

Diversity of coelomycetous fungi in human infections: a 10-year experience of two European reference centres.

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Running title: Coelomycetous fungi of clinical interest in Europe.

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25 **ABSTRACT**

26 The coelomycetous fungi are difficult to properly identify from their phenotypic
 27 characterization and their role as etiologic agents of human infections is not clear. We
 28 studied the species distribution of these fungi among clinical isolates that had been
 29 collected and stored over a ten-year period in two European reference laboratories
 30 (France and Spain). We identified phenotypically and molecularly 97 isolates by
 31 sequencing the D1-D2 fragment of the 28S nrRNA (LSU) gene. Species of the orders
 32 *Pleosporales* and *Glomerellales* were present in both collections, and *Botryosphaeriales*
 33 and *Diaporthales* only in the French one. The most prevalent species were *Medicopsis*
 34 *romeroi*, *Neocucurbitaria keratinophila*, *Neocucurbitaria unguis-hominis* and
 35 *Paraconiothyrium cyclothyrioides*, which had been recovered primarily from superficial
 36 tissues. The *Didymellaceae* was the most common family represented, with 27 isolates
 37 distributed into five genera.

38

39 **Keywords:** *Colletotrichum*, coelomycetes, coelomycetous fungi, *Didymella*,
 40 *Medicopsis*, mycosis, *Neocucurbitaria*, *Paraconiothyrium*, *Phoma*.

41 INTRODUCTION

42 Human infections by coelomycetous fungi are rare and poorly characterized due
 43 to the difficulty in identifying these fungi using only phenotypic tools. The
 44 coelomycetous fungi are characterized by the production of conidia into fruiting bodies
 45 (= conidiomata), and were originally included in the orders *Sphaeropsidales* and
 46 *Melanconiales* of the class Coelomycetes, taxa which today lack scientific validity due
 47 to the demonstrated polyphyletic character of this sort of fungus (1–3). They cause
 48 superficial or subcutaneous infections, mostly following a traumatic inoculation of
 49 contaminated plant material or soil particles during agricultural work in tropical and
 50 subtropical areas (4–6). The most common coelomycetous fungi involved in these
 51 infections are the etiologic agents of black-grain eumycetoma, such as *Biatrispora*
 52 *mackinnonii*; *Falciformispora* spp., *Medicopsis* *romeroi*, and
 53 *Pseudochaetosphaeronema* *lareense*. Other common coelomycetous fungi include
 54 *Lasiodiplodia* *theobromae* and *Neoscytalidium* *dimidiatum* (synanamorph of
 55 *Hendersonula* *toruloidea*) (7–11), which typically cause onychomycosis, subcutaneous
 56 phaeohyphomycosis (12–15), and eumycetoma (16). In addition, many species of
 57 *Phoma* and *Pyrenochaeta* have been reported as occasional agents of localized and
 58 systemic infections in humans (9, 17–20). The taxonomy of several coelomycetous
 59 genera mentioned before have been revised recently but they still constitute a group of
 60 highly polyphyletic taxa that are usually difficult to identify phenotypically (2, 21–24).

61 In a recent study conducted in the USA, Valenzuela-Lopez *et al.* (6) identified
 62 230 fungal strains by sequencing the D1-D2 domains of the 28S rRNA gene (LSU),
 63 from which 152 (66.1%) strains belonged to the order *Pleosporales*, the rest being
 64 distributed in several orders of the phylum Ascomycota. Most of these strains were
 65 recovered from superficial tissue. *Neoscytalidium* *dimidiatum*, *Paraconiothyrium*

cyclothyrioides and members of the family *Didymellaceae* were the most prevalent taxa. In addition, those authors demonstrated the usefulness of the LSU as a good molecular marker for a preliminary identification of coelomycetous fungi at genus level. In fact, such locus is easily amplified and many sequences are available in the GenBank database. However, the nucleotide sequences of more phylogenetically informative genes need analysing in order to identify the fungi at species level. Genes such as the RNA polymerase II subunit 2 (*rpb2*), translation elongation factor 1-alpha (*tef1*), beta-tubulin (*tub2*) and the ribosomal internal transcribed spacer region (ITS), combined in a multi-locus analysis, have all been recommended for this purpose (25)

Until now, the coelomycetous fungi involved in invasive fungal infections (IFIs) are poorly known in Europe, probably due to the infrequency of these fungi and the complexity of their identification in the absence of characteristic fruiting bodies when grown on culture media used in the clinical lab. In a recent French study, eighteen proven cases of cutaneous and subcutaneous primary infections by coelomycetous fungi were reported and analysed in patients from tropical and subtropical regions (26).

For a better knowledge of the diversity of coelomycetous fungi involved in human infections, we studied a large set of clinical isolates that had been identified in two mycology reference centres in France and Spain, and determined their *in vitro* antifungal susceptibility pattern.

RESULTS

Locations of infections

The majority of the isolates were recovered from superficial tissue, mainly skin (44%; 43/97), eyes (27%; 26/97), nails/hairs (18%; 17/97) and mouth/sinus (2%; 2/97). A few were recovered from deeper sites: bones (4%, 4/97), blood (2%, 2/97), cerebrospinal fluid (n=1), bone marrow (n=1) and lung (n=1) (Table 1 & 2).

Phylogenetic analyses

The maximum-likelihood (ML) phylogenetic analysis of the LSU sequences (approximately 584 pb) demonstrated that the 97 isolates were distributed into four orders, but scattered into fourteen clades (Fig. 1). Most of the isolates (81%; 78/97) belonged to the order *Pleosporales*, which were distributed into nine clades corresponding to 23 species of twelve genera, followed by those of the *Botryosphaerales* (8%; 8/97), the *Diaporthales* (6%; 6/97) and the *Glomerellales* (5%; 5/97).

The most common species identified was *Medicopsis romeroi* (11%; 11/97), followed by *Paraconiothyrium cyclothyrioides*, *Neocucurbitaria keratinophila* and *N. unguis-hominis* (8% each; 8/97). These species were mostly isolated from cutaneous lesions (Table 2).

Clade 1 of the *Pleosporales* corresponded to the family *Didymellaceae*, which included 27 isolates distributed into five genera, morphologically characterized by their production of pycnidial conidiomata and hyaline, aseptate conidia. The five genera were *Didymella*, *Epicoccum*, *Neoascochyta*, *Phoma* and *Xenodidymella*. *Didymella* was represented by 13 isolates, six of them clustering with the type strain of *D. gardeniae* (CBS 626.68), and the other seven clustered with a reference strain of *D. glomerata*

(CBS 528.66). The genus *Epicoccum* grouped five of the isolates, three of them clustering with a reference strain of *E. sorghinum* (CBS 179.80) and the other two with the type strain of the type species of the genus, *E. nigrum* (CBS 173.73). The genus *Phoma* was represented by seven clinical isolates and a reference strain of *Phoma herbarum* (CBS 615.75). Two additional isolates included in this clade (CNRMA 16.76 and CNM-CM 6201) grouped with the type strains of *Xenodidymella saxea* (CBS 419.92) and *Neosascochyta desmazieri* (CBS 297.69), respectively.

Clade 2 had two species of *Preussia*: CNM-CM 7335 grouped with a reference strain of *P. typharum* (CBS 107.69), while CNM-CM 7343 represented an unknown species forming a sister clade with the type strain of *P. terricola* (CBS 317.65).

Clade 3 grouped three isolates of *Paraphoma*, one of them (CNM-CM 8075) clustered with the type strain of *P. fimeti* (CBS 170.70), and the remaining two (CNRMA 15.665 and CNRMA 9.467) representing unidentified phoma-like species.

Clade 4 had two sister clades of the genus *Tintelnotia*, which produced pycnidia and hyaline, aseptate conidia. The isolate CNM-CM 7430 was identified as *T. destructans*. However, the other two isolates (CNM-CM 7080 and CNM-CM 7981) did not cluster with any known species of the genus and might represent new species.

Clade 5 had 20 isolates of *Neocucurbitaria*. *Neocucurbitaria keratinophila* and *N. unguis-hominis* were the most common species, both with eight isolates each. *Neocucurbitaria cava*, with a single isolate (CNRMA 15.708), was also included in this clade. Three Spanish isolates, CNM-CM 6489, CNM-CM 7025 and CNM-CM 7132 were identified as *Neocucurbitaria* sp. due to being phylogenetically different from the other isolates and, again, might be a new species of the genus. *Neocucurbitaria* spp.

produces pycnidia, ornamented or not, with bristle-like setose structures, and hyaline, aseptate conidia.

Clade 6 had eleven isolates of *Medicopsis romeroi* (syn. *Pyrenochaeta romeroi*), which produces pycnidia and hyaline, aseptate conidia.

Clade 7 is represented by a single isolate (CNRMA 11.1115), phylogenetically distinct from the known pleosporalean fungi, possibly representing a novel taxon.

Clades 8 and 9 belonged to the family *Didymosphaeriaceae*. Clade 8 included a single isolate (CNM-CM 6000) phylogenetically related to a reference strain of *Paraphaeosphaeria michotii* (MFLUCC 13-0349). Clade 9 grouped ten isolates, two related to a reference strain of *Paraconiothyrium fuckelii* (CBS 797.95) and eight with the type strain of *Paraconiothyrium cyclothyrioides* (CBS 972.95). Members of the *Didymosphaeriaceae* form pycnidia and pale brown, 0-1 septate conidia.

The order *Botryosphaeriales* are present in Clades 10 to 12. Clade 10 had only one isolate (CNRMA 12.597) which clustered with a reference strain of *Neofusicoccum luteum* (CBS 110299); Clade 11 also had a single isolate (CNRMA 6.1007) that clustered with the type strain of *Diplodia seriata* (CBS 112555), and Clade 12 grouped six isolates, five of them clustering with the type strain of *Lasiodiplodia theobromae*, and CNRMA 15.383 identified as *Lasiodiplodia* sp. These fungi produce stromatic conidiomata and aseptate, hyaline to brown, thick-walled conidia.

Clade 13 included the type strain of *Diaporthe sclerotioides* (CBS 296.67) and six isolates corresponding to unidentified species of the genus *Diaporthe* (*Diaporthales*), none of them able to be morphologically distinguished since they produce pycnidia and small hyaline conidia.

Clade 14, corresponding to the *Glomerellales*, was used as outgroup. Five isolates nested in the *Colletotrichum* clade, two clustering with reference strains of *C. gigasporum* (CBS 159.75) and *C. gloeosporioides* (CBS 122687), respectively; and the other three, could not be identified. All the isolates showed the typical morphology of *Colletotrichum*, i.e., acervuli, conidia variable in shape, flattened with thickened tip branches (appressoria).

Antifungal susceptibility testing

The minimum inhibitory concentration (MIC) was determined for 46 of the isolates included here (16 from Spain and 30 from France) (Table 3, Table S1). Globally, the geometric mean (GM) and MIC₅₀ values of itraconazole and caspofungin were the highest (Table 3). The MIC of amphotericin B (0.06-1 mg/L) was generally low among the *Pleosporales* with the exception of one isolate of *M. romeroi* and one of *D. gardeniae*, with MICs of 8 and 32 mg/L, respectively. The azole MIC ranged between 0.03 and 1 mg/L for isolates belonging to the genera *Paraconiothyrium*, *Paraphoma*, *Tintelnotia* and *Neocucurbitaria*, with the exception of two isolates of *N. unguis-hominis*, which showed higher values (16 mg/L). The terbinafine MIC was low except for *Diaporthe* spp. and a few isolates of *Colletotrichum* spp. and *M. romeroi*.

DISCUSSION

The present study is the largest on this taxonomically complex group of fungi from clinical origin, with almost a hundred isolates morphologically and molecularly characterized from two southern European countries (France and Spain). Most of these coelomycetous fungi belonged to the order *Pleosporales* and were most commonly recovered from superficial infections. Similar results were observed in a previous work that focused on coelomycetous fungi collected at a North American reference centre (6). However, the diversity of the fungi identified in that study was higher, i.e. eleven orders were represented against four here.

In the present study, *Medicopsis romeroi* was the most frequently isolated species whereas the most common taxon in the American study was *Neoscytalidium dimidiatum*. Interestingly, while *M. romeroi* is usually reported as an etiologic agent of black grain eumycetoma (4, 11, 26–29), our isolates were mainly recovered from eye and non-mycetoma subcutaneous infections.

The second most frequently isolated species were *Paraconiothyrium cyclothyrioides*, *Neocucurbitaria unguis-hominis* and *N. keratinophila*. *Paraconiothyrium cyclothyrioides* is an emerging pathogen (6, 26, 30, 31) and was represented by eight isolates recovered from skin or superficial locations and mainly from tropical regions. *Neocucurbitaria unguis-hominis*, initially described as an agent of human onychomycosis (17), was equally distributed across both centres (n=8 isolates). Regarding *N. keratinophila*, this species was reported for the first time from a corneal infection in Spain (18, 19). Interestingly, as well as being the first case reported for this species, all the isolates of *N. keratinophila* were recovered in Spain from superficial tissue.

Other coelomycetous fungi we identified in the present work were *Didymella glomerata* and *Phoma herbarum*. Although *Phoma* spp. are commonly reported as a coelomycete involved in human infections (9, 20, 32–39), recent extensive changes in taxonomy and nomenclature have spread all but one of the species into different genera of the *Didymellaceae*, *Phoma herbarum* remaining as the unique species of the genus (22–24). Interestingly, *Didymella gardeniae* was commonly found in our study (five isolates from Spain and one from France).

Recently, Ahmed *et al.* (40) proposed *Tintelnotia destructans*, a new phoma-like fungus belonging to the *Phaeosphaeriaceae* able to cause eye and nail infections. They reported the successful use of terbinafine against a case of keratitis by this species. Two of the Spanish isolates recovered from superficial specimens (one cutaneous exudate and one nail sample) were molecularly related to the above-mentioned species but phylogenetically different and might represent a new taxon.

Lasiodiplodia theobromae (order *Botryosphaerales*) is the only species of this genus involved in human opportunistic infections (41–46). Valenzuela-Lopez *et al.* (6) found a higher species diversity in the North American study than we report here, since five of the French isolates were identified as *L. theobromae*. The other three isolates of the *Botryosphaerales* we found were related, one to a different species of *Lasiodiplodia* and the other two to other genera, specifically *Neofusicoccum* and *Diplodia*.

Four species of the genus *Diaporthe* (formerly *Phomopsis*; order *Diaporthales*), i.e. *D. bougainvilleicola*, *D. longicolla*, *D. phaseolorum* and *D. phoenicicola*, are considered opportunistic pathogens that cause mycoses that range from superficial to deep infections (47–51). Six isolates from France were phylogenetically placed into the latter genus. However, our results are only preliminary since only one phylogenetic

marker was analysed. Similar was observed in several polyphyletic genera of the coelomycetes (52, 53).

We also report the finding of five clinical isolates of *Colletotrichum*. Two of the isolates corresponded to *C. gigasporum* (formerly *C. crassipes*) and *C. gloeosporioides*, taxa that have previously been reported as agents of keratitis, endophthalmitis and phaeohyphomycotic cyst; the other three isolates could not be identified at species level. This genus encompasses numerous plant pathogens that are found worldwide, although mainly in tropical and subtropical regions (54). The taxonomy of *Colletotrichum* is complicated and the genus is organized in species-complexes (55–59). Species such as *C. coccodes*, *C. crassipes*, *C. dematium*, *C. gloeosporioides*, *C. graminicola* and *C. truncatum* cause superficial and deep infections (endophthalmitis, keratitis, subcutaneous cyst or more rarely arthritis) (60–65). Further studies, including different phylogenetic markers, are needed to delimit the different species and clarify their pathogenic role.

The antifungal susceptibility of coelomycetous fungi involved in human infections is poorly known, mainly because they do not easily sporulate. In spite of the limited number of isolates tested here, amphotericin B seemed the most active drug *in vitro* together with terbinafine, in agreement with Valenzuela-Lopez *et al.* (6). Until more *in vitro* data is available, the antifungal treatment of the infection by this sort of fungus remains purely empirical. In a recent study, Guégan *et al.* (26) recommended extensive surgical resection of affected tissues as a first-line treatment for solitary subcutaneous lesions by coelomycetous fungi, followed by an antifungal therapy (posaconazole or voriconazole) in the case of relapse or amphotericin B in refractory cases.

Since our study is based on isolates from the two reference centres, we cannot comment on the incidence of infections due to coelomycetes nor compare their epidemiology between France and Spain. However, we still provide a good picture of the great diversity of coelomycetous fungi in the clinical context, and the basis for future studies on this interesting but neglected group of fungi.

MATERIAL AND METHODS

Fungal isolates

We studied 97 isolates of coelomycetous fungi recovered from clinical specimens, 51 of which were provided by the French National Reference Centre for Invasive Mycoses and Antifungals (NRCMA) at the *Institut Pasteur*, Paris (CNRMA isolates, n=51). The NRCMA offers expertise on difficult-to-identify fungi and the epidemiological surveillance of all cases of IFIs, which are notified on a voluntary basis either through active or passive surveillance programmes. The Spanish National Centre of Microbiology at the *Instituto de Salud Carlos III*, Madrid provided 46 isolates (CNM-CM isolates, n=46). This mycology reference laboratory receives isolates from the National Health System on a voluntary basis, the main aim of which is to support it by identifying and profiling the antifungal susceptibility of fungal isolates. The isolates were collected between 2005 and 2015. Table 1 gives information about the country of isolation and the location of the infection in the body.

Morphological and physiological characterization

For morphology studies, the isolates were cultured on oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 L tap water) and malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 L distilled water) at $20 \pm 1^\circ\text{C}$ for 14 days in darkness.

270 The morphological features of the vegetative and reproductive structures were studied
 271 using an Olympus CH2 bright-field microscope (Olympus Corporation, Tokyo, Japan)
 272 in wet mounts (on water and lactic acid) and slide cultures (by growing the isolates on
 273 OA and MEA) of the fungal isolates, following Valenzuela-Lopez *et al.* (6). Colour
 274 standards by Kornerup & Wanscher (66) were used in colony description.
 275 Photomicrographs were taken with an Axio-Imager M1 microscope (Zeiss, Oberkochen,
 276 Germany).

277 **DNA extraction, amplification and sequencing**

278 Total genomic DNA was extracted from colonies grown on potato dextrose agar
 279 (PDA; 4 g of potato infusion, 20 g dextrose, 15 g of agar-agar, 1 L tap water) after seven
 280 days of incubation at $20 \pm 1^\circ\text{C}$, using the FastDNA kit protocol (Bio101, Vista, CA),
 281 with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) following the
 282 manufacturer's protocol. DNA was quantified using the Nanodrop 2000 (Thermo
 283 Scientific, Madrid, Spain). LSU was amplified with the primer pair LR0R and LR5
 284 (67). The amplicons were sequenced in both directions with the same primer pair used
 285 for amplification at Macrogen Europe (Macrogen Inc., Amsterdam, The Netherlands).
 286 The consensus sequences were obtained using the SeqMan software version 7.0.0
 287 (DNASar Lasergene, Madison, WI, USA).

288 **Molecular identification and phylogenetic analysis**

289 Preliminary molecular identification of the isolates was made using LSU
 290 nucleotide sequences in BLAST_N searches. Twenty-eight LSU sequences of type or
 291 reference strains deposited in the GenBank database by the Westerdijk Fungal
 292 Biodiversity Institute (CBS) and the Mae Fah Luang University (MFLUCC) culture
 293 collections were used for identification and phylogenetic purposes. DNA sequences

generated in this study were deposited in GenBank (accession numbers are given in Table 1).

For the phylogenetic study, sequences were aligned using the ClustalW application (68) of the MEGA 6.06 (69) computer program, and manually adjusted using the same software platform. Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) with MEGA 6.06 and MrBayes 3.2.4 (70), respectively. The best substitution model for the gene matrix (TN93+G) was estimated using MEGA 6.06. For ML analyses, nearest-neighbour interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) of ≥ 70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried out with four million 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 burn-in. A PP value of ≥ 0.95 was considered significant. Reference strains of *Colletotrichum gigasporum* (CBS 159.75), *C. gloeosporioides* (CBS 122687) and *C. hippeastri* (CBS 241.78) were used as outgroup.

Antifungal susceptibility testing

The *in vitro* susceptibility testing in both reference centres (n= 46 isolates) followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) procedure (71, 72). The antifungals used were amphotericin B (Sigma-Aldrich Química, Madrid, Spain), itraconazole (Sigma-Aldrich Química, Madrid, Spain), posaconazole (Schering-Plough Research Institute, Kenilworth, N.J.), voriconazole (Pfizer S.A., Madrid, Spain), caspofungin (Merck & Co., Inc., Rahway, N.J.), micafungin (Astellas

318 Pharma Inc, Tokyo, Japan) and terbinafine (Novartis, Basel, Switzerland). For the
319 NCRMA, all antifungal drugs were obtained from ALSACHIM, Strasbourg, France.

320 The isolates were cultured on potato carrot agar (PCA; 20 g each of filtered
321 potatoes and carrots, 20 g of agar, 1 L of distilled water) or OA for seven to 30 days at
322 25°C and 30°C to obtain sporulation. Conidia were then collected in sterile water
323 containing 0.01% (v/v) Tween 80 (Sigma-Aldrich, St. Louis, MO, USA), and the
324 suspension was adjusted to $2-5 \times 10^5$ conidia/mL. The minimal effective concentration
325 (MEC) was determined for each echinocandin and the minimal inhibitory concentration
326 (MIC) for the other drugs (90% inhibition for amphotericin B and 80% for the azoles)
327 after 24 h and 48 h of incubation at 35°C. *Aspergillus flavus* ATCC 204304 and
328 *Aspergillus fumigatus* ATCC 204305 were used as quality control strains in all tests
329 carried out. Susceptibility profiles were determined for 46 isolates since non-sporulating
330 isolates were excluded at the NRCMA.

331

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560

561 **FIGURE LEGEND**

562 **FIG 1** Maximum likelihood tree obtained from the D1-D2 of LSU (584 bp) sequences
 563 of the 125 strains, where 28 belong to type or reference strains. The branch lengths are
 564 proportional to phylogenetic distance. Bayesian posterior probability scores ≥ 0.95 and
 565 Bootstrap support values $\geq 70\%$ are indicated on the nodes. Some branches were
 566 shortened to fit them to the page, these are indicated by two diagonal lines with the
 567 number of times a branch was shortened. The species of the genus *Colletotrichum* were
 568 used to root the tree. Superscript ^T indicated the type strains.

569

570

TABLE 1 Taxonomical identification of the isolates studied, origin and GenBank accession numbers. New sequences generated are indicate in bold.

Order	Species	Strain no. ^a	Origin	Country	GenBank accession no. ^b
<i>Botryosphaeriales</i>	<i>Diplodia seriata</i>	CBS 112555 ^T	<i>Vitis vinifera</i> dead plant	Portugal	KF766327
		CNRMA 6.1007	bone	France	LT965964
	<i>Lasiodiplodia</i> sp.	CNRMA 15.383	eye	France (West Indies, Guadeloupe)	LT965965
	<i>Lasiodiplodia theobromae</i>	CBS 164.96 ^T	fruit along coral reef coast	Papua New Guinea	NG_042460
		CNRMA 10.1369	skin	France (West Indies, Martinique)	LT965966
		CNRMA 10.813	eye	France (West Indies, Martinique)	LT965967
		CNRMA 11.360	eye	France (West Indies, Martinique)	LT965968
		CNRMA 13.891	skin	France	LT965969
		CNRMA 14.708	eye	France (West Indies, Guadeloupe)	LT965970
	<i>Neofusicoccum luteum</i>	CBS 110299	<i>Vitis vinifera</i> cane	Portugal	AY928043
		CNRMA 12.597	eye	France	LT965971
<i>Diaporthales</i>	<i>Diaporthe sclerotioides</i>	CBS 296.67 ^T	<i>Cucumis sativus</i> root	The Netherlands	AF439628
	<i>Diaporthe</i> sp.	CBS 477	<i>Cucumis sativus</i>	USA	AF439631
		CNRMA 8.522	eye	France	LT965972
		CNRMA 9.205	eye	France (West Indies, Guadeloupe)	LT965973
		CNRMA 11.385	eye	France (West Indies, Martinique)	LT965974
		CNRMA 12.311	blood	France	LT965975
		CNRMA 13.515	skin	France	LT965976
		CNRMA 14.198	skin	France	LT965977

Glomerellales	<i>Colletotrichum gigasporum</i>	CBS 159.75	air and stored grains	India	DQ286206
		CNRMA 16.553	skin	France (West Indies, Guadeloupe)	LT965978
	<i>Colletotrichum gloeosporioides</i>	CBS 122687	<i>Leucospermum</i> sp. leaf litter	South Africa	EU552111
		CNRMA 15.504	eye	France (West Indies, Martinique)	LT965979
	<i>Colletotrichum hippeastri</i>	CBS 241.78	<i>Hippeastrum</i> sp.	The Netherlands	DQ286167
	<i>Colletotrichum</i> sp.	CNM-CM4760	corneal swab	Spain	LT965980
		CNM-CM 6116	conjunctival	Spain	LT965981
		CNM-CM 7345	humor acuosus	Spain	LT965982
Pleosporales	<i>Didymella gardeniae</i>	CBS 626.68 ^T	<i>Gardenia jasminoides</i> leaf	India	GQ387595
		CNM-CM 3697	nail	Spain	LT965983
		CNM-CM 3895	nail	Spain	LT965984
		CNM-CM 5036	scales	Spain	LT965985
		CNM-CM 5814	conjunctival exudate	Spain	LT965986
		CNM-CM 7499	conjunctival exudate	Spain	LT965987
		CNRMA 11.794	skin	France	LT965988
	<i>Didymella glomerata</i>	CBS 528.66	<i>Chrysanthemum</i> sp. cutting	The Netherlands	EU754184
		CNM-CM 3356	toenail	Spain	LT965989
		CNM-CM 3546	nail	Spain	LT965990
		CNM-CM 4675	nail	Spain	LT965991
		CNM-CM 7099	cutaneous exudate	Spain	LT965992
		CNRMA 9.1046	skin	France	LT965993

	CNRMA 10.867	skin	France	LT965994
	CNRMA 15.6	mouth/sinus	France	LT965995
<i>Epicoccum nigrum</i>	CBS 173.73 ^T	<i>Dactylis glomerata</i> seed	USA	GU237975
	CNM-CM 5281	skin	Spain	LT965996
	CNM-CM 5724	vitreous humor	Spain	LT965997
<i>Epicoccum sorghinum</i>	CBS 179.80	<i>Sorghum vulgare</i>	Puerto Rico	GU237978
	CNRMA 7.167	bone	France (New Caledonia)	LT965998
	CNRMA 10.947	skin	France (New Caledonia)	LT965999
	CNRMA 10.948	skin	France (New Caledonia)	LT966000
<i>Medicopsis romeroi</i>	CBS 252.60 ^T	maduromycosis	Venezuela	EU754207
	CNM-CM 3387	knee ulcer	Spain	LT966001
	CNM-CM 7645	cutaneous exudate	Spain	LT966002
	CNRMA 4.200	eye	France	LT966003
	CNRMA 5.321	skin	France	LT966005
	CNRMA 7.1225	skin	France	LT966007
	CNRMA 8.1363	skin	France	LT966008
	CNRMA 11.680	skin	France	LT966010
	CNRMA 11.949	bone	France	LT966011
	CNRMA 14.407	skin	France	LT966013
	CNRMA 15.461	bone	France	LT966014
	CNRMA 15.7	skin	France	LT966015
<i>Neoscochyta desmazieri</i>	CBS 297.69 ^T	<i>Lolium perenne</i>	Germany	KT389726
	CNM-CM 6201	nail	Spain	LT966016

<i>Neocucurbitaria cava</i>	CBS 257.68 ^T	wheat-field soil	Germany	EU754199
	CNRMA 15.708	mouth/sinus	France	LT966017
<i>Neocucurbitaria keratinophila</i>	CBS 121759 ^T	corneal scrapings	Spain	LT623215
	CNM-CM 5882	cutaneous exudate	Spain	LT966018
	CNM-CM 6401	finger nail	Spain	LT966019
	CNM-CM 6455	cutaneous exudate	Spain	LT966020
	CNM-CM 7013	cutaneous exudate	Spain	LT966021
	CNM-CM 7457	cutaneous exudate	Spain	LT966022
	CNM-CM 7731	cutaneous exudate	Spain	LT966023
	CNM-CM 8010	conjunctival exudate	Spain	LT966024
<i>Neocucurbitaria unguis-hominis</i>	CNM-CM 8674	toenail	Spain	LT966025
	CBS 112.79	airborn	Wales	GQ387622
	CNM-CM 7037	nail	Spain	LT966026
	CNM-CM 7089	cutaneous lesion	Spain	LT966027
	CNM-CM 8717	urine	Spain	LT966028
	CNM-CM 8743	toenail	Spain	LT966029
	CNRMA 4.1112	eye	France	LT966030
	CNRMA 6.243	eye	France	LT966031
	CNRMA 16.153	eye	France	LT966032
	CNRMA 16.19	lung	France	LT966033
<i>Neocucurbitaria</i> sp.	CNM-CM 6489	wound exudate	Spain	LT966034
	CNM-CM 7025	hair	Spain	LT966035
	CNM-CM 7132	toenail	Spain	LT966036

<i>Paraconiothyrium cyclothyrioides</i>	CBS 972.95 ^T	soil	Papua New Guinea	JX496232
	CNM-CM 6313	conjunctival exudate	Spain	LT966037
	CNM-CM 6513	nail	Spain	LT966038
	CNM-CM 4767	abscess	Spain	LT966039
	CNRMA 11.383	skin	France (West Indies, Martinique)	LT966041
	CNRMA 11.855	skin	France	LT966042
	CNRMA 13.245	skin	France	LT966043
	CNRMA 16.374	skin	France (West Indies, Guadeloupe)	LT966044
	CNRMA 16.556	skin	France (West Indies, Guadeloupe)	LT966045
<i>Paraconiothyrium fuckelii</i>	CBS 797.95	<i>Rubus</i> sp. dead stem	Denmark	JX496226
	CNRMA 3.240	eye	France	LT966046
	CNRMA 4.493	eye	France	LT966047
<i>Paraphaeosphaeria michotii</i>	MFLUCC 13-0349	<i>Poaceae</i> dead leaves	Italy	KJ939282
	CNM-CM 6000	skin	Spain	LT966048
<i>Paraphoma fimeti</i>	CBS 170.70 ^T	<i>Apium graveolens</i> seeds	The Netherlands	GQ387584
	CNM-CM 8075	wound exudate	Spain	LT966049
<i>Paraphoma</i> sp.	CNRMA 9.467	skin	France	LT966050
	CNRMA 15.665	skin	France	LT966051
<i>Phaeosphaeriopsis obtusispora</i>	CBS 246.64	<i>Aloe arborescens</i> dead leaf	Portugal	JX681119
<i>Phoma herbarum</i>	CBS 615.75	<i>Rosa multiflora</i> dead stem	The Netherlands	EU754186

	CNM-CM 2132	right toe	Spain	LT966052
	CNM-CM 3526	bone marrow	Spain	LT966053
	CNM-CM 3597	blood culture	Spain	LT966054
	CNM-CM 8031	nail	Spain	LT966055
	CNRMA 9.1095	skin	France	LT966056
	CNRMA 11.1097	eye	France	LT966057
	CNRMA 12.1227	eye	France	LT966058
pleosporelean fungus	CNRMA 11.1115	skin	France	LT966059
<i>Preussia</i> sp.	CNM-CM 7343	nail	Spain	LT966060
<i>Preussia terricola</i>	CBS 317.65 ^T	<i>Musa sapientum</i> rhizosphere	Honduras	GQ203725
<i>Preussia typharum</i>	CBS 107.69	Dung of deer	Japan	GQ203726
	CNM-CM 7335	nail	Spain	LT966061
<i>Pseudophaeosphaeria rubi</i>	MFLUCC 14-0259	<i>Rubus idaeus</i> dead branch	Italy	KX765299
<i>Tintelnotia destructans</i>	CBS 127737 ^T	anterior eye chamber cornea	Germany	KY090664
	CNM-CM 7430	Unknown	Spain	LT966062
<i>Tintelnotia</i> sp.	CNM-CM 7080	nail	Spain	LT966063
	CNM-CM 7981	cutaneous exudate	Spain	LT966064
<i>Xenodidymella saxea</i>	CBS 419.92 ^T	Corroded mediterranean marble	Unknown	GU238141

CNRMA 16.76

Cerebrospinal fluid

France

LT966065

^a **CBS**: Strains from Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CNM-CM**: Isolates from the National Centre for Microbiology, Instituto Carlos III, Madrid, Spain; **CNRMA**: Isolates from the National Reference Center for Invasive Mycoses and Antifungals; Institut Pasteur, Paris, France; **MFLUCC**: Strains from Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. Type strains are indicated by a superscript ^T

^b LSU, large subunit ribosomal DNA sequences

TABLE 2 Localization of infections due to coelomycetous fungi isolates

Orders	no. of isolates obtained from:		
	Superficial infection	Deep infection	Total no. of isolates
<i>Botryosphaeriales</i>	7	1	8
<i>Diaporthales</i>	5	1	6
<i>Glomerellales</i>	5		5
<i>Pleosporales</i>	71	7	78
Total no. of isolates (%)	88 (91)	9 (9)	97 (100)

TABLE 3 Overall *in vitro* antifungal activity against the 46 coelomycetous isolates as determined by EUCAST^a methodology

Antifungal agent	MIC/MEC values (mg/L) ^b				
	range	median	GM	MIC ₅₀	MIC ₉₀
Amphotericin B	0.03 -16	0.5	0.41	0.25	1
Itraconazole	0.014 -16	2	1.72	0.5	16
Voriconazole	0.03 -16	0.5	0.70	0.6	4
Posaconazole	0.014 -16	0.5	0.58	0.25	8
Caspofungin	0.125- 16	2	2.17	1	8
Micafungin	0.015- 16	0.5	0.53	0.125	8
Terbinafin	0.014- 16	0.25	0.39	0.25	2

^aEUCAST, European Committee on Antimicrobial Susceptibility Testing procedure (71);

^bMIC, minimum inhibitory concentration; MEC, minimal effective concentration; MIC₅₀ and MIC₉₀, MIC encompassing 50 and 90% of isolates tested, respectively.



