PROFESSOR JOSÉ F. F. CANO-LIRA (Orcid ID : 0000-0003-4495-4394)

Article type : Review Article

DNA sequencing to clarify the taxonomical conundrum of the clinical coelomycetes

Nicomedes Valenzuela-Lopez[1,2], José F. Cano-Lira[1*], Alberto M. Stchigel[1], Josep Guarro[1]

[1]Mycology Unit, Medical School and IISPV, University Rovira i Virgili, C/ Sant Llorenç 21, 43201 Reus, Spain.

[2]Microbiology Unit, Medical Technology Department, Faculty of Health Science, University of Antofagasta, Chile.

Running head: Coelomycetes of clinical interest.

[*]Corresponding author. E-mail: jose.cano@urv.cat. Unitat de Micologia, Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, 21 Sant Llorenç St., 43201, Reus, Spain.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/myc.12785

Summary

The taxonomy of the fungi that produce human infections and that develop asexual fruiting bodies in culture has become very complex. Recent molecular studies have produced dramatic changes in their classification. Currently, the coelomycetes traditionally included in *Sphaeropsidales* and *Melanconiales* are in fact distributed across at least three different classes of the Phylum Ascomycota. Approximately 1,000 genera and 7,000 species have been grouped in the classes *Dothideomycetes*, *Leotiomycetes* and *Sordariomycetes* and their proper identification can only be made by analysing their DNA sequences and comparing them with those corresponding to type strains available in the adequate databases. To facilitate this task for scientists and clinicians involved in the study of these complex, and every day more numerous taxa, we have updated the knowledge about the taxonomy of the commonest coelomycetes of clinical interest with the aim of improving their identification and antifungal treatment.

Keywords: Coelomycetes, *Medicopsis*, mycosis, *Neocucurbitaria*, *Phoma*, *Pyrenochaeta*.

INTRODUCTION

The number of species of coelomycetes involved in human infections has increased enormously in recent years[1-4]. This has been a consequence of the current use of molecular biology approaches in taxonomy that have allowed more stable systematic criteria to be established and a redefinition of modern concepts of genera and species. On that basis, numerous taxa have recently been delimited. For instance, two of the most relevant genera of coelomycetes such as *Phoma* and *Pyrenochaeta* have undergone major changes. The former

has been reduced to only one species within the family *Didymellaceae*; while *Pyrenochaeta* has been excluded from *Cucurbitariaceae* and maintained as *incertae sedis*, its species mainly being distributed into the genus *Neocucurbitaria*.[5,6] The coelomycetes have been classified, traditionally, by their morphological features.[3,4] However, its classification turned out to be obsolete, and these fungi have been considered, based on phylogenetic analyses in three different classes, i.e. *Dothideomycetes* (bitunicate ascostromatic-like fruiting bodies), *Leotiomycetes* (apothecium-like fruiting bodies), and *Sordariomycetes* (unitunicate ascomata-like fruiting bodies) of the phylum Ascomycota.[4,7-9] Nevertheless, in the clinical setting the term "Coelomycete" is still used to refer to fungi morphologically characterized by producing conidia within fruiting bodies (= conidiomata) of two types, acervular (cup-shaped) or pycnidial (globose to pyriform) conidiomata.[10,11] Taxonomically, species with clinical prevalence are distributed mainly within the two first classes mentioned above, as was shown by Valenzuela-Lopez *et al*. [4], in a study of a large number of coelomycetous isolates from the USA, which demonstrated that the majority of them were distributed across at least eleven orders, being the *Pleosporales* the most prevalent.

With an ability to cause human infections, the coelomycetes have been involved in numerous opportunistic mycoses ranging from superficial to deep infections, most of them acquired by traumatic implantation of plant material or soil particles mainly in subtropical and tropical areas.[1,3-4,12-14] The most frequent are reduced to a specific group of taxa that includes *Colletotrichum* spp., *Medicopsis romeroi* and *Neoscytalidum dimidiatum*, although apart from these a huge number of species are occasionally also involved in human pathologies (see Table 1). Approximately 50 species have been reported in human mycoses, mainly causing subcutaneous infections.

Identification of the coelomycetes in the clinical laboratory is not easy because of their difficulty in sporulating. Recognizing and characterizing the most representative morphological structures of these fungi requires some expertise and even then these microorganisms remain sterile in many cases.[3] The use of molecular techniques based on the amplification and sequencing of appropriate phylogenetic markers is very important in the identification in this group of fungi.

Treatments are still not established due to the lack of clinical breakpoints for these fungi and to the difficulties in performing antifungal susceptibility testing against these fungi that hardly sporulate. Only few studies on coelomycetes have demonstrated that the use of surgical resection and a few drugs such as amphotericin B and triazoles have shown some efficacy.[14-15]

The aim of this paper is to update the current knowledge of the taxonomy of these taxa involved in human infections.

LABORATORY IDENTIFICATION

Isolation and morphological identification of clinical coelomycetous isolates

For cultural isolation and characterization the isolates should be inoculated on the following culture media: malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 L distilled water), oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 L tap water) and potato dextrose agar (PDA, 4 g of potato infusion, 20 g dextrose, 15 g of agar-agar, 1 L tap water) at 25 ± 1°C for 14 days in darkness. If the isolate does not sporulate, its incubation under near ultraviolet (UV) light (12 hours light, 12 hours dark) or on carnation leaf agar (CLA) to induce sporulation can be useful.[16-17] For micromorphological characterization, the use of wet mounts prepared in Shear's mounting medium (potassium acetate 3 g, distilled

water 150 mL, glycerin 60 mL, ethanol (95 %) 90 mL) or 85% lactic acid are recommended. Table 1 summarizes the most relevant morphological features of the most prevalent coelomycetes in the clinical setting.

DNA extraction, amplification, and sequencing

For identification purposes the fungal genomic DNA should be extracted from colonies grown on PDA after 7 days of incubation at 25 ± 1°C, following the protocols by Valenzuela-Lopez *et al*. [4].

Molecular identification of coelomycetes

To this purpose, the fragment of the 28S nrRNA gene (LSU) and internal transcribed spacer region (ITS) should be amplified, and depending on the fungus secondary phylogenetic markers such as beta-tubulin gene (*tub*2) and/or RNA polymerase II subunit 2 gene (*rpb*2) should be additionally tested (see Table 2).

Preliminary identification of coelomycetes can be carried out using the BLAST nucleotide tool of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast/), the Westerdijk Fungal Biodiversity Institute (former CBS) (http://www.westerdijkinstitute.nl/Collections/) or the Q-Bank (http://www.q-bank.eu/Fungi/) databases. For accurate identification of the isolates, the sequences must be compared with those of type or reference strains. It is also recommended to perform an alignment and its phylogenetic analysis using appropriate softwares such as MrBayes, RAxML, MEGA or several other useful programs.[18-20]

We suggest, depending on the fungi in order to identify, to follow the scheme of figure 1 and the primers listed in Table 2. In general, the genes ITS, LSU, *tub*2 and translation elongation factor 1-alpha (*tef*1) are easy to amplify; and also the *rpb*2, which is a bit more difficult to amplify; however, it is one of the most informative marker. [5-6] Nowadays, most of ITS and LSU genes of these species are sequenced and available in the public databases. Recently,

additional phylogenetic markers have been sequenced, which increase the possibility of a better taxonomic classification and identification of the fungi.

In figure 2 we provide an example of phylogenetic analysis with a tree performed by using the MEGA software with a set of LSU sequences of clinical coelomycetes available. However, the precise identification always depends on the phylogenetic markers used (see above). Unfortunately, most of the coelomycetes information in public databases it is not updated and in many cases is necessary an exhaustive literature search for a correct taxonomic placement of the fungus.

Recently, the use of matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry for identifying microorganisms in the clinical laboratory has quickly become important.[21-23] This technique was used previously mostly for yeasts, but more recently it has been also adapted for identifying molds. A recent study by Fraser *et al*. [23] also demonstrated the usefulness of this technique for coelomycetes that produce black grain mycetoma.

Conclusions

The taxonomy of the coelomycetes that have produced human infections is confusing because they are currently distributed across a high number of genera and species. They can only be properly identified by DNA sequencing and comparison with reference strains. However, their identity is crucial for their correct antifungal management, which is still little known.

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministerio de Economía y Competitividad,

grant CGL2017-88094-P.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Sutton DA. Coelomycetous fungi in human disease. A review: clinical entities, pathogenesis, identification and therapy. *Rev Iberoam Micol*. 1999;16:171–179.
2. de Hoog, Guarro J, Gene J, Figueras MJ. Atlas of clinical fungi. Utrecht/Reus: Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, 2000.
3. Stchigel AM, Sutton DA. Coelomycete fungi in the clinical lab. *Curr Fungal Infect Rep*. 2013;7:171–191.
4. Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, *et al*. Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. *J Clin Microbiol*. 2017;55:552–567.
5. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. Resolving the *Phoma* enigma. *Stud Mycol*. 2015;82:137–217.
6. Valenzuela-Lopez N, Cano-Lira JF, Guarro J, *et al*. Coelomycetous Dothideomycetes with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. *Stud Mycol*. 2018;90:1–69.
7. Schoch CL, Crous PW, Groenewald JZ, *et al*. A class-wide phylogenetic assessment of Dothideomycetes. *Stud Mycol*. 2009;64:1–15.
8. Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. *Pestalotiopsis* revisited. *Stud Mycol*. 2014;79:121–186.
9. Wijayawardene NN, Hyde KD, Wanasinghe DN, *et al*. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers*. 2016;77:1–316.

10. Sutton BC. *The Coelomycetes: Fungi Imperfecti with pycnidia, acervuli and stromata*. Kew, Surrey, England, Commonwealth Mycological Institute; 1980.
11. Kirk PM, Cannon PF, Stalpers JA, Minter DW. *Ainsworth & Bisby's dictionary of the fungi*. 10th ed. Wallingford, United Kingdom: CAB International; 2008.
12. Ahmed SA, Van De Sande WW, Stevens DA, *et al*. Revision of agents of black-grain eumycetoma in the order Pleosporales. *Persoonia*. 2014;33:141–154.
13. Vasant JA, Maggiani F, Borman AM. Subcutaneous mycotic cyst caused by *Roussoella percutanea* in a UK renal transplant patient. *Mycopathologia*. 2017;182:721–725.
14. Guégan S, Garcia-Hermoso D, Sitbon K, *et al*. Ten-year experience of cutaneous and/or subcutaneous infections due to coelomycetes in France. *Open Forum Infect Dis*. 2016;3:ofw106.
15. Chowdhary A, Meis JF, Guarro J, *et al*. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. *Clin Microbiol Infect*. 2014;20:47–75.
16. Fisher NL, Burgess LW, Toussoun TA, Nelson PE. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*. 1982;72:151–153.
17. Su YY, Qi YL, Cai L. Induction of sporulation in plant pathogenic fungi. *Mycology*. 2012;3:195–200.
18. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*. 2001;17:754–755.
19. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30:1312–1313.
20. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol*. 2013;30:2725–2729.

21. Havlicek V, Lemr K, Schug KA. Current trends in microbial diagnostics based on mass spectrometry. *Anal Chem*. 2013;85:790–797.
22. Sanguinetti M, Posteraro B. Identification of molds by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol*. 2017;55:369–379.
23. Fraser M, Borman AM, Johnson EM. Rapid and robust identification of the agents of black-grain mycetoma by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol*. 2017;55:2521–2528.
24. Campbell CK, Mulder JL. Skin and nail infection by Scytalidium hyalinum sp. nov. *Sabouraudia*. 1977;15:161–166.
25. Moore MK. Morphological and physiological studies of isolates of *Hendersonula toruloidea* Nattrass cultured from human skin and nail samples. *J Med Vet Mycol*. 1988;26:25–39.
26. Kombila M, Martz M, Gomez de Diaz M, de Bievre C, Richard-Lenoble D. *Hendersonula toruloidea* as an agent of mycotic foot infection in Gabon. *J Med Vet Mycol*. 1990;28:215–223.
27. Al-Rajhi AA, Awad AH, Al-Hedaithy SS, Forster RK, Caldwell KC. *Scytalidium dimidiatum* fungal endophthalmitis. *Br J Ophthalmol*. 1993;77:388–390.
28. Benne CA, Neeleman C, Bruin M, de Hoog G, Fleer A. Disseminating infection with *Scytalidium dimidiatum* in a granulocytopenic child. *Eur J Clin Microbiol Infect Dis*. 1993;12:118–121.
29. Geramishoar M, Zomorodian K, Zaini F, *et al*. First case of cerebral phaeohyphomycosis caused by *Nattrassia mangiferae* in Iran. *Jpn J Infect Dis*. 2004;57:285–286.

30. Jabbarvand M, Hashemian MR, Abedinifar Z, Amini A. *Nattrassia mangiferae* keratitis after laser in situ keratomileusis. *J Cataract Refract Surg*. 2004;30:268–272.
31. Padhi S, Uppin SG, Uppin MS, *et al*. Mycetoma in South India: retrospective analysis of 13 cases and description of two cases caused by unusual pathogens: *Neoscytalidium dimidiatum* and *Aspergillus flavus*. *Int J Dermatol*. 2010;49:1289–1296.
32. Jagielski T, Zak I, Tyrak J, Bryk A. First probable case of subcutaneous infection due to *Truncatella angustata*: a new fungal pathogen of Humans?. *J Clin Microbiol*. 2015;53:1961–1964.
33. Rebell G, Forster RK. *Lasiodiplodia theobromae* as a cause of keratomycoses. *Sabouraudia*. 1976;14:155–170.
34. Restrepo A, Arango M, Velez H, Uribe L. The isolation of *Botryodiplodia theobromae* from a nail lesion. *Sabouraudia*. 1976;14:1–4.
35. Slomovic AR, Forster RK, Gelender H. *Lasiodiplodia theobromae* panophthalmitis. *Can J Ophthalmol*. 1985;20:225–228.
36. Maslen MM, Collis T, Stuart R. *Lasiodiplodia theobromae* isolated from a subcutaneous abscess in a Cambodian immigrant to Australia. *J Med Vet Mycol*. 1996;34:279–283.
37. Woo PC, Lau SK, Ngan AH, Tse H, Tung ET, Yuen KY. *Lasiodiplodia theobromae* pneumonia in a liver transplant recipient. *J Clin Microbiol*. 2008;46:380–384.
38. Kindo AJ, Pramod C, Anita S, Mohanty S. Maxillary sinusitis caused by *Lasiodiplodia theobromae*. *Indian J Med Microbiol*. 2010;28:167–169.
39. Papacostas LJ, Henderson A, Choong K, Sowden D. An unusual skin lesion caused by *Lasiodiplodia theobromae*. *Med Mycol Case Rep*. 2015;8: 44–46.

40. Tan DH, Sigler L, Gibas CF, Fong IW. Disseminated fungal infection in a renal transplant recipient involving *Macrophomina phaseolina* and *Scytalidium dimidiatum*: case report and review of taxonomic changes among medically important members of the *Botryosphaeriaceae*. *Med Mycol*. 2008;46:285–292.
41. Srinivasan A, Wickes BL, Romanelli AM, et al. Cutaneous infection caused by Macrophomina phaseolina in a child with acute myeloid leukemia. J Clin Microbiol. 2009;47:1969–72.
42. Premamalini T, Ambujavalli BT, Vijayakumar R, Rajyoganandh SV, Kalpana S, Kindo AJ. Fungal keratitis caused by *Macrophomina phaseolina* - A case report. *Med Mycol Case Rep*. 2012;1:123-126.
43. Kirkness CM, Seal DV, Clayton YM, Punithalingam E. *Sphaeropsis subglobosa* keratomycosis - First reported case. *Cornea*. 1991;10:85–89.
44. Cariello PF, Wickes BL, Sutton DA, *et al*. *Phomopsis bougainvilleicola* prepatellar bursitis in a renal transplant recipient. *J Clin Microbiol*. 2013;51:692–695.
45. Iriart X, Binois R, Fior A, *et al*. Eumycetoma caused by *Diaporthe phaseolorum* (*Phomopsis phaseoli*): a case report and a mini-review of *Diaporthe*/*Phomopsis* spp invasive infections in humans. *Clin Microbiol Infect*. 2001;17:1492–1494.
46. Gajjar DU, Pal AK, Parmar TJ, *et al*. Fungal scleral keratitis caused by *Phomopsis phoenicicola*. *J Clin Microbiol*. 2011;49:2365–2368.
47. Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Africa. *Am J Ophthalmol*. 1980;90:38–47.
48. O'Quinn RP, Hoffmann JL, Boyd AS. *Colletotrichum* species as emerging opportunistic fungal pathogens: a report of 3 cases of phaeohyphomycosis and review. *J Am Acad Dermatol*. 2001;45:56–61.

49. Liao WQ, Shao JZ, Li SQ, *et al*. Colletotrichum dematium caused keratitis. *Chin Med J*. 1983;96:391–394.

50. Mendiratta DK, Thamke D, Shukla AK, Narang P. Keratitis due to *Colletotrichum dematium* - A case report. *Indian J Med Microbiol*. 2005;23:56–58.

51. Singh R, Gupta V, Chakarborty A, Nijhawan R, Arora S, Gupta A. Fungal endophthalmitis complicating subconjunctival injection of triamcinolone acetonide in anterior scleritis. *Ann Ophthalmol* (Skokie). 2006;38:253–256.

52. Castro LG, da Silva LC, Guarro J, *et al*. Phaeohyphomycotic cyst caused by *Colletotrichum crassipes*. *J Clin Microbiol*. 2001;39:2321–2324.

53. Shukla PK, Khan ZA, Lal B, Agrawal PK, Srivastava OP. Clinical and experimental keratitis caused by *Colletotrichum* state of *Glomerella cingulata* and *Acrophialophora fusispora*. *Sabouraudia*. 1983;21:137–147.

54. Guarro J, Svidzinski TE, Zaror L, Forjaz MH, Gené J, Fischman O. Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. *J Clin Microbiol*. 1998;36:3060–3065.

55. Potea ST, Chakraborty A, Lahiri KK, Patolea MS, Deshmukhc RA, Shah SR. Keratitis by a rare pathogen *Colletotrichum gloeosporioides*: A case report. *J Mycol Med*. 2017;27:407–411.

56. Ritterband DC, Mahendra S, Seedor JA. *Colletotrichum graminicola*: A new corneal pathogen. *Cornea*. 1997;16:362–364.

57. Shivaprakash MR, Appannanavar SB, Dhaliwal M, *et al*. *Colletotrichum truncatum*: an unusual pathogen causing mycotic keratitis and endophthalmitis. *J Clin Microbiol*. 2011;49:2894–2898.

58. Chowdhary A, Guarro J, Randhawa HS, *et al*. A rare case of chromoblastomycosis in a renal transplant recipient caused by a non-sporulating species of *Rhytidhysteron*. *Med Mycol*. 2008;46:163–166.
59. Mahajan VK, Sharma V, Prabha N, *et al*. A rare case of subcutaneous phaeohyphomycosis caused by a *Rhytidhysteron* species: a clinico-therapeutic experience. *Int J Dermatol*. 2014;53:1485–1489.
60. Mishra K, Das S, Goyal S, *et al*. Subcutaneous mycoses caused by *Rhytidhysteron* species in an immunocompetent patient. *Med Mycol Case Rep*. 2014;5:32–34.
61. Guarro J, Höfling-Lima AL, Gené J, *et al*. Corneal ulcer caused by the new fungal species *Sarcopodium oculorum*. *J Clin Microbiol*. 2002;40:3071–3075.
62. Perdomo H, García D, Gené J, *et al*. *Phialemoniopsis*, a new genus of Sordariomycetes, and new species of *Phialemonium* and *Lecythophora*. *Mycologia*. 2013;105:398–421.
63. Zaitz C, Heins-Vaccari EM, Freitas RS, *et al*. Subcutaneous phaeohyphomycosis caused by *Phoma cava*. Report of a case and review of the literature. *Rev Inst Med trop S Paulo*. 1997;39:43–48.
64. Ferrer C, Pérez-Santonja JJ, Rodríguez AE, *et al*. New Pyrenochaeta species causing keratitis. *J Clin Microbiol*. 2009;47:1596–1598.
65. Verkley GJ, Gené J, Guarro J, *et al*. *Pyrenochaeta keratinophila* sp. nov., isolated from an ocular infection in Spain. *Rev Iberoam Micol*. 2010;27:22–24.
66. English MP, Atkinson R. Onychomycosis in elderly chiropody patients. *Br J Dermatol*. 1974;91:67–72.
67. Punithalingam E, English MP. *Pyrenochaeta unguis-hominis* sp.nov. on human toe-nails. *Trans Br Mycol Soc*. 1975;64:539–541.

68. Toh YF, Yew SM, Chan CL, *et al*. Genome anatomy of *Pyrenochaeta unguis-hominis* UM 256, a multidrug resistant strain isolated from skin scraping. *PLoS One*. 2016;11:e0162095.

69. Balis E, Velegraki A, Fragou A, Pefanis A, Kalabokas T, Mountokalakis T. Lung mass caused by Phoma exigua. *Scand J Infect Dis*. 2006;38:552–555.

70. Punithalingam E. Sphaeropsidales in culture from humans. *Nova Hedwigia*. 1979;31:119–158.

71. Rai MK. *Phoma sorghina* infection in human being. *Mycopathologia*. 1989;105:167–170.

72. Bakerspigel A, Lowe D, Rostas A. The isolation of *Phoma eupyrena* from a human lesion. *Arch Dermatol*. 1981;117:362–363.

73. Punithalingam E. *Phoma oculo-hominis* sp.nov. from corneal ulcer. *Trans Br Mycol Soc*. 1976;67:142–143.

74. Colombier MA, Alanio A, Denis B, *et al*. Dual Invasive Infection with *Phaeoacremonium parasiticum* and *Paraconiothyrium cyclothyrioides* in a Renal Transplant Recipient: Case Report and Comprehensive Review of the Literature of *Phaeoacremonium* Phaeohyphomycosis. *J Clin Microbiol*. 2015;53:2084–2094.

75. Kiehn TE, Polsky B, Punithalingam E, Edwards FF, Brown AE, Armstrong D. Liver infection caused by Coniothyrium fuckelii in a patient with acute myelogenous leukemia. *J Clin Microbiol*. 1987;25:2410–2412.

76. Borelli D. *Pyrenochaeta romeroi* nova especie. *Dermatol Venez*. 1959;1:325–326.

77. Girard C, Dereure O, Rispail P, Durand L, Guilhou JJ. Subcutaneous phaeohyphomycosis due to Pyrenochaeta romeroi in a patient with leprosy. *Acta Derm Venereol*. 2004;84:154–155.

78. Ocampo MA, Kanitakis J, Bienvenu AL, Chauvet C, Euvrard S. Phaeohyphomycosis caused by *Pyrenochaeta romeroi* mimicking a plantar wart in a kidney transplant recipient. *Transpl Infect Dis*. 2012;14:173–174.
79. Borelli D, Zamora R, Senabre G. *Chaetosphaeronema larense* nova species agente de micetoma. *Gaceta Med Caracas*. 1976;84:307–318.
80. Ahmed SA, Desbois N, Quist D, *et al*. Phaeohyphomycosis caused by a novel species, *Pseudochaetosphaeronema martinelli*. *J Clin Microbiol*. 2015;53:2927–2934.
81. Chabasse D, de Bievre C, Legrand E, *et al*. Subcutaneous abscess caused by *Pleurophomopsis lignicola* Petr: first case. *J Med Vet Mycol*. 1995;33:415–417.
82. Padhye AA, Karpati A, Rosenthal SA, Punithalingam E. Subcutaneous phaeohyphomycotic abscess caused by *Pleurophomopsis lignicola*. *Med Mycol*. 2004;42:129–134.
83. Borelli D. *Pyrenochaeta mackinnonii* nova species agente de micetoma. *Castellania*. 1976;4:227–234.
84. Ahmed SA, Hofmüller W, Seibold M, *et al*. *Tintelnotia*, a new genus in *Phaeosphaeriaceae* harbouring agents of cornea and nail infections in humans. *Mycoses*. 2017;60:244–253.
85. Shukla NP, Rajak RK, Agarwal GP, Gupta DK. *Phoma minutispora* as a Human Pathogen. *Mycoses*. 1984;27:255–258.
86. Ahmed SA, Stevens DA, van de Sande WW, Meis JF, de Hoog GS. *Roussoella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. *Med Mycol*. 2014;52:689–698.
87. Borman AM, Desnos-Ollivier M, Campbella CK, Bridge PD, Dannaoui E, Johnson EM. Novel taxa associated with Human fungal black-grain Mycetomas: *Emarellia*

grisea gen. nov., sp. nov., and *Emarellia paragrisea* sp. nov. *J Clin Microbiol*. 2016;54:1738–1745.

88. Segretain G, Destombes P. Mycetomas caused by *Madurella grisea* and *Pyrenochaeta romeroi*. *Sabouraudia*. 1969;7:51–61.
89. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. Orlando, Florida: Academic Press; 1990:315–322.
90. Rehner SA, Samuels GJ. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol Res*. 1994;98:625–634.
91. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J Bacteriol*. 1990;172:4238–4246.
92. Carbone I, Kohn L. A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia*. 1999;91:553–556.
93. O'Donnell K, Cigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol Phylogenet Evol*. 1997;7:103–116.
94. Glass NL, Donaldson G. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. *Appl Environ Microb*. 1995;61:1323–1330.
95. Woudenberg JHC, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia*. 2009;22:56–62.

96. Guerber JC, Liu B, Correll JC, Johnston PR. Characterization of diversity in *Colletotrichum acutatum* sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. *Mycologia*. 2003;95:872–895.
97. Crous PW, Groenewald JZ, Risede JM, Hywel-Jones NL. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Stud Mycol*. 2004;50:415–430.
98. Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. *Mol Biol Evol*. 1999;16:1799–1808.
99. Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia*. 2006;98:1041–1052.

Figure legend

Figure 1. Flow scheme showing the phylogenetic markers for coelomycete identification ("?" means that ITS not always it's an useful tool to resolve the identification at species level). Data referred to genes are included in Table 2.

Figure 2. Maximum likelihood tree obtained from the D1-D2 of LSU (588 bp) sequences of 40 coelomycetous isolates. Bootstrap support values of ≥70 are indicated on the nodes. The GenBank accession numbers are given for each isolate. *Chaetomella oblonga* ATCC 12718 and *C. zambiensis* CBS 137978 were used to root the tree.

Table 1 Species of coelomycetes of clinical interest and their morphological characteristics

Order & Family	Species (former name)	Human infection	Reference	Conidiomata	Conidia	Other features
Amphisphaeriales						
Bartaliniaceae	*Truncatella angustata*	Subcutaneous	23	Acervular to pycnidial	Fusiform, 3-septate, 15–23 × 6–8 µm, with apical appendages; basal cell hyaline, obconic, thin-walled; mediun cells brown, doliiform to subcylindrical, thick-walled	
Botryosphaeriales						
Botryosphaeriaceae	*Hendersonula toruloidea* synanamorph: *Neoscytalidium dimidiatum* (*Scytalidium hyalinum*)	Cutaneous, keratitis, onychomycosis, black grain eumycetoma, endophthalmitis, subcutaneous, Cerebral, systemic	24-31	Pycnidial	Initially hyaline, aseptate, becoming dark brown and septate with age, 12–20 × 4–8 µm	The synanamorph *N. dimidiatum* produces arthroconidia
	Lasiodiplodia theobromae (*Botryodiplodia theobromae*)	Keratitis, onychomycosis, endophthalmitis, subcutaneous, pneumonia, sinusitus	32-38	Pycnidial	Initially hyaline, becoming dark brown with longitudinal striations , ellipsoidal, thick-walled, 1-septate, 20–30 × 10–15 µm	Sexual morph on vegetal material. Paraphyses hyaline, cylindrical, septate
	Macrophomina phaseolina	Cutaneous, keratitis, systemic	39-41	Pycnidial	Hyaline, ellipsoid to obovoid, 14–30 × 5–10	Sclerotia on vegetal material

					µm	
	Neodeightonia subglobosa (*Sphaeropsis subglobosa*)	Keratitis	42	Stromatic	Brown, obovoidal or subspherical to spherical, with truncate base, thick-walled, aseptate, 9–12 × 6–9 µm	
Diaporthales						
Diaporthaceae	*Diaporthe bougainvilleicola* (*Phomopsis bougainvilleicola*)	Bursitis	43	Stromatic	Hyaline, of two types: alpha, ellipsoidal or fusiform, 5–8.4 × 1.2–2 µm, and beta, filiform, curved, 16–31 × 0.5–0.9 µm	
	Diaporthe phaseolorum (*Phomopsis phaseoli*)	Black grain eumycetoma	44	Pycnidial	Hyaline, of two types: alpha, ovoid, 6.7–7 × 2.4 µm, and beta, filiform, 13.3–22.5 × 0.5–0.9 µm	
	Diaporthe phoenicicola (*Phomopsis phoenicicola*)	Keratitis	45	Pycnidial	Hyaline, only alpha-conidia, ovoid to fusiform, 8–12 × 2–2.5 µm	
Glomerellales						
Glomerellaceae	*Colletotrichum coccodes* (*C. atramentarium*)	Keratitis, subcutaneous	46-47	Acervular	Hyaline, straight, fusiform, 16–22 × 3–4 µm	Apressoria brown, clavate, 11–16.5 × 6–9.5 µm

	Colletotrichum dematium	Keratitis, endophthalmitis	48-50	Acervular	Hyaline, falcate, acute apex, 20–30 × 3–5 µm	Apressoria brown, clavate to circular, 8–11.5 × 6.5–8 µm
	Colletotrichum gigasporum (*C. crassipes*)	Subcutaneous	51	Acervular	Hyaline, cylindrical with rounded ends, 27–30 × 9–10 µm	Apressoria brown to dark brown, variable in shape including ovoid, clavate or slightly irregular, 15–30 × 7–14 µm
	Colletotrichum gloeosporioides	Keratitis, subcutaneous	52-54	Acervular	Hyaline, straight, cylindrical, obtuse at the apex, 9–24 × 3–4.5 µm	Apressoria brown, clavate or irregular, 6–20 × 4–12 µm
	Colletotrichum graminicola	Keratitis	55	Acervular	Hyaline, fusiform to falcate, 23–29 × 3.5–5 µm	Apressoria brown, irregular, 17–20 × 12–14 µm
	Colletotrichum truncatum	Keratitis	56	Acervular	Hyaline, fusiform, 20–23.5 × 3.5–4 µm	Appressoria light brown, solitary or in groups, ellipsoidal or clavate, 6.5–13 × 5.5–7.5 µm
Hysteriales						
Hysteriaceae	*Rhytidhysteron rufulum*	Subcutaneous	57-59	Pycnidial	Hyaline, globose to subglobose, smooth- and thin-walled	Sexual morph on vegetal material

Incertae sedis	*Phialemoniopsis ocularis* (*Sarcopodium oculorum*)	Keratitis	60-61	Pycnidial or sporodochial	Hyaline, ellipsoidal, smooth-walled, aseptate, 2–3.5 × 1–1.5 µm	Chlamydospores, brown, thick- and rough-walled, intercallary, solitary or in chains, globose to pyriform, 5–9 × 3–6 µm
Pleosporales						
Cucurbitariaceae	*Neocucurbitaria cava* (*Pyrenochaeta cava*)	Subcutaneous	62	Pycnidial	Hyaline, cylindrical to slightly allantoid, smooth- and thin-walled, aseptate, 2.5–3.5 × 1–1.5 µm	Growth at 37ºC[a]
	Neocucurbitaria keratinophila (*Pyrenochaeta keratinophila*)	Keratitis	63-64	Pycnidial	Hyaline, ellipsoidal, smooth- and thin-walled, aseptate, 2.5–3 × 1–2 µm	Growth at 37ºC[a]
	Neocucurbitaria unguis-hominis (*Pyrenochaeta unguis-hominis*)	Onychomycosis	65-67	Pycnidial	Hyaline, cylindrical, smooth- and thin-walled, aseptate, 2–3.5 × 1–1.5 µm	Growth at 37ºC[a]
Didymellaceae	*Boeremia exigua* var. *exigua* (*Phoma exigua*)	Lung mass	68	Pycnidial	Hyaline, smooth- and thin-walled, mainly aseptate or 1(–2)-septate, 4.5–8 × 2.5–4 µm	

Didymella glomerata (*Phoma glomerata*)	Subcutaneous	69	Pycnidial	Hyaline, variable in shape, smooth- and thin-walled, aseptate, 4–8.5 × 1.5–3 µm	Chlamydospores dark brown, solitary or in chains, multicellular-dictyosporous, 30–65 × 15–25 µm
Epicoccum sorghinum (*Phoma sorghina*)	Subcutaneous	70	Pycnidial	Hyaline, variable in shape mostly ovoid-ellipsoidal, smooth- and thin-walled, aseptate, 4.5–7 × 2–3 µm	Chlamydospores dark brown, solitary or in chains, irregular, dictyosporous, 8–35 µm diam.
Juxtiphoma eupyrena (*Phoma eupyrena*)	Subcutaneous	71	Pycnidial	Hyaline, ellipsoidal, smooth- and thin-walled, aseptate, 4–5.5 × 2–2.5 µm	Chlamydospores dark brown, barrel shaped, 4–15 µm diam.
Phoma herbarum (*Phoma cruris-hominis*)	Subcutaneous	69	Pycnidial	Hyaline, ellipsoidal to ovoid, smooth- and thin-walled, aseptate, 4.5–6 × 2–3 µm	
Stagonosporopsis oculi-hominis (*Phoma oculi-homini*)	Keratitis	72	Pycnidial	Hyaline to brown, cylindrical, smooth- and thin-walled, aseptate (3–7 × 1–2 µm) or 1-septate(9–16 × 3–4.5 µm)	

Didymosphaeriaceae	*Paraconiothyrium cyclothyrioides*	Systemic	73	Stromatic	Initially hyaline, yellowish brown with age, cylindrical, thin-walled, aseptate, 3–4.2 × 1.2–1.5 µm	
	Paraconiothyrium fuckelii (*Coniothyrium fuckelii*)	Systemic	74	Pycnidial	Pale brown, cylindrical, smooth- and thin-walled, aseptate, 2.5–4 × 1.5–2 µm	
Incertae sedis	*Medicopsis romeroi* (*Pyrenochaeta romeroi*)	Cutaneous, black grain eumycetoma, subcutaneous	11,75-77	Pycnidial	Hyaline, cylindrical to ellipsoidal, smooth- and thin-walled, aseptate, 2–2.8 × 1.2–1.5 µm	Growth at 37ºC[a]
Macrodiplodiopsidaceae	*Pseudochaetosphaeronema larense* (*Chaetosphaeronema larense*)	Black grain eumycetoma	78	Pycnidial	Hyaline, cylindrical, smooth- and thin-walled, aseptate, 2–3 × 1–1.8 µm	
	Pseudochaetosphaeronema martinelli	Subcutaneous	79			Culture sterile
Melanommataceae	*Pleurophomopsis lignicola*	Subcutaneous, sinusitus	80-81	Pycnidial	Hyaline, ellipsoidal, smooth- and thin-walled, aseptate, 2–3 × 0.5–1 µm	
Nigrograna ceae	*Nigrograna mackinnonii* (*Pyrenochaeta mackinnonii*)	Black grain eumycetoma	82	Pycnidial	Subhyaline, ellipsoidal, smooth, aseptate, 2.5–3 × 1.5–2 µm	

Phaeosphaeriaceae	*Tintelnotia destructans*	Onychomycosis	83	Pycnidial	Hyaline to pale brown, ellipsoidal, smooth- and thin-walled, aseptate, 2–3.2 × 1–2 µm	Growth at 37ºC[a]
Sporormiaceae	*Westerdykella minutispora* (*Phoma minutispora*)	Subcutaneous	84	Pycnidial	Hyaline, subglobose to ellipsoidal, smooth- and thin-walled, aseptate, 2–2.5 × 1.5–2 µm	Chlamydospores dark brown, subglobose or irregular, solitary mostly terminally on hyphae, 6–15 µm diam. Can produce sexual morph
Thyridariaceae	*Parathyridaria percutanea* (*Roussoella percutanea*)	Subcutaneous	85	Pycnidial	Hyaline to pale brown, ellipsoidal, smooth- and thin-walled, aseptate, 1.2–2 × 0.7–0.9 µm	Growth at 37ºC[a]
Trematosphaeriaceae	*Emarellia grisea*	Black grain eumycetoma	86			Culture sterile
	Emarellia paragrisea	Black grain eumycetoma	86			Culture sterile
	Trematosphaeria grisea (*Madurella grisea*)	Black grain eumycetoma	11,87-88	Pycnidial	Hyaline to pale brown, clavate to ellipsoidal, smooth- and thin-walled, aseptate, 4–5.4 × 2–2.4 µm	Growth at 37ºC[a]

[a] species that was previously tested at different temperature conditions

Table 2 Primers used for coelomycetes identification

Gene	Product name	Primer	Direction	Sequence (5'–3')	Reference	Used in
Internal transcribed spacer (complete)	ITS	ITS-5	Forward	GGA AGT AAA AGT CGT AAC AAG G	White *et al*.[89]	All coelomycetes
		ITS-4	Reverse	TCC TCC GCT TAT TGA TAT GC	White *et al*.[89]	
28S ribosomal RNA	LSU	LR0R	Forward	GTA CCC GCT GAA CTT AAG C	Rehner & Samuels[90]	All coelomycetes
		LR5	Reverse	TCC TGA GGG AAA CTT CG	Vilgalys & Hester[91]	
Actin	ACT	ACT-512F	Forward	ATG TGC AAG GCC GGT TTC GC	Carbone & Kohn[92]	*Colletotrichum*, *Didymella*, *Phoma*
		ACT-783R	Reverse	TAC GAG TCC TTC TGG CCC AT	Carbone & Kohn[92]	
Beta-tubulin	TUB2	T1	Forward	AAC ATG CGT GAG ATT GTA AGT	O'Donnell & Cigelnik[93]	*Colletotrichum*, *Diaporthe*
		Bt-2b	Reverse	ACC CTC AGT GTA GTG ACC CTT GGC	Glass & Donaldson[94]	
		TUB2Fd	Forward	GTB CAC CTY CAR ACC GGY CAR TG	Woudenberg *et al*.[95]	*Didymellaceae*, Pleosporalean coelomycetes
		TUB4Rd	Reverse	CCR GAY TGR CCR AAR ACR AAG TTG TC	Woudenberg *et al*.[95]	
Calmodulin	CAL	CAL-228F	Forward	GAG TTC AAG GAG GCC TTC TCC C	Carbone & Kohn[92]	*Colletotrichum*, *Diaporthe*
		CAL-737R	Reverse	CAT CTT TCT GGC CAT CAT GG	Carbone & Kohn[92]	
Chitin synthase 1	CHS-1	CHS-79F	Forward	TGG GGC AAG GAT GCT TGG AAG AAG	Carbone & Kohn[92]	*Colletotrichum*

		CHS-354R	Reverse	TGG AAG AAC CAT CTG TGA GAG TTG	Carbone & Kohn[92]	
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	GDF1	Forward	GCC GTC AAC GAC CCC TTC ATT GA	Guerber *et al*.[96]	*Colletotrichum*
		GDR1	Reverse	GGG TGG AGT CGT ACT TGA GCA TGT	Guerber *et al*.[96]	
Histone H3	HIS3	CYLH3F	Forward	AGG TCC ACT GGT GGC AAG	Crous *et al*.[97]	*Colletotrichum*
		CYLH3R	Reverse	AGC TGG ATG TCC TTG GAC TG	Crous *et al*.[97]	
RNA polymerase II second largest subunit	RPB2	fRPB2-5F	Forward	GAY GAY MGW GAT CAY TTY GG	Liu *et al*.[98]	All coelomycetes
		fRPB2-7R	Reverse	CCC ATW GCY TGC TTM CCC AT	Liu *et al*.[98]	
Translation elongation factor 1-alpha	TEF	EF1-728F	Forward	CAT CGA GAA GTT CGA GAA GG	Carbone & Kohn[92]	*Diaporthe*
		EF1-986R	Reverse	TAC TTG AAG GAA CCC TTA CC	Carbone & Kohn[92]	
		TEF1-983F	Forward	GCY CCY GGH CAY CGT GAY TTY AT	Schoch *et al*.[99]	All coelomycetes
		TEF1-2218R	Reverse	AT GAC ACC RAC RGC RAC RGT YTG	Schoch *et al*.[99]