



Phylogeography and evolutionary patterns in *Sporothrix* spanning more than 14 000 human and animal case reports

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Key words

epidemiology
historical biogeography
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saprosonis
Sporothrix
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transmission routes
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zoonosis

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 zoonosis 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.

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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).

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

Current/obsolete name	Strain	Country	Source	Genbank accession no.			
				ITS	CAL	TEF1	TEF3
<i>S. brasiliensis</i>	CBS 120339(T)	Brazil	Human	KP017087	KP101421	KP016994	KP017184
	CBS 130106	Brazil	Human	–	KP101422	KP016985	KP017204
	CBS 130107	Brazil	Human	–	KP101456	KP016995	KP017205
	CBS 130109	Brazil	Human	KP017088	KP101455	KP016996	KP017138
	CBS 130110	Brazil	Human	KP017089	KP101423	KP016997	KP017152
	CBS 132985	Brazil	Cat	–	KP101424	KP016998	KP017185
	CBS 132987	Brazil	Human	–	KP101425	KP016999	KP017194
	CBS 132988	Brazil	Human	–	KP101457	KP016986	KP017153
	CBS 132989	Brazil	Cat	–	KP101426	KP016987	KP017195
	CBS 132990	Brazil	Cat	–	KP101427	KP016988	KP017154
	CBS 132991	Brazil	Human	–	KP101428	KP017000	KP017155
	CBS 132992	Brazil	Human	–	KP101429	KP017001	KP017206
	CBS 132993	Brazil	Human	–	KP101430	KP017002	KP017186
	CBS 132994	Brazil	Dog	–	KP101431	KP016989	KP017156
	CBS 132995	Brazil	Cat	–	KP101432	KP016990	KP017157
	CBS 132996	Brazil	Cat	–	KP101433	KP016991	KP017158
	CBS 132997	Brazil	Cat	–	KP101449	KP017003	KP017139
	CBS 132998	Brazil	Cat	KP017090	KP101434	KP016992	KP017159
	CBS 132999	Brazil	Cat	–	KP101453	KP017004	KP017187
	CBS 133000	Brazil	Cat	–	KP101450	KP017005	KP017140
	CBS 133010	Brazil	Cat	–	KP101440	KP017013	KP017145
	CBS 133015	Brazil	Cat	–	KP101445	KP017018	KP017149
	CBS 133017	Brazil	Cat	–	KP101458	KP017020	KP017150
	CBS 133001	Brazil	Cat	–	KP101451	KP017005	KP017141
	CBS 133002	Brazil	Cat	–	KP101452	KP017005	KP017142
	CBS 133003	Brazil	Cat	–	KP101454	KP016982	KP017188
	CBS 133004	Brazil	Dog	–	KP101435	KP017008	KP017189
	CBS 133006	Brazil	Cat	–	KP101436	KP017009	KP017143
	CBS 133007	Brazil	Cat	–	KP101437	KP017010	KP017190
	CBS 133008	Brazil	Cat	–	KP101438	KP017011	KP017144
	CBS 133009	Brazil	Cat	–	KP101439	KP017012	KP017191
	CBS 133011	Brazil	Cat	–	KP101441	KP017014	KP017146
	CBS 133012	Brazil	Cat	–	KP101442	KP017015	KP017147
CBS 133013	Brazil	Cat	–	KP101443	KP017016	KP017192	
CBS 133014	Brazil	Cat	–	KP101444	KP017017	KP017148	
CBS 133016	Brazil	Cat	–	KP101446	KP017019	KP017193	
CBS 133019	Brazil	Cat	–	KP101447	KP016993	KP017196	
CBS 133021	Brazil	Cat	–	KP101448	KP017021	KP017151	
<i>S. globosa</i>	CBS 120340(T)	Spain	Human	KP017084	KP101459	KP016949	KP017165
	CBS 129717	China	Human	–	KP101460	KP016962	KP017166
	CBS 129718	China	Human	–	KP101461	KP016950	KP017167
	CBS 129719	China	Human	KP017085	KP101462	KP016951	KP017168
	CBS 129720	China	Human	–	KP101463	KP016952	KP017169
	CBS 129721	China	Human	–	KP101478	KP016953	KP017170
	CBS 129722	China	Human	–	KP101464	KP016964	KP017183
	CBS 129723	China	Human	–	KP101465	KP016963	KP017171
	CBS 129724	China	Human	–	KP101466	KP016954	KP017172
	CBS 129725	China	Human	–	KP101467	KP016955	KP017173
	CBS 130104	Spain	Human	–	KP101468	KP016965	KP017174
	CBS 130105	Spain	Human	–	KP101469	KP016956	KP017175
	CBS 130115	Spain	Human	–	KP101470	KP016966	KP017176
	CBS 130116	Spain	Human	–	KP101471	KP016957	KP017177
	CBS 130117	Japan	Human	–	KP101472	KP016967	KP017178
	CBS 132923	Brazil	Human	–	KP101473	KP016958	KP017179
	CBS 132924	Brazil	Human	KP017083	KP101474	KP016968	KP017180
	CBS 132925	Brazil	Human	–	KP101475	KP016959	KP017203
	CBS 292.55	UK	Human	KP017086	KP101476	KP016960	KP017181
	CBS 340.35	Japan	Human	–	KP101477	KP016961	KP017182
<i>S. luriei</i>	CBS 937.72(T)	South Africa	Human	AB128012	AM747302	KP016948	KP017207
	<i>S. mexicana</i>	CBS 120341(T)	Mexico	Soil, rose tree	KP017072	AM398393	KP016931
<i>S. schenckii</i>	CBS 120342	Mexico	Carnation	KP017073	AM398392	KP016932	KP017231
	CBS 132927	Brazil	Human	–	JF811340	KP016933	KP017232
	CBS 132928	Brazil	Human	–	JF811341	KP016934	KP017233
	CBS 133192	Italy	Dog	KP017075	JX080721	KP016935	KP017234
	CBS 125601	Colombia	Human	–	KP101395	KP016973	KP017120
	CBS 117440	South Africa	Human	KP017098	KP101386	KP017026	KP017114
	CBS 117842	South Africa	Human	–	KP101387	KP017027	KP017115
<i>S. sp. / S. schenckii</i>	CBS 115870	South Africa	NK	–	–	KP016930	KP017229
	CBS 130101	Peru	Human	KP017095	KP101390	KP017022	KP017197
	CBS 130103	Argentina	Human	–	KP101388	KP017025	KP017117
	CBS 130111	Colombia	Human	–	KP101401	KP016974	KP017123
	CBS 130112	Peru	Human	KP017096	KP101391	KP017023	KP017132
	CBS 130114	Peru	Human	–	KP101392	KP017024	KP017133
	CBS 130097	Bolivia	Human	–	KP101396	KP016969	KP017137
	CBS 130098	Peru	Human	KP017091	KP101397	KP016979	KP017121
	CBS 130099	Peru	Human	KP017092	KP101398	KP016980	KP017122
	CBS 132926	Brazil	Human	–	KP101404	KP016981	KP017202
	CBS 132961	Brazil	Cat	–	KP101402	KP016970	KP017124

Table 1 (cont.)

Current/obsolete name	Strain	Country	Source	Genbank accession no.			
				ITS	CAL	TEF1	TEF3
<i>S. schenckii</i> (cont.)	CBS 132962	Brazil	Human	–	KP101403	KP016975	KP017125
	CBS 132964	Brazil	Human	–	KP101407	KP017031	KP017118
	CBS 132966	Brazil	Human	–	KP101400	KP017032	KP017126
	CBS 132969	Brazil	Human	–	KP101409	KP017029	KP017198
	CBS 132970	Brazil	Human	–	KP101405	KP016976	KP017127
	CBS 132971	Brazil	Human	–	KP101411	KP016977	KP017160
	CBS 132972	Brazil	Human	–	KP101412	KP016978	KP017199
	CBS 132973	Brazil	Human	–	KP101408	KP017033	KP017200
	CBS 132974	Brazil	Human	–	KP101410	KP016971	KP017128
	CBS 132975	Brazil	Human	–	KP101406	KP017034	KP017119
	CBS 132976	Japan	Human	–	KP101415	KP017035	KP017201
	CBS 132977	Mexico	Plant debris	–	KP101399	KP017030	KP017134
	CBS 132981	Brazil	Human	–	KP101414	KP017036	KP017129
	CBS 132982	Brazil	Human	–	KP101416	KP016972	KP017130
	CBS 211.61	South Africa	NK	KP017093	KP101393	KP016983	KP017135
	CBS 345.53	The Netherlands	Human	–	KP101417	KP017038	KP017161
	CBS 356.29	Germany	NK	–	KP101413	KP017037	KP017131
	CBS 359.36(T)	USA	NK	KP017100	KP101420	KP017041	KP017163
	CBS 444.67	Mozambique	Human	KP017099	KP101389	KP017028	KP017116
	CBS 472.48	NK	NK	KP017097	KP101418	KP017039	KP017162
<i>S. schenckii</i> / <i>S. sp.</i>	CBS 498.86	NK	NK	–	KP101419	KP017040	KP017164
<i>S. schenckii</i>	CBS 938.72	France	Human	KP017094	KP101394	KP016984	KP017136
<i>S. abietinum</i>	CBS 125.89	Mexico	<i>Abies vejarii</i>	AF484453	–	–	–
<i>S. africanum</i>	CBS 116566	South Africa	<i>Protea caffra</i>	DQ316200	–	–	–
<i>S. aurorae</i>	CBS 118837(T)	South Africa	<i>Pinus eliottii</i>	DQ396796	–	–	–
<i>S. brunneoviolacea</i>	CBS 793.73	Germany	Meadow soil	KP017069	KP017106	KP017061	KP017112
<i>S. brunneoviolacea</i> / <i>S. inflata</i>	CBS 101570	USA	Endophyte in <i>Vitis vinifera</i>	KP017068	KP017101	KP017057	KP017108
<i>S. brunneoviolacea</i>	CBS 110895	Austria	Root of <i>Quercus petraea</i>	–	KP017104	KP017062	KP017109
	CBS 110896	Austria	Root of <i>Quercus robur</i>	–	KP017102	KP017058	KP017110
	CBS 124561(T)	Spain	Soil	FN546959	KP017103	KP017059	KP017113
	CBS 124562	Spain	Soil	FN546957	–	–	–
	CBS 124564	Spain	Soil	FN546958	KP017105	KP017060	KP017111
	CBS 959.73(T)	NK	NK	–	–	KP017047	KP017241
<i>S. sp.</i> / <i>S. curviconia</i>	CBS 145.94	NK	NK	KP017071	–	–	–
<i>S. sp.</i> / <i>S. curviconia</i>	CBS 541.84	Chile	<i>Pinus radiata</i> log	KC113234	KP101483	KP017046	KP017239
<i>S. dentifundum</i>	CBS 115790(T)	Hungary	<i>Quercus</i> wood	AY495434	–	–	–
<i>S. dimorphospora</i> / <i>S. inflata</i>	CBS 553.74(T)	Canada	Soil	KP017082	–	KP017052	KP017209
	CBS 840.73	Chile	Wood	–	–	KP017050	KP017210
<i>S. dimorphospora</i>	CBS 125439	USA	Soil	KP017080	–	KP017048	KP017208
	CBS 125440	Spain	Soil	KP017081	–	–	–
	CBS 125442	Spain	Soil	FN546961	–	KP017051	KP017212
<i>S. foliorum</i>	CBS 326.37	The Netherlands	Industrial strain	KP017067	–	KP016929	KP017240
<i>S. fusiforme</i>	CBS 112912	Azerbaijan	<i>Populus nigra</i>	AY280481	–	–	–
<i>S. gemellus</i>	CBS 121959(T)	South Africa	<i>Tarsonemus</i> sp. from <i>Protea caffra</i>	DQ821560	–	–	–
	CBS 239.68(T)	Germany	Soil, wheat field	AY495426	–	KP017054	KP017213
<i>S. inflata</i>	CBS 794.73	Sweden	Humus in <i>Picea</i> forest	KP017079	–	KP017053	KP017216
	CBS 841.73	Chile	Soil	AY495431	–	KP017055	KP017214
	CBS 156.72	The Netherlands	Greenhouse soil	–	–	KP017056	KP017215
<i>S. sp.</i> / <i>S. inflata</i>	CBS 119147	South Africa	<i>Eucalyptus</i> wood pole	KP017064	KP017107	KP017063	KP017242
<i>S. lignivora</i>	CBS 119148(T)	South Africa	<i>Eucalyptus</i> wood	KP017065	–	–	–
	CBS 119149	South Africa	<i>Eucalyptus</i> wood pole	KP017066	–	–	–
<i>S. lunatum</i>	CBS 112927(T)	Austria	<i>Carpinus betulus</i>	AY280485	–	–	–
<i>S. pallida</i>	CBS 111110	Germany	Insect, <i>Zootermopsis nevadensis</i>	–	AM398382	KP016937	KP017217
<i>S. pallida</i> / <i>S. stylites</i>	CBS 115868	South Africa	<i>Eucalyptus camaldulensis</i>	EF127881	–	KP016941	KP017221
	CBS 115869	South Africa	Wood utility pole	EF127884	–	KP016942	KP017222
	CBS 115872	South Africa	Wood pole	EF127882	–	KP016938	KP017220
<i>S. pallida</i> / <i>S. humicola</i>	CBS 118129(T)	South Africa	Soil	KP017076	–	KP016939	KP017223
<i>S. pallida</i> / <i>S. stylites</i>	CBS 118848(T)	South Africa	Wood pole	KP017077	–	KP016943	KP017224
<i>S. pallida</i> / <i>S. nivea</i>	CBS 131.56(T)	Japan	<i>Stemonitis fusca</i>	EF127880	–	KP016944	KP017227
<i>S. pallida</i>	CBS 182.63	The Netherlands	Soil	KC113233	–	KP016940	KP017228
<i>S. pallida</i> / <i>S. schenckii</i>	CBS 201.53	South Africa	Decaying grass	–	–	KP016945	KP017225
<i>S. pallida</i> / <i>S. albicans</i>	CBS 302.73(T)	UK	Soil	KP017078	AM398396	KP016946	KP017226
<i>S. pallida</i> / <i>S. sp.</i>	CBS 622.95	NK	NK	–	–	KP016947	KP017218
<i>S. pallida</i> / <i>S. sp.</i>	CBS 623.95	NK	NK	–	–	KP016936	KP017219
<i>S. palmiculminatum</i>	CBS 119590(T)	South Africa	<i>Protea repens</i>	DQ316191	–	–	–
<i>S. phasma</i>	CBS 119721(T)	South Africa	<i>Protea laurifolia</i>	DQ316219	–	–	–
<i>S. protearum</i>	CBS 116567	South Africa	<i>Protea caffra</i>	DQ316203	–	–	–
<i>S. splendens</i>	CBS 116569	South Africa	<i>Protea repens</i>	DQ316215	–	–	–
<i>S. stenoceras</i>	CBS 237.32(T)	Norway	Pine pulp	AF484462	–	–	–
<i>S. variecibatus</i>	CBS 121960	South Africa	<i>Protea longifolia</i>	DQ821569	KP101479	KP017042	KP017235
	CBS 121961(T)	South Africa	<i>Oodinychus</i> sp. mite from <i>Protea repens</i>	KP017070	KP101481	KP017043	KP017236
	CBS 121962	South Africa	<i>Eucalyptus</i> sp.	DQ821567	KP101482	KP017044	KP017237
	CBS 123288	NK	NK	–	KP101480	KP017045	KP017238

Table 1 (cont.)

Current/obsolete name	Strain	Country	Source	Genbank accession no.			
				ITS	CAL	TEF1	TEF3
<i>Ceratocystiopsis minuta</i>	RJ705	Poland	<i>Picea abies</i>	EU913697	–	–	–
<i>O. ainoae</i>	CMW 1903	Norway	<i>Picea abies</i>	HM031495	–	–	–
<i>O. angusticollis</i>	CBS 186.86	USA	<i>Pinus banksiana</i>	AY924383	–	–	–
<i>O. arduennense</i>	MUCL 44866	Belgium	<i>Fagus sylvatica</i>	AY573241	–	–	–
<i>O. bacillisporum</i>	MUCL 45378	Belgium	<i>Fagus sylvatica</i>	AY573258	–	–	–
<i>O. bicolor</i>	CBS 492.77	USA	Gallery of <i>Ips</i> sp. in <i>Picea</i> sp.	DQ268604	–	–	–
<i>O. braganthinum</i>	CBS 430.92	Brazil	Soil	FN546964	–	–	–
	CBS 474.91(T)	Brazil	Soil	FN546965	–	–	–
<i>O. brevisculum</i>	YCC-522, JCM 12501	Japan	Single ascospore isolate from YCC-494	AB200423	–	–	–
<i>O. canum</i>	CBS 133.51	Sweden	<i>Pinus sylvestris</i>	HM031489	–	–	–
<i>O. catonianum</i>	C1084	Italy	<i>Pyrus</i>	AF198243	–	–	–
<i>O. conicola</i>	CBS 127.89	Mexico	Cone with <i>Conophthorus</i> <i>cembroides</i>	AY924384	–	–	–
<i>O. coronatum</i>	CBS 497.77	NK	NK	AY924385	–	–	–
<i>O. denticiliatum</i>	CMW 29493	Norway	<i>Scolytus ratzeburgi</i> on <i>Betula</i> sp.	FJ804490	–	–	–
<i>O. fasciatum</i>	UM 56	Canada	<i>Pseudotsuga menziesii</i>	EU913720	–	–	–
<i>O. flexuosum</i>	CBS 208.83	Norway	<i>Picea abies</i>	AY924387	–	–	–
<i>O. floccosum</i>	CBS 799.73	Sweden	Soil	AF198231	–	–	–
<i>O. fumeum</i>	CMW 26813	South Africa	<i>Eucalyptus cloeziana</i>	HM051412	–	–	–
<i>O. fuscum</i>	CMW 23196	Finland	<i>Pityogenes chalcographus</i> on <i>Picea abies</i>	HM031504	–	–	–
<i>O. ips</i>	CMW 7075	NK	NK	AY546704	–	–	–
<i>O. japonicum</i>	YCC-099	NK	NK	GU134169	–	–	–
<i>O. karelicum</i>	CMW 23099	Russia	<i>Scolytus ratzeburgi</i> on <i>Betula pendula</i>	EU443762	–	–	–
<i>O. kryptum</i>	DAOM229701	NK	NK	AY304436	–	–	–
<i>O. minus</i>	AU58.4	Canada	Lodgepole pine lumber	AF234834	–	–	–
	CCMA12	NK	NK	AY934511	–	–	–
<i>O. montium</i>	CMW13221	NK	NK	AY546711	–	–	–
<i>O. multiannulatum</i>	MUCL19062	NK	NK	AY934512	–	–	–
<i>O. nigrocarpum</i>	ATCC22391	USA	<i>Dendroctonus</i> sp.	AF484474	–	–	–
	CBS 637.66(T)	USA	<i>Abies</i> sp.	AY280489	–	–	–
<i>O. nikkoense</i>	YCC-430	Japan	NK	AB506674	–	–	–
<i>O. novoulmi</i>	C510	USA	<i>Ulmus</i> sp.	AF198236	–	–	–
<i>O. piceae</i>	CBS108.21(T)	Germany	NK	AF198226	–	–	–
<i>O. piliferum</i>	CBS 129.32	The Netherlands	Scots pine	AF221070	–	–	–
<i>O. pluriannulatum</i>	MUCL18372	NK	NK	AY934517	–	–	–
<i>O. quercus</i>	CMW2467	France	<i>Quercus</i> sp.	AY466626	–	–	–
<i>O. rostrocoronatum</i>	CBS 434.77(T)	USA	Woodpulp	AY194509	–	–	–
<i>O. saponiodorum</i>	CMW29497	Finland	<i>Ips typographus</i> on <i>Picea abies</i>	HM031507	–	–	–
<i>O. sejunctum</i>	Ophi 1A	NK	NK	AY934519	–	–	–
<i>O. setosum</i>	AU160-38	NK	NK	AF128929	–	–	–
<i>O. subannulatum</i>	CBS 188.86	USA	<i>Pinus</i>	AY934522	–	–	–
<i>O. tapionis</i>	CMW23266	Finland	<i>Hylastes brunneus</i> on <i>Pinus sylvestris</i>	HM031493	–	–	–
<i>O. tenellum</i>	CBS 189.86	USA	<i>Pinus banksiana</i>	AY934523	–	–	–
<i>O. tetropii</i>	CBS 428.94	Austria	Breeding system of <i>Tetropium</i> sp., <i>Picea abies</i>	AY934524	–	–	–
<i>O. triangulosporum</i>	DSMZ4934	NK	NK	AY934525	–	–	–
<i>Pesotum australiae</i>	CMW6606	Australia	<i>Acacia mearnsii</i>	EF408603	–	–	–
<i>Pesotum cupulatum</i>	C1194	USA	<i>Pseudotsuga</i>	AF198230	–	–	–

O = *Ophiostoma*, S = *Sporothrix*, NK = Not known, T = type culture.

Recently de Beer et al. (unpubl. data) delimited the genera *Sporothrix* and *Ophiostoma* on the basis of sequence data of four loci (LSU, ITS, CAL, BT2). Their classification matched with main ecological trends, i.e. *Ophiostoma* species were prevalently associated with bark beetles behind bark, whereas *Sporothrix* contained all major pathogens and/or occurred in plant debris or in soil. Some intermediate clades showed diverse ecologies and were phylogenetically ambiguous.

Traditionally, our understanding of the evolutionary history and phylogenetic relationships within *Sporothrix* has been fragmented due to the tendency of studying human pathogens and environmental species separately. Only a few studies attempted to integrate strains with dissimilar sources as well as geographic origins, moreover, sampling strategies are usually restricted to a short period of time (Dixon et al. 1991, Vismer & Hull 1997, Mesa-Arango et al. 2002, de Beer et al. 2003). Despite this

segregation of information, focal outbreaks due to *Sporothrix* species are often connected to an environmental source, mainly involving traumatic inoculation of plant material into the cutaneous and subcutaneous tissues of subjects. Furthermore, assumptions of species distribution and ecological niche have been historically based on knowledge of morphological traits. 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

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 14 000 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 (Feeney 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

Table 2 Success rates of sequencing for each gene.

Contigs (%)	<i>S. brasiliensis</i> (n = 44)	<i>S. schenckii</i> (n = 43)	<i>S. globosa</i> (n = 22)	Environmental species (n = 96)
ITS (%)	81.8	100	100	70.8
CAL (%)	95.5	100	90.9	62.5
TEF1 (%)	95.5	79.1	100	96.9
TEF3 (%)	97.7	97.7	100	100

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 multi-locus 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 (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× 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 v. 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 1×10^7 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 (AFLP) genotyping using a previously described procedure (Chowdhary et al. 2013). However, for the amplification of the DNA fragments the selective cytosine residue of the *Eco*RI primer was replaced by an adenine residue (5'-Flu-GACTGCGTACCAATTCAA-3'), while the *Mse*I primer remained the same with one selective residue (5'-GATGAGTC-CTGACTAAG-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 1898 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 two 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 χ^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

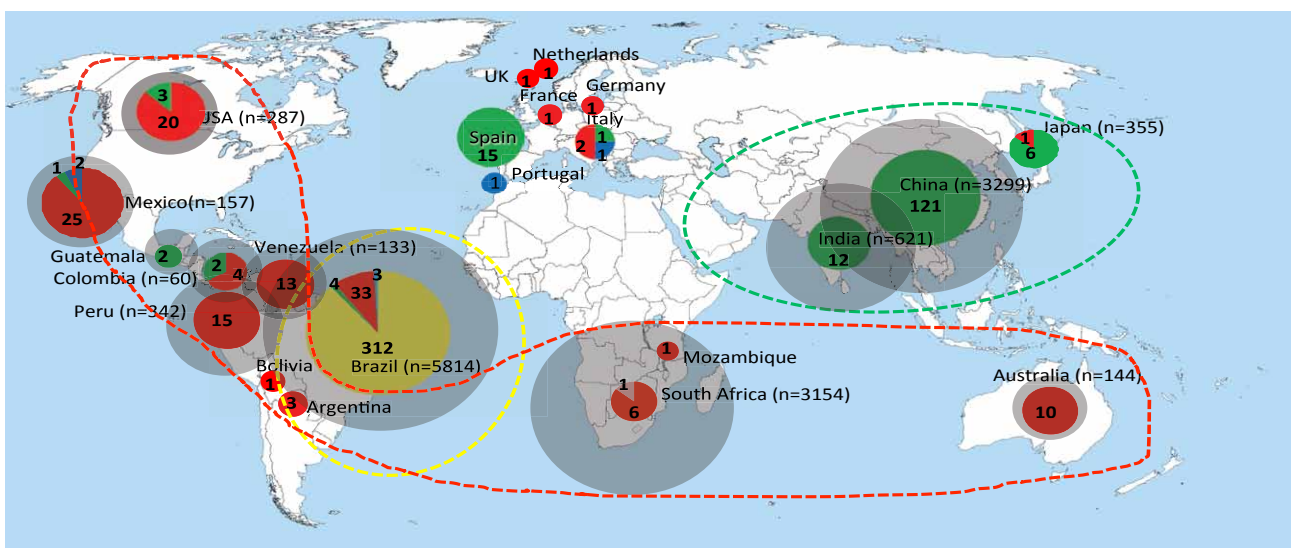


Fig. 1 Geographic distribution of sporotrichosis caused by *S. brasiliensis*, *S. schenckii*, and *S. globosa* according to case reports published over 70 years, compared with sequenced isolates and with expression of statistical probabilities that the prevalent endemic species was concerned in historical 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.

- *S. schenckii*
- *S. brasiliensis*
- *S. globosa*
- *S. mexicana*
- *S. luriei*

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.

Continent / region	Country	Reported cases	Sequenced isolates	<i>S. brasiliensis</i> %	<i>S. globosa</i> %	<i>S. schenckii</i> %	<i>S. mexicana</i> %	<i>S. luriei</i>
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: 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		1: 4 %	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		17: 71 %	5: 21 %	2: 8 %	
Total			625	312	168	137	7	1

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 *Ceratomyces 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 invariable characters, 246 variable parsimony-informative sites (34 %), and 72 singletons. 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 *Protea* 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 99 % 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 part 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

Table 4 Variability of loci used in 136 *Sporothrix* isolates examined.

Locus	No. of bp sequenced	% variable sites	% parsimony-informative sites	% singleton sites
<i>CAL</i>	792	54.5	42.8	11.7
<i>TEF1</i>	526	17.5	13.5	4.0
<i>TEF3</i>	255	33.7	21.2	12.5
Combined genes	1573	38.8	29.5	9.28

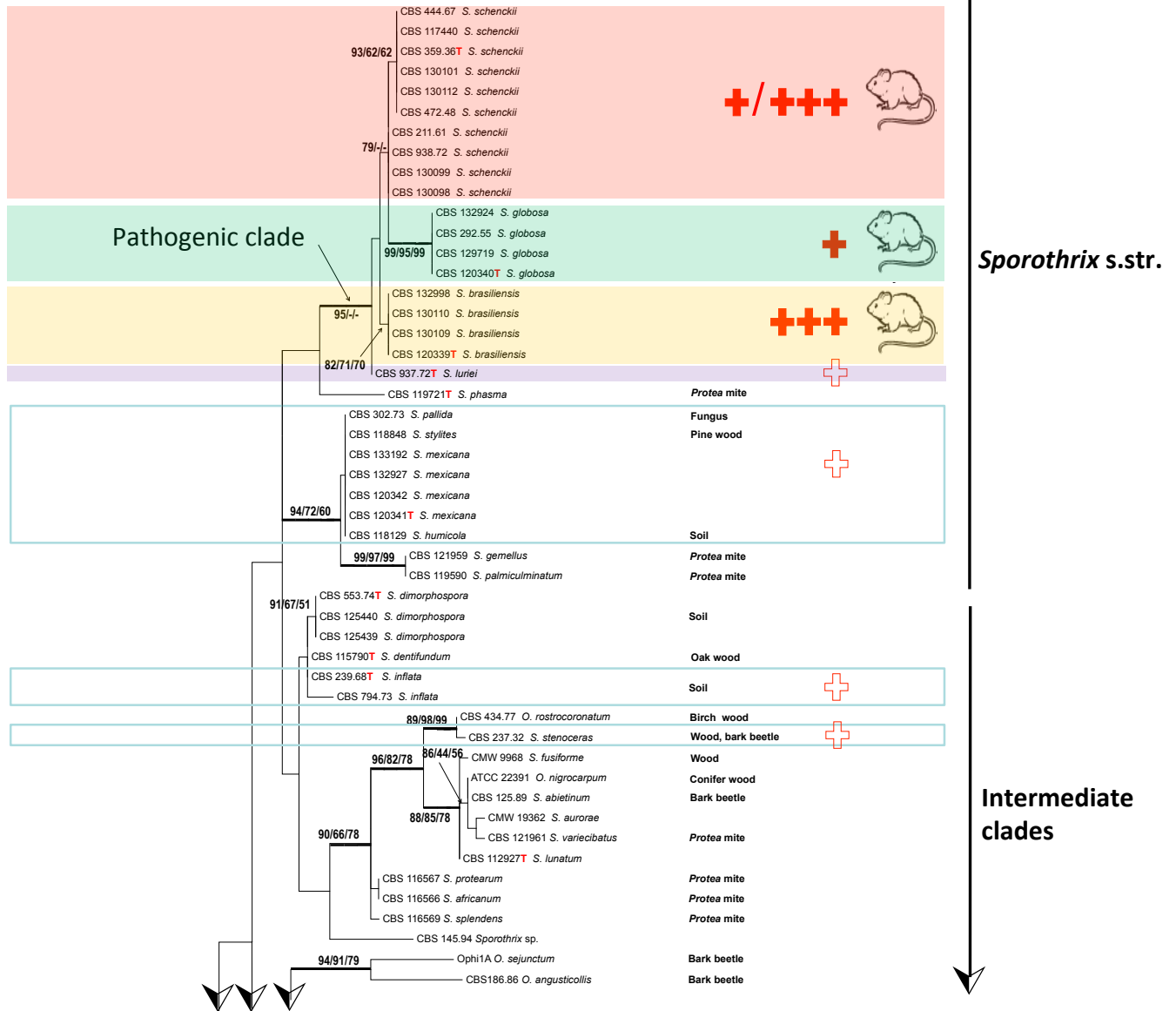


Fig. 2 Phylogenetic relationships inferred from PhyML based on ITS sequences of 101 strains belonging to *Sporothrix* and *Ophiostoma*. The numbers close to the branches represent indices of support (ML/NJ/MP) based on 1 000 bootstrap replications. Branches with bootstrap support value higher than 80 % are indicated in **bold**.

clustered according to their geographic origin at low geographic distances, but numerous strains of *S. globosa* with identical profiles were repeatedly found to originate from different continents (Fig. 4).

Table 3 shows the number of sequences analysed, relative to the geography of isolation of the respective strain. In most cases, one species is predominant in each defined area. On the basis of this predominance, the probability of attribution of a given strain from that area was calculated. Using this probability, historical case reports without sequence data could be attributed to either *S. brasiliensis*, *S. globosa*, or *S. schenckii*; cases reported as *S. luriei* and *S. mexicana* were too rare for this calculation and were left blank in Table 3. Probability of Chinese strains as *S. globosa*, for example, was 100 %, whereas in Europe none of the species was predominant and thus such calculation was impossible. Sporotrichosis is rare in Europe, and etiologic agents may have been imported, which would explain the relatively high species diversity in this continent.

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 ($p = 10^{-6}$). 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*,

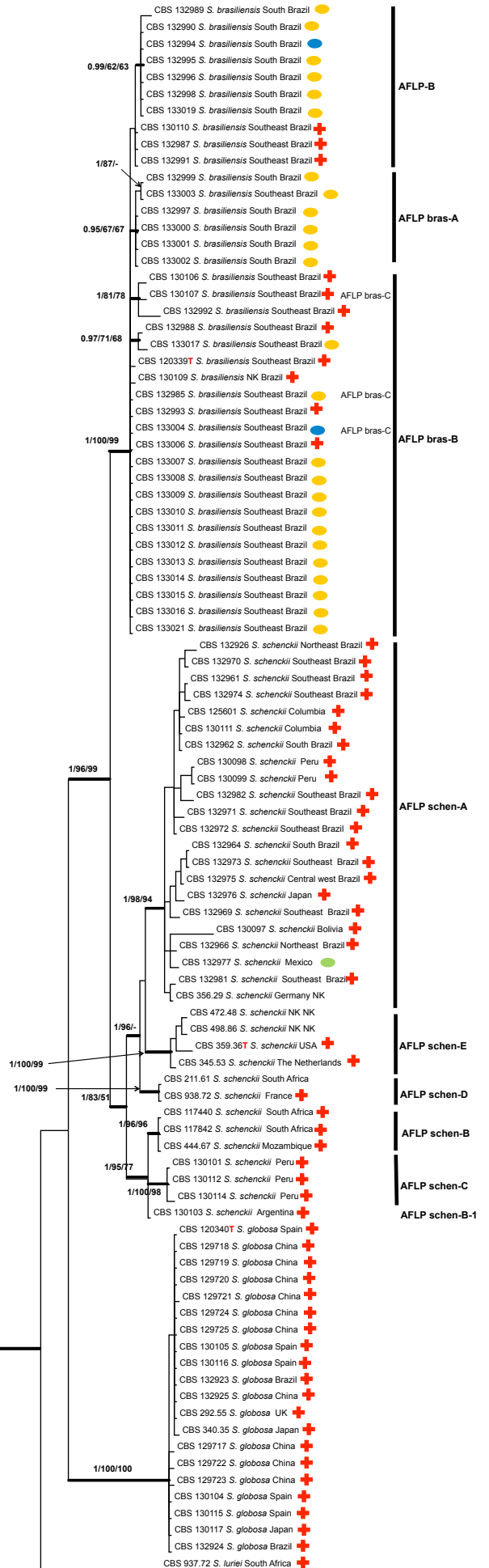
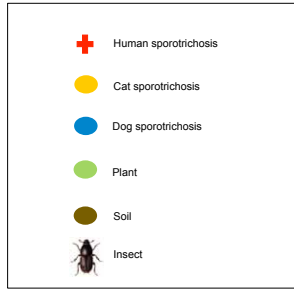


Fig. 3 Phylogenetic relationship inferred from Bayesian statistics based on concatenated *CAL*, *TEF1* and *TEF3* sequences of 135 strains of *Sporothrix* species. Bootstrap and posterior probabilities values were added to respective branches (BI/ML/NJ). Branches with bootstrap support values higher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



Fig. 3 (cont.)

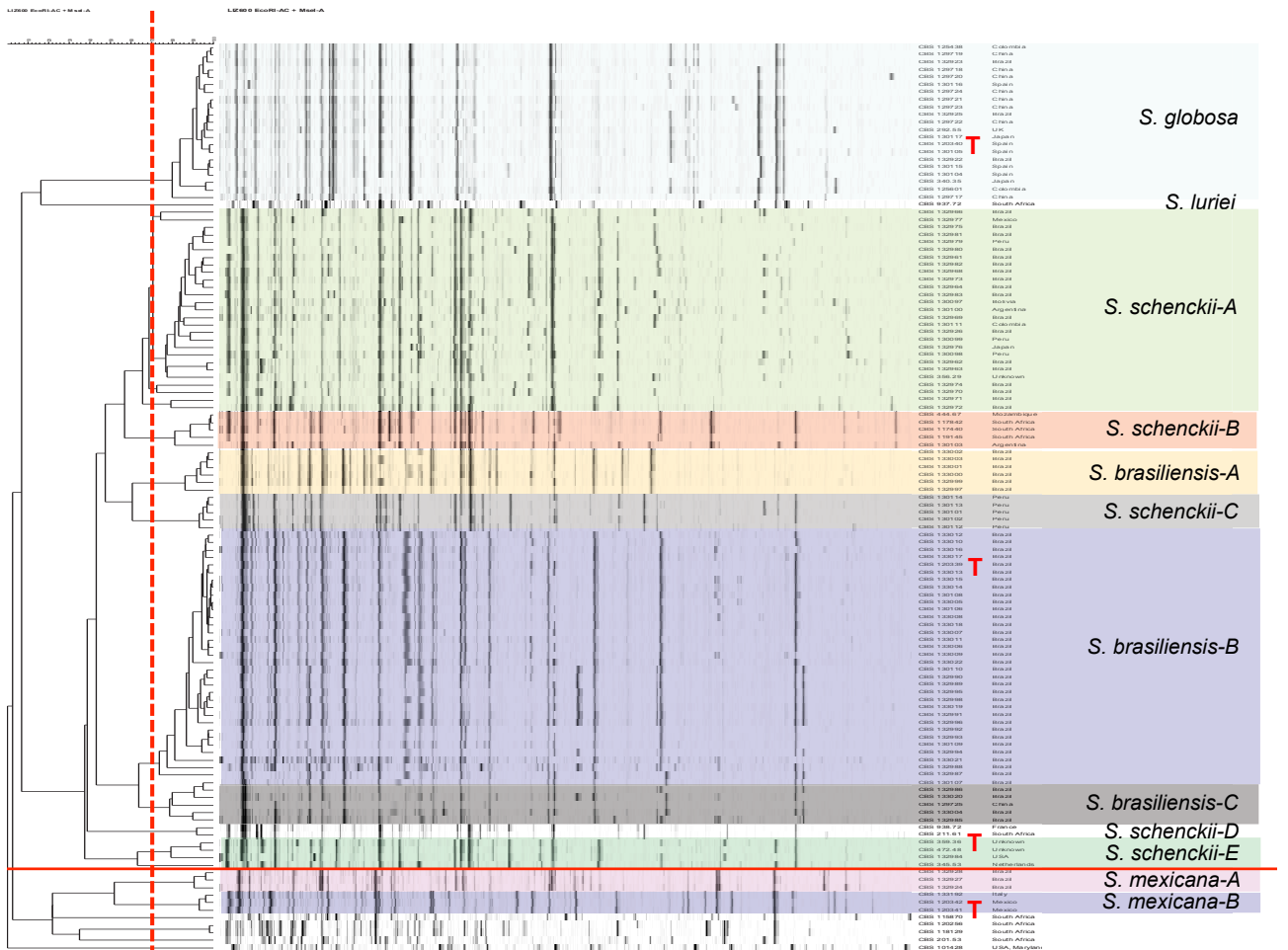


Fig. 4 Amplified fragment length polymorphism (AFLP) profiles of 122 strains of *Sporothrix*. Clustering of AFLP banding pattern of isolates of *Sporothrix* was done by UPGMA. Red vertical bars represents cut-off for distinction of clusters. Strains of *S. mexicana* and below are phylogenetically unrelated.

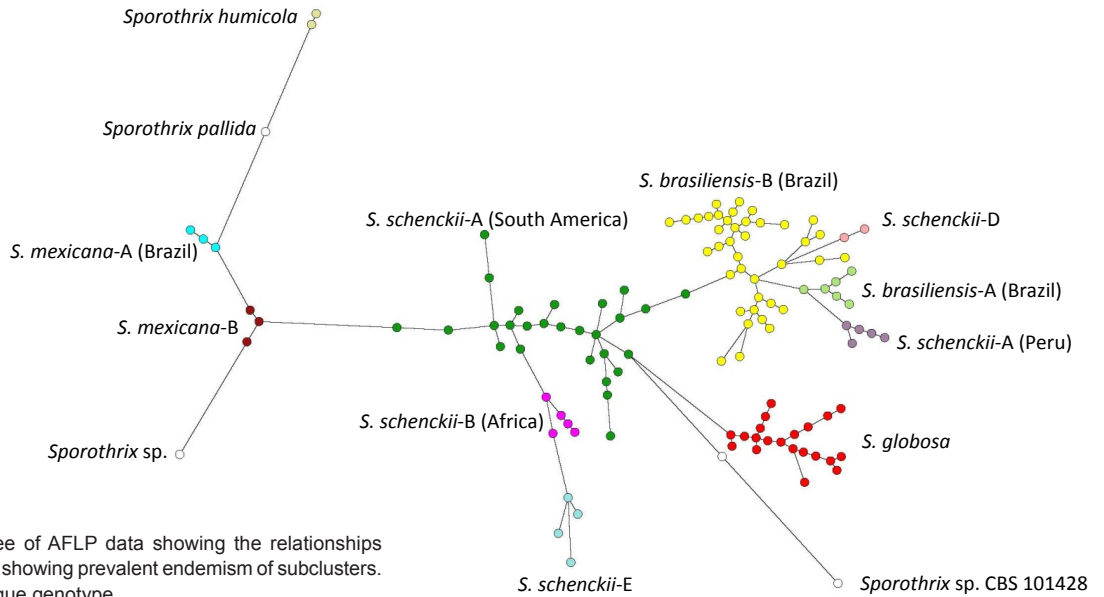


Fig. 5 Minimum spanning tree of AFLP data showing the relationships among 135 *Sporothrix* isolates, showing prevalent endemism of subclusters. Each dot corresponds to a unique genotype.

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

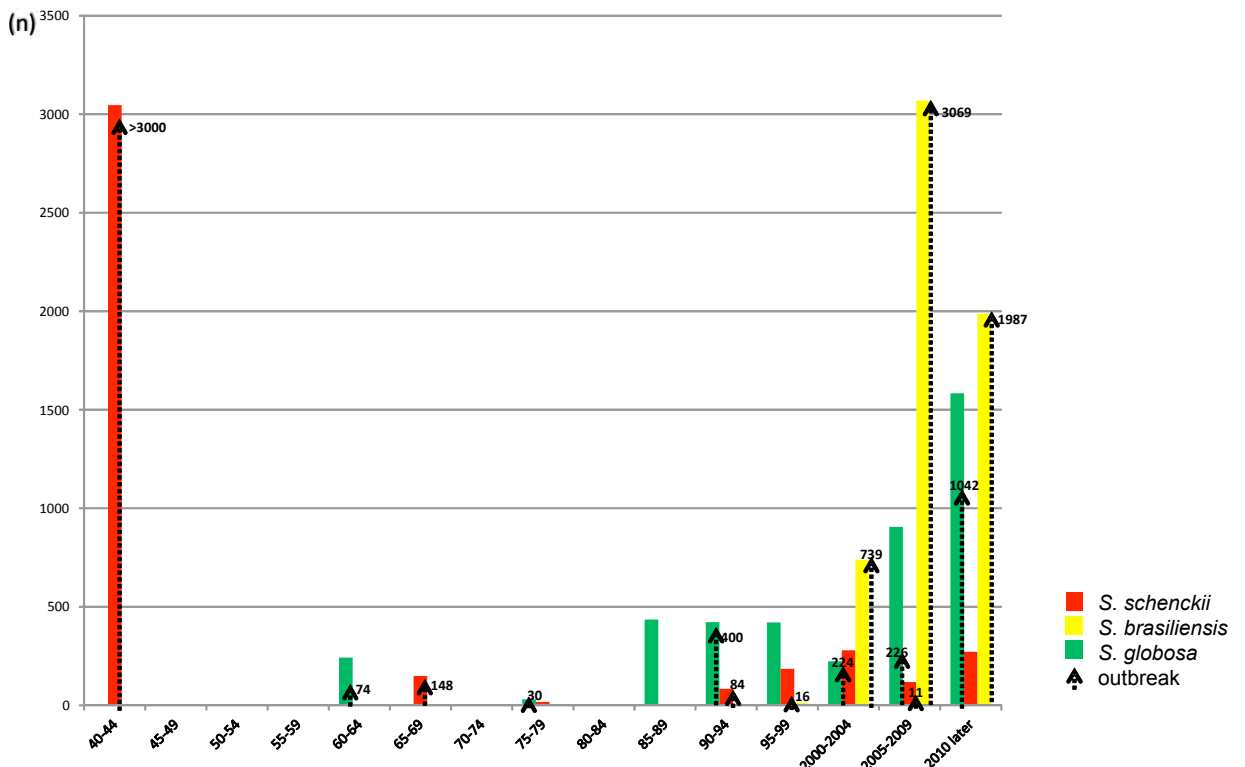


Fig. 6 Timeline of epidemics and case series caused by three main pathogenic *Sporothrix* species since 1940. Vertical bars represent gross number of cases, vertical arrows denote case series and sapronoses or zoonoses.

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* infructescences. 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 leukemic 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. pallida* 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 (Arrillaga-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 degree 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 virulent isolates of *Sporothrix* (Fernandes et al. 2013). Further, this molecule was also shown to be a component of the cell wall of *S. brasiliensis* and *S. schenckii* (Castro et al. 2013) and appears to be relevant during infection.

Sporothrix brasiliensis shows low degrees of variability and has been suggested to be clonal (Rodrigues et al. 2013b, 2014d). The low variability of this taxon is also supported by low chromosomal polymorphisms (Sasaki et al. 2014) and homogeneous susceptibility profiles to antifungal agents (Rodrigues et al. 2014c). In our dataset (Fig. 3) a supported clade matched with AFLP group A and mainly contained strains from southern Brazil; strains of the top clade had the preponderant AFLP type B. The occurrence of separate genotypes among strains indicated that the Brazilian *S. brasiliensis* epidemic has at least two distinct sources as was proposed earlier by Rodrigues et al. (2013b) and confirmed here by AFLP data.

Sporothrix globosa strains analysed were strictly identical. Isolates studied by Yu et al. (2013) were divided into two highly supported subclades (*S. globosa* I and *S. globosa* II). Group I comprised the majority of Chinese clinical isolates, three Chinese environmental isolates from reed, corn stalks, and soil, the type strain of *S. globosa*, and some isolates from the USA, India, Japan, Brazil, and the UK. *Sporothrix globosa* II included

a small part of Chinese clinical isolates and a single isolate from Italy. More material is needed to establish whether *S. globosa* is preponderantly clonal.

As noticed earlier (Marimón et al. 2006, 2007), *S. schenckii* is the likely ancestral, most variable species within the pathogenic clade. This genetic variability is reflected in significant differences in genome size and chromosome profiles generated by pulsed-field gel electrophoresis that usually display 4 to 7 chromosomal bands, ranging from 2.0 to 7.0 Mb (Sasaki et al. 2014). In our dataset, five supported subgroups were visible in all partitions (Fig. 3), which were also recognisable in AFLP data (Fig. 4). This suggests that the groups are separate lineages with limited gene flow. Nearly all lineages A–E of *S. schenckii* have restricted geographic distributions (Fig. 5). Four of the clusters are concurrent with groups distinguished by Marimón et al. (2006, 2007).

As can be concluded from combined AFLP and MLST data, remarkable differences between species are noted. Sporotrichosis in Brazil dates back to 1907, when the disease was first diagnosed in naturally infected mice (Lutz & Splendore 1907). *Sporothrix brasiliensis* strains with closely similar AFLP profiles have very limited distribution. Despite the large expansion of populations during the Rio de Janeiro zoonotic epidemic, strains with identical genotypes are found at small geographic distances (Rodrigues et al. 2014d). The species thus seems to have a slow vector of dispersal. Several authors (Barros et al. 2004, Madrid et al. 2012, Rodrigues et al. 2013b) have demonstrated a role of felines in transmission of *S. brasiliensis*. Cats are relatively sedentary, and thus the zoonosis is expected to expand geographically at a slow pace. *Sporothrix globosa* also shows low degrees of variation, but a significant difference with *S. brasiliensis* is observed in that identical multilocus genotypes are repeatedly found at very large geographic distances. For example, molecularly identical strains CBS 125438, CBS 129719, and CBS 132923 originated from Colombia, China, and Brazil, respectively, while also strains from UK, Japan, Spain, Brazil, and China had identical genotypes. Obviously a rapid vector of dispersal is at work in *S. globosa*. Given the large distances between identical strains, airborne distribution 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 'reed 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 Hajjeh et al. (1997) in an outbreak of sporotrichosis from *Sphagnum* moss among nine out of 65 nursery workers. Feeney et al. (2007) described 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 creosote (Helm & Berman 1947).

<i>S. schenckii</i> (cont.)	1997	USA	5	Human	M	Temperate	Hay bale	LC	Dooley et al. 1997
	2002	USA	1	Human	M	Temperate	Fire ant	F, I, arynx	Miller & Keeling 2002
	2003	USA	1	Infant	F	Temperate		D	Khable et al. 2003
	2007	USA	1	Human(HIV)	F	Temperate		F	Vilela et al. 2007
	2007	USA	1	Dog	F	Temperate		F	Whittemore & Webb 2007
	2009	USA	14	Cat		Temperate		F, D, LC	Crothers et al. 2009
			4	Dog		Temperate		F, D, LC	
			4	Horse		Temperate		F, LC	
			1	Donkey		Temperate		LC	
	2009	USA	1	Infant		Temperate	Cat	F	Tougan et al. 2009
	2011	USA	1	Human	F	Temperate	Mouldy hay	F, LC	Rees & Swartzberg 2011
	1998	Australia	16	Human	M	Warm, dry		F, LC	Conias & Wilson 1998
	2007	Australia	11	Human	M	Warm, dry	Hay		Feeney et al. 2007
	2012	Australia	31	Human	M	Warm, dry	Plant debris		Sivagnanam et al. 2012
	2013	Australia	86	Human (lower immune)	M	Humid		Pulmonary	Aung et al. 2013
	1994	Mexico	4	Human	F	Warm, dry	Rust-stained tent		Campos et al. 1994
	2001	Mexico	50	Human	F			LC	Espinosa-Texis et al. 2001
	2006	Mexico	55	Human	M		Farmer, housewife	F, LC	Macotela-Ruiz & Nochebuena-Ramos 2006
	2007	Mexico	25	Child	M		Plant, soil, squirrel, cat, rat	D, F, LC	Bonifaz et al. 2007
	2007	Mexico	13	Human	M			LC	Arenas et al. 2007
	1943	Uruguay	46	Human	M	Cool, humid			Mackinnon 1943
	1969	Uruguay	157	Human	M	Warm	Armadillo		Mackinnon et al. 1969
	2004	Uruguay	42	Human	M	Warm	Armadillo	F, LC	Civila et al. 2004
	2000	Peru	238	Human	M	Cool, dry	Farmer, student, housewife, infant	D, F, LC	Pappas et al. 2000
	2011	Peru	20	Human	M	Cool, dry		F	Ramirez-Soto et al. 2011
	2010	Columbia	60	Human	M	Warm, humid	Farmer	F, LC	Rubio et al. 2010
	2013	Venezuela	87	Human	M	Warm, humid	Farmer, student, housewife	F, LC	Mata-Essayag et al. 2013

NC = No comparison; NK = Not known; D = disseminated; F = fixed; LC = lymphocutaneous; SYS = systemic; S = *Sporothrix*.

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 (saprozoses) 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

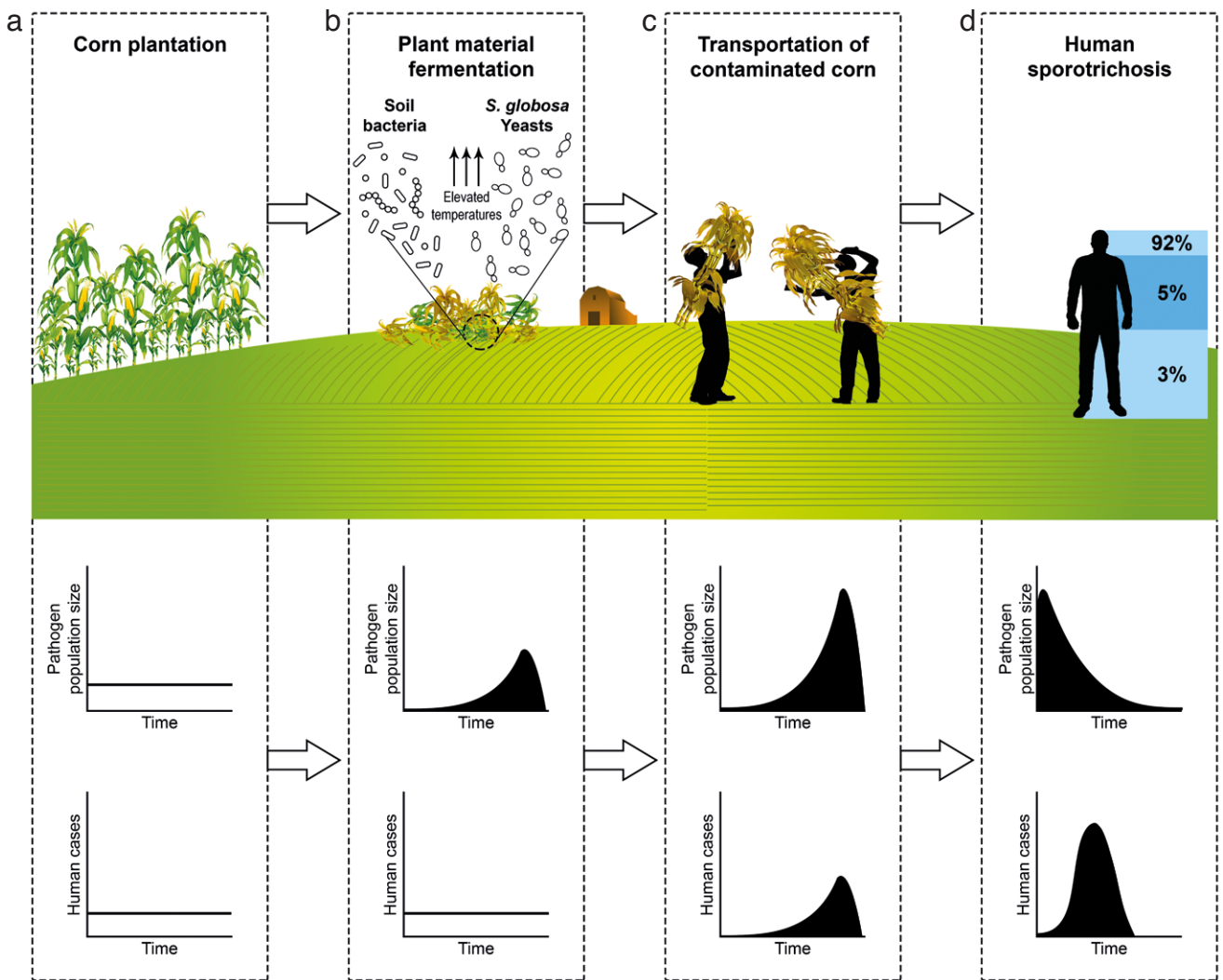


Fig. 7 Schematic overview of the hypothesis for the dissemination of the plant-born pathogen *Sporothrix globosa*, applied to the large human epidemics occurring in corn crops in north-east China. a. The species has not been found as an endophyte. b. Specific conditions in decaying plant material, such as fermentation, may stimulate excessive growth of the thermodependent yeasts, which facilitates infections in mammals. c. Onset of facial infections during transportation of corn debris. d. Delayed development of human infection during decline of growth in plant debris.

human sapronosis 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 di-

gestive 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-

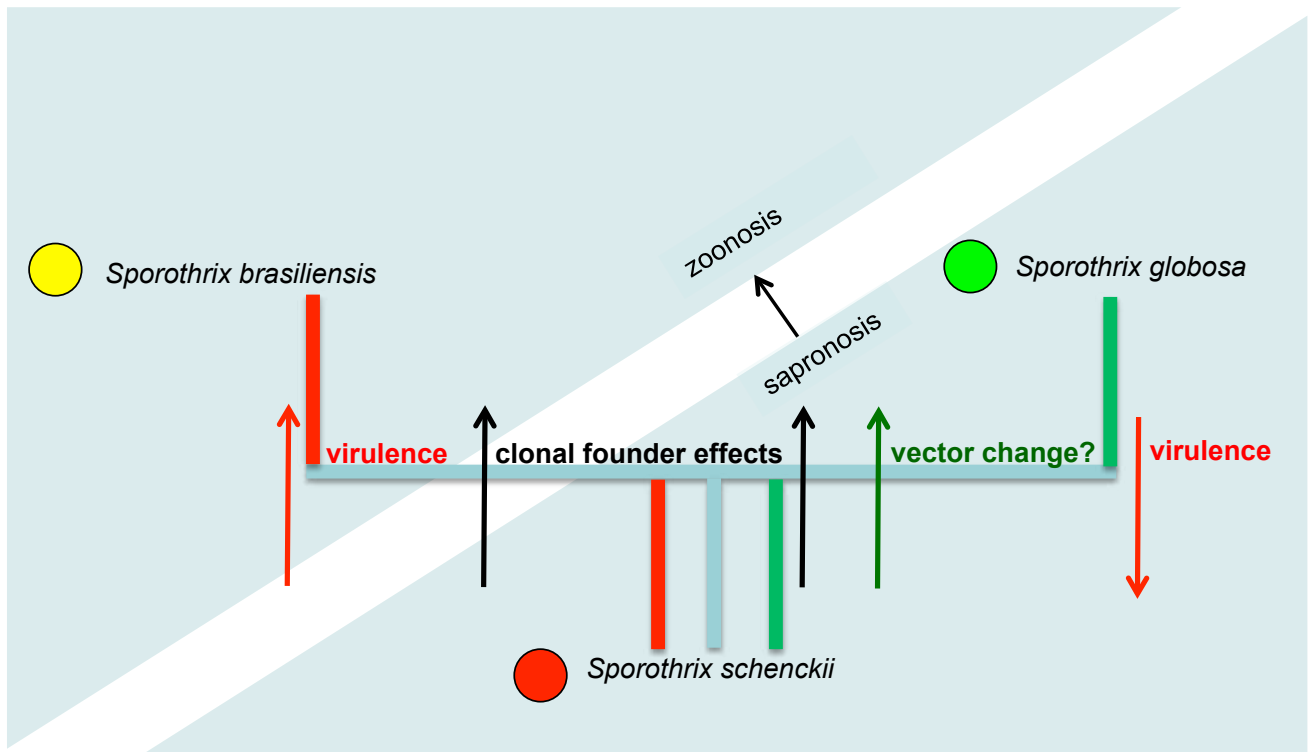


Fig. 8 Diagram of hypothetical processes of evolution from highly diverse ancestral species *S. schenckii* and clonal offshoots *S. globosa* and *S. brasiliensis*. Due to differential selection processes, the derived species differ significantly in virulence, transmission and type of epidemic caused.

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. *Sporothrix mexicana* 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. terbinafin, 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.

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