Two novel species of Mesophoma gen. nov. from PR China and a potential biocontrol of the invasive weed Ageratina adenophora

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Abstract

During an investigation of the fungal pathogens associated with the invasive weed *Ageratina adenophora* from China, some interesting isolates were collected. Among them, a novel genus *Mesophoma* containing two novel species, *M. speciosa* and *M. ageratinae*, from healthy leaf, leaf spot, and roots of *Ag. adenophora* was found. Phylogenetic analysis of the combined the internal transcribed spacer (ITS), large nuclear subunit ribosomal DNA (LSU), the RNA polymerase II second largest subunit (*rpb2*), and the partial β-tubulin (*tub2*) sequences showed that *M. speciosa* and *M. ageratinae* each formed a distinct clade, separating from all genera previously described in Didymellaceae. Combined morphological characters allowed us to describe them as novel species belonging to a novel genus *Mesophoma*. The full descriptions, illustrations, and a phylogenetic tree showing the position of *M. speciosa* and *M. ageratinae* were provided in this study. The potential for these strains to be developed into a biocontrol for the spread of the invasive weed *Ag. adenophora* was also discussed.

Introduction

Didymellaceae was established by de Gruyter et al. in 2009. It is one of the largest families in the fungal kingdom, which again belongs to the largest Dothideomycetes order, Pleosporales (Pleosporomycetidae, Dothideomycetes, Pezizomycotina, Ascomycota). This family is extremely species-rich and more than 5 400 species belonging to at least 37 genera have been recorded (Hou et al. 2020a; Hou et al. 2020b; Phukhamsakda et al. 2020; Crous et al. 2021).

Didymellaceae previously only included three main genera, viz. *Ascochyta* Lib., *Didymella* Speg., and *Phoma* Fr., as well as several related phoma-like genera (De Gruyter et al. 2009). The phoma-like circumscription is a pervasive and general concept, including species that produce pycnidia with aseptate, hyaline conidia occurring on herbaceous stems. The previously broad and ambiguous concept of the genus *Phoma* and the host-orientated nomenclature, together with the wide host range and occurrence, often resulted in incorrect taxonomic placements.

After more than 50 years of studying this group of fungi, Boerema and co-workers proposed that Phoma should be divided into nine sections, later studies showed five of them: *Macrospora* Fuckel, *Peyronellaea* Goid., *Phoma*, *Phyllostictoides* (Desm.) Keissl. and *Sclerophomella* Höhn in Didymellaceae (De Gruyter et al. 2009; De Gruyter et al. 2013).

In 2010, Aveskamp et al. established the genus *Boeremia*, redefined the genera *Epicoccum* Link and *Stagonosporopsis* Died, and confirmed two sexual genera, namely *Leptosphaerulina* McAlpine and *Macroventuria* Aa. Despite these studies, the polyphyly of *Ascochyta*, *Didymella* and *Phoma* remained unresolved (Aveskamp et al. 2010).

In a further study, Chen et al. provided an updated understanding of the taxonomy and evolution of Didymellaceae by including the *rpb2* locus in a four-locus phylogenetic analysis. The study revealed 17 well-supported monophyletic clades, leading to the introduction of nine genera. (Chen et al. 2015).

Subsequently, several additional genera have been introduced (Chen et al. 2017), such as *Briansuttonomyces* Crous, *Neomicrosphaeropsis* Thambug and *Pseudoascochyta* Valenz.-Lopez from plant materials (Crous and Groenewald 2016; Crous et al. 2016). After that, Valenzuela-Lopez et al. added six genera into the family based on clinical specimens and several reference species of *Phoma* from prior studies were also confirmed (Aveskamp et al. 2010; Valenzuela-Lopez et al. 2018).

In 2020, the family was expanded and added 9 well-defined genera, *Vandijckomycella* Hernández-Restrepo (Hou et al. 2020a), *Dimorphoma*, *Ectodidymella*, *Longididymella*, *Macroascochyta*, *Paramicrosphaeropsis*, *Pseudopeyronellaea*, *Sclerotiophoma* (Hou et al. 2020b) and *Anthodidymella* (Phukhamsakda et al. 2020). Crous introduced the genus *Neoscirrhia* from *Sasa veitchii* diseased stem and the genus *Nothomicrosphaeropsis* from *Welwitschia mirabilis* dead leaves (Crous et al. 2021).

The Didymellaceae includes plant pathogens, opportunists, endophytes, and saprobes from a wide range of substrates, such as asbestos, cement, crockery (Aveskamp et al. 2008), soil, oceans and their fauna, glaciers, and even deep-sea sediments (Zucconi et al. 1996; Yarden 2014; Zhang et al. 2014; Chen et al. 2017; Hou et al. 2020a). More than 50% of the species in this family have been reported as plant pathogens, causing great losses to a wide range of economic crops (Aveskamp et al. 2008).

Ageratina adenophora (Spreng.) R.M.King & H.Rob. (Compositae) is a perennial herb native to Central America, and has invaded more than 40 countries worldwide in tropical to temperate regions (Poudel et al. 2019). Since the first record in China in the 1940s, the plant has been widely distributed in the provinces of Yunnan, Sichuan, Guizhou, Guangxi and Tibet, and it has continuously spread east- and northward with clear invasive history records (Wang and Wang 2006). There is evidence that *Ag. adenophora* can be infected by fungal

pathogens in introduced ranges(Zhou et al. 2010; Poudel et al. 2019). To determine if the fungal pathogens of *Ag. adenophora* accumulated in the introduced ranges can be developed into a biocontrol to slow down its invasion, the foliar fungi have been intensely investigated for decades (Zhou et al. 2010; Mei et al. 2014; Fang et al. 2019)

Our recent study indicates that *Ag. adenophora* accumulates diverse foliar pathogens from neighbors (horizontally transmitted); however, a dominant pathogen but unclassified OTU (OTU515) belonging to the plant pathogen-rich family Didymellaceae does not occur on surrounding native plants (Chen et al. 2020). Interestingly, the ITS locus of this group is highly concurrent with those found in fungal DNA extracted from *Ag. adenophora* seeds (Fang et al. 2021), suggesteing that this fungus may co-spread with *Ag. adenophora* in a seed-borne manner (vertically transmitted). These fungi were previously classified as *Allophoma cylindrispora* based on ITS locus by using the UNITE database (Fang et al. 2021), however, the further phylogenetic analysis of the multiple loci showed that these fungi formed a distinct clade. Therefore, it remains to be determined for the phylogenetic position for these pathogens. In this study, combined morphological characters allowed us to describe them as novel species belonging to a novel genus. Here we provided their descriptions and illustrations based on these newly recovered isolates. In addition, their phylogenetic positions were determined by combining sequences of the internal transcribed spacer (ITS), the large subunit nuclear ribosomal RNA gene (LSU rRNA), the RNA polymerase II second largest subunit (*rpb2*), and the partial β-tubulin (*tub2*). We also discussed the potential for these strains to be developed into a biocontrol for the spread of the invasive weed.

Materials & Methods

Sampling and isolation

From 2015 to 2017, we sampled healthy, diseased and rotten leaves and roots of the invasive plant *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. in Yunnan Province, Pu 'er, Dali, Kunming. These plant samples were placed into sterile self-sealing plastic bags. All the samples were transported to the lab and stored at 4 ℃ until processing.

Endophytic fungi were isolated by incubating surface-disinfected tissue segments according to the method described by Arnold and Lutzoni (2007). The healthy leaves were rinsed with tap water and then surface sterilized (0.5% sodium hypochlorite for 2 min s and 75% ethanol for 2 min and rinsed with sterile water 3 times). For the isolation of leaf spot fungi, healthy leaf tissues and the margins of diseased tissues of each leaf spot were cut into 6 mm^2 sections. Meanwhile, for the isolation of healthy and rotten leaves fungi, leaf tissues were cut into 6 mm^2 sections.

The roots were rinsed with tap water and then surface sterilized (0.5% sodium hypochlorite for 5 min s and 75% ethanol for 5 min and rinsed with sterile water 3 times). Sterilized root tissues were cut into 2mm sections.

The disinfected fragments above were subsequently plated onto potato dextrose agar (PDA; 200 g potato, 20 g glucose, 18 g agar, 1 L distilled water) and incubated at ambient temperature for 6–8 days or until mycelia growing from the leaf fragments were observed. A total of 213 Didymellaceae strains were collected.

The pure strains were incubated on PDA, oatmeal agar (OA, 40 g oatmeal, 18 g agar, 1 L distilled wate) and malt extract agar (MEA; 30 g malt powder, 3 g peptone, 18 g agar, 1 L distilled water) at 28°C. Morphological observations were conducted on cultures growing on PDA, OA and MEA after incubation at 28°C for one week. Observation was performed using an Olympus BX51 microscope (Olympus, Tokyo, Japan), and sterile water was used as a mounting medium for microscopy.

Pure cultures were deposited in the China General Microbiological Culture Collection (CGMCC), Guangdong Microbial Culture Collection Center (GDMCC).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the fresh mycelia using the CTAB method (Murray and Thompson 1980). The internal transcribed spacer (ITS) was amplified with ITS4 and ITS5 primers (White et al. 1990). LR7 and LR5 (Vilgalys and Hester 1990) were used for large nuclear subunit ribosomal DNA (LSU) amplification; Btub2Fd and Btub4Rd (Woudenberg et al. 2009) for the partial β-tubulin (*tub2*) region, and RPB2-5F2 (Sung et al. 2007) and fRPB2-7cR (Liu et al. 1999) for the RNA polymerase II second largest subunit (*rpb2*). Amplification was performed in a 50 µl reaction volume, which contained 1.0 µL DNA template, 1.0 µL of each forward and reverses primers, 25 µL 2 × MasterMix (Tsingke Biological technology Co. Ltd. Beijing, China) and 22 µL dd H_2O. Amplicons for each locus were generated following

the protocols listed in the previous method (Chen et al. 2017). The sequences were deposited in GenBank database at the National Center for Biotechnology Information (NCBI) and the accession numbers are listed in Table 1.

Phylogenetic analysis

The ITS sequences generated in this study were used as queries to search similar DNA sequences in GenBank using BLAST. The results indicated that the new taxa had the highest ITS sequence similarity to Didymellaceae spp. Therefore, we selected 89 strains representing 74 species belonging to 37 genera in Didymellaceae and retrieved their respective ITS, LSU, *rpb2* and *tub2* sequences from GenBank (Table 1). We also retrieved GenBank accessions from *Leptosphaeria doliolum* (Pers.) Ces. & De Not. (CBS 505.75) of Pleosporales as outgroup for phylogenetic analyses. All sequences analyzed in this study were listed in Table 1. All nucleotide sequences generated from different primer pairs were used to generate consensus sequences by Seq-Man v.7.0.0 (DNASTAR, Madison, WI) and were aligned through BioEdit v.7.0 (Hall 1999). Manual gap adjustments were done to improve the alignment and ambiguously aligned regions were also excluded. Then, the combined sequence alignment was converted to a NEXUS file using ClustalX 1.83 (Higgins 1994).

Maximum-likelihood (ML) analysis was computed using RAxML (Stamatakis 2006) with the PHY files generated with CLUSTAL_X version 1.83 (Thompson et al. 1997), using the GTR-GAMMA model. ML bootstrap proportions (MLBPs) were computed with 1000 replicates. Bayesian (BI) analyses were performed on MrBayes v.3.2.1 (Ronquist et al. 2012) based on the models selected by the MrModeltest according to the protocol described by Chen et al. (Chen et al. 2017) The best-fit models of evolution for the four loci tested (GTR + I + G for ITS, LSU, *rpb2* and *tub2*) were estimated by MrModeltest v. 2.3.

Metropolis-coupled Markov chain Monte Carlo (MCMCMC) searches were run for 5000 000 generations, sampling every100th generation. Two independent analyses with four chains each (one cold and three heated) were run until the average standard deviation of the split frequencies dropped below 0.01. The initial 25% of the generations of MCMC sampling were discarded as burn-in. The refinement of the phylogenetic tree was used for estimating BI posterior probability (BIPP) values. The tree was viewed in FigTree version 1.4 (Rambaut 2012)

Results

Phylogenetic analysis

The final alignment comprised 2052 base pairs with combined sequences of ITS 425bp, LSU 754bp, *rpb2* 589bp and *tub2* 284bp. The combined dataset was analyzed by using BI and ML methods. The topology of the tree was shown in Fig. 1, with the Bayesian posterior probabilities (≥ 0.8) and ML bootstrap support (≥ 50%) indicated for respective clades (Fig. 1). In this tree, 37 known genera were clustered as 37 unique clades.

We found that our strains formed an independent lineage in Didymellaceae, being clearly separated from other genera. Besides, strains were separated into two clades with 1.00 Bayesian posterior probability and 100% ml bootstrap support. Combined with morphological differences, we determined that our strains were two novel species of *Mesophoma.* The *Mesophoma* is close to *Allophoma* Qian Chen & L. Cai, *Heterophoma* Qian Chen & L. Cai and *Boeremia, Stagonosporopsis*. while distant from *Epicoccum*. The descriptions and illustrations based on these newly recovered isolates were provided as follows.

Taxonomy

Mesophoma H.B. Zhang, A.L. Yang & L. Chen, **gen. nov.**

Etymology

Greek,. (*Meso-*) meaning middle, between, intermediate, or moderate. – *phoma*, referring to the genus *Phoma*

MycoBank: MB 843556

Type species. *Mesophoma speciosa* H.B. Zhang et al.

Conidiomata pycnidial, solitary or aggregated, globose to subglobose, black or brown-yellowish, covered with hyphae, superficial or immersed. Ostiole single, slightly papillate. Pycnidial wall consisting of 2–6 layers of cells of textura angularis. Conidiogenous cells phialidic, hyaline, smooth-walled, ampulliform to doliiform. Conidia oblong to cylindrical, obovoid, sometimes slightly curved to reniform, aseptate, smooth, thin-walled, guttulate, hyaline.

Mesophoma speciosa H.B. Zhang, A.L. Yang & L. Chen, **sp. nov.**

Etymology: Latin, speciosa, meaning splendid, referring to its elegant appearance

MycoBank: MB 843557

Conidiomata pycnidial, solitary or aggregated, globose to subglobose, black or brown-yellowish, covered with hyphae, superficial or semimmersed, 79–156 × 63–168 µm. Ostiole single, slightly papillate. Pycnidial wall consisting of 4–6 layers of cells of textura angularis. Conidiogenous cells phialidic, hyaline, smooth-walled, ampulliform to doliiform, 5–7 × 8–10 µm. Conidia oblong to cylindrical, obovoid, sometimes slightly curved to reniform, aseptate, smooth, thin-walled, guttulate, hyaline, 3–6 × 1–2 µm, aggregated in cream masses (Fig. 2).

Cultural characteristics: Colonies on PDA, 84–95 mm diam after 7 d, margin regular, aerial mycelia felted, white to grey. Colonies on OA, 62–67 mm diam after 7 d, margin regular, aerial mycelia felted, grey to reddish brown. Colonies on MEA, 56–69 mm diam after 7 d, margin regular, aerial mycelia felted, black to grey. NaOH (1mol/L) spot test negative on OA (Fig. 2).

Distribution: The Lancang county, Yunnan Province, China.

Habitat: Living in leaf spot of *Ageratina adenophora*.

Holotype: CGMCC 3.20982, isolated from leaf spots of *Ageratina adenophora* (Spreng.) R.M. King & H. Rob., 20August 2017, H.B. Zhang, preserved by lyophilization (a metabolically inactive state) in the State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan. GenBank: ITS ON124744, LSU ON124751, *rpb2* ON156007 and *tub2* ON113859.

Note The new species *M. speciosa* is genetically distinct from the other genera and *M. ageratinae* in genus of *Mesophoma*. Morphologically *M. speciosa* can be distinguished from them in having smaller conidia and 0 septum, larger conidiogenous cells.

Mesophoma ageratinae H.B. Zhang, A.L. Yang & L. Chen, **sp. nov.**

Etymology: Latin, *ageratinae*, referring to the host *Ageratina adenophora*.

MycoBank: MB 843558

Conidiomata pycnidial, scatteredor aggregated, globose to subglobose, black or yellow, covered with hyphae, superficialor (semi-) immersed, 65–214 × 51–223 µm. Ostiole single, slightly papillate. Pycnidial wall consisting of 3–5 layers of cells of textura angularis. Conidiogenous cells phialidic, ampulliform to doliiform, hyaline, smooth, 5–7 × 6–8 µm. Conidia oblong to cylindrical, obovoid, sometimes slightly curved, or reniform, smooth, thin-walled, guttulate, hyaline, aseptate, 3–5 × 1–2 µm, aggregated in cream masses (Fig. 2).

Cultural characteristics: Colonies on PDA, 84–85 mm diam after 7 d, margin regular, aerial mycelia felted, white to grey. Colonies on OA, 62–67 mm diam after 7 d, margin regular, aerial mycelia felted, grey to leaden-black. Colonies on MEA, 48–50 mm diam after 7 d, margin regular, aerial mycelia felted, grey to leaden-black. NaOH (1mol/L) spot test negative on OA (Fig. 2).

Distribution: Kunming county, Yunnan Province, China.

Habitat: Living in leaf spot of *Ageratina adenophora*.

Holotype: CGMCC 3.20981, isolated from leaf spots of *Ageratina adenophora* (Spreng.) R.M. King & H. Rob., 22August 2017, H.B. Zhang, preserved by lyophilization (a metabolically inactive state) in the State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan. GenBank: ITS ON124745, LSU ON124752, *rpb2* ON156008 and *tub2* ON113860.

Note Phylogenetically, *M. ageratinae* forms a clade separated from all species previously described. Morphologically, *M. ageratinae* also can be distinguished from them in having smaller and thinner conidia and 0 septum, larger conidiogenous cells.

Discussion

The ITS, LSU, *rpb2* and *tub2* locus in phylogenetic analyses of phoma-like taxa has revealed a largely improved phylogeny of Didymellaceae in several previous studies, and the recent segregation of this particular genus (*Phoma*) has resulted in a large number of

new genera. Those genera were mainly established based on the *rpb2* marker. In this study, we obtained a series of isolates, which are grouped into a novel genus *Mesophoma* based on the phylogenetic analysis of concatenated sequences at 4 markers (Valenzuela-Lopez et al. 2018; Jayasiri et al. 2019)

The novel genus *Mesophoma* phylogenetically formed a distinct clade, which separates from all genera previously described in the Didymellaceae (Fig. 1). Although these two novel species of the genus, *M. speciosa* and *M. ageratinae* are close to these genera, such as *Allophoma*, *Heterophoma, Boeremia and Stagonosporopsis*, they distinguished from them by morphology. Conidia of *M. speciosa* and *M. ageratinae* (3–6 × 1–2 µm, 3–5 × 1–2 µm) are much smaller and thinner than *Stagonosporopsis* and *Boeremia* (3.5–10 × 1.5–3.5 µm, 2.5–12 × 2–4 µm) (Fig. 2–3)(Aveskamp et al. 2010). Conidiogenous cells of *M. speciosa* and *M. ageratinae* (5–7 × 8–10 µm, 5–7 × 6–7 µm) are much larger than *Allophoma* (3–4.5 × 3.5–4.5 µm) (Fig. 2–3)(Chen et al. 2015). Conidia of Heterphoma are 0–1(–2) septate while that of *M. ageratinae* and *M. ageratinae* almost all 0 septate(Fig. 2–3)(Chen et al. 2015).

Phylogenetic analysis showed that *M. ageratinae* and *M. ageratinae* formed two distinct clades (Fig. 1). Pycnidia of *M. ageratinae* are larger than that of *M. ageratinae*, with average size of 127 × 121µm vs 115 × 116 µm (Fig. 2–3). Conidia of *M. ageratinae* are thinner than *M. ageratinae*, with average size of 4 × 2 µm vs 4 × 1 µm (Fig. 2–3). Therefore, we identified them as two novel species belonging to novel genus *Mesophoma*.

These two species including seven strains were found in the investigation of fungi from the healthy, diseased and rotten leaves and roots of the invasive plant *Ag. adenophora*. In our recently investigation of fungi associated with *Ag. adenophora*, we also found that these fungi belonging to Didymellaceae family may be co-spread with the host through seeds (and thus can be vertically transmitted) (Fang et al. 2021), because *Ag. adenophora* disperses minute asexual seeds primarily by wind and water. In addition, we isolated two strains of *Epicoccum* from this family, A68 and K16; disease experiment verified that these two strains were pathogenic to not only *Ag. adenophora* but also most to Cucurbita plants including *Cucumis melo* and *Cucurbita moschata*. Nonetheless, two strains (Y122, S188?) from the novel genus *Mesophoma* were only pathogenic to *Ag. adenophora*, not to Cucurbita plants, as well as most tested native plant species. These data suggested that these newly reported strains might be host-specific pathogens of *Ag. Adenophora*, and thus could be candidates for biocontrol of *Ag. adenophora*.

Declarations

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Contributions

Z.H.B. and Y.Z.F. designed the research and project outline. Y.A.L., C.L., Z.Z.Y. performed isolation and phylogenetic analysis. C. Lu. and L.J.P performed the disease experiment. Y.A.L. performed morphological analyses of the strain and prepared the figures and tables. Y.A.L. collaborated with C.L. for supervision of the study and preparation of the manuscript. All authors read and approved the final manuscript

Conflict of interest

The authors declare that there are no conflicts of interest.

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Tables

Table 1 Isolates used in this study and their GenBank accession numbers. New taxa are indicated in bold

Species	Strain number	Host, substrate	Country	GenBank accession numbers			
				LSU	ITS	*rpb2*	*tub2*
Mesophoma speciosa	**CGMCC 3.20982**	***Ageratina adenophora*, diseased leaf**	**China**	**ON124751**	**ON124744**	**ON156007**	**ON113859**
Mesophoma speciosa	**S188**	***Ageratina adenophora*, root**	**China**	**ON124755**	**ON124748**	**ON156011**	**ON113863**
Mesophoma speciosa	**G503**	***Ageratina adenophora*, diseased leaf**	**China**	**ON124754**	**ON124747**	**ON156010**	**ON113862**
Mesophoma speciosa	**Y188**	***Ageratina adenophora*,dead leaf**	**China**	**ON124757**	**ON124750**	**ON156013**	**ON113865**
Mesophoma ageratinae	**CGMCC 3.20981**	***Ageratina adenophora*, diseased leaf**	**China**	**ON124752**	**ON124745**	**ON156008**	**ON113860**
Mesophoma ageratinae	**G215**	***Ageratina adenophora*, diseased leaf**	**China**	**ON124753**	**ON124746**	**ON156009**	**ON113861**
Mesophoma ageratinae	**Y122**	***Ageratina adenophora*, healthy leaf**	**China**	**ON124756**	**ON124749**	**ON156012**	**ON113864**
Allophoma piperis	CBS 268.93	*Peperomia pereskiifolia*	Netherlands	GU238129	GU237816	KT389554	GU237644
Allophoma nicaraguensis	CBS 506.91	*Coffea arabica*	Nicaragua	GU238058	GU237876	KT389551	GU237596
Allophoma oligotrophica	CGMCC 3.18114	Air	China	KY742194	KY742040	KY742128	KY742282
Heterophoma nobilis	CBS 507.91	*Dictamnus albus*	Netherlands	GU238065	GU237877	KT389638	GU237603
Heterophoma verbasci-densiflori	CBS 127.93	*Verbascum densiflorum*	Netherlands	GU238120	GU237774	—	GU237639
Heterophoma sylvatica	CBS 874.97	*Melampyrum pratense*	Netherlands	GU238148	GU237907	—	GU237662
Boeremia diversispora	CBS 101194	*Phaseolus vulgaris*	Netherlands	GU237929	GU237716	KT389564	GU237491
Boeremia hedericola	CBS 367.91	*Hedera helix*	Netherlands	GU237949	GU237842	KT389579	GU237511
Boeremia foveata	CBS 109176	*Solanum tuberosum*	Bulgaria	GU237946	GU237742	KT389578	GU237508
Stagonosporopsis actaeae	CBS 106.96	*Actaea spicata*	Netherlands	GU238166	GU237734	KT389672	GU237671
Stagonosporopsis crystalliniformis	CBS 713.85	*Lycopersicon esculentum*	Colombia	GU238178	GU237903	KT389675	GU237683
Stagonosporopsis ajacis	CBS 177.93	*Delphinium* sp.	Kenya	GU238168	GU237791	KT389673	GU237673
Epicoccum huancayense	CBS 105.80	*Solanum* sp.	Peru	GU238084	GU237732	KT389630	GU237615

Table 1. (Continued).

Species	Strain number	Host, substrate	Country	GenBank accession numbers			
				LSU	ITS	*rpb2*	*tub2*
Epicoccum plurivorum	CBS 558.81	*Setaria* sp.	New Zealand	GU238132	GU237888	KT389634	GU237647
Epicoccum poae	CGMCC 3.18363	*Poa annua*	USA	KY742267	KY742113	KY742182	KY742355
Didymella acetosellae	CBS 179.97	*Rumex hydrolapathum*	Netherlands	GU238034	GU237793	KP330415	GU237575
Didymella suiyangensis	CGMCC 3.18352	Air	China	KY742243	KY742089	KY742168	KY742330
Didymella heteroderae	CBS 109.92	*Undefined food material*	Netherlands	GU238002	FJ426983	KT389601	FJ427098
Macroventuria anomochaeta	CBS 502.72	*Medicago sativa*	South Africa	GU237985	GU237873	—	GU237545
Macroventuria anomochaeta	CBS 525.71	*Decayed canvas*	South Africa	GU237984	GU237881	GU456346	GU237544
Macroventuria wentii	CBS 526.71	*Plant litter*	USA	GU237986	GU237884	KT389642	GU237546
Paraboeremia camellae	CGMCC 3.18106	*Camellia* sp.	China	KX829042	KX829034	KX829050	KX829058
Paraboeremia litseae	CGMCC 3.18109	*Litsea* sp.	China	KX829037	KX829029	KX829045	KX829053
Paraboeremia oligotrophica	CGMCC 3.18111	*Carbonatite*	China	KX829039	KX829031	KX829047	KX829055
Nothophoma anigozanthi	CBS 381.91	*Anigozanthus maugleisii*	Netherlands	GU238039	GU237852	KT389655	GU237580
Nothophoma quercina	CBS 633.92	*Quercus* sp.	Ukraine	EU754127	GU237900	KT389657	GU237609
Nothophoma arachidis-hypogaeae	CBS 125.93	*Arachis hypogaea*	India	GU238043	GU237771	KT389656	GU237583
Ascochyta boeremae	CBS 373.84	*Pisum sativum*	Australia	KT389698	KT389481	KT389560	KT389775
Ascochyta herbicola	CBS 629.97	Water	USA	GU238083	GU237898	KP330421	GU237614
Ascochyta medicaginicola var. *macrospora*	BRIP 45051	*Medicago sativa*	Australia	KY742198	KY742044	KY742132	KY742286
Briansuttonomyces eucalypti	CBS 114879	*Eucalyptus* sp.	South Africa	KU728519	KU728479	—	KU728595
Briansuttonomyces eucalypti	CBS 114887	*Eucalyptus* sp.	South Africa	KU728520	KU728480	—	KU728596

Table 1. (Continued).

Species	Strain number	Host, substrate	Country	GenBank accession numbers			
				LSU	ITS	*rpb2*	*tub2*
Pseudoascochyta novae-zelandiea	CBS 141689	*Cordyline australis*	New Zealand	LT592893	LT592892	LT592895	LT592894
Pseudoascochyta pratensis	CBS 141688	Soil	Spain	LT223131	LT223130	LT223133	LT223132
Leptosphaerulina americana	CBS 213.55	*Trifolium pratense*	USA	GU237981	GU237799	KT389641	GU237539
Leptosphaerulina arachidicola	CBS 275.59	*Arachis hypogaea*	Taiwan, China	GU237983	GU237820	—	GU237543
Leptosphaerulina australis	CBS 317.83	*Eugenia aromatica*	Indonesia	EU754166	GU237829	GU371790	GU237540
Phoma herbarum	CBS 127589	*Polytrichum juniperinum*	USA	KT389757	KT389539	KT389664	KT389838
Phoma herbarum	CBS 274.37	*Picea excelsa*	UK	KT389754	KT389537	KT389662	KT389835
Phoma herbarum	CBS 134.96	*Delphinium* sp.	Netherlands	KT389753	KT389535	KT389661	KT389834
Phomatodes aubrietiae	CBS 383.67	*Aubrietia hybrida* cv. *Superbissima*	Netherlands	GU238044	GU237854	—	GU237584
Phomatodes nebulosa	CBS 100191	*Thlaspi arvense*	Poland	KP330446	KP330434	KT389666	KP330390
Phomatodes nebulosa	CBS 117.93	*Mercurialis perennis*	Netherlands	GU238114	GU237757	KP330425	GU237633
Neomicrosphaeropsis italica	MFLUCC 15-0484	*Tamarix* sp.	Italy	KU729853	KU900319	KU695539	KX453298
Neomicrosphaeropsis rossica	MFLUCC 14-0586	*Tamarix ramosissima*	Russia	KU729855	KU752192	—	—
Neomicrosphaeropsis novorossica	MFLUCC 14-0578	*Tamarix ramosissima*	Russia	KX198710	KX198709	—	—
Calophoma glaucii	CBS 112.96	*Dicentra* sp.	Netherlands	GU238077	GU237750	—	GU237610
Calophoma clematidina	CBS 102.66	*Clematis* sp.	UK	FJ515630	FJ426988	KT389587	FJ427099
Calophoma rosae	CGMCC 3.18347	*Rosa* sp.	China	KY742203	KY742049	KY742135	KY742291
Neodidymelliopsis polemonii	CBS 109181	*Polemonium caeruleum*	Netherlands	GU238133	GU237746	KP330427	GU237648
Neodidymelliopsis cannabis	CBS 121.75	*Urtica dioica*	Netherlands	GU237972	GU237761	—	GU237535

Table 1. (Continued).

Species	Strain number	Host, substrate	Country	GenBank accession numbers			
				LSU	ITS	*rpb2*	*tub2*
Neodidymelliopsis achlydis	CBS 256.77	*Achlys triphylla*	Canada	KT389749	KT389531	—	KT389829
Xenodidymella applanata	CBS 115577	*Rubus idaeus*	Sweden	KT389762	KT389546	KT389688	KT389850
Xenodidymella catariae	CBS 102635	*Nepeta cataria*	Netherlands	GU237962	GU237727	KP330404	GU237524
Xenodidymella humicola	CBS 220.85	*Franseria* sp.	USA	GU238086	GU237800	KP330422	GU237617
Neoascochyta europaea	CBS 819.84	*Hordeum vulgare*	Germany	KT389728	KT389510	KT389645	KT389808
Neoascochyta graminicola	CBS 102789	*Lolium perenne*	New Zealand	KT389736	KT389518	KT389649	KT389816
Neoascochyta Soli	CGMCC 3.18365	Soil	China	KY742275	KY742121	—	KY742363
Ectophoma multirostrata	CBS 110.79	Cucumis sativus	Netherlands	GU238110	FJ427030	LT623264	FJ427140
Ectophoma multirostrata	CBS 274.60	Soil	Maharashtra	GU238111	FJ427031	LT623265	FJ427141
Cumuliphoma indica	CBS 654.77	Unknown	India	GU238122	FJ427043	LT623261	FJ427153
Cumuliphoma indica	CBS 991.95	Soil	Papua New Guinea	GU238121	FJ427044	LT623262	FJ427154
Juxtiphoma eupyrena	CBS527.66	Wheat field soil	Germany	GU238073	FJ427000	LT623269	FJ427111
Juxtiphoma eupyrena	CBS 374.91	Solanum tuberosum	The Netherlands	GU238072	FJ426999	LT623268	FJ427110
Remotididymella bauhiniae	MFLU 18–2118	*Bauhinia*	Thaland	MK347954	MK347737	MK434914	MK412884
Remotididymella destructiva	CBS 133.93	*Solanum lycopersicon*	Guadeloupe	GU238064	GU237779	LT623257	GU237602
Vacuiphoma bulgarica	CBS 357.84	Trachystemon orientale	Bulgaria	GU238050	GU237837	LT623256	GU237589
Vacuiphoma oculihominis	FMR 13801	Human superficial	USA	LN907451	LT592954	LT593093	LT593023
Similiphoma crystallifera	CBS 193.82	Chamaespartium sagittale	Austria	GU238060	GU237797	LT623267	GU237598

Table 1. (Continued).

Species	Strain number	Host, substrate	Country	GenBank accession numbers			
				LSU	ITS	*rpb2*	*tub2*
Vandijckomycella joseae	CBS 144948	Garden soil	The Netherlands	MN823440	MN823589	MN824614	MN824763
Vandijckomycella joseae	CBS 143011	Garden soil	The Netherlands	MN823441	MN823590	MN824615	MN824764
Vandijckomycella snoekiae	CBS 144954	Garden soil	The Netherlands	MN823442	MN823591	MN824616	MN824765
Longididymella clematidis	CBS 123705	*Clematis ligusticifolia*	USA	FJ515634	FJ515593	MT018076	FJ515611
Longididymella vitalbae	CBS 123707	*Clematis vitalba*	Switzerland	MH874853	MH863321	MT018075	FJ515613
Sclerotiophoma versabilis	CBS 124689	Human toenail	Denmark	MN943723	MN973517	MT018123	MT005617
Sclerotiophoma versabilis	CBS 876.97	*Silene* sp.	The Netherlands	GU238152	GU237909	MT018124	GU237664
Paramicrosphaeropsis ellipsoidea	CBS 197.97	*Quercus ilex*	Spain	MN943780	MN973574	MT018224	MT005680
Paramicrosphaeropsis ellipsoidea	CBS 194.97	*Quercus ilex*	Spain	MN943781	MN973575	MT018225	MT005681
Ectodidymella nigrificans	CBS 100190	*Brassica napus*	Germany	GU237967	GU237708	MT018078	GU237530
Ectodidymella nigrificans	PD 84/512	Crucifer	The Netherlands	GU237966	GU237919	MT018077	GU237529
Pseudopeyronellaea eucalypti	CBS 142522	*Eucalyptus pellita*	Malaysia	KY979810	KY979755	KY979848	KY979921
Pseudopeyronellaea eucalypti	CPC 27682	*Eucalyptus pellita*	Malaysia	KY979811	KY979756	KY979849	KY979922
Dimorphoma saxea	CBS 298.89	Limestone	Germany	MH873865	MH862175	MT018299	GU237654
Dimorphoma saxea	CBS 419.92	Corroded mediterranean marble	Germany	MH874030	MH862364	KP330429	MT005727
Macroascochyta grandis	CBS 100409	*Tradescantia* sp.	New Zealand	GU238057	GU237712	MT018063	GU237593
Anthodidymella ranunculacearum	MFLUCC 17–2184	*Clematis vitalba*	Italy	MT214550	MT310597	MT394681	—
Anthodidymella ranunculacearum	MFLUCC 17–2209	*Clematis vitalba*	Italy	MT214551	MT310598	—	—

Table 1. (Continued).

Species	Strain number	Host, substrate	Country	GenBank accession numbers			
				LSU	ITS	*rpb2*	*tub2*
Neoscirrhia osmundae	CPC 38085	*Sasa veitchii*	Netherlands	MW883822	MW883430	MW890066	MW890135
Nothomicrosphaeropsis welwitschiae	CPC 38879	*Welwitschia mirabilis*	Namibia	MW883826	MW883434	MW890067	MW890138
Leptosphaeria doliolum	CBS 505.75	*Urtica dioica*	Netherlands	GQ387576	JF740205	KT389640	JF740144

CBS: Westerdijk Fungal Biodiversity Institute (formerly CBSKNAW), Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection, Beijing, China; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; FMR, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain;LC: Cai's personal collection deposited in laboratory, housed at CAS, China; YMF: Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China; CPC: Culture collection of Pedro Crous housed at the CBS.

Figures

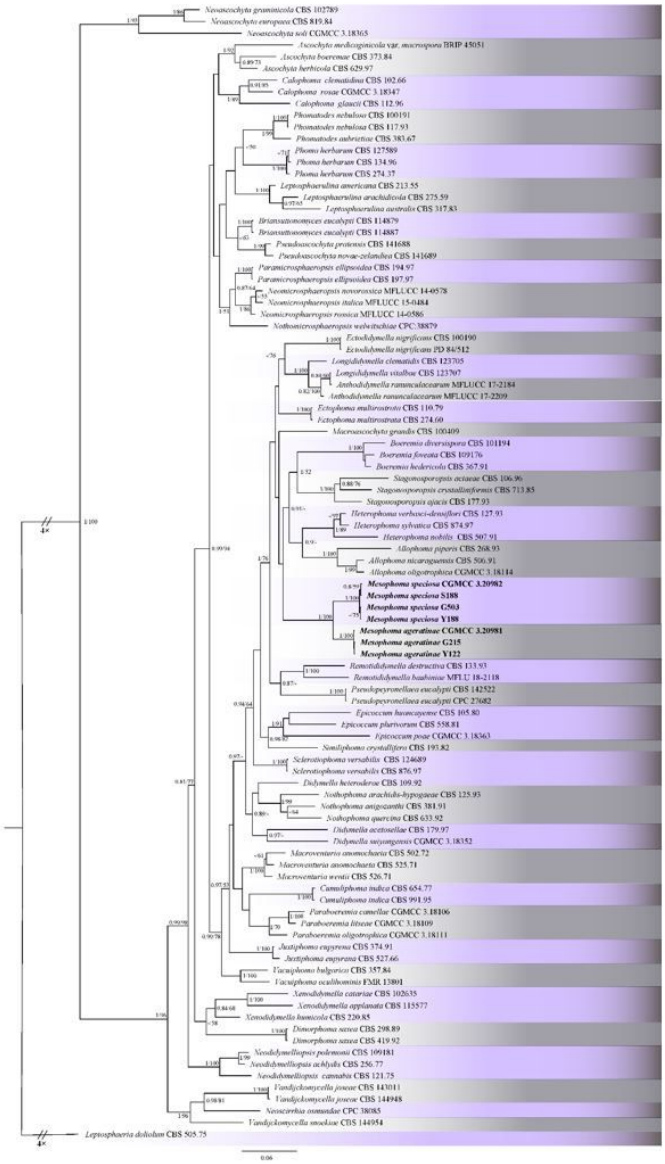

Figure 1

Phylogenetic tree derived from Bayesian analysis based on combined LSU, ITS, *rpb2* and *tub2* sequences of 89 strains representing species in Didymellaceae. The numbers above branches represent Bayesian posterior probabilities (left) and maximum-likelihood bootstrap percentages (right). Bootstrap percentages over 50% and significant Bayesian posterior probability (0.8) are shown on the respective branches. Some branches were shortened to fit them to the page – these are indicated by two diagonal lines with the number of times a branch was shortened indicated next to the lines. New taxa introduced in this study are formatted in bold. The tree was rooted to *Leptosphaeria doliolum* (CBS 505.75).

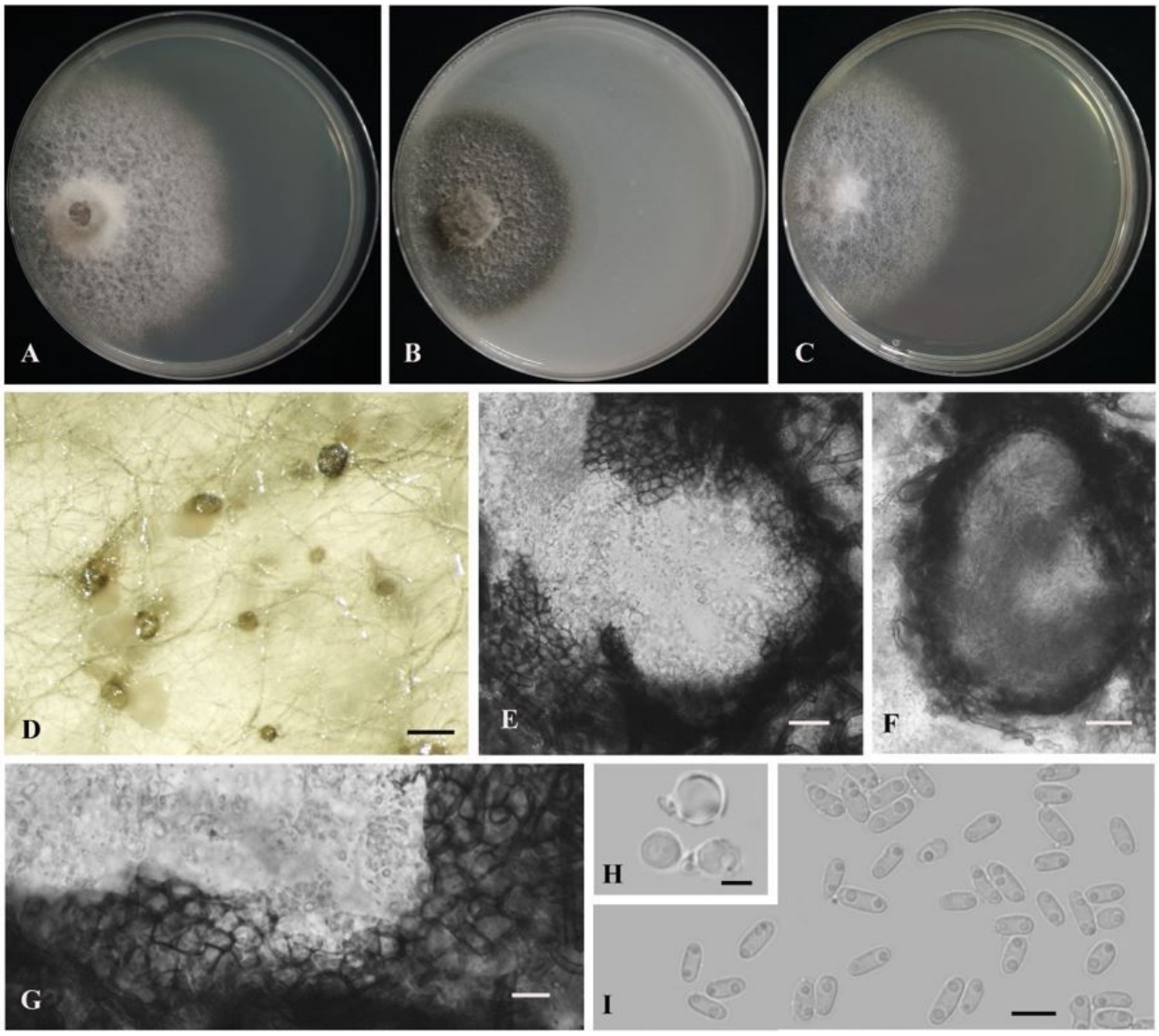

Figure 2

M. speciosa (CGMCC 3.20982, holotype). A. Colony on PDA (front). B. Colony on OA (front). C. Colony on MEA (front). D. Pycnidia forming on OA. E-F. Pycnidium. G. Section of pycnidial wall. H. Conidiogenous cells. I. Conidia. Scale bars: D = 200 μm; E-F = 20 μm; G-I = 5 μm.

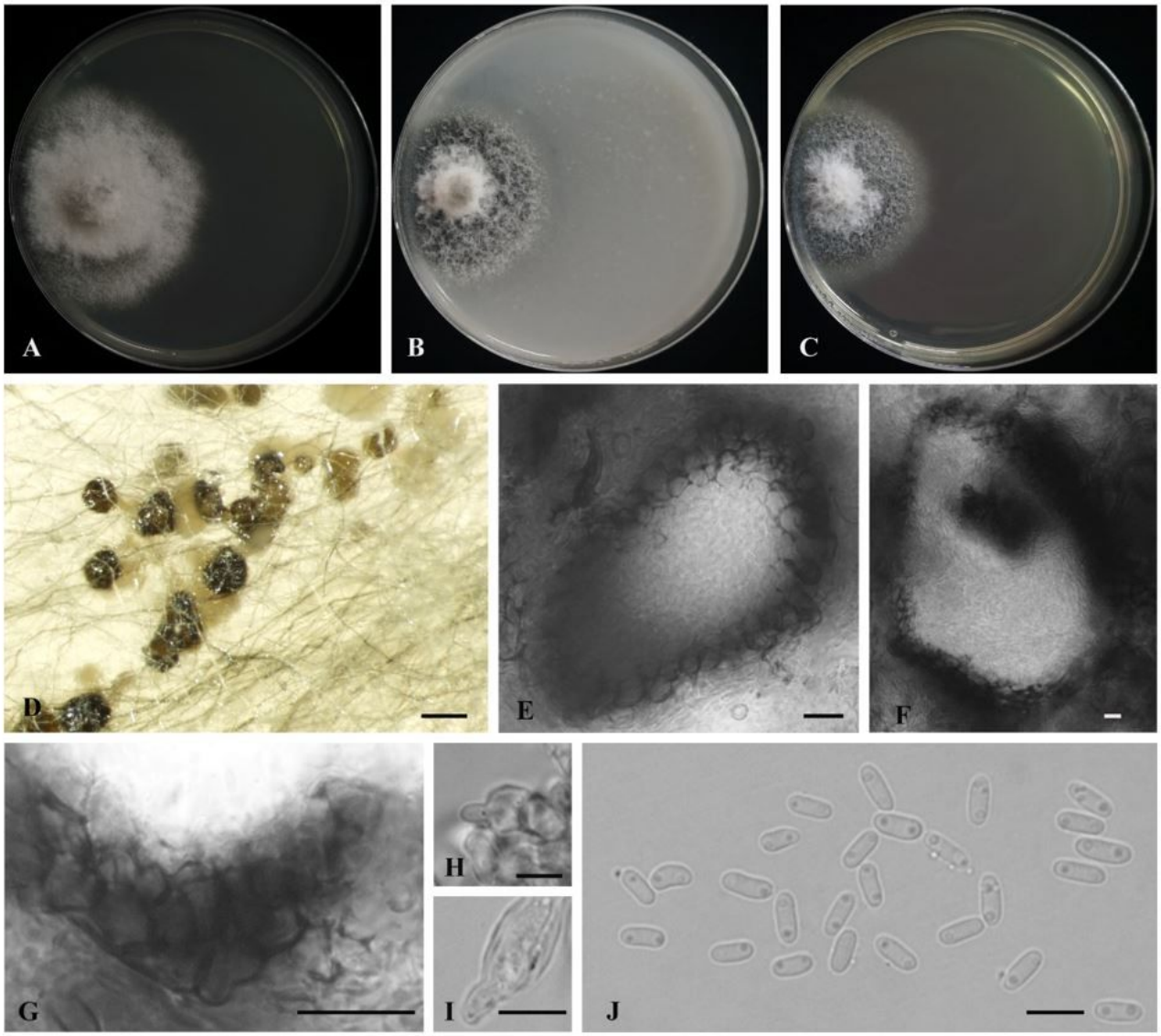

Figure 3

M. ageratinae (CGMCC 3.20981, holotype). A. Colony on PDA (front). B. Colony on OA (front). C. Colony on MEA (front). D. Pycnidia forming on OA. E-F. Pycnidium. G. Section of pycnidial wall. H-I. Conidiogenous cells. J. Conidia. Scale bars: D = 200 μm; E-G = 10 μm; H-J = 5 μm.