

A new pleosporalean fungus isolated from superficial to deep human clinical specimens.

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Keyword:	Ascomycota, filamentous fungi, infection, mycoses, Pleosporales
Abstract:	Human infections by pleosporalean fungi (class <i>Dothideomycetes</i> , phylum <i>Ascomycota</i>) are rarely reported. Because their identification is challenging using morphological characterization, several phylogenetic markers must be sequenced for an accurate identification and taxonomical placement of the isolates. Three isolates of clinical origin were phenotypically characterized, but due to the absence of relevant morphological traits, D1-D2 domains of the 28S nrRNA gene (LSU), the internal transcribed spacer region (ITS) of the nrRNA, and fragments of the RNA polymerase II subunit 2 (rpb2) and translation elongation factor 1-alpha (tef1) genes were sequenced to allow a phylogenetic analysis that would solve their phylogenetic placement. That analysis revealed that these isolates did not match any previously known pleosporalean genera, and they are proposed here as the new fungal genus, <i>Gambiomyces</i> . Unfortunately, the isolates remained sterile, which, consequently, made the morphological description of the reproductive structures impossible. Future studies should try to understand the behaviour of this fungus in nature as well as its characteristics as an opportunistic fungal pathogen. Molecular identification is becoming an essential tool for proper identification of Dothideomycetes of clinical origin.

SCHOLARONE™ Manuscripts A new pleosporalean fungus isolated from superficial to deep human clinical specimens.

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Running title: New pleosporalean fungus of clinical interest in Europe.

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ABSTRACT

Human infections by pleosporalean fungi (class Dothideomycetes, phylum Ascomycota) are rarely reported. Because their identification is challenging using morphological characterization, several phylogenetic markers must be sequenced for an accurate identification and taxonomical placement of the isolates. Three isolates of clinical origin were phenotypically characterized, but due to the absence of relevant morphological traits, D1-D2 domains of the 28S nrRNA gene (LSU), the internal transcribed spacer region (ITS) of the nrRNA, and fragments of the RNA polymerase II subunit 2 (rpb2) and translation elongation factor 1-alpha (tef1) genes were sequenced to allow a phylogenetic analysis that would solve their phylogenetic placement. That analysis revealed that these isolates did not match any previously known pleosporalean genera, and they are proposed here as the new fungal genus, Gambiomyces. Unfortunately, the isolates remained sterile, which, consequently, made the morphological description of the reproductive structures impossible. Future studies should try to understand the behaviour of this fungus in nature as well as its characteristics as an opportunistic fungal pathogen. Molecular identification is becoming an essential tool for proper identification of Dothideomycetes of clinical origin.

LAY ABSTRACT

We describe a new pleosporalen pathogenic fungus, *Gambiomyces profunda*, found in superficial to deep samples from a human patient. Because all strains remained sterile, the fungus was finally identified following a phylogenetic analysis by using four different molecular markers.

Keywords: Ascomycota, filamentous fungi, infection, mycoses, *Pleosporales*.

Introduction

Pleosporales encompasses approximately 55 families, and is the largest order in the class Dothideomycetes. In humans, pleosporalean fungi produce mainly superficial and subcutaneous infections, mostly due to a traumatic implantation of contaminated plant material or soil particles.² Pleosporalean taxa responsible for opportunistic human infections can be divided based on their asexual reproduction in hyphomycetous (whose conidia are borne from free conidiophores), encompassing the genera Alternaria, Bipolaris, Curvularia, and Exserohilum, and coelomycetous (whose conidia are formed within a cavity composed of fungal or fungal-host tissues), encompassing a large number of genera, such as Boeremia, Didymella, Epicoccum, Medicopsis, Neocurcubitaria, Paraconiothyrium, Phoma, and Stagonosporopsis, among others.^{3,4} Many of these taxa are characterized by having melanin in their cell wall, which is one of their virulence factors. Recent studies on coelomycetous fungi conducted in the USA demonstrated that members of *Pleosporales* are the most frequently involved in human mycoses.⁵ Similar results have been reported by Garcia-Hermoso et al. (2019)⁶ in Europe. Pleosporalean fungi implicated in human infections usually are not well documented, as the culture conditions routinely used in clinical laboratories do not always favour the development of their fertile reproductive structures, essential for the morphological characterization of isolates. Consequently, a PCR-based amplification and sequencing of several phylogenetic markers are necessary for an accurate identification at species-level.^{2,3,5} In the present study, we describe three isolates of a melanized filamentous fungus recovered from biopsy samples taken from a patient presenting with subcutaneous phaeophyphomycosis. As the isolates did not produce any reproductive structure under several culture conditions, the molecular identification was carried out. Four phylogenetic markers were sequenced (LSU, ITS, rpb2 and tef1) and a phylogenetic analysis was made

in order to ascertain its taxonomic placement, finding that this fungus was within the *Pleosporales* but did not closely match any previously described genetic sequences.

Methods

Three fungal strains isolated from the deep layer of skin, joint fluid and bone biopsy samples of the same patient (see Table 1), were studied morphologically following the protocols of Valenzuela-Lopez *et al.* (2017).⁵ Additionally, potato-carrot agar (PCA; 20 g each of filtered potatoes and carrots, 20 g of agar, 1 L of distilled water) was used to obtain the reproductive structures. Their growth at cardinal temperatures were determined on potato dextrose agar (PDA; Pronadisa, Madrid) after 7 d in darkness, ranging from 5 to 35 °C, at 5 °C intervals, and including 37 °C. The production of metabolite E+ (NaOH spot test) was carried out by the application of a droplet of 1N NaOH on a colony grown on MEA.^{7,8}

A preliminary molecular identification of the isolates was made using four loci: LSU with the primers pair LR0R/LR5, ITS (ITS5/TS4), *rpb*2 (RPB2-5F2/fRPB2-7cR) and *tef*1 (TEF1-983F/TEF1-2218R).⁹⁻¹⁴ Those were sequenced in both directions with the same primer pair used for amplification at Macrogen Europe (Macrogen Inc., Madrid, Spain). The consensus sequences were obtained using the SeqMan software v. 7 (DNAStar Lasergene, Madison, WI, USA). The four loci sequences were first analysed in BLASTn searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the CBS database (www.cbs.knaw.nl). Only the sequences of type or reference strains deposited in GenBank /CBS databases were considered for identification purposes. A level of identity ≥98% was considered for species-level identification.

In order to determine the taxonomic placement of these strains, a phylogenetic analysis was made using sequences of species obtained from GenBank, listed in Table 1. For the phylogenetic study, the sequences were aligned through MEGA v. 6.06,¹⁵ using the

ClustalW¹6 application, refined with MUSCLE¹7 and manually adjusted using the same software platform. Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) with RAxML v. 8.2.10¹8 and MrBayes v. 3.2.6,¹9 respectively. The best substitution model for each gene matrix corresponded to GTR+I+G, and it was estimated using MrModelTest v. 2.3.²0 Support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) ≥70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried out with 10 M generations, with samples taken every 1,000 generations. The 50 % majority rule consensus trees and posterior probability values (PP) were calculated after removing the first 25 % of the resulting trees for burnin. A PP value ≥0.95 was considered as significant. Both ML and BS analyses were run in CIPRES.²¹ Sequences generated in this study were deposited in GenBank (see Table 1), and the taxonomic novelties in the MycoBank (www.mycobank.org).²²

Results

The histopathological study of the patient's lesions revealed a presence of hyphae associated with necrotizing granulomas and chronic osteomyelitis. Despite being inoculated onto different culture media, the strains recovered from clinical specimens failed to form fertile reproductive (asexual and/or sexual) structures, although they were able to grow temperatures ranging between 15 and 37 °C. Molecular identification based on the BLASTn search did not match any of the previously known pleosporalean fungi, indicating that our strains likely represented a novel species. The closest hits using the LSU, ITS, *rpb2*, and *tef1* sequences were *Subplenodomus valerianae* CBS 630.68 (GenBank MH870914; Identities = 838/863 (97%), gaps = 1/863 (0%)), *Medicopsis romeroi* CNRMA4.200 (GenBank KP132404; Identities = 411/482 (85%), gaps = 27/482 (5%)), *Aposphaeria corallinolutea* MFLU 15-2752 (GenBank KY554207; Identities =

644/841 (77%), gaps = 6/841 (0%)) and *Medicopsis romeroi* UTHSC DI16-315 (GenBank LT797119; Identities = 897/979 (92%), gaps = 2/979 (0%)), respectively. The ITS sequences of our three strains revealed that they all corresponded to the same fungus. For the phylogenetic study, the final concatenated dataset with three phylogenetic markers (LSU, *rpb*2 and *tef*1) were used to perform the Maximum likelihood (ML) and the Bayesian inference (BI). A phylogenetic analysis included 164 strains with a total of 2,000 characters including gaps (833 for LSU, 441 for *rpb*2 and 726 for *tef*1), of which 805 were parsimony informative (306 for LSU, 249 for *rpb*2 and 250 for *tef*1). The ML analysis showed a similar tree topology and was congruent with that obtained by BI analysis. For the BI multi-locus analysis, a total of 117,428 trees were sampled after the burn-in with a stop value of 0.01. In the phylogenetic tree (Fig. 1), which includes 28 of the closest families of the 70 accepted into the order *Pleosporales*, the strain FMR 17178 formed a distinct and distant clade together with *Massariosphaeria phaeospora*, a species that had previously been included in the family *Cyclothyriellaceae* by Jaklitsch and Voglmayr.²³ Therefore, we propose our fungal isolates are of a new genus *Gambiomyces*.

Taxonomy

Gambiomyces Valenzuela-Lopez, Stchigel, Martin-Gomez & Cano, gen. nov. MycoBank MB 835155.

Etymology: The name *Gambio*- refers to the country of origin of the patient from whom the fungus was isolated (The Gambia, Africa), and from the Greek *-mykēs*, fungus.

Colonies remaining sterile. Hyphae septate, smooth- to nodose, thin- to thick-walled, hyaline at first, becoming brown with the age, forming big masses of tightly hyphae forming dark brown exopigment. Chlamydospore-like and setae-like structures present.

Type species: Gambiomyces profunda Valenzuela-Lopez, Stchigel, Martin-Gomez & Cano.

Gambiomyces profunda Valenzuela-Lopez, Stchigel, Martin-Gomez & Cano, sp. nov. MycoBank MB 835156. (Fig. 2)

Etymology: From Latin -profundus, deep, because the fungus infects deep tissues.

Colonies remaining sterile. Hyphae septate, smooth to nodose, thin- to thick-walled, hyaline at first, becoming brown with the age, 2–6 µm wide, anastomosing to form large masses (up to 800 µm diam.) of closely tight hyphae producing a dark brown exopigment. from which arise not septate, dark brown, short, sinuous setae-like structures with a rounded tip. *Chlamydospore*-like cells present. NaOH spot test negative. Crystals absent. Optimal, minimum and maximum temperatures of growth: 25, 15 and 37 °C, respectively. Culture characteristics (Fig. 2): Colonies on malt extract agar (MEA) reaching 5–8 mm diam after 7 d at 25±1 °C, slightly floccose, white (M. 3A1); reverse white (M. 3A1) to pale yellow (M. 3A3). Colonies on oatmeal agar (OA) reaching 6–11 mm diam after 7 d at 25±1 °C, flattened, front and reverse white (M. 3A1). Colonies on potato-carrot agar (PCA) reaching 10–11 mm diam after 7 d at 25±1 °C, slightly floccose, white (M. 6A1) to brown (M. 6E5); reverse brownish orange (M. 6C3). Colonies on PDA reaching 10 mm diam after 7 d at 25±1 °C, slightly floccose, white (M. 3A1) to pale-yellow (M. 3A3); reverse white (M. 6A1) to dark brown (M. 6F3). NaOH spot test negative. Crystals absent. Optimal, minimum and maximum temperatures of growth: 25, 15 and 37 °C, respectively. Material examined: **Spain**, from human joint fluid, 2018, isolated by MT Martin-Gomez (holotype FMR H-17178, ex-type living culture FMR 17178); from human skin biopsy,

2018, MT Martin-Gomez, living culture FMR 17177; from human bone, 2018, MT Martin-Gomez, living culture FMR 17179.

Notes: To our knowledge, these strains recovered from a case of human mycosis are phylogenetically distinct to other taxa of the order *Pleosporales*. Unfortunately, those isolates remained sterile, so a more detailed morphological characterization was not possible. However, they correspond to a new opportunistic fungus able to grow at 37°C and even infect deep human tissues.

Discussion

In the last thirty years the reports of coelomycetous fungi from clinical samples, although infrequent, have been increasing. Most of these are phoma-like and pyrenochaeta-like fungi that are involved in superficial mycoses, and are occasionally involved in subcutaneous and deep tissue infections.^{5,24} Regarding human infections, about 17 coelomycetous genera and more than 22 species within the *Pleosporales* have already been reported, in contrast to hyphomycetous pelosporalean fungi, whose presence is restricted to only four genera.^{3,25}

The three strains described here were obtained from a patient who had been resident in Spain for several years but born in Gambia, a tropical African country. The strains studied were isolated from three different clinical specimens affecting the lower limb at different levels of tissue penetration (skin, joint fluid and bone). Morphological identification posed a challenge for the clinical laboratory due to the difficulties in producing reproductive structures, which could have provided a clue to its identification. This is commonplace in human melanized fungi recovered from clinical species, making it difficult to correctly assign to a particular genus and species.

The current use of DNA sequences comparison in fungal taxonomy has improved the identification of the *Pleosporales* and their classification. The taxonomy of several genera of coelomycetous pleosporalean fungi involved in human infections, such as *Phoma* and *Pyrenochaeta* has been reviewed recently, resulting in major changes in their nomenclature.^{24,26}

This taxonomic reassessments have been driven by the fact that their original taxon concepts resulted in polyphyletic taxa, where the morphological features of the fertile reproductive structures are not informative enough for identification.

DNA sequences comparison of four different molecular markers provided support that the melanized fungus isolated from human mycoses, ranging from superficially to deeply infected tissues, represents a new opportunistic human pathogen. Importantly, the fungus has the ability to grow at 37°C. Other new fungi recovered from human infections that are unable to generate reproductive structures and have been described recently, i.e. *Emarellia grisea* and *Emarellia paragrisea*. These species can produce eumycetoma, which are characterized by their chronic progressive destruction of soft tissues provoking tumefaction and drainage of fungal dark grains. ²⁷ *Pseudochaetosphaeronema martinelli* has been recently described. It is another sterile, melanized fungus that has been involved in cases of subcutaneous phaeohyphomycosis by itsdirect implantation into subcutaneous tissues by injuries probably caused by contaminated plant material. ²⁸ These species have been isolated in various geographical regions (India, Martinique and Sri Lanka) having a tropical climate in common.

DNA sequence comparisons of phylogenetic informative markers have improved fungal identification, especially from clinical sources. In our work the isolates could only be assessed by sequencing multiple phylogenetic markers. The ribosomal markers ITS and

LSU are often used by clinicians, but additional markers such as *rpb2*, *tef1*, *tub2* and others are required in many cases to achieve an acceptable level of accuracy. ^{29,30} Despite the fact that our strains could initially be distinguished by sequencing the nrRNA genes, a phylogenetic analysis was need to understand their affiliation when compared comprehensively against other members in the order *Pleosporales*. Furthermore, the present study provides new sequences of the ITS, *rpb2* and *tef1* markers of this new fungus to help identification in the future using DNA barcode data. Despite the *Pleosporales* comprising more than seventy families, our isolates could not be placed in any of them, and so remains in an uncertain position (*incertae sedis*) in the same terminal clade as CBS 611.86, which has been identified previously as *Massariosphaeria phaeospora*. Despite *M. phaeospora* being the type species of the genus, it is clearly polyphyletic, ³¹ and CBS 611.86 is not the ex-type strain of such species. Consequently, further studies are need to perform in order to clarify the taxonomic placement of *Massariosphaeria* and our fungus. Further studies are needed to understand the biological cycle of this potential human pathogen, and its source and manner of infection.

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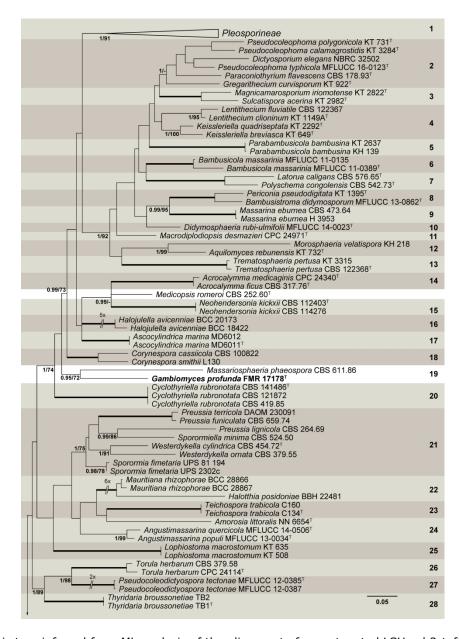
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FIGURE LEGEND

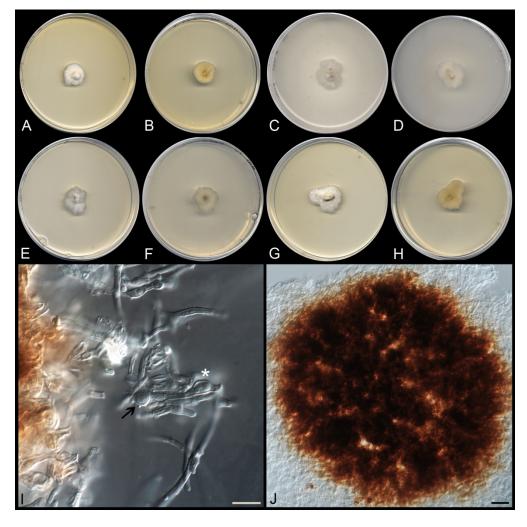
FIG 1 Phylogenetic tree inferred from ML analysis of the alignment of concatenated LSU -rpb2-tef1 nucleotide sequences of 164 strains representing different taxa within the Pleosporales. The Bayesian posterior probabilities (PP) above 0.95 and the RAxML bootstrap support values (BS) above 70 % are given at the nodes (PP/BS). Fully supported branched (1 PP/100 BS) are indicated in bold. Some branches were shortened to fit them to the page, and these are indicated by two diagonal lines with the number of times a branch was shortened. The families within the order Pleosporales are represented by the numbered clades. Clade 1 represents the suborder Pleosporineae. The novel taxa are given in bold. Type strains are indicated by a superscript "T". The tree was rooted with the type strains of Chaetomella zambiensis CBS 137978 and Pilidium pseudoconcavum CPC 21642 both members of Chaetomellaceae family in the order Chaetomellales.

FIG 2 Colonies characteristic of strain FMR 17178 after 14 d at 25±1 °C in different culture media. A-B. MEA (front in the left and reverse in the right). C-D. OA (front and reverse). E-F. PCA (front and reverse). G-H. PDA (front and reverse). I. Chlamydospore-like cells (indicated by an arrow), inflated hypha (indicated by an asterisk). J. Anastomosing large masses (up to 800 μm diam.) of closely tight hyphae producing a dark brown exopigment, from which arise not septate, dark brown, short, sinuous setae-like structures with a rounded tip. Scale bars: $I = 10 \mu m$, $J = 25 \mu m$.



Phylogenetic tree inferred from ML analysis of the alignment of concatenated LSU-rpb2-tef1 nucleotide sequences of 164 strains representing different taxa within the *Pleosporales*. The Bayesian posterior probabilities (PP) above 0.95 and the RAxML bootstrap support values (BS) above 70 % are given at the nodes (PP/BS). Fully supported branched (1 PP/100 BS) are indicated in bold. Some branches were shortened to fit them to the page, and these are indicated by two diagonal lines with the number of times a branch was shortened. The families within the order *Pleosporales* are represented by numbered clades. Clade 1 represents the suborder *Pleosporineae*. The novel taxa are given in bold. Type strains are indicated by a superscript "T". The tree was rooted with the type strains of *Chaetomella zambiensis* CBS 137978 and *Pilidium pseudoconcavum* CPC 21642 both members of *Chaetomellaceae* family in the order *Chaetomellales*.

210x297mm (300 x 300 DPI)



Colonies characteristic of strain FMR 17178 after 14 d at 25 ± 1 °C in different culture media. A-B. MEA (front in the left and reverse in the right). C-D. OA (front and reverse). E-F. PCA (front and reverse). G-H. PDA (front and reverse). I. Chlamydospore-like cells (indicated by an arrow), inflated hypha (indicated by an asterisk). J. Anastomosing large masses (up to 800 μ m diam.) of closely tight hyphae producing a dark brown exopigment, from which arise not septate, dark brown, short, sinuous setae-like structures with a rounded tip. Scale bars: I = 10 μ m, J = 25 μ m.

165x165mm (300 x 300 DPI)

Table 1. Isolates used in this study and their GenBank accession numbers. Newly generated sequences and proposed species are indicated in bold.

Family (clade number in	Species	Strain number	Host, substrate	Country	I	GenBank accession numbers				
Fig. 1)					LSU	ITS	RPB2	TEF-1α		
Acrocalymmaceae (14)	Acrocalymma ficus	CBS 317.76 ^T	Ficus sp.	India	KP170712	NR_137953	-	-		
	Acrocalymma medicaginis	CPC 24340 ^T	Medicago sativa	Australia	KP170713	KP170620	-	-		
Aigialaceae (39)	Aigialus grandis	BCC 18419	Mangrove wood	Malaysia	GU479774	-	GU479813	GU479838		
	Aigialus grandis	BCC 20000	Mangrove wood	Malaysia	GU479775	-	GU479814	GU479839		
Amniculicolaceae (37)	Amniculicola lignicola	CBS 123094 ^T	Submerged wood of <i>Fraxinus</i> excelsior	France	EF493861	-	EF493862	GU456278		
Amorosiaceae (24)	Amorosia littoralis	NN 6654 ^T	Marine inter-tidal sediment	Bahamas	AM292055	-	-	-		
	Angustimassarina populi	MFLUCC 13-0034 ^T	Populus sp.	Italy	KP888642	KP899137	-	KR075164		
	Angustimassarina quercicola	MFLUCC 14-0506 ^T	Quercus robur	Germany	KP888638	KP899133	-	KR075169		
Anteagloniaceae (48)	Anteaglonium abbreviatum	ANM925a	Decorticated woody substrate	USA	GQ221877	-	-	GQ221924		
	Anteaglonium latirostrum	GKM1119 ^T	Decorticated woody substrate	Kenya	GQ221874	-	-	GQ221937		
	Anteaglonium latirostrum	GKML100Nb	Decorticated woody substrate	Kenya	GQ221876	-	-	GQ221938		
	Anteaglonium parvulum	GKM219N	Decorticated woody substrate	Kenya	GQ221881	-	-	GQ221916		
Aquasubmersaceae (45)	Aquasubmersa mircensis	MFLUCC 11-0401 ^T	Submerged wood	Thailand	NG_042699	NR_121545	-	-		
Ascocylindricaceae (17)	Ascocylindrica marina	$MD6011^{T}$	Decayed wood	Saudi Arabia	KT252905	-	-	-		
	Ascocylindrica marina	MD6012	Submerged decayed wood of Avicennia marina	Egypt	KT252906	-	-	-		
Astrosphaeriellaceae (43)	Astrosphaeriella fusispora	MFLUCC 10-0555	Dead stem of bamboo	Thailand	KT955462	-	-	-		
Bambusicolaceae (6)	Bambusicola massarinia	MFLUCC 11-0135	Bamboo	Thailand	KU863111	KU940122	KU940169	KU940192		
	Bambusicola massarinia	MFLUCC 11-0389 ^T	Decaying culm of bamboo	Thailand	JX442037	NR_121548	-	-		
Camarosporiaceae (1)	Camarosporomyces flavigenus	CBS 314.80^{T}	Water	Romania	GU238076	KY929138	-	-		
	Camarosporium quaternatum	CPC 31081 ^T	Lycium barbarum	Hungary	KY929171	KY929136	-	KY929201		
	Camarosporium quaternatum	CPC 31518	Lycium barbarum	Hungary	KY929172	KY929137	-	KY929202		
Camarosporidiellaceae (1)	Camarosporidiella aborescentis	MFLUCC 17-0738	Amorpha sp.	Russia	MF434204	MF434117	-	MF434380		
	Camarosporidiella caraganicola	MFLUCC 14-0605 ^T	Caragana frutex	Russia	KP711381	KP711380	-	-		
Caryosporaceae (42)	Caryospora minima	Unknown	Fresh water	China	EU196550	-	-	-		

Chaetomellaceae (outgroup)	Chaetomella zambiensis	CBS 137978 ^T	Unidentified Fabaceae	Zambia	KJ869187	KJ869130	-	-
	Pilidium pseudoconcavum	CPC 21642 ^T	Leaves of Greyia radlkoferi	South Africa	KF777236	KF777184	-	-
Coniothyriaceae (1)	Coniothyrium palmarum	CBS 758.73	Phoenix dactylifera	Israel	EU754154	-	-	-
	Coniothyrium palmarum	CBS 400.71	Chamaerops humilis	Italy	EU754153	AY720708	KT389592	-
	Ochrocladosporium elatum	CBS 146.33 ^T	Wood pulp	Sweden	EU040233	EU040233	-	-
	Ochrocladosporium frigidarii	CBS 103.81 ^T	Cooled room	Germany	EU040234	EU040234	-	-
	Staurosphaeria aptrootii	CBS 483.95 ^T	Lycium sp.	The Netherlands	GU301806	NR_155186	-	GU349044
	Staurosphaeria lycii	MFLUCC 17-0210 ^T	Lycium barbarum	Russia	MF434284	NR_154460	-	MF434460
	Staurosphaeria rhamnicola	MFLUCC 17-0813	Rhamnus sp.	Uzbekistan	MF434288	MF434200	-	MF434462
	Staurosphaeria rhamnicola	MFLUCC 17-0814 ^T	Rhamnus sp.	Uzbekistan	MF434289	NR_154461	-	MF434463
Corynesporaceae (18)	Corynespora cassiicola	CBS 100822	Sainopaulia ionantha	The Netherlands	GU301808	-	GU371742	GU349052
	Corynespora smithii	L130	Fagus sylvatica	Austria	KY984298	KY984298	KY984362	KY984436
Cryptocoryneaceae (46)	Cryptocoryneum condensatum	CBS 113959	Salix fragilis	Sweden	LC194350	LC096156	LC194432	LC096138
	Cryptocoryneum japonicum	KT 3300 ^T	Fagus crenata	Japan	LC194356	LC096162	LC194438	LC096144
Cucurbitariaceae (1)	Cucurbitaria berberidis	CBS 130007 ^T	Berberis vulgaris	Austria	KC506793	LT717673	LT854936	-
	Neocucurbitaria cava	CBS 257.68 ^T	Wheat-field soil	Germany	EU754199	JF740260	LT717681	-
Cyclothyriellaceae (20)	Cyclothyriella rubronotata	CBS 419.85	Dead branch of <i>Acer</i> pseudoplatanus	The Netherlands	GU301875	-	GU371728	GU349002
	Cyclothyriella rubronotata	CBS 121872	branches of Acer pseudoplatanus	Austria	KX650541	KX650541	KX650571	KX650516
	Cyclothyriella rubronotata	CBS 141486 ^T	Ulmus glabra	Austria	KX650544	KX650544	KX650574	KX650519
Dacampiaceae (1)	Dacampia hookeri	Hafellner 73897	Solorina saccata	Austria	KT383792	-	-	-
	Dacampia hookeri	Hafellner 75980	Solorina saccata	Austria	KT383794	-	-	-
Delitschiaceae (40)	Delitschia didyma	UME 31411	Animal dung	Sweden	AY853366	-	-	-
	Delitschia winteri	AFTOL-ID 1599	Dung of rabbit	The Netherlands	DQ678077	-	DQ677975	DQ677922
Dictyosporiaceae (2)	Dictyosporium elegans	NBRC 32502	Submerged wood of Rhizophora stylosa	Japan	DQ018100	DQ018087	-	-
	Gregarithecium curvisporum	KT 922 ^T	Sasa sp.	Japan	AB807547	AB809644	-	AB808523
	Paraconiothyrium flavescens	CBS 178.93 ^T	Solanum tuberosum	The Netherlands	GU238075	-	-	-

	Pseudocoleophoma calamagrostidis	KT 3284 ^T	Calamagrostis matsumurae	Japan	LC014609	LC014592	-	LC014614
	Pseudocoleophoma polygonicola	KT 731 ^T	Polygonaceous plant	Japan	AB807546	AB809634	-	AB808522
	Pseudocoleophoma typhicola	MFLUCC 16-0123 ^T	Submerged stems of <i>Typha</i> latifolia	UK	KX576656	KX576655	-	-
Didymellaceae (1)	Didymella exigua	CBS 183.55 ^T	Rumex arifolius	France	EU754155	GU237794	EU874850	-
	Neoascochyta cylindrispora	CBS 142456 ^T	Human superficial tissue	USA	LN907495	LT592963	LT593102	-
Didymosphaeriaceae (10)	Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023 ^T	Rubus ulmifolius	Italy	KJ436586	-	-	-
Dothidotthiaceae (1)	Dothidotthia aspera	CPC 12932	Acer negundo	USA	EU673275	-	-	-
	Dothidotthia symphoricarpi	CPC 12929 ^T	Symphoricarpos rotundifolius	USA	EU673273	-	-	-
Halojullaceae (16)	Halojulella avicenniae	BCC 18422	Mangrove wood	Thailand	GU371823	-	GU371787	GU371816
	Halojulella avicenniae	BCC 20173	Mangrove wood	Thailand	GU371822	-	GU371786	GU371815
Halotthiaceae (22)	Halotthia posidoniae	BBH 22481	Posidoniae oceanica	Cyprus	GU479786	-	-	-
	Mauritiana rhizophorae	BCC 28866	Mangrove wood	Thailand	GU371824	-	-	GU371817
	Mauritiana rhizophorae	BCC 28867	Mangrove wood	Thailand	GU371825	-	-	GU371818
Hermatomycetaceae (44)	Hermatomyces tectonae	KH 356	Woody plant	Japan	LC194372	LC194488	LC194454	LC194399
	Hermatomyces tectonae	MFLUCC 14-1140 ^T	Tectona grandis	Thailand	KU764695	KU144917	KU712486	-
Incertae sedis	Gambiomyces profunda	FMR 17177	Skin plate biopsy	Spain	-	LR215843	-	-
	Gambiomyces profunda	FMR 17178 ^T	Joint fluid	Spain	LR215842	LR215841	LR215845	LR215846
	Gambiomyces profunda	FMR 17179	Bone	Spain	-	LR215844	-	-
	Massariosphaeria phaeospora	CBS 611.86	Trisetum distichophyllum	Switzerland	GU301843	-	GU371794	-
	Medicopsis romeroi	CBS 252.60 ^T	Human mycetoma	Venezuela	EU754207	NR_130697	KF015708	KF015678
	Pyrenochaeta nobilis	CBS 407.76 ^T	Laurus nobilis	Italy	EU754206	MH860989	LT623276	MF795880
Latoruaceae (7)	Latorua caligans	CBS 576.65 ^T	Soil	Brazil	KR873266	NR_132923	-	-
	Polyschema congolensis	CBS 542.73 ^T	Soil	Zaire	EF204502	-	EF204486	-
Lentitheciaceae (4)	Lentithecium clioninum	KT 1149A ^T	Submerged woody plant	Japan	AB807540	LC014566	-	AB808515
	Lentithecium fluviatile	CBS 122367	Populus sp.	France	GU301825	-	-	GU349074
	Keissleriella breviasca	KT 649 ^T	Dactylis glomerata	Japan	AB807588	NR_155211	-	AB808567
	Keissleriella quadriseptata	KT 2292 ^T	Dactylis glomerata	Japan	AB807593	AB811456	-	AB808572

L	eptosphaeriaceae (1)	Leptosphaeria conoidea	CBS 616.75	Lunaria annua	The Netherlands	JF740279	JF740201	KT389639	-
		Leptosphaeria doliolum	CBS 505.75 ^T	Urtica dioica	The Netherlands	GQ387576	JF740205	KT389640	GU349069
		Paraleptosphaeria dryadis	CBS 643.86	Dryas octopetala	Switzerland	GU301828	MH862007	GU371733	GU349009
L	ibertasomycetaceae (1)	Libertasomyces myopori	CPC 27354 ^T	Myoporum serratum	South Africa	KX228332	NR_145200	-	-
		Libertasomyces platani	CPC 29609 ^T	Platanus sp.	New Zealand	KY173507	KY173416	KY173585	-
L	igninsphaeriaceae (53)	Ligninsphaeria jonesii	GZCC 15-0080	Bamboo	Thailand	KU221038	-	-	-
		Ligninsphaeria jonesii	MFLUCC 15-0641 ^T	Bamboo	Thailand	KU221037	-	-	-
L	indgomycetaceae (36)	Lindgomyces ingoldianus	ATCC 200398 ^T	Submerged wood	USA	NG_042321	NR_119938	-	-
		Lindgomyces ingoldianus	KH 100	Submerged wood	Japan	AB521737	JF419899	-	-
		Phyllosticta flevolandica	CBS 998.72 ^T	Soil	The Netherlands	DQ678090	-	DQ677988	-
L	ophiostomataceae (25)	Lophiostoma macrostomum	KT 508	Morus bombycis	Japan	AB619010	JN942961	JN993491	LC001751
		Lophiostoma macrostomum	KT 635	Herbaceous plant	Japan	AB433273	-	JN993484	LC001752
L	ophiotremataceae (47)	Lophiotrema nucula	CBS 627.86	Acer platanoides	Sweden	GU301837	LC194497	LC194465	LC194410
	Aacrodiplodiopsidaceae	Macrodiplodiopsis desmazieri	CPC 24971 ^T	Platanus sp.	Switzerland	KR873272	NR_132924	-	-
À	Massariaceae (41)	Massaria inquinans	WU 30527	Acer pseudoplatanus	Austria	HQ599402	HQ599402	HQ599460	HQ599342
Λ	Iassarinaceae (9)	Massarina eburnea	CBS 473.64	Fagus sylvatica	Switzerland	GU301840	-	GU371732	GU349040
		Massarina eburnea	Н 3953	Fagus sp.	UK	AB521735	LC014569	-	AB808517
Λ	Ielanommataceae (34)	Melanomma pulvis-pyrius	CBS 124080 ^T	Salix caprea	France	GU456323	-	GU456350	GU456265
Λ	Microsphaeropsidaceae (1)	Microsphaeropsis olivacea	CBS 233.77	Pinus laricio	France	GU237988	GU237803	KT389643	-
N	Morosphaeriaceae (12)	Aquilomyces rebunensis	KT 732 ^T	Submerged twigs of woody plant	Japan	AB807542	AB809630	-	AB808518
		Morosphaeria velatispora	KH 218	Rhizophora mucronata	Japan	AB807555	LC014571	-	AB808531
Ν	leocamarosporiaceae (1)	Neocamarosporium betae	CBS 523.66	Beta vulgaris	The Netherlands	EU754179	FJ426981	KT389670	-
		Neocamarosporium goegapense	CBS 138008 ^T	Mesembryanthemum sp.	South Africa	KJ869220	KJ869163	-	-
Ν	Teohendersoniaceae (15)	Neohendersonia kickxii	CBS 112403 ^T	Fagus sylvatica	Italy	KX820266	KX820255	-	-
		Neohendersonia kickxii	CBS 114276	Fagus sp.	Sweden	KX820267	KX820256	-	-
Ν	Teophaeosphaeriaceae (1)	Neophaeosphaeria agaves	CPC 21264 ^T	Agave tequilana var. azul	Mexico	KF777227	NR_137833	-	-
		Neophaeosphaeria filamentosa	CBS 102202	Yucca rostrata	Mexico	GQ387577	JF740259	GU371773	-

Neopyrenochaetaceae (1)	Neopyrenochaeta acicola	CBS 812.95 ^T	Waterpipe	The Netherlands	GQ387602	LT623218	LT623271	-
	Neopyrenochaeta telephoni	CBS 139022 ^T	Screen of a mobile phone	India	KM516290	KM516291	LT717685	-
Nigrogranaceae (32)	Nigrograna mackinnonii	CBS 674.75 ^T	Human mycetoma	Venezuela	GQ387613	NR_132037	KF015703	KF407986
Occultibambusaceae (31)	Occultibambusa bambusae	MFLUCC 13-0855 ^T	Bamboo	Thailand	KU863112	KU940123	KU940170	KU940193
Ohleriaceae (30)	Ohleria modesta	CBS 141480	Chamaecytisus proliferus	Spain	KX650563	KX650563	KX650583	KX650534
	Ohleria modesta	MGC	Chamaecytisus proliferus	Spain	KX650562	KX650562	KX650582	KX650533
Parabambusicolaceae (5)	Parabambusicola bambusina	KH 139	Sasa sp.	Japan	AB807537	LC014579	-	AB808512
	Parabambusicola bambusina	KT 2637	Sasa kurilensis	Japan	AB807538	LC014580	-	AB808513
Paradictyoarthriniaceae	Paradictyoarthrinium diffractum	MFLUCC 12-0557	Tectona grandis	Thailand	KP744497	KP744454	KX437765	-
(29)	Paradictyoarthrinium diffractum	MFLUCC 13-0466	Tectona grandis	Thailand	KP744498	KP744455	KX437764	-
Parapyrenochaetaceae (1)	Parapyrenochaeta acaciae	CPC 25527 ^T	Acacia sp.	Australia	KX228316	KX228265	LT717686	-
	Parapyrenochaeta protearum	CBS 131315 ^T	Protea mundii	South Africa	JQ044453	JQ044434	LT717683	-
Periconiaceae (8)	Bambusistroma didymosporum	MFLUCC 13-0862 ^T	Bamboo	Thailand	KP761730	KP761733	-	KP761727
	Periconia pseudodigitata	KT 1395 ^T	Phragmites australis	Japan	AB807564	NR_153490	-	AB808540
Phaeosphaeriaceae (1)	Phaeosphaeria oryzae	CBS 110110^{T}	Oryza sativa	South Korea	GQ387591	KF251186	-	-
	Phaeosphaeria oryzae	MFLUCC 11-0170	Etlingera sp.	Thailand	KM434279	KM434269	KM434306	-
Pleomassariaceae (35)	Pleomassaria siparia	CBS 279.74	Betula verrucosa	The Netherlands	DQ678078	-	KT216532	DQ677923
Pleosporaceae (1)	Alternaria alternata	MFLUCC 14-1184	dead stem	Italy	KP334701	KP334711	KP334737	KP334735
	Stemphylium vesicarium	CBS 191.86 ^T	Medicago sativa	India	JX681120	NR_111243	KC584471	-
Pseudoastrosphaeriellaceae (52)	Pseudoastrosphaeriella thailandensis	MFLUCC 11-0144	Bamboo	Thailand	KT955478	-	KT955416	KT955440
Pseudocoleodictyosporaceae	Pseudocoleodictyospora	MFLUCC 12-0385 ^T	Tectona grandis	Thailand	KU764709	KU712443	KU712491	-
(27)	tectonae Pseudocoleodictyospora tectonae	MFLUCC 12-0387	Tectona grandis	Thailand	KU764704	KU712444	KU712492	-
Pseudodidymellaceae (33)	Pseudodidymella fagi	H 2579 ^T	Fagus crenata	Japan	LC203356	LC150787	LC203412	LC203384
Pseudopyrenochaetaceae (1)	Pseudopyrenochaeta lycopersici	CBS 306.65 ^T	Lycopersicon esculentum	Germany	EU754205	NR_103581	LT717680	-
	Pseudopyrenochaeta terrestris	CBS 282.72 ^T	Soil	The Netherlands	LT623216	LT623228	LT623287	-
Pyrenochaetopsidaceae (1)	Pyrenochaetopsis leptospora	CBS 101635 ^T	Secale cereale	Unknow	GQ387627	JF740262	LT623282	-

	Xenopyrenochaetopsis pratorum	CBS 445.81 ^T	Lolium perenne	New Zealand	GU238136	JF740263	KT389671	-
Salsugineaceae (51)	Salsuginea ramicola	KT 2597.1	Mangrove wood	Japan	GU479800	-	GU479833	GU479861
Sulcatisporaceae (3)	Magnicamarosporium	KT 2822 ^T	Diplospora dubia	Japan	AB807509	AB809640	-	AB808485
	iriomotense Sulcatispora acerina	KT 2982 ^T	Acer palmatum	Japan	LC014610	LC014597	-	LC014615
Shiraiaceae (1)	Shiraia bambusicola	NBRC 30753	Phyllostachys sp.	Japan	AB354968	AB354987	-	-
	Shiraia bambusicola	NBRC 30754	Phyllostachys sp.	Japan	AB354969	AB354988	-	-
Sporormiaceae (21)	Preussia funiculata	CBS 659.74	Soil under Adansonia sp.	Senegal	GU301864	-	GU371799	GU349032
	Preussia lignicola	CBS 264.69	Rabbit dung	The Netherlands	GU301872	-	GU371765	GU349027
	Preussia terricola	DAOM 230091	Unknown	Unknown	AY544686	KT225529	DQ470895	-
	Sporormia fimetaria	UPS 2302c	Cow dung	Sweden	GQ203728	-	-	-
	Sporormia fimetaria	UPS 81 194	Sheep dung	Greenland	GQ203729	-	-	-
	Sporormiella minima	CBS 524.50	Goat dung	Panama	DQ678056	KT389543	DQ677950	DQ677897
	Westerdykella cylindrica	CBS 454.72 ^T	Cow dung	Kenya	NG_027595	DQ491519	DQ470925	DQ497610
	Westerdykella ornata	CBS 379.55	Mangrove mud	Mozambique	GU301880	NR_103587	GU371803	GU349021
Teichosporaceae (23)	Teichospora trabicola	C134 ^T	Robinia pseudoacacia	Austria	KU601594	KU601594	-	KU601602
	Teichospora trabicola	C160	Robinia pseudoacacia	Germany	KU601591	KU601591	KU601600	KU601601
Testudinaceae (50)	Lepidosphaeria nicotiae	AFTOL-ID 1576	Soil	Iraq	DQ678067	-	DQ677963	DQ677910
	Ulospora bilgramii	AFTOL-ID 1598	Unknown	Unknown	DQ678076		DQ677974	DQ677921
Tetraplosphaeriaceae (49)	Tetraplosphaeria sasicola	KT 563 ^T	Sasa senanensis	Japan	AB524631	AB524807	-	-
Thyridariaceae (28)	Thyridaria broussonetiae	$TB1^T$	Amorpha fruticosa	Hungary	KX650568	KX650568	KX650586	KX650539
	Thyridaria broussonetiae	TB2	Broussonetia papyrifera	Italy	KX650570	KX650570	KX650587	KX650540
Torulaceae (26)	Torula herbarum	CBS 379.58	Dung of racoon	Canada	KF443383	-	-	KF443400
	Torula herbarum	CPC 24114 ^T	Phragmites australis	The Netherlands	KR873288	KR873288	-	-
Trematosphaeriaceae (13)	Trematosphaeria pertusa	CBS 122368 ^T	Fraxinus excelsior	France	FJ201990	NR_132040	FJ795476	KF015701
	Trematosphaeria pertusa	KT 3315	Submerged twigs of woody plant	Japan	LC014612	LC014602	LC014617	-
Wicklowiaceae (38)	Wicklowia aquatica	AF289 1	Fresh water	Costa Rica	GU045446	-	-	-
	Wicklowia aquatica	F76 2	Submerged decorticated woody debris	USA	GU045445	-	-	-

T ex-type strain

