

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

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16 **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 *Biatriospora*  
52 *mackinnonii*; *Falciformispora* spp., *Medicopsis romeroi*, and  
53 *Pseudochaetosphaeronema larense*. 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*

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

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

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

85

## 86 RESULTS

### 87 Locations of infections

88 The majority of the isolates were recovered from superficial tissue, mainly skin  
89 (44%; 43/97), eyes (27%; 26/97), nails/hairs (18%; 17/97) and mouth/sinus (2%; 2/97).  
90 A few were recovered from deeper sites: bones (4%, 4/97), blood (2%, 2/97),  
91 cerebrospinal fluid (n=1), bone marrow (n=1) and lung (n=1) (Table 1 & 2).

### 92 Phylogenetic analyses

93 The maximum-likelihood (ML) phylogenetic analysis of the LSU sequences  
94 (approximately 584 pb) demonstrated that the 97 isolates were distributed into four  
95 orders, but scattered into fourteen clades (Fig. 1). Most of the isolates (81%; 78/97)  
96 belonged to the order *Pleosporales*, which were distributed into nine clades  
97 corresponding to 23 species of twelve genera, followed by those of the  
98 *Botryosphaerales* (8%; 8/97), the *Diaporthales* (6%; 6/97) and the *Glomerellales* (5%;  
99 5/97).

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

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

110 (CBS 528.66). The genus *Epicoccum* grouped five of the isolates, three of them  
111 clustering with a reference strain of *E. sorghinum* (CBS 179.80) and the other two with  
112 the type strain of the type species of the genus, *E. nigrum* (CBS 173.73). The genus  
113 *Phoma* was represented by seven clinical isolates and a reference strain of *Phoma*  
114 *herbarum* (CBS 615.75). Two additional isolates included in this clade (CNRMA 16.76  
115 and CNM-CM 6201) grouped with the type strains of *Xenodidymella saxea* (CBS  
116 419.92) and *Neoscochyta desmazieri* (CBS 297.69), respectively.

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

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

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

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

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

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

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

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

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

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

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

### 162 **Antifungal susceptibility testing**

163 The minimum inhibitory concentration (MIC) was determined for 46 of the  
164 isolates included here (16 from Spain and 30 from France) (Table 3, Table S1).  
165 Globally, the geometric mean (GM) and MIC<sub>50</sub> values of itraconazole and caspofungin  
166 were the highest (Table 3). The MIC of amphotericin B (0.06-1 mg/L) was generally  
167 low among the *Pleosporales* with the exception of one isolate of *M. romeroi* and one of  
168 *D. gardeniae*, with MICs of 8 and 32 mg/L, respectively. The azole MIC ranged  
169 between 0.03 and 1 mg/L for isolates belonging to the genera *Paraconiothyrium*,  
170 *Paraphoma*, *Tintelnotia* and *Neocucurbitaria*, with the exception of two isolates of *N.*  
171 *unguis-hominis*, which showed higher values (16 mg/L). The terbinafine MIC was low  
172 except for *Diaporthe* spp. and a few isolates of *Colletotrichum* spp. and *M. romeroi*.

173

174 **DISCUSSION**

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

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

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

198 Other coelomycetous fungi we identified in the present work were *Didymella*  
199 *glomerata* and *Phoma herbarum*. Although *Phoma* spp. are commonly reported as a  
200 coelomycete involved in human infections (9, 20, 32–39), recent extensive changes in  
201 taxonomy and nomenclature have spread all but one of the species into different genera  
202 of the *Didymellaceae*, *Phoma herbarum* remaining as the unique species of the genus  
203 (22–24). Interestingly, *Didymella gardeniae* was commonly found in our study (five  
204 isolates from Spain and one from France).

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

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

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

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

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

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

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

251

## 252 **MATERIAL AND METHODS**

### 253 **Fungal isolates**

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

### 266 **Morphological and physiological characterization**

267           For morphology studies, the isolates were cultured on oatmeal agar (OA; 30 g of  
268 filtered oat flakes, 15 g of agar-agar, 1 L tap water) and malt extract agar (MEA; 40 g of  
269 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 (DNASStar 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<sub>N</sub> 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

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

296 For the phylogenetic study, sequences were aligned using the ClustalW  
297 application (68) of the MEGA 6.06 (69) computer program, and manually adjusted  
298 using the same software platform. Phylogenetic reconstructions were made by  
299 maximum-likelihood (ML) and Bayesian inference (BI) with MEGA 6.06 and MrBayes  
300 3.2.4 (70), respectively. The best substitution model for the gene matrix (TN93+G) was  
301 estimated using MEGA 6.06. For ML analyses, nearest-neighbour interchange was used  
302 as the heuristic method for tree inference. Support for internal branches was assessed by  
303 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) of  $\geq 70$  was considered  
304 significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried  
305 out with four million generations, with samples taken every 1,000 generations. The 50%  
306 majority rule consensus trees and posterior probability values (PP) were calculated after  
307 removing the first 25% of the resulting trees for burn-in. A PP value of  $\geq 0.95$  was  
308 considered significant. Reference strains of *Colletotrichum gigasporum* (CBS 159.75),  
309 *C. gloeosporioides* (CBS 122687) and *C. hippeastri* (CBS 241.78) were used as  
310 outgroup.

### 311 **Antifungal susceptibility testing**

312 The *in vitro* susceptibility testing in both reference centres (n= 46 isolates)  
313 followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST)  
314 procedure (71, 72). The antifungals used were amphotericin B (Sigma-Aldrich Química,  
315 Madrid, Spain), itraconazole (Sigma-Aldrich Química, Madrid, Spain), posaconazole  
316 (Schering-Plough Research Institute, Kenilworth, N.J.), voriconazole (Pfizer S.A.,  
317 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  $\geq 0.95$  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 <sup>T</sup> 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. <sup>a</sup>	Origin	Country	GenBank accession no. <sup>b</sup>	
<i>Botryosphaerales</i>	<i>Diplodia seriata</i>	CBS 112555 <sup>T</sup>	<i>Vitis vinifera</i> dead plant	Portugal	KF766327	
		CNRMA 6.1007	bone	France	<b>LT965964</b>	
	<i>Lasiodiplodia</i> sp.	CNRMA 15.383	eye	France (West Indies, Guadeloupe)	<b>LT965965</b>	
	<i>Lasiodiplodia theobromae</i>	CBS 164.96 <sup>T</sup>	fruit along coral reef coast	Papua New Guinea	NG_042460	
		CNRMA 10.1369	skin	France (West Indies, Martinique)	<b>LT965966</b>	
		CNRMA 10.813	eye	France (West Indies, Martinique)	<b>LT965967</b>	
		CNRMA 11.360	eye	France (West Indies, Martinique)	<b>LT965968</b>	
		CNRMA 13.891	skin	France	<b>LT965969</b>	
		CNRMA 14.708	eye	France (West Indies, Guadeloupe)	<b>LT965970</b>	
	<i>Neofusicoccum luteum</i>	CBS 110299	<i>Vitis vinifera</i> cane	Portugal	AY928043	
		CNRMA 12.597	eye	France	<b>LT965971</b>	
	<i>Diaporthales</i>	<i>Diaporthe sclerotioides</i>	CBS 296.67 <sup>T</sup>	<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	<b>LT965972</b>	
CNRMA 9.205			eye	France (West Indies, Guadeloupe)	<b>LT965973</b>	
CNRMA 11.385			eye	France (West Indies, Martinique)	<b>LT965974</b>	
CNRMA 12.311			blood	France	<b>LT965975</b>	
CNRMA 13.515			skin	France	<b>LT965976</b>	
CNRMA 14.198			skin	France	<b>LT965977</b>	

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

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

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

<i>Paraconiothyrium cyclothyrioides</i>	CBS 972.95 <sup>T</sup>	soil	Papua New Guinea	JX496232
	CNM-CM 6313	conjunctival exudate	Spain	<b>LT966037</b>
	CNM-CM 6513	nail	Spain	<b>LT966038</b>
	CNM-CM 4767	abscess	Spain	<b>LT966039</b>
	CNRMA 11.383	skin	France (West Indies, Martinique)	<b>LT966041</b>
	CNRMA 11.855	skin	France	<b>LT966042</b>
	CNRMA 13.245	skin	France	<b>LT966043</b>
	CNRMA 16.374	skin	France (West Indies, Guadeloupe)	<b>LT966044</b>
	CNRMA 16.556	skin	France (West Indies, Guadeloupe)	<b>LT966045</b>
<i>Paraconiothyrium fuckelii</i>	CBS 797.95	<i>Rubus</i> sp. dead stem	Denmark	JX496226
	CNRMA 3.240	eye	France	<b>LT966046</b>
	CNRMA 4.493	eye	France	<b>LT966047</b>
<i>Paraphaeosphaeria michotii</i>	MFLUCC 13-0349	<i>Poaceae</i> dead leaves	Italy	KJ939282
	CNM-CM 6000	skin	Spain	<b>LT966048</b>
<i>Paraphoma fimeti</i>	CBS 170.70 <sup>T</sup>	<i>Apium graveolens</i> seeds	The Netherlands	GQ387584
	CNM-CM 8075	wound exudate	Spain	<b>LT966049</b>
<i>Paraphoma</i> sp.	CNRMA 9.467	skin	France	<b>LT966050</b>
	CNRMA 15.665	skin	France	<b>LT966051</b>
<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	<b>LT966052</b>
	CNM-CM 3526	bone marrow	Spain	<b>LT966053</b>
	CNM-CM 3597	blood culture	Spain	<b>LT966054</b>
	CNM-CM 8031	nail	Spain	<b>LT966055</b>
	CNRMA 9.1095	skin	France	<b>LT966056</b>
	CNRMA 11.1097	eye	France	<b>LT966057</b>
	CNRMA 12.1227	eye	France	<b>LT966058</b>
pleosporelean fungus	CNRMA 11.1115	skin	France	<b>LT966059</b>
<i>Preussia</i> sp.	CNM-CM 7343	nail	Spain	<b>LT966060</b>
<i>Preussia terricola</i>	CBS 317.65 <sup>T</sup>	<i>Musa sapientum</i> rhizosphere	Honduras	GQ203725
<i>Preussia typharum</i>	CBS 107.69	Dung of deer	Japan	GQ203726
	CNM-CM 7335	nail	Spain	<b>LT966061</b>
<i>Pseudophaeosphaeria rubi</i>	MFLUCC 14-0259	<i>Rubus idaeus</i> dead branch	Italy	KX765299
<i>Tintelnotia destructans</i>	CBS 127737 <sup>T</sup>	anterior eye chamber cornea	Germany	KY090664
	CNM-CM 7430	Unknown	Spain	<b>LT966062</b>
<i>Tintelnotia</i> sp.	CNM-CM 7080	nail	Spain	<b>LT966063</b>
	CNM-CM 7981	cutaneous exudate	Spain	<b>LT966064</b>
<i>Xenodidymella saxea</i>	CBS 419.92 <sup>T</sup>	Corroded mediterranean marble	Unknown	GU238141

CNRMA 16.76

Cerebrospinal fluid

France

**LT966065**

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<sup>a</sup> **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 <sup>T</sup>

<sup>b</sup> LSU, large subunit ribosomal DNA sequences

**TABLE 2** Localization of infections due to coelomycetous fungi isolates

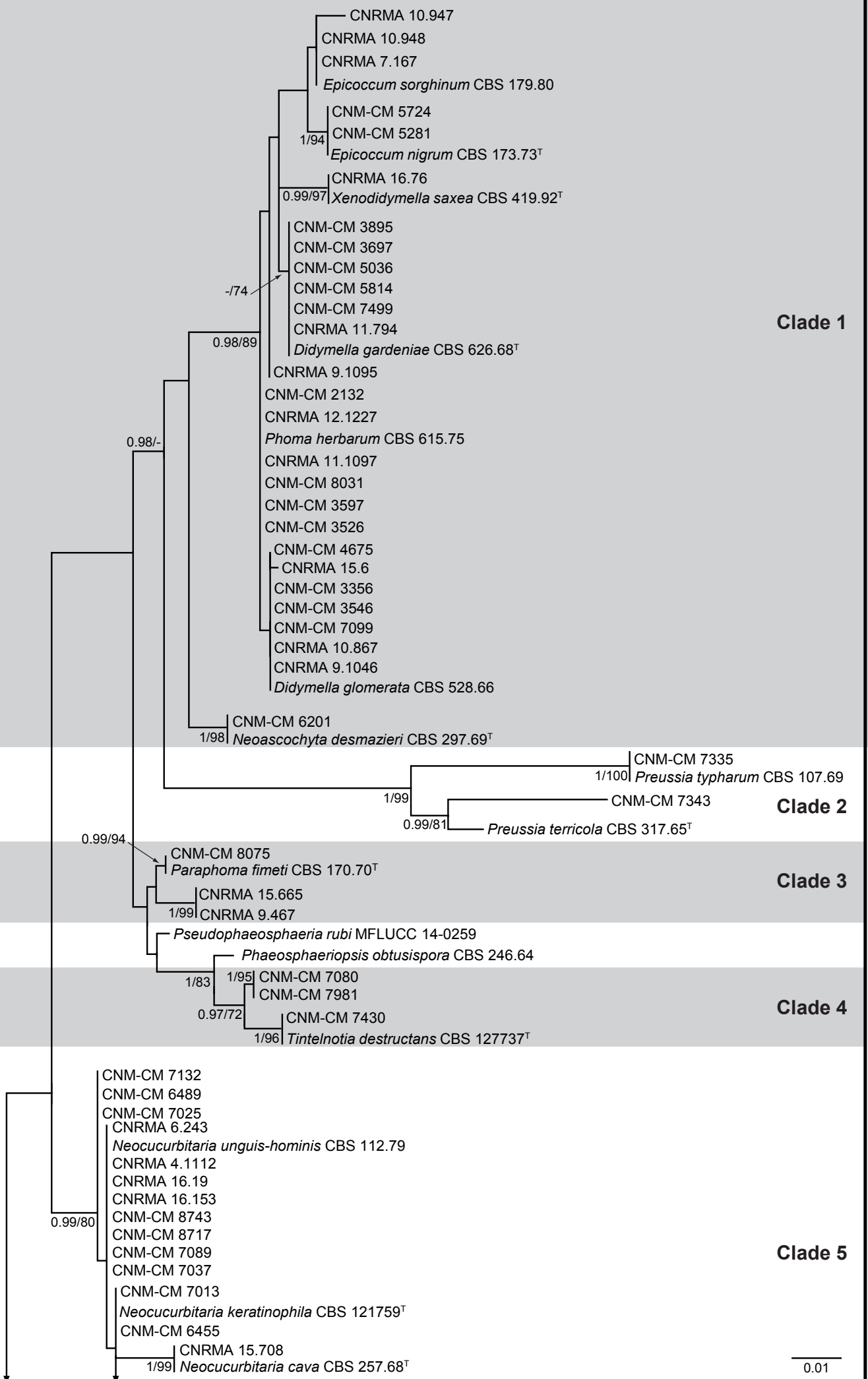
<b>Orders</b>	<b>no. of isolates obtained from:</b>		
	<b>Superficial infection</b>	<b>Deep infection</b>	<b>Total no. of isolates</b>
<i>Botryosphaerales</i>	7	1	8
<i>Diaporthales</i>	5	1	6
<i>Glomerellales</i>	5		5
<i>Pleosporales</i>	71	7	78
<b>Total no. of isolates (%)</b>	<b>88 (91)</b>	<b>9 (9)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

**TABLE 3** Overall *in vitro* antifungal activity against the 46 coelomycetous isolates as determined by EUCAST<sup>a</sup> methodology

Antifungal agent	MIC/MEC values (mg/L) <sup>b</sup>				
	range	median	GM	MIC <sub>50</sub>	MIC <sub>90</sub>
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

<sup>a</sup>EUCAST, European Committee on Antimicrobial Susceptibility Testing procedure (71);

<sup>b</sup>MIC, minimum inhibitory concentration; MEC, minimal effective concentration; MIC<sub>50</sub> and MIC<sub>90</sub>, MIC encompassing 50 and 90% of isolates tested, respectively.



Pleosporales

**Clade 1**

**Clade 2**

**Clade 3**

**Clade 4**

**Clade 5**

0.01

