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Research Letter

RESEARCH LETTER – Taxonomy & Systematics

Agricultural systems as potential sources of emerging human mycoses caused by *Trichoderma*: a successful, common phylotype of *Trichoderma longibrachiatum* in the frontline

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One sentence summary: Agricultural environments as potential sources of opportunistic *Trichoderma* infections.

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ABSTRACT

Trichoderma species are abundant in different agricultural habitats, but some representatives of this genus, mainly clade *Longibrachiatum* members are also emerging as causative agents of various human diseases with even fatal outcome. Strains of these species frequently show resistance to commonly used azole antifungals. Based on previous data it is hypothesized that *Trichoderma* isolates identified in human infections derive from environmental—including agricultural—origins.
We examined *Trichoderma longibrachiatum* Rifai and *Trichoderma bissettii* Sandoval-Denis & Guarro strains recovered from four novel cases of human mycoses, along with isolates from previous case reports and different agricultural habitats, using multilocus phylogenetic analysis, BIOLOG Phenotype Microarrays and Etest. Strains attributed to *T. bissettii* were more abundant in both clinical and agricultural specimens compared to *T. longibrachiatum*. The majority of the isolates of both taxa could tolerate >256, >32 and >32 μg/ml fluconazole, itraconazole and posaconazole, respectively. None of the obtained results revealed characteristic differences between strains of clinical and agricultural origin, nor between the two taxa, supporting that agricultural environments may be significant sources of infections caused by these emerging human fungal pathogens. Furthermore, based on our findings we propose the re-classification of *T. bissettii* as *T. longibrachiatum* f. sp. *bissettii*.

Keywords: *Trichoderma*; emerging mycoses; agricultural environments; multilocus phylogenetic analysis; azole resistance; GCPSR concept; *Trichoderma longibrachiatum* f. sp. *bissettii* nom. prov.

INTRODUCTION

Members of the filamentous fungal genus *Trichoderma*, including *Trichoderma longibrachiatum* Rifai, are commonly isolated from agricultural environments, such as cultivated soils and mushroom farms (Castle *et al.* 1998, Hatvani *et al.* 2007; Druzhinina *et al.* 2008; Choi *et al.* 2010; Błaszczyk *et al.* 2011; Poosapati *et al.* 2014; Al-Sadi *et al.* 2015; Jiang *et al.* 2016; Wang *et al.* 2016). Furthermore, certain isolates were shown to have the potential to be used in various agricultural applications, e.g. for plant growth promotion or the biological control of different plant pathogens (Djian *et al.* 1991; Srivastava *et al.* 2012; Al-Shammari *et al.* 2013; Poosapati *et al.* 2014; Zhang, Gan and Xu 2014, 2016; Nandini *et al.* 2017; Zhang *et al.* 2017). At the same time, case reports about a range of human diseases caused by *T. longibrachiatum*, e.g. skin and lung infections, allergic sinusitis, peritonitis, sinusitis sphenoidalis, as well as disseminated infections in the stomach, heart, brain and liver of hosts mainly with impaired immune system are being published in an increasing number (Kredics *et al.* 2011; Hatvani *et al.* 2013; Molnár-Gábor *et al.* 2013; Rodríguez Peralta *et al.* 2013; Tascini *et al.* 2016; Akagi *et al.* 2017; Piecková *et al.* 2017).

Formerly *T. longibrachiatum* was considered as a uniform species, however, in a recent study it has been divided into two distinct groups, and beside *T. longibrachiatum*, a new species, *Trichoderma bissettii* Sandoval-Denis & Guarro has been described and introduced (Sandoval-Denis *et al.* 2014).

Environmental isolates of *T. longibrachiatum* and *T. bissettii*—including strains originated from agricultural specimens—are presumed to have the potential to cause mycoses in humans. Therefore, the aims of the present study were the identification of *Trichoderma* strains recovered from four recent cases of human infections, characterization of the isolates in comparison with strains from previous cases and those obtained from agricultural samples using different methods, as well as the comprehensive revision of previously published data.

MATERIALS AND METHODS

Fungal isolates

The four clinical *Trichoderma* strains involved in this study were isolated from the ear swab taken from a chronic external otitis patient in Croatia (Case 1), the wall of the aorta of a Hungarian patient with previous aortic valve implantation (Case 2), the inflammable fluid excreted from the pacemaker sac of another cardiology patient from Hungary (Case 3) and the corneal scraping taken from a fungal keratitis patient in India (Case 4), and deposited in the Szeged Microbiology Collection (SZMC, www.szmc.hu) at the Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary. Several *T. longibrachiatum* isolates of the SZMC collection, which had been recovered previously from agricultural environments (wheat rhizosphere and the growing substrate of cultivated mushrooms), were also involved in the study. The complete list of the examined fungal strains is shown in Table 1. The strains were maintained on PDA (Potato Dextrose Agar) medium at 37°C.

Species identification

Species identification of the four *Trichoderma* isolates was carried out based on the PCR amplification and sequence analysis of the internal transcribed spacers (ITS1 and 2) of the rRNA gene cluster and a fragment of the translation elongation factor 1-alpha (*tef1*) gene. The applied primers and PCR conditions are shown in Table 2. Amplification was performed in an MJ Mini™ Personal Thermal Cycler (BIO-RAD). Sequencing of the amplicons was carried out by the sequencing service of the Biological Research Centre, Szeged, Hungary. The obtained sequence chromatograms were subjected to visual checking and correction, and the sequences were then deposited in the GenBank database under the accession numbers shown in Table 1. ITS 1 and 2 sequences were analyzed by *Trich*Okey 2.0 (Druzhinina *et al.* 2005) at www.isth.info. The *tef1* sequences were aligned using the software ClustalX2 (Larkin *et al.* 2007).

Phylogenetic analysis

Besides ITS and *tef1*, partial sequences of four other loci, namely hydrophobin 4 (*hfb4*), calmodulin (*cal1*), actin (*act1*) and RNA polymerase B subunit II (*rpb2*) were also included in the phylogenetic analysis (Table 2). The corresponding sequences of the six phylogenetic markers from the newly presented

Table 1. Strains and sequences analyzed in the study.

Species	Strain number	Specimen	Reference	GenBank accession numbers					
				ITS	*tef1*	*hfb4*	*cal1*	*act1*	*rpb2*
Clinical isolates of *T. longibrachiatum* and *T. bissettii*									
T. longibrachiatum	**SZMC 23386**	keratitis	This study	**MH236156**	**MH249953**	**MN688311**	**MN650682**	**MN641012**	**MN650665**
	TUCIM 1695 (CNM-CM 1798)	blood culture	Druzhinina *et al.* (2008)	AY920396	MH249945	MN688312	EU401473	MN641013	MN650666
	TUCIM 1689 (ATCC 201044)	neutropenic child	Druzhinina *et al.* (2008)	AY585879	EU401600	MN688313	EU401468	MN641014	MN650667
	TUCIM 1696 (CNM-CM 2171)	foot skin	Druzhinina *et al.* (2008)	AY920397	EU401606	MN688314	EU401474	MN641023	MN650668
	UTHSC 06-3659	cerebrospinal fluid	Sandoval-Denis *et al.* (2014)	KJ174214	HG931245	–	–	–	–
	UTHSC 08-1222	lung tissue	Sandoval-Denis *et al.* (2014)	KJ174219	HG931250	–	–	–	–
T. bissettii	**SZMC 21467**	otitis externa	This study	**MH236159**	**MH249952**	**MN688318**	**MN641030**	**MN641022**	**MN650672**
	SZMC 23615	endocarditis	This study	**MH236160**	**MH249954**	–	–	–	–
	SZMC 23681	pacemaker	This study	**MH236161**	**MH249955**	**MN688319**	**MN641031**	**MN641015**	**MN650673**
	TUCIM 1685* (UAMH 7955)	sinus	Druzhinina *et al.* (2008)	AY328040	EU401596	–	EU401464	–	–
	TUCIM 1686* (UAMH 7956)	lung tissue	Druzhinina *et al.* (2008)	AY328041	EU401597	–	EU401465	–	–
	TUCIM 1687* (UAMH 9515)	peritoneal effluent	Druzhinina *et al.* (2008)	AY328035	EU401598	–	EU401466	–	–
	TUCIM 1690* (ATCC 208859)	HIV-positive patient	Druzhinina *et al.* (2008)	AY328042	EU401601	–	EU401469	–	–
	TUCIM 1691* (CBS 446.95)	lung tissue	Druzhinina *et al.* (2008)	AY328039	EU401602	MN688320	EU401470	MN641016	MN650674
	TUCIM 1692* (IP 2110.92)	lung, heart	Druzhinina *et al.* (2008)	Z82902	EU401603	MN688321	EU401471	MN641017	MN650675
	TUCIM 1693* (CNM-CM 382)	peritoneal fluid	Druzhinina *et al.* (2008)	AY328034	EU401604	–	EU401472	–	–
	TUCIM 1697* (CNM-CM 2277)	sputum	Druzhinina *et al.* (2008)	AY920398	EU401607	–	EU401475	–	–
	TUCIM 2882* (SZMC IM3)	sinus lavage	Druzhinina *et al.* (2008)	EU401576	EU401627	–	EU401495	–	–
	UTHSC 08-2443 (CBS 137447) ex-type strain	sinus	Sandoval-Denis *et al.* (2014)	KJ174235	HG931266	–	–	–	–
	UTHSC 08-615	wound	Sandoval-Denis *et al.* (2014)	KJ174234	HG931265	–	–	–	–

Table 1. Continued

Species	Strain number	Specimen	Reference	GenBank accession numbers					
Environmental isolates of *T. longibrachiatum* and *T. bissettii*									
T. longibrachiatum	TUCIM 1254 (CBS 816.68, DAOM 167 674) ex-type strain	nr	Druzhinina *et al.* (2008)	EU401556	EU401591	–	EU401459	–	–
	TUCIM 1301	nr	Druzhinina *et al.* (2008)	EU401559	EU401594	–	EU401462	–	–
	TUCIM 1303	nr	Druzhinina *et al.* (2008)	EU401560	EU401595	–	EU401463	–	–
	TUCIM 680	flower soil	Druzhinina *et al.* (2008)	AY857222	EU401583	–	EU401450	–	–
	TUCIM 1890	soil, coffee-growing area	Druzhinina *et al.* (2008)	EU401568	EU401618	MN688315	EU401486	MN641024	MN650669
	TUCIM 1815	soil, coffee-growing area	Druzhinina *et al.* (2008)	EU401564	EU401613	MN688316	EU401481	MN641025	MN650670
	TUCIM 42	tea plantation	Druzhinina *et al.* (2008)	EU401547	EU401577	MN688317	EU401444	MN641026	MN650671
	TaDOR673	sorghum cultivation	Poosapati *et al.* (2014)	–	KM190859	–	–	–	–
T. bissettii	SZMC 1767*	*Agaricus* compost	This study	MH236157	MH249950	MN688322	MN650683	MN641018	MN650676
	SZMC 20810*	*Pleurotus* substrate	This study	MH236158	MH249951	–	–	–	–
	SZMC 0887*	wheat rhizosphere	Kredics *et al.* (2012)	DQ345823	MH249946	MN688323	MN650684	MN641019	MN650677
	SZMC 1012*	wheat rhizosphere	Kredics *et al.* (2012)	DQ345803	MH249947	MN688324	MN641032	MN641021	MN650678
	SZMC 1158*	wheat rhizosphere	Kredics *et al.* (2012)	DQ345810	MH249948	MN688325	MN641033	MN641020	MN650679
	SZMC 1159*	wheat rhizosphere	Kredics *et al.* (2012)	DQ345812	MH249949	MN688326	MN650685	MN641027	MN650680
	SZMC 25718	*Agaricus* compost	This study	MN329160	MN641029	MN688327	MN650686	MN641028	MN650681
	TUCIM 2879*	nr	Druzhinina *et al.* (2008)	EU401573	EU401624	–	EU401492	–	–
	TUCIM 1698*	biocontrol agent	Druzhinina *et al.* (2008)	AY585880	EU401608	–	EU401476	–	–
	TUCIM 2058*	*Agaricus* compost	Druzhinina *et al.* (2008)	–	EU401620	–	EU401488	–	–
	TUCIM 850*	soil, guava plantation	Druzhinina *et al.* (2008)	EU401555	EU401590	–	EU401458	–	–
	TUCIM 1749*	nr	Druzhinina *et al.* (2008)	EU401562	EU401611	–	EU401479	–	–
	TUCIM 1750*	nr	Druzhinina *et al.* (2008)	EU401563	EU401612	–	EU401480	–	–
	TUCIM 2056*	*Pleurotus* substrate	Druzhinina *et al.* (2008)	EU401569	EU401619	–	EU401487	–	–
	TUCIM 624*	nr	Druzhinina *et al.* (2008)	EU401551	EU401582	–	EU401449	–	–
	TUCIM 45*	nr	Druzhinina *et al.* (2008)	–	EU401578	–	EU401445	–	–
	TUCIM 2062*	mushroom compost	Druzhinina *et al.* (2008)	EU401572	EU401623	–	EU401491	–	–
	TUCIM 2059*	*Agaricus* compost	Druzhinina *et al.* (2008)	EU401570	EU401621	–	EU401489	–	–
	TUCIM 1707*	nr	Druzhinina *et al.* (2008)	EU401561	EU401610	–	EU401478	–	–
	TUCIM 710*	soil, artificial rain forest	Druzhinina *et al.* (2008)	AY857241	EU401586	–	EU401454	–	–
	TUCIM 842*	soil, clover field	Druzhinina *et al.* (2008)	EU401552	EU401587	–	EU401455	–	–
	TUCIM 849*	soil, wheat field	Druzhinina *et al.* (2008)	EU401554	EU401589	–	EU401457	–	–
ND	TUCIM 848	soil under orange tree	Druzhinina *et al.* (2008)	EU401553	EU401588	–	EU401456	–	–
	TUCIM 1889	soil, coffee-growing area	Druzhinina *et al.* (2008)	EU401567	EU401617	–	EU401485	–	–
	B29	*Agaricus* compost	Hatvani *et al.* (2007)	DQ328887	DQ364635	–	–	–	–
	T52	potting media	Al-Sadi *et al.* (2015)	LN846726	LN846778	–	–	–	–

Table 1. Continued

Species	Strain number	Specimen	Reference	GenBank accession numbers					
Further sequences used for phylogenetic analysis									
T. orientale	TUCIM 166 (GJS 88-81, ATCC 90550) ex-type strain	nr	Druzhinina *et al.* (2008)	EU401550	EU401581	–	EU401448	–	–
T. orientale	GJS 09-784	nr	Druzhinina *et al.* (2012)	–	JN175578	–	JN175402	–	JN175522
T. ghanense	GJS 08-208	nr	–	JN133556	JN133573	–	JN133530	JN133591	JN133562
T. citrinoviride	DAOM 139758	nr	Degenkolb *et al.* (2008) Druzhinina *et al.* (2012)	EU330960	EU338334	–	JQ389878	JN133585	JN175543
T. aethiopicum	PPRC W4	nr	Druzhinina *et al.* (2008) Atanasova *et al.* (2010)	EU401566	EU401615	–	EU401483	–	HM182986
T. aethiopicum	PPRC J11	nr	Druzhinina *et al.* (2008)	EU401565	EU401614	–	EU401482	–	–
T. pinnatum	GJS 04-100	nr	Druzhinina *et al.* (2012)	–	JN175571	–	JN175395	–	JN175515
T. pinnatum	GJS 02-120	nr	Druzhinina *et al.* (2012)	–	JN175572	–	JN175396	–	JN175516
T. harzianum	CBS 226.95 neotype strain	nr	Druzhinina *et al.* (2010) Chaverri *et al.* (2003)	AY605713	AY605833	MF527129	FJ577684	FJ442567	AF545549

Clinical isolates first reported in this study are set in bold.
*Originally identified as *T. longibrachiatum*, proposed as *T. longibrachiatum* f. *bissettii* nom. prov. in this study.
TUCIM: corresponding to former C.P.K. identifier (Druzhinina *et al.* 2008).
ND: The available *tef1* sequence does not allow differentiation.
nr: not relevant.

Table 2. Examined markers and PCR conditions used for species identification and phylogenetic analysis.

Locus	Primers	Temperature profile
Internal transcribed spacer (ITS)	ITS1: TCCGTAGGTGAACCTGCGG ITS4: TCCTCCGCTTATTGATATGC (Andersson *et al.* 2009)	1 cycle: 94°C, 2 min 35 cycles: 94°C, 30 s; 48°C, 40 s; 72°C, 40 s 1 cycle: 72°C, 2 min
Translation elongation factor 1α (*tef1*)	EF1-728F: CATCGAGAAGTTCGAGAAGG TEF-LLErev: AACTTGCAGGCAATGTGG (Hatvani *et al.* 2007)	1 cycle: 94°C, 1 min 30 cycles: 94°C, 1 min; 59°C, 1 min; 74°C, 50 s 1 cycle: 74°C, 7 min
Calmodulin (*cal1*)	CMD5: CCGAGTACAAGGARGCCTTC CMD6: CCGATRGAGGTCATRACGTGG (Hong *et al.* 2005)	1 cycle: 94°C, 1 min 30 cycles: 94°C, 30 s; 56°C, 30 s; 72°C, 30 s 1 cycle: 72°C, 2 min
Hydrophobin 4 (*hfb4*)	hfb4a fw: GCCTCTCTGGCCATTGCCGCGCCYGC hfb rev: XXAGCATCCTGGCACAAAACACC (Espino-Rammer *et al.* 2013)	1 cycle: 94°C, 1 min 30 cycles: 94°C, 1 min; 64°C, 1 min; 72°C, 50 s 1 cycle: 72°C, 7 min
Actin (*act1*)	Act-1: TGGGACGATATGGAlAAIATCTGGCA Act-4r: TCITCGTATTCTTGCTTIGAIATCCACAT (Voigt and Wöstemeyer 2000)	40 cycles: 95°C, 1 min; 52°C, 30 s; 72°C, 1 min
RNA polymerase B subunit II (*rpb2*)	fRPB2-5F: GAYGAYMGWGATCAY TTY GG fRPB2-7cR: CCCATRGCT TGY TTR CCC AT (Liu *et al.* 1999)	1 cycle: 95°C, 5 min 35 cycles: 95°C, 1 min; 55°C, 90 s; 72°C, 90 s 1 cycle: 72°C, 7 min

clinical isolates were aligned with previously published sequences of clinical and environmental *T. longibrachiatum* and *T. bissettii* strains (Hatvani *et al.* 2007; Druzhinina *et al.* 2008; Poosapati *et al.* 2014; Sandoval-Denis *et al.* 2014; Al-Sadi *et al.* 2015) (Table 1) using MAFFT v7.312 with the G-INS-i strategy (Katoh and Standley 2013). Alignments of each loci were concatenated and Maximum likelihood analysis on the concatenated dataset was conducted by RAxML v8.121 (Stamatakis 2014) using the GTR model with G rate heterogeneity. The final length of the dataset was 3722 bp long with 211 parsimony informative sites, alignment was partitioned by loci. Statistical support of the best ML tree was obtained with 1000 thorough bootstrap replicates.

Metabolic profiling

Carbon source assimilation assays of clinical and environmental isolates (Druzhinina *et al.* 2008) were performed using BIOLOG FF Microplates at 25, 30 and 35°C as described previously (Druzhinina *et al.* 2006; Friedl, Kubicek and Druzhinina 2008; Atanasova and Druzhinina 2010). Two-way ANOVA was used to assess the differences between previously reported *T. bissettii* and *T. longibrachiatum* strains. Principal Component Analysis was performed using the ClustVis online tool (https://biit.cs.ut.ee/clustvis, Metsalu and Vilo 2015). Growth curves for every species cultivated on individual carbon sources were plotted using R (version 3.2.2).

Antifungal susceptibility testing

The MIC (Minimum Inhibitory Concentration) values of various antifungal drugs (amphotericin B (AMB), fluconazole (FLC), voriconazole (VRC), itraconazole (ITC), posaconazole (PSC), caspofungin (CSP), anidulafungin (AND) and micafungin (MCF)) against *Trichoderma* isolates were determined using the Etest method modified for moulds, according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France, http://www.biomerieux-diagnostics.com/etest).

RESULTS AND DISCUSSION

Clinical description of four patients with Trichoderma *infection*

Case 1: A 56-year-old, otherwise healthy female patient in Croatia had chronic external otitis infection for 2 months in 2010. Based on the clinical observations, she was initially treated with azithromycin. However, the patient was continued to have symptoms such as erythema, discomfort, scaling and mild pruritus limited to the external auditory canal along with fluffy and grayish-green discharge. The ear swab was obtained and cultured on blood agar (BA) and Sabouraud dextrose agar (SDA) medium supplemented with 50 μg/ml chloramphenicol. The pure mould colonies observed within 72 hours upon incubation at room temperature on both media were becoming green with age. Initial identification of the fungal isolate (SZMC 21467, Table 1) as a member of the genus *Trichoderma* was performed according to microscopic features. Subsequently, the patient was treated with terbinafine as a topical therapy, and the hygiene of the colonized external ear canal was also improved, which resulted in complete recovery from the infection.

Case 2: A 71-year-old Hungarian man with previous aortic valve implantation in his anamnesis presented in outpatient department in 2015 with increased inflammation parameters, profuse diarrhea, fever, reduced renal function and elevated liver enzymes. Besides several, small, filament-like mobile structures, transesophageal echocardiography revealed a mass of undulating vegetation 8 × 4 mm in size (Fig. 1). All blood cultures were negative for fungi. However, culturing clinical samples—taken from the removed aortic valve and the wall of the aorta during his reoperation—revealed filamentous fungal growth (SZMC 23615, Table 1) after 48 hours of incubation. In spite of adequate antifungal therapy by intravenous voriconazole (Pfizer), the patient has died due to another cause.

Case 3: A 75-year-old Hungarian woman underwent pacemaker implant surgery in 2009. Her pacemaker's sac became enlarged in 2016. She was presented with erythematosus and

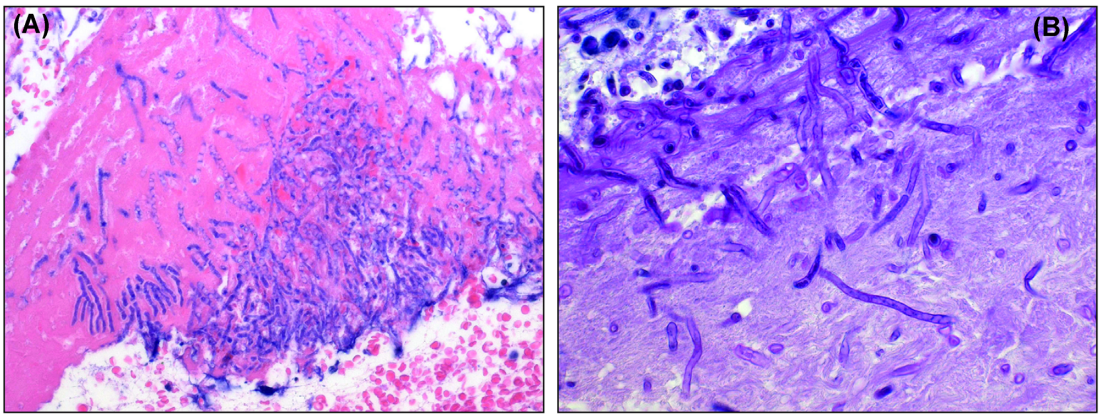

Figure 1. Microscopic visualization of hyphal elements in infected tissues (homograft resecatum, Case 2). **(A)** 40× and **(B)** 63× magnification.

warm skin surface above her implant. She had chills with no fever. Based on the clinical findings, microbial infection of pacemaker sac was suspected. The device was extracted and the collected fluid from the implant was sent for culture, which revealed the growth of a filamentous fungus (SZMC 23681, Table 1) identified as *Trichoderma* sp. based on morphological examinations.

Case 4: A 65-year-old male patient was presented to the Aravind Eye Hospital, Coimbatore, Tamilnadu, India, with complaints of pain and redness in the right eye of 2 days duration in 2014. The visual acuity was counting fingers for the right eye and 6/18 for the left eye. Examination of the right eye showed a central full thickness corneal infiltrate with feathery margins surrounded by an immune ring with hypopyon and cataractous lens. B scan ultrasonography of the right eye was within normal limits. Left eye examination revealed an immature cataract with normal fundus. Microbiological workup revealed fungal filaments in both 10% KOH wet mount and Gram staining of the smear. The culture (SZMC 23386, Table 1) of the corneal infiltrate showed the characteristics of *Trichoderma* species. The patient's treatment was started with topical 1% voriconazole (Vozole, Aurolab, India), 5% natamycin (Natamet, Sun Pharma, India) and 2% homatropine (Homide, Warren, India). The patient was a known diabetic and asthmatic on irregular treatment. His random blood sugar was 3.84 mg/ml, which was controlled well with oral hypoglycaemic agents. The patient was admitted in the hospital and intrastromal and intracameral 1 mg/ml voriconazole (Vozole-PF, Aurolab, India) was given once in 3 days. Secondary glaucoma was controlled by topical 0.5% betaxalol (Iobet, FDC, India), 0.2% brimonidine (Brimo, Alcon, India) and 1% brinzolamide (Brinolar, Sun Paharma, India) administered twice per day. Oral ketoconazole 200 mg, twice per day (Nizral, Janssen-Cilag Pharma, India) was also added to the treatment regimen. The patient showed healing infiltrate with peripheral scarring and active endothelial plaque. Due to persistent endothelial plaque, therapeutic keratoplasty along with lens extraction and anterior vitrectomy was performed. The postoperative period was uneventful. The culture of the excised corneal button also resembled *Trichoderma* spp. The patient was discharged with topical antifungals and 2% cyclosporine drops. At the 8-month follow-up visit, the visual acuity was 2/60 with graft edema and deep vessels, and aphakia in the right eye. Antiglaucoma medicines were stopped since the intraocular pressure was within normal limits.

Species identification

The four clinical isolates were identified based on the analysis of their ITS1 and 2 and *tef1* sequences. ITS sequence analysis by *Trich*Okey 2.0 did not allow an exact species level identification, as *T. longibrachiatum* shares the same ITS1 and 2 sequence with several closely related species. Therefore, an alignment was prepared from the *tef1* sequences of the four clinical isolates as well as *T. longibrachiatum* and *T. bissettii* strains reported in previous studies (Druzhinina *et al.* 2008; Kredics *et al.* 2012; Poosapati *et al.* 2014; Sandoval-Denis *et al.* 2014; Al-Sadi *et al.* 2015) (Table 1). The only difference of diagnostic value between the *tef1* sequences of *T. longibrachiatum* and *T. bissettii* was identified as a 28-bp indel within the fourth large intron of the gene: this region is present in *T. longibrachiatum* strains but missing from those known as *T. bissettii*, and can therefore be used as a hallmark for DNA BarCoding identification (Supplementary Figure S1, Supporting Information).

Among the four novel human mycoses reported in this study, *T. bissettii* could be identified as the causal agent in three cases. In Case 1 this phylotype was diagnosed from otitis externa in Croatia, where this is the first documented case of human infection due to *Trichoderma* spp. (SZMC 21467, Table 1). The inflammation of the external auditory canal can be caused by a wide range of fungal and bacterial species (Franco-Vidal *et al.* 2007; Parize *et al.* 2009; Hariga *et al.* 2010; Chen *et al.* 2011; Tarazi, Al-Tawfiq and Abdi 2012; Szigeti *et al.* 2012a, 2012b; Lee, Kim and Nguyen 2013; Song *et al.* 2014; Marchionni *et al.* 2016; Matos, Tomasin and Battelino 2016), but only a single case has been attributed to *Trichoderma* spp. so far (Hennequin *et al.* 2000), where the causal agent was identified as *T. longibrachiatum* based on ITS1 and 2 sequence analyses, therefore the exact taxonomic position of the isolate cannot be determined. The *Trichoderma* strains recovered from two different cases of heart infection in Hungary (Cases 2 and 3) also proved to be *T. bissettii* (SZMC 23615 and SZMC 23681, Table 1). The first documented case of endocarditis due to *Trichoderma* spp. was published by Bustamante-Labarta *et al.* (2000), but the isolate was not identified at the species level. Rodríguez Peralta *et al.* (2013) and Tascini *et al.* (2016) reported about cases of endocarditis caused by *T. longibrachiatum,* however, sequences enabling exact species identification have not been provided. In contrast, the fungal keratitis in India (Case 4) was attributed to *T. longibrachiatum* (SZMC 23386, Table 1). Mycotic keratitis can occur due to infection by a broad range of opportunistic fungi, such as *Aspergillus*, *Fusarium*, *Curvularia*, *Penicillium*,

Candida and *Corynespora* species, in several cases by strains of agricultural origin (Thomas and Kaliamurthy 2013; Yamada *et al.* 2013; Kredics *et al.* 2015; Słowik *et al.* 2015; Maharana *et al.* 2016; Chung, Lee and You 2018; Oostra, Schoenfield and Mauger 2018). Most of the few cases of keratitis caused by *Trichoderma* species mentioned so far in the literature lack detailed descriptions (Venugopal *et al.* 1989; Mohd-Tahir *et al.* 2012; Gharamah *et al.* 2014). In the studies of Venugopal *et al.* (1989) and Mohd-Tahir *et al.* (2012), the infection was attributed to *Trichoderma* sp., while Gharamah *et al.* (2014) classified their isolate as *Trichoderma hamatum* (Bonord.) Bain. However, Piecková *et al.* (2017) identified the causal agent of ulcerative keratitis as *Trichoderma citrinoviride* Bissett, another representative of the *Longibrachiatum* clade, presented in a well-documented case report. The same identification strategy was also performed for a set of previously collected agricultural isolates from wheat rhizosphere, Hungary (SZMC 0887, SZMC 1012, SZMC 1158, SZMC 1159); *Agaricus bisporus* (J.E. Lange) Imbach compost, Hungary (SZMC 1767) and Poland (SZMC 25718); as well as *Pleurotus ostreatus* (Jacq.) P. Kumm. growing substrate, Iran (SZMC 20810), which were originally identified as *T. longibrachiatum*—all of these isolates were found to belong to *T. bissettii* (Table 1). In addition, the majority of both clinical and environmental isolates identified as *T. longibrachiatum* in our previous study (Druzhinina *et al.* 2008) also proved to represent *T. bissettii* (9 out of 12 and 15 out of 24, respectively) (Table 1). The available *tef1* sequences of strains TUCIM 848 (Druzhinina *et al.* 2008) and B29 (Hatvani *et al.* 2007) did not contain the diagnostic regions, whereas, the *tef1* sequences have not been provided in the case of other clinical isolates (Seguin *et al.* 1995; Tanis *et al.* 1995; Munoz *et al.* 1997; Furukawa *et al.* 1998; Kuhls *et al.* 1999; Richter *et al.* 1999; Hennequin *et al.* 2000; Chouaki *et al.* 2002; Myoken *et al.* 2002; Tang *et al.* 2003; Aroca, Piontelli and Cruz 2004; Lee *et al.* 2007; Alanio *et al.* 2008; Lagrange-Xélot *et al.* 2008; Santillan Salas *et al.* 2011; Tascini *et al.* 2016; Akagi *et al.* 2017) and agricultural strains (Castle *et al.* 1998; Choi *et al.* 2010; Błaszczyk *et al.* 2011; Srivastava *et al.* 2012; Al-Shammari *et al.* 2013; Zhang, Gan and Xu 2014; Jiang *et al.* 2016; Wang *et al.* 2016; Nandini *et al.* 2017; Zhang *et al.* 2017) reported previously as *T. longibrachiatum*. Furthermore, based on the *tef1* sequence provided for strain T52 (originally reported as *T. longibrachiatum*; Al-Sadi *et al.* 2015), it does not represent either *T. longibrachiatum* or *T. bissettii*, therefore a repeated analysis is recommended in order to identify the isolate more adequately.

Phylogenetic analysis

Previously *T. longibrachiatum* was known as a single agamospecies (Druzinina *et al.* 2008) but Sandoval-Denis *et al.* (2014) separated it into two species by attributing several strains to the new species *T. bissettii* based on the topology of *tef1* phylogram. In this study we used the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept (Dettman, Jacobson and Taylor 2003) that implies that a species should be supported by the topologies of at least two unlinked loci and not contradicted by several others. Consequently, as addition to the ITS1 and 2 and *tef1* phylogenetic markers, we used the hyperpolymorfic sequence of the gene encoding hydrophobin 4 (*hfb4*), which is part of the core genome of *Trichoderma* spp. (Espino-Rammer *et al.* 2013) and therefore may be used for differentiation in uncertain cases, as well as fragments of the *cal1* (Hong *et al.* 2005), *act1* (Voigt and Wöstemeyer 2000) and *rpb2* (Liu, Whelen and Hall 1999) genes. Similarly to ITS1 and 2, *cal1* also proved inappropriate to differentiate between *T. longibrachiatum* and *T. bissettii sensu* Sandoval-Denis *et al.* (2014) due to the lack of sequence variability (data not shown). The concatenated phylogram (Fig. 2) shows that although *T. longibrachiatum* could be distinguished from *T. bissettii* based also on *hfb4*, *act1* and *rpb2* as single locus markers, altogether their separation to two different species is not supported. Therefore, we propose the placement of *T. bissettii sensu* Sandoval-Denis *et al.* (2014) to the rank of 'phylotype' under the name *T. longibrachiatum* f. sp. *bissettii* nom. prov., and suggest the characteristic 28-bp indel inside the fourth large intron of *tef1* (Supplementary Figure S1, Supporting Information) as a DNA BarCoding hallmark to differentiate between this and other *T. longibrachiatum* phylotypes. Our results indicate that *T. longibrachiatum* f. sp. *bissettii* very frequently occurs in agricultural environments: all putative *T. longibrachiatum* isolates of agricultural origin (wheat rhizosphere and *A. bisporus* compost, Hungary and Poland; *P. ostreatus* substrate, Iran) included in this study proved to be *T. longibrachiatum* f. sp. *bissettii*. Altogether, the majority of both clinical and environmental isolates originally identified as *T. longibrachiatum* represent *T. longibrachiatum* f. sp. *bissettii*, which can therefore be considered as a succesful, commonly occurring phylotype of *T. longibrachiatum sensu lato*.

Metabolic profile analysis

The carbon source assimilation profile of clinical and environmental isolates of *T. longibrachiatum* s. st. (*sensu stricto*) and the phylotype *T. longibrachiatum* f. sp. *bissettii* nom. prov. was assessed using BIOLOG FF Microplates at 25, 30 and 35°C (Supplementary Figure S2, Supporting Information). Statistical analysis reveal no significant difference between the two phylotypes as well as between clinical and environmental isolates at three tested temperatures (ANOVA, $P > 0.05$). Principal component analysis performed based on the cumulative dataset also confirmed overlapping metabolic profiles between *T. longibrachiatum* f. sp. *bissettii* and *T. longibrachiatum* (Fig. 3).

Antifungal susceptibility testing

The antifungal susceptibility data revealed minor differences among the isolates. However, all the examined strains were found to be susceptible to MCF and VRC (MIC: 0.0275-2 and 0.75-5 μg/mL, respectively), while they showed tolerance to ITC, FLC and PSC (MIC: 32 μg/mL or higher, 64–256 and 32 μg/mL or higher, respectively). Furthermore, the strain *T. longibrachiatum* f. sp. *bissettii* SZMC 21467 was found to tolerate AMB and CSP (MICs: 32 μg/mL), while *T. longibrachiatum* f. sp. *bissettii* SZMC 23615 showed resistance to AND (MIC: >32 μg/mL) and its MIC value for MCF was also higher (2 μg/mL) in comparison to the rest of the examined strains. Clinical and agricultural isolates of *T. longibrachiatum* s. st. and *T. longibrachiatum* f. sp. *bissettii* did not show characteristic differences in susceptibility to the tested antifungal drugs (Table 3). Espinel-Ingroff (2001) used the same method to determine the MIC values of AMB and ITC in five clinical *T. longibrachiatum* isolates and observed values of 1.0–4.0 and >8 μg/mL, respectively. Antal *et al.* (2005) compared the potential virulence factors, including the antifungal drug resistance of clinical and agricultural isolates originally reported or reidentified as *T. longibrachiatum*. All isolates showed resistance to FLC (MIC: 64–256μg/mL), while diversity was observed in their tolerance to ITC and AMB (MIC: 0.5–32 and 0.016–8 μg/mL, respectively). They were found to be most susceptible to ketoconazole (MIC: 0.008–1 μg/mL) but the highest MIC value (1 μg/mL) was observed in the case of *T. longibrachiatum* f. sp. *bissettii*

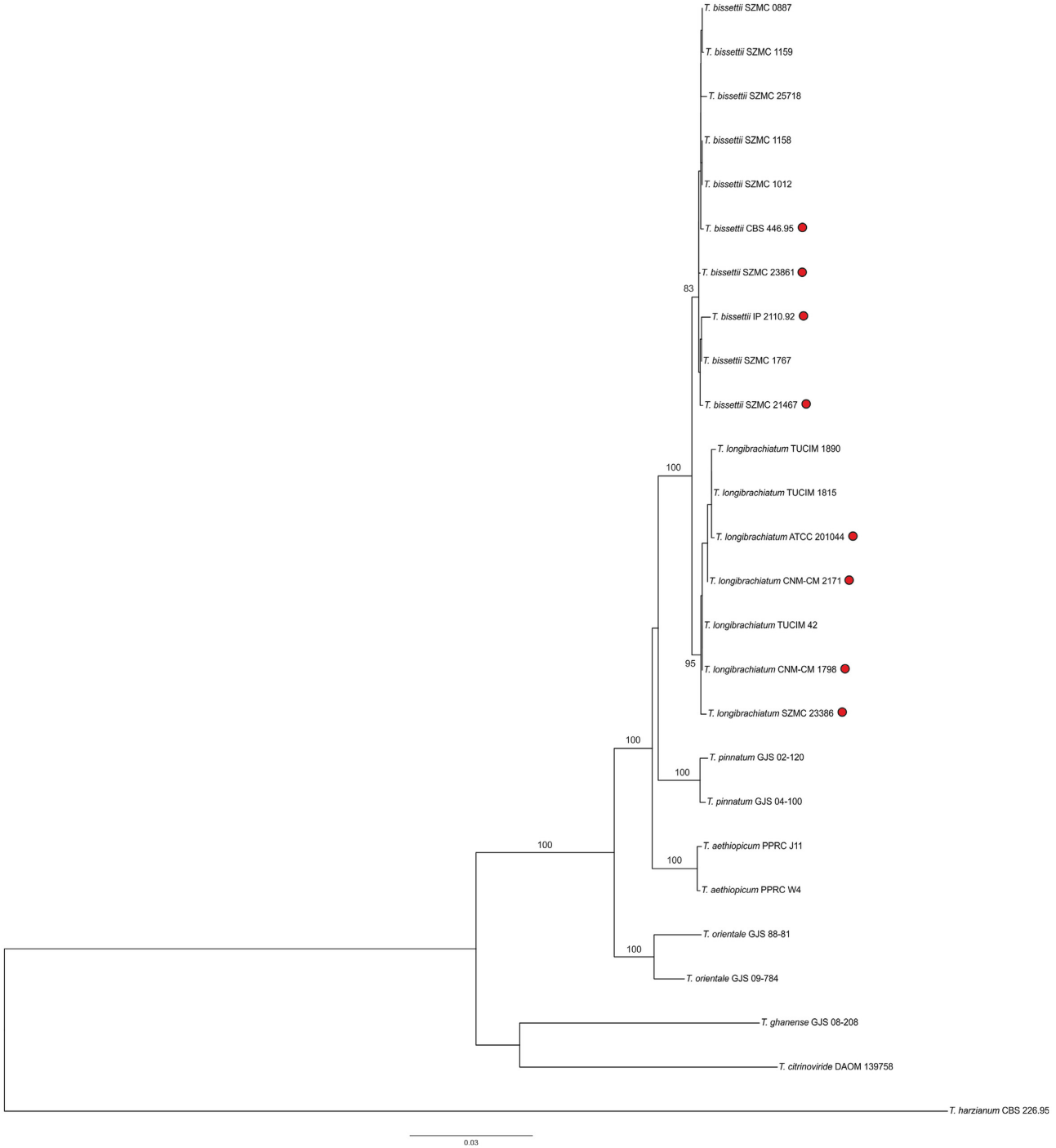

Figure 2. Maximum likelihood tree showing the phylogenetic relationship between environmental and clinical isolates of *T. longibrachiatum* and *T. bissettii* based on the concatenated loci ITS1 and 2, *tef1*, *hfb4*, *act1*, *cal1* and *rpb2*. Statistical support of the best ML tree was obtained with 1000 thorough bootstrap replicates. Clinical isolates are marked with red dot.

strains. Molnár-Gábor *et al.* (2013) reported *T. longibrachiatum* SZMC IM3 (re-identified as *T. longibrachiatum* f. sp. *bissettii* in the present study) as the causal agent of sinusitis sphenoidalis. The strain was resistant to FLC and ITC, while low MIC values for AMB, VRC and CSP were observed (MIC: >256, > 2, 0.5, 0.5 and 0.25 μg/mL, respectively). In the study of Tascini *et al.* (2016) a case of endocarditis was attributed to *T. longibrachiatum*, which was found to be susceptible to VRC, AMB and CSP (MIC: 0.5, 1 and 1 μg/mL, respectively). Other authors determined the antifungal susceptibility of clinical isolates reported as *T. longibrachiatum* and *T. bissettii sensu* Sandoval-Denis *et al.* (2014) using different techniques, such as the broth dilution or disk method, but high level of tolerance to azoles, especially FLC, ITC and PSC were detected (Kredics *et al.* 2011; Sandoval-Denis *et al.* 2014). Although there is no clear evidence for the phenomenon (Hollomon 2017), the emerging triazole resistance among clinical isolates of different filamentous fungi, such as *Aspergillus, Fusarium* and *Alternaria* species is proposed to be a potential

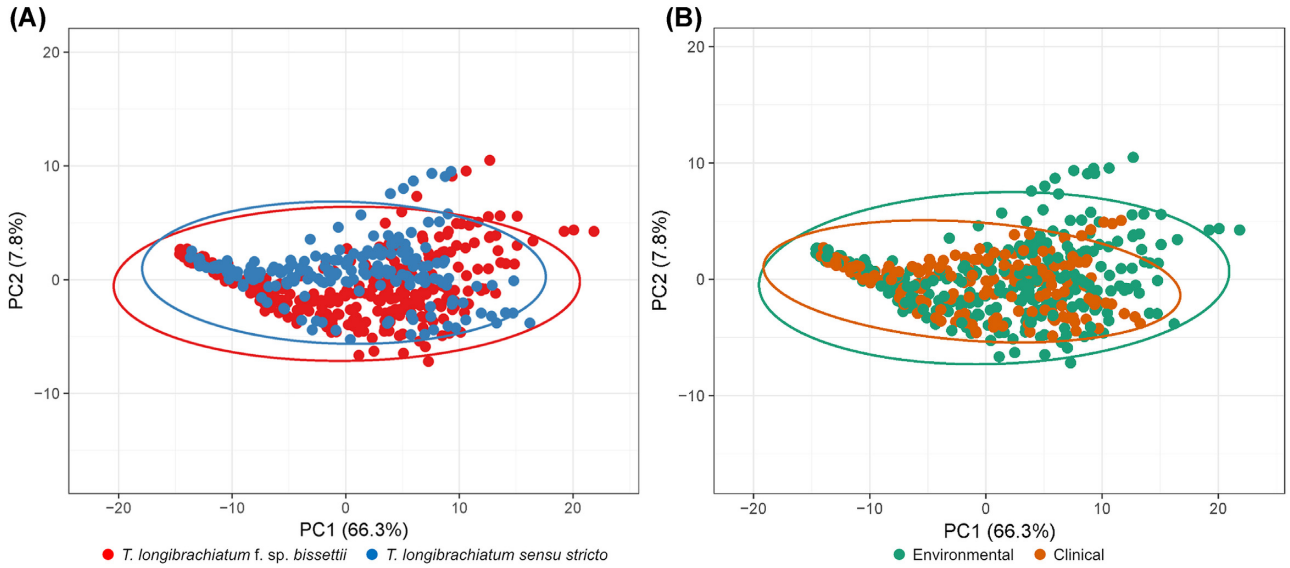

Figure 3. Principal component analysis performed by ClustVis on the complete dataset deriving from carbon source assimilation assays of clinical and environmental *T. longibrachiatum* s. st. and *T. longibrachiatum* f. sp. *bissettii* isolates performed on BIOLOG FF Microplates at 25, 30 and 35°C.

Table 3. Minimum inhibitory concentration (MIC: μg/mL) of antifungal drugs determined by the Etest method modified for moulds.

Strain	Source	AMB	AND	CSP	MCF	ITC	FLC	PSC	VRC
T. longibrachiatum SZMC 23386	keratitis	1	0.44*	0.29*	0.04*	>32	>256	>32	5
T. longibrachiatum TUCIM 42	tea plantation	2.5	0.38*	0.38*	0.064*	>32	64*	>32	2
T. bissettii SZMC 21467	otitis externa	32	0.0435*	32	0.0275*	32	>256	32	1
T. bissettii SZMC 23615	endocarditis	4	>32	0.23*	2*	>32	>256	>32	1.5
T. bissettii SZMC 23681	pacemaker	1.5	0.25*	0.078*	0.064*	>32	>256	>32	0.75
T. bissettii SZMC 1158	wheat rhizosphere	4	0.25*	0.38*	0.032*	>32	>256	>32	3
T. bissettii SZMC 1767	mushroom compost	7	0.25*	0.5*	0.064*	>32	>256	>32	2

AMB: amphotericin B, AND: anidulafungin, CSP: caspofungin, MCF: micafungin, ITC: itraconazole, FLC: fluconazole, PSC: posaconazole, VRC: voriconazole.
*Microcolonies in the inhibition ellipse.
The clinical isolates reported in this study are set in bold.

consequence of the extensive use of azole pesticides in agriculture (O'Donnell *et al.* 2008; Chowdhary *et al.* 2013; Verweij *et al.* 2013; Ribas *et al.* 2016; Berger *et al.* 2017), which is supposed to apply to *T. longibrachiatum* s. st. and *T. longibrachiatum* f. sp. *bissettii* as well.

CONCLUSION

The findings presented in this study strongly support the hypothesis that isolates of the species *T. longibrachiatum* and its newly proposed phylotype *T. longibrachiatum* f. sp. *bissettii* nom. prov. recovered from human mycoses may originate from agricultural environments.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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Conflict of Interests. None declared.

REFERENCES

Akagi T, Kawamura C, Terasawa N *et al.* Suspected pulmonary infection with *Trichoderma longibrachiatum* after allogeneic stem cell transplantation. *Internal Med* 2017;**56**: 215–9.

Alanio A, Brethon B, Feuilhade de Chauvin M *et al*. Invasive pulmonary infection due to *Trichoderma longibrachiatum* mimicking invasive Aspergillosis in a neutropenic patient successfully treated with voriconazole combined with caspofungin. *Clin Infect Dis* 2008;**46**:e116–8.

Al-Sadi AM, Al-Oweisi FA, Edwards SG *et al*. Genetic analysis reveals diversity and genetic relationship among *Trichoderma* isolates from potting media, cultivated soil and uncultivated soil. *BMC Microbiol* 2015;**15**:147.

Al-Shammari TA, Bahkali AH, Elgorban AM *et al*. The use of *Trichoderma longibrachiatum* and *Mortierella alpina* against root-knot nematode, *Meloidogyne javanica* on tomato. *J Pure Appl Microbiol* 2013;**7**:199–207.

Andersson MA, Mikkola R, Raulio M *et al*. Acrebol, a novel toxic peptaibol produced by an *Acremonium exuviarum* indoor isolate. *J Appl Microbiol* 2009;**106**:909–23.

Antal Z, Kredics L, Pakarinen J *et al*. Comparative study of potential virulence factors in human pathogenic and saprophytic *Trichoderma longibrachiatum* strains. *Acta Microbiol Immunol Hung* 2005;**52**:341–50.

Aroca TS, Piontelli LE, Cruz CHR. Case Report: *Trichoderma longibrachiatum* infections in a pediatric patient with peritoneal dialysis. *Bol Micol* 2004;**19**:13–7.

Atanasova L, Druzhinina IS. Review: global nutrient profiling by Phenotype MicroArrays: a tool complementing genomic and proteomic studies in conidial fungi. *J Zhejiang Univ Sci B* 2010;**11**:151–68.

Atanasova L, Jaklitsch WM, Komon-Zelazowska M *et al*. Clonal species *Trichoderma parareesei* sp. nov. likely resembles the ancestor of the cellulase producer *Hypocrea jecorina/T. reesei*. *Appl Environ Microbiol* 2010;**76**:7259–67.

Berger S, El Chazli Y, Babu AF *et al*. Azole resistance in *Aspergillus fumigatus*: a consequence of antifungal use in agriculture? *Front Microbiol* 2017;**8**:1024.

Błaszczyk L, Popiel D, Chełkowski J *et al*. Species diversity of *Trichoderma* in Poland. *J Appl Genet* 2011;**52**:233–43.

Bustamante-Labarta MH, Caramutti V, Allende GN *et al*. Unsuspected embolic fungal endocarditis of an aortic conduit diagnosed by transesophageal echocardiography. *J Am Soc Echocardiogr* 2000;**13**:953–4.

Castle A, Speranzini D, Rghei N *et al*. Morphological and molecular identification of *Trichoderma* isolates on North American mushroom farms. *Appl Environ Microbiol* 1998;**64**:133–7.

Chaverri P, Castlebury LA, Overton BE *et al*. *Hypocrea/Trichoderma*: species with conidiophore elongations and green conidia. *Mycologia* 2003;**95**:1100–40.

Chen YA, Chan KC, Chen CK *et al*. Differential diagnosis and treatments of necrotizing otitis externa: a report of 19 cases. *Auris Nasus Larynx* 2011;**38**:666–70.

Choi IY, Choi JN, Sharma PK *et al*. Isolation and identification of mushroom pathogens from *Agrocybe aegerita*. *Mycobiology* 2010;**38**:310–5.

Chouaki T, Lavarde V, Lachaud L *et al*. Invasive infections due to *Trichoderma* species: report of 2 cases, findings of *in vitro* susceptibility testing, and review of the literature. *Clin Infect Dis* 2002;**35**:1360–7.

Chowdhary A, Kathuria S, Xu J *et al*. Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. *PLoS Pathog* 2013;**9**:e1003633.

Chung SR, Lee HS, You IC. Fungal keratitis caused by *Corynespora cassiicola*, a plant pathogen. *J Mycol Infect* 2018;**23**:24–6.

Degenkolb T, Dieckmann R, Nielsen KF *et al*. The *Trichoderma brevicompactum* clade: a separate lineage with new species, new peptaibiotics, and mycotoxins. *Mycol Prog* 2008;**7**:177–219.

Dettman JR, Jacobson DJ, Taylor JW. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* 2003;**57**:2703–20.

Djian C, Pijarowski L, Ponchet M *et al*. Acetic acid: a selective nematocidal metabolite from culture filterates of *Paecilomyces lilacinus* (Thom) Samson and *Trichoderma longibrachiatum* Rifai. *Nematologica* 1991;**37**:101–12.

Druzhinina IS, Komon-Zelazowska M, Ismaiel A *et al*. Molecular phylogeny and species delimitation in the section *Longibrachiatum* of *Trichoderma*. *Fungal Genet Biol* 2012;**49**:358–68.

Druzhinina IS, Komoń-Zelazowska M, Kredics L *et al*. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. *Microbiology* 2008;**154**:3447–59.

Druzhinina IS, Kopchinskiy AG, Komoń M *et al*. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genet Biol* 2005;**42**:813–28.

Druzhinina IS, Kubicek CP, Komon-Zelazowska M *et al*. The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. *BMC Evol Biol* 2010;**10**:94

Druzhinina IS, Schmoll M, Seiboth B *et al*. Global carbon utilization profiles of wild-type, mutant, and transformant strains of *Hypocrea jecorina*. *Appl Environ Microbiol* 2006;**72**:2126–33.

Espinel-Ingroff A. Comparison of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi. *J Clin Microbiol* 2001;**39**:1360–7.

Espino-Rammer L, Ribitsch D, Przylucka A *et al*. Two novel class II hydrophobins from *Trichoderma* spp. stimulate enzymatic hydrolysis of poly(ethylene terephthalate) when expressed as fusion proteins. *Appl Environ Microbiol* 2013;**79**:4230–8.

Franco-Vidal V, Blanchet H, Bebear C *et al*. Necrotizing external otitis: a report of 46 cases. *Otol Neurotol* 2007;**28**:771–3.

Friedl MA, Kubicek CP, Druzhinina IS. Carbon source dependence and photostimulation of conidiation in *Hypocrea atroviridis*. *Appl Environ Microbiol* 2008;**74**:245–50.

Furukawa H, Kusne S, Sutton DA *et al*. Acute invasive sinusitis due to *Trichoderma longibrachiatum* in a liver and small bowel transplant recipient. *Clin Infect Dis* 1998;**26**:487–9.

Gharamah AA, Moharram AM, Ismail MA *et al*. Bacterial and fungal keratitis in Upper Egypt: *in vitro* screening of enzymes, toxins and antifungal activity. *Indian J Ophthalmol* 2014;**62**:196–203.

Hariga I, Mardassi A, Belhaj Younes F *et al*. Necrotizing otitis externa: 19 cases' report. *Eur Arch Otorhinolaryngol* 2010;**267**:1193–8.

Hatvani L, Antal Z, Manczinger L *et al*. Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma* species. *Phytopathology* 2007;**97**:532–7.

Hatvani L, Manczinger L, Vágvölgyi C *et al*. *Trichoderma* as a human pathogen. In: Mukherjee PK , Horwitz BA , Singh US , Mukherjee M , Schmoll M (eds.). *Trichoderma - Biology and Applications*. Wallingford: CABI, 2013, 292–313.

Hennequin C, Chouaki T, Pichon JC *et al*. Otitis externa due to *Trichoderma longibrachiatum*. *Eur J Clin Microbiol Infect Dis* 2000;**19**:641–2.

Hollomon D. Does agricultural use of azole fungicides contribute to resistance in the human pathogen *Aspergillus fumigatus*? *Pest Manag Sci* 2017;**73**:1987–93.

Hong SB, Go SJ, Shin HD *et al*. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. *Mycologia* 2005;**97**:1316–29.

Jiang Y, Wang JL, Chen J *et al*. *Trichoderma* biodiversity of agricultural fields in East China reveals a gradient distribution of species. *PLoS One* 2016;**11**:e0160613.

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;**30**:772–80.

Kredics L, Hatvani L, Manczinger L *et al*. Chapter 62. *Trichoderma*. In: Liu D (ed.). *Molecular Detection of Human Fungal Pathogens*. London: Taylor & Francis Group, 2011, 509–26.

Kredics L, Láday M, Körmöczi P *et al*. Genetic and biochemical diversity among *Trichoderma* isolates in soil samples from winter wheat fields of the Great Hungarian Plain. *Acta Biol Szeged* 2012;**56**:141–9.

Kredics L, Narendran V, Shobana CS *et al*. Filamentous fungal infections of the cornea: a global overview of epidemiology and drug sensitivity. *Mycoses* 2015;**58**:243–60.

Kuhls K, Lieckfeldt E, Börner T *et al*. Molecular reidentification of human pathogenic *Trichoderma* isolates as *Trichoderma longibrachiatum* and *Trichoderma citrinoviride*. *Med Mycol* 1999;**37**:25–33.

Lagrange-Xélot M, Schlemmer F, Gallien S *et al*. *Trichoderma* fungaemia in a neutropenic patient with pulmonary cancer and human immunodeficiency virus infection. *Clin Microbiol Infect* 2008;**14**:1190–2.

Larkin MA, Blackshields G, Brown NP *et al*. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007;**23**:2947–8.

Lee HJ, Kim DW, Cho HS *et al*. Peritonitis and intra-abdominal abscess by *Trichoderma longibrachiatum* in a patient undergoing continous ambulatory peritoneal dialysis (CAPD). *Korean J Nephrol* 2007;**26**:254–7.

Lee H, Kim J, Nguyen V. Ear infections: otitis externa and otitis media. *Prim Care* 2013;**40**:671–86.

Liu YL, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol Biol Evol* 1999;**16**:1799–808.

Maharana PK, Sharma N, Nagpal R *et al*. Recent advances in diagnosis and management of mycotic keratitis. *Indian J Ophthalmol* 2016;**64**:346–57.

Marchionni E, Parize P, Lefevre A *et al*. *Aspergillus* spp. invasive external otitis: favourable outcome with a medical approach. *Clin Microbiol Infect* 2016;**22**:434–7.

Matos T, Tomazin R, Battelino S. First report of otitis externa caused by *Schizophyllum commune* and review of the literature. *Wien Klin Wochenschr* 2016;**128**:387–90.

Metsalu T, Vilo J. Clustvis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Res* 2015;**43**:W566–70.

Mohd-Tahir F, Norhayati A, Siti-Raihan I *et al*. A 5-year retrospective review of fungal keratitis at Hospital Universiti Sains Malaysia. *Interd Perspect Infect Dis* 2012,**2012**:851563.

Molnár-Gábor E, Dóczi I, Hatvani L *et al*. Isolated sinusitis sphenoidalis caused by *Trichoderma longibrachiatum* in an immunocompetent patient with headache. *J Med Microbiol* 2013;**62**:1249–52.

Munoz FM, Demmler GJ, Travis WR *et al*. *Trichoderma longibrachiatum* infection in a pediatric patient with aplastic anemia. *J Clin Microbiol* 1997;**35**:499–503.

Myoken Y, Sugata T, Fujita Y *et al*. Fatal necrotizing stomatitis due to *Trichoderma longibrachiatum* in a neutropenic patient with malignant lymphoma: a case report. *Int J Oral Maxillofac Surg* 2002;**31**:688–91.

Nandini B, Hariprasad P, Prakash HS *et al*. Trichogenic-selenium nanoparticles enhance disease suppressive ability of *Trichoderma* against downy mildew disease caused by *Sclerospora graminicola* in pearl millet. *Sci Rep* 2017;**7**:2612.

O'Donnell K, Sutton DA, Fothergill A *et al*. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and *in vitro* antifungal resistance within the *Fusarium solani* species complex. *J Clin Microbiol* 2008;**46**:2477–90.

Oostra TD, Schoenfield LR, Mauger TF. *Candida dubliniensis*: a novel cause of fungal keratitis. *IDCases* 2018;**14**:e00440.

Parize P, Chandesris MO, Lanternier F.*et al*. Antifungal therapy of *Aspergillus* invasive otitis externa: efficacy of voriconazole and review. *Antimicrob Agents Chemother* 2009;**53**:1048–53.

Piecková E, Globanová M, Pol'anová M *et al*. *Trichoderma citrinoviride* Bissett from the section Longibrachiatum caused ulcerative keratitis - a case study. *Derma* 2017;**17**:3–6.

Poosapati S, Ravulapalli PD, Tippirishetty N *et al*. Selection of high temperature and salinity tolerant *Trichoderma* isolates with antagonistic activity against *Sclerotium rolfsii*. *Springerplus* 2014;**3**:641.

Ribas ADR, Spolti P, Del Ponte EM *et al*. Is the emergence of fungal resistance to medical triazoles related to their use in the agroecosystems? A mini review. *Braz J Microbiol* 2016;**47**:793–9.

Richter S, Cormican MG, Pfaller MA *et al*. Fatal disseminated *Trichoderma longibrachiatum* infection in an adult bone marrow transplant patient: species identification and review of the literature. *J Clin Microbiol* 1999;**37**:1154–60.

Rodríguez Peralta LI, Mañas Vera MR, García Delgado MJ *et al*. Endocarditis caused by *Trichoderma longibrachiatum* in a patient receiving home parenteral nutrition. *Nutr Hosp* 2013;**28**:961–4.

Sandoval-Denis M, Sutton DA, Cano-Lira JF *et al*. Phylogeny of the clinically relevant species of the emerging fungus *Trichoderma* and their antifungal susceptibilities. *J Clin Microbiol* 2014;**52**:2112–25.

Santillan Salas CF, Joshi AY, Dhiman N *et al*. Fatal postoperative *Trichoderma longibrachiatum* mediastinitis and peritonitis in a paediatric patient with complex congenital cardiac disease on peritoneal dialysis. *J Med Microbiol* 2011;**60**:1869–71.

Seguin P, Degeilh B, Grulois I *et al*. Successful treatment of a brain abscess due to *Trichoderma longibrachiatum* after surgical resection. *Eur J Clin Microbiol Infect Dis* 1995;**14**:445–8.

Słowik M, Biernat MM, Urbaniak-Kujda D *et al*. Mycotic infections of the eye. *Adv Clin Exp Med* 2015;**24**:1113–7.

Song JE, Haberkamp TJ, Patel R *et al*. Fungal otitis externa as a cause of tympanic membrane perforation: a case series. *Ear Nose Throat J* 2014;**93**:332–6.

Srivastava PK, Shenoy BD, Gupta M *et al*. Stimulatory effects of arsenic-tolerant soil fungi on plant growth promotion and soil properties. *Microbes Environ* 2012;**27**:477–82.

Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;**30**:1312–3.

Szigeti G, Kocsubé S, Dóczi I *et al*. Molecular identification and antifungal susceptibilities of black *Aspergillus* isolates from otomycosis cases in Hungary. *Mycopathologia* 2012a;**174**:143–7.

Szigeti G, Sedaghati E, Mahmoudabadi AZ *et al*. Species assignment and antifungal susceptibilities of black aspergilli recovered from otomycosis cases in Iran. *Mycoses* 2012b;**55**:333–8.

Tang P, Mohan S, Sigler L *et al.* Allergic fungal sinusitis associated with *Trichoderma longibrachiatum*. *J Clin Microbiol* 2003;**41**:5333–6.

Tanis BC, van der Pijl H, van Ogtrop ML *et al.* Fatal fungal peritonitis by *Trichoderma longibrachiatum* complicating peritoneal dialysis. *Nephrol Dial Transplant* 1995;**10**:114–6.

Tarazi AE, Al-Tawfiq JA, Abdi RF. Fungal malignant otitis externa: pitfalls, diagnosis, and treatment. *Otol Neurotol* 2012;**33**:769–73.

Tascini C, Cardinali G, Barletta V *et al.* First case of *Trichoderma longibrachiatum* CIED (Cardiac Implantable Electronic Device)-associated endocarditis in a non-immunocompromised host: biofilm removal and diagnostic problems in the light of the current literature. *Mycopathologia* 2016;**181**:297–303.

Thomas PA, Kaliamurthy J. Mycotic keratitis: epidemiology, diagnosis and management. *Clin Microbiol Infect* 2013;**19**:210–20.

Venugopal PL, Venugopal TL, Gomathi A *et al.* Mycotic keratitis in Madras. *Indian J Pathol Microbiol* 1989;**32**:190–7.

Verweij PE, Kema GH, Zwaan B *et al.* Triazole fungicides and the selection of resistance to medical triazoles in the opportunistic mould *Aspergillus fumigatus*. *Pest Manag Sci* 2013;**69**:165–70.

Voigt K, Wöstemeyer J. Reliable amplification of actin genes facilitates deep-level phylogeny. *Microbiol Res* 2000;**155**:179–95.

Wang G, Cao X, Ma X *et al.* Diversity and effect of *Trichoderma* spp. associated with green mold disease on *Lentinula edodes* in China. *Microbiologyopen* 2016;**3**:641.

Yamada H, Takahashi N, Hori N *et al.* Rare case of fungal keratitis caused by *Corynespora cassiicola*. *J Infect Chemother* 2013;**19**:1167–9.

Zhang S, Gan Y, Xu B. Efficacy of *Trichoderma longibrachiatum* in the control of *Heterodera avenae*. *BioControl* 2014;**59**:319–31.

Zhang S, Gan Y, Xu B. Application of plant-growth promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front Plant Sci* 2016;**7**:1405.

Zhang S, Gan Y, Ji W *et al.* Mechanisms and characterization of *Trichoderma longibrachiatum* T6 in suppressing nematodes (*Heterodera avenae*) in wheat. *Front Plant Sci* 2017;**8**:1491.