Survey, Identification and Characterization of cylindrocarpon-like asexual morphs in Spanish forest nurseries

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Abstract

Cylindrocarpon-like asexual morphs infect herbaceous and woody plants, mainly in agricultural scenarios, but also in forestry systems. The aim of the present study was to characterize a collection of cylindrocarpon-like isolates recovered from the roots of a broad range of forest hosts from nurseries showing decline by morphological and molecular studies. Between 2009 and 2012, 17 forest nurseries in Spain were surveyed and a total of 103 cylindrocarpon-like isolates were obtained. Isolates were identified based on DNA sequences of the partial gene regions histone H3 (*his3*). For the new species, the internal transcribed spacer and intervening 5.8S nrRNA gene (ITS) region, β-tubulin (*tub2*), and translation elongation factor 1-α (*tef1*) were also used to determine their phylogenetic position. Twelve species belonging to the genera *Cylindrodendrum*, *Dactylonectria* and *Ilyonectria* were identified from damaged roots of 15 different host

genera. The species *C. alicantinum*, *D. macrodidyma*, *D. novozelandica*, *D. pauciseptata*, *D. pinicola, D. torresensis*, *I. capensis, I. cyclaminicola, I. liriodendri*, *I. pseudodestructans*, *I. robusta* and *I. rufa* were identified. In addition, two *Dactylonectria* species (*D. hispanica* sp. nov., and *D. valentina* sp. nov.), one *Ilyonectria* species (*I. ilicicola* sp. nov.) and one *Neonectria* species (*N. quercicola* sp. nov.) are newly described. The present study demonstrates the prevalence of this fungal group associated with seedlings of diverse hosts showing decline symptoms in forest nurseries in Spain.

Introduction

Spain has the second largest forest area in the European Union (EU), 27.7 M ha, which accounts for the 15.4% of the total European forest. The most extensive forest systems in Spain include holm oak forests (*Quercus ilex*) (2.8 M ha, 15.3% of the forested area), oak range-lands consisting mainly of holm oaks (2.4 M ha), and Aleppo pines (*Pinus halepensis*) (2 M ha) (MAGRAMA 2014).

Forest systems not only offer market valuable services such as timber, hunting, fishing and tourism, but also important environmental and social contributions such as carbon capture, hydric and soil regulation, hosting biodiversity and regulating climate. Climate change has substantially built public awareness about the need of developing forest management strategies to counterbalance the deforestation rate (Trumbore et al. 2015).According to FAO (2015), in 2010, the deforestation rate in Spain was 57,000 ha per year, while the reforestation rate shed a positive balance with 89,000 ha per year.

Forest nurseries provide woody plants for the afforestation process, but they are also a key point to prevent and control early infections by fungal pathogens, thus guaranteeing the phytosanitary quality of planting materials. An increasing incidence of invasive pathogens related to global tree-planting projects has been reported worldwide (Wingfield

et al. 2015). Numerous examples of alien invasions have been reported in the forestry sector, such as: *i*) *Phytophthora cinnamomi* root disease or Jarrah dieback; *ii*) Sudden Oak Death caused by *P. ramorum*; *iii*) Dutch elm disease caused by *Ophiostoma ulmi*; *iv*) Chestnut blight caused by *Cryphonectria parasitica*; *v*) Cedar root rot caused by *P. lateralis* (Brasier 2008). Thus, nursery regulations should be implemented at a global scale and updated phytosanitary measures must be considered as the cornerstone of the all nursery systems (Wingfield et al. 2015).

Cylindrocarpon-like asexual morphs infect herbaceous and woody plants, mainly in agricultural scenarios, but also in forest systems (Agustí-Brisach and Armengol 2013). This group of fungi has been commonly associated with damping-off, root rot or bark necrosis in forest nurseries and also with cankers on forest stands (Jankowiak et al. 2016).In Swedish conifer nurseries, *Cylindrocarpon* (*Cy*.) *destructans*, was the main pathogen isolated from damaged root tissues (Beyer-Ericsonet al. 1991). Lilja et al. (1992) reported the presence of *Cy. cylindroides*, *Cy. destructans*, *Cy. didymum*, *Cy. magnusiarum*, *Cy. obtusisporum* and *Cy. pineum* in seedlings of *Pinus* (*P.*) *sylvestris* and *Picea* (*Pc.*) *abies* in Finnish nurseries. None of these asexual morphs seemed to be pathogenic but they predisposed *P. sylvestris* to be colonised by the most common saprophytic *Cy. destructans*. Dumroese and James (2005) stated that in forest and conservation nurseries in the Pacific Northwest of USA, cylindrocarpon-like asexual morphs were among the most ubiquous root pathogens, with *Cy. destructans* being the most frequently isolated. Menkis et al. (2006) confirmed the presence of *Nectria* species (*N. gliocladioides*, *N. inventa*, *N. lucida*, *N. macrodydima* and *N. radicicola*) in decayed roots of *P. sylvestris* and *Pc. abies* nursery seedlings in Lithuania. In 2009, damping off of *P. radiata* seedlings was observed in a pine nursery in Spain. *Dactylonectria* (*D*.)

pauciseptata was described as the causal agent of damping-off, extensive root necrosis, and root death, exhibited by the pine seedlings (Agustí-Brisach et al. 2011).

In forests, cylindrocarpon-like asexual morphs have also been reported as the dominant fungi on roots of *Fraxinus excelsior*, *Fagus* (*F.*) *sylvatica* and *Quercus* spp. (Kubíková 1963; Krzan 1987; Halmschlager and Kowalski 2004).Cylindrocarpon-like asexual morphs can hinder the natural regeneration of different tree species. *Cylindrocarpon destructans* arose as the main root pathogen implicated in the absence of regeneration of *Taxus baccata* and *Abies* (*A.*) *alba* in Poland (Manka et al. 1968; Kowalski 1982). Damping off caused by *Cy. destructans* was observed in the natural regeneration of *Eucalyptus* trees in Australia (Mwanza and Kellas 1987;Iles et al. 2010). In Canada, Axelrood et al.(1998)isolated cylindrocarpon–like asexual morphs from the roots of naturally regenerating seedlings of *Pseudotsuga* (*Ps.*) *menziesii*. Szewczyk and Szwagrzyk, (2010) reported that this complex of asexual morphs affected the regeneration of old stands of *F. sylvatica* and *A. alba* in Western Carpathians, Poland. In 2012, *Neonectria candida* (syn. *N. ramulariae*) was described as a pathogen of *F. crenata* seeds in Japan (Hirooka et al. 2012), also affecting its natural regeneration. Jankowiak et al.(2016) characterised a collection of cylindrocarpon-like fungi associated with beech litter in Austria and Poland and identified five species from *F. sylvatica* and *P. sylvestris: Ilyonectria crassa*, *I. pseudodestructans*, *I. rufa, N. candida* and *N. obtusispora*, and seven species were identified to genus level (*Ilyonectria* or *Neonectria* species).

The taxonomy of cylindrocarpon-like asexual morphs has been revised several times since the genus *Cylindrocarpon* was first introduced in 1913 by Wollenweber to describe the asexual morphs of the *Nectria* section *Willkomiotes* Wollenw., which included species without chlamydospores (Brayford 1993;Halleen et al. 2006). Afterwards, in 1917, the term *Cylindrocarpon* also embraced species with mycelial chlamydospores in culture,

with *Cy. destructans* becoming the most important species of this group. Booth (1966) split the genus into four groups based on the presence or absence of microconidia and chlamydospores (Brayford 1993; Halleen et al. 2006).Further studies transferred species of the *Nectria* group with cylindrocarpon-like asexual morphs into *Neonectria* (Rossman et al. 1999;Martiri et al. 2001; Brayford et al. 2004). In 2004, the new asexual morph genus, *Campylocarpon*, was described by Halleen et al. (2004). Later, Chaverri et al. (2011) recognized five novel genera within *Neonectria* based on characters associated with perithecial anatomy and conidial septation: *Campylocarpon, Ilyonectria, Neonectria* (*Cylindrocarpon s. s.*), *Rugonectria*, and *Thelonectria*. Finally, in 2014, Lombard et al. stated that the genus *Ilyonectria* was paraphyletic, and therefore designating a new genus *Dactylonectria*, to resolve this. At the same time, the genus *Cylindrodendrum* was shown to form a well-supported monophyletic sister clade to the *Ilyonectria* clade.

Within *I. destructans* complex, twelve new taxa were delineated mainly based on isolates previously describe as *C.destructans s.l.* from a diverse host range (Cabral et al. 2012a). This study comprised, isolates of *I. liriodendri* from *Quercus suber* (*Q. suber*); isolates of *I. robusta* from *Quercus* sp. and *Quercus robur* (*Q. robur*); *I. rufa* from *A. alba*, *Ps. menziesii* and *Pc. glauca*; *I. pseudodestructans* from *Quercus* sp., and *I. europaea* from *Aesculus hippocastanum*.

Dactylonectria species have also been isolated from forest species; *D. estremocensis* was isolated from *Quercus* sp. and *Pc. glauca*, *D. torresensis* from *A. nordmanniana* and *Quercus* sp. (Cabral *et al*. 2012b), and *D. pinicola* from *P. laricio* (Lombard et al. 2014). Several *Neonectria* species have been associated with forest species, which include *N. coccinea, N. ditissima, N. faginata*, *N. fuckeliana*, *N. lugdunensis*, *N. major*, *N. neomacrospora, N. obtusispora* and *N. tsugae* (Castlebury et al. 2006; Lombard et al. 2014).

In 2002, Sánchez *et al*. reported high mortality levels of *Quercus* seedlings (*Q. ilex*, *Q. suber*, and *Q. faginea*) caused by *C. destructans* in a nursery in southeastern Spain, but no further studies have explored the occurrence of species with cylindrocarpon-like asexual morphs associated with root rot and dieback in Spanish forest nurseries. There is a lack of knowledge about the relevance of this group of fungi in forest nurseries. Thus, the aim of the present study was to characterize a large collection of cylindrocarpon-like isolates recovered from forest nurseries and a broad range of hosts displaying decline symptoms, by means of phenotypical characterization and DNA analysis.

Materials and methods

Fungal isolation

Between 2009 and 2012, extensive surveys were conducted in 17 Spanish forest nurseries located in the provinces of Alicante, Castellón, Tarragona and Valencia (Eastern Spain), and León, Logroño, Soria, and Madrid (Central-northern Spain). The survey was focused on plants showing symptoms such as wilting, dieback, chlorosis, foliage discoloration, defoliation, growth reduction and general decline (Fig. 1 A-E).

Affected plants showed root rot and loss of the feeder roots with the presence of necrotic lesions. These symptoms led to a reduction of the root biomass and root hairs, diminishing the volume of the root system and its feeder abilities (Fig. 1 F, G), which resulted in plant collapse (Fig. 1 A-E). At least three plants per symptomatic species were collected in each nursery and transported to the laboratory for fungal isolation. Affected roots were washed under running tap water, surface disinfested for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Small pieces of discolored tissues were plated on potato dextrose agar (PDA) (Biokar-Diagnostics, Zac de Ther, France)

amended with 0.5 g liter^{-1} of streptomycin sulphate (Sigma-Aldrich, St. Louis, MO, USA) (PDAS). Plates were incubated for 5 to 10 days at 25°C in darkness.

According to morphological characters (mycelium aspect and colony colour), 103 isolates of cylindrocarpon-like asexual morphs representative of different hosts and geographical origins were selected for further analysis (Table 1). These isolates were single-spored with the serial dilution method prior to morphological and molecular characterization (Dhingra and Sinclair 1995). For long-term storage, agar plugs with mycelium and conidia from cultures were stored in 15% glycerol solution at -80ºC in1.5 mL cryovials.

Morphological characterization

Single conidial cultures were grown for up to 5 weeks at 20ºC on synthetic nutrient-poor agar (SNA; Nirenberg 1976) with or without the addition of two 1cm^2 pieces of sterile filter paper on the medium surface, PDA, and oatmeal agar (OA; Crous et al. 2009) under continuous near-UV fluorescent light (NUV; 400-315 nm; Philips TL 8W BLB, The Netherlands). To induce perithecia of new species, homothallic and heterothallic crosses (for the cases that there is more than one isolate in the species) were performed as described by Cabral et al. (2012a).

Fungal structures were measured at a 1,000× magnification using a Leica DM2500 and images were captured using a Leica DFC295 digital camera with the Leica Application Suite (LAS) version 3.3.0. For this purpose, an agar square was removed and placed on a microscope slide, to which a drop of water was added and overlaid with a cover slip. For each isolate, 30 measurements were obtained for each informative structure. Measurements were obtained with LAS software and round to the nearest 0.5 µm. The 95% confidence intervals were determined and the extremes of the conidial

measurements are shown in parenthesis. For the other structures, only the extremes are presented.

Culture characteristics (texture, density, color, growth front, transparency and zonation) were described on PDA and OA after incubation at 20ºC in the dark for 14 days. Color (surface and reverse) was described using the color charts of Rayner (1970).

Cardinal growth temperatures were assessed by inoculating 90 mm diameter PDA dishes with a 6 mm diameter plug cut from the edge of an actively growing colony. Growth was determined after 7 days in two orthogonal directions. Trials were conducted at 5 to 35ºC with 5ºC intervals, with three replicates per strain at each temperature.

DNA isolation, sequencing and phylogenetic analysis

For DNA extraction, fungal mycelium, from pure cultures grown on PDA for 2 to 3 weeks at 25ºC in darkness, were scraped and grinded to a fine powder with liquid nitrogen using a mortar and pestle. Total genomic DNA was extracted using the E.Z.N.A. Plant Miniprep Kit (Omega Bio-tek, Doraville, USA) following manufacturer's instructions. DNA was visualized by electrophoresis on 1% agarose gels stained with REALSAFE (REALSAFE Nucleic Acid Staining Solution 20,000x, Durviz S. L., Valencia, Spain) and stored at –20ºC.

In order to identify the species involved, partial sequences of the histone H3 (*his3*) gene region was amplified according to Cabral et al. (2012a). Six isolates (Cy-FO-3, Cy-FO - 45, Cy-FO-133, Cy-FO-224, Cy-FO-225 and Cy-FO-226) were additionally sequenced for the internal transcribed spacer and intervening 5.8S gene (ITS) region, partial regions of the β-tubulin (*tub2*) and translation elongation factor 1-α (*tef1*) genes to better resolve their phylogenetic position. PCR amplifications were carried out using 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4mM of each primer, 1 U of Taq polymerase

(Canvax Biotech, S.L., Córdoba, Spain), and 1 µL of template DNA (20 ng/µL). The PCR reaction mix was adjusted to a final volume of 25 µL with ultrapure sterile water (Chromasolv Plus®, Sigma-Aldrich, Steinheim, Germany). The cycle conditions in a Peltier Thermal Cycler-200 (MJ Research) were: 94ºC for 3 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, elongation at 72ºC for 45 s, and a final extension at 72ºC for 10 min. Primers used were CYLH3F and CYLH3R (Crous et al. 2004b) for *his3*, ITS1F (Gardes and Bruns 1993) and ITS4 (White et al.1990) for ITS, T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) for *tub2*, and CylEF-1 (5'- ATG GGT AAG GAV GAV AAG AC-3'; J.Z. Groenewald, unpublished) and CylEF-R2 (Crous et al. 2004b) for *tef1*. After confirmation by agarose gel electrophoresis, PCR products were sequenced in both directions by Macrogen Inc., Sequencing Center (The Netherlands, Europe). Sequences were assembled and edited to resolve ambiguities and consensus sequences for all isolates were compiled into a single file (Fasta format) using Sequencher software v. 5.3 (Gene Codes Corporation, Ann Arbor, MI, USA).

Phylogenetic analysis was first conducted on the *his3* single-locus alignment for all isolates obtained in this study, as this locus were reported to be a very informative locus (Cabral et al. 2012a). For the cases, that was not possible to infer species level for a specific isolate with only the *his3* single-locus phylogeny a combined alignment of the four loci (*his3*, ITS, *tub2* and *tef1*) was also analyzed. GenBank sequences (Table 1) from different species of *Cylindrodendrum*, *Dactylonectria*, *Ilyonectria* and *Neonectria* were selected based on their high similarity with our query sequences using MegaBLAST. These were added to the sequences obtained and aligned using MAFFT version 7.305 implemented on CIPRES Science Gateway V 3.3 (Miller et al. 2010) and edited manually, if necessary, using MEGA 7.0.26 (Kumar et al. 2016). The alignments for each locus

were combined in a single file using the program SequenceMatrix 1.8 (Vaidya et al. 2011).The best nucleotide substitution model settings for each locus were determined by jModelTest 2.1.10 (Darriba et al. 2012), with the following likelihood settings: number of substitution schemes = 3 (24 models), base frequencies (+F), proportion of invariable sites (+I) and rate variation among sites (+G) (nCat = 4), using the Akaike information criterion (AIC). The Bayesian analyses of the combined four-loci dataset and individual locus data were performed with MrBayes v. 3.2.1 (Ronquist et al. 2012) based on the results of the jModelTest. The Markov Chain Monte Carlo sampling (MCMC) analysis of four chains started in parallel from a random tree topology. The number of generations was set at 10 M and the run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. Trees were saved each 1,000 generations. Burn-in was set at 25% after which the likelihood values were stationary and the remaining trees were used to calculate posterior probabilities. Trees from different runs were then combined and summarized in a majority rule 50% consensus tree. Maximum likelihood (ML) was implemented in the CIPRES Science Gateway V 3.3 (Miller et al. 2010) using RAxML-HPC v.8 on XSEDE (8.2.9) using the GTRCAT model and 1,000 rapid bootstrap inferences were done.

Both analyses were performed, rooting the trees to *Campylocarpon* (*Ca.*) *fasciculare* (CBS 112613) and *Ca. pseudofasciculare* (CBS 112679) and tree topologies were compared on http://phylo.io (Robinson et al. 2016).

Sequences derived in this study were lodged in GenBank, the alignments and phylogenetic trees in TreeBASE under study number S22022 (http://purl.org/phylo/treebase/phylows/study/TB2:S22022), and taxonomic novelties in MycoBank (www.MycoBank.org) (Crous et al. 2004a). GenBank accession numbers of the isolates collected during this study are listed in Table 1.

Results

Phylogenetic analysis

One hundred and three isolates were amplified with the primers CYLH3F and CYLH3R and approximately 500 bp were obtained for all. The *his3*single-locus alignment contains 427 aligned characters (including gaps), from which 195 characters were parsimony-informative, 17 were variable but parsimony-uninformative and 212 were constant. The AIC best-fit nucleotide substitution model identify by jModelTest was general time reversible model with inverse gamma rates (GTR+I+G). The Bayesian consensus tree and Maximum Likelihood tree had similar topology, and therefore only the Bayesian consensus tree is presented with bootstrap support values (BS) and posterior probability values (PP). The phylogenetic analysis contained a total of 174 ingroup taxa and two outgroup taxa [*Ca. fasciculare*(CBS112613),and *Ca. pseudofasciculare* (CBS112679)]. The phylogeny obtained with *his3*alignmentresulted in four major clades: the first major clade, comprised the isolates from the genus *Ilyonectria*; the second, isolates from the genus *Neonectria*; the third, isolates from the genus *Cylindrodendrum* and the fourth, isolates from the genus *Dactylonectria* (Fig. 2). About 71% of the isolates obtained in this study belonged to the genus *Dactyonectria* and included: *D. hispanica* (0.97%), *D. macrodidyma* (24.27%), *D .novozelandica* (29.13%), *D. pauciseptata* (2.91%), *D. pinicola* (0.97%), *D. torresensis* (11.65%) and *D. valentina* (0.97%). The genus *Ilyonectria* included 27.18% of the isolates: *I. capensis* (2.91%), *I. cyclaminicola* (0.97%), *I. ilicicola* (2.91%), *I. liriodendri* (8.74%), *I. pseudodestructans* (0.97%), *I robusta* (1.94%) and *I. rufa* (8.74%). The genus *Neonectria* contained one species,

Neonectria quercicola (0.97%). *Cylindrodendrum alicantinum* (*C. alicantinum*) (0.97%) was the only species of the genus *Cylindrodendrum*.

Six isolates (Cy-FO-3, Cy-FO-45, Cy-FO-133, Cy-FO-224, Cy-FO-225 and Cy-FO-226) could not be identified to the species level employing the *his3* sequences (Fig. 2).Therefore the ITS, *tub2*and *tef1*regions were analyzed additionally, and these sequences were concatenated with those obtained from the *his3*region for their identification (Fig. 3). The four loci alignment contained 79 taxa (including the two outgroups) and 1,935 aligned characters (including gaps), from which 718 characters were parsimony-informative, 83 were variable but parsimony-uninformative and 1,101 were constant. The AIC best-fit nucleotide substitution model identify by jModelTest was GTR+I+G model for ITS and *his3*and GTR+G for *tub2*and *tef1*.

The Bayesian and ML consensus trees obtained with the four-loci alignment confirmed the existence of four novel taxa within our set of isolates.

Taxonomy

Based on the phylogenetic analysis and morphological characters, two new species of *Dactylonectria*, one species of *Ilyonectria* and one species of *Neonectria* are described (Fig. 2, Fig. 3and Fig. 4). No perithecia were observed in the homothallic or heterothallic crosses performed.

Dactylonectria hispanica B. Mora-Sala, A. Cabral, J. Armengol &P. Abad-Campos, **sp. nov**. MycoBank MB822023 (Fig. 5).

Etymology: Name refers to Spain, where the fungus was isolated.

Diagnosis: Morphologically *D. hispanica,* can be distinguished by its slightly larger 3-septate macroconidia when compared to *D. vitis* and *D. valentina*. Fourteen polymorphisms can distinguish *D. hispanica* from *D. valentina:* five in *tub2* locus at position 27 (A:T), 136 (G:A), 206 (A:G), 335 (T:C) and 434(C:A), five in *his3* locusatposition94(C:T), 104 (T:C); 214 (T:C); 291 (T:C) and 395 (C:T); and four in *tef1* locus at position 224(G:A), 266 (A:T), 289 (T:A) and 291 (A:C).

Typus: **Spain**: Valencia, Ayora, on *Pinus halepensis* (complete roots), 2011, *B. Mora-Sala* (CBS H-23154 – holotype; CBS 142827 = Cy-FO-45 – ex-type culture).

Conidiophores simple. Complex conidiophores not observed. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to aggregated, unbranched or sparsely branched with up to four phialides, 1 to 2-septate, 40 to 75 µm long; phialides monophialidic, cylindrical, tapering towards the apex, 16 to 28 µm long, 2 to 3.0 µm wide at the base, 3 to 4 µm at the widest point, 1.5 to 2.5 µm near the aperture.

Macroconidia (1 to) 3-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, mostly with a visible centrally located to laterally displaced hilum; 1-septate (26–)31 to 37(–53) × (7.0–)7.5 to 8.5(–9.0) µm (av. 34 × 8 µm) L/W ratio (3–)3.9 to 4.7(–6.5) (av. 4.3), 2-septate (30.5–)37 to 43(–53) × (6–)7.5 to 8.5(–9) µm (av. 40 × 8 µm) L/W ratio (3.5–)4.5 to 5.5(–7) (av. 5.0), and 3-septate macroconidia (39–)45 to 47(–58) × (6.5–)7.8 to 8.2(–9.5) µm (av. 46 × 8 µm), L/W ratio (4.5–)5.5 to 6(–7.5) (av. 5.8). Macroconidia formed in heads or as flat domes of slimy masses.

Microconidia rarely formed, aseptate to 1-septate with a minutely or clearly laterally displaced hilum; aseptate microconidia ellipsoidal to fusiform (9.5–)10.5 to 14(–17)×(5.5–)6 to 7(–7.5) (av. 12.2×6.5 µm) L/W ratio (1.5–)1.7 to 2.1(–2.2) (av. 1.8); 1-septate, fusiform to subcylindrical (14–)19 to 21.5(–24.5)×(6–)7 to 7.5(–8.5) (av. 20.2×7.2 µm) L/W ratio (2–)2.6 to 3.0(–3.5) (av. 2.8). *Chlamydospores* observed on

SNA; globose to subglobose to ellipsoidal, 7 to 10×6 to 9 µm diameter, smooth but often appearing rough due to deposits, thick-walled, in chains or in clumps, hyaline, becoming slightly brown.

Culture characteristics: Mycelium felty with low to average density (OA) or average to strong density (PDA). Surface on OA buff to sepia with sparse cinnamon aerial mycelium; margin luteous. Surface on PDA honey to buff; margin buff. Zonation absent, transparency homogeneous and margins even (OA) and uneven (PDA). Reverse similar to surface, except in color, sepia to cinnamon (PDA). Colonies on PDA grow poorly, less than 1 mm diameter at 5°C after 7 days. Optimum temperature at 25°C, when colonies reach 28 mm diameter, after 7 days. Colony diameter was 9 mm at 30°C after 7days. No growth was observed at 35°C.

Host and distribution: *Pinus halepensis* (roots) (Spain, Valencia)

Notes: *Dactylonectria hispanica* is closely related to *D. valentina* and *D. vitis* based on phylogenetic inference. The morphology of these species is very similar, but *D. hispanica* can be distinguished by its slightly larger 3-septate macroconidia when compared to *D. vitis* (34.9–)41.6 to 43.5(–51.6) × (6.2–)7.9 to 8.2(–9.5) µm (av. =42.5 × 8.0 µm; Cabral et al. 2012a) and *D. valentina* (30–)35.5 to 37(–44) × (6–)7.5 to 8(–9.0) µm (av. 36.3 × 7.6 µm); this study). No complex conidiophores or penicillate conidiophores with aseptate microconidia were observed in *D. hispanica*. The isolate Cy228 is classify as *D. hispanica*, as it form a clade very well supported (BS = 100% and PP =1.0) with the isolate Cy-FO-45.

Dactylonectria valentina B. Mora-Sala, A. Cabral, J. Armengol &P. Abad-Campos, **sp. nov**.MycoBank MB822024(Fig. 6).

Etymology: Name refers to the province of Valencia, Spain, where the fungus was isolated.

Diagnosis: The morphologically *D. valentina* can be distinguished by its slightly smaller 3-septate macroconidia when compare to *D. vitis* and *D. valentina,* and for the absence of luteous margin in OA plates. Fourteen nucleotide differences can distinguish *D. valentina* from *D. hispanica* (as describe in diagnosis of *D. hispanica*).

Typus: **Spain**: Valencia, El Puig, 2009, on *Ilex aquifolium* (complete roots), *B. Mora-Sala* (CBS H-23155 – holotype; CBS 142826 = Cy-FO-133 – ex-type culture).

Conidiophores simple or complex. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to aggregated, unbranched or sparsely branched with up to four phialides, 1 to 4–septate, 55 to 130 µm long; phialides monophialidic, cylindrical, tapering towards the apex, 15 to 30.5 µm long, 2.1 to 3.2 µm wide at the base, 3.1 to 4.5 µm at the widest point, 1.5 to 3 µm near the aperture. Conidiophores forming aseptate microconidia arising from mycelium on agar surface, 1 to 4–septate, with a terminal arrangement of phialides, ranging from 2 to a dense cluster; sparsely branched or penicillate; monophialides narrowly flask-shaped, typically with widest point near the middle, 9 to 17 µm long, 1.5 to 3.0 µm wide at the base, 2 to 3.5 µm at widest point, 1 to 2 µm near the apex. *Sporodochial conidiophores* irregularly branched; phialides more or less cylindrical but slightly tapering towards the tip, or narrowly flask-shaped, with widest point near the middle, 14 to 20 µm long, 2.5 to 3.5 µm wide at the base, 3.0 to 4.5 µm at widest point, 1.5 to 2.5 µm near the apex. *Macroconidia* (1 to)3-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, mostly with a visible centrally located to laterally displaced hilum; 1-septate (18–)25 to 28(–33.5) × (5.5–)6.5 to 7(–8.5) µm (av. 26.3×6.9 µm) L/W ratio (3–)3.7 to 4.2(–5) (av. 3.9), 2-septate (26–)30 to 32(–35.5) × (6.5–)7.5 to 8(–9) µm (av. 31.1×7.6 µm) L/W ratio (3.0–)3.9 to

4.3(–5) (av. 4.1),and 3-septate macroconidia (30–)35.5 to 37(–44) × (6–)7.5 to 8(–9.0) µm (av. 36.3×7.6 µm), L/W ratio (3.5–)4.5 to 5(–6.5) (av. 4.8). Macroconidia formed in heads or as flat domes of slimy masses. *Microconidia* aseptate to 1-septate with a minutely or clearly laterally displaced hilum; aseptate microconidia subglobose to oval (3.5–)5 to 5.5(–7.5)×(3–)3.9 to 4.1(–4.5) (av. 5.2×4 µm) L/W ratio (1–)1.2 to 1.4(–2) (av. 1.3); 1-septate microconidia, rarely formed, fusiform to subcylindrical (14–)15 to 17(–18.5)×(4.5–)4.7 to 5.5(–6) (av. 16×5µm) L/W ratio (2.5–)2.8 to 3.3(–3.5) (av. 3.0).

Chlamydospores observed in the bottom of SNA plate; globose to subglobose to ellipsoidal, 9 to 19×7 to 12 µm diameter, smooth but often appearing rough due to deposits, thick-walled, mainly in chains or in clumps, hyaline, becoming slightly brown in the outer wall.

Culture characteristics: Mycelium felty with density low to average (OA) and average to strong (PDA). Surface on OA sienna, with sparse, saffron aerial mycelium, and buff growth at margin. Surface on PDA chestnut, with sienna aerial mycelium, with buff margin. Zonation was absent, transparency was homogeneous and growth margin even. Reverse similar to surface, except in color, chestnut to sienna on PDA. Colonies on PDA grow poorly, 1 mm diameter at 5°C after 7 days. Optimum temperature at 25°C, when colonies reach 35 mm diameter after 7 days. Colony diameter was 8 mm at 30°C after 7days. No growth was observed at 35°C.

Host and distribution: Ilex aquifolium (roots) (Spain, Valencia).

Ilyonectria ilicicola B. Mora-Sala, A. Cabral, J. Armengol&P. Abad-Campos, **sp. nov**. MycoBank MB822025(Fig. 7)

Etymology: Name refers to the plant host genus, *Ilex*, from which this fungus was isolated.

Diagnosis: *Ilyonectria ilicicola* can be distinguish morphologically from *I. cyclaminicola, I. leucospermi* and *I. protearum* by having slightly larger and narrower macroconidia. This taxa is best distinguish by *tub2* and *his3* genes.

Typus: **Spain**: Tarragona, 2012, on *Ilex* sp. roots, *B. Mora-Sala* (CBS H-23156 – holotype; CBS 142828=Cy-FO-225 – ex-type culture)

Conidiophores simple or complex. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to aggregated, unbranched or sparsely branched with up to three phialides, 1 to 3-septate, 49 to 178 µm long; phialides monophialidic, cylindrical, tapering towards the apex, 26 to 66 µm long, 2 to 4 µm wide at the base, 2.5 to 4.5 µm at the widest point, 1.5 to 3 µm near the aperture.

Complex conidiophores aggregated in sporodochia. Sporodochia consist of a pulvinate mass of short conidiophores, irregularly branched; phialides cylindrical, tapering towards the apex, 12 to 30 µm long, 1.5 to 2.5 µm wide at the base, 2.0 to 2.5 µm at the widest point, and 1 to 2 µm wide at the apex.

Macroconidia 1(to 3)-septate, straight, cylindrical, with both ends obtusely rounded, base sometimes with a visible, centrally located to laterally displaced hilum; 1-septate macroconidia (19.5–)25 to 26(–32.5)×(3.5–)5 to 5.5(-6.5) (av. 25.5×5.2µm), L/W ratio (3.5–)4.9 to 5.1(–7) (av. 5.0 µm); 2-septate macroconidia (26.5–)30 to 31.5(–35.5)×(5–)5.5 to 6(–7) (av. 30.7×5.7 µm), L/W ratio (4.5–)5.2 to 5.7(-7) (av. 5.4 µm); and 3-septate macroconidia (28–)31.5 to 34(–40.5)×(4.5–)5.7 to 6.2(–7) (av. 32.9×5.9 µm) L/W ratio (4.5–)5.3 to 5.9(–7.75) (av. 5.6 µm). Macroconidia predominant, formed by both types of conidiophores, forming flat domes of slimy masses.

Microconidia aseptate to 1-septate, with a minutely or clearly laterally displaced hilum; aseptate microconidia formed in simple conidiphores ellipsoidal to oval to fusiform (5–)9 to 9.5(–14.5)×(2.5–)3.4 to 3.6(–5) (av. 9.3×3.5 µm), L/W ratio (1.5–)2.6 to 2.8(–4.3)

(av. 2.7µm); aseptate microconidia globose to subglobose formed in complex conidiophores 4.5 to 6×4 to 4.5 µm; 1-septate microconidia fusiform to ellipsoidal, (12–)15.5 to 16.5(–20)×(3.5–)4.3 to 4.6(–5.5) (av. 16×4.5µm), L/W ratio (2.5–)3.5 to 3.8(–4.5) (av. 3.7µm); microconidia formed in heads on simple conidiophores or as masses on complex conidiophores.

Chlamydospores globose to subglobose, 11 to 20×10 to 19 µm diam., smooth, but often appearing rough due to deposits, thick-walled, formed in lateral branches, rarely intercalary, mostly isolated, hyaline, becoming medium brown.

Culture characteristics: Mycelium felty with average density. Surface on OA fawn to cinnamon with aerial mycelium dark buff, with a buff margin. On PDA sepia with aerial mycelium, vinaceous buff, and margin buff. Zonation absent, with homogeneous transparency and margins even (OA) or lobate (PDA). Colonies similar in reverse, except in color, greyish sepia (OA) and dark brick to sepia (PDA). Colonies on PDA grow 6–7 mm diameter at 5°C after 7 days. Optimum temperature at 25°C, when colonies reach 62-64 mm diameter after 7 days. Colony diameter was 13-16 mm at 30°C after 7days. No growth was observed at 35°C.

Additional cultures examined: Cy-FO-224 and Cy-FO-226. Spain: Tarragona, isolated from *Ilex* sp. roots, 2012, B. Mora-Sala.

Host and distribution: *Ilex* sp. (roots) (Spain, Tarragona).

Notes: *Ilyonectria ilicicola* is phylogenetically closely related to *I. protearum*, *I. leucospermi* and *I. cyclaminicola* based on the phylogenetic inference in this study. The morphology of these four species overlap. *Ilyonectria ilicicola, I. protearum* and *I. cyclaminicola* formed sporodochia within 5 weeks, and have solitary chlamydospores and can be distinguished from *I. leucospermi* that failed to form sporodochia after 8 weeks

incubation at 24°C under continuous UV light and have intercalary chlamydospores (Cabral et al. 2012a; Lombard et. al. 2013).

Neonectria quercicola B. Mora-Sala, A. Cabral, J. Armengol & P. Abad-Campos, **sp. nov**. MycoBank MB 823852(Fig. 8).

Etymology: Name refers to the plant host genus, *Quercus*, from which this fungus was isolated.

Diagnosis: Neonectria quercicola can be distinguish morphologically by having long conidiophores that terminate in a whorl of phialides, and for production only 1-septate macroconidia. Phylogenetically it is better distinguished with the genes *his3* and *tef1*

Typus: **Spain**: Alicante, Alcoi, 2011, on *Quercus ilex* roots, *P. Abad-Campos* (CBS H-23353– holotype; CBS 143704=Cy-FO-3 – ex-type culture

Conidiophores simple or complex. Simple conidiophores short and sparsely branched 1 to 2-septate and 30 to 60 µm long, or long with 4 to 7-septate, 150 to 390 µm long and terminating in a whorl of phialides; phialides monophialidic, cylindrical, tapering towards the apex, 15 to 25 µm long, 1.5 to 4 µm wide at the base, 2 to 4.0 µm at the widest point, and 1.0 to 2.5 µm near the aperture. *Sporodochial conidiophores* irregularly branched.

Macroconidia 1-septate, straight, cylindrical with both ends more or less broadly rounded, mostly with a visible centrally located to laterally displaced hilum; 1-septate (17.5–)21.5 to 22.5(–26.5) × (4–)4.5 to 5(–6) (av. 22 × 4.7 µm) L/W ratio (3.5–)4.6–4.8(–6) (av. 4.7µm). Macroconidia formed in heads or as flat domes of slimy masses.

Microconidia rarely formed (0 to) 1-septate; 1-septate microconidia, mostly without a visible hilum ellipsoidal to oblong (10–)12 to 13.5(–15) × (4–)4.5to 5(–5.5) (av. 13 × 4.8 µm) L/W ratio (2–)2.5 to 3(–3.5) (av. 2.7µm).

Chlamydospores not observed.

Culture characteristics: Mycelium felty with low density (OA) or strong density (PDA). Surface on OA buff with aerial mycelium cinnamon. Surface on PDA pale buff; with a pale luteous concentric ring. Zonation absent (OA) to concentric (PDA), transparency homogeneous and margins even. Reverse similar to surface, except in color, light cinnamon (OA) and buff with center sepia (PDA).

Colonies on PDA grow 5.7 mm diameter at 5°C after 7 days. Optimum temperature at 25°C, when colonies reach 17.2 mm diameter, after 7 days. Colony diameter was 6.4 mm at 30°C after 7days. No growth was observed at 35°C.

Host and distribution: Quercus ilex (Spain, Alicante, Alcoi)

Notes: Based on the phylogenetic inference in this study, *Neonectria quercicola* is closely related to other three isolates with no description available (CPC 13530, CPC 13531 and CR21). These isolates should also be considered *N. quercicola*, as they form a clade very well supported with 100% bootstrap support and a Bayesian posterior probability of 1.0.

Host distribution

The fungal species identified in this study were found associated with15 host genera: *Abies* (2.9%), *Arbutus* (5.8%), *Cistus* (1.9%), *Crataegus* (1%), *Ilex* (4.9%), *Juglans* (2.9%), *Juniperus* (6.8%), *Lonicera* (1%), *Myrtus* (1%), *Pinus*(37.9%), *Pistacia* (1.9%), *Pyracantha* (1%), *Quercus* (26.2%), *Rosmarinus* (3.9%) and *Santolina* (1%) (Table 2). *Pinus* was the genus with the highest number of isolates recovered and from which eight species were identified: *D. hispanica, D. macrodidyma, D. novozelandica, D. pauciseptata, D. torresensis, I. capensis, I. liriodendri* and *I. rufa*. *Quercus* was the only host from which the four fungal genera were isolated, and the host with the highest number of fungal species isolated: *D. macrodidyma, D. novozelandica, D. torresensis, I. cyclaminicola, I. liriodendri*, *I. pseudodestructans, I. rufa*, *C. alicantinum* and *N.*

quercicola. The third host regarding the number of species isolated was *Juniperus*: *D. macrodidyma, D. novozelandica, D. capensis, I. liriodendri* and *I. rufa*, followed by *Arbutus*, from which four fungal species were recovered, and *Abies* and *Rosmarinus*, from which three fungal species were recovered. In *Ilex* and *Juglans* only two fungal species were recovered and only one from *Cistus, Crataegus, Lonicera, Myrtus, Pistacia, Pyracantha* and *Santolina*.

Regarding the species, *D. novozelandica* was recovered from 66.67% of the hosts, followed by *D. macrodidyma* (60%), *D. torresensis* and *I. rufa* (40%), *I. liriodendri* (33.33%), *I. capensis* (20%) and *D. pauciseptata* (13.33%). The remaining fungal species were only recovered from one host, which represented 6.67% of the total number of hosts surveyed in this study (Table 2).

DISCUSSION

The present study represents the first attempt to characterize a wide collection of cylindrocarpon–like asexual morphs collected from forest nurseries in Spain. This clearly demonstrates the prevalence of this fungal group associated with seedlings of diverse number of hosts showing decline symptoms. Cylindrocarpon–like asexual morphs are ubiquitous and can be easily found in soil or associated with plant roots, some of them having also a potential role as latent pathogens or endophytic organisms (Halleen et al. 2006, Agustí-Brisach and Armengol 2013).

Sixteen species belonging to the genera *Cylindrodendrum*, *Dactylonectria*, *Ilyonectria*, and *Neonectria* were identified from damaged roots of 15 forest plant genera. Six isolates were not identified to the species level with the *his3* data. Although *his3* region has previously showed to be a very informative locus (Cabral et al. 2012a), a combined

analysis with ITS, *tub2* and *tef1* regions better resolved and confirmed that these isolates represented novel phylogenetic species, newly described as: *D. hispanica*, *D. valentina*. *I. ilicicola* and *N. quercicola*.

This is the first report of *C. alicantinum* on *Q. ilex*; of *D. macrodydima* on: *Ilex aquifolium, Juniperus phoenicea, Lonicera* sp., *Myrtus communis, P. halepensis, Pyracantha* sp., *Q. faginea, Q. ilex* and *Rosmarinus officinalis*; of *D. novozelandica* on: *Crataegus azarolus, J. phoenicea, Pinus* sp., *P. halepensis, Pistacia lentiscus, Quercus* sp., *Q. ilex, Q. suber, R. officinalis* and *Santolina chamaecyparissus*; of *D. pauciseptata* on: *Abies nordmanniana* and *P. halepensis*; of *D.pinicola* on: *A. concolor*, of *D. torresensis* on: *Ar. unedo*, *Cistus albidus*, *Ju. regia*, *P. halepensis*, *Q. ilex* and *R. officinalis; of I. capensis* in *Arbutus unedo, Juniperus* sp. and *P. halepensis*; of *I. cyclaminicola* on *Quercus* sp.; of *I. liriodendra* on: *Ar. unedo*, *Juniperus* sp., *P. halepensis*; of *I. pseudodestructans* in *Q. ilex*; of *I. robusta* in *Juglans regia*; and of *I. rufa* on: *A. nordmanniana, Ar. unedo, Juniperus* sp., *P. halepensis, Q. faginea* and *Q. ilex.* Furthermore, this is the first report of *I. capensis* in Europe because, to our knowledge, this fungus had only been recorded affecting *Protea* in South Africa (Lombard et al. 2013).

To date *D. novozelandica, D. macrodidyma, D. torresensis, I. liriodendri, I. robusta* and *C. alicantinum* had been reported only in cultivated crops such as grapevine (*Vitis vinifera*) (Agustí-Brisach and Armengol 2013) or loquat (*Eriobotrya japonica*)(Agustí-Brisach et al.2016) in Spain, but never affecting forest plants.

Dactylonectria pauciseptata had been reported as a pathogen of grapevines in Slovenia and New Zealand (Schroers et al. 2008), Uruguay (Abreo et al. 2010), Spain (Martín et al. 2011), Portugal (Cabral et al. 2012a), Brazil (dos Santos et al. 2014), and British Columbia, Canada (Úrbez-Torres et al. 2014). It has also been reported as a pathogen of

apple trees in South Africa and of peach trees in Italy (Tewoldemedhin et al. 2011;Yaseen et al. 2012).This fungus had also been recorded in forest hosts: *Viburnum tinus* in Italy (Aiello 2015) and *P. radiata* in Spain (Agustí-Brisach et al. 2011). Thus, this study increases the range of forest hosts in nursery for this species, representing the first report of *D. pauciseptata* on *A. nordmanniana* and *P. halepensis.*

In our study, no correlation was found between the fungal pathogens isolated and nurseries from which they were collected. The fungal species did not show any distribution pattern among the different locations surveyed probably because the small sample size, as 3 plants per host is probably not enough to look for these correlations. Furthermore, it should be taken into account that there were locations (provinces) in which a higher number of nurseries were surveyed that included Valencia, Castellón and Alicante. Likewise, some of the surveyed hosts, in particular *Pinus* and *Quercus*, were the target hosts of the survey undertaken due to the importance of these genera in Spanish forests (MAGRAMA 2014). Therefore, these hosts had a higher number of sampled plants, also corresponding with a higher number of cylindrocarpon-like isolates compared to other hosts. These two genera prevail in the Mediterranean landscape, as they constitute the characteristic vegetation of the Mediterranean forests. In this regard, the presence of nine cylindrocarpon-like species on *Quercus* and eight on *Pinus* trees highlight the need for better management of nursery diseases to avoid the dispersal of these fungi through planting materials used for reforestation purposes. Management of cylindrocarpon-like asexual morphs associated with black-foot disease has been intensively studied on grapevine nurseries, where the incorporation of holistic and integrated control measures such as cultural practices and sanitation, chemical and biological control, has been shown as the best approach to improve the phytosanitary quality of planting material (Gramaje

and Armengol 2011; Gramaje et al. 2018). Implementing a similar strategy could be advisable in forest nurseries.

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Table 1.*Campylocarpon*, *Cylindrodendrum*, *Dactylonectria* and *Ilyonectria* isolates used in this study.

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
Campylocarpon fasciculare	**CBS 112613**; STE-U 3970; C 76	*Vitis vinifera*	F. Halleen	South Africa, Western Cape, Riebeeck Kasteel	AY677301	AY677221	JF735502	JF735691
C. pseudofasciculare	**CBS 112679**; CPC5472; HJS-1227	*V. vinifera*	F. Halleen	South Africa, Western Cape, Wellington	AY677306	AY677214	JF735503	JF735692
Cylindrodendrum album	CBS 110655; VC-51	Pine forest soil	F.X. Prenafeta-Boldú	The Netherlands, De Veluwe	KM231765	KM232022	KM231485	KM231890
	CBS 301.83; ATCC 46842; IMI 255534; TRTC 49165; UBC 8265	*Fucus distichus*	R.C. Summerbell	Canada, British Columbia, Vancouver, Wreck Beach	KM231764	KM532021	KM231484	KM231889
C. alicantinum	**CBS 139518**; Cyl-3	*Eriobotrya japonica*	J. Armengol	Spain, Alicante, Callosa d'En Sarrià	KP456014	KP400578	KP639555	KP452501
	Cyl-11	*E. japonica*	J. Armengol	Spain, Alicante, Callosa d'En Sarrià	KP456017	KP400581	KP639558	KP452504
	Cy-FO-25	*Quercus ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709593	-
C. hubeiense	**CBS 124071**; HMAS 98331, 5620	*Rhododendron*	W.P. Wu, W.Y. Zhuang & Y. Nong	China, Hubei	FJ560439	FJ860056	KR909093	HM054090
	CBS 129.97	*Viscum album*	W. Gams	France, Dép. Jura, Châtelneuf near St. Laurent	KM231766	KM232023	KM231486	KM231891
Dactylonectria alcacerensis	**CBS 129087**; Cy159	*V. vinifera*	A. Cabral & H. Oliveira	Portugal, Alcácer de Sol Torrão	JF735333	AM419111	JF735630	JF735819
	Cy134; IAFM Cy20-1	*V. vinifera*	J. Armengol	Spain, Ciudad Real, Villarrubia de los Ojos	JF735332	AM419104	JF735629	JF735818
D. anthuriicola	**CBS 564.95**; PD 95/1577	*Anthurium* sp.	R. Pieters, 1995	Netherlands, Bleiswijk	JF735302	JF735430	JF735579	JF735768

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
D. estremocensis	**CBS 129085**; Cy145	*V. vinifera*	C. Rego & T. Nascimento	Portugal, Estremoz	JF735320	JF735448	JF735617	JF735806
	CPC 13539; 94–1685; CCFC226730	*Picea glauca*	R. C. Hamelin, 1994	Canada, Quebec	JF735330	JF735458	JF735627	JF735816
D. hispanica	**CBS 142827**;Cy-FO-45	*Pinus halepensis*	B. Mora-Sala. 2011	Spain, Valencia, Ayora	KY676882	KY676876	KY676864	KY676870
	Cy228	*Ficus* sp.	F. Caetano, 2003	Portugal, Lisbon	JF735301	JF735429	JF735578	JF735767
D. hordeicola	**CBS 162.89**	*Hordeum vulgare*	M. Barth	Netherlands, Noordoostpolder, Marknesse, Lovinhhoeve	AM419060	AM419084	JF735610	JF735799
D. macrodidyma	**CBS 112615**; STE-U 3976; C98; CPC 20709	*V. vinifera*	F. Halleen	South Africa, Western Cape, Malmesbury, Jakkalsfontein	AY677290	AY677233	JF735647	JF735836
	CBS 112601; STE-U 3983; C 82	*V. vinifera*	F. Halleen, 1999	South Africa, Western Cape, Tulbagh	AY677284	AY677229	JF735644	JF735833
	Cy-FO-1	*Q. faginea*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709497	-
	Cy-FO-9	*Q. ilex*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709498	-
	Cy-FO-10	*P. halepensis*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709499	-
	Cy-FO-13	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709500	-
	Cy-FO-18	*Q. faginea*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709501	-
D. macrodidyma	Cy-FO-19	*J. phoenicea*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709502	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-20	*J. phoenicea*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709503	-
	Cy-FO-23	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709504	-
	Cy-FO-24	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709505	-
	Cy-FO-26	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709506	-
	Cy-FO-29	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709507	-
	Cy-FO-30	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709508	-
	Cy-FO-31	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709509	-
	Cy-FO-32	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709510	-
	Cy-FO-34	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709511	-
	Cy-FO-48	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709512	-
	Cy-FO-51	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709513	-
	Cy-FO-55	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709514	-
D. macrodidyma	Cy-FO-61	*P. halepensis*	B. Mora-Sala. 2011	Spain, Valencia, Ayora	-	-	KX709515	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-134	*I. aquifolium*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709516	-
	Cy-FO-147	*Rosmarinus officinalis*	B. Mora-Sala. 2009	Spain, Valencia, Cheste	-	-	KX709517	-
	Cy-FO-154	*Lonicera* sp.	B. Mora-Sala. 2009	Spain, Valencia, Torrente	-	-	KX709518	-
	Cy-FO-160	*Pyracantha* sp.	B. Mora-Sala. 2009	Spain, Castellon, Segorbe	-	-	KX709519	-
	Cy-FO-195	*Myrtus communis*	B. Mora-Sala. 2009	Spain,Valencia, Chiva	-	-	KX709520	-
	Cy-FO-223	*P. halepensis*	B. Mora-Sala. 2010	Spain, La Rioja, Projano	-	-	KX709521	-
D. novozelandica	CBS 112608; STE-U 3987; C 62	*V. vinifera*	F. Halleen	South Africa, Western Cape, Citrusdal	AY677288	AY677235	JF735632	JF735821
	CBS 113552; STE-U 5713; HJS-1306; NZ C 41	*Vitis* sp.	R. Bonfiglioli	New Zealand, Candy P New Ground	JF735334	AY677237	JF735633	JF735822
	Cy-FO-5	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709522	-
	Cy-FO-6	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709523	-
	Cy-FO-11	*P. halepensis*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709524	-
	Cy-FO-14	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709525	-
D. novozelandica	Cy-FO-15	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709526	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-16	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709527	-
	Cy-FO-21	*J. phoenicea*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709528	-
	Cy-FO-22	*J. phoenicea*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709529	-
	Cy-FO-27	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709530	-
	Cy-FO-33	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709531	-
	Cy-FO-35	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709532	-
	Cy-FO-36	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709533	-
	Cy-FO-40	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709534	-
	Cy-FO-41	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709535 KX709536	-
	Cy-FO-44	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709537	-
	Cy-FO-46	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709538	-
D. novozelandica	Cy-FO-47	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709539	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-56	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709540	-
	Cy-FO-59	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709541	-
	Cy-FO-60	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709542	-
	Cy-FO-66	*Quercus* sp.	P. Abad-Campos. 2011	Spain, Castellón, Pobla de Benifassà	-	-	KX709543	-
	Cy-FO-137	*Q. suber*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709544	-
	Cy-FO-146	*Santolina chamaecyparissus*	B. Mora-Sala. 2009	Spain, Valencia, Cheste	-	-	KX709545	-
	Cy-FO-153	*R. officinalis*	B. Mora-Sala. 2009	Spain, Valencia, Rugat	-	-	KX709546	-
	Cy-FO-180	*R. officinalis*	B. Mora-Sala. 2009	Spain, Valencia, Picasent	-	-	KX709547	-
	Cy-FO-188	*Crataegus azarolus*	B. Mora-Sala. 2009	Spain,Valencia, Chiva	-	-	KX709536	-
	Cy-FO-191	*Pinus* sp.	B. Mora-Sala. 2009	Spain, Castellon, Segorbe	-	-	KX709548	-
	Cy-FO-210	*Pistacia lentiscus*	B. Mora-Sala. 2009	Spain,Valencia, Chiva	-	-	KX709549	-
D. novozelandica	Cy-FO-211	*Pi. lentiscus*	B. Mora-Sala. 2009	Spain,Valencia, Chiva	-	-	KX709550	-
	Cy-FO-222	*P. halepensis*	B. Mora-Sala. 2010	Spain, La Rioja, Projano	-	-	KX709551	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
D. pauciseptata	CBS 100819; LYN 16202/2	*Erica melanthera*	H.M. Dance, 1998	New Zealand, Tauranga	EF607090	EF607067	JF735582	JF735771
	CBS 120171; KIS 10467	*Vitis* sp.	M. Žerjav, 2005	Slovenia, Krŝko	EF607089	EF607066	JF735587	JF735776
	Cy-FO-37	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709552	-
	Cy-FO-38	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709553	-
	Cy-FO-178	*Abies nordmanniana*	B. Mora-Sala. 2009	Spain, León	-	-	KX709554	-
D. pinicola	**CBS 173.37**; IMI 090176	*P. laricio*	T. R. Peace	UK, England, Devon, Haldon	JF735319	JF735447	JF735614	JF735803
	CBS 159.34; IMI 113891; MUCL 4084; VKM F-2656	-	H.W. Wollenweber, 1934	Germany	JF735318	JF735446	JF735613	JF735802
	Cy-FO-177	*A. concolor*	B. Mora-Sala. 2009	Spain, León	-	-	KX709555	-
D. torresensis	**CBS 129086**; Cy218	*V. vinifera*	A. Cabral	Portugal, Torres Vedras	JF735362	JF735492	JF735681	JF735870
	CBS 119.41	*Fragaria* sp.	H.C. Koning	Netherlands, Baarn	JF735349	JF735478	JF735657	JF735846
	Cy-FO-2	*Q. ilex*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709556	-
D. torresensis	Cy-FO-12	*P. halepensis*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709557	-
	Cy-FO-28	*Q. ilex*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709558	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-43	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709559	-
	Cy-FO-54	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709560	-
	Cy-FO-64	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709561	-
	Cy-FO-65	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709562	-
	Cy-FO-127	*Arbutus unedo*	B. Mora-Sala. 2009	Spain,Valencia, Chiva	-	-	KX709563	-
	Cy-FO-205	*Cistus albidus*	B. Mora-Sala. 2009	Spain, Castellón, Pobla de Benifassà	-	-	KX709564	-
	Cy-FO-206	*C. albidus*	B. Mora-Sala. 2009	Spain, Castellón, Pobla de Benifassà	-	-	KX709565	-
	Cy-FO-218	*Juglans regia*	B. Mora-Sala. 2010	Spain, Soria, Rejas de S. Este	-	-	KX709566	-
	Cy-FO-227	*R. officinalis*	B. Mora-Sala. 2009	Spain, Valencia, Llaurí	-	-	KX709567	-
D. vitis	**CBS 129082**; Cy233	*V. vinifera*	C. Rego, 2008	Portugal, Vidigueira	JF735303	JF735431	JF735580	JF735769
D. valentina	**CBS 142826;** Cy-FO-133	*Ilex aquifolium*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	KY676881	KY676875	KY676863	KY676869
Ilyonectria capensis	**CBS 132815**; CPC 20695	*Protea* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231151	JX231103	JX231135	JX231119

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	CBS 132816; CPC 20700	*Protea* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231160	JX231112	JX231144	JX231128
	Cy-FO-63	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709568	-
	Cy-FO-129	*Ar. unedo*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709569	-
	Cy-FO-184	*Juniperus* sp.	B. Mora-Sala. 2009	Spain, La Rioja, Logroño	-	-	KX709570	-
I. coprosmae	CBS 119606; GJS 85-39	*Metrosideros* sp.	G. J. Samuels	Canada, Ontario	JF735260	JF735373	JF735505	JF735694
I. crassa	CBS 129083; NSAC-SH-1	*Panax quinquefolium*	S. Hong, 1998	Canada, Nova Scotia	AY295311	JF735395	JF735536	JF735725
	CBS 158.31; IMI 061536; NRRL 6149	*Narcissus* sp.	W. F. van Hell	The Netherlands	JF735276	JF735394	JF735535	JF735724
I. cyclaminicola	**CBS 302.93**	*Cyclamen* sp.	M. Hooftman	The Netherlands, Roelofarendsveen	JF735304	JF735432	JF735581	JF735770
	Cy-FO-67	*Quercus* sp.	P. Abad-Campos. 2011	Spain, Castellón, Pobla de Benifassà	-	-	KX709571	-
I. destructans	**CBS 264.65**	*Cyclamen persicum*	L. Nilsson	Sweden, Skåne, Bjärred	AY677273	AY677256	JF735506	JF735695
I. europaea	CBS 102892	*Phragmites australis*	W. Leibinger	Germany, Lake Constance	JF735295	JF735422	JF735569	JF735758
	CBS 129078; Cy241	*V. vinifera*	C. Rego	Portugal, Vidigueira	JF735294	JF735421	JF735567	JF735756

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
I. gamsii	**CBS 940.97**	Soil	J. T. Poll	The Netherlands, Lelystad	AM419065	AM419089	JF735577	JF735766
I. ilicicola	Cy-FO-224	*Ilex* sp.	B. Mora-Sala. 2012	Spain,Tarragona	KY676883	KY676877	KY676865	KY676871
	CBS 142828; Cy-FO-225	*Ilex* sp.	B. Mora-Sala. 2012	Spain,Tarragona	KY676884	KY676878	KY676866	KY676872
	Cy-FO-226	*Ilex* sp.	B. Mora-Sala. 2012	Spain,Tarragona	KY676885	KY676879	KY676867	KY676873
I. leucospermi	**CBS 132809**; CPC 20701	*Leucospermum* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231161	JX231113	JX231145	JX231129
	CBS 132810; CPC 20703	*Protea* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231162	JX231114	JX231146	JX231130
I. liliigena	**CBS 189.49**; IMI 113882	*Lilium regale*	M.A.A. Schippers	The Netherlands, Hoorn	JF735297	JF735425	JF735573	JF735762
	CBS 732.74	*Lilium* sp.	G. J. Bollen	The Netherlands, Heemskerk	JF735298	JF735426	JF735574	JF735763
I. liriodendri	**CBS 110.81**; IMI 303645	*Liriodendron tulipifera*	J.D. MacDonald & E.E.	USA, California	DQ178163	DQ178170	JF735507	JF735696
	CBS 117527; Cy76	*V. vinifera*	C. Rego, 1999	Portugal, Ribatejo e Oeste	DQ178165	DQ178172	JF735509	JF735698
I. liriodendri	Cy-FO-50	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709572	-
	Cy-FO-52	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709573	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-57	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709574	-
	Cy-FO-68	*Quercus* sp.	P. Abad-Campos. 2011	Spain, Castellón, Pobla de Benifassà	-	-	KX709575	-
	Cy-FO-132	*Ar. unedo*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709576	-
	Cy-FO-136	*Q.suber*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709577	-
	Cy-FO-142	*Ar. unedo*	B. Mora-Sala. 2009	Spain, Valencia, Cheste	-	-	KX709578	-
	Cy-FO-183	*Juniperus* sp.	B. Mora-Sala. 2009	Spain, La Rioja, Logroño	-	-	KX709579	-
	Cy-FO-221	*P. halepensis*	B. Mora-Sala. 2010	Spain, La Rioja, Projano	-	-	KX709580	-
I. lusitanica	**CBS 129080**; Cy197	*V. vinifera*	N. Cruz	Portugal, Melgaço	JF735296	JF735423	JF735570	JF735759
I. mors-panacis	CBS 124662; NBRC 31881; SUF 811	*Pa. ginseng*	Y. Myazawa	Japan, Nagano, Kitasaku-gun	JF735290	JF735416	JF735559	JF735748
	CBS 306.35	*Pa. quinquefolium*	A. A. Hildebrand	Canada, Ontario	JF735288	JF735414	JF735557	JF735746
I. palmarum	CBS 135753; CPC 22088; DiGeSA-HF7	*Howea forsteriana*	G. Polizzi	Italy, Sicily, Catania province, Aci Castello	HF937432	HF922609	HF922621	HF922615
I. palmarum	**CBS 135754**; CPC 22087; DiGeSA-HF3	*H. forsteriana*	G. Polizzi	Italy, Sicily, Catania province, Aci Castello	HF937431	HF922608	HF922620	HF922614
I. panacis	**CBS 129079**; CDC-N-9a	*Pa. quinquefolium*	K. F. Chang	Canada, Alberta	AY295316	JF735424	JF735572	JF735761

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
I. protearum	**CBS 132811**; CPC 20707	*Protea* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231157	JX231109	JX231141	JX231125
	CBS 132812; CPC 20711	*Protea* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231165	JX231117	JX231149	JX231133
I. pseudodestructans	CBS 117824	*Quercus* sp.	E. Halmschlager	Austria, Patzmannsdorf	JF735292	JF735419	JF735562	JF735751
	CBS 129081; Cy20	*V.vinifera*	C. Rego	Portugal, Gouveia,São Paio	AJ875330	AM419091	JF735563	JF735752
	Cy-FO-71	*Q. ilex*	B. Mora-Sala. 2015	Spain, Valencia, Ayora	-	-	KX709581	-
I. robusta	CBS 129084; Cy192	*V. vinifera*	N. Cruz, 2005	Portugal, Monção	JF735273	JF735391	JF735532	JF735721
	CBS 308.35	*Pa. quinquefolium*	A. A. Hildebrand	Canada, Ontario	JF735264	JF735377	JF735518	JF735707
	Cy-FO-217	*Ju. regia*	B. Mora-Sala. 2010	Spain, Soria, Rejas de S. Este	-	-	KX709582	-
	Cy-FO-219	*Ju. regia*	B. Mora-Sala. 2010	Spain, Soria, Rejas de S. Este	-	-	KX709583	-
I. rufa	**CBS 153.37**	Dune sand	F. Moreau	France	AY677271	AY677251	JF735540	JF735729
I. rufa	CBS 640.77	*A. alba*	F. Gourbière, 1977	France, Villeurbanne	JF735277	JF735399	JF735542	JF735731
	Cy-FO-4	*Q. ilex*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709584	-

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	Cy-FO-7	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Quart de Poblet	-	-	KX709585	-
	Cy-FO-8	*Q. ilex*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	-	-	KX709586	-
	Cy-FO-17	*Q. faginea*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709587	-
	Cy-FO-53	*P. halepensis*	P. Abad-Campos. 2011	Spain, Valencia, Ayora	-	-	KX709588	-
	Cy-FO-128	*Ar. unedo*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709589	-
	Cy-FO-130	*Ar. unedo*	B. Mora-Sala. 2009	Spain, Valencia, El Puig	-	-	KX709590	-
	Cy-FO-161	*Juniperus* sp.	B. Mora-Sala. 2009	Spain, Castellon, Segorbe	-	-	KX709591	-
	Cy-FO-179	*A. nordmanniana*	B. Mora-Sala. 2009	Spain, León	-	-	KX709592	-
I. venezuelensis	**CBS 102032**; ATCC 208837; AR2553	Bark	A. Rossman	Venezuela, Amazonas, Cerro de la Neblina	AM419059	AY677255	JF735571	JF735760
I. vredehoekensis	**CBS 132807**; CPC 20699	*Protea* sp.	C. M. Bezuidenhout	South Africa, Western Cape, Stanford	JX231155	JX231107	JX231139	JX231123
	CBS 132814; CPC 20690[b]	*Protea* sp.	C.M. Bezuidenhout	South Africa	JX231158	JX231110	JX231142	JX231126
Neonectria candida	CBS 182.36; IMI 113893; UPSC 1903	*Malus sylvestris*	H.W. Wollenweber	-	JF735314	JF735439	JF735603	JF735792

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
N. candida, authentic strain of *C. obtusiusculum* (=*C. magnusianum*)	**CBS 151.29**; IMI 113894; MUCL 28083; MUCL 28094	*Ma. sylvestris*	H.W. Wollenweber	UK, England, Cambridge	JF735313	JF735438	JF735602	JF735791
N. ditissima, authentic strain of *C. willkommii*	**CBS 226.31**; IMI 113922	*Fagus sylvatica*	H.W. Wollenweber	Germany, Tharandt	JF735309	DQ789869	JF735594	JF735783
N. ditissima, representative strain of *N. galligena*	CBS 835.97	*Salix cinerea*	W. Gams, 1997	Belgium, Marais de Sampant	JF735310	DQ789880	JF735595	JF735784
N. major, type strain	**CBS 240.29**; IMI 113909	*Alnus incana*	H.W. Wollenweber	Norway	JF735308	DQ789872	JF735593	JF735782
N. neomacrospora representative strain	**CBS 324.61**; DSM 62489; IMB 9628	*A. concolor*	J.A. von Arx	Netherlands, Zwolle	JF735312	DQ789875	JF735599	JF735788
	CBS 503.67	*A. alba*, wood	F. Roll-Hansen	Norway, Hordaland, Fana	AY677261	JF735436	JF735600	JF735789
,	CBS 118984; GJS 03-28	*Arceuthobium tsugense*	L. Reitman, 2005	Canada, British Columbia, Vancouver Island, Spider Lake	JF735311	DQ789882	JF735598	JF735787
N. obtusispora	CBS 183.36; IMI 113895	*Solanum tuberosum*	H.W. Wollenweber, 1936	Germany	AM419061	AM419085	JF735607	JF735796
	CPC 13544; DAOM 182772; JAT 1366	*Prunus armenica*	J.A. Traquair, 1982	Canada, Ontario, Ruthven	AY295306	JF735443	JF735608	JF735797
N.quercicola	**CBS 143704**; Cy-FO-3	*Q. ilex*	P. Abad-Campos. 2011	Spain, Alicante, Alcoi	KY676880	KY676874	KY676862	KY676868
	CPC 13530; DAOM 185722; JAT 1591	*Pyrus* sp.	J.A. Traquair, 1983	Canada, Ontario, Harrow	AY295302	JF735441	JF735605	JF735794
N. quercicola	CPC 13531; CCFC 226722; DAOM 226722; CR6	*Pseudotsuga menziesii*	P. Axelrood	Canada, British Columbia	AY295301	JF735442	JF735606	JF735795

Species	Strain number	Host	Collected/isolated by/year	Location	GenBank Accession Numbers.			
					ITS	*tub2*	*his3*	*tef1*
	CR21	*Ps. menziesii*	P. Axelrood	Canada, British Columbia	JF735315	JF735440	JF735604	JF735793
Neonectria sp.1	CPC 13545; DAOM 185212; # 5	*Pyrus* sp.	J.A. Traquair & B. Harrison, 1982	Canada, Ontario, Harrow	AY295303	JF735437	JF735601	JF735790

AR: Amy Y. Rossman personal collection;**ATCC**: American Type Culture Collection, USA; **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CCFC**: Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; **CDC**: Centers for Disease Control and Prevention, Atlanta, GA, USA; **CPC**: Culture collection of Pedro Crous, housed at CBS; **Cy**: Cylindrocarpon collection housed at Laboratório de Patologia Vegetal "Veríssimo de Almeida" - ISA, Lisbon, Portugal; **DAOMC**: Canadian Collection of Fungal Cultures, Canada; **DiGeSA**: Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Catania, Italy;**DSM**: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; **GJS**: Gary J. Samuels collection; **HJS**: Hans-Josef Schroers collection; **HMAS**: Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences;**IAFM**: Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Spain; **ICMP**: International Collection of Microorganisms from Plants, Auckland, New Zealand; **IMI**: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; **JAT**: J. A. Traquair collection; **KIS**: Agricultural Institute of Slovenia, Ljubljana, Slovenia; **LYN:** Lynchburg College, Biology Department, USA; **MUCL**: Mycothèque de l'Université Catholique de Louvain, Belgium; **NBRC**: NITE Biological Resource Center, Japan; **NRRL**: Agricultural Research Service Culture Collection, USA; **NZ**: Collection of L. Castlebury; **PD**: Collection of the Dutch National Plant Protection Organization (NPPO-NL), Wageningen, The Netherlands;**STE-U**: Stellenbosch University, South Africa; **TRTC**: Royal Ontario Museum Fungarium, Toronto, Ontario, Canada;**UPSC**: Fungal Culture Collection at the Botanical Museum, Uppsala University, Uppsala, Sweden; **VKM**: All-Russian Collection of Microorganisms, Russia.

Ex-type culture indicated in bold.

Table 2. Distribution of the *Cylindrocarpon*-like isolates according to the fungal species identified in the study. Number and percentages of isolates, hosts and forest nurseries.

Fungal Species	Isolates [a]		Host [b]			Nursery [c]		
	No.	%	No.	%	Genera	No.	%	Location (Province)
Cylindrodendrum alicantinum	1	0.97	1	6.67	*Quercus*	1	5.88	Valencia
Dactylonectria hispanica	1	0.97	1	6.67	*Pinus*	1	5.88	Valencia
Dactylonectria macrodidyma	25	24.27	9	60.00	*Juniperus, Ilex, Lonicera*, *Myrtus, Pyracantha, Quercus, Pinus,Rosmarinus*	9	52.94	Alicante, Castellón, Logroño, Valencia
Dactylonectria novozelandica	30	29.13	10	66.67	*Crataegus, Juniperus, Pinus*, *Pistacia, Quercus, Rosmarinus,Santolina*	11	64.71	Alicante, Castellón, Logroño, Valencia
Dactylonectria pauciseptata	3	2.91	2	13.33	*Abies,Pinus*	2	11.76	Valencia, León
Dactylonectria pinicola	1	0.97	1	6.67	*Abies*	1	5.88	León
Dactylonectria torresensis	12	11.65	6	40.00	*Arbutus, Cistus, Juglans, Pinus, Quercus,Rosmarinus*	6	35.29	Alicante, Castellón, Soria, Valencia
Dactylonectria valentina	1	0.97	1	6.67	*Ilex*	1	5.88	Valencia
Ilyonectria capensis	3	2.91	3	20.00	*Arbutus, Juniperus*, *Pinus*	3	17.65	Logroño, Valencia
Ilyonectria cyclaminicola	1	0.97	1	6.67	*Quercus*	1	5.88	Castellón
Ilyonectria ilicicola	3	2.91	1	6.67	*Ilex*	1	5.88	Tarragona
Ilyonectria liriodendri	9	8.74	5	33.33	*Arbutus, Juniperus, Pinus, Quercus*	6	35.29	Castellón, Logroño, Valencia
Ilyonectria pseudodestructans	1	0.97	1	6.67	*Quercus*	1	5.88	Valencia
Ilyonectria robusta	2	1.94	1	6.67	*Juglans*	1	5.88	Soria
Ilyonectria rufa	9	8.74	6	40.00	*Abies, Arbutus, Juniperus, Quercus,Pinus*	6	35.29	Alicante, Castellón, León, Valencia
Neonectria quercicola	1	0.97	1	6.67	*Quercus*	1	5.88	Alicante

[a] Number of isolates and percentages were calculated on the basis of a total of 103 isolates.

[b] Number of hosts and percentages were calculated on the basis of a total of 15 plant genera.

[c] Number of nurseries and percentages were calculated on the basis of a total of 17 surveyed nurseries.

Figure captions

Figure 1 – Symptomatology of nursery plants from which *Cylindrocarpon*-like anamorphs were isolated. **A**, **D** and **E**: shoot dieback on *Myrtus* sp. (A), *Juniperus* sp. (D) and *Rosmarinus officinalis* (E). **B** and **C**: *Juniperus* sp. and *Pinus* sp. dieback; apical dieback (B) and internal basal dieback (C). **F**: *Pinus* sp. seedlings showing reduced root system, including loss and rot of the feeder roots. **G**: *Quercus ilex* seedlings showing decline aerial symptoms, uneven growth and a reduction of the root system.

Figure 2 –Fifty percent majority rule consensus tree from a Bayesian analysis based on the alignment of partial histone H3 gene sequences from the 103 cylindrocarpon-like asexual morphs obtained from forest nurseries, and additional sequences of *Cylindrodendrum*, *Dactylonectria*, *Ilyonectria* and *Neonectria* species. The RAxML bootstrap support and Bayesian posterior probability values are indicated at the nodes (ML/PP). The tree was rooted to *Campylocarpon fasciculare* (CBS 112613) and *C. pseudofasciculare* (CBS 112679). The scale bar indicates 0.1 expected changes per site. Ex-type cultures are indicated in **bold**. Colors are used to indicate clades from the same genera *Ca. – Campylocarpon*, *C. – Cylindrodendrum*, *D. – Dactylonectria*, *I. – Ilyonectria*, *N. – Neonectria*. Tentative new species are indicated in colored boxes.

Figure 3 –Fifty percent majority rule consensus tree from a Bayesian analysis based on the combined four gene dataset (ITS, *tub2, his3* and *tef1*). The RAxML bootstrap support and Bayesian posterior probability values are indicated at the nodes (ML/PP). The tree was rooted to *Campylocarpon fasciculare* (CBS 112613) and *C. pseudofasciculare* (CBS 112679). The scale bar indicates 0.1 expected changes per site. Ex-type cultures are indicated in **bold**. Colors are used to indicate clades from the same genera *Ca. –*

Campylocarpon, *C. – Cylindrodendrum*, *D.– Dactylonectria*, *I. – lyonectria*, *N. – Neonectria*. New species are indicated in colored boxes.

Figure 4 - Ten-day-old colonies grown at 20ºC in darkness on PDA (A-F upper face; G-L bottom face) and Oat-meal agar (M-R upper face; S-Y bottom face) of: *Dactylonectria hispanica* isolate Cy-FO-45 (A, G, M and S); *D. valentina* isolate Cy-FO-133 (B, H, N and T); *Ilyonectria ilicicola* isolates Cy-FO-224 (C, I, O and U), Cy-FO-225 (D, J, P and V) and Cy-FO-226 (E, K, Q and X) and *Neonectria quercicola* Cy-FO-3 (F, L, R and Y).

Figure 5 - *Dactylonectria hispanica* (ex-type culture Cy-FO-45*).* **A–D** Simple, sparsely branched conidiophores of the aerial mycelium. **E–G** Micro- and macroconidia. **H** Chlamydospores in mycelium. Scale bars: C, D = 20 µm; A–B, E–H = 10 µm.

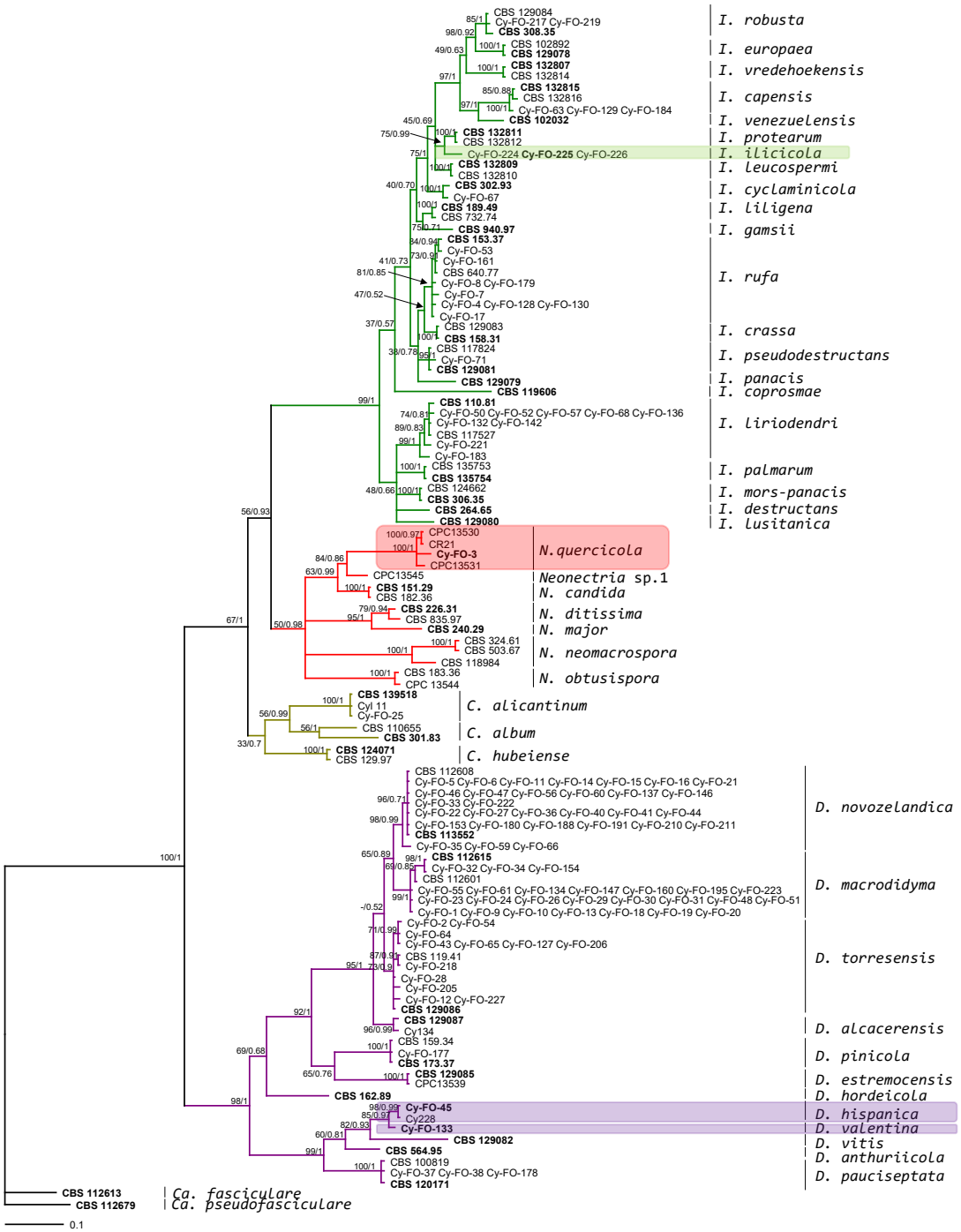
Figure 6 - *Dactylonectria valentina.* **A–E** Simple, sparsely branched conidiophores of the aerial mycelium. **E–G** Conidiophores forming microconidia arising from mycelium at agar surface, with a terminal arrangement of phialides, ranging from 2 to a dense cluster; sparsely branched or penicillate. **H** Sporodochial conidiophores. **I–K** Micro- and macroconidia. **L–M** Chlamydospores in mycelium. Scale bars: H = 50 µm, A–B, F, L = 20 µm; C–E, G, I–K, M = 10 µm.

Figure 7 - *Ilyonectria ilicicola* (ex-type culture Cy-FO-225). **A–E** Simple, sparsely branched conidiophores of the aerial mycelium. **F–H** Complex conidiophores. **I–K** Micro- and macroconidia. **L–M** Chlamydospores in mycelium. Scale bars: F, G = 50 µm, A–C, L = 20 µm; D–E, I–K, M = 10 µm; E, H, M from Cy-FO-224 and A–D, F–G, I–L from Cy-FO-225.

Figure 8 – *Neonectria quercicola* **A** Simple, sparsely branched conidiophores of the aerial mycelium. **B–D** Long conidiophores of the aerial mycelium. **E** Sporodochial

conidiophores. **F–I** Micro- and macroconidia. Scale bars: B, E, I = 50 μm; C = 20 μm; A, D, F–H = 10 μm.All from Cy-FO-3.

Figure 1


I. robusta
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I. vredehoekensis
I. capensis
I. venezuelensis
I. protearum
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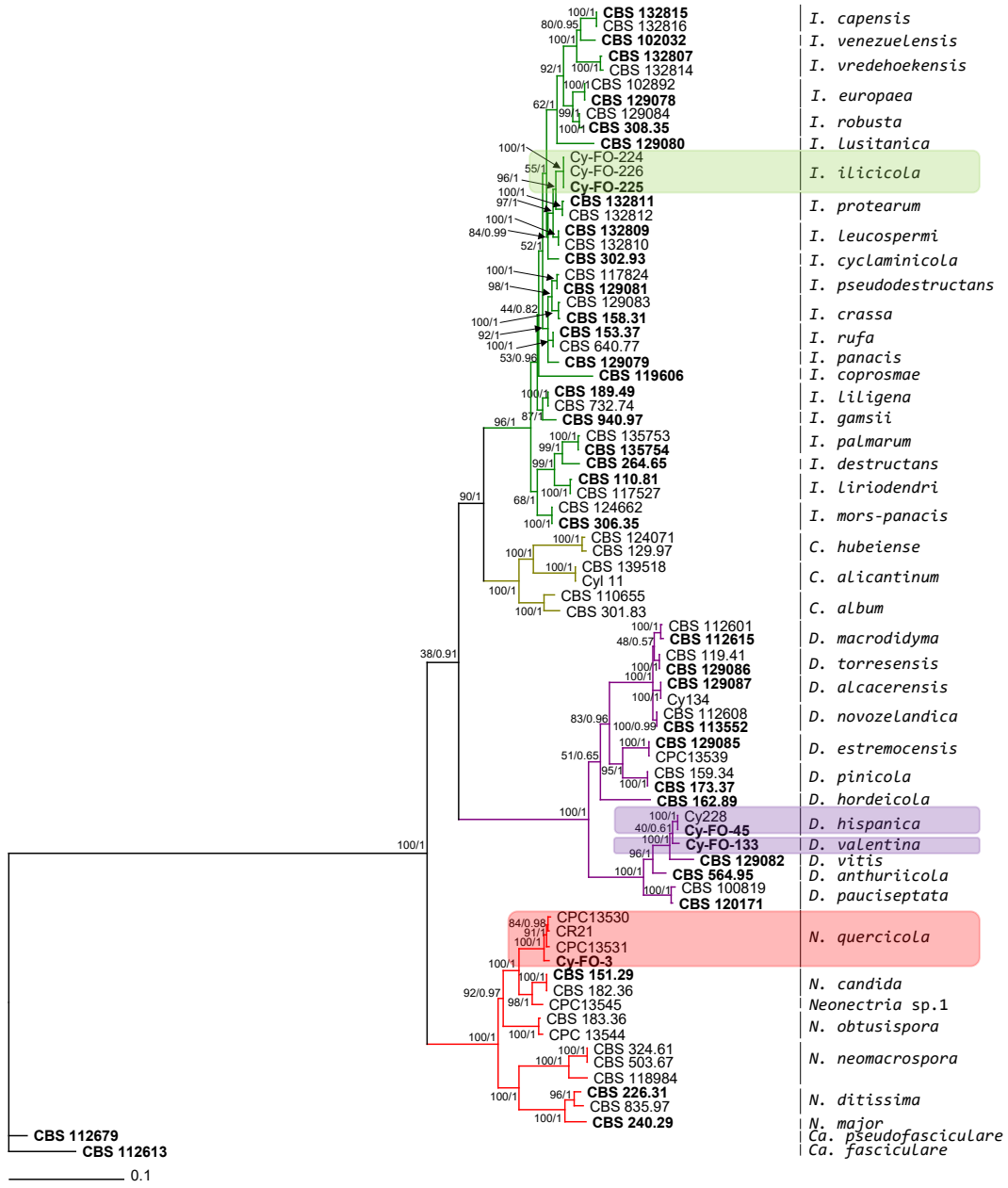
I. leucospermi
I. cyclaminicola
I. liligena
I. gamsii
I. rufa
I. crassa
I. pseudodestructans
I. panacis
I. coprosmae
I. liriodendri
I. palmarum
I. mors-panacis
I. destructans
I. lusitanica
N.quercicola
Neonectria sp.1
N. candida
N. ditissima
N. major
N. neomacrospora
N. obtusispora
C. alicantinum
C. album
C. hubeiense
D. novozelandica
D. macrodidyma
D. torresensis
D. alcacerensis
D. pinicola
D. estremocensis
D. hordeicola
D. hispanica
D. valentina
D. vitis
D. anthuriicola
D. pauciseptata
Ca. fasciculare
Ca. pseudofasciculare
0.1

Figure 2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Cy-FO-226
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CBS 640.77
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CBS 940.97
CBS 135753
CBS 135754
CBS 264.65
CBS 110.81
CBS 117527
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CBS 306.35
CBS 124071
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CBS 139518
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Cy134
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Cy-FO-133
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CPC13531
Cy-FO-3
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CBS 182.36
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I. capensis
I. venezuelensis
I. vredehoekensis
I. europaea
I. robusta
I. lusitanica
I. ilicicola
I. protearum
I. leucospermi
I. cyclaminicola
I. pseudodestructans
I. crassa
I. rufa
I. panacis
I. coprosmae
I. liligena
I. gamsii
I. palmarum
I. destructans
I. liriodendri
I. mors-panacis
C. hubeiense
C. alicantinum
C. album
D. macrodidyma
D. torresensis
D. alcacerensis
D. novozelandica
D. estremocensis
D. pinicola
D. hordeicola
D. hispanica
D. valentina
D. vitis
D. anthuriicola
D. pauciseptata
N. quercicola
N. candida
Neonectria sp.1
N. obtusispora
N. neomacrospora
N. ditissima
N. major
Ca. pseudofasciculare
Ca. fasciculare

Figure 3

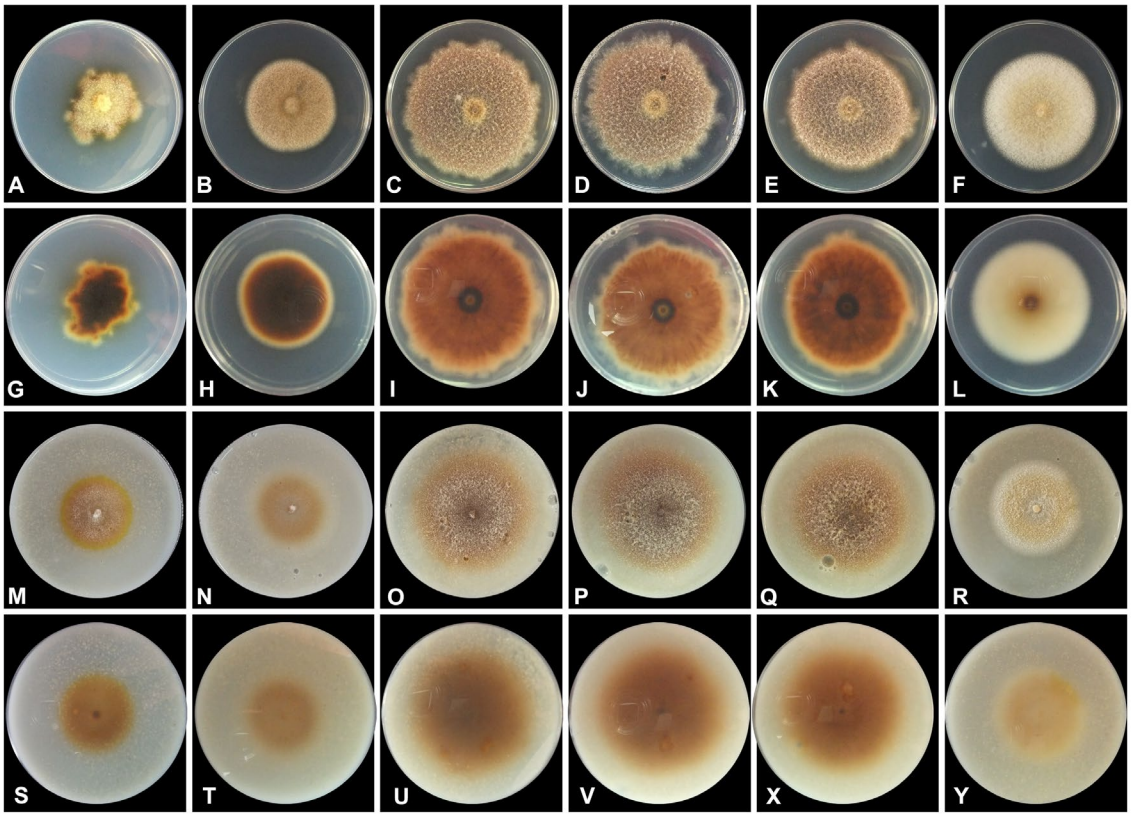

Figure 4

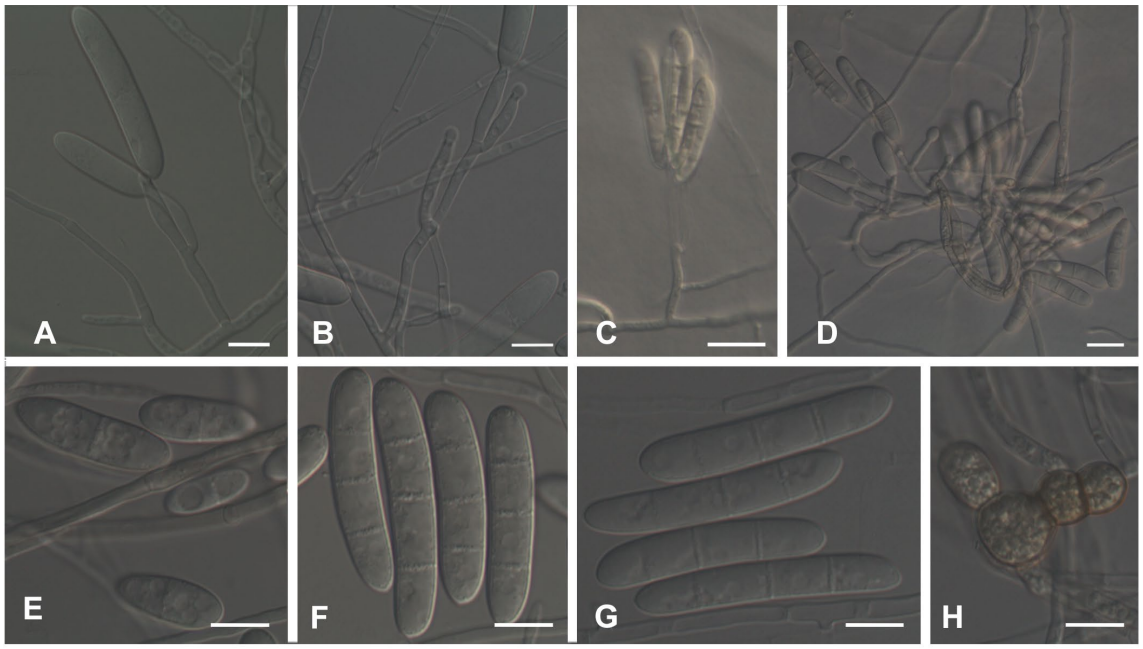

Figure 5

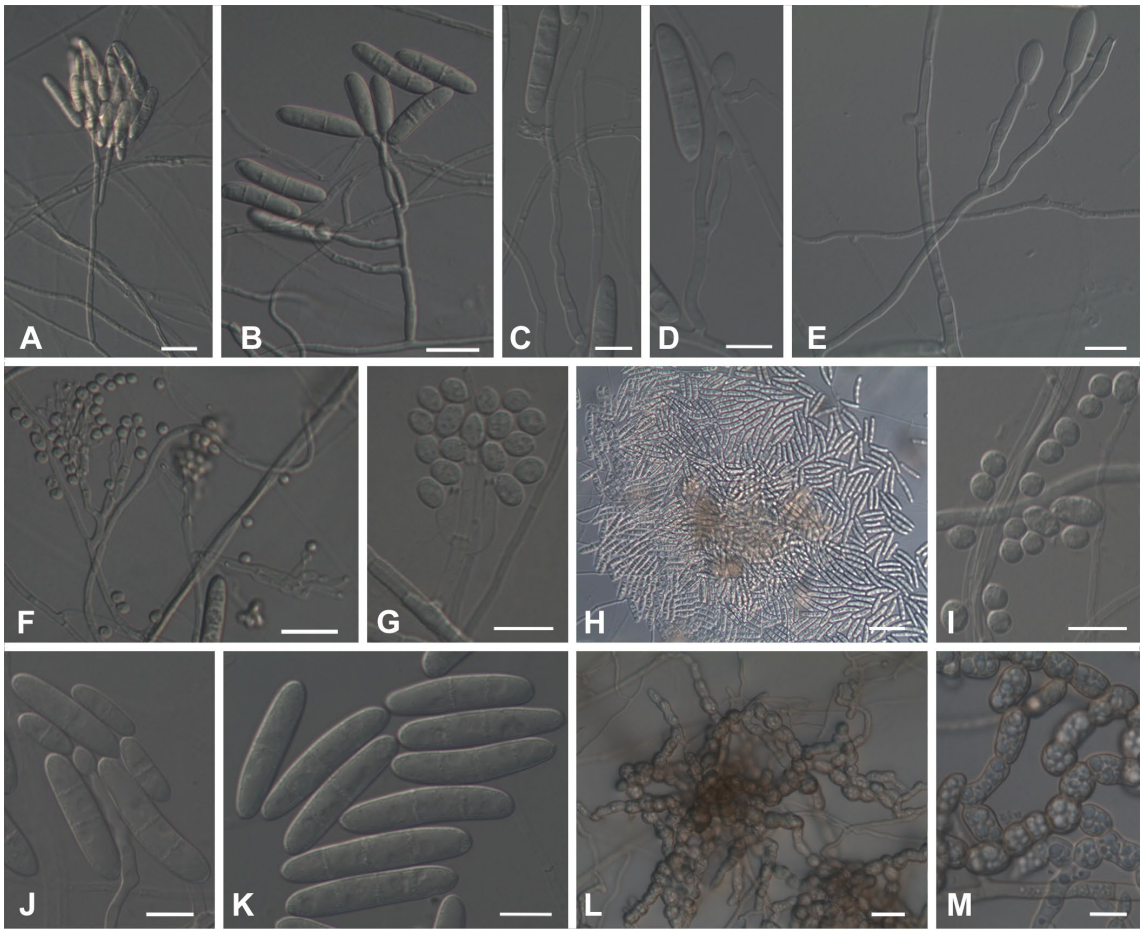

Figure 6

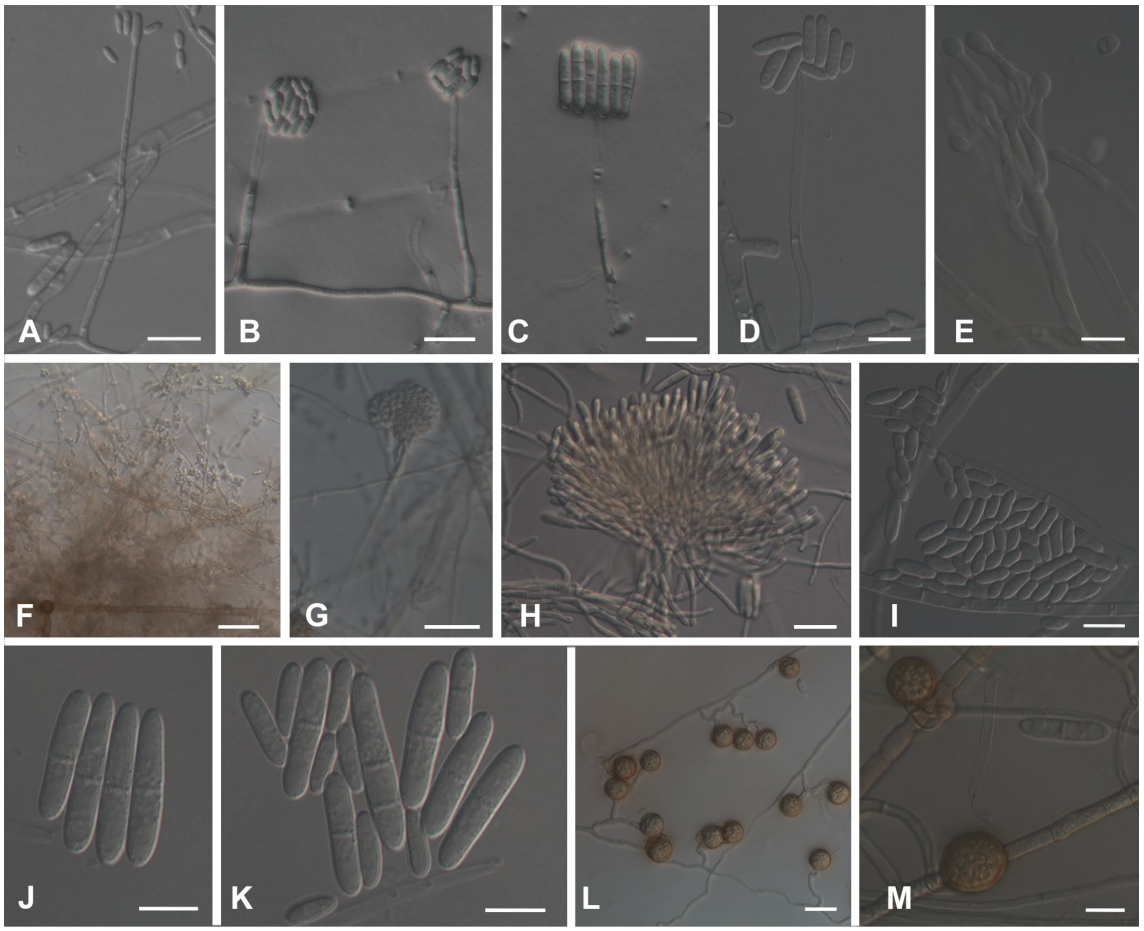

Figure 7

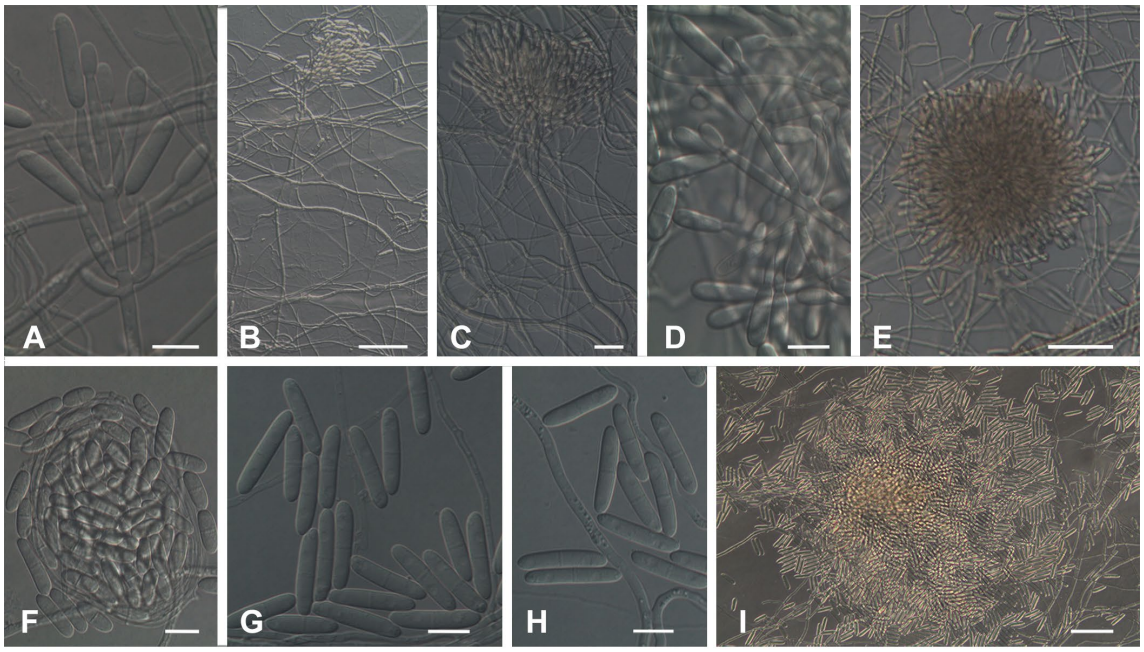

Figure 8