Phylogeny and evolutionary patterns in Sporothrix spanning more than 14,000 human and animal case reports

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Key words
epidemiology
historical biogeography
phylogeny
saprosonis
Sporothrix
sporotrichosis
transmission routes
yeast conversion
zoososis

Abstract Pathology to vertebrate hosts has emerged repeatedly in the order Ophiostomatales. Occasional infections have been observed in Sporothrix mexicana at a low level of virulence, while the main pathogenic species cluster in a derived clade around S. schenckii s.str. In this paper, phylogeny and epidemiology of the members of this clade were investigated for 99 clinical and 36 environmental strains using four genetic loci, viz. rDNA ITS and partial CAL, TEF1, and TEF3; data are compared with amplified fragment length polymorphism (AFLP) genotyping. The four main species of the pathogenic clade were recognised. The species proved to show high degrees of endemicity, which enabled interpretation of literature data where live material or genetic information is lacking. The clade of four species comprised nine subclusters, which often had limited geographic distribution and were separate from each other in all partitions, suggesting low degrees of interbreeding between populations. In contrast, S. globosa exhibited consistent global distribution of identical AFLP types, suggesting another type of dispersal. Sporothrix brasiliensis is known to be involved in an expanding zoosonosis and transmitted by cats, whereas S. globosa infections originated from putrid plant material, causing a saprosonis. Sporothrix schenckii s.str., the most variable species within the clade, also had a plant origin, with ecological similarities to that of S. globosa. A hypothesis was put forward that highly specific conditions in the plant material are required to promote the growth of Sporothrix. Fermented, self-heated plant debris may stimulate the thermodependent yeast-like invasive form of the fungus, which facilitates repeated infection of mammals.

INTRODUCTION

Sporotrichosis is a subcutaneous or cutaneous infection caused by traumatic inoculation of contaminated materials carrying inocula of Sporothrix species. Classically, the infection is known as rose ‘gardener’s disease’ (Engle et al. 2007) or ‘reed toxin’ (Song et al. 2013), as plants are often the source of the disease. The infection was first reported in 1898 in the USA by Benjamin R. Schenck (Schenck 1898). A second, similar case was described from Chicago two years thereafter (Hektoen & Perkins 1900) which led to the description of the pathogen as Sporothrix schenckii. During the century that followed (for a review, see Travassos & Lloyd 1980), the etiologic agent was supposed to be a single species that displayed a large diversity of virulence (de Lima et al. 2003), clinical features and routes of infection. Marimón et al. (2006, 2007), however, using molecular phylogenetic analyses showed that several sibling species were concerned. This was later confirmed by others applying additional gene regions (Criseo & Romeo 2010, Madrid et al. 2010, Rodrigues et al. 2014a, d). In retrospect, main groups recognized with multi-locus sequence data proved to correspond with phenotypic characters. Nowadays, the Sporothrix schenckii s.l. clade contains the clinically relevant species S. brasiliensis, S. globosa, and S. luriei in addition to S. schenckii sensu stricto (s.str.), while S. mexicana takes a remote phylogenetic position in the Ophiostoma-Sporothrix complex which is nested within the order Ophiostomatales (de Beer et al. 2003, de Beer & Wingfield 2013, Zhou et al. 2013).
| Current/obsolete name | Strain | Country | Source | Genbank accession no. |
|----------------------|--------|---------|--------|----------------------|
| **S. brasiliensis**  | CBS 120339(T) | Brazil | Human | – | Monitor 1784 |
|                     | CBS 130106 | Brazil | Human | – | Monitor 204 |
|                     | CBS 130107 | Brazil | Human | – | Monitor 205 |
|                     | CBS 130109 | Brazil | Human | – | Monitor 138 |
|                     | CBS 130110 | Brazil | Human | – | Monitor 152 |
|                     | CBS 132985 | Brazil | Cat | – | Monitor 185 |
|                     | CBS 132987 | Brazil | Human | – | Monitor 194 |
|                     | CBS 132988 | Brazil | Human | – | Monitor 153 |
|                     | CBS 132989 | Brazil | Cat | – | Monitor 195 |
|                     | CBS 132990 | Brazil | Cat | KP101427 | Monitor 154 |
|                     | CBS 132991 | Brazil | Human | – | Monitor 155 |
|                     | CBS 132992 | Brazil | Human | – | Monitor 206 |
|                     | CBS 132993 | Brazil | Human | – | Monitor 186 |
|                     | CBS 132994 | Brazil | Dog | – | Monitor 156 |
|                     | CBS 132995 | Brazil | Cat | – | Monitor 157 |
|                     | CBS 132996 | Brazil | Cat | – | Monitor 158 |
|                     | CBS 132997 | Brazil | Cat | – | Monitor 139 |
|                     | CBS 132998 | Brazil | Cat | KP101790 | Monitor 159 |
|                     | CBS 133000 | Brazil | Cat | – | Monitor 187 |
|                     | CBS 133010 | Brazil | Cat | – | Monitor 140 |
|                     | CBS 133015 | Brazil | Cat | – | Monitor 145 |
|                     | CBS 133017 | Brazil | Cat | – | Monitor 149 |
|                     | CBS 133001 | Brazil | Cat | KP101705 | Monitor 141 |
|                     | CBS 133002 | Brazil | Cat | – | Monitor 142 |
|                     | CBS 133003 | Brazil | Cat | – | Monitor 188 |
|                     | CBS 133004 | Brazil | Dog | – | Monitor 189 |
|                     | CBS 133006 | Brazil | Cat | – | Monitor 143 |
|                     | CBS 133007 | Brazil | Cat | – | Monitor 190 |
|                     | CBS 133008 | Brazil | Cat | – | Monitor 144 |
|                     | CBS 133009 | Brazil | Cat | – | Monitor 191 |
|                     | CBS 133011 | Brazil | Cat | – | Monitor 146 |
|                     | CBS 133012 | Brazil | Cat | – | Monitor 147 |
|                     | CBS 133013 | Brazil | Cat | – | Monitor 192 |
|                     | CBS 133014 | Brazil | Cat | – | Monitor 148 |
|                     | CBS 133016 | Brazil | Cat | – | Monitor 193 |
|                     | CBS 133019 | Brazil | Cat | – | Monitor 196 |
|                     | CBS 133021 | Brazil | Cat | – | Monitor 151 |
| **S. globosa**      | CBS 120340(T) | Spain | Human | KP17084 | Monitor 165 |
|                     | CBS 129717 | China | Human | – | Monitor 166 |
|                     | CBS 129718 | China | Human | – | Monitor 167 |
|                     | CBS 129719 | China | Human | KP17085 | Monitor 168 |
|                     | CBS 129720 | China | Human | KP1463 | Monitor 169 |
|                     | CBS 129721 | China | Human | – | Monitor 170 |
|                     | CBS 129722 | China | Human | – | Monitor 183 |
|                     | CBS 129723 | China | Human | – | Monitor 171 |
|                     | CBS 129724 | China | Human | – | Monitor 172 |
|                     | CBS 129725 | China | Human | – | Monitor 173 |
|                     | CBS 130104 | Spain | Human | – | Monitor 174 |
|                     | CBS 130105 | Spain | Human | – | Monitor 175 |
|                     | CBS 130115 | Spain | Human | – | Monitor 176 |
|                     | CBS 130116 | Spain | Human | – | Monitor 177 |
|                     | CBS 130117 | Japan | Human | – | Monitor 178 |
|                     | CBS 132923 | Brazil | Human | – | Monitor 179 |
|                     | CBS 132924 | Brazil | Human | KP17083 | Monitor 180 |
|                     | CBS 132925 | Brazil | Human | – | Monitor 203 |
|                     | CBS 292.55 | UK | Human | KP17086 | Monitor 181 |
|                     | CBS 340.35 | Japan | Human | – | Monitor 182 |
| **S. luriei**       | CBS 937.72(T) | South Africa | Human | AB128012 | Monitor 207 |
| **S. mexicana**     | CBS 120341(T) | Mexico | Soil, rose tree | KP17072 | Monitor 230 |
|                     | CBS 120342 | Mexico | Carnation | KP17073 | Monitor 231 |
|                     | CBS 132927 | Brazil | Human | – | Monitor 232 |
|                     | CBS 132928 | Brazil | Human | – | Monitor 234 |
|                     | CBS 133192 | Italy | Dog | KP17075 | Monitor 233 |
| **S. schenckii**    | CBS 125601 | Colombia | Human | – | Monitor 120 |
|                     | CBS 117440 | South Africa | Human | KP17098 | Monitor 114 |
|                     | CBS 117442 | South Africa | Human | – | Monitor 115 |
| **S. sp. / S. schenckii** | CBS 115670 | South Africa | NK | – | Monitor 229 |
| **S. sp.**          | CBS 130101 | Peru | Human | KP17095 | Monitor 197 |
|                     | CBS 130103 | Argentina | Human | – | Monitor 117 |
|                     | CBS 130111 | Colombia | Human | – | Monitor 123 |
|                     | CBS 130112 | Peru | Human | KP17096 | Monitor 132 |
|                     | CBS 130114 | Peru | Human | – | Monitor 133 |
|                     | CBS 130097 | Bolivia | Human | – | Monitor 137 |
|                     | CBS 130098 | Peru | Human | KP17091 | Monitor 121 |
|                     | CBS 130099 | Peru | Human | KP17092 | Monitor 122 |
|                     | CBS 132926 | Brazil | Human | – | Monitor 202 |
|                     | CBS 132961 | Brazil | Cat | – | Monitor 124 |
| Current/obsolete name | Strain | Country | Source | Genbank accession no. |
|----------------------|--------|---------|--------|----------------------|
| **S. abietinum**     | CBS 125.89 | Mexico | Abies vejarii | AF484453 – – – |
| **S. africanum**     | CBS 116566 | South Africa | Protea caffra | DQ316200 – – – |
| **S. auroae**        | CBS 118837 | South Africa | Pinus radiata | DQ396796 – – – |
| **S. brunneoviolacea** | CBS 793.73 | Germany | Meadow soil | KP017069 KP017106 KP017061 |
| **S. brunneoviolacea**/ **S. inflata** | CBS 101570 | USA | Endophyte in Vitis vinifera | KP017068 KP017101 KP017057 |
| **S. dentifundum**   | CBS 115790 | South Africa | Protea caffra | KP017071 |
| **S. dimorphospora** | CBS 125439 | USA | Soil | KP017080 |
| **S. dimorphospora** | CBS 125440 | Spanish | Soil | KP017081 |
| **S. foliolum**      | CBS 125454 | The Netherlands | Soil | FN546957 – – – |
| **S. fusiforme**     | CBS 112912 | Azerbaijan | Populus nigra | AY280481 – – – |
| **S. gemellus**      | CBS 121959(T) | UK | Industrial strain | KP017053 – – – |
| **S. inflata**       | CBS 239.68(T) | Germany | Soil, wheat field | KP017054 |
| **S. laurifolia**    | CBS 794.73 | Sweden | Humus in Picea forest | KP017053 |
| **S. lignivora**     | CBS 124560 | The Netherlands | Protea caffra | KP017054 |
| **S. lundatum**      | CBS 119147 | South Africa | Eucalyptus wood soil | KP017064 |
| **S. palida**        | CBS 121959(T) | South Africa | Taranetum sp. from Protea caffra | KP017066 |
| **S. phasma**        | CBS 125.89 | Mexico | Abies vejarii | KP017071 |
| **S. proteus**       | CBS 118837 | South Africa | Pinus radiata log | KC113234 KP017043 KP017046 |
| **S. scabridum**     | CBS 541.84 | Chile | Soil | KP017070 |
| **S. sp.**/ **S. curvicolla** | CBS 154.94 | NK | NK | KP017071 |
| **S. setigerum**     | CBS 115790 | South Africa | Soil | KP017071 |
| **S. sp.**/ **S. inflata** | CBS 156.72 | The Netherlands | Eucalyptus wood pole | KP017072 |
| **S. simplodes**     | CBS 119147 | South Africa | Eucalyptus wood pole | KP017064 |
| **S. splendens**     | CBS 131.56(T) | Japan | Stemnotis fusca | KP017054 |
| **S. stenoceras**    | CBS 131.56(T) | Japan | Stemnotis fusca | KP017054 |
| **S. tennesseensis** | CBS 182.63 | The Netherlands | Soil | KC113233 |
| **S. thomsoniae**    | CBS 201.53 | South Africa | Decaying grass | KP017054 |
| **S. thomsoniae**    | CBS 201.53 | South Africa | Decaying grass | KP017054 |
| **S. variicolor**    | CBS 302.73(T) | UK | Soil | KP017078 |
| **S. variolorum**    | CBS 252.95 | NK | NK | KP017071 |
| **S. variolorum**    | CBS 629.95 | NK | NK | KP017071 |
| **S. variolorum**    | CBS 629.95 | NK | NK | KP017071 |
| **S. variolorum**    | CBS 629.95 | NK | NK | KP017071 |
| **S. vulcanorum**    | CBS 115869 | South Africa | Protea repens | DQ821569 KP017047 KP017048 KP017049 |
| **S. vultuosus**     | CBS 115869 | South Africa | Protea repens | DQ821569 KP017047 KP017048 KP017049 |
| **S. xenochilus**    | CBS 115869 | South Africa | Protea repens | DQ821569 KP017047 KP017048 KP017049 |
Sporothrix four loci (LSU, ITS, with main ecological trends, i.e. Traditionally, our understanding of the evolutionary history and ecological trends and were phylogenetically ambiguous. However, common features used to recognize species, such as single-celled conidia disposed on clusters of denticles, are known to overlap among clinical and environmental Sporothrix species. As a result, cryptic entities were long-time overlooked throughout the taxonomic history of this genus. Today, multi-locus sequencing provides a more reliable classification and enables in-depth studies of distribution and ecology. When a species described with classical parameters is subdivided into a series of molecular siblings, and material for re-identification is not available, the older literature about this

### Table 1 (cont.)

| Current/obsolete name | Strain | Country | Source | ITS Genbank accession no. | CAL Genbank accession no. | TEF1 Genbank accession no. | TEF3 Genbank accession no. |
|----------------------|--------|---------|--------|---------------------------|---------------------------|---------------------------|---------------------------|
| Ceratocystis minuta  | RJ705  | Poland  | Picea abies | EU913697 | – | – | – |
| O. ainoae            | CMW 1903 | Norway | Picea abies | HMO31495 | – | – | – |
| O. angusticollis     | CBS 186.86 | USA | Pinus banksiana | AYS24383 | – | – | – |
| O. arduennense       | MUCL 44666 | Belgium | Fagus sylvatica | AYS73214 | – | – | – |
| O. basillicaporum    | MUCL 45378 | Belgium | Fagus sylvatica | AYS73258 | – | – | – |
| O. bicolor           | CBS 492.77 | USA | Gallery of Ips sp. in Picea sp. | DQ269804 | – | – | – |
| O. bragantium        | CBS 430.92 | Brazil | Soil | FNS46964 | – | – | – |
| O. breviusculum      | CBS 474.91(T) | Brazil | Soil | FNS46965 | – | – | – |
| O. canum             | CBS 133.51 | Sweden | Pinus sylvestris | HMO31489 | – | – | – |
| O. cationianum       | C1084 | Italy | Pyrus | AF198243 | – | – | – |
| O. conicola          | CBS 127.89 | Mexico | Cone with Conophthorus cervicoides | AYS24384 | – | – | – |
| O. coronatum         | CBS 497.77 | NK | NK | AYS24385 | – | – | – |
| O. denticillatum     | CMW 29493 | Norway | Scolytus ratzburgi on Betula sp. | FJ804490 | – | – | – |
| O. fasciatus         | UM 56 | Canada | Pseudeotsuga menziesii | EU913720 | – | – | – |
| O. flexuosum         | CBS 208.83 | Norway | Picea abies | AYS24387 | – | – | – |
| O. floccosum         | CBS 799.73 | Sweden | Soil | AF198231 | – | – | – |
| O. fumecum           | CMW 26813 | South Africa | Eucalyptus citriana | HMO51412 | – | – | – |
| O. fuscum            | CMW 23196 | Finland | Pityogenes chalcographus on Picea abies | HMO31504 | – | – | – |
| O. fuscum            | CMW 7075 | NK | NK | AYS546704 | – | – | – |
| O. japonicum         | YCC-099 | NK | NK | GU134169 | – | – | – |
| O. karelicum         | CMW 23099 | Finland | Scolytus ratzburgi on Betula pendula | EU443762 | – | – | – |
| O. kryptum           | DAOM229701 | NK | NK | AY304436 | – | – | – |
| O. minus             | AU84.34 | Canada | Lodgepole pine lumber | AF234534 | – | – | – |
| O. montium           | CMW13221 | NK | NK | AYS546711 | – | – | – |
| O. multilannulatum   | MUC19062 | NK | NK | AYS34512 | – | – | – |
| O. nitrocarponum     | ATCC22291 | USA | Dendroctonus sp. | AF84474 | – | – | – |
| O. nikkense          | YCC-430 | Japan | NK | AY506674 | – | – | – |
| O. novoluni          | C510 | USA | Ulmus sp. | AF198236 | – | – | – |
| O. piceae            | CBS108.21(T) | Germany | NK | AF198226 | – | – | – |
| O. pilleferum        | CBS 129.32 | The Netherlands | Scots pine | AF221070 | – | – | – |
| O. pluriannulatum    | MUC18372 | NK | NK | AY934517 | – | – | – |
| O. quercus           | CMW2467 | France | Quercus sp. | AY466626 | – | – | – |
| O. rostrocoronatum   | CBS 434.77(T) | USA | Woodpulp | AY194509 | – | – | – |
| O. saponiodorum      | CMW29497 | Finland | Ips typographus on Picea abies | HM031507 | – | – | – |
| O. sequentum         | Ophi 1A | NK | NK | AY393519 | – | – | – |
| O. selotessum        | AU160-38 | NK | NK | AY329029 | – | – | – |
| O. subannulatum      | CBS 188.86 | USA | Pinus | AY393522 | – | – | – |
| O. tanipionis        | CMW23266 | Finland | Hylastes brunneus on Pinus sylvestris | HMO31493 | – | – | – |
| O. tenellum          | CBS 189.86 | USA | Pinus banksiana | AY393523 | – | – | – |
| O. tetrupii          | CBS 428.94 | Austria | Breeding system of Tetropium sp., Picea abies | AY393524 | – | – | – |
| O. triangulosporum   | DSMZ4934 | NK | NK | AYS934525 | – | – | – |
| Pesotum australiae   | CMW6006 | Australia | Acacia mearnsii | EF408603 | – | – | – |
| Pesotum cupulatum    | C1194 | USA | Pseudotsuga | AY938230 | – | – | – |

O = Ophiostoma, S = Sporothrix, NK = Not known, T = type culture.
species becomes uninterpretable. This is a rather general consequence of drastic changes of taxonomic criteria. One of the first examples of such a new starting point was the case of Trichosporon, where the commonly used, physiologically defined species T. beigeli became obsolete after a molecular revision of the genus (Guého et al. 1992). In the case of sporotrichosis, abandoning existing literature would be highly inappropriate. Over the past century, large amounts of information have been collected on a worldwide scale. Barros et al. (2011) listed several hundreds of cases published during the last decade alone. In the current study 90 publications with case reports, case series and outbreaks, with a total of over 4,000 patients involved, are analysed. An early sporotrichosis epidemic that included over 200 cases during a 6-year period was reported from France by de Beurmann & Gougerot (1912). In the 1940s, the largest outbreak thus far took place in a gold mine in South Africa, involving over 3,000 miners developing the disease after having been infected via untreated wood contaminated by Sporothrix (Helm & Berman 1947). Other large epidemics were those related to plant materials like Sphagnum moss used in a nursery in the USA (Centers for Disease Control and Prevention 1988), rotten hay in Australia (Feeeny et al. 2007), and reed and cornstalks in China (Wang & Sun 1982, Li et al. 1995, Song et al. 2013), with thousands of patients involved. The most recent outbreak of sporotrichosis was reported from south-east Brazil and was found to be caused by S. brasiliensis (Rodrigues et al. 2013b, 2014d). To understand the mechanisms behind the emergence of epidemics, outbreak data should be compared to historical information on Sporothrix infections. Re-interpretation of historical data in the light of modern molecular phylogeny is therefore compulsory.

The aim of the present study is to introduce a hypothetical system that enables to interpret and use at least part of the literature where sequence data are lacking. We collected pre-molecular papers, which contained interpretable case reports and geographical information. Available strains from each of these regions were sequenced and identified, and these data were compared to published materials. Frequencies of each of the identified species were compared with the assumption that their distributions in each region had largely remained unaltered. Additionally, clinical and environmental isolates deposited during the last century in the CBS culture collection (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands) were re-identified with molecular techniques, which enables phylogenetic analysis of the human-pathogenic Sporothrix species in relation to other fungi that belong to the order Ophiostomatales.

**MATERIAL AND METHODS**

**Fungal strains**

A total of 205 strains were analysed, of which 109 were of clinical origin and 96 were environmental; all were maintained under the name ‘Sporothrix’ in the reference collection of the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands. Of these, 135 isolates comprising clinical (n = 99) and environmental (n = 36) strains belonged to the main pathogenic Sporothrix clade. Data on geographic origins and sources of isolation were collected and are listed in Table 1. All available type strains were included. Stock cultures were maintained on slants of 2% malt extract agar (MEA) at 24 °C. Data on Ophiostoma species that are included in the current study were collected from GenBank, the accession numbers of used sequences are listed in Table 1.

**DNA extraction**

DNA was extracted following the Quick CTAB protocol. 1–10 mm³ fungal material was transferred to 2 mL screw-capped tubes filled with 490 μL CTAB-buffer 2× and 6–10 acid-washed glass beads. Ten μL proteinase K [10 mg/mL] (Sigma-Aldrich, St Louis, MO, USA) were added and mixed thoroughly for 10 min using a MoBio vortex (MoBio, Carlsbad, CA, USA). After that, 500 μL chloroform : isoamylalcohol (24 : 1) was added and shaken for 2 min followed by incubation for 60 min at 60 °C. Tubes were centrifuged for 10 min at 14 000 × g. The supernatant was collected in a new tube. To ~400 μL DNA sample ~270 μL of ice-cold iso-propanol (Sigma) was added and centrifuged again at 14 000 × g for 10 min and the upper layer was dissolved in 1 mL ice-cold ethanol 70 %. Tubes were centrifuged again at 14 000 × g for 2 min, air-dried and re-suspended in 50 μL TE-buffer (pH 8.0). Quality of genomic DNA was verified by running 2 μL DNA sample in a 0.8 % agarose gel. DNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA). Samples were stored at −20 °C until further use.

**DNA amplification and sequencing**

Four gene regions were amplified for inclusion in the multilocus sequence data analysis, i.e. rDNA internal transcribed spacer (ITS), and the partial genes calmodulin (CAL), translation elongation factor-1 (TEF1) and -3 (TEF3). Primers used for amplification and sequencing of CAL were CL1 and CL2a (D’O donnell et al. 2000). TEF primers were developed by B. Stielow (unpubl. data). PCR reactions were performed in a mixture containing 1.25 μL 10 x PCR buffer, 6.7 μL ddH₂O, 1 μL dNTP mix (2.5 mM), 0.25 μL of each primer (10 pmol), 0.06 μL Taq DNA polymerase (5 U/μL), 0.625 μL DMSO (Sigma), and 2.5 μL template DNA (100 ng/μL). PCR reactions were performed in a Hybaid Touchdown PCR machine (Hybaid, Middlesex, UK); the used annealing temperatures are listed in Table 2. PCR products were visualized by electrophoresis on a 1 % (w/v) agarose gel. Amplicons were purified using exoSAP-IT (Affymetrix, Santa Clara, CA, USA). The ABI Prism BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA) was applied according to the instructions provided by the manufacturer. Reactions were purified by using Sephadex G-50 ultrafine (GE Healthcare Bio-Sciences, Uppsala, Sweden) and sequencing was performed by using an ABI 3730xL automatic sequencer (Applied Biosystems).

**Phylogenetic analyses**

Consensus sequences were assembled by using SeqMan package of Lasergene software v. 8.1 (DNASTar, Madison, WI, USA) and alignments were made in BioEdit v. 7.0.5.2 software (Hall 1999). The genetically diverse ITS sequences were aligned by using MUSCLE program (www.ebi.ac.uk/Tools/msa/muscle), while sequences of the CAL, TEF1 and TEF3 sequences were aligned by using the server version of the MAFFT program v. 7.0 (www.ebi.ac.uk/Tools/msa/mafft/) (Katoh & Standley 2013). Retrieved alignments were manually checked to avoid mis-paired bases. All sequences determined in this study were deposited in GenBank and the accession numbers are listed in Table 1.

The best-fit evolutionary model was determined by application of ModelTest v. 0.1.1. Bayesian analysis was performed with
MrBayes v. 3.1.2 (Ronquist et al. 2012). Four MCMC chains were run simultaneously for $1 \times 10^7$ generations. Bootstrapped Maximum Likelihood analysis was performed by using RAxML-VI-HPC v. 7.0.3 (Stamatakis et al. 2008) as implemented on the Cipres portal (www.phylo.org/) with non-parametric bootstrapping using 1 000 replicates.

Amplified fragment length polymorphism genotyping

The Sporothrix isolates were subjected to amplified fragment length polymorphism (APL) genotyping using a previously described procedure (Chowdhary et al. 2013). However, for the amplification of the DNA fragments the selective cytosine residue of the EcoRI primer was replaced by an adenine residue (5′-Flu-GACTGCTACCAATTCAA-3′), while the Msel primer remained the same with one selective residue (5′-GATGAGTCCTGACTAAGG-3′). After amplification, amplicons were 50× diluted using ddH2O; 1 µL of the diluted amplicon was then added to a mixture of 8.9 µL ddH2O and 0.1 µL LIZ600 (Applied Biosystems) followed by a heating step for 1 min at 96 °C followed by cooling down to 4 °C. Fragment analysis was carried out using an ABI3500xL Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instructions. Raw data were then inspected visually after importation into BioNumerics v. 6.6 (Applied Maths, St. Martens-Latem, Belgium) and analysed by UPGMA clustering using the Pearson correlation coefficient.

Meta-analysis

We analysed the existing medical and veterinary literature on human and veterinary cases of sporotrichosis from the first publication in 1899 till present. A search was initiated using the PubMed database for which the MeSH terms ‘Sporothrix’ and ‘sporotrichosis’, yielded in total 705 results. Reports on treatment, immunology, antifungals and virulence factors, as well as book chapters and reports that also include other diseases were neglected. The focus was then placed on cases and case series from 1940 up to now; case reports with insufficient data were discarded. Over 14 000 cases published in 90 reports were collected; the selection covered countries all over the world. The search also included ~2 827 cases published in Chinese language. Numbers are approximate because some cases had been used in repeated publications; we tried to exclude duplicates when individual cases were numbered. Cases were listed geographically on the basis of identifiable entities, such as Europe, China, or Brazil. The statistical method for Table 5 is the $\chi^2$-test.

RESULTS

Judging from literature data, the most endemic regions are China (3 299 cases), South Africa (3 154 cases), and Brazil (5 814 cases). Less frequently, sporotrichosis occurs in Japan, Australia, India, and the remaining Americas outside the eastern part of South America. The disease is less prevalent in Europe, except for the unique outbreak involving 200 cases occurring in France over a period of six years at the beginning of last century (Beurmann & Gougerot 1912).

Outside the recent Brazilian epidemic, nearly all cases and case series were published to be caused by Sporothrix schenckii s.l.; the subdivision of this taxon into four molecular siblings occurred only in 2006 (Marimón et al. 2006). We aimed to recognise the individual siblings retrospectively by comparing contemporary distributions of molecular species with historical biogeography abstracted from published data. A comparison of the number of published cases (Fig. 1; grey circles) and the number of sequenced strains in the same area (Fig. 1; coloured) is given. Distributions of molecular species as percentages of the total numbers of sequenced cases in the same defined area are given in Table 3 and Fig. 1. In most of the defined areas a single molecular species is preponderant (> 80 %). Main calculated endemic areas with their prevalent species are as follows: Asia S. globosa (99.3 %), Australia and southern Africa S. schenckii (94 %), south-eastern South America S. brasiliensis (88 %), western part of South America and Central and North America S. schenckii (89 %). The percentages indicate statistical probabilities that the prevalent endemic species was concerned in historical publications without sequence data. In European countries the low number of cases hinders to ascertain predominant species.

In order to have a complete overview of molecular species occurring in humans and animals and their potential routes of transmission, we sequenced all strains deposited in the CBS collection over the last hundred years under the name ‘Sporothrix’. Using standard primers, sequencing efficiency proved to differ slightly between species. Success rates of sequencing for

![Fig. 1 Geographic distribution of sporotrichosis caused by S. brasiliensis, S. schenckii, and S. globosa according to case reports published over 70 years, compared with sequenced isolates and with expression of statistical probabilities that the prevalent endemic species was concerned in historical publications without sequence data. Samples were categorised as sequenced and non-sequenced specimens. The sizes of circumferences are roughly proportional to the numbers of cases / strains included. Numbers reported within the pies denote the number of strains examined. Main endemic areas indicated by dotted lines.]
each gene are listed in Table 2. In clinical strains, the largest percentages of poor sequences were encountered in TEF1 and ITS in S. schenckii and S. brasiliensis, viz. 20.9 % and 18.2 %, respectively. Strains of environmental species outside the S. schenckii clade generally generated good results with TEF1 and TEF3, but a somewhat higher percentages of failure were obtained with ITS and CAL (Table 2).

ITS sequences could be aligned confidently over the entire order Ophiostomatales; a general tree is presented in Fig. 2, using Ceratocystis minuta RJ705 as outgroup. The complete alignment included 101 sequences for ITS, 37 generated in this study and 64 retrieved from GenBank. ITS sequences produced an 807 bp-long alignment (327 for ITS1, 191 for ITS2), and included 334 invariant characters, 246 variable parsimony-informative sites (34 %), and 72 singleton. Several highly confident clades could be recognized, one of which (Fig. 2) consisted of Ophiostoma species associated with bark beetles (bootstrap support 86 %). Outside this clade several more bark beetle-associated species were noted, at significant distance and separated by Ophiostoma species with other habitats. Several intermediary lineages were found in soil and in wood-inhabiting infructescences, intermingled with occasional wood-inhabiting taxa (Fig. 2). The ultimate clade contained four potentially human-pathogenic Sporothrix species. Sporothrix stenoceras represented a separate clade, with 9 % statistical support and distinct from the clades containing pathogenic species (Marimón Clades I‒III and VI) and a saprophytic clade (Marimón Clade IV) (Marimón et al. 2006).

A multilocus tree (Fig. 3) excluding the major clade of Ophiostoma was based on 135 selected isolates including 99 clinical strains and 36 representative environmental strains of species having all three genes for multilocus studies. Lengths of generated amplicons were 792 bp, 526 bp, and 255 bp for CAL, TEF1, and TEF3, respectively. Of the 1 573 nucleotides sequenced, 942 (59.9 %) were constant, 464 (29.5 %) were parsimony-informative, and 146 (9.3 %) were variably parsimony non-informative sites. Over the entire dataset, the lowest number of variable sites was 92 (17.5 %) in the TEF1 fragment, and the highest was 432 (54.5 %) in the CAL fragment; extended data per gene are provided in Table 4.

Phylogenetic trees were analysed using independent and combined datasets. All 135 sequences generated in this study, except for one CAL which was retrieved from GenBank. Gene trees of CAL, TEF1, and TEF3 presented a higher discriminatory power and similar topologies to the ITS tree. Sequences could be aligned confidently over the entire dataset. The combined tree based on CAL, TEF1, and TEF3 data of 135 strains is given in Fig. 3, basically comprising the upper part (Sporothrix s.str.) of the ITS tree (Fig. 2) and using Sporothrix foliorum, CBS 326.37 as outgroup. The best-fit model of evolution was estimated to be HKY+GAMMA. Fig. 3 shows the majority-rule consensus trees, deduced by Bayesian inferences sampled by MCMC and were selected to demonstrate the tree topology.

The combined tree of 135 isolates was subdivided into several main groups (Fig. 3), five of which were also observed in previous studies (Marimón et al. 2006, 2007, Madrid et al. 2009, Rodrigues et al. 2013b, 2014d). Each described species could be distinguished at high bootstrap values. Five subclades were discernible within S. schenckii, which almost entirely matched with AFLP groups A–E (below; Fig. 4) and were mostly geographically restricted (Fig. 5). CBS 130103 was the single strain from Argentina clustering in AFLP group B from South Africa with small deviations in AFLP profile (Fig. 4) as well as in sequence data (Fig. 3). In S. brasiliensis several small sets of strains had bootstrap support, but groups were too similar to allow meaningful distinction. Sporothrix globosa was homogeneous. Sporothrix mexicana was found to be nested in an environmental clade comprising saprobic species (Fig. 2; Marimón Clade IV).

AFLP data of 116 strains of the potentially human- and animal-pathogenic species (Pathogenic clade in Fig. 2) are listed in Fig. 4. At a cut-off level of 70 % 13 groups could be recognised. All strains of S. globosa clustered in a single group. Sporothrix brasiliensis consisted of three groups, and S. schenckii of five groups (AFLP A–E; Fig. 4), the members of which matched with the groups found with multilocus sequence data (Fig. 3). In AFLP, Sporothrix mexicana contained two groups that could not be seen in the combined tree, and the single available strain of S. luriei deviated from all remaining species. In each of the groups of S. brasiliensis and S. schenckii, strains were

| Continent / region | Country | Reported cases | Sequenced isolates | S. brasiliensis % | S. globosa % | S. schenckii % | S. mexicana % | S. luriei % |
|--------------------|---------|----------------|--------------------|------------------|-------------|---------------|--------------|-------------|
| Asia               | China   | 3299           | 121                | 121: 100 %       |             |               |              |             |
|                    | India   | 621            | 12                 | 12: 100 %        |             |               |              |             |
|                    | Japan   | 355            | 7                  | 6.86 %           | 1: 14 %     |               |              |             |
| Australia          | Australia | 144           | 10                 | 10: 100 %        |             |               |              |             |
| Africa             | South Africa | 3154        | 7                  | 6: 86 %          |             | 1             |              |             |
|                    | Mozambique | 1             | 1                  | 1: 100 %         |             |               |              |             |
| Western and southern parts of South America, Central and North America | Peru | 342 | 15 | 15: 100 % | | | |
|                    | Argentina | 3             |                    | 3: 100%          | | | |
|                    | Bolivia | 1             |                    | 1: 100%          | | | |
|                    | Mexico | 157 | 28 | 25: 89 % | 2: 7 % | | |
|                    | Venezuela | 133 | 13 | 13: 100 % | | | |
|                    | Guatemala | 55 | 2 | 2: 100 % | | | |
|                    | Columbia | 60 | 6 | 2: 33 % | 4: 67 % | | |
|                    | USA | 287 | 23 | 3: 13 % | 20: 87 % | | |
| Eastern South America | Brazil | 5814 | 352 | 312: 88.4 % | 4: 1.1 % | 33: 9.3 % | 3: 0.8 % | |
| Europe             | 6       | 24             | 5: 21 %           | 2: 8 %           | | | |
| Total              | 625     | 312            | 168               | 137             | 7           | 1             | | |

Table 3 Estimated distributions of molecular species on the basis of percentages of sequenced strains compared to the total number of published cases in the respective area.

| Locus   | No. of bp sequenced | % variable sites | % parsimony-informative sites | % singleton sites |
|---------|---------------------|------------------|------------------------------|-----------------|
| CAL     | 792                 | 54.5             | 42.8                         | 11.7            |
| TEF1    | 526                 | 17.5             | 13.5                         | 4.0             |
| TEF3    | 255                 | 33.7             | 21.2                         | 12.5            |
| Combined genes | 1573             | 38.8             | 29.5                         | 9.28            |
Table 5 provides an overview of cases of sporotrichosis published in the world literature with an accent on case series and including the great majority of cases published to date. Sporotrichosis classically was reported to occur in temperate and subtropical climates with a relatively high humidity. From Table 5 it appears that this holds true for S. schenckii. Hyperendemic areas are Brazil, Peru, Uruguay, Venezuela, and South Africa. In the hyperendemic area of north-east China and Japan, where S. globosa is prevalent, the climate is relatively cold (p = 10^-6). In Asian countries, where S. globosa is prevalent, a preponderance of female patients is noted (p = 10^-4). In Australia and South Africa more males are involved, partly associated with outdoor work, such as was the case with the miner epidemic in Witwatersrand by S. schenckii. In American countries male : female ratios are variable; no significant deviation from an equal ratio was noted in S. brasiliensis (Table 5).

Globally the most common clinical form is lymphocutaneous (LC) sporotrichosis, but in China the fixed cutaneous form is prevalent (p = 0.037). The mode of transmission of sporotrichosis often remains unclear. Trauma was mentioned in many cases, but was difficult to define in some cases because small traumata are easily neglected by patients. In some cases absence of trauma was explicitly mentioned (Table 5) and thus subcutaneous inoculation does not need to be apparent for the onset of sporotrichosis. Contact with decaying plant material was frequently noted in S. globosa and S. schenckii.
Many of the reports listed in Table 5 were case series from various sources (Table 5). The total number of outbreaks during a fixed period. These outbreaks comprised >14,000 cases, while the number of reports of single cases is relatively low; this low number is only partially influenced by the fact that single cases with insufficient data were discarded from our meta-analysis. The outbreak-character of sporotrichosis is further demonstrated in Fig. 6, where case series during intervals of 5 years are listed. Several large outbreaks, such as by *S. brasiliensis* in south-east Brazil and *S. globosa* in Jilin, north-east China, are still ongoing. Fig. 6 does not list the case series of 200 infections in France during the years 1904–1911 (Beermann & Gougerot 1912).

DISCUSSION

For the present study, four gene regions were analysed: the *rDNA* ITS domain, the partial calmodulin (*CAL*) gene, and two regions in the translation elongation factor (*TEF1* and *TEF3*). Molecular taxonomy of *Sporothrix* is particularly based on *CAL* (Marimón et al. 2006), although ITS performs equally well in distinguishing the main species, as demonstrated by Zhou et al. (2013) and Rodrigues et al. (2014b, d) and confirmed with a larger dataset in the present study. The tree topology of three combined genes *CAL*, *TEF1*, and *TEF3* proved to be similar to that of the ITS tree. The most variable gene is *CAL* (variable sites 54.5%) followed by *TEF3* (33.7%) and *TEF1* (17.5%). A similar range of variability was found with the number of parsimony-informative sites (*CAL → TEF3 → TEF1*). Diagnostics with *CAL* is optimal because intraspecific variability is small compared to barcoding gaps between species, yielding a highly resolved phylogenetic tree (data not shown). The genes analysed tend to differ in PCR performance, although ITS may

**Fig. 2** (cont.)
Fig. 3 Phylogenetic relationship inferred from Bayesian statistics based on concatenated CAL, TEF1 and TEF3 sequences of 135 strains of Sporothrix species. Bootstrap and posterior probabilities values were added to respective branches (BI/ML/NJ). Branches with bootstrap support values higher than 80% are indicated in bold.
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Fig. 3 (cont.)

Fig. 4 Amplified fragment length polymorphism (AFLP) profiles of 122 strains of Sporothrix. Clustering of AFLP banding pattern of isolates of Sporothrix was done by UPGMA. Red vertical bars represents cut-off for distinction of clusters. Strains of S. mexicana and below are phylogenetically unrelated.
present a higher number of negatives than usual in fungi (Table 2) (Schoch et al. 2012).

The genus *Sporothrix* is embedded in the order *Ophiostomatales*. The core genus is *Ophiostoma*, which is classically known to comprise fungi that live in association with bark beetles. De Beer et al. (unpubl. data) delimited the two genera, maintaining nearly all arthropod-associated species in *Ophiostoma*. Ecologies of the 32 accepted *Sporothrix* species were quite diverse. Virulence to mammals is nearly exclusively found in a small group of species around *S. schenckii*. The ITS tree combining these groups demonstrate that phylogenetic distances are moderate and comply with classification in a single order; ITS was alignable with reasonable confidence over the entire dataset. The maximum ITS distance (measured by similarity in BioNumerics) from *S. brasiliensis* CBS 133019 to *S. foliorum* CBS 326.37 was 15%. The span over diversity within the clade with pathogenic species (*S. brasiliensis* CBS 133019 to *S. mexicana* CBS 120342) was 4.3% for ITS and 8% for three genes analysed. We randomly sequenced all strains deposited in the CBS collection over a century and found that only 3 isolates might represent undescribed species (Table 1). It is questionable whether *S. inflata* and *S. dimorphospora* are different species. Within the habitats analysed sampling has apparently been sufficient to cover extant biodiversity.

The occurrence of two pronounced types of ecology within the *Ophiostomatales*, viz. human pathogenicity and bark beetle

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**Fig. 5** Minimum spanning tree of AFLP data showing the relationships among 135 *Sporothrix* isolates, showing prevalent endemicum of subclusters. Each dot corresponds to a unique genotype.

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**Fig. 6** Timeline of epidemics and case series caused by three main pathogenic *Sporothrix* species since 1940. Vertical bars represent gross number of cases, vertical arrows denote case series and sapronoses or zoonoses.
association, is remarkable. In the ITS tree (Fig. 2) the species living inside bark beetle galleries constitute a well-supported clade, matching *Ophiostoma* (de Beer et al. 2003). Some of the species in Fig. 2 have been isolated from sapwood and a role of the bark beetle has then usually not been proven. Outside this clade explicit bark beetle-association is uncommon. Arthropod dispersal remains common in other clades, however, as exemplified by clades with species from mites in *Protea* in-fructescences. As the intermediate species in the phylogenetic trees (Fig. 2) exhibit other types of ecology, there is no obvious link between bark beetle-association and human pathogenicity. The remaining groups compose a polytomy from an unresolved backbone with bootstrap values below 80 % (Fig. 2). Comparing the clades over the entire tree, we observe a consistent decrease of bark beetle association outside *Ophiostoma*, concomitantly with an increasing vertebrate infectivity. Inside *Ophiostoma*, a single case of human infection in a leukaemic patient was described in *Ophiostoma piceae* (Bommer et al. 2009). Outside the pathogenic clade (Fig. 3), two cases were reported by S. *stenoceras* (Mariat et al. 1968), while *S. palida* infections are represented by sporadic cases related to impairment of the immune system, e.g. in transplant recipients (Morrison et al. 2013). In all these species, human pathology is highly exceptional (white crosses in Fig. 2), except for several *S. mexicana* cases (Dias et al. 2011, Rodrigues et al. 2013a) where human infection is relatively common, but mild. *Sporothrix luriei* is a very rare species, with a single proven case from South Africa (Ajello & Kaplan 1969) and two cases with unproved culture from Italy and India, respectively (Alberici et al. 1989, Padhye et al. 1992). The species is reported to be highly virulent (Fernández-Silva et al. 2012). The remaining species of the pathogenic clade (Guarro et al. 1999), *S. schenckii*, *S. globosa* and *S. brasiliensis* occur in epidemic proportions with several thousands of cases each. Virulence has been tested in animal inoculations (Amillaga-Moncrieff et al. 2009, Castro et al. 2013, Fernandes et al. 2013) using mice as model animal. *Sporothrix brasiliensis* presented more fungal burden, dissemination capacity and massive infiltration in infected tissues compared to *S. schenckii* and *S. globosa*. Highest virulence was observed in *S. brasiliensis* which correlates with high degrees of pathogenicity in felines (Rodrigues et al. 2013b) and humans (Silva-Vergara et al. 2012). A connection among disease severity, humoral response and protein secretion revealed a common immunogenic protein of 60 kDa recognised by antisera in all cat isolates.*Sporothrix* presents more genetic variability, but a significant difference seems likely. The absence of *S. globosa* from Africa and Australia then remains puzzling, but this perhaps can be explained by sampling effects. Notably, *S. globosa* infections are derived from plant debris (Wang & Sun 1982, Li et al. 1995, Song et al. 2013) and is classically known as ‘red toxin’ (Song et al. 2013). Cats have never been observed as sources of infection in endemic areas of *S. globosa*. Conversely, plants have never been observed as sources of infection by *S. brasiliensis*. In this respect, *S. schenckii* seems intermediate. Classically the infection is known as ‘rose gardener’s disease’, suggesting a plant source of infection and traumatic inoculation (Rodrigues et al. 2014d). In our dataset, CBS 132977 in group A originated from plant material in Mexico, while the remaining strains of that group were of clinical origin. In the literature a connection between *S. schenckii* and plants has been made many times. Dixon et al. (1991) described a sapronosis of 84 cases, studying 21 clinical isolates which proved to be identical to strains from *Sphagnum* moss by RFLP. A similar report was that of Hajieh et al. (1997) in an outbreak of sporotrichosis from hay in Australia, where *S. schenckii* was shown to be the etiologic agent by ITS sequencing. The large epidemic from South Africa in the forties of the previous century, with more than 3 000 cases, was proven to have untreated mining wood as source of infection, and disappeared after the wood had been impregnated with cresote (Helm & Berman 1947).
| Species  | Year | Country  | n  | Host | M/F | Climate     | Occupation                        | Probable transmission | Clinical form (D, F, LC, SYS, other) | Reference |
|----------|------|----------|----|------|-----|-------------|------------------------------------|------------------------|--------------------------------------|-----------|
|          |      |          |    |      |     | Warm, humid | Cat owner, veterinarian             | Cat                    | LC                                   | Larsson et al. 1989 |
|          |      |          |    |      |     | Warm, humid | Housewife, student                  | Cat                    | D, F, LC                              | Al-Tawfiq & Vicolis 1998 |
|          |      |          |    |      |     | Warm, humid | Farmer, teacher, student             | Cat                    | F, LC                                | Barros et al. 2004 |
|          |      |          |    |      |     | Warm, humid | Cat                                 | Cat                    | F, LC                                | da Rosa et al. 2005 |
|          |      |          |    |      |     | Warm, humid | Arthritis                           | Cat                    | D, F, LC, SYS                        | Appenheimer et al. 2006 |
|          |      |          |    |      |     | Warm, humid | Housewife                           | Cat                    | D, F, LC, SYS                        | Barros et al. 2008a |
|          |      |          |    |      |     | Warm, humid | Cat                                 | Cat                    | Schubach et al. 2005                |
|          |      |          |    |      |     | Warm, humid | Armadillo hunting                   | LC                     | Freitas et al. 2012                 |
|          |      |          |    |      |     | Warm, humid | Cat                                 | Cat                    | Silva et al. 2012                   |
|          |      |          |    |      |     | Warm, humid | Cat                                 | Cat                    | Freitas et al. 2013                 |
|          |      |          |    |      |     | Cool       | Worker in paper factory              | Decaying reed           | F, LC                                | Peereira et al. 2014 |
|          |      |          |    |      |     | Cool       | Horticulure, forest farming          | Cat                    | Wu 1986                              |
|          |      |          |    |      |     | Cool       | Farmer                              | Reed                   | Ran et al. 1999                      |
|          |      |          |    |      |     | Cool       | F, LC                               | F, LC                  | Yang et al. 2005                     |
|          |      |          |    |      |     | Cool       | F, LC                               | Gao et al. 2007         |
|          |      |          |    |      |     | Cool       | F, LC                               | F, LC                  | Zhang & Lin 2008                     |
|          |      |          |    |      |     | Cool       | F, LC                               | Fu et al. 2008          |
|          |      |          |    |      |     | Cool       | F, LC                               | Li et al. 2011           |
|          |      |          |    |      |     | Cool       | F, LC                               | Song et al. 2011        |
|          |      |          |    |      |     | Cool       | D, F, LC                            | D, F, LC               | Song et al. 2013                     |
|          |      |          |    |      |     | Cool       | F, LC                               | F, LC                  | Pulmonary                             |
|          |      |          |    |      |     | Cool       | Farmer                              | Cat                    | Padhye et al. 1992                   |
|          |      |          |    |      |     | Cool       | Humid                               | Cat                    | Khaitan et al. 1998                  |
|          |      |          |    |      |     | Cool       | Horticulture, forest farming         | Cat                    | Ghosh et al. 1999                    |
|          |      |          |    |      |     | Cool       | F, Cool                             | F, LC                  | Mehta et al. 2007                    |
|          |      |          |    |      |     | Cool       | F, Cool                             | F, LC                  | Aganjia et al. 2008                   |
|          |      |          |    |      |     | Cool       | Medical attendant                    | Cat                    | Yegneswaran et al. 2009              |
|          |      |          |    |      |     | Cool       | Plant                               | F, LC                  | Bhuta et al. 2011                    |
|          |      |          |    |      |     | Cool       | F                                  | F, LC                  | Tilak et al. 2012                     |
|          |      |          |    |      |     | Cool       | F, Cool                             | F, LC                  | Verma et al. 2012                     |
|          |      |          |    |      |     | Cool       | Farmer                              | Plant                  | Iloth et al. 1986                     |
|          |      |          |    |      |     | Cool       | F, LC                               | F, LC                  | Takenaka et al. 2009                 |
|          |      |          |    |      |     | Cool       | Vet student, cat owner               | Cat                    | Zamri-Saad et al. 1990              |
|          |      |          |    |      |     | Cool       | Farmer                              | Cat                    | Ng et al. 2012                        |
|          |      |          |    |      |     | Cool       | Unrestituted mine wood               | Cat                    | Newton et al. 2005                   |
|          |      |          |    |      |     | Cool       | Farmer                              | Wood                   | D                                    |
|          |      |          |    |      |     | Cool       | Sphagnum moss                       | F, LC                  | Helm & Berman 1947                   |
|          |      |          |    |      |     | Cool       | Sphagnum moss                       | F, LC                  | Visser & Holt 1997                   |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F, LC                  | Barros et al. 2010                   |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F, LC                  | Alessio et al. 1965                  |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F, LC                  | Thompson & Kaplan 1977               |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F, LC                  | Pulmonary                             |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F, LC                  | SY S                     |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F, LC                  | Dixon et al. 1991                    |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | F                      | Cooper et al. 1992                   |
|          |      |          |    |      |     | Warm, dry  | Sphagnum moss                       | LC                     | Haji et al. 1997                      |
From the above it is obvious that historical outbreak data are needed to understand the behaviour of individual Sporothrix species. Data of Feeney et al. (2007) and Yu et al. (2013) could be verified as *S. schenckii* and *S. globosa*, respectively by GenBank submissions, but in many cases neither molecular data nor strains were available for study. Given the geographic structuring of almost all *Sporothrix* populations, we used geographically defined sets of sequenced strains (Fig. 1) to deduce the most probable identity of historical strains in the same region. Ratios of numbers of strains sequenced per region, compared to the number of reported cases from the same region, are illustrated in Fig. 1. For example, all strains from China sequenced thus far, i.e., 112 strains from Yu et al. (2013) and Tan et al. (2013), plus nine from the present study, were identified as *S. globosa*. Thus, the historical probability in China to be *S. globosa* is 100%, while in the USA the strains are expected to be *S. schenckii* with a probability of 87%. Data are summarised in Table 3, where we took 80% as cut-off below which percentage historical data could not be interpreted. Summarising published cases since 1940 (Table 5), a remarkable phenomenon becomes apparent. Most of the published cases concerned case series or outbreaks, either with plant origins (sapronoses) or feline origins (zoonoses). Fig. 6 shows all cases since 1940 in a histogram accumulatively in 5-year intervals. The great majority of cases were part of an outbreak, the numbers of cases per series varying from 5 to 3,069. The smallest outbreak concerns patients being infected from the same heap of hay stored in an old house (Dooley et al. 1997). Although neglected by the present study, individual cases are uncommon, even in older literature when only few cases had been published. The oldest outbreak is that in France during the period 1906–1911, which started 8 yr after the first description of *Sporothrix* by Hektoen & Perkins in 1900. Since then, sporotrichosis has remained rare in Europe, and part of the etiologic agents may have been imported, which would explain the relatively high species diversity in this continent.

How can we explain this outbreak behaviour observed in all *Sporothrix* species irrespective of their different modes of transmission? The plant-borne species (*S. schenckii* and *S. globosa*) are found on decaying plant material or wood. In each plant-borne species, large differences are observed in type of plant material. In *S. schenckii*, for example, this was mining wood, rose thorns, and *Sphagnum* moss. In *Sporothrix* species with plant material as source of infection we have to assume that not the host plant species, but the condition of the plant material is significant. This condition has to be highly special. Notably, *Sphagnum* moss is used worldwide in large quantities, but only a few outbreaks of sporotrichosis have been described. As another example, decaying hay is ubiquitous material, and thus infections with regular intervals over time would be expected, but we consistently observe occasional infections from a common source, i.e., outbreaks. Therefore we hypothesise that *Sporothrix* species are not plant pathogens, but require particular conditions in decaying plant material, which are reached only occasionally. We postulate that a particular state of decay and fermentation of the plant material promotes excessive growth of *Sporothrix*. High temperature and humidity, associated with metabolic changes (induction of respiratory system) and oxidative stress due decay and fermentation may shift the morphology, favouring the invasive yeast growth form (Klein & Tebbets 2007). This hypothesis is illustrated in Fig. 7. In a small-scale study in The Netherlands (Y. Zhang, unpubl. data) we were unable to detect *Sporothrix* in growing corn plants. The species is hypothesised to grow exponentially in corn debris (Fig. 7b) serving as a potential inoculum for corn harvesters; the prevalent clinical type in north-east China is facial (Xia et al. 2009). At disappearance of the infectious material, the
human saprophytosis will die out with some delay (Fig. 7d), matching with the observation that most cases in China’s Jilin Province become apparent during winter (Song et al. 2013).

Occasionally, Sporothrix infections have been described that transmitted by animals very different from cats, which may be warm-blooded vertebrates but also arthropods: bites by squirrels, bats, fire ants, and spiders have been recorded (Moaven et al. 1999, Miller & Keeling 2002, Alves et al. 2010, Rodrigues et al. 2014a). The fungus was also isolated from plant material in armadillo burrows (Mackinnon et al. 1969, Rodrigues et al. 2014a). The conditions of plant decay in the burrow may be suitable for the development of Sporothrix, and subsequent dispersal by the armadillo may be expected.

Thus, despite the preponderance of cat vectors, the animal host species may vary, just as the plant host species did. This leads us to a hypothesis of wild animals occasionally providing conditions similar to those in fermented plant material. Cats take up propagules from the soil and easily transmit them to their mouth by licking. Conditions in animal saliva at the feline body temperature (normal range 37.7–39.1 °C) might be a stimulating factor for the production of the Sporothrix yeast phase. Cat saliva has a pH of 7.5–8.0, which is similar to that of self-heating bulk corn debris (around 8.0) and optimal for the mould-to-yeast conversion. With a hypothesis of conditional similarities between fermenting plant material and animal digestive tracts, the unique host shift of Sporothrix from plant to animal becomes understandable.

In south-east Brazil, transmission occurs nearly always by cats. Cat saliva is a stable environment, and despite the presence of antibodies – which generally have a low impact on fungal infections – repeated colonisation by Sporothrix once it has adapted to these conditions may be expected. The large outbreak in this area (da Rosa et al. 2005, Schubach et al. 2004, 2008, Silva et al. 2012, Pereira et al. 2014) suggests that the number of cases increases relative to the number of patients and cat vectors. Several peculiarities of cats may facilitate the dispersal of the fungus in the environment within limited endemic areas. Firstly, they are the most common pet animals with close contact to humans. Secondly, given the hypothesized origin of Sporothrix in cat saliva and its transmission to claws during licking, cat mobility and clawing enable them to take up and transmit the fungus, either each other during play or fight with house cats or stray cats, or transmit the fungus to human hosts via scratches or bites (Madrid et al. 2012).

Cat-transmitted cases also occur in S. schenckii, but at a much lower frequency (Rodrigues et al. 2013b), suggesting that this is not an exclusive relationship between S. brasiliensis and the feline host. In 1952, a first cat-transmitted case was reported from the USA. Until 1988, cat-associated cases and reports of transmission to human were sporadic. Four Malay-
sian veterinary students and one cat owner developed lesions of sporotrichosis after being bitten or scratched by cats with apparent fight wounds (Zamri-Saad et al. 1990). Crothers et al. (2009) described a series of 14 cases in cats, six of which were disseminated. The few outbreaks involving cats during this period invariably was limited to people in close contact with cats (Zamri-Saad et al. 1990, Cooper et al. 1992). This condition changed considerably since around 1998 in Brazil, when the incidence of cat and cat-transmitted cases increased dramatically to more than 5,000 human and feline cases in an expanding area around Rio de Janeiro and São Paulo (Barros et al. 2004, 2008b, Schubach et al. 2008, Pereira et al. 2014).

Sporothrix schenckii has been supposed to be the ancestral species on phylogenetic grounds, mainly because of its high degree of variability in all markers, its wide distribution, and hypothetical presence of sexuality as judged from a balanced mating type distribution (Teixeira et al. In press). The species seems preponderantly plant-transmitted. The share of cat-transmitted cases is much lower than in S. brasiliensis, and much higher than in S. globosa, where it is zero. In humans, disseminated cases occur almost only in immunocompromised patients, while cats seem to be relatively susceptible to infection. Host-shifts from plant material to animals are likely to have occurred already within S. schenckii, which diminishes the value of genomic comparisons between species. The main (preponderantly) clonal offshoots S. brasiliensis and S. globosa seem to have adapted successfully to their respective new habitats. This evolutionary hypothesis is summarised in Fig. 8. The ancestral species S. schenckii contains divergent genotypes with different behaviour. The clonal offshoot S. brasiliensis on average has increased virulence and is cat-transmitted; thus a shift from sapronoses to zoonoses takes place. The clonal offshoot S. globosa has lower virulence and has maintained sapronotic behaviour, but its vector of distribution seems to have changed. S. brasiliensis is phylogenetically distant to the main pathogenic clade of S. schenckii, S. globosa, and S. brasiliensis, and thus is unlikely to have played a role in the evolutionary host shift described above. Information on the few clinical strains available from the 1950s and 1970s decade is very limited (Rodrigues et al. 2013a). Dias et al. (2011) described one disseminated case without history of trauma from Portugal in an immunocompetent patient.

A certain degree of gender predominance was discernible which differed between species (Table 5). Considering all publications with >1 case, we noticed that in S. globosa in 11 case series female hosts were predominant, vs zero times male hosts. This is in agreement with Verma et al. (2012) who reported a female preponderance in the epidemiology of sporotrichosis in Himachal Pradesh, a small hill state in north-west India, usually related to agriculture practice. In S. schenckii these figures were opposite: one female vs eight males. In S. brasiliensis female/male ratios were more or less equal (7/9). Gender ratios have been reported to differ, mainly related to urban migration. In Brazil, many housewives stay at home and tend to be in charge of caring cats, while males have a larger chance to be infected during outdoor activities (Schubach et al. 2008). In rural areas of north-east China, most males go to cities to find jobs, leaving females, elderly and children at home, having increased chance of infection. Sporothrix schenckii infection classically seems to be connected with male agricultural activities (Rodrigues et al. 2014d). As most publications are unclear about gender ratios, more epidemiological study is necessary.

Conclusions
Our data and the review of the existing literature have shown that Sporothrix is unique in the fungal kingdom by its prevalent occurrence in the form of outbreaks, and that these outbreaks differ fundamentally from each other. In S. brasiliensis a huge zoonosis is taking place today, while the contemporary outbreak of similar dimensions in China is a sapronosis. In the ancestral species S. schenckii most outbreaks are sapronoses, but small zoonoses with cats as prime susceptible hosts have also been observed. It is significant to public health to consider these distinctions. Sapronoses, providing very special conditions...
promoting fungal growth, basically can be controlled by removal of the plant biomass allowing this contamination. In contrast, the zoonoses of cats compose a much more diffuse source of infection, which is more difficult to control. In addition, the ancestral species S. schenckii contains a mixture of strains that are susceptible to antifungals widely used in cutaneous infections, e.g. terbinafine, ketoconazole, and itraconazole, and strains with decreased susceptibility (Stopiglia et al. 2014). A large difference is noted particularly with azoles between the highly susceptible species S. brasiliensis and the resistant species S. globosa (Marimón et al. 2008). Selection and clonal expansion of resistant strains during epidemics may increase the significance of sporotrichosis as a human disease.

Acknowledgements

The research presented in this paper was supported by the KNAW - FES project ‘Barcoding the CBS Collections’, by KNAW China Desk Project 11CDP009, and the project was co-funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (1-965-34-HiCi) which the authors gratefully acknowledge. Bert Gerrits van den Ende is thanked for technical assistance.

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