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Original

Sporothrix globosa, a pathogenic fungus with widespread geographical distribution

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ABSTRACT

Sporothrix globosa, reported from the USA, Europe, and Asia, is a recently described pathogenic species morphologically similar to *Sporothrix schenckii*. In this study, the phylogenetic affinities of 32 clinical and environmental isolates morphologically identified as *S. schenckii*, from Mexico, Guatemala, and Colombia, were assessed by cladistic analysis of partial sequences of the calmodulin gene using the maximum parsimony and neighbor-joining methods. The study revealed that one out of 25 isolates from Mexico (4%), one out of three isolates from Guatemala (33.3%), and two out of four isolates from Colombia (50%) belonged to *S. globosa*, while the other isolates belonged to *S. schenckii sensu stricto*. This is the first record of *S. globosa* from Mexico, and Central and South America.

Sporothrix globosa, un hongo patógeno con amplia distribución geográfica

Palabras clave:
Filogenia
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Distribución

RESUMEN

Sporothrix globosa es un hongo patógeno recientemente descrito. Esta especie, morfológicamente similar a *Sporothrix schenckii*, se ha descrito en EE.UU., Europa y Asia. En este trabajo se investigaron las relaciones filogenéticas de 32 aislamientos clínicos y ambientales, identificados morfológicamente como *S. schenckii*, procedentes de México, Guatemala y Colombia, mediante análisis cladístico de secuencias parciales del gen de la calmodulina usando los métodos de máxima parsimonia y *neighbor-joining*. El estudio reveló que uno de los 25 aislamientos de México (4%), uno de los tres aislamientos de Guatemala (33%) y dos de los cuatro aislamientos de Colombia (50%) correspondían a *S. globosa*, mientras que los demás aislamientos pertenecían a *S. schenckii sensu stricto*. La presencia de *S. globosa* en México, América Central y del Sur se describe por primera vez.

Sporotrichosis is a chronic infectious disease that typically involves the skin and subcutaneous tissue.[7,12] Cases of arthritis, meningitis, and other deep-seated forms have been reported less frequently.[1,3–5,10,25] Infection is acquired via traumatic implantation or less frequently by inhalation of propagules of the etiological agent living in soil, plant material, and other substrata.[14,19] Sporotrichosis is distributed worldwide, but most cases occur in temperate, warm, and tropical countries. The largest number of reports comes from North America, but it is also common in areas of Central and South America, Asia, and South Africa.[8,12]

For several decades, sporotrichosis has been attributed to a single pathogen, *Sporothrix schenckii* Hektoen & Perkins, an anamorphic fungus related to the ascomycetous genus *Ophiostoma* H. & P. Syd.[2] However, isolates identified as *S. schenckii* showed a great deal of phenotypic[6,12] and genetic[9,13,20] variability, suggesting that this taxon was a species complex. In a recent phylogenetic study based on the analysis of sequences of the chitin-synthase, ß-tubulin, and calmodulin (CAL) genes, numerous isolates phenotypically identified as *S. schenckii* were tested.[17] The strains were distributed into at least six distinct groups, which were considered as putative phylogenetic species. Later, the same authors found diagnostic features to separate phenotypically

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and genetically three of these clades, which were formally proposed as new species. They were *Sporothrix brasiliensis* Marimon, Gené, Cano & Guarro, *Sporothrix globosa* Marimon, Gené, Cano & Guarro, and *Sporothrix mexicana* Marimon, Gené, Cano & Guarro.[16]

Since variations in antifungal susceptibility have been demonstrated among the different species of the *S. schenckii* complex, their identification is clinically relevant.[18] Furthermore, considering that the taxonomy of the fungi causing sporotrichosis has been reevaluated, it becomes necessary to identify clinical isolates at the species level in order to study their epidemiology and geographical distribution, and to determine if different clinical patterns are associated with each of these taxa. Recently we have had the opportunity of studying numerous isolates from Mexico, Colombia, and Guatemala and our interest was to assess if a given species is predominantly implicated in cases of human infection in these countries, or, by contrast, a range of species can be present. To do this, we analyzed partial sequences of the CAL locus, which had previously proven to be the most informative gene.[16,17]

Materials and methods

Fungal isolates

Thirty-two clinical and environmental isolates morphologically identified as *S. schenckii*, from Colombia, Guatemala, and Mexico were included in this study (Table 1). These isolates were obtained from culture collections located at the *Servicio de Dermatología y Departamento de Micología*, in *Hospital General de México*, Mexico, and at the *Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Autónoma de México*, Mexico. The isolates were subcultured on potato dextrose agar (PDA; Difco Laboratories, USA) plates and incubated at 25 °C for 14 days in the dark. Isolates were stored at 4–7 °C and in slant cultures submerged in mineral oil at room temperature.

DNA extraction, amplification, and sequencing

DNA extraction, amplification, and sequencing of the CAL locus of the 32 isolates were performed as described previously,[16,17] using primers CL1 and CL2A.[21]

DNA sequence alignments

The program Autoassembler vers. 1.40 (Applied Biosystems) was used to obtain consensus sequences from the two complementary sequences of each isolate. The consensus sequences of the 32 isolates sequenced here were aligned with CAL sequences of 39 other isolates of *S. schenckii sensu stricto* and the related species *S. brasiliensis*, *S. globosa*, *S. mexicana*, and *Sporothrix albicans* S.B. Saksena determined in a previous study,[16] using ClustalX vers. 1.81,[24] followed by manual adjustments with a text editor.

Phylogenetic analysis

A phylogenetic analysis was performed by the maximum parsimony method using the PAUP* version 4.0b10 software.[23] Briefly, the most parsimonious trees were obtained after 100 heuristic searches with random sequence addition and tree

Table 1
Collection number, fungal species, source, origin, and EMBL accession numbers of the isolates studied.

Isolate no.	Species	Source	Origin	EMBL accession no.
FMR 9549	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9550	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9551	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9553	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9554	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9555	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9556	*S. globosa*	Human, lymphocutaneous sporotrichosis	Mexico	FM179331
FMR 9557	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9559	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9560	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9561	*S. schenckii*	Human, fungaemia	Mexico	–
FMR 9562	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	FM179332
FMR 9563	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9564	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9565	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9566	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9567	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9568	*S. schenckii*	Human, fixed cutaneous sporotrichosis	Mexico	–
FMR 9570	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9572	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9616	*S. schenckii*	Human, fixed cutaneous sporotrichosis, hand	Colombia	–
FMR 9617	*S. globosa*	Human, fixed cutaneous sporotrichosis, wrist	Colombia	FM179329
FMR 9619	*S. globosa*	Human, fixed cutaneous sporotrichosis, cheek	Colombia	FM179330
FMR 9620	*S. schenckii*	Human, lymphocutaneous sporotrichosis, arm	Colombia	–
FMR 9621	*S. schenckii*	Human, lymphocutaneous sporotrichosis	Mexico	–
FMR 9622	*S. schenckii*	Soil under *Coffea* sp.	Mexico	–
FMR 9624	*S. globosa*	Human, lymphocutaneous sporotrichosis, finger	Guatemala	FM207489
FMR 9625	*S. schenckii*	Human, fixed cutaneous sporotrichosis, forearm	Guatemala	–
FMR 9626	*S. schenckii*	Human, lymphocutaneous sporotrichosis foot	Guatemala	–
FMR 9629	*S. schenckii*	Soil	Mexico	–
FMR 9631	*S. schenckii*	Soil	Mexico	FM179333
FMR 9632	*S. schenckii*	Soil	Mexico	–

FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain.

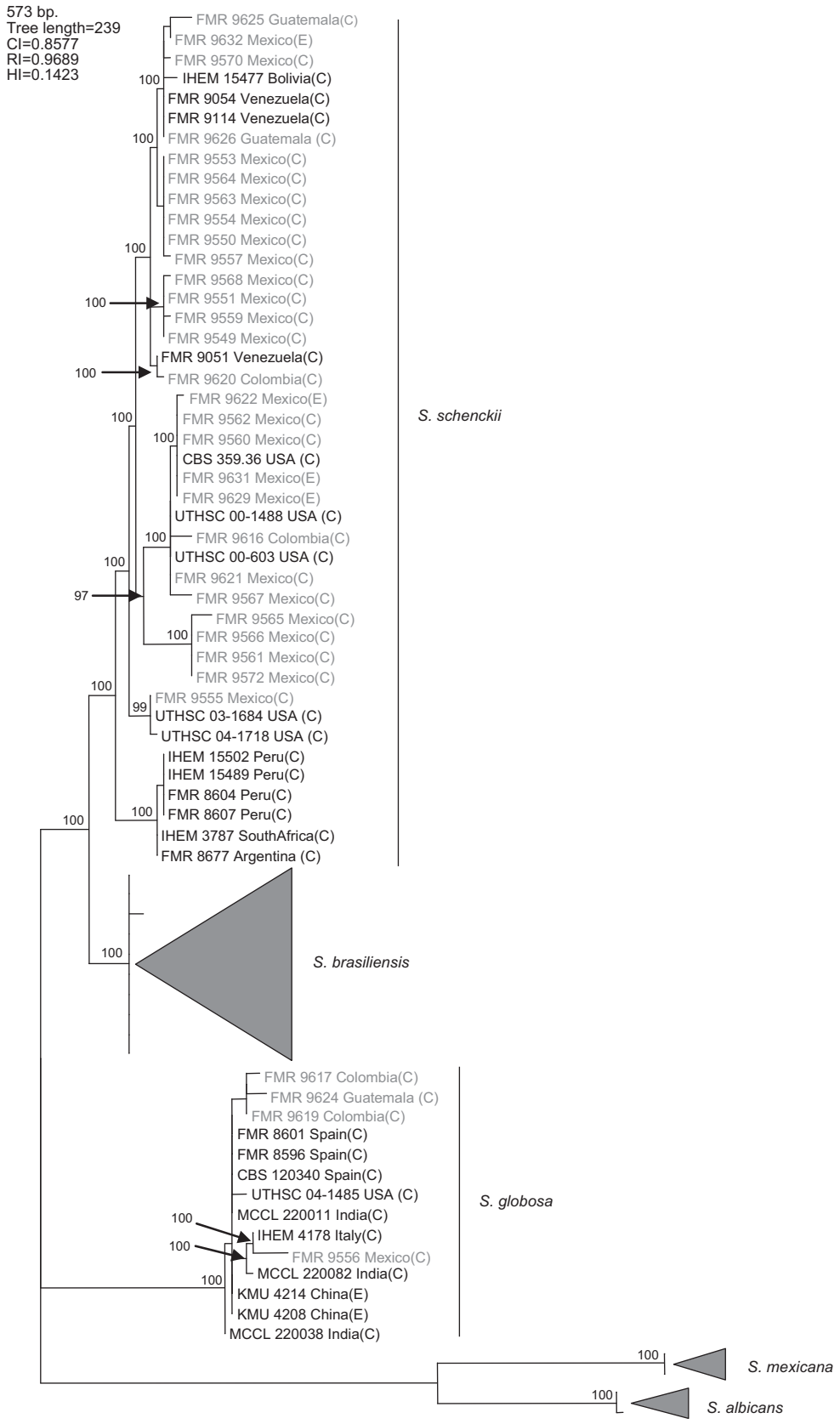

Figure 1. One of the 5000 most parsimonious trees obtained from heuristic searches based on an analysis of the CAL locus. Bootstrap values above 80% are indicated at the nodes. Type strains are indicated in bold type. Isolates for which new CAL sequences were generated during this study are highlighted in blue. CI, consistency index; RI, retention index; HI, homoplasy index; (E), environmental isolate; (C), clinical isolate; FMR, Facultat de Medicina i Ciències de la Salut, Reus, Spain; IHEM, The BCCM/IHEM Biomedical Fungi and Yeasts Collection, Brussels, Belgium; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; UTHSC, Fungus Testing Laboratory, University of Texas Health Science Center; MCCL, Mycology Culture Collection Laboratory, Postgraduate Institute of Medical Education and Research, Chandigarh, India; KMU, Kanazawa Medical University, Ishikawa, Japan.

bisection–reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MulTrees). Also, a neighbor-joining phylogeny[22] was constructed using the Kimura-2-parameter substitution model with pairwise deletion of gaps, as implemented in the MEGA3 computer program.[11] The robustness of branches was assessed by bootstrap analysis of 1000 replicates.

Results and discussion

With the primers used, a fragment of 573 bp of the CAL locus was amplified and sequenced. The complete alignment included 71 sequences, 32 generated in this study and 39 retrieved from a previous study,[16] the latter belonging to *S. schenckii s. str.* (15), *S. brasiliensis* (10), *S. globosa* (10), *S. mexicana* (2), and *S. albicans* (2), resulting in a data set of 573 characters, including 403 constant, 160 variable parsimony informative (39.7%), and 10 variable parsimony noninformative sites. Cladistic analysis by the neighbor-joining and maximum parsimony methods generated trees with identical topology. Maximum parsimony analysis of the CAL data set yielded 5000 trees, 239 steps in length, in which 20 nodes received 100% bootstrap support. One of the most parsimonious trees is shown in figure 1. The 71 sequences were distributed into the five main groups detected in previous studies,[16,17] representing *S. brasiliensis*, *S. schenckii sensu stricto*, *S. globosa*, *S. mexicana*, and *S. albicans*. One out of 25 isolates from Mexico (4%), one out of three isolates from Guatemala (33.3%), and two out of four (50%) isolates from Colombia grouped into the *S. globosa* clade, which also included another 10 sequences belonging to isolates from India, China, Italy, USA, and Spain. The other isolates from Colombia, Mexico, and Guatemala grouped into the *S. schenckii s. str.* clade, which also included sequences of another 15 isolates from Bolivia, Venezuela, USA, Peru, South Africa, and Argentina. Since none of the sequences generated in this study grouped into the *S. mexicana*, *S. brasiliensis* or *S. albicans* clades, the isolates belonging to these clades are not detailed in figure 1. The 24 isolates from Mexico in the *S. schenckii* clade were distributed among 14 different haplotypes. Two clinical (FMR 9560 and FMR 9562) and two environmental isolates from Mexico (FMR 9629 and FMR 9631) exhibited the same haplotype as the type strain of *S. schenckii*, CBS 359.36. The *S. schenckii* isolates from Guatemala and Colombia were distributed among four different haplotypes. The *S. globosa* isolates from Mexico, Guatemala, and Colombia were distributed among four haplotypes different from that of the type strain.

A previous study revealed the existence of differences in the geographical distribution among the members of the *S. schenckii* complex, including widespread as well as geographically restricted species.[16] *S. brasiliensis* and *S. mexicana* occurred only in Brazil and Mexico, respectively, and these taxa grouped all the isolates from Brazil ($N = 29$) and Mexico ($N = 2$) tested in that study. Based on these observations, we first thought the 25 isolates from Mexico studied here could follow the same pattern of close phylogenetic affinity observed in isolates from Brazil. However, none of these isolates grouped into the *S. mexicana* clade, a species originally reported from soil and from carnation leaves. On the other hand, *S. schenckii* and *S. globosa* are widespread fungi showing transoceanic distributions.[16] Until now, 36 isolates of *S. globosa* have been reported from United Kingdom, Spain, Italy, China, Japan, USA, and India. This is the first record of this species from Mexico, and Central and South America.

An experimental model of disseminated infection by different *Sporothrix* species in immunocompetent mice showed that, while *S. schenckii s. str.* and *S. brasiliensis* were able to kill immunocompetent animals inoculated intravenously, *S. globosa* did not cause any apparent lesion, suggesting that it might be less virulent than the former species.[15] Interestingly, in contrast with *S. schenckii s. str.* and *S. brasiliensis*, which have been associated with both localized and invasive disease,[16,17] no cases of invasive infections have been attributed to *S. globosa*. This apparently lower virulence might be related to the inability of the fungus to grow at 37 °C.[16]

Marimon et al.[16] proposed as key features for the differentiation of the clinically relevant *Sporothrix* species the presence or absence of pigmented sessile conidia, growth rates at different temperatures, and carbohydrate assimilation test results. Although morphologically similar to other taxa within the *S. schenckii* complex, *S. globosa* is the only member of the complex unable to grow at 37 °C on PDA. Moreover, among the four *Sporothrix* species with pigmented sessile conidia treated, only *S. globosa* showed the combination of positive sucrose and negative raffinose assimilations. These easily diagnosed features allow the identification of *S. globosa* and related taxa by simple inexpensive laboratory procedures.

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