix schenckii*. J Fungi 2022; https://doi.org/10.3390/jof8030318

Gómez-Gaviria M, Martínez-Álvarez JA, Mora-Montes HM. Current progress in *Sporothrix brasiliensis* basic aspects. *J Fungi* 2023; https://doi.org/10.3390/jof9050533

Gonsales FF, Fernandes NCCA, Mansho W, Montenegro H, Benites NR. Direct PCR of lesions suggestive of sporotrichosis in felines. Arq Bras Med Vet Zootec 2020; https://doi.org/10.1590/1678-4162-11743

Gonsales FF, Fernandes NCCA, Mansho W, Montenegro H, Guerra JM, Araújo LJT, Silva SMP, Benites NR. Feline *Sporothrix* spp. detection using cell blocks from brushings and fine-needle aspirates: performance and comparisons with culture and histopathology. Vet Clin Pathol 2019; https://doi.org/10.1111/vcp.12708

Gremião IDF, Miranda LHM, Reis EG, Rodrigues AM, Pereira SA. Zoonotic epidemic of sporotrichosis: cat to human transmission. PLoS Pathog 2017; https://doi.org/10.1371/journal.ppat.1006077

Gremião IDF, Rocha EMS, Montenegro H, Carneiro AJB, Xavier MO, Farias MR, Monti F, Mansho W, Pereira RHMA, Pereira SA, Lopes-Bezerra LM. Guideline for the management of feline sporotrichosis caused by *Sporothrix brasiliensis* and literature revision. Braz J Microbiol 2021; https://doi.org/10.1007/s42770-020-00365-3

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucl Acid 1999;41:95-98.

Hernández-Castro R, Pinto-Almazán R, Arenas R, Sánchez-Cárdenas CD, Espinosa-Hernández VM, Sierra-Maeda KY, Conde-Cuevas E, Juárez-Durán ER, Xicohtencatl-Cortes J, Carrillo-Casas EM, Steven-Velásquez J, Martínez-

Herrera E, Rodríguez-Cerdeira C. Epidemiology of clinical sporotrichosis in the Americas in the last ten years. J Fungi (Basel) 2022; https://doi.org/10.3390/jof8060588

Kimura M. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980; https://doi.org/10.1007/BF01731581

Kumar S, Tamura K, Nei M. MEGA: molecular evolutionary genetics analysis software for microcomputers. Bioinformatics 1994; https://doi.org/10.1093/bioinformatics/10.2.189

Lopes-Bezerra LM, Mora-Montes HM, Zhang Y, Nino-Vega G, Rodrigues AM, De Camargo ZP, De Hoog S. Sporotrichosis between 1898 and 2017: the evolution of knowledge on a changeable disease and on emerging etiological agents. Med Mycol 2018; https://doi.org/10.1093/mmy/myx103

Macêdo-Sales PA, Souto SRLS, Destefani CA, Lucena RP, Rocha EMS, Baptista ARS. Diagnóstico laboratorial da esporotricose felina em amostras coletadas no estado do Rio de Janeiro, Brasil: limitações da citopatologia por imprint. Revista Pan-Amazônica Saúde 2018; https://doi.org/10.5123/s2176-62232018000200002

Marimon R, Cano J, Gené J, Sutton DA, Kawasaki M, Guarro J. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 2007; https://doi.org/10.1128/jcm.00808-07

Marques-Melo EH, Lessa DFS, Nunes ACBT, Chaves KP, Porto WJN, Notomi MK, Garrido LHA. Felino doméstico como agente transmissor de esporotricose para humano: relato do primeiro caso no estado de Alagoas. Rev Baiana Saúde Pública 2014; https://doi.org/10.22278/2318-2660.2014.v38.n2.a535

Miranda LHM, Meli M, Conceição-Silva F, Novacco M, Menezes RC, Pereira SA, Sugiarto S, Reis ÉG, Gremião IDF, Hofmann-Lehmann R. Co-infection with feline retrovirus is related to changes in immunological parameters of cats with sporotrichosis. PLoS One 2018; https://doi.org/10.1371/journal.pone.0207644

Mora-Montes HM, Dantas AS, Trujillo-Esquivel E, Baptista ARS, Lopes-Bezerra LM. Current progress in the biology of members of the *Sporothrix Schenckii* complex following the genomic era. FEMS Yeast Research 2015; https://doi.org/10.1093/femsyr/fov065

Moreira SM, Andrade EHP, Paiva MT, Zibaoui HM, Salvato LA, Azevedo MI, Oliveira CSF, Soares DFM, Keller KM, Magalhães SL, Morais MHF, Costa JRR, Bastos CV. Implementation of an animal sporotrichosis surveillance and control program, southeastern Brazil. Emerg Infect Dis 2021; https://doi.org/10.3201/eid2703.202863

Nakasu CCT, Waller SB, Ripoll MK, Ferreira MRA, Conceição FR, Gomes AR, Osório LG, Faria RO, Cleff MB. Feline sporotrichosis: a case series of itraconazole-resistant *Sporothrix brasiliensis* infection. Braz J Microbiol 2021; https://doi.org/10.1007/s42770-020-00290-5

Nunes GDL, Carneiro RL, Filgueira KD, Filgueira FGF, Fernandes THT. Esporotricose felina no município de itaporanga, estado da Paraíba, Brasil: relato de um caso. Arq Ciênc Vet Zool 2011;14(2):157-161.

Orofino-Costa R, Freitas DFS, Bernardes-Engemann AR, Rodrigues AM, Talhari C, Ferraz CE, Veasey JV, Quintella L, Sousa MSLA, Vettorato R, Almeida-Paes R, Macedo PM. Human sporotrichosis: recommendations from the Brazilian Society of Dermatology for the clinical, diagnostic and therapeutic management. An Bras Dematol 2022; https://doi.org/10.1016/j.abd.2022.07.001

Orofino-Costa R, Macedo PM, Rodrigues AM, Bernardes-Engemann AR. Sporotrichosis: An update on epidemiology, etiopathogenesis, laboratory and clinical therapeutics. An Bras Dermatol 2017; https://doi.org/10.1590/abd1806-4841.2017279

Rocha LCS, Rauber AL. Amazônia legal brasileira: a incidência de focos de calor entre os anos de 2001 e 2020 e a correlação com o desmatamento de corte raso. Revista Equador 2023;12(1):199-226.

Rodrigues AM, Hoog GS, Camargo ZP. Molecular diagnosis of pathogenic *Sporothrix* species. PLoS Negl Trop Dis 2015; https://doi.org/10.1371/journal.pntd.0004190

Rodrigues AM, Teixeira MM, Hoog GS, Schubach TMP, Pereira SA, Fernandes GF, Bezerra LML, Felipe MS, Camargo ZP. Phylogenetic analysis reveals a high prevalence of *Sporothrix Brasiliensis* in feline sporotrichosis outbreaks. PLoS Negl Trop Dis 2013; https://doi.org/10.1371/journal.pntd.0002281

Rossow JA, Queiroz-Telles F, Caceres DH, Beer KD, Jackson BR, Pereira JG, Gremião IDF, Pereira SA. A one health approach to combatting Sporothrix brasiliensis: narrative review of an emerging zoonotic fungal pathogen in South America. J Fungi 2020; https://doi.org/10.3390/jof6040247

Sambrook J, Fritsch ER, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.

Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U.S.A. 1977; https://doi.org/10.1073/pnas.74.12.5463

SES. Cenário Epidemiológico: Esporotricose no Estado RJ. Boletim epidemiológico esporotricose nº 001/2019. Rio de Janeiro: Secretaria de Saúde do Estado do Rio de Janeiro, 2019. Accessed May 18, 2024. http://www.riocomsaude.rj.gov.br/Publico/MostrarArquivo.aspx?C=qEn%2BgM7lw8A%3D#:~:text=obtidos%20no%20FORMSUS.-,CENÁRIO%20EPIDEMIOLÓGICO%3A%20ESPOROTRICOSE%20NO%20ESTADO%20RJ.,casos%20por%20100%20mil%20habitantes

Silva AG, Matos AFC, Sousa BR, Ferraz CE, Luiz RLF, Neves RP, Lima-Neto RG, Oliveira MME. Rapid molecular diagnosis of sporotrichosis directly from biological samples from a reference center in Brazil. J Fungi 2024; https://doi.org/10.3390/jof10060432

Silva FS, Cunha SCS, Moraes VA, Leite JS, Ferreira AMR. Refractory feline sporotrichosis: a comparative analysis on the clinical, histopathological, and cytopathological. Pesq Vet Bras 2022; https://doi.org/10.1590/1678-5150-PVB-6923

Silva GM, Howes JCF, Leal CAS, Mesquita EP, Pedrosa CM, Oliveira AAF, Silva LBG, Mota RA. Surto de esporotricose felina na região metropolitana do Recife. Pesq Vet Bras 2018a; https://doi.org/10.1590/1678-5150-PVB-5027

Silva JN, Miranda LHM, Menezes RC, Gremião IDF, Oliveira RVC, Vieira SMM, Conceição-Silva F, Ferreiro L, Pereira SA. Comparison of the sensitivity of three methods for the early diagnosis of sporotrichosis in cats. J Comp Pathol 2018b; https://doi.org/10.1016/j.jcpa.2018.03.002

Silva JN, Passos SRL, Menezes RC, Gremião IDF, Schubach TMP, Oliveira JC, Figueiredo ABF, Pereira SA. Diagnostic accuracy assessment of cytopathological examination of feline sporotrichosis. Med Mycol 2015; https://doi.org/10.1093/mmy/myv038

Smith DFQ, Casadevall A. The role of melanin in fungal pathogenesis for animal hosts. In: Rodrigues ML. Fungal Physiology and Immunopathogenesis. Cham: Springer; 2019.

Spinelli TP, Bezerra LM, Souza BOF, Rocha A, Neto JE, Sá FB. Primary conjunctival sporotrichosis in three cats from northeastern Brazil. Vet Ophthalmol 2021; https://doi.org/10.1111/vop.12865

Stefaniszen AG, Ferreira LVO, Guimarães-Okamoto PTC, Melchert A. Recent advances in the management of feline sporotrichosis. J Adv Vet Res 2023;13(5):850-856.

WHO. Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. World Health Organization, 2021. Accessed Aug 12, 2024. https://www.who.int/publications/i/item/9789240010352

Xavier MO, Poester VR, Trápaga MR, Stevens DA. *Sporothrix brasiliensis*: epidemiology, therapy, and recent developments. J Fungi 2023; https://doi.org/10.3390/jof9090921

Zhang Y, Hagen F, Stielow B, Rodrigues AM, Samerpitak K, Zhou X, Feng P, Yang L, Chen M, Deng S, Li S, Liao W, Li R, Li F, Meis JF, Guarro J, Teixeira M, Al-Zahrani HS, Camargo ZP, Zhang L, Hoog GS. Phylogeography and evolutionary patterns in *Sporothrix* spanning more than 14,000 human and animal case reports. Persoonia, Int Mycol Evol Fungi 2015; https://doi.org/10.3767/003158515X687416

Supplementary material

Table S1. Clinical data of animals with feline sporotrichosis from São Luís Island, Maranhão state, Brazil

Animal	Breed	Sex	Age	Clinical presentation	1st lesion aspect	1st lesion spot	Sneeze	Ocular secretion	Location of the lesions
1	No defined breed	Male	2 years	Disseminated cutaneous	n.a.	Thoracic limb	n.a.	No	Right thoracic limb and dorsum pelvic region
2	No defined breed	Male	3 years	Disseminated cutaneous	Ulcerate	Right thoracic limb	No	No	Ears, upper left eyelid, lower right eyelid and right thoracic limb
3	No defined breed	Female	1 year	Disseminated cutaneous	Nodular	Abdominal region	No	No	Ventral abdominal region and pelvic limbs
4	No defined breed	Female	n.a.	Disseminated cutaneous	Ulcerate	Abdominal region	Yes	No	Nose, digit thoracic limb (left), digit pelvic limb (right) and abdominal region
5	No defined breed	Male	2 years	Disseminated cutaneous	n.a.	n.a.	No	No	Ears, nose, lower left eyelid, upper left eyelid and cervical region
6	No defined breed	Male	2 years	Disseminated cutaneous	Ulcerate	Face	Yes	No	Mandible, ears, forehead and thoracic limbs
7	No defined breed	Male	3 years	Disseminated cutaneous	Ulcerate	n.a.	Yes	No	Nose, ears, left thoracic limb, dorsum thoracic region, left pelvic limb, left eyelids and right pelvic limb
8	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a	Yes	No	Scrotum, nose, upper left eyelid, right pelvic limb, thoracic limbs and taiface
9	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	n.a. No	Forehead, ears, thoracic limbs and pelvic limbs
10	No defined breed	Male	2 years	Disseminated cutaneous	Ulcerate	Eyelid	Yes	Yes	Right thoracic limb, cervical region. thoracic region, nose and eyelid
11	No defined breed	Male	9 years	Disseminated cutaneous	Ulcerate	Near the ear	Yes	Yes	Right thoracic limb, cervical region, upper right eyelid, nose and forehead
12	No defined breed	Female	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Nose, upper left eyelid, ears, pelvic limb, left thoracic limb and thoracic region
13	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Ears, nose, right thoracic limb and right pelvic limb
14	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	Yes	Upper left eyelid, ears, tail and pelvic limbs
15	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Ears, nose, upper left eyelid, upper right eyelid, thoracic limbs, left pelvic limb and tail
16	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	Yes	Ears, nose, left eyelid and lower right eyelid
17	No defined breed	Male	3 years	Disseminated cutaneous	Ulcerate	Thoracic limb	No	No	Thoracic limbs, pelvic limbs
18	No defined breed	Male	3 years	Disseminated cutaneous	Ulcerate	Near the eye	No	No	Left thoracic limb, left ear, lower left eyelid and tail
19	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Ears, nose, thoracic limbs, left pelvic limb and tail

Animal	Breed	Sex	Age	Clinical presentation	1st lesion aspect	1st lesion spot	Sneeze	Ocular secretion	Location of the lesions
20	No defined breed	Female	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Ears, nose, thoracic limbs and left pelvic limb
21	No defined breed	Male	4 years	Disseminated cutaneous	Ulcerate	Cervical region	No	No	Ears, cervical region and thoracic region
22	No defined breed	Male	4 years	Disseminated cutaneous	Ulcerate	Face	No	No	Thoracic limbs, cervical region, and near the left ear
23	No defined breed	Female	15 years	Disseminated cutaneous	Nodular	Right thoracic limb	Yes	No	Right thoracic limb, right pelvic limb and nose
24	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Right pelvic limb, left ear and left thoracic limb
25	No defined breed	Male	7 months	Disseminated cutaneous	Ulcerate	Right thoracic limb	Yes	No	Right pelvic limb, nose and mandible
26	No defined breed	Male	n.a.	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Forehead, ears and upper left eyelid
27	No defined breed	Female	4 years	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Tail and pelvic limbs
28	No defined breed	Male	4 years	Disseminated cutaneous	n.a.	n.a.	n.a.	No	Right ear, upper left eyelid, lower right eyelid, thoracic limbs and left ear
29	No defined breed	Male	4 years	Disseminated cutaneous	Ulcerate	Left eyelid	No	Yes	Thoracic limbs, left eyelids and left ear
30	Siamese	Female	3 years	Disseminated cutaneous	Ulcerate	Thoracic limb	No	No	Nose, thoracic limb, thoracic region, right pelvic limb, axillary region (left) and right thoracic limb
31	No defined breed	Male	7 years	Disseminated cutaneous	Ulcerate	Eyelid	No	No	Thoracic limbs, upper right eyelid, lower left eyelid and left ear
32	No defined breed	Male	1 year	Disseminated cutaneous	Nodular	Tail	Yes	No	Left pelvic limb, tail, thoracic limbs, nose and ear
33	No defined breed	Male	8 years	Fixed cutaneous	n.a.	Nose	n.a.	Yes	Upper eyelids, nose and lower left eyelid
34	No defined breed	Female	1 year	Fixed cutaneous	Ulcerate	Abdominal region	No	No	Abdominal region (*linea alba*)
35	No defined breed	Male	1 year	Fixed cutaneous	Ulcerate	Cervical region	No	No	Left ear, forehead, upper right eyelid
36	No defined breed	Female	10 months	Fixed cutaneous	Ulcerate	Abdominal region	No	No	Ventral abdominal region
37	No defined breed	Male	2 years	Fixed cutaneous	n.a.	n.a	n.a.	No	Left thoracic limb
38	No defined breed	Female	1 year	Fixed cutaneous	Ulcerate	Nose	Yes	No	Nose
39	No defined breed	Male	n.a.	Fixed cutaneous	n.a.	Nose	Yes	No	Nose

Animal	Breed	Sex	Age	Clinical presentation	1st lesion aspect	1st lesion spot	Sneeze	Ocular secretion	Location of the lesions
40	No defined breed	Female	2 years	Fixed cutaneous	Nodular	Right pelvic limb	No	No	Pelvic limbs
41	No defined breed	Female	7 years	Fixed cutaneous	Ulcerate	Pelvic region	No	No	Right thoracic limb
42	No defined breed	Male	1year	Fixed cutaneous	Nodular	Scrotum	No	No	Scrotum
43	No defined breed	Male	5 years	Fixed cutaneous	Ulcerate	Left thoracic limb	No	No	Left thoracic limb
44	No defined breed	Male	7 years	Fixed cutaneous	Ulcerate	Right pelvic limb	Yes	Yes	Right pelvic limb
45	No defined breed	Female	2 years	Fixed cutaneous	Ulcerate	Pelvic limbs	No	No	Pelvic limbs
46	No defined breed	Male	1 year	Fixed cutaneous	Ulcerate	Right Thoracic limb	No	No	Right thoracic limb

n.a. = not accessed data.