HHS Public Access

Author manuscript

Lancet Microbe. Author manuscript; available in PMC 2025 March 01.

Published in final edited form as:

Lancet Microbe. 2024 March ; 5(3): e282–e290. doi:10.1016/S2666-5247(23)00364-6.

Emergence of zoonotic sporotrichosis in Brazil: a genomic epidemiology study

Amanda Ribeiro dos Santos,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA; School of Medicine, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil

Elizabeth Misas,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Brian Min,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Ngoc Le,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Ujwal R Bagal,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Lindsay A Parnell,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

D Joseph Sexton,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Shawn R Lockhart,

Correspondence to: Dr Nancy A Chow, Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA 30329, USA, nchow@cdc.gov.

Contributors

ARdS did literature search, figures, study design, data curation, data analysis, data interpretation, and writing. NAC did study design, project administration, resources, supervision, software, visualisation, data interpretation, and writing (original draft, review, and editing). APL did literature search, data interpretation, conceptualisation, and writing (review and editing). EM, URB, and LAP did bioinformatics methodology, data analysis, data interpretation, software, and figures. BM and NL did laboratory methodology, data analysis, and data interpretation. DJS and SRL did project administration, resources, supervision, and writing (review and editing). MdSCM, JPFT, GMO, LXB, PC, JLMS, LSA, and HLAF did laboratory methodology, data collection and investigation. MdSCM, PC, JV, and TMC did supervision, funding acquisition, and writing (review and editing). ARdS, NAC, EM, BM, and URB verified all the underlying data that were reported in this study. All authors had full access to all the data in the study, read and approved the final manuscript, as well as having the final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

For more on **FastTree** see https://ubuntu.pkgs.org/20.04/ubuntu-universe-amd64/fasttree_2.1.11-1_amd64.deb.html

For more on **MEGA** see https://www.megasoftware.net/

For more on **Microreact** see http://microreact.org

For more on **BLAST** see http://blast.ncbi.nlm.nih.gov/Blast.cgi

For more on **MycoSNP** see https://github.com/CDCgov/mycosnp-nf/

For an **interactive map of the geographical distribution of the clades in the different Brazilian states** see https://microreact.org/project/7sL7k1VNqVVu2fQchMLTBZ-figure2d

Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Marcia de Souza Carvalho Melhem,
School of Medicine, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil; Parasitology and Mycology Center, Instituto Adolfo Lutz, São Paulo, Brazil

Juliana Possatto Fernandes Takahashi,
School of Medicine, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil; Parasitology and Mycology Center, Instituto Adolfo Lutz, São Paulo, Brazil

Gabriel Manzi Oliboni,
Coordenadoria de Controle de Doenças, Secretaria de Estado de Saúde, São Paulo, Brazil

Lucas Xavier Bonfieti,
Parasitology and Mycology Center, Instituto Adolfo Lutz, São Paulo, Brazil

Paola Cappellano,
Microbiology Section, Grupo Fleury, São Paulo, Brazil

Jorge Luiz Mello Sampaio,
Microbiology Section, Grupo Fleury, São Paulo, Brazil

Lisandra Siufi Araujo,
School of Medicine, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil; Central Public Health Laboratory of Mato Grosso do Sul, Secretaria de Estado de Saúde de Mato Grosso do Sul, Campo Grande, Brazil

Hilton L Alves Filho,
School of Medicine, Universidade Federal de Pelotas, Pelotas, Brazil

James Venturini,
School of Medicine, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil

Tom M Chiller,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Anastasia P Litvintseva,
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Nancy A Chow
Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA 30329, USA

Summary

Background—Zoonotic sporotrichosis is a neglected fungal disease, whereby outbreaks are primarily driven by *Sporothrix brasiliensis* and linked to cat-to-human transmission. To understand the emergence and spread of sporotrichosis in Brazil, the epicentre of the current epidemic in South America, we aimed to conduct whole-genome sequencing (WGS) to describe the genomic epidemiology.

Methods—In this genomic epidemiology study, we included *Sporothrix* spp isolates from sporotrichosis cases from Brazil, Colombia, and the USA. We conducted WGS using Illumina NovaSeq on isolates collected by three laboratories in Brazil from humans and cats with

sporotrichosis between 2013 and 2022. All isolates that were confirmed to be *Sporothrix* genus by internal transcribed spacer or beta-tubulin PCR sequencing were included in this study. We downloaded eight *Sporothrix* genome sequences from the National Center for Biotechnology Information (six from Brazil, two from Colombia). Three *Sporothrix* spp genome sequences from the USA were generated by the US Centers for Disease Control and Prevention as part of this study. We did phylogenetic analyses and correlated geographical and temporal case distribution with genotypic features of *Sporothrix* spp isolates.

Findings—72 *Sporothrix* spp isolates from 55 human and 17 animal sporotrichosis cases were included: 67 (93%) were from Brazil, two (3%) from Colombia, and three (4%) from the USA. Cases spanned from 1999 to 2022. Most (61 [85%]) isolates were *S brasiliensis*, and all were reported from Brazil. Ten (14%) were *Sporothrix schenckii* and were reported from Brazil, USA, and Colombia. For *S schenckii* isolates, two distinct clades were observed wherein isolates clustered by geography. For *S brasiliensis* isolates, five clades separated by more than 100 000 single-nucleotide polymorphisms were observed. Among the five *S brasiliensis* clades, clades A and C contained isolates from both human and cat cases, and clade A contained isolates from six different states in Brazil. Compared with *S brasiliensis* isolates, larger genetic diversity was observed among *S schenckii* isolates from animal and human cases within a clade.

Interpretation—Our results suggest that the ongoing epidemic driven by *S brasiliensis* in Brazil represents several, independent emergence events followed by animal-to-animal and animal-to human transmission within and between Brazilian states. These results describe how *S brasiliensis* can emerge and spread within a country.

Funding—Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil; the São Paulo Research Foundation; Productivity in Research fellowships by the National Council for Scientific and Technological Development, and Ministry of Science and Technology of Brazil.

Introduction

Sporotrichosis is a fungal disease caused by dimorphic fungi found in the environment of the *Sporothrix schenckii* species complex: *S schenckii* sensu stricto, *Sporothrix brasiliensis*, *Sporothrix globosa*, and *Sporothrix luriei*.[1] Transmission routes vary by species; specifically, *S schenckii* and *S globosa* are primarily transmitted via traumatic inoculation of contaminated plant debris. Alternatively, *S brasiliensis* is primarily associated with animal infections and zoonotic transmission through deep scratches and bites from infected domestic and feral cats.[2] Additionally, transmission through the sneeze of infected cats has been suggested in a 2022 study.[3] Infection often presents as cutaneous or lymphocutaneous lesions, and for some cases, infection can progress to a life-threatening disseminated disease, which has been described in patients who are immunosuppressed due to advanced HIV, chronic alcoholism, and diabetes.[4] Several antifungal therapy strategies are available to treat infections caused by *Sporothrix* spp; itraconazole is the first-line therapy, with terbinafine and potassium iodide as alternatives and amphotericin B used in cases of severe infection.[5]

Calculating incidence of sporotrichosis and describing the global geographical distribution of *Sporothrix* spp are difficult due to an absence of robust surveillance systems. Bongomin and colleagues,[6] estimated worldwide incidence of sporotrichosis based on published reports

and reported annual incidence to be over 40 000 cases per year. Regarding geographical distribution of *Sporothrix* spp, Zhang and colleagues[7] speciated clinical isolates from major geographical areas using *CAL*, *TEF1*, and *TEF3* genes and found that 139 (99%) of 140 isolates in Asia were *S globosa*, all ten isolates included from Australia were *S schenckii*, seven (88%) of eight isolates in Africa were *S schenckii*,[7,8] and 81 (89%) of 91 isolates in the Americas (except for Brazil) including Colombia and USA, were *S schenckii*. In Brazil, 312 (89%) of 352 isolates were *S brasiliensis*.

A large sporotrichosis epidemic is ongoing in Brazil (1998 to present) with human and cat cases reported in 25 of 26 Brazilian states.[8] The dominant species is *S brasiliensis*. In São Paulo state, located in southeastern Brazil, the São Paulo Zoonosis Control Center conducted veterinary surveillance between 2008 and 2013 among 132 cats and identified only one sporotrichosis case in 2008.[9] However, during 2011–13, 163 cat cases had culture-confirmed *S brasiliensis* in São Paulo and its metropolitan region.[10] In Rio de Janeiro, also located in southeastern Brazil, sporotrichosis outbreaks began in the 1990s with an abrupt and continuous increase starting in 1998.[11] Rio de Janeiro now reports the largest number of cases in Brazil: 4517 human, 4916 cat, and 244 canine cases were reported from Rio de Janeiro with specimen collection dates from 1991 to 2017.[12] Additionally, sporotrichosis has been classified as a notifiable disease in Rio de Janeiro, and in 2019, 1720 annual cases were reported, corresponding to an annual incidence of 9·96 per 100 000 inhabitants.[13]

How sporotrichosis emerged and spread in Brazil is not well understood, but two major competing hypotheses have been proposed. One hypothesis suggests a single origin of the outbreak lineage that first emerged in Rio de Janeiro state and subsequently spread into other Brazilian states.[2,14,15] The other hypothesis proposes independent emergence of genetically distinct *S brasiliensis* lineages in different Brazilian states over time, all capable of infecting cats and humans and contributing to the ongoing outbreak.[16] This latter hypothesis was supported by a genomic epidemiologic study published in 2023, reporting short tandem repeat analysis of 173 *S brasiliensis* isolates and whole-genome sequencing (WGS) of 21 representative strains.[17] We aimed to conduct a genomic epidemiology study to understand the emergence and spread of sporotrichosis in Brazil and provide insight for future spread of this fungal infection.

Methods

Study design and samples

In this genomic epidemiology study, we analysed the genomes of *Sporothrix* spp isolates that were collected primarily in Brazil as well as some isolates from the USA and Colombia.

We conducted WGS on isolates from three different laboratories in Brazil: Microbiology Section of Grupo Fleury (São Paulo, Brazil), Parasitology and Mycology Center of Adolfo Lutz Institute (São Paulo, Brazil) serving as the reference laboratory for São Paulo State, and the Central Public Health Laboratory of Mato Grosso do Sul (Campo Grande, Brazil) serving as the reference laboratory for Mato Grosso do Sul State. These isolates were from clinical samples from human and animal patients with clinical suspicion of sporotrichosis received by the three reference laboratories for diagnosis purposes and were kept as part

of the isolate collection bank for each laboratory. All culture-confirmed *Sporothrix* isolates collected from 2013 to 2022 by the three different laboratories were sent to the US Centers for Disease Control and Prevention (CDC) Mycotic Diseases Branch laboratory (Atlanta, GA, USA). All isolates that were confirmed to be *Sporothrix* genus by internal transcribed spacer or beta-tubulin PCR sequencing at the CDC Mycotic Diseases Branch laboratory were included in this study.

We searched the National Center for Biotechnology Information (NCBI) Sequence Read Archive from database inception to Oct 13, 2022, for whole-genome sequences generated by Illumina, using the search term "*Sporothrix*". Only six *Sporothrix* spp genome sequences from Brazil and two *S schenckii* genome sequences from Colombia passed our quality control threshold and were included in this study (appendix 2 p 6). The quality control threshold parameters used in this study are in the appendix 2 (p 7).

The CDC Mycotic Diseases Branch laboratory has a historical collection of *S schenckii* isolates; we randomly chose three isolates, and all three were from the USA.

This study did not qualify for ethics committee approval in Brazil as there was no use of experimental animals or involvement of human samples. The isolates used in this study came from a culture collection and do not share any sensitive human information.

Species identification and genome sequencing

Species identification was done at the CDC Mycotic Diseases Branch laboratory. DNA was extracted using ZR Fungal/Bacterial DNA MiniPrep kit (ZYMO Research, Irvine, CA, USA). The isolated DNA was measured by a nanospectrophotometer Nanodrop 2000c (Thermo-Scientific, Waltham, MA, USA), and 10 ng DNA was used in PCR amplification of the nuclear ribosomal internal transcribed spacer 4 and 5 using 5′-TCCTCCGCTTATTGATATGC and 5′-GGAAGTAAAAGTCGTAACAAGG primers and amplification of beta-tubulin 2 A and B using 5′-GGTAACCAAATCGGTGCTGCTTTC and 5′-ACCCTCAGTGTAGTGACCCTTGGC primers as described by Maubon and colleagues.[18] For genus confirmation, all sequences obtained from PCR amplification regions were compared with published sequences in GenBank using the Basic Local Alignment Search Tool (BLAST). Next, genomic libraries were constructed using NEBNext Ultra DNA Library Prep kit (New England Biolabs, Ipswich, MA, USA) for Illumina and sequenced on Illumina NovaSeq 6000SP reagent kit (500 cycles).

Single-nucleotide polymorphism (SNP) and phylogenetic analysis

SNPs were identified using MycoSNP version 1.4 as described by Bagal and colleagues.[19] This method prepares the reference, performs quality control, and calls variants using a reference indexed. Analyses were conducted using *S brasiliensis* strain 5110 reference genome (NCBI taxonomy identification: 1398154).[20]The MycoSNP pipeline generated the maximum likelihood tree from the filtered SNPs calling file, using FastTree version 2.1.11. The consensus phylogenomic tree of 1000 ultrafast bootstraps coupled with 1000 Shimodaira–Hasegawa-like approximate likelihood ratio tests was used to assess support for each clade. Genetic distance calculations and neighbour-joining tree construction was performed by MEGA version 11, using a multi-FASTA file generated by MycoSNP

v1.4. The neighbour-joining tree and maps were visualised together with metadata containing additional epidemiological data for each sample using Microreact version 252. A multidimensional scaling analysis was performed. For multidimensional scaling, a patristic distance matrix was built from the maximum likelihood tree using the dendrophy function version 3.12.0 in python version 2.7.11. The values in the patristic distance matrix were used directly without scaling or normalisation for performing multidimensional scaling using cmdscale R function, R version 4.0.3. A plot was created to visualise the differences between samples using the qplot function in R (appendix 2 pp 2–4).

Characterisation of the mating type locus

A simplified de novo assembly was performed with SPAdes v.3.1.3, using half of the reads and the following parameters for each isolate: -k 127 and –only-assembler. To identify diverged regions between two mating type idiomorphs (*MAT1-1* and *MAT1-2*), two sets of primers that target the α box region from *MAT1-1* and high-mobility group domain from *MAT1-2* loci[21] were searched using nucleotide BLAST (BLASTn) against the sequences: *MAT1-1-1* in *S schenkii* (GenBank accession number: XM_016732606.1) and *MAT1-2-1* in *S brasiliensis* (GenBank accession number: JX101596). The PCR products (673bp product corresponding to *MAT1-1* and 291bp product corresponding to *MAT1-2*) were searched using BLASTn against the assembled scaffolds of each isolate to identify their mating type. For two genome assemblies (B22144 and B22096) that showed BLASTn hits with both PCR products, an additional reference assembly using Burrows-Wheeler Aligner version 0.7.17 was performed. Format conversion, sorting and index were generated with Samtools v1.15.1. Integrative Genomics Viewer v2.16.2 was used to visualise the binary alignment map files. Based on read mapped coverage, only one matting type idiomorph was confirmed in each isolate.

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

72 *Sporothrix* spp isolates from human and animal sporotrichosis cases were included in our study: 67 (93%) were from Brazil, two (3%) from Colombia, and three (4%) from the USA (table). 61 genomes (all from Brazil) were sequenced as part of this study. Characteristics of the human-associated *Sporothrix* isolates collected in Brazil are in the appendix 2 (p 5). Specimen collection dates ranged from 1999 to 2022 (figure 1A). Of the 17 isolates from animal sporotrichosis cases, 16 (94%) were collected from skin lesions. We provide a browsable version for sporotrichosis genomic epidemiology in Brazil through Microreact to explore phylogeny, geographical distribution, and timelines.

Genomic analysis of the 72 *Sporothrix* spp isolates revealed that in Brazil, 61 (91%) of 67 isolates were *S brasiiensis*, five (7%) were *S schenckii*, and one (1%) was *S globosa*. Isolates from Colombia (two [3%] of 72) and the USA (three [4%] of 72) were all *S schenckii*

(figure 1B; table). *S schenckii* and *S brasiliensis* isolates were more than 1·5 million SNPs different from *S globosa* and nearly 400 000 SNPs different from each other (figure 1 B).

Among *S schenckii* isolates, large genetic diversity was observed (maximum pairwise SNP difference: 594 599 SNPs) with two distinct clades separated by 443 443 SNPs. One clade (referred to as *S schenckii* clade A) comprised three isolates (two human isolates and one animal isolate) in the USA with a maximum pairwise SNP difference of 64 542 SNPs. The second clade (referred to as *S schenckii* clade B) comprised a subclade of five isolates (five human isolates and two animal isolates) in Brazil (São Paulo and Distrito Federal) and another subclade of two human isolates from Colombia. These subclades were separated by 67 169 SNPs (figure 1B; appendix 2 p 10). Mating type was assessed and four of ten *S schenckii* isolates were *MAT1-2*, and six were *MAT1-1* (appendix 2 p 10).

Of the 61 *S brasiliensis* isolates from Brazil, most were from the southeastern states: 22 (36%) were from São Paulo and four (7%) were from Rio de Janeiro. The remaining isolates were from the midwestern regions, Mato Grosso do Sul, Corumbá city (nine [15%]) and Distrito Federal, Brasília city (two [3%]); and the northeast states Rio Grande do Norte (three [5%]), Pernambuco (one [2%]), and Bahia (one [2%]). The state of collection for 19 (31%) isolates was unknown (table). The phylogenetic tree and multidimensional scaling analysis revealed five distinct clades (referred to as *S brasiliensis* clades A–E) separated by a minimum 115 937 and a maximum of 309 321 SNPs (figure 2A, B). Each clade was supported with bootstrap values of 100%.

S brasiliensis clade A consisted of 39 (64%) of the 61 isolates from Brazil with a maximum pairwise SNP difference of 247 SNPs (figure 3A). Of these, 28 (72%) isolates were from human cases, and 11 (28%) were from cat cases. Isolates from six cat cases collected from 2021 to 2022 in the city of Ilhabela, São Paulo state, were highly genetically related to each other (maximum pairwise SNP difference of 21 SNPs) as well as to the isolates from human cases collected between 2013 and 2021 in São Paulo and Rio de Janeiro states (maximum pairwise SNP difference between human and cat isolates in this subcluster was 58 SNPs; figure 2C, D; figure 3A). Isolates from the remaining five cat cases had unknown origins and did not cluster with those from other cat cases (figure 3A). Eight of nine isolates from human cases from Corumbá city in Mato Grosso do Sul state, clustered together at maximum pairwise SNP difference of 98 SNPs. To note, the oldest *S brasiliensis* isolate included in this analysis (SRR12483729) was collected from a human case in 1999 in the Rio de Janeiro state and clustered with clade A at a maximum pairwise SNP difference of 149 SNPs from other humans and cat isolates from the five Brazilian states that comprised this clade (figure 3A).

S brasiliensis clade B comprised two subclades separated by 1155 SNPs (figure 3B). One subclade comprised three isolates from human cases collected in 2021 in Rio Grande do Norte state with maximum pairwise SNP difference of 124 SNPs (figure 2C, D; figure 3B). The second subclade comprised two isolates from human cases collected in 2021 from São Paulo state and unknown geographical origin, with a SNP difference of 268 SNPs (figure 2C, D; figure 3B). *S brasiliensis* clade C also comprised two subclades separated by 68 566 SNPs (figure 3C). One subclade comprised six isolates (one from cat and five from

human cases) with a maximum pairwise SNP difference of 68 SNPs. For those with known geographical origin, all were reported from São Paulo state from 2019 to 2021 (figure 2C, D; figure 3C). The second subclade comprised eight isolates from human cases with a maximum pairwise SNP difference of 164 SNPs. Geographical origin was known for five of the cases; all were reported from São Paulo state from 2015 to 2021 (figure 2C, D; figure 3C). *S brasiliensis* clade D and clade E were the most genetically distant clades, separated by at least 55 864–234 021 SNPs from clades A, B, and C (figure 2A). Clade D comprised two isolates from a cat case (collected 2016) and a dog case (collected 2018) in Distrito Federal state, separated by 66 213 SNPs (figure 2C, D; figure 3C). Clade E comprised one isolate from a human case collected in 2017 with unknown state of origin (figure 2).

Mating type was assessed and 46 (75%) of the 61 *S brasiliensis* isolates were *MAT1-2*, 15 (25%) were *MAT1-1*. As shown in appendix 2 (p 10), isolates from clade A were *MAT1-2*, and all isolates from clades B and E were *MAT-1-1*. Isolates from clades C and D had both *MAT1-1* and *MAT1-2*, clustering by subclade (appendix 2 p 10).

Discussion

In this study, WGS of 72 *Sporothrix* spp isolates was used to describe the genomic epidemiology of sporotrichosis in Brazil. Our results support previous work showing *S brasilienisis* as the primary driver of the ongoing epidemic. Additionally, our study provides evidence for the independent emergence of several genetically distinct *S brasiliensis* lineages and clonality among animal and human isolates within some clades, indicative of animal-to-animal and animal-to-human transmission.

Historically, Rio de Janeiro state is considered the centre of origin of *S brasiliensis* in Brazil, where zoonotic transmission has been reported since the late 1990s.[11] Molecular studies from the past 10 years using more traditional genotyping methods like amplified fragment length polymorphism, simple sequence repeats, and short tandem repeats, as well as advanced technologies like WGS suggest that *S brasiliensis* emerged independently in at least seven different regions of Brazil: Rio de Janeiro, Ceará, Distrito Federal, Rio Grande do Sul, Minas Gerais, Paraná, and São Paulo state.[14-17] Our results using, to our knowledge, the largest sample of *S brasiliensis* WGS to date corroborate these previous findings. Overall, the analysis of *S brasiliensis* isolates recovered from seven different Brazilian states from 1999 to 2022 revealed several genetically different clades that emerged independently in at least three Brazilian states (Rio de Janeiro, Sao Paulo, and Distrito Federal).

Our analysis identified five distinct genetic clades, separated by over 100 000 SNPs, (*S brasiliensis* clades A–E). Large genetic distance between clades suggests independent emergence of these lineages. In contrast, the extremely low genetic diversity among human and cat isolates within clade A and a subclade of clade C indicate the ongoing cat-to-cat and cat-to-human transmission, further supporting an active epidemic. Clade A contained 64% of all *S brasiliensis* isolates from Brazil in the study and was found in five different states. Clade B consisted of two subclades, one of which included three clonal isolates from the northeastern part of Brazil, and the other clade included an isolate of unknown origin and an isolate from São Paulo. Clade C consisted of two subclades with different mating types

separated by 68 566 SNPs, all of which were from São Paulo. We found very few isolates comprising clade D and clade E; this finding might indicate that isolates in these clades do not contribute substantially to the ongoing epidemic, or that they were not well captured in our convenience sample.

The presence of genetically related isolates in different geographical areas suggests movement of strains across state lines, probably caused by the movement of infected animals causing new outbreaks. For example, in this study, all samples from Mato Grosso do Sul state, specifically samples from the 2019–20 sporotrichosis outbreak in Corumbá city, clustered together with isolates from Rio de Janeiro state (clade A). Interestingly, Corumbá has a long history of high mobility of citizens from Rio de Janeiro, and vice versa, due to the frequent stationing of military personnel, particularly from the Navy. Additionally, the cultural similarities between Corumbá and Rio de Janeiro city are consistent with travel of citizens from the two cities. Considering this epidemiological aspect, an introduction from Rio de Janeiro to Mato Grosso do Sul state might have occurred.

In our study, we observed 1:3 distribution of mating types in *S brasiliensis* with 75% of isolates carrying *MAT1-2* allele, which agrees with previous studies.[21] Mating type was associated with clades except for clades C and D, in which two genetically distinct subclades were associated with different mating types. Isolates with different mating types were found within the same broader geographical areas suggesting a possibility of mating; although, we did not observe any evidence of recombination on the phylogenetic tree. Overall, low genetic diversity among isolates and the presence of a single mating type within some clades strongly supports clonal propagation within clades, which has been proposed previously.[15,22]

Notably, the observed *S brasiliensis* population was similar to that described in another emerging pathogenic fungus, *Candida auris*, in which four genetically distinct clades were shown to emerge simultaneously in different geographical areas.[23] The timings of both *S brasiliensis* and *C auris* emergences are also similar and date back to the 1990s. It has been suggested that increasing temperatures have the potential to select for environmental fungi adapted for growth at temperatures approaching that of the human body.[24] As proposed by Casadevall and colleagues[25] for *C auris*, zoonotic sporotrichosis might be another example of new fungal diseases emerging following climate and environmental changes. More research is needed to understand the underlying environmental and epidemiologic reasons for emergence of these pathogens.

The reasons why *S brasiliensis* is geographically restricted to South America are unknown, but one possible explanation might be that this fungus has a unique ecological niche that is restricted to this region. Little is known about *S brasiliensis* natural life cycle; however, the presence of a specialised infectious yeast form suggests an evolutionary adaptation to infection and points to the presence of a natural host species. For example, a small mammal that is endemic to Brazil, widespread in the urban environment, and encountered by cats to acquire infection fits this description; however, more research is needed to better understand the natural reservoirs of this pathogen. Additionally, Argentina, Paraguay, Chile, and Uruguay[26-28] have also reported cases of *S brasiliensis* in cats. In Paraguay, two cases of probable *S brasiliensis* were reported to be transmitted by infected cats brought from

Brazil by their owners in 2017.[29] Additionally, *S brasiliensis* infections linked to a cat brought from Brazil was identified in the UK in 2021; however, no widespread transmission was detected.[30] Whether the *S brasiliensis* cases in Argentina, Chile, and Uruguay are independent emergences or introductions from Brazil remain to be seen. Establishing sanitary barriers to contain the migration of infected cats is a fundamental measure for controlling the expansion of *S brasiliensis*.

Although most isolates in our study were *S brasiliensis*, four *S schenckii* isolates and one *S globosa* isolate were also identified among cases in Brazil, which allowed only limited comparative analyses between species. However, we found two genetically distinct clades in *S schenkii*, one of which was found in South America and another in the USA. All isolates within these clades were genetically distinct and no evidence of clonality was identified; nevertheless, a larger sample of *S schenckii* isolates is needed to better understand the genomic epidemiology of this species.

Although infections with *S schenkii* have been reported in Brazil and other countries, both cat and human cases of *S schenkii* remain rare. It is not clear why *S brasiliensis* is more likely to cause infection compared with the other two species. One possible explanation might be the presence of unique genetic features that enable *S brasiliensis* to readily infect cats and produce large quantities of infectious yeasts or conidia that drive transmission. Current evidence suggest that zoonotic transmission is more common in *S brasiliensis* compared with other *Sporothrix* species: yeasts are directly inoculated into the host by bites or deep scratching providing a highly effective route of transmission.[31] However, a different explanation is also possible: *S brasiliensis* might be more widespread in the environment compared with *S schenkii* and *S globosa* and therefore, more likely to be acquired by feral cats, which are common in Brazilian cities and are probably the main driver of transmission. The observed independent emergence of several genetic clades is consistent with this second hypothesis; however, both hypotheses are not mutually exclusive. Overall, the species of *S brasiliensis* and *S schenkii* are closely genetically related and both are capable of infecting cats and humans. More studies are needed to investigate the reasons for emergence of *S brasiliensis* to control the ongoing epidemic and help control future emergences.

Our study has the following limitations: (1) the number of isolates from different states and counties were relatively small, and collected at different times and constituted a convenience sample, which might not accurately reflect the population of *S brasiliensis* in Brazil, (2) isolates from other South American countries were not publicly available, (3) demographic (eg, age and sex) and clinical outcome data were missing for many human and animal cases, and (4) the state or country of each human and animal case might not represent the exposure location or the original environmental source of *Sporothrix* spp. Many of these limitations highlight the need for more robust, systematic laboratory surveillance for *S brasiliensis* in Brazil. In the future, a more systematic and representative population genomic study of *Sporothrix* species will be informative for better understanding the emergence of these fungi in South America and beyond.

Overall, our results increase our knowledge of how *S brasiliensis* can emerge and spread within a country. The identification of transmission between states in Brazil might help guide future public health interventions to control the spread of zoonotic sporotrichosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (Finance Code 001) and by the São Paulo Research Foundation (grant number 2017/50333). MSCM and JV are funded by Productivity in Research fellowships by the National Council for Scientific and Technological Development, and Ministry of Science and Technology of Brazil. We thank Centers for Disease Control and Prevention Office of Advanced Molecular Detection for the support with the genomic techniques development used in this study. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Data sharing

Read data has been deposited into the Sequence Read Archive database (BioProject ID PRJNA957313). The genomic analysis linked with epidemiologic data can also be found in the Microreact project at https://microreact.org/project/72YTFfH7ST9T6ZVMU8nmXN-genomic-epidemiology-of-sporotrichosis-brazil. Any additional data are presented in the Article and are available from the corresponding author on reasonable request.

References

1. 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; 45: 3198–206. [PubMed: 17687013]
2. Rodrigues AM, de Melo Teixeira M, de Hoog GS, et al. Phylogenetic analysis reveals a high prevalence of *Sporothrix brasiliensis* in feline sporotrichosis outbreaks. PLoS Negl Trop Dis 2013; 7: e2281. [PubMed: 23818999]
3. de Andrade Galliano Daros Bastos F, Raimundo Cognialli RC, Rodrigues de Farias M, Dos Santos Monti F, Wu K, Queiroz-Telles F. Spread of *Sporothrix* spp. through respiratory droplets from infected cats: a potential route of transmission. Med Mycol 2022; 60: myac079. [PubMed: 36318452]
4. Rossow JA, Queiroz-Telles F, Caceres DH, et al. A One Health approach to combatting *Sporothrix brasiliensis*: narrative review of an emerging zoonotic fungal pathogen in South America. J Fungi (Basel) 2020; 6: 247. [PubMed: 33114609]
5. Kauffman CA, Bustamante B, Chapman SW, Pappas PG, Infectious Diseases Society of America. Clinical practice guidelines for the management of sporotrichosis: 2007 update by the Infectious Diseases Society of America. Clin Infect Dis 2007; 45: 1255–65. [PubMed: 17968818]
6. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi (Basel) 2017; 3: 57. [PubMed: 29371573]
7. Zhang Y, Hagen F, Stielow B, et al. Phylogeography and evolutionary patterns in *Sporothrix* spanning more than 14 000 human and animal case reports. Persoonia 2015; 35: 1–20. [PubMed: 26823625]
8. Rodrigues AM, Gonçalves SS, de Carvalho JA, Borba-Santos LP, Rozental S, Camargo ZPD. Current progress on epidemiology, diagnosis, and treatment of sporotrichosis and their future trends. J Fungi (Basel) 2022; 8: 776. [PubMed: 35893145]

9. Borges TS, Rossi CN, Fedullo JDL, Taborda CP, Larsson CE. Isolation of *Sporothrix schenckii* from the claws of domestic cats (indoor and outdoor) and in captivity in São Paulo (Brazil). Mycopathologia 2013; 176: 129–37. [PubMed: 23729233]
10. Montenegro H, Rodrigues AM, Dias MAG, da Silva EA, Bernardi F, de Camargo ZP. Feline sporotrichosis due to *Sporothrix brasiliensis*: an emerging animal infection in São Paulo, Brazil. BMC Vet Res 2014; 10: 269. [PubMed: 25407096]
11. de Lima Barros MB, Schubach TM, Galhardo MC, et al. Sporotrichosis: an emergent zoonosis in Rio De Janeiro. Mem Inst Oswaldo Cruz 2001; 96: 777–79. [PubMed: 11562701]
12. Lopes-Bezerra LM, Mora-Montes HM, Zhang Y, et al. Sporotrichosis between 1898 and 2017: the evolution of knowledge on a changeable disease and on emerging etiological agents. Med Mycol 2018; 56 (suppl 1): 126–243. [PubMed: 29538731]
13. Casz Schechtman R, Falcão EMM, Carard M, García MSC, Mercado DS, Hay RJ. Esporotricose: hiperendêmica por transmissão zoonótica, com apresentações atípicas, reações de hipersensibilidade e maior gravidade. An Bras Dermatol 2022; 97: 1–13. [PubMed: 34893422]
14. de Carvalho JA, Beale MA, Hagen F, et al. Trends in the molecular epidemiology and population genetics of emerging *Sporothrix* species. Stud Mycol 2021; 100: 100129. [PubMed: 35027980]
15. Losada LCML, Monteiro RC, de Carvalho JA, et al. High-throughput microsatellite markers development for genetic characterization of emerging *Sporothrix* species. J Fungi (Basel) 2023; 9: 354. [PubMed: 36983522]
16. Eudes Filho J, Santos IBD, Reis CMS, et al. A novel *Sporothrix brasiliensis* genomic variant in midwestern Brazil: evidence for an older and wider sporotrichosis epidemic. Emerg Microbes Infect 2020; 9: 2515–25. [PubMed: 33155518]
17. Spruijtenburg B, Bombassaro A, Meijer EFJ, et al. *Sporothrix brasiliensis* genotyping reveals numerous independent zoonotic introductions in Brazil. J Infect 2023; 86: 610–13. [PubMed: 36841342]
18. Maubon D, Garnaud C, Ramarozatovo LS, Fahafahantsoa RR, Cornet M, Rasamoelina T. Molecular diagnosis of two major implantation mycoses: chromoblastomycosis and sporotrichosis. J Fungi (Basel) 2022; 8: 382. [PubMed: 35448613]
19. Bagal UR, Phan J, Welsh RM, et al. MycoSNP: a portable workflow for performing whole-genome sequencing analysis of *Candida auris*. Methods Mol Biol 2022; 2517: 215–28. [PubMed: 35674957]
20. Teixeira MM, de Almeida LG, Kubitschek-Barreira P, et al. Comparative genomics of the major fungal agents of human and animal Sporotrichosis: *Sporothrix schenckii* and *Sporothrix brasiliensis*. BMC Genomics 2014; 15: 943. [PubMed: 25351875]
21. de Carvalho JA, Pinheiro BG, Hagen F, et al. A new duplex PCR assay for the rapid screening of mating-type idiomorphs of pathogenic *Sporothrix* species. Fungal Biol 2021; 125: 834–43. [PubMed: 34537179]
22. Teixeira MM, Rodrigues AM, Tsui CKM, et al. Asexual propagation of a virulent clone complex in a human and feline outbreak of sporotrichosis. Eukaryot Cell 2015; 14: 158–69. [PubMed: 25480940]
23. Lockhart SR, Etienne KA, Vallabhaneni S, et al. Simultaneous emergence of multidrug-resistant Candida auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis 2017; 64: 134–40. [PubMed: 27988485]
24. Garcia-Solache MA, Casadevall A. Global warming will bring new fungal diseases for mammals. M Bio 2010; 1: 1.
25. Casadevall A, Kontoyiannis DP, Robert V. On the emergence of *Candida auris*: climate change, azoles, swamps, and birds. M Bio 2019; 10: e01397–19.
26. Cognialli RCR, Cáceres DH, Bastos FAGD, et al. Rising incidence of *Sporothrix brasiliensis* infections, Curitiba, Brazil, 2011-2022. Emerg Infect Dis 2023; 29: 1330–39. [PubMed: 37347527]
27. Thomson P, González C, Blank O, et al. Sporotrichosis outbreak due to *Sporothrix brasiliensis* in domestic cats at Magallanes, Chile: a One-Health-Approach study. J Fungi (Basel) 2023; 9: 226. [PubMed: 36836340]

28. Etchecopaz AN, Lanza N, Toscanini MA, et al. Sporotrichosis caused by *Sporothrix brasiliensis* in Argentina: case report, molecular identification and in vitro susceptibility pattern to antifungal drugs. J Mycol Med 2020; 30: 100908. [PubMed: 31732417]

29. García Duarte JM, Wattiez Acosta VR, Forneron Viera PML, et al. Esporotricosis transmitida por gato doméstico. Reporte de un caso familiar. Rev Nac 2017; 9: 67–76 (in Spanish).

30. Rachman R, Ligaj M, Chinthapalli S, Serafino Wani R. Zoonotic acquisition of cutaneous *Sporothrix braziliensis* infection in the UK. BMJ Case Rep 2022; 15: e248418.

31. Rodrigues AM, de Hoog GS, de Camargo ZP. *Sporothrix* species causing outbreaks in animals and humans driven by animal-animal transmission. PLoS Pathog 2016; 12: e1005638. [PubMed: 27415796]

Research in context

Evidence before this study

We searched PubMed, from database inception to July 31, 2023, for published studies assessing the genomic epidemiology of sporotrichosis in Brazil. The search terms used were *Sporothrix brasiliensis* AND (genomic epidemiology) OR (molecular epidemiology). No language restrictions were applied to this search. The search returned 33 results, with two studies of *Sporothrix* isolates from Brazil that used whole-genome sequencing (WGS). The first study (Eudes Filho and colleagues) was published in 2020 and included seven *S brasiliensis* isolates from two Brazilian states (Distrito Federal and Rio de Janeiro). The second study (Spruijtenburg and colleagues) was published in 2023 and included 21 *S brasiliensis* isolates from six Brazilian states (Distrito Federal, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, and São Paulo) and 173 *S brasiliensis* isolates analysed by short tandem repeat analysis. Spruijtenburg and colleagues suggested that there were numerous independent introductions of *S brasiliensis*, with one clade accompanied by large clonal spread in the cities of Rio de Janeiro (Rio de Janeiro state) and Curitiba (Parana state), Brazil.

Added value of this study

In this study, we performed WGS on 67 *Sporothrix* spp isolates from Brazil and used genomic epidemiology to show multiple, independent emergences of genetically distinct lineages of *S brasiliensis*. We also compared the genetic variability within species of *S brasiliensis* and *Sporothrix schenckii* isolates from human and animal cases and showed that, in contrast to *S schenckii*, *S brasiliensis* showed clonality among isolates from different geographical areas and within some clades. This data highlights two important points: (1) it confirms the ongoing cat-to-cat and cat-to-human *S brasiliensis* transmission, and (2) it supports multiple independent introductions between states and cities. Additionally, this is the first published study reporting the 2019 to present sporotrichosis outbreak in Corumbá city in Mato Grosso do Sul state, Brazil.

Implications of all the available evidence

Our results indicate that the cat-to-cat and cat-to-human transmission is not restricted to a single genetic lineage and was acquired independently by several lineages. The data describing the independent emergence of five *S brasiliensis* strains raise concerns that new strains of *S brasiliensis* might continue to emerge and spread to different geographical areas.

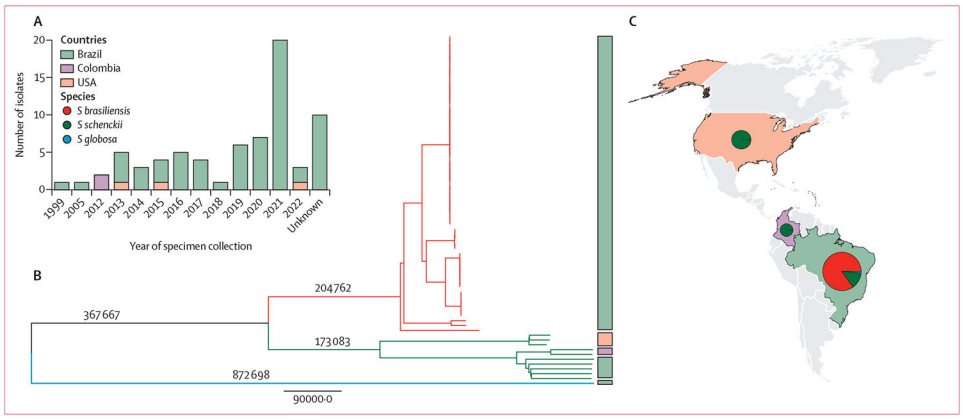

Figure 1: Genomic diversity among isolates and geographical distribution of three clinically relevant *Sporothrix* species: *Sporothrix schenckii*, *Sporothrix brasiliensis*, and *Sporothrix globosa*

(A) Temporal distribution of *Sporothrix* spp isolates recovery from human and animal sporotrichosis cases by country. (B) Neighbour-joining phylogenetic tree of isolates from Brazil, Colombia, and the USA. Branch colours indicate species, and branch lengths represent single-nucleotide polymorphisms, whereby pairwise single-nucleotide polymorphism differences between the major clades are shown. (C) The geographical origin of isolates by species.

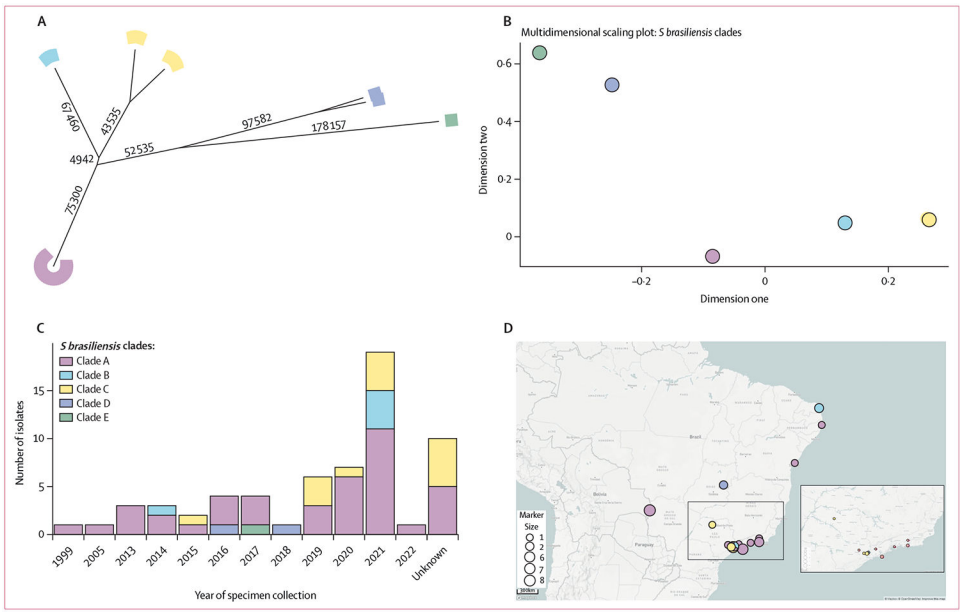

Figure 2: Genomic, temporal, and geographical distribution of five *Sporothrix brasiliensis* clades

(A) Neighbour-joining phylogenetic tree of the *S brasiliensis* isolates showing the single-nucleotide polymorphism distance between clades. (B) Multidimensional scaling analysis of the genetic differences between *S brasiliensis* isolates. The circles represent multiple isolates clustering together. Among different clusters, bootstrap values were 100%. The percentage of dissimilarity explained by dimension one is 41·7% and by dimension two is 31·4%. (C) Temporal distribution of *S brasiliensis* clades isolates by year. (D) The geographical distribution of the clades in the different Brazilian states. The size of the circles is proportional to the number of cases or isolates. The colours correspond to different clades. Clade E is not represented in the map due to unknown isolate state of origin. An interactive map of the geographical distribution of the clades in the different Brazilian states is also available.

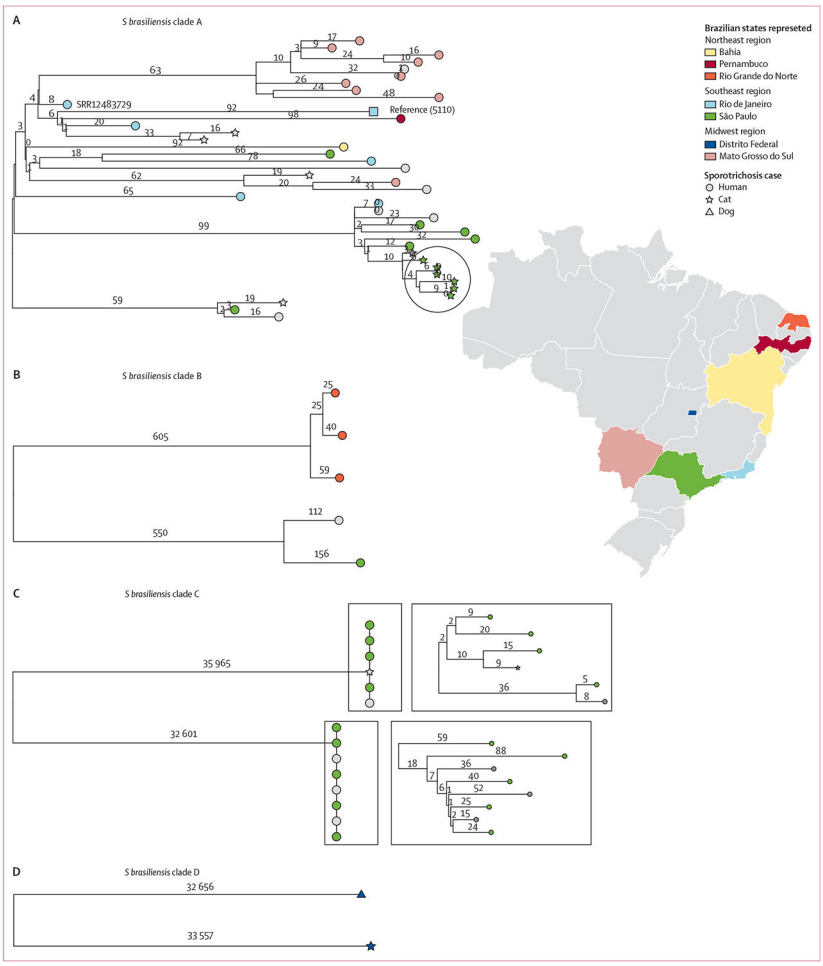

Figure 3: Phylogenetic characterisation of the five *Sporothrix brasiliensis* clades

Neighbour-joining phylogenetic tree of the *S brasiliensis* clade A (A), *S brasiliensis* clade B (B), *S brasiliensis* clade C (C), and *S brasiliensis* clade D (D). Branch lengths represent the single-nucleotide polymorphism distance between isolates and leaf colours correspond to the different Brazilian states where the *S brasiliesis* isolate was collected. The shapes represent the *S brasiliensis* host (human, cat, or canine case), with the square representing the *S brasiliensis* strain 5110 reference genome (National Center for Biotechnology Information taxonomy identification number 1398154).[20] Clade E is not represented in the figure because it was composed of one sample (identification number B22068) with unknown state of origin.

Table:

Characteristics of *Sporothrix* isolates

	Sporothrix brasiliensis **(n=6l)**	***Sporothrix schenckii*** **(n=10)**	***Sporothrix*** **spp*** **(n=72)**
Country of collection			
Brazil	61 (100%)	5 (50%)	67 (93%)
São Paulo	22 (36%)	2 (20%)	24 (33%)
Mato Grosso do Sul	9 (15%)	0	9 (12%)
Rio de Janeiro	4 (7%)	0	4 (6%)
Rio Grande do Norte	3 (5%)	0	3 (4%)
Brasília	2 (3%)	1 (10%)	3 (4%)
Bahia	1 (2%)	0	1 (1%)
Pernambuco	1 (2%)	0	1 (1%)
Unknown	19 (31%)	2 (20%)	22 (31%)
USA	0	3 (30%)	3 (4%)
Colombia	0	2 (20%)	2 (3%)
Human cases			
Total	47 (77%)	7 (70%)	55 (76%)
With sex reported	31 (51%)	1 (10%)	32 (44%)
Female	17 (28%)	0	17 (24%)
Male	14 (23%)	1 (10%)	15 (21%)
Age, years	44 (36–53)	Unknown	44 (36–53)
Animal cases			
Total	14 (23%)	3 (30%)	17 (24%)
Cats	13 (21%)	3 (30%)	16 (22%)
Dogs	1 (2%)	0	1 (1%)

Data are n (%) or median (IQR). *Includes *Sporothrix brasiliensis* (n=61), *Sporothrix schenckii* (n=10), and *Sporothrix globosa* (n=1) isolates.