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Origin and distribution of *Sporothrix globosa* causing sapronoses in Asia

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Abstract

Purpose. The aim of the study was to evaluate the main sources and epidemiological patterns and speculate on the evolutionary origin of *Sporothrix globosa* in Asia.

Methodology. Case and case series literature on sporotrichosis in Asia from January 2007 onwards were reviewed using meta-analysis. Phylogenetic analysis of relevant *S. globosa* was carried out on the basis of concatenated sequences of ITS, *TEF3* and *CAL*. A haplotype network of *CAL* sequences of 281 *Sporothrix* isolates was analysed to determine the population structure of *S. globosa*.

Results. Nearly all cases of sporotrichosis caused by *S. globosa* in Asia were human. In contrast to the remaining pathogenic *Sporothrix* species, feline transmission was exceptional; nearly all regional cat-associated cases were caused by *Sporothrix schenckii*. While the latter species was highly variable and showed recombination, *S. globosa* seemed to be a clonal offshoot, as was *Sporothrix brasiliensis*. The origin of the segregants was located in an area of high variability in *S. schenckii* with a relatively high frequency of Asian strains.

Conclusion. In Asia, *S. globosa* was the prevalent species. The low diversity of *S. globosa* suggested a recent divergence with a founder effect of low variability from the variable ancestral species, *S. schenckii*.

INTRODUCTION

Sporotrichosis has gained importance in recent years due to its worldwide prevalence and the recognition of multiple cryptic species within the originally described species, *Sporothrix schenckii*, all molecular species having distinctive ecology, distribution and epidemiology. *S. schenckii* had been regarded as the single agent of sporotrichosis for many decades, since its first isolation by Benjamin Schenck in 1898. Recent phenotypic and genotypic studies have segregated *S. schenckii sensu stricto*, *Sporothrix brasiliensis*, *Sporothrix globosa* and *Sporothrix luriei* [1]. Later *Sporothrix mexicana* [2], *Sporothrix pallida* [3] and *Sporothrix chilensis* [4] were added as potential causes of infection, while also some published cases have been caused by *Ophiostoma* species with sporothrix anamorphs [5]. A wide variation in geographical distribution, virulence and antifungal susceptibility has been demonstrated between these newly identified species. In addition, the source of infection deviates between species; therefore, identification down to the species level is critical for public health and appropriate patient management [6].

Among the implantation mycoses, occupation seems to play an important role in sporotrichosis. In sapronoses, professionals handling plants or plant materials, such as farmers, gardeners, foresters and nursery workers, are particularly at high risk. Traumatic inoculation is the obvious

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Abbreviation: Hd, haplotype diversity.

reason that exposed body parts are involved most frequently. In North-East China, this has been explained by the high exposure of corn harvesters to corn debris during the harvesting season [7]. Similarly, in India, infection was frequently reported on the hands and face, and 90 % of the patients were involved in agricultural work [8]. In contrast, zoonotic sporotrichosis appears to be occupation independent and any contact with an infected animal can predispose to the infection. In recent epidemics in Rio de Janeiro, Brazil, 421 patients reported a history of cat scratch or contact with an infected cat [9].

Both genders and all age groups can become infected with sporotrichosis, and no specific host factors or susceptible population have been reported. The onset of the disorder mainly depends on exposure to the fungus in the environment, and anyone living in an endemic area is at risk. However, a certain degree of gender predominance has been observed, which differed between species. In Asian countries, Zhang *et al.* reported a preponderance of female patients in a case series of patients with confirmed *S. globosa* infection [7]. In India, a male to female ratio of 1 : 1.7 was observed, similar to that in North-East China (male : female=1 : 1.42) [10]. The male : female ratio for *S. brasiliensis* in Brazil was 1 : 2.46, while in *S. schenckii* male patients predominated [11]; this gender difference in *S. schenckii* might be due to increased exposure rather than to a difference in susceptibility. In urban settings, sporotrichosis is prevalent among adults. However, in tropical regions and in areas of hyperendemicity, the disease may be more common in children and adolescents. Case-controlled studies indicate that playing in crop fields and on dirty floors in houses provides possible modes of exposure in children [12]. This may hold true for the unique outbreak of sporotrichosis in infants aged <10 months in China. Contaminated cornstalks stacked by families for cooking and heating during the winter were regarded as the source of this outbreak. Most infants start crawling around the age of 6 months, and unnoticed falling on the face may lead to infection. Fixed cutaneous-type infection of the face, especially on the nose, was noted in these cases, all caused by *S. globosa*.

S. globosa has a wide geographical distribution, having been isolated from the Americas, Europe and Asia. However, it is remarkable that in Asian countries *S. globosa* is the preponderant endemic species, with a prevalence of 99.3 % based on historical data [7]. In South-East Brazil, *S. brasiliensis* is currently causing a large epidemic. The species as yet has a limited distribution in the Rio de Janeiro–São Paulo area [6]. *S. schenckii* seems to be replaced by *S. brasiliensis* when the epidemic front reaches the original endemic areas of this species [13]. In Asia, *S. schenckii* is very rare and *S. brasiliensis* has not yet been reported. In the present article, we will review the main sources of infection, epidemiological patterns and possible natural habitat, and speculate on the evolutionary origin of *S. globosa*.

METHODS

Strains and cultivation

The clinical strains of *S. globosa* were obtained from the CBS-KNAW (Fungal Biodiversity Centre, The Netherlands) reference collection (www.cbs.knaw.nl) and supplemented with fresh isolates recovered from patients with sporotrichosis (Table 1). Strains were cultivated on 5 % malt extract agar (MEA) (Oxoid) in 8 cm culture plates incubated at 24 °C for 7 days. For further studies the same medium was used. Photo plates were prepared using slide cultures, grown on MEA for 7 days at 24 °C, and microscopic slide preparation was carried out using water as the mounting fluid. Photos were made by light microscopy using a Nikon Eclipse 80i equipped with differential interference contrast, and a Nikon digital DS-5M114780 camera.

Molecular analysis

DNA extraction was performed according to Rodrigues *et al.* [6]. Briefly, cultures were grown for 7 days on 5 % MEA (Oxoid) at 24 °C. Fungal material was suspended in 400 µl 2× CTAB (cetyl-trimethylammonium bromide) buffer containing 6–10 acid-washed glass beads (1.5–2 mm); 100 µl polyvinylpyrrolidone 10 % was added and mixed thoroughly with a Mo Bio vortex for 10 min. After 60 min incubation at 60 °C, 500 µl chloroform:isoamylalcohol (24 : 1) was added, shaken for 2 min and centrifuged at 20 817 ***g*** for 10 min. The aqueous layer was collected, and 50 µl RNAse solution was added and incubated for 30 min at 37 °C. A two-thirds volume of ice-cold isopropanol was added, mixed and centrifuged at 20 817 ***g*** for 10 min. The supernatant was removed, and 1 ml ice-cold 70 % ethanol was added, mixed gently and centrifuged again at 20 817 ***g*** for 2 min. Samples were air-dried or dried using a Speed Vac. Pellets were resuspended in 50 µl TE buffer and stored at −20 °C. DNA quality was verified by electrophoresis in 0.8 % agarose.

Amplification and sequencing

For molecular identification, three markers were considered, i.e. the rDNA internal transcribed spacer (ITS), and the partial genes calmodulin (*CAL*) and translation elongation factor 3 (*TEF3*). Primer sets V9G and LS266 were used for ITS, CL1 and CL2a for CAL [7], and EF3-3185F and EF3-3538R for TEF3 [14]. PCR amplification was performed in a 12.5 µl reaction mixture containing 7 µl ddH_2O, 0.5 µl BSA, 0.5 µl (10 pmol) of each primer, 1.25 µl PCR buffer, 1.25 µl deoxynucleotide triphosphate, 0.5 µl $MgCl_2$ solution (25 mM), 0.5 µl (5 U) BioTaq polymerase (GC Biotech) and 1 µl template DNA. PCRs for ITS were carried out according to Zhou *et al.* [15], for *CAL* according to Zhang *et al.* [7] and for TEF3 according to Stielow *et al.* [14]. Amplification was performed in a 9700 thermal cycler (Applied Biosystems). Concentrations of amplicons were estimated with 1.2 % agarose gels, photographed and analysed by the Gel Doc XR system (BioRad), with Smart Ladder (Eurogentec) as a size and concentration marker. Sequencing reactions were performed with a BigDye terminator cycle sequence

Table 1. Isolates of *Sporothrix* included in the study

No.	CBS no.	Alternative no.	Taxon name	Locality/country	Clinical type	GenBank no.		
						ITS	*CAL*	*TEF3*
1	CBS 140741	SY11021702	*Sporothrix globosa*	Liuhe, Jilin, China	Fixed cutaneous	KY387616	KY387689	KY387746
2	CBS 140742	SY11021303	*Sporothrix globosa*	Fuyu, Jilin, China	Fixed cutaneous	KY387627	KY387690	KY387757
3	CBS 140743	SY11022105	*Sporothrix globosa*	Changchun, Jilin, China	Disseminated	KY387629	KY387691	KY387759
4	–	SY11022201	*Sporothrix globosa*	Gongzhuling, Jilin, China	Lymphocutaneous	KY387679	KY387734	KY387796
5	–	SY11021406	*Sporothrix globosa*	Panshi, Jilin, China	Fixed cutaneous	KY387678	KY387733	KY387795
6	–	SY11021003	*Sporothrix globosa*	Meihekou, Jilin, China	Fixed cutaneous	KY387677	KY387732	KY387794
7	CBS 140744	SY11021504	*Sporothrix globosa*	Dongfeng, Jilin, China	Lymphocutaneous	KY387633	KY387695	KY387763
8	CBS 140745	SY11020808	*Sporothrix globosa*	Yitong, Jilin, China	Fixed cutaneous	KY387634	KY387696	KY387764
9	CBS 140746	SY11020802	*Sporothrix globosa*	Yitong, Jilin, China	Fixed cutaneous	KY387635	KY387697	KY387765
10	CBS 140747	SY11022403	*Sporothrix globosa*	Yushu, Jilin, China	Lymphocutaneous	KY387617	KY387698	KY387747
11	CBS 140748	SY11102001	*Sporothrix globosa*	Jilin, Jilin, China	Fixed cutaneous	KY387618	KY387699	KY387748
12	CBS 140749	SY11110301	*Sporothrix globosa*	Daan, Jilin, China	Fixed cutaneous	KY387619	KY387700	KY387749
13	CBS 140750	SY11103101	*Sporothrix globosa*	Jilin, Jilin, China	Fixed cutaneous	KY387620	KY387701	KY387750
14	CBS 140751	SY11121203	*Sporothrix globosa*	Jiutai, Jilin, China	Fixed cutaneous	KY387621	KY387702	KY387751
15	CBS 140752	SY11120802	*Sporothrix globosa*	Huaide, Jilin, China	Lymphocutaneous	KY387622	KY387703	KY387752
16	–	SY11120901	*Sporothrix globosa*	Shulan, Jilin, China	Lymphocutaneous	KY387675	KY387731	KY387792
17	CBS 140753	SY11111901	*Sporothrix globosa*	Jiutai, Jilin, China	Fixed cutaneous	KY387624	KY387705	KY387754
18	–	SY11121502	*Sporothrix globosa*	Changlin, Jilin, China	Fixed cutaneous	KY387674	KY387735	KY387791
19	CBS 140754	SY11121201	*Sporothrix globosa*	Fuyu, Jilin, China	Fixed cutaneous	KY387626	KY387707	KY387756
20	CBS 140755	SY11122901	*Sporothrix globosa*	Jiache, Jilin, China	Fixed cutaneous	KY387628	KY387708	KY387758
21	CBS 138657	LFQ2	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387651	KY387711	KY387767
22	CBS 138658	LFQ3	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387652	KY387712	KY387768
23	CBS 138659	LFQ4	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387653	KY387713	KY387769
24	CBS 138660	LFQ5	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387654	KY387714	KY387770
25	CBS 138661	LFQ6	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387655	KY387715	KY387771
26	CBS 138662	LFQ7	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387656	KY387716	KY387772
27	CBS 138663	LFQ8	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387657	KY387717	KY387773
28	CBS 138664	LFQ9	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387658	KY387718	KY387774
29	CBS 138665	LFQ1	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387672	KY387730	KY387789
30	CBS 138666	LFQ10	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387660	KY387719	KY387776
31	CBS 138667	LFQ11	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387661	KY387720	KY387777
32	CBS 138668	LFQ12	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387662	KY387721	KY387778
33	CBS 138669	LFQ13	*Sporothrix globosa*	Jilin, China	Lymphocutaneous	KY387663	KY387722	KY387779
34	CBS 138670	LFQ14	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387664	KY387723	KY387780
35	CBS 138671	LFQ15	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387665	KY387724	KY387781
36	CBS 138672	LFQ16	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387666	KY387725	KY387782
37	CBS 138673	LFQ17	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387667	KY387726	KY387783
38	CBS 138674	LFQ18	*Sporothrix globosa*	Jilin, China	Fixed cutaneous	KY387668	KY387727	KY387784
39	CBS 129723	ZX 17788	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387641	KP101465	KP017171
40	CBS 129724	ZX 17634	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387642	KP101466	KP017172
41	CBS 129720	ZX 18715	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387638	KP101463	KP017169
42	CBS 129718	ZX 19151	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	–	KY387710	KP017167
43	CBS 129719	ZX 18933	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387637	KP101462	KP017168
44	CBS 129717	ZX 22172	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	–	KY387709	KP017166
45	CBS 129722	ZX 18072	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387640	KP101464	KP017183
46	CBS 129725	ZX 17518	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387643	KP101467	KP017173
47	CBS 129721	ZX 18624	*Sporothrix globosa*	Chongqing, China	Human sporotrichosis	KY387639	KP101478	KP017170
48	CBS 130117	FMR 9022	*Sporothrix globosa*	Zaragoza, Spain	Human sporotrichosis	KY387648	KP101472	KP017178
49	CBS 130105	FMR 8597	*Sporothrix globosa*	Zaragoza, Spain	Human sporotrichosis	KY387645	KP101469	KP017175
50	CBS 130116	FMR 5898	*Sporothrix globosa*	Zaragoza, Spain	Human sporotrichosis	KY387647	KP101471	KP017177
51	CBS 130104	FMR 8595	*Sporothrix globosa*	Zaragoza, Spain	Human sporotrichosis	KY387644	KP101468	KP017174

Table 1. cont.

No.	CBS no.	Alternative no.	Taxon name	Locality/country	Clinical type	GenBank no.		
						ITS	*CAL*	*TEF3*
52	CBS 120340 (T)	FMR 8600	*Sporothrix globosa*	Zaragoza, Spain	Human sporotrichosis	KP017084	KP101459	KP017165
53	CBS 130115	FMR 8596	*Sporothrix globosa*	Zaragoza, Spain	Human sporotrichosis	KC113228	KP101470	KP017176
54	CBS 132924	–	*Sporothrix globosa*	Goiania, Brazil	Lymphocutaneous	KY387650	KP101474	KP017180
55	CBS 132923	–	*Sporothrix globosa*	Fortaleza, Brazil	Fixed cutaneous	–	KP101473	KP017179
56	CBS 292.55	–	*Sporothrix globosa*	England	Human sporotrichosis	KY387669	KP101476	KP017181
57	CBS 340.35	–	*Sporothrix globosa*	Japan	Human sporotrichosis	–	KP101476	KP017181
58	CBS 140866	NUBS 12003	*Sporothrix globosa*	Kanagawa, Japan	Human sporotrichosis	KY387680	KY387736	KY387745
59	CBS 140867	NUBS 12004	*Sporothrix globosa*	Chiba, Japan	Feline sporotrichosis	KY387681	KY387737	KY387797
60	CBS 140868	NUBS 12005	*Sporothrix globosa*	Japan	Human sporotrichosis	KY387682	KY387738	KY387798
61	CBS 141042	dH 24381	*Sporothrix globosa*	Japan	Human sporotrichosis	KY387683	AB759542	KY387799
62	CBS 141043	dH 24382	*Sporothrix globosa*	Japan	Human sporotrichosis	KY387684	–	KY387800
63	CBS 141044	NUD 0080305	*Sporothrix globosa*	Japan	Human sporotrichosis	KY387685	KY387742	KY387801
64	CBS 141045	NUD 0120810	*Sporothrix globosa*	Japan	Human sporotrichosis	KY387686	KY387743	KY387802
65	CBS 140869	–	*Sporothrix globosa*	Japan	Human sporotrichosis	KY387687	KY387744	KY387803
66	CBS 120339 (T)	FMR 8309	*Sporothrix brasiliensis*	Rio de Janeiro, Brazil	Human sporotrichosis	KP017087	KP101421	KP017184
67	CBS 359.36 (T)	–	*Sporothrix schenckii*	USA	Notknown	KP017100	KP101420	KP017163
68	CBS 120341 (T)	–	*Sporothrix mexicana*	Mexico	Soil, rose tree	KP017072	AM398393	KP017230
69	CBS 132928	–	*Sporothrix mexicana*	Minas Gerais, Brazil	Human sporotrichosis	KY387688	JF811341	KP017234

ready reaction kit (Applied Biosystems) and analysed on an ABI Prism 3730XL sequencer.

Phylogenetic analysis

Phylogenetic analysis was carried out on the basis of concatenated sequences of ITS, *TEF3* and *CAL*. Sequences were aligned with the web version of MUSCLE and adjusted in BIOEDIT v. 7.1.3. The combined matrix was prepared in DATACONVERT 1.0 and maximum-likelihood analysis was performed using MEGA v6 with 500 bootstrap replications and TL91+G as best model. *S. mexicana* CBS 120341 and CBS 132928 were used as outgroups. Bootstrap values >70 % were considered statistically significant, and values are shown with the branches.

Population analysis

In order to compare the population structure of *S. globosa* with other *Sporothrix* species, sequences of *CAL* of 71 *S. brasiliensis* and 132 *S. schenckii* were acquired from GenBank and included in the analysis. Furthermore, 88 additional *S. globosa CAL* sequences were retrieved from GenBank and analysed. The *CAL* sequences were collected in BIOEDIT v. 7.1.3 software and alignments were made with MUSCLE. The extent of DNA polymorphism in the dataset (excluding gaps/missing data) was determined using DNASP v. 5.10. These polymorphisms included the number of polymorphic sites (S), the haplotype diversity (Hd) and nucleotide diversity (π). A network was constructed for haplotypes using Network 4.6.1.0 (Fluxus-Technology) with the application of the median-joining method. Splitstree v. 4.8 was used to determine the possibility of recombination in the *S. globosa* and *S. schenckii* population.

Meta-analysis

All available case and case series literature from January 2007 onwards were searched systematically in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) with different combinations of the key words *Sporothrix*, sporotrichosis and Asia [1]. Resulting papers in English and Chinese were included. Reports on treatment, immunology, antifungals and virulence factors, as well as book chapters and reports that also included other diseases, were disregarded, and case reports with insufficient data were discarded. Numbers are approximate because some cases had been used in repeated publications; we tried to exclude duplicates when individual cases were numbered.

Thermal behaviour

The thermotolerance of five strains was tested by incubation for 10 days at 33, 35, 37 and 40 °C on brain-heart infusion agar (BHI; Oxoid). Colony diameters were measured at regular intervals. Thermostability was tested by three-point inoculations of approximately 1 mm-diameter fungal material placed upside-down in each culture plate, for the five randomly selected isolates; morphology was verified microscopically. Strains were incubated on BHI at 37 °C for 10 days, then returned to 25 °C for 7 days. Colony diameters were measured to determine thermostability, and growth was checked microscopically.

RESULTS

A concatenated tree of ITS, *CAL* and *TEF3* (n=69) was made using MEGA v6 (Fig. 1). The total alignment length was 1434 bp. Strains belonging to *S. globosa* were closely

Fig. 1. Maximum-likelihood tree based on concatenated sequences of ITS, *TEF3* and *CAL*. Bootstrap values >70 % based on 500 replications are indicated with the branches. *S. mexicana* CBS 120341(T) and CBS 132928 were used as outgroups.

similar in all partitions, clustering with a high bootstrap of 100 % and with a substitution rate of 0.01. No significant difference between *S. globosa* strains was found for geographical distance, isolates from Europe and South America being randomly dispersed between Asian *S. globosa* in the tree. Strain CBS 140867 from a feline infection in Japan was identical in all partitions to the prevalent *S. globosa* genotype, while strain dH 24432 from a feline leg infection in Hong Kong proved to be *S. schenckii*. It is worth noting that morphologically strain dH 24432 was indistinguishable from *S. globosa*, with characteristic spherical hyaline and brown conidia (Figs 2 and 3). Four Chinese strains, i.e. a group of three from Nanchang and a single isolate from Hubei (not included in the study), proved to be *S. schenckii* [7, 16].

The genetic diversity of *S. globosa*, *S. schenckii* and *S. brasiliensis* was assessed using sequences of calmodulin (n=356) (Fig. 4). In the total dataset, 76 polymorphic sites were detected of which 18 were singletons and 58 were parsimonious informative sites. In *S. globosa* (n=149), 17 polymorphic sites (S) were observed, of which 9 were singletons and 8 were parsimonious informative sites. The haplotype analysis of the total dataset divided the isolates into 42 haplotype groups with an Hd of 0.8153 and nucleotide diversity (π) of 0.0500. The highest diversity was observed in *S. schenckii* with a total of 28 haplotypes (Hd=0.732, π=0.01521), whereas low diversity was observed in the *S. brasiliensis* population with only 5 haplotypes (Hd=0.34, π=0.00127). The *S. globosa* population showed also a low diversity compared to *S. schenckii* with a total of 9 haplotypes (Hd=0.33, π=0.00164). Within the *S. globosa* population the PHI test did not show statistically significant evidence for recombination (P=1.0), whereas evidence for recombination was observed in *S. schenckii* (P=0.02146).

Yeast conversion of strains of *S. globosa* in BHI broth was obtained at 35 °C, yielding large spherical yeast cells and multilateral, fusiform daughter cells. Strains showed very limited or no expansion growth at 37 °C, but all isolates tested resumed growth when returned to 25 °C (4 to 10 mm diameter after 7 days). Colonies were white, non-pigmented, with a waxy appearance and with a growth speed that was significantly lower than with the original culture. Microscopically dense hyphae without conidia were seen.

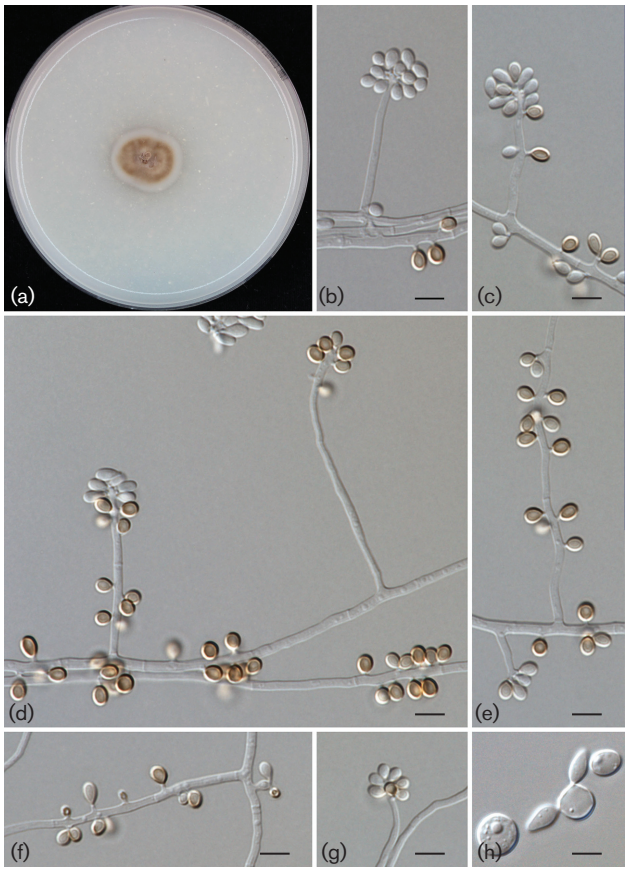

Fig. 2. Morphology of *S. globosa* CBS 120340 (T). (a) A colony on CMA. (b–g) Conidiogenous cells, sympodial conidia and sessile conidia. (h) Yeast-like cells at 37 °C. Scale Bars, 5 µm.

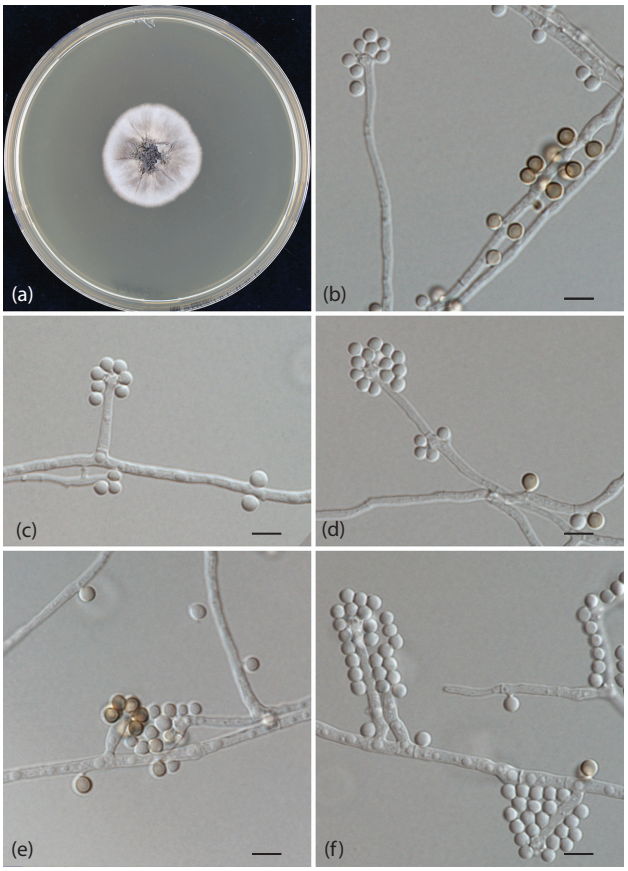

Fig. 3. Morphology of *S. schenckii* dH 24432 from a Hong Kong cat. (a) A colony on MEA. (b–f) Conidiogenous cells, sympodial conidia and sessile conidia. Scale Bars, 5 µm.

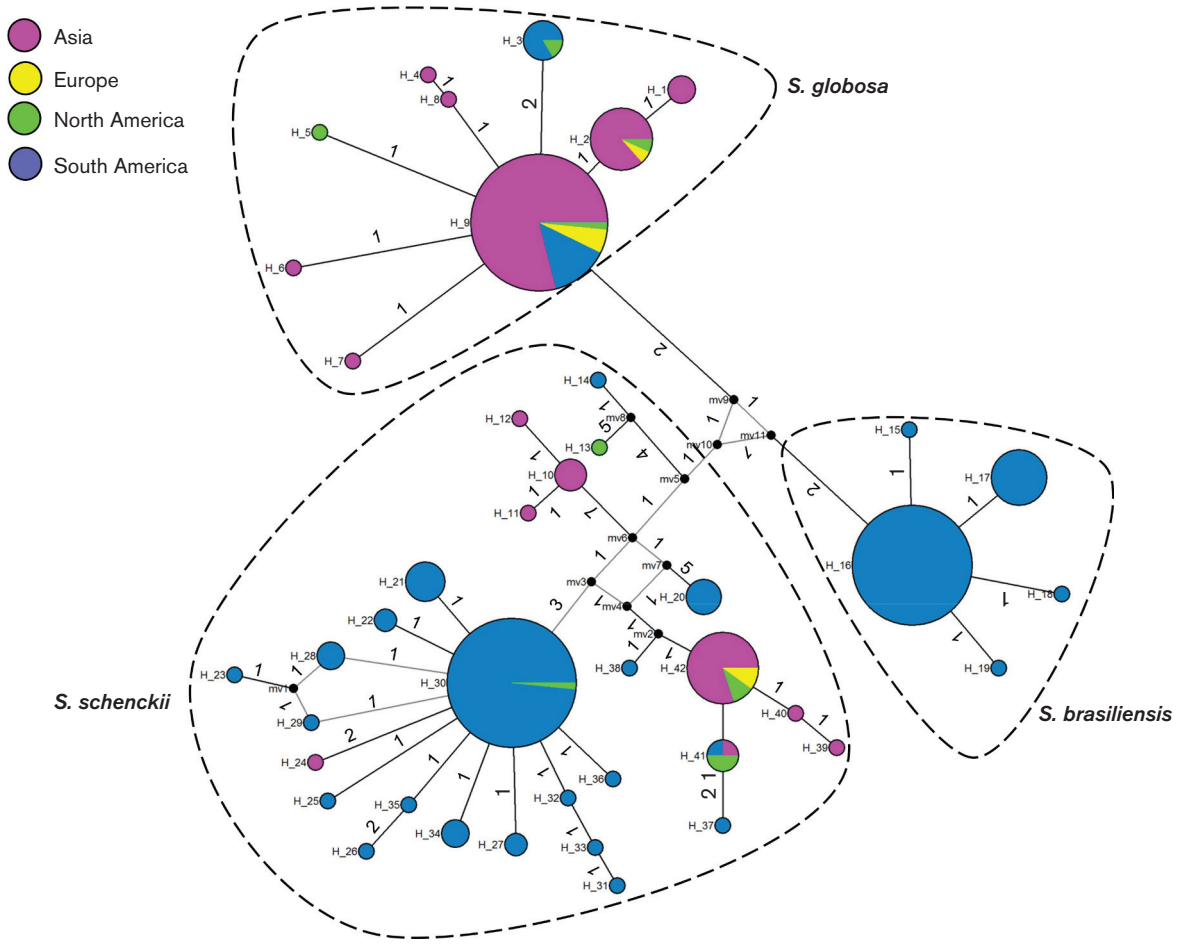

Fig. 4. Haplotype network of *CAL* sequences of 281 *Sporothrix* isolates. Haplotypes are represented by pie charts with the circumference proportional to the haplotype frequency. The small black dots [median vectors (mv)] represent internal haplotypes not present in the dataset. Numbers on the lines indicate mutation steps between the haplotypes.

DISCUSSION

Members of the ascomycete order *Ophiostomatales* are particularly known for their association with bark beetles that live in galleries behind the bark of woody plants and serve as vectors of distribution [17]. The numerous species of the genus *Ophiostoma* nearly all show this ecology. The genus *Sporothrix* contains some members in a single clade that are consistently able to cause human infection [7]. A number of *Sporothrix* species between these ecological groups have other types of arthropod-associated life cycles, such as *Sporothrix phasma* and *Sporothrix gemmellus*, which are associated with mites in *Protea* flowers that remain closed over prolonged periods and provide a special microclimate. Zhang *et al.* combined the notions that human infection is often acquired via plant debris and that the species have a thermodependent yeast phase and suggested that the source of contamination might be self-heated fermenting plant debris [7]. This type of material contains numerous small arthropods, which might link the ecology of human pathogens in *Sporothrix* to that of beetle-associated species in *Ophiostoma*.

S. globosa is characterized by the production of both hyaline and sessile brown to dark brown conidia; both terminal and lateral conidia are spherical (Figs 2 and 3). Barros *et al.* identified strains by colony colour on cornmeal agar, assimilation of sucrose and raffinose, and by measuring colony diameters at 30 and 37 °C [18]. Significant growth inhibition is observed above 35 °C for *S. globosa*, and some isolates are unable to grow or grow extremely slowly at 37 °C, which might partly explain its lower virulence and its prevalence in the most superficial, fixed cutaneous type of infection. Fungi normally tolerate ambient environmental temperatures with a certain bandwidth, defining mesophily. Prolonged incubation at maximum temperature is usually lethal, unless the fungus is thermostable, i.e. able to resume growth upon incubation at the permissive temperature. Regrowth at 25 °C, however, yields restricted colonies without sporulation, indicating a marked negative

impact of elevated temperatures. Zhang *et al.* hypothesized self-heated composting material as a possible environmental niche of *S. globosa*, which seems to correspond with the species' temperature profile [7]. In comparison, *S. brasiliensis* is better supported by the normal body temperature of humans (36.5–37.5 °C) and cats (37.7–39.1 °C), and is highly virulent, with dissemination and massive infiltration of infected tissues compared to *S. schenckii* and *S. globosa* [19–21]. As a result, *S. brasiliensis* is associated with high degrees of pathogenicity in both felines [22], and humans [23].

Despite the world wide distribution and the medical importance of pathogenic *Sporothrix* species, which have wide host ranges in plant materials and animals, their ecology and mode of dispersal are still poorly understood. *S. globosa* has been extensively reported in Asian countries, including China, Japan and India; in addition, some cases of sporotrichosis by *S. globosa* were reported from Europe and from the Americas [1, 24–28]. In China, where the largest outbreak of *S. globosa* was reported, efforts have been made to isolate this fungus from the environment. However, most of the environmental strains recovered so far were identified as non-pathogenic *Sporothrix* species and only four strains of *S. globosa* have been successfully isolated, from wheat, reed and soil [27, 29]. Human pathogenic *Sporothrix* species have never been observed as plant pathogens, as they fail to grow on living plants [30]. This not only hampered the recognition of the environmental niches of *S. globosa,* but also has raised a question on the mode of transmission. Cat-to-cat and cat-to-human transmission is very common in *S. brasiliensis* [6], has occasionally been confirmed in *S. schenckii* [22] and is extremely rare in *S. globosa*. Our

Fig. 5. Distribution of *Sporothrix* species in East Asia. Green, local populations of *S. globosa*; red, local populations of *S. schenckii*; grey, local populations of unsequenced *Sporothrix* species. Cat cartoons denote feline cases of sporotrichosis. Numbers in brackets represent literature reports of sporotrichosis without sequence data but presumed identity (compare with the report by Zhang *et al.* [7]).

isolate dH 24432 from a cat leg-wound lesion in Hong Kong, together with 28 feline isolates from Malaysia, proved to be *S. schenckii*, while CBS 140867 from a cat in Chiba, Japan, proved that *S. globosa* is indeed able to cause this kind of infection. Older reports of infection by cats, dogs and armadillos, and occasionally by birds, fish, dolphins, squirrels and invertebrates, have usually been ascribed to *S. schenckii*. This species is also found in mining wood, rose thorns and *Sphagnum* moss, regarded as the common source of outbreaks by this species [24, 31, 32].

In Asia, *S. globosa* is prevalent, *S. schenckii* very rare and *S. brasiliensis* completely absent. Focus areas of *S. globosa* are found in a belt from India diagonally over China to Japan (Fig. 5). Nearly all cases are human. Only one feline case of sporotrichosis by *S. globosa* is known, which was encountered in Japan. In our haplotype network involving 281 strains (Fig. 4), *S. schenckii* was the most variable species, primarily occurring outside Asia. It is regarded as the ancestral species of the nearly clonal taxa *S. brasiliensis* and *S. globosa* [7]. This hypothesis matches with our data, where recombination was detected in *S. schenckii* but not in the other species, and *S. schenckii* was by far the most variable species. The Asian strains of this species mainly came from a zoonosis in cats in Malaysia [33]. Both *S. brasiliensis* and *S. globosa* are derived from an area with increased variability in the haplotype network, but several median vectors are necessary to connect the Chinese *S. schenckii* with *S. globosa* strains from the same region. The ancestral species has mixed sources of infection, whereas the segregants differ widely in their modes of transmission. Rodrigues *et al.* [6] also noted that *S. brasiliensis* has highly structured populations suggesting local cat-vectors of dispersal, whereas *S. globosa* populations showed global occurrence [7], possibly matching with airborne dispersal with plant dust. As opportunists, *Sporothrix* species have a wide sloppy fitness space that enables them to reside in non-optimal habitats and that may lead to punctuated host shifts [34]. *S. brasiliensis* was shown to have accessory small chromosomes presumably linked to virulence, and suggesting a lifestyle that is able to accommodate rapid environmental changes with transient windows of opportunity [35]. The low diversity of *S. brasiliensis*, as well as of *S. globosa*, suggests a recent divergence with a founder effect of low variability compared to the variable ancestral species *S. schenckii*.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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