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Feline sporotrichosis due to Sporothrix brasiliensis: an emerging animal infection in São Paulo, Brazil

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Abstract

Background: Sporotrichosis is a mycotic infectious disease that is generally acquired by traumatic inoculation of contaminated materials especially from plant debris or through bites and scratches from diseased animals, such as domestic cats. It affects the skin, lymphatic system, and other organs in the warm-blooded host. Etiological agents are embedded in the plant-associated order Ophiostomatales. With essential differences between possible outbreak sources and ecological niche, host-environment interactions are classic determinants of risk factors for disease acquisition. Sporotrichosis outbreaks with zoonotic transmission, such as those that are ongoing in southern and southeastern Brazil, have highlighted the threat of cross-species pathogen transmission. Sporothrix brasiliensis has emerged as a human threat owing to the intimate contact pattern between diseased cats and humans in endemic areas.

Results: We describe the recent emergence of feline sporotrichosis in the metropolitan region of São Paulo, Brazil, with an overwhelming occurrence of S. brasiliensis as the etiological agent. A phylogenetic and a haplotype approach were used to investigate the origin of this epidemic and the impact of feline transmission on genetic diversity. During the last 3-year period, 163 cases of feline sporotrichosis were reported in São Paulo with proven S. brasiliensis culture. The haplotype diversity of feline S. brasiliensis isolates revealed the expansion of a clonal population with low genetic diversity. Haplotype analysis confirmed that isolates from São Paulo shared the haplotype originated in the long-lasting outbreak of cat-transmitted sporotrichosis in Rio de Janeiro, which differed from the haplotype circulating in the Rio Grande do Sul epidemic.

Conclusions: The fast spread of sporotrichosis in a short period of time highlights the potential for outbreaks and suggests that the mycosis may affect an urban population with a high concentration of susceptible felines. The feline sporotrichosis epidemic shows no signs of slowing, and this epidemiological pattern may require specific public health strategies to control future outbreaks.

Keywords: Sporotrichosis, Feline, Sporothrix brasiliensis, Zoonosis, Emerging infectious diseases, Epidemiology, Cat, Sporothrix schenckii, Mycosis, Outbreak

Background

Epidemics caused by new and old fungal agents have emerged and re-emerged over time as a threat to the health of vertebrate hosts [1]. The great global burden of fungal infections in animals is specially observed as a result of a pathogen-host shift or a recent introduction of a pathogen in a susceptible host population [2-4]. Domestic animals are at risk of developing several mycotic diseases that can be directly transmitted to humans; however, such diseases are often neglected by health systems. Because domestic animals have intimate contact with their owners, they play an important role in the emergence of human infections; this situation is also common in the developing world, where environmental conditions are juxtaposed with inadequate public health infrastructure. Reducing the public health risks from zoonosis outbreaks in urban areas requires different prevention and control strategies, as their increased frequency during recent decades may be related to poverty, poor sanitation, and anthropogenic changes in the environment.

Sporotrichosis is a neglected disease of humans and animals. The disease occurs worldwide in the form of...
sapronoses and zoonoses, mainly in tropical and subtropical regions [4-7], and is the most frequent subcutaneous mycosis in Latin America [8]. Since it was first noted in the United States in 1898, this mycosis has been described as a disease of occupational risk, affecting farmers, gardeners, and agricultural workers. However, recent epidemics have demonstrated the potential for zoonotic transmission of the disease, and have nearly always involved cats as the main source of infection [9,10].

Around the world, the classical type of transmission relies on the traumatic inoculation of contaminated plant material in the environment. In contrast, in the alternative type of transmission, bites and scratches from a diseased cat effectively disseminate the fungus. Either route of infection begins by affecting the skin locally with the development of a nodular ulcerated lesion, and eventually spreads out from the site of trauma through the lymphatic system and causes damage to other organs of the warm-blooded host [11,12]. Zoonotic sporotrichosis is highly frequent in the southern [4,5,13] and southeastern [4,5,14,15] regions of Brazil, and animals usually experience the severe form of sporotrichosis.

Traditionally, sporotrichosis has been attributed to the dimorphic fungus *Sporothrix schenckii sensu lato* (s.l.). Multigene phylogenies have clarified species boundaries within cryptic isolates [16] and led to the proposal of the *S. schenckii* complex, which comprises a clinically important clade that includes *S. brasiliensis* (clade I), *S. schenckii sensu stricto* (s. str.) (clade II), *S. globosa* (clade III), and *S. luriei* (clade VI) [17,18]. Host susceptibility, species distribution, and sensitivity profile to antifungal agents are all divergent among closely related species [4,5,19,20]. A high prevalence of *S. brasiliensis*, the most virulent species in the complex [20,21] and geographically restricted to Brazil [4,5,7,22], has been reported in cats [4]. Rodrigues *et al.* [4] suggests that the thermal resistance exhibited by *S. brasiliensis* may be an important mechanism of adaptation to the feline body, and may partially explain the success of *S. brasiliensis* infection over the remaining species in the complex. Indeed, the cat-cat contact pattern during fights and the cat-human contact pattern of scratches and bites may also support the success of horizontal disease transmission in a short period of time [4,5], because the fungus does not die with the feline, and can be transmitted to the next warm-blooded host. The increased proximity between cats and humans favors the emergence of sporotrichosis in Brazil.

Since the 1990's, the epidemiological profile of sporotrichosis has changed from a low-prevalence disease to a major health problem that affects people living in neglected urban areas [4,5]. Its prevalence may reach epidemic proportions over time. In the metropolitan area of Rio de Janeiro, sporotrichosis is estimated to account for more than 3,800 feline, 4,000 human, and 120 canine cases in the period from 1998 to 2012 [23-25]. Massive zoonotic transmission has also been detected in the southern region of Brazil [5,13,26], with characteristics similar to the ongoing epidemic in Rio de Janeiro.

In contrast to the major ongoing epidemics in other provinces of Brazil, during the past 20 years São Paulo state has reported a basal number of sporotrichosis cases, nearly always unrelated to feline transmission types [5,27]. The Zoonosis Control Center of São Paulo (ZCC-SP) has performed an epidemiological surveillance service among feral cats since 2008. In December 2010, a few cases of sporotrichosis in cats were reported to our service; since then, an increasing number of feline cases have been identified in São Paulo and in two of its neighboring cities. Here, we report the molecular epidemiology of *Sporothrix* species as an emerging pathogen among felines in the metropolitan area of São Paulo and discuss its relevance in one of the most populous regions of the Americas.

**Results**

The first suspected cases of feline sporotrichosis emerged in March 2011 in the region of Itaquera, an urban area with a high population density. Cases are ongoing in the most neglected areas, which have limited access to basic sanitation and public health services (Figure 1). One hundred sixty-three out of 279 clinical samples from cats (58%) and 1 out of 11 samples from dogs (8%) were positive for several *Sporothrix* spp. in the city of São Paulo. Figure 2 shows the clinical aspects of feline sporotrichosis. In the metropolitan area of São Paulo, in the cities of Diadema and Guarulhos, 10 (100%) and 17 of 40 (43%) feline clinical samples were positive for *Sporothrix*, respectively (Table 1). Judging from the number of diseased animals with proven cultures for several *Sporothrix* spp., the sporotrichosis epizootic has shown no signs of slowing during a 3-year period (2011-2013) (Figure 3).

Typical *Sporothrix* colonies were grown from the liver and spleen of animal #1, while fragments from lung and gut were negative. In animal #2, only fragments from the gut were positive for *Sporothrix*. Molecular analysis revealed that the etiological agent was *S. brasiliensis*. Cultures of several *Sporothrix* spp. were obtained from fecal samples from both necropsied cats (M1727/11 and M1732/11). A single environmental sample of feces collected in a sand heap was also positive for *S. brasiliensis* (M039/12).

Positivity for environmental samples was low. All 17 samples from sand piles and a soil sample were negative for *Sporothrix* spp. out of five samples of bark from trees with signs of cat scratches, only one (M1753/11) was positive for *Sporothrix* species. A sample (M1239/11) of decaying wood from a tree branch where a feline with sporotrichosis climbed and fell was also positive for *Sporothrix*. Unfortunately, we were unable to provide a pure culture because of
the constant growth of contaminants; therefore, we were unable to identify the exact *Sporothrix* species.

Restriction fragment length polymorphism patterns were obtained from *Sporothrix* isolates (Additional file 1) after enzymatic digestion of the *CAL* products with *Hha*I enzyme. Fragments were 251, 232, 198, 96, and 85 bp in length, which is compatible with the restriction profile of *S. brasiliensis* (CBS 120339). A representative gel containing 16 clinical samples is shown in Figure 4.

A single *CAL* amplicon of approximately 800-900 bp was observed for all isolates. The complete alignment included 95 sequences (47 generated in this study and 48 retrieved from previous investigations). Aligned sequences of *CAL* were 729 bp long, including 355 invariable characters, 216 variable parsimony-informative (29.6%), and 133 singletons. A phylogenetic tree was constructed using Maximum likelihood (model T92 + I) with 1,000 bootstrap replications (Figure 5). The 95 operational taxonomic units were distributed into 7 main groups, 6 of which had been detected in previous studies [4,5,22]. We used the fungus *Grossmannia serpens* (CBS 141.36) as an outgroup [28]. Phylogenetic analyses of the 47 evaluated *Sporothrix* spp. revealed that they all belonged to the species *S. brasiliensis*, and that all were closely related to the type strain CBS 120339.

After speciation, the haplotype diversity of feline *S. brasiliensis* isolates was assessed regarding the *EF1*-α dataset (see Additional file 1). We compared the sequences generated from the recently isolated samples from São Paulo to the ongoing epidemics in Rio de Janeiro and Rio Grande do Sul. The haplotype number for *EF1*-α was low (3 haplotypes: H9, H11 and H12) [4]. Haplotype analysis revealed that isolates from São Paulo shared the same haplotype (H9) from the Rio de Janeiro epidemic (see Additional file 2), which differed from the haplotype circulating in the Rio Grande do Sul epidemic (H11 and H12).

**Discussion**

We used molecular tools to investigate the sudden emergence of feline sporotrichosis caused by *S. brasiliensis* in the metropolitan region of São Paulo, the most populated city in Brazil. Sporotrichosis is the most important subcutaneous mycosis that affects animals [4,5,9,10]. Infections caused by *S. brasiliensis* are remarkable among the genus *Sporothrix* because of their intense pathogenicity to the vertebrate host [20,21]. Thus far, *S. brasiliensis* is geographically restricted to Brazil [4,5,22]. To the best of our knowledge, this is the first report of a cat-transmitted epizootic in this area.

Since the identification of the first cases of animal sporotrichosis in São Paulo 3 years ago (2011), there has been a predominance of cases in the southeastern area, specifically in Itaquera and Itaim Paulista districts, denoting an epidemic character (Figure 1). Based on this initial outbreak,
the ZCC-SP conducted active searches in households, which resulted in finding other cases of feline sporotrichosis. To date, a total of 83 feline cases and one canine case have been identified in Itaquera, and 56 feline cases have been recorded in Itaim Paulista (Figure 3). As new active searches are conducted in São Paulo, new cases are detected, suggesting that the epidemic is unlikely to end spontaneously. It is important to note that a small number of cats with sporotrichosis have also been found in other districts of the city, as well as in other cities of the same metropolitan area of São Paulo, such as Diadema (10 feline cases) and Guarulhos (17 feline cases) (Table 1; Figure 1). These findings indicate the spread of the epidemic, and lead us to believe that the transmission of this disease might have a silent character.

Molecular data for 48 animals revealed that the outbreak is caused by *S. brasiliensis*. In this study, we have successfully identified the isolates of feline sporotrichosis by CAL-RFLP, as suggested by Rodrigues et al. [29]. The use of CAL-RFLP considerably reduced the cost of molecular identification in this epidemic scenario. The overwhelming prevalence of *S. brasiliensis* during outbreaks is in agreement with results obtained by Rodrigues et al. [4] regarding feline-transmitted epidemics in Rio de Janeiro and Rio Grande do Sul. In addition, low genetic diversity was observed during the early phase of this epidemic, in agreement with previous results [4,5]. A haplotype network based on EF1-α suggests that the predominant haplotype among felines in São Paulo and the haplotype observed in Rio de Janeiro are the same. Although this information may not be conclusive because of the low number of markers used, it still indicates that

| Table 1 Sporotrichosis in feline and canine samples collected from different cities of São Paulo State, Brazil (March 2011 to April 2014) |
|---|---|---|---|---|
| City | District | Host | Positive | Negative | Positivity |
| --- | --- | --- | --- | --- | --- |
| São Paulo | Cidade Ademar | Feline | 8 | 4 | 67% |
| | Guianazes | Feline | 2 | 0 | 100% |
| | Itaim Paulista | Canine | 0 | 2 | 0% |
| | | Feline | 56 | 23 | 71% |
| | Itaquera | Canine | 1 | 9 | 10% |
| | | Feline | 83 | 80 | 51% |
| | Pedreira | Feline | 1 | 1 | 50% |
| | Pirituba | Feline | 1 | 0 | 100% |
| | Socorro | Feline | 2 | 3 | 40% |
| | Tremembé | Feline | 3 | 0 | 100% |
| | Vila Maria | Feline | 5 | 3 | 63% |
| | Vila Matilde | Feline | 2 | 2 | 50% |
| **Subtotal** | | | | | |
| | Canine | 1 | 11 | 8% |
| | Feline | 163 | 116 | 58% |
| Diadema | Canine | 0 | 1 | 0% |
| | Feline | 10 | 0 | 100% |
| Guarulhos | Canine | 0 | 3 | 0% |
| | Feline | 17 | 23 | 43% |
| **Total** | | | | | |
| | Canine | 1 | 15 | 6% |
| | Feline | 190 | 139 | 58% |

Figure 2 Clinical aspects of feline sporotrichosis. (A) Wet, ulcerated skin lesions, often particularly concentrated in the cephalic region. (B) Weight loss during the evolution of the disease.

Figure 3 Temporal evolution of the feline sporotrichosis epidemic in the metropolitan region of São Paulo. The constant number of positive cats indicates the maintenance of cat-transmitted sporotrichosis.
The disease is spreading from Rio de Janeiro. The state of Rio de Janeiro is bordered by Minas Gerais, Espírito Santo, and São Paulo, and their geographic proximity may support this finding. With particular differences in intensity and frequency of cases, the occurrence of feline sporotrichosis has also been recorded in Minas Gerais [4,5] and Espírito Santo [30,31].

The prevalence of *S. brasiliensis* in cats, but not dogs, in the same geographic area is remarkable. This epidemic profile observed in São Paulo was also detected earlier in Rio de Janeiro and Rio Grande do Sul [5,13-15,32-34]. The success of *S. brasiliensis* epidemics must consider a complexity pathogen-host-environment interplay, including: (a) high susceptibility of the feline host and high virulence of the pathogen; (b) feline habits; and (c) recent introduction of *S. brasiliensis* in a susceptible urban population of felines. Animal sporotrichosis was first described in São Paulo in 1907 by Lutz and Splendore in naturally infected rats [35]. Freitas *et al.* [36,37] reported a series of feline and canine cases in São Paulo, however, we cannot conclude that *S. brasiliensis* was the species involved since Rodrigues *et al.* [4,5] reported that *S. schenckii s. str.* may also infect cats, however with a significantly lower frequency.

The role of cats in the fungal transmission is a key factor in understanding the evolution of disease transmission and emergence in urban areas. This may require the development of specific surveillance programs and control measures by the appropriate authorities. Characteristics of cats’ behavior, particularly fighting during copulation, territorial dispute, or intimate contact, lead to deep scratches and bites, which enable traumatic inoculation of the fungus. The possibility of transmission (and, consequently, transmission to humans) is intensified in areas where non-sterilized animals roam freely, resulting in intimate contact. In Itaquera region, where the majority of the sporotrichosis cases were identified, dogs and cats usually have free access to the street, and most of them are not sterilized.

In the present study, it was possible to isolate *S. brasiliensis* from the organs of necropsied animals at ZCC-SP, in agreement with previous reports from Schubach *et al.* [38], who described the isolation of *S. schenckii s.l.* from tissue samples from lung, liver, spleen, lymph nodes,
Figure 5 (See legend on next page.)
Sporothrix brasiliensis were also isolated from feces collected from the small intestine of both necropsied cats, as well as from feces collected from a pile of sand in Itaquera. These findings introduce new insights regarding the ecology of S. brasiliensis. Feces from diseased cats may contaminate the soil, creating an environmental reservoir for S. brasiliensis and becoming a new source of contamination for animals or humans. Furthermore, cats habitually bury their feces in sand or soil and sharpen their claws on tree bark, enabling the initial contamination of the claw by the fungus. Cats have retractable claws and the fungus may be retained superficially in the animal’s body [9,10]. Moreover, cats’ habit of cleaning themselves by licking can lead to contamination of the oral mucosa, which renders biting and scratching effective for deep implantation of the fungus in cutaneous and subcutaneous sites on other animals and humans. Another possibility that may render soil a source of contamination is the inappropriate disposal of carcasses of animals that died with sporotrichosis, such as backyard burial, or even throwing the animals into the wastelands [4,33].

Sporothrix was also isolated from a sample of decaying wood and from the bark of a tree, showing that the fungus is present in the environment within the studied transmission area. However, the rate of positivity among environmental samples was low, which may be related to the sampling strategies, seasonality, and number of evaluated samples. Failure to isolate pathogenic Sporothrix spp. embedded in the S. schenckii complex from the original source of infection in the environment is not unusual [39-41].

Conclusions
The recent introduction of S. brasiliensis to the metropolitan area of São Paulo has resulted in the permanent infection of felines during the 3 years since it was first detected. We observed striking similarities between the São Paulo epidemic and the long-lasting outbreak of cat-transmitted sporotrichosis and those observed in Rio de Janeiro and Rio Grande do Sul. Moreover, it is unlikely that the epidemic remains limited to cats. The threat of cross-species pathogen transmission can lead to the risk of a massive epidemic for humans in these areas [5] and poses a significant challenge for public health systems. Strategies to control the spreading of the disease may include the education of population about the main aspects of Sporothrix transmission, animal sterilization programs, treatment, and prophylaxis, as well as development of campaigns to avoid abandonment of diseased animals by their owners in the most affected areas.

Methods
Animal and clinical samples
From March 2011 until April 2014, we studied a total of 345 animals from São Paulo city and neighboring cities that were suspected of having sporotrichosis. Pet owners were informed about the risk of zoonotic transmission of sporotrichosis, and verbal informed consent was obtained by a professional from the ZCC-SP, before the collection of the samples. Suspected animals had apparent cutaneous lesions throughout the body, especially in the cephalic region. The lesions were usually wet, with secretion, but were dry in rare cases. Clinical samples were collected from wet lesions with sterile swabs and sent to the laboratory on the same day. However, if it was not possible to send the samples to the laboratory on the day of collection, they were placed in transport media (Stuart Media) and refrigerated at 4°C. Dry lesions were scraped, and the crusts were collected in sterile flasks. A professional from the ZCC-SP collected all samples. This study was performed in accordance with guidelines for good laboratory practice, and every effort was made to minimize suffering. Ethical approval was provided by the Institutional Committee (Universidade Federal de São Paulo 0244/11).

Sporothrix spp. isolation and identification
Clinical samples were directly inoculated on Mycosel Agar slants (Becton Dickinson, Sparks, MD, USA) in duplicate and incubated at 25°C for 30 days. Suspected colonies were subcultured on Sabouraud dextrose agar plates (Becton Dickinson, Sparks, MD, USA). Macroscopic and microscopic characteristics were applied to the dichotomous key to clinical species of the S. schenckii complex [18].

Organs
Two cats that presented with terminal disseminated sporotrichosis were subject to gross necropsy under aseptic conditions after euthanasia at ZCC-SP. The cats’ organs (liver, lung, spleen, and gut) were removed aseptically, cut into small fragments, and macerated in 2 mL of sterile saline solution; 0.5 mL were plated in Mycosel agar plates (in duplicate) and incubated at 25°C for 30 days. Gut samples were well rinsed with sterile saline solution...
before sample processing. The suspected colonies were isolated and identified as described above.

Fecal samples
Fecal samples from necropsied cats were collected from the gut. In addition, two environmental feces samples were collected from a sand heap in the Itaquera region, in the backyard of a residence where diseased cats lived. Each fecal sample was diluted in sterile saline solution (1:10 dilution) with chloramphenicol (200 mg/L), vigorously homogenized for 5 min, and allowed to settle for 15 min. Samples of the supernatant (0.5 mL) were plated on Mycosel agar plates (Becton Dickinson, Sparks, MD, USA) in duplicate and incubated at 25°C for 30 days. The suspected colonies were isolated and identified as described above.

Environmental samples
Environmental samples (n =24) were collected in order to identify potential reservoirs and sources of contamination in neighboring Itaquera, where most of the cases of feline sporotrichosis were found. Sand samples (n =17) were collected from sand piles that contained cat feces; a soil sample (n =1) was collected from a square to which the suspected colonies were isolated and identified as described above and incubated at 25°C for 30 days.

Molecular characterization
Sporothrix colonies were grown on potato dextrose agar slants (Becton Dickinson, Sparks, MD, USA) for 10 days at 25°C. DNA was extracted and purified from fungal colonies by following the Fast DNA kit protocol (MP Biomedicals, Vista, CA, USA) [22]. The calmodulin (CAL) locus region was amplified directly from genomic DNA by polymerase chain reaction (PCR) using the degenerated primers CL1 (5′-GAR TWC AAG GAG GCC TTC TC-3′) and CL2A (5′-TTT TTG CAT CAT GAG TTG GAC-3′) [42], which amplified an 800-bp amplicon corresponding to exons 3 through 5. The CAL sequence was used for taxonomy purposes. The translation elongation factor-1 alpha (EF1-α) locus region was amplified and sequenced using the primers EF1-F (5′-CTG AGG CTC GTT ACC AGG AG-3′) and EF1-R (5′-CGA CTT GAT GAC ACC GAC AG-3′), as described by Rodrigues et al. [4]. We used EF1-α information to compare the ongoing epidemics in Rio de Janeiro, Rio Grande do Sul, and São Paulo. Only PCR products that produced single bands were sequenced. Amplified products were gel purified with the Wizard™ SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), following the manufacturer’s instructions. PCR products were sequenced directly in two reactions with forward and reverse primers to increase the quality of the sequence data (Phred >30).

The sequencing reactions were conducted using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA, USA) and the sequencing products were determined using an ABI 3730 DNA Analyzer 48-well capillary sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Sequences generated in both senses were assembled into single sequences via CAP3 implemented in BioEdit software [43]. Sequences were aligned with MAFFT version 7 [44], and retrieved alignments were manually edited to avoid mis-paired bases. Sequences were exported as FASTA files for BLAST search at http://www.ncbi.nlm.nih.gov/BLAST. All sequences were deposited online at GenBank (Additional file 1).

Phylogenetic reconstructions
Relationships among Sporothrix isolates collected during the São Paulo outbreak were determined by phylogenetic analysis of CAL sequences and comparison to reference strains (see Additional file 1) [4,5,16-18,22,45]. Maximum Likelihood and Neighbor-joining methods were employed to complete phylogenetic analyses using MEGA6 [46]. Considering the Bayesian information criterion (BIC) and Akaiki information criterion (AIC) [47], the Tamura 3-parameter model (T92 model) [48] was found to be the best evolutionary model for the CAL sequence. The model was applied assuming that a certain fraction of sites are evolutionarily invariant. Trees were estimated using 1,000 bootstrap replicates [49]; gaps and missing data were not included in the analysis.

Haplotype network
Haplotype analysis based on EF1-α sequences (see Additional file 1) were estimated using DnaSP software version 5.10 [50] in order to visualize differences and diversity among S. brasiliensis isolates recovered from zoonotic outbreaks in São Paulo, Rio de Janeiro, and Rio Grande do Sul [4]. Gaps and missing data were excluded from the calculations. Median-joining networks [51] were obtained and visualized using Network 4.610 software (Fluxus Technology).

CAL restriction fragment length polymorphism
Molecular characterization was also performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as an alternative molecular approach. The partial CAL gene was amplified using the primers CL1 and CL2A [42] as described above and digested with HhaI as described elsewhere [29]. Digested products were electrophoresed on 2.5% (w/v) agarose gels for 90 min at 100 V in the presence of GelRed™ (Biotium, Hayward, CA, USA).
We included a lane loaded with 100-bp DNA Step Ladder (Promega, Madison, WI, USA), as well as one positive control from each of the following reference strains: S. brasiliensis (CBS 120359), S. schenckii (CBS 359.36), S. globosa (CBS 120340), and S. mexicana (CBS 120341). The bands were visualized using the L-Pix Touch (Loccus Biotecnologia, São Paulo, Brazil) imaging system under UV illumination.

Availability of supporting data
All the supporting information is included as additional files.

Additional files

Additional file 1: Strains, species, origin, CAL and EF1-a, and GenBank accession numbers of Sporothrix spp. isolates used in this study. All sequences were deposited online at Genbank (http://www.ncbi.nlm.nih.gov/genbank).

Additional file 2: Median-joining haplotype network of Sporothrix schenckii complex isolates, comparing all EF1-a haplotypes described in the ongoing epidemics in Rio de Janeiro, Rio Grande do Sul, and São Paulo. Isolates recovered in the São Paulo epidemics (2011-2013) share the same haplotype (H9) as previous outbreaks in Rio de Janeiro (1998-2012) reported by Rodrigues et al. [4]. The size of the circumference is proportional to the haplotype frequency. Isolates are coded, and their frequencies are represented by geographic region of isolation. Black dots (median vectors) represent unsampled or extinct haplotypes in the population.

Abbreviations
s.l.: sensu lato, s. str.: sensu stricto; ZCC-SP: Zoonosis Control Center of São Paulo; CAL: Calmodulin; EF1-a: Translation elongation factor-1 alpha; ML: Maximum likelihood; NJ: Neighbor-joining; BIC: Bayesian information criterion; AIC: Akaike information criterion; T92: Tamura 3-parameter method; PCR-RFLP: Polymerase chain reaction–restriction fragment length polymorphism.

Competing interests
The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

Authors’ contributions
Study development and design: AMR, HM, and ZPC. Epidemiological surveillance, clinical observations and post-mortem examination: HM, MAGD, and ZPC. Molecular genetic studies and phylogenetic analysis: AMR. Drafting of the paper: AMR, HM, and ZPC. All authors read and approved the final manuscript.

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References
1. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ: Emerging fungal threats to animal, plant and ecosystem health. Nature 2012, 484:186–194.
2. Olson DH, Anensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, Gater T, Weaver G, Fisher MC: Mapping the global emergence of Batrachochytrium dendrobatidis, the amphibian chytrid fungus. PLoS One 2013, 8:e56802.
3. Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, Hicks AC, Ballmann AE, Coleman JT, Reddell DN, Reeder DM, Blehert DS: Experimental infection of bats with Geomyces destructans causes white-nose syndrome. Nature 2011, 480:376–378.
4. Rodrigues AM, de Melo TM, de Hoog GS, Schubach TMP, Pereira SA, Fernandes GF, Bezerra LML, Feliipe MS, de Camargo ZP: Phylogenetic analysis reveals a high prevalence of Sporothrix brasiliensis in feline sporotrichosis outbreaks. PloS Negl Trop Dis 2013, 7:e2281.
5. Rodrigues AM, de Hoog GS, Zhang Y, Camargo ZP: Emerging sporotrichosis is driven by clonal and recombinant Sporothrix species. Emerg Microbes Infect 2014, 3:52.
6. Verma S, Verma GK, Singh G, Kanga A, Shanker V, Singh D, Gupta P, Mokta K, Sharma V: Sporotrichosis in Sub-Himalayan India. PLoS Negl Trop Dis 2012, 6:e1673.
7. Zhou X, Rodrigues AM, Feng P, Hoog GS: Global ITS diversity in the Sporothrix schenckii complex. Fungal Divers 2014, 66:153–165.
8. Queiroz-Telles F, Nucci M, Colombo AL, Tobón A, Restrepo A: Mycoses of implantation in Latin America: an overview of epidemiology, clinical manifestations, diagnosis and treatment. Med Mycol 2011, 49:225–236.
9. Schubach A, Barros MB, Wanke B: Epidemic sporotrichosis. Curr Opin Infect Dis 2008, 21:129–133.
10. Barros MB, de Almeida PR, Schubach AO: Sporothrix schenckii and sporotrichosis. Clin Microbiol Rev 2011, 24:633–654.
11. Silva-Vergara ML, de Camargo ZP, Silva PF, Abdalla MR, Sgarbiere RN, Rodrigues AM, dos Santos KC, Barata CH, Ferreira-Palm KM: Disseminated Sporothrix brasiliensis infection with endocardial and ocular involvement in an HIV-infected patient. Am J Trop Med Hyg 2012, 86:477–480.
12. Bonifaz A, Vázquez-González D: Diagnosis and treatment of lymphocutaneous sporotrichosis: What are the options? Curr Fungal Infect Rep 2013, 7:252–259.
13. Madrid W, Mattai AS, Fernandes CG, Oliveira Nobre M, Meireles MCA: Epidemiological findings and laboratory evaluation of sporotrichosis: A description of 103 cases in cats and dogs in Southern Brazil. Mycopathologia 2012, 173:265–273.
14. Barros MBL, Schubach AO, Schubach TMP, Wanke B, Lambert-Passos SR: An epidemic of sporotrichosis in Rio de Janeiro, Brazil: epidemiological aspects of a series of cases. Epidemiol Infect 2006, 136:1192–1196.
15. Schubach A, Schubach TM, Barros MB, Wanke B: Cat-transmitted sporotrichosis, Rio de Janeiro, Brazil. Emerg Infect Dis 2005, 11:1952–1954.
16. Marimon R, Gené J, Cano J, Trilles L, Dos Santos LM, Guarro J: Molecular phylogeny of Sporothrix schenckii. J Clin Microbiol 2006, 44:3251–3256.
17. 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, 45:3198–3205.
18. Marimon R, Gené J, Cano J, Guarro J: Sporothrix lutea: a rare fungus from clinical origin. Med Mycol 2008, 46:621–625.
19. Rodrigues AM, de Hoog GS, de Cassia PD, Birhanee RSN, da Costa Sidrim JJ, Gadelha MF, Colombo AL, de Camargo ZP: Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. BMC Infect Dis 2014, 14:219.
20. Fernandes GF, dos Santos PO, Rodrigues AM, Sasaki AA, Burger E, de Camargo ZP: Characterization of virulence profile, protein secretion and immunogenicity of different Sporothrix schenckii sensu stricto isolates compared with S. globosa and S. brasiliensis species. Virulence 2013, 4:241–249.
21. Antillaga-Moncrieff J, Capilla J, Mayayo E, Marimon R, Maríné M, Gené J, Cano J, Guarro J: Different virulence levels of the species of Sporothrix in a murine model. Clin Microbiol Infect 2009, 15:651–655.
22. Rodrigues AM, de Hoog S, de Camargo ZP: Emergence of pathogenicity in the Sporothrix schenckii complex. Med Mycol 2013, 51:405–412.
23. Barros MBL, Schubach TP, Coll JO, Gremião ID, Wanke B, Schubach A: Sporotrichosis: development and challenges of an epidemic. Rev Panam Salud Publica 2010, 27:455–460 (in Portuguese).
24. Pereira SA, Gremião ID, Kitada AA, Boechat JS, Viana PG, Schubach TM: The epidemiological scenario of feline sporotrichosis in Rio de Janeiro, State of Rio de Janeiro, Brazil. Rev Soc Bras Med Trop 2014, 47:392–393.

25. Silva MB, Costa MM, Torres CC, Galhardo MC, Valle AC, Magalhaes Mde A, Sabroza PC, Oliveira RM: Urban sporotrichosis: a neglected epidemic in Rio de Janeiro, Brazil. Cad Saúde Publica 2012, 14:75–1800 [in Portuguese].

26. da Rosa ACM, Szwierkiewski ML, Vettorato R, Gervasi RL, Vettorato G, Weber A: Epidemiology of sporotrichosis: a study of 304 cases in Brazil. J Am Acad Dermatol 2005, 52:541–549.

27. Borges TS, Rossi CN, Fedullo JD, Taborda CP, Larson CE: Isolation of Sporothrix schenckii from the claws of domestic cats (indoor and outdoor) and in captivity in São Paulo (Brazil). Mycopathologia 2013, 176:619–637.

28. Duong TA, de Beer ZW, Wingfield BD, Wingfield MJ: Spontaneous case in domestic cat (in Portuguese). Rev Soc Bras Med Trop 2014, 175.

29. Rodrigues AM, de Hoog GS, Camargo ZP: Study of extra-urban outbreak of sporotrichosis associated with hay bale props in a farm of four cats in Rio de Janeiro. Zoonoses Public Health 2003, 50:434–448.

30. Oliveira MM, Mafredre SB, Ribeiro MA, Zancope-Oliveira RM: Unusual clinical presentation of sporotrichosis in three members of one family. Int J Dermatol 2012, 51:354–358.

31. Falquetto A, Bravim Maifrede S, Araújo Ribeiro M: Isolation of Sporothrix species complex in Portuguese. Rev Fac Med Vet Univ Sao Paulo 2013, 381.

32. Barros MBL, Schubach AO, Do Valle ACF, Galhardo MC, Conceição-Silva F, de O Schubach A: Isolation of species involved in the first familial outbreak of sporotrichosis in the state of Espírito Santo, Southeastern Brazil. Mem Inst Oswaldo Cruz 2013, 108:936–938.

33. Chaves AR, de Campos MF, Do Carmo CN, Gremião IDF, Pereira SA, Schubach TM: Treatment abandonment in feline sporotrichosis – Study of 147 cases. Zoonoses Public Health 2013, 60:149–153.

34. dos Santos IB, Schubach TMP, Leme LRP, Okamoto T, Figueiredo FB, Pereira SA, Quintella LP, Madeira MF, Coelho F, Reis RS, de O Schubach A: Sporotrichosis—The main differential diagnosis with tegumentary leishmaniosis in dogs from Rio de Janeiro, Brazil. Vet Parasitol 2007, 143:71–76.

35. Luiz A, Splendore A: Contribution to the knowledge of the so-called sporotrichosis. Revista Medica de São Paulo 1907, 21:443–450 [in Portuguese].

36. Freitas DC, Moreno G, Saliba AM, Botelho JA, Mós EM: Sporotrichosis in dogs and cats. Rev Fac Med Vet Univ Sao Paulo 1965, 7:381–387 [in Portuguese].

37. Freitas DC, Migliano MF, Zani Neto L: Sporotrichosis. Observation of spontaneous case in domestic cat (Felis catus). Rev Fac Med Vet Univ Sao Paulo 1956, 5:601–604 [in Portuguese].

38. Schubach TM, Schubach Ade Q, Guzzi-Maya T, Okamoto T, Reis RS, Monteiro PC, Gutierrez-Galhardo MC, Wanke B: Pathology of sporotrichosis in 10 cats in Rio de Janeiro. Vet Rec 2003, 152:172–175.

39. Dooley DP, Bostic PS, Beckius ML: Spook house sporotrichosis. A point-source outbreak of sporotrichosis associated with hay bale props in a Halloween haunted-house. Arch Intern Med 1997, 157:1885–1887.

40. Mehra KS, Sharma NL, Kangia AK, Mahajan VK, Ranjan N: Isolation of Sporothrix schenckii from the environmental sources of cutaneous sporotrichosis patients in Himachal Pradesh, India: results of a pilot study, Mycoses 2007, 50:496–501.

41. Rodrigues AM, Baggal E, de Carnago ZP, Boscio SMG: Sporothrix schenckii sensu stricto isolated from soil in an armadillo’s burrow. Mycopathologia 2014, 177:199–206.

42. O’Donnell K, Nirenberg H, Aoki T, Cigelnik E: A multigene phylogeny of the Gibberella fujikuroi species complex: Detection of additional phylogenetically distinct species. Mycoscience 2000, 41:51–78.

43. Hall TA: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999, 41:95–98.

44. Kato H, Standley DM: MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 2013, 30:772–780.

45. Romeo O, Scordino F, Criseo G: New insight into molecular phylogeny and epidemiology of Sporothrix schenckii species complex based on calmodulin-encoding gene analysis of Italian isolates. Mycopathologia 2011, 172:179–186.

46. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S: MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 2013, 30:2725–2729.

47. Akaike H: A new look at the statistical model identification. Automatic Control, IEEE Transactions on 1974, 19:716–723.

48. Tamura K: Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Mol Biol Evol 1992, 9:678–687.

49. Felsenstein J: Evolution confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985, 39:783–791.

50. Librado P, Rozas J: DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 2009, 25:1451–1452.

51. Bandelt H, Forster P, Rohl A: Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 1999, 16:37–48.