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The phoma-like dilemma

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Abstract: Species of *Didymellaceae* have a cosmopolitan distribution and are geographically widespread, occurring in diverse ecosystems. The family includes several important plant pathogenic fungi associated with fruit, leaf, stem and root diseases on a wide variety of hosts, as well as endophytic, saprobic and clinically relevant species. The *Didymellaceae* was recently revised based on morphological and phylogenetic analyses of ex-type strains subjected to DNA sequencing of partial gene data of the LSU, ITS, *rpb2* and *tub2* loci. Several poly- and paraphyletic genera, including *Ascochyta*, *Didymella* and *Phoma* were redefined, along with the introduction of new genera. In the present study, a global collection of 1 124 *Didymellaceae* strains from 92 countries, 121 plant families and 55 other substrates, including air, coral, human tissues, house dust, fungi, insects, soil, and water were examined via multi-locus phylogenetic analyses and detailed morphological comparisons, representing the broadest sampling of *Didymellaceae* to date. Among these, 97 isolates representing seven new genera, 40 new species and 21 new combinations were newly introduced in *Didymellaceae*. In addition, six epitypes and six neotypes were designated to stabilise the taxonomy and use of older names. A robust, multi-locus reference phylogenetic tree of *Didymellaceae* was generated. In addition, *rpb2* was revealed as the most effective locus for the identification of *Didymellaceae* at species level, and is proposed as a secondary DNA marker for the family.

Key words: Multi-locus phylogeny, New taxa, *Phoma*, *rpb2*, Taxonomy.

Taxonomic novelties: New genera: ***Dimorphoma*** L.W. Hou, L. Cai & Crous, ***Ectodidymella*** L.W. Hou, L. Cai & Crous, ***Longididymella*** L.W. Hou, L. Cai & Crous, ***Macroascochyta*** L.W. Hou, L. Cai & Crous, ***Paramicrosphaeropsis*** L.W. Hou, L. Cai & Crous, ***Pseudopeyronellaea*** L.W. Hou, L. Cai & Crous, ***Sclerotiophoma*** L.W. Hou, L. Cai & Crous. **New species:** ***Allophoma*** *alba* L.W. Hou, Pfenning, L. Cai & Crous, *Al. anatii* L.W. Hou & O. Yarden, ***Ascochyta*** *pilosella* L.W. Hou, L. Cai & Crous, ***Calophoma*** *parvula* L.W. Hou, L. Cai & Crous, ***Chaetasbolisia*** *argentina* L.W. Hou, L. Cai & Crous, ***Didymella*** *aloeicola* L.W. Hou, L. Cai & Crous, *Did. guttulata* L.W. Hou, L. Cai & Crous, *Did. indica* L.W. Hou, L. Cai & Crous, *Did. mitis* L.W. Hou, L. Cai & Crous, *Did. prolaticolla* L.W. Hou, L. Cai & Crous, *Did. subglobispora* L.W. Hou, L. Cai & Crous, *Did. subrosea* L.W. Hou, L. Cai & Crous, *Did. variabilis* L.W. Hou, L. Cai & Crous, ***Epicoccum*** *brahmansense* L.W. Hou, L. Cai & Crous, *Ep. dickmanii* L.W. Hou & O. Yarden, *Ep. longiostiolatum* L.W. Hou, L. Cai & Crous, *Ep. multiceps* L.W. Hou, L. Cai & Crous, *Ep. polychromum* L.W. Hou, L. Cai & Crous, *Ep. variabile* L.W. Hou, L. Cai & Crous, ***Leptosphaerulina*** *obtusispora* L.W. Hou, L. Cai & Crous, *Le. sisyrinchiicola* L.W. Hou, L. Cai & Crous, ***Macroascochyta*** *grandis* L.W. Hou, L. Cai & Crous, ***Macroventuria*** *angustispora* L.W. Hou, L. Cai & Crous, *Ma. terrestris* L.W. Hou, L. Cai & Crous, ***Microsphaeropsis*** *fusca* L.W. Hou, L. Cai & Crous, *Mi. taxicola* L.W. Hou, L. Cai & Crous, *Mi. viridis* L.W. Hou, L. Cai & Crous, ***Neoascochyta*** *fusiformis* L.W. Hou, L. Cai & Crous, *Neoa. humicola* L.W. Hou, L. Cai & Crous, *Neoa. longispora* L.W. Hou, L. Cai & Crous, *Neoa. mortariensis* L.W. Hou, L. Cai & Crous, ***Neodidymelliopsis*** *tiliae* L.W. Hou, L. Cai & Crous, ***Nothophoma*** *infuscata* L.W. Hou, L. Cai & Crous, ***Paramicrosphaeropsis*** *ellipsoidea* L.W. Hou, L. Cai & Crous, ***Phomatodes*** *pilosa* L.W. Hou, L. Cai & Crous, ***Remotididymella*** *brunnea* L.W. Hou, L. Cai & Crous, *R. humicola* L.W. Hou, L. Cai & Crous, ***Xenodidymella*** *glycyrrhizicola* L.W. Hou, L. Cai & Crous, ***Stagonosporopsis*** *cucumeris* L.W. Hou, L. Cai & Crous, *St. sambucella* L.W. Hou, L. Cai & Crous. **New combinations:** ***Ascochyta*** *astragalina* (Rehm ex Sacc.) L.W. Hou, L. Cai & Crous, *As. koolunga* (J.A. Davidson *et al.*) L.W. Hou, L. Cai & Crous, ***Calophoma*** *vincetoxici* (De Not.) L.W. Hou, L. Cai & Crous, ***Chaetasbolisia*** *eupatorii* (Died.) L.W. Hou, L. Cai & Crous, ***Didymella*** *combreti* (Crous) L.W. Hou, L. Cai & Crous, *Did. prosopidis* (Crous & A.R. Wood) L.W. Hou, L. Cai & Crous, ***Dimorphoma*** *saxea* (Aveskamp *et al.*) L.W. Hou, L. Cai & Crous, ***Ectodidymella*** *nigrificans* (P. Karst.) L.W. Hou, L. Cai & Crous, ***Ectophoma*** *insulana* (Mont.) L.W. Hou, L. Cai & Crous, ***Epicoccum*** *tobaicum* (Szilv.) L.W. Hou, L. Cai & Crous, ***Longididymella*** *clematidis* (Woudenb. *et al.*) L.W. Hou, L. Cai & Crous, *Lo. vitalbae* (Briard & Har.) L.W. Hou, L. Cai & Crous, ***Nothophoma*** *acaciae* (Crous) L.W. Hou, L. Cai & Crous, *No. eucalyptigena* (Crous) L.W. Hou, L. Cai & Crous, *No. prosopidis* (Crous & A.R. Wood) L.W. Hou, L. Cai & Crous, ***Pseudopeyronellaea*** *eucalypti* (Crous & M.J. Wingf.) L.W. Hou, L. Cai & Crous, ***Remotididymella*** *capsici* (Bond.-Mont.) L.W. Hou, L. Cai & Crous, ***Sclerotiophoma*** *versabilis* (Boerema *et al.*) L.W. Hou, L. Cai & Crous, ***Stagonosporopsis*** *nemophilae* (Neerg). L.W. Hou, L. Cai & Crous, ***Vacuiphoma*** *ferulae* (Pat.) L.W. Hou, L. Cai & Crous, *Va. laurina* (Thüm.) L.W. Hou, L. Cai & Crous. **New name:** *Heterophoma verbasci-densiflori* L.W. Hou, L. Cai & Crous, *Nothophoma nullicana* L.W. Hou, L. Cai & Crous. **Epitypes (basionyms):** *Amphisphaeria vincetoxici* De Not., *Phoma eupatorii* Died., *Phyllosticta acetosellae* A.L. Sm. & Ramsb., *Phyllosticta verbascicola* Ellis & Kellerm., *Phyllosticta arachidis-hypogaeae* V.G. Rao, *Pleosphaerulina briosiana* Pollacci. **Neotypes (basionyms):** *Ascochyta ferulae* Pat., *Ascochyta nobilis* Kabát & Bubák, *Epicoccum oryzae* S. Ito & Iwadare, *Phoma laurina* Thüm., *Phoma nemophilae* Neerg., *Phyllosticta insulana* Mont. **Lectotypes (basionyms):** *Amphisphaeria vincetoxici* De Not., *Phoma eupatorii* Died. **Resurrected names:** *Epicoccum mezzettii* Goid., *Ep. oryzae* Ito & Iwadare, *Ep. purpurascens* Ehrenb., *Toruloidea tobaica* Szilv.

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INTRODUCTION

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 (Boerema *et al.* 2004, De Gruyter *et al.* 2009, Aveskamp *et al.* 2010, Chen *et al.* 2015). Species lacking good characteristics to be identified to genus level were often collectively deposited in collections as "phoma-like", making this genus a "repository" to which more than 3 000 species epithets are associated in the MycoBank database (Crous *et al.* 2004). After more than 50 years of studying this group of fungi, Boerema and co-workers proposed that *Phoma* should be divided into nine sections (Boerema *et al.* 2004), although later studies again showed these sections to be poly- and paraphyletic (Aveskamp *et al.* 2008, 2010). Recent progress in molecular phylogenetic analyses has greatly contributed to a substantial revision of phoma-like genera. This group of fungi has been elevated to family level and is now recognised as *Didymellaceae* based on the type genus *Didymella* (De Gruyter *et al.* 2009), which was also neotypified by de Gruyter in the same study.

Currently, *Didymellaceae* 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. To date, more than 5 400 species belonging to at least 31 genera have been recorded, including recently established genera such as *Neoascochyta* and *Paraboeremia* and historical genera such as *Ascochyta*, *Didymella* and *Phoma* that remain highly problematic for the identification of new isolates (De Gruyter *et al.* 2009, Chen *et al.* 2015, 2017). Molecular characteristics have become increasingly essential in the identification of species in *Didymellaceae*. Based on sequence data obtained from 324 strains, Aveskamp *et al.* (2010) divided the family into 18 distinct clusters, and demonstrated that some important genera, such as *Ascochyta*, *Didymella* and *Phoma*, were highly polyphyletic. At the same time, phoma-like species that are located outside of this family were revealed to have affiliations to more than 25 families after phylogenetic analyses (De Gruyter *et al.* 2009, 2010, 2013). In a further study, Chen *et al.* (2015) provided an updated understanding of the taxonomy and evolution of *Didymellaceae* by including the *rpb2* locus in a four-locus phylogenetic analysis. Several additional new genera, namely, *Briansuttonomyces*, *Cumuliphoma*, *Ectophoma*, *Juxtiphoma*, *Neomicrosphaeropsis*, *Pseudoascochyta*, *Remotididymella*, *Similiphoma*, and *Vacuiphoma* and a large number of new species have recently been introduced and accepted in the family (Ariyawansa *et al.* 2015a, Crous & Groenewald 2016, Thambugala *et al.* 2016, Wijayawardene *et al.* 2016, Valenzuela-Lopez *et al.* 2018), revealing the great diversity and wide distribution of *Didymellaceae*.

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 (Dorenbosch 1970, Zucconi *et al.* 1996, Clipson *et al.* 2001, Yarden 2014, Zhang *et al.* 2014, 2016, Chen *et al.* 2017, Hou *et al.* 2020). As pathogens, species in *Didymellaceae* infect plants and also parasitise fungi, lichens, insects and vertebrates (Perrotta & Graniti 1988, Costa *et al.* 1993, Hutchison *et al.* 1994, Sullivan & White 2000, Hawksworth & Cole 2004, Aveskamp *et al.* 2008).

Some species of *Didymellaceae* are recognised as important quarantine species, posing a threat to a diverse range of crops; for example, *Phoma foveata* was considered to pose a high risk to the potato industry and was subsequently added to the quarantine organism list in Europe in 1975 (De Gruyter 2012). Although it was eventually removed from the European quarantine list in 1999, *Phoma foveata* is still a quarantine organism in many countries outside Europe, *e.g.* in South America (Mendes *et al.* 2007, EPPO Global Database 2019). In addition, six *Phoma*-related species are listed as quarantine species in China (Announcement No. 862 of the Ministry of Agriculture of the People's Republic of China 2007), as well as *Phoma andigena* (as *Stagonosporopsis andigena*) and *Phoma tracheiphila* (as *Plenodomus tracheiphilus*) in the European and Mediterranean regions (Smith *et al.* 1992, EPPO Global Database 2019) and *Phoma macdonaldii* in Australia (Miric *et al.* 1999). As time for examination of potentially infected plants/animals is always limited, accurate and fast identification of quarantine organisms is of extreme importance. Detection and identification based on morphological characteristics are time-consuming and require a high degree of taxonomic experience, reducing the chance to successfully identify phoma-like species to species level in the laboratory (Aveskamp *et al.* 2008). Considering the importance of correct identification of *Didymellaceae* pathogens from intercepted material and the limitation of traditional morphological methods, authentic reference sequences with high discriminatory power are urgently needed.

As the most widely sequenced marker for fungi, the ITS locus has been proposed as a universal DNA barcode for fungi (Schoch *et al.* 2012). In *Didymellaceae*, however, the nuclear ribosomal RNA gene sequences (ITS, LSU and SSU) are insufficient to distinguish closely related species (Aveskamp *et al.* 2008, Chen *et al.* 2015). Therefore, identifying a different DNA locus (or secondary DNA marker) (Hebert *et al.* 2002, Summerbell *et al.* 2005, Stielow *et al.* 2015) has been a promising initiative for the rapid detection of potentially serious plant pathogens (Armstrong & Ball 2005).

The objectives of the present study were: 1) to elucidate the taxonomy of a global collection of 1 124 phoma-like strains of *Didymellaceae*, including all isolates used in the monograph of Boerema *et al.* (2004) deposited in the CBS culture collection of the Westerdijk Institute; 2) to revise the taxonomy of previously introduced genera, as well as designate epitypes/neotypes to fix the application of older names where possible; and 3) to evaluate *rpb2* as potential secondary species-level DNA barcode across all genera included in *Didymellaceae*.

MATERIALS AND METHODS

Isolates

All isolates in this study were obtained from the CBS culture collection of the Westerdijk Fungal Biodiversity Institute (WI; Utrecht, the Netherlands, formerly CBS-KNAW). A total of 1 124 isolates identified as *Didymellaceae* based on morphological characters or preliminary DNA sequence data were included in this study, including 209 ex-type strains (Table S1 and Table S2). Freeze-dried strains from the CBS culture collection were revived in 2 mL of malt/peptone (50 %/50 %) liquid medium and subsequently transferred to oatmeal agar (OA) (Crous *et al.* 2019b). Strains stored in liquid nitrogen were transferred to OA directly from straws (Stalpers *et al.* 1987). Some of the cultures were incubated under near-ultraviolet (UV) light (12 h

light, 12 h dark) on pine needle agar (PNA) (Smith *et al.* 1996, Su *et al.* 2012) to promote sporulation if necessary.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh mycelia harvested from cultures grown on OA for 7–14 d at room temperature using the Wizard® Genomic DNA purification kit (Promega, Madison, USA) following the manufacturers' protocols. Four loci were amplified, including the internal transcribed spacer regions 1 and 2 and 5.8S nuclear ribosomal RNA gene (ITS), partial beta-tubulin gene (*tub2*), partial large subunit nrRNA gene (28S nrDNA; LSU) and partial DNA-directed RNA polymerase II second largest subunit (*rpb2*) gene using the primer pairs ITS5/ITS4 (White *et al.* 1990, de Hoog & Gerrits van den Ende 1998), Btub2Fd/Btub4Rd (Woudenberg *et al.* 2009), LR0R/LR5 or LR0R/LR7 (Vilgalys & Hester 1990, Rehner & Samuels 1994), and RPB2-5F2/RPB2-7cR (Liu *et al.* 1999, Sung *et al.* 2007), respectively. The PCR amplifications, except for that of *rpb2*, were performed following the method of Chen *et al.* (2015). The amplification for *rpb2* was performed according to an improved protocol with bovine serum albumin (BSA): a total volume of 12.5 µL containing 1.25 µL of EasyTaq Buffer (Bioline, Luckenwalde, Germany), 0.5 µL of dNTPs (40 µM), 0.5 µL of $MgCl_2$ (2 mM), 0.5 µL of BSA (1 µg/µL), 0.25 µL of each primer (0.2 µM), 0.1 µL of *Taq* DNA polymerase (Bioline) and 1 µL of genomic DNA was used. The PCR conditions for *rpb2* were: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 56 °C for 80 s and extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. The PCR products for four loci were purified and sequenced in both directions as explained in Crous *et al.* (2013a). The consensus sequences were assembled from forward and reverse sequences using Seqman Pro v. 10.0.1 (DNASTAR, Madison, USA). Novel sequences generated in this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov, Supplementary Table S1).

Morphology

Morphological studies of living cultures were conducted following the methods described by Chen *et al.* (2015, 2017). Isolates were grown on fresh oatmeal agar (OA), malt extract agar (MEA) and potato dextrose agar (PDA) (Crous *et al.* 2019b) and incubated at 25 °C, under near-ultraviolet (UV) light (12 h light/12 h dark) to induce sporulation. Colony diameter was measured and the NaOH spot test was performed by addition of a drop of 1N NaOH to determine the secretion of metabolite E on OA cultures after 7 d (Boerema *et al.* 2004). Colony characters (upper surface and reverse) and colours were rated following the colour charts of Rayner (1970) after 14 d of incubation. Micromorphological observation was carried out from cultures grown on OA after 7 d of incubation, and included the characteristics of conidiomata/ascomata, the presence of mycelium/setae outside of the conidiomata/ascomata, the number of ostioles, the thickness of the pycnidial/pseudothecial wall, the shape of cells of the pycnidial/ pseudothecial wall, and the length and width of conidiogenous cells/asci and conidia/ascospores. Slide preparations were mounted in distilled water to study the micromorphological structures of mature ascomata/conidiomata, ascospores/conidia and asci/conidiogenous cells (Aveskamp *et al.* 2010, Chen *et al.* 2015, 2017). At least 30 measurements were made for all morphologically informative features. Micromorphological observations were processed with a Nikon Eclipse 80i compound microscope with differential interference contrast (DIC) optics and a Nikon AZ100 dissecting microscope, both equipped with a Nikon DS-Ri2 high-definition colour digital camera. The structure of the mature pseudothecial/pycnidial wall was studied using microtome sections of 8–10 µm thickness, prepared with a Leica CM 1100 freezing cryostat microtome and mounted in lactic acid (Aveskamp *et al.* 2010, Chen *et al.* 2015). Descriptions of novelties and taxonomic recombinations were deposited in MycoBank, and specimens were deposited in the CBS fungarium.

Sequence alignment and molecular phylogenetic analysis

Among all the isolates in this study, partial genes of 431 isolates that were sequenced in previous studies and deposited in GenBank (Yarden *et al.* 2007, Aveskamp *et al.* 2009a, 2010, De Gruyter *et al.* 2010, 2013, Chen *et al.* 2015, 2017) were treated as reference sequences. Together with the sequences of related species described in recent studies (Thambugala *et al.* 2016, Wijayawardene *et al.* 2016) and outgroup species (*Coniothyrium palmarum* CBS 400.71, *Neocucurbitaria aquatica* CBS 297.74 and *Pleiochaeta setosa* CBS 496.63, CBS 118.25), all reference sequences were downloaded from GenBank and Q-Bank (Bonants *et al.* 2013), and listed in Table S1 and Table S2. Subsequent alignments for four individual loci (ITS, LSU, *rpb2* and *tub2*) were generated with MAFFT v. 7 using the default settings on the web server of the European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/Tools/msa/mafft/) (Katoh & Standley 2013, Li *et al.* 2015), and were manually edited in MEGA v. 6.0 when necessary (Tamura *et al.* 2013). A multi-locus gene dataset was generated using SequenceMatrix v. 1.8 (Vaidya *et al.* 2011).

To investigate the phylogenetic relationships between different isolates and to establish the identity of the isolates at species level, both Bayesian inference (BI) and Maximum Likelihood (ML) methods were used for phylogenetic analyses of individual sequence alignments, followed by the concatenated alignments. The best substitution model of evolution for each of the four data partitions were estimated using jModeltest v. 2.1.4 (Darriba *et al.* 2012) according to the Akaike information criterion before the Bayesian analysis. Bayesian analyses were performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012) as described by Chen *et al.* (2015). Markov Chain Monte Carlo sampling (MCMC) analyses of four chains were started in parallel from a random tree topology. Four simultaneous Markov chains were run for 10 M generations with a sampling frequency set to the 1 000th generation (resulting in 10 000 total trees per parallel run) or until the run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. The first 25 % of the trees were discarded as the burn-in phase of each analysis, and the remaining trees were used to calculate posterior probabilities (Chen *et al.* 2015). Maximum Likelihood analyses including 1 000 bootstrap replicates, which were conducted using RAxML v. 7.2.6 (Stamatakis & Alachiotis 2010). A general time reversible (GTR) model was applied with a gamma-distributed rate variation. The resulting trees were viewed using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). Alignments and the phylogenetic trees derived from this study were uploaded to TreeBASE (accession number: S25826; www.treebase.org). Three trees were generated: the phylogenetic

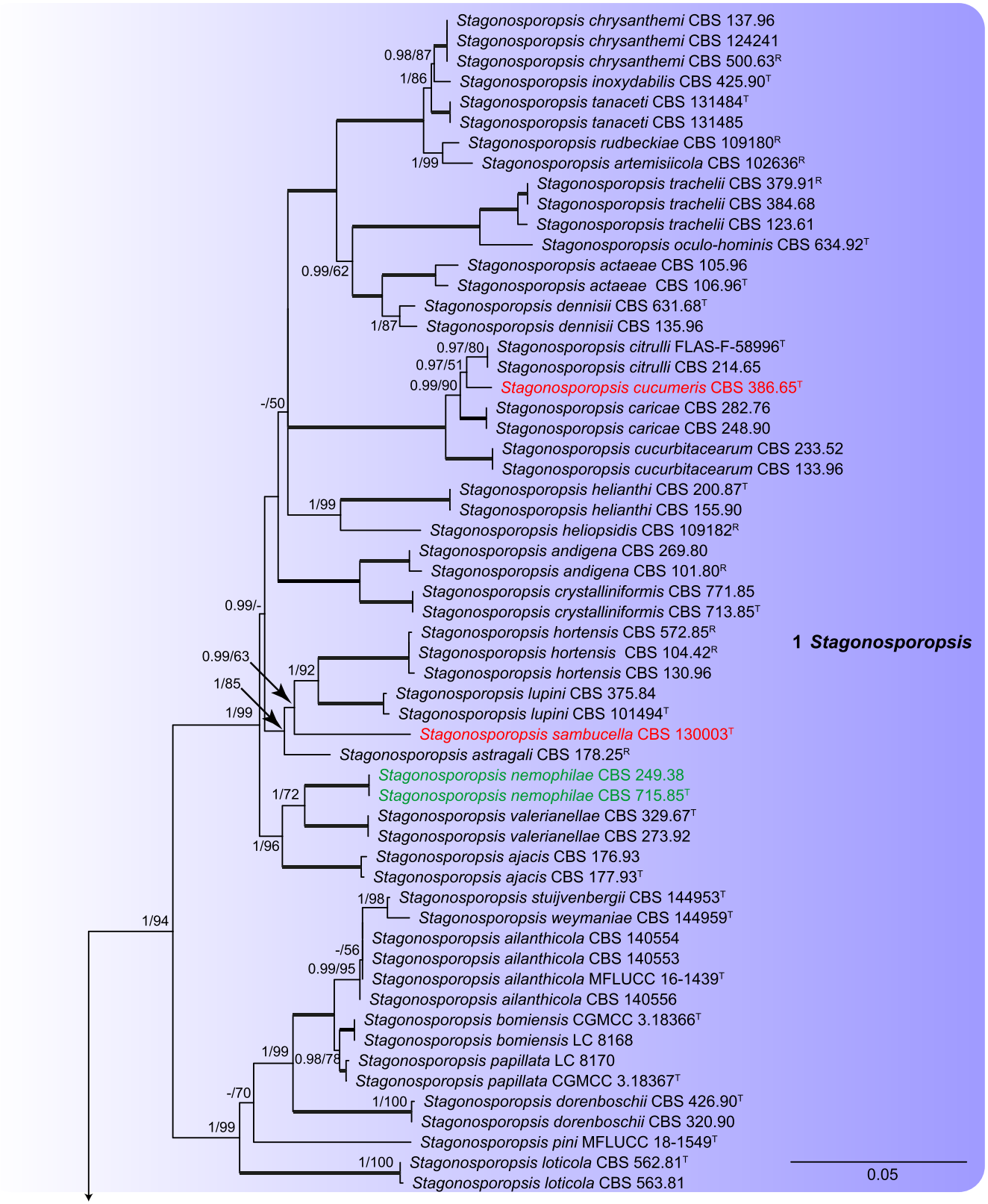


Fig. 1. Phylogenetic tree inferred from a Maximum Likelihood analysis based on a concatenated alignment of LSU, ITS, *rpb2* and *tub2* sequences of 597 strains representing *Didymellaceae* and outgroup sequences. The RAxML bootstrap support values (MLBS) above 50 % and Bayesian posterior probabilities (BPP) above 0.80 are given at the nodes (BPP/MLBS). Some of the basal branches were shortened to facilitate layout (the fraction in round parentheses refers to the presented length compared to the actual length of the branch). The scale bar represents the expected number of changes per site. Genera are delimited in coloured boxes, with the genus name indicated to the right. Strains with special status are indicated with a superscript letter after the accession number (R: representative; T: ex-type). The new species are printed in red font and new combinations in green font. The tree is rooted to *Coniothyrium palmarum* culture CBS 400.71, *Neocucurbitaria aquatica* culture CBS 297.74 and *Pleiochaeta setosa* cultures CBS 496.63 and CBS 118.25.

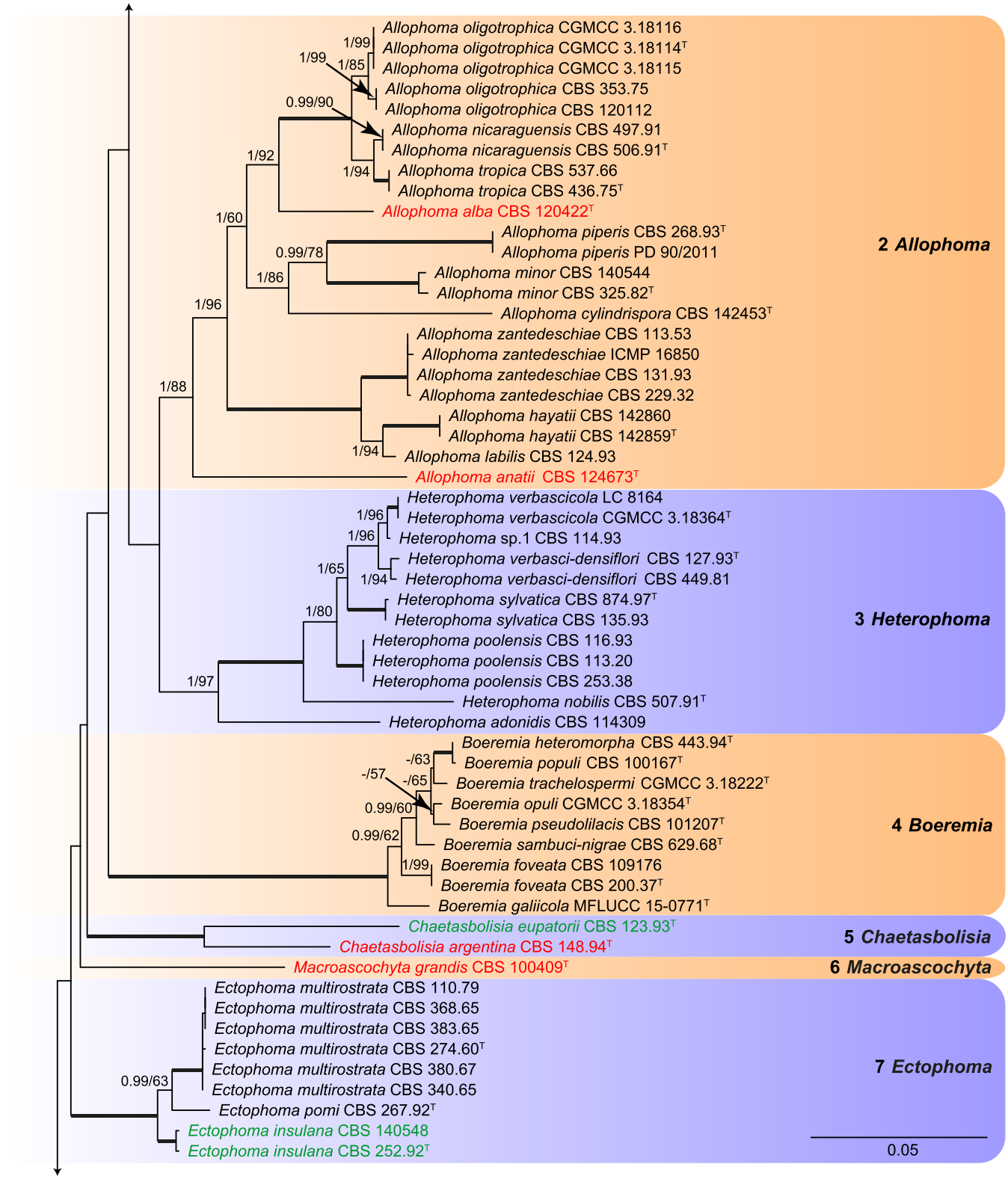
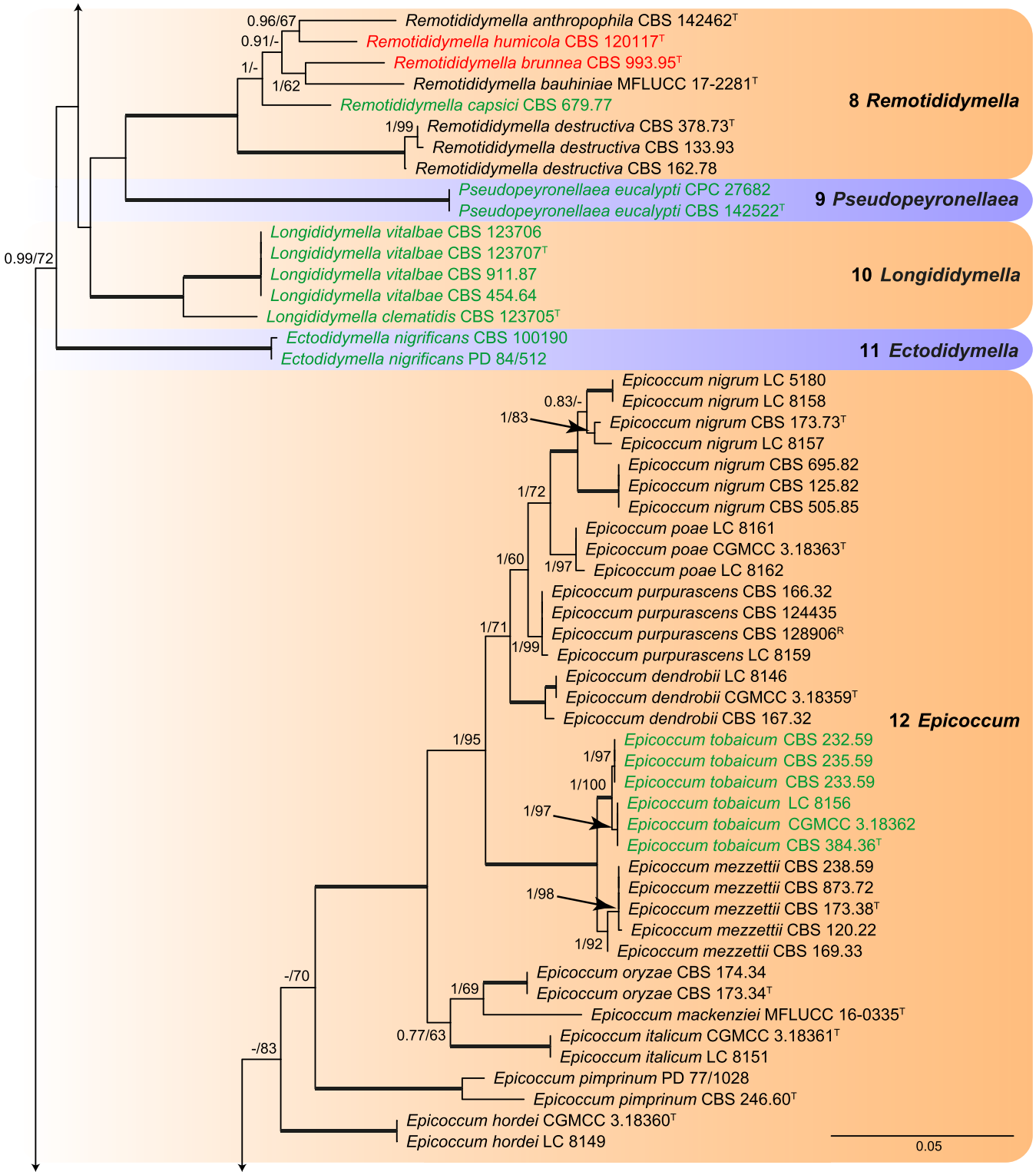


Fig. 1. (Continued).



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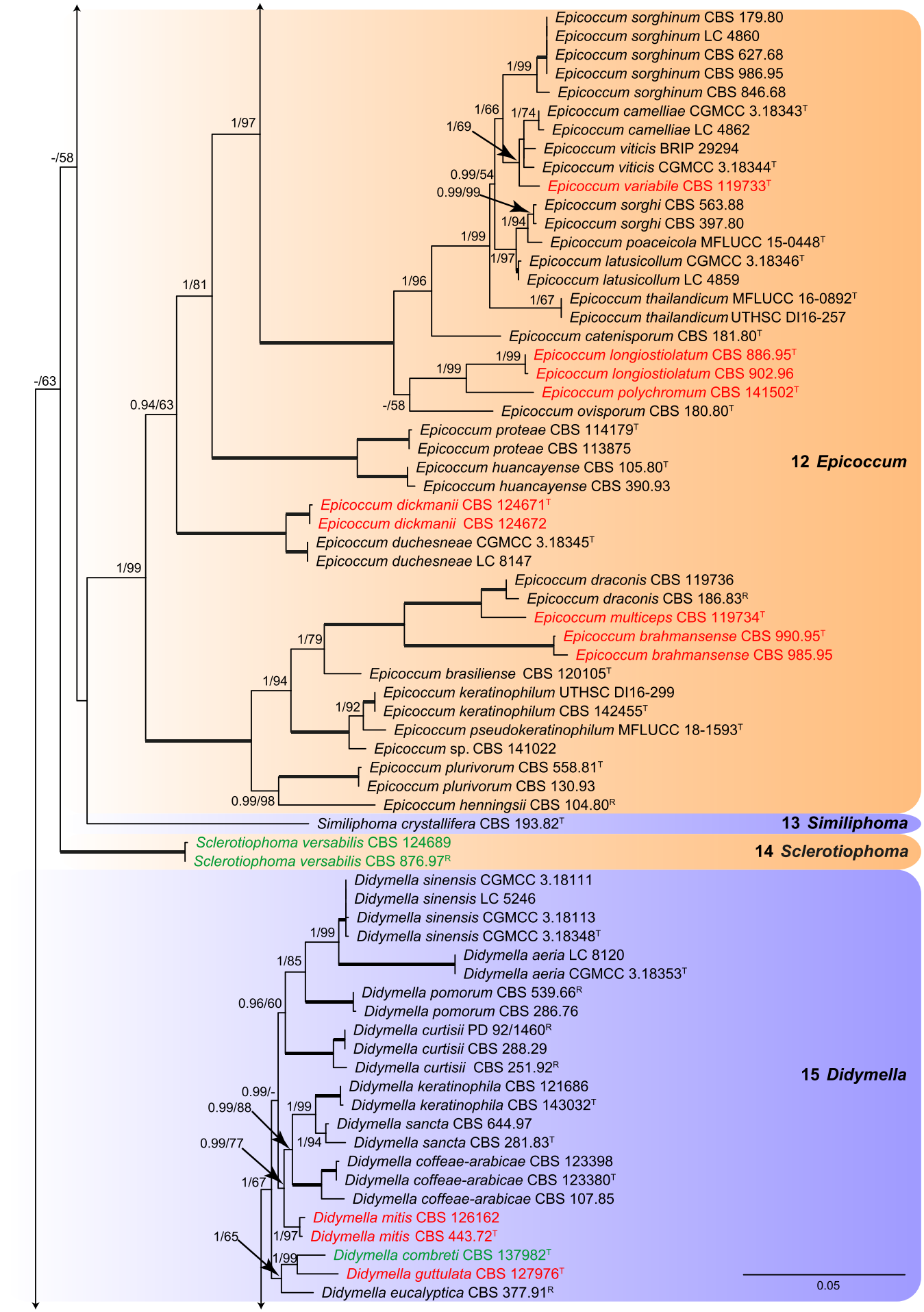

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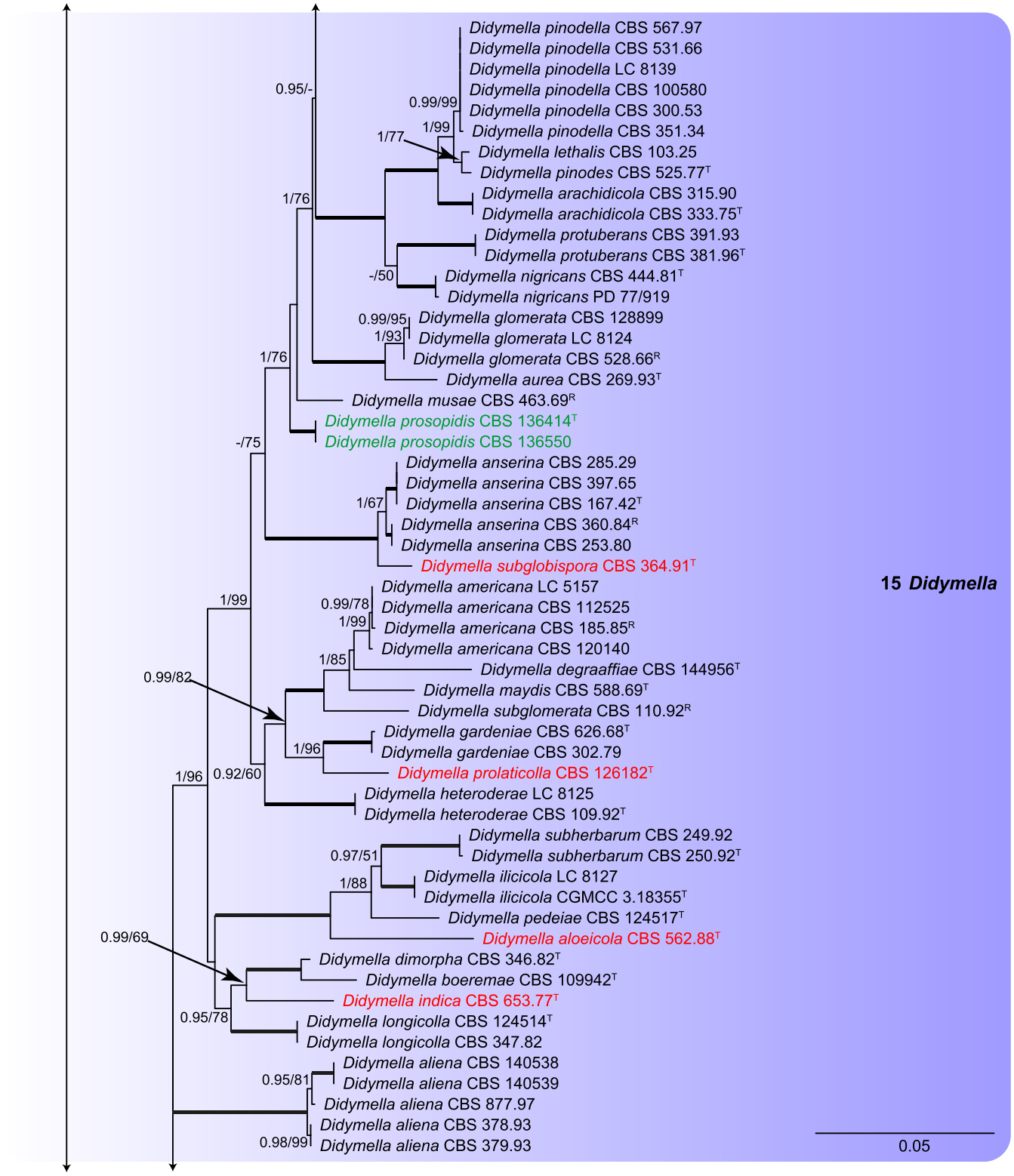

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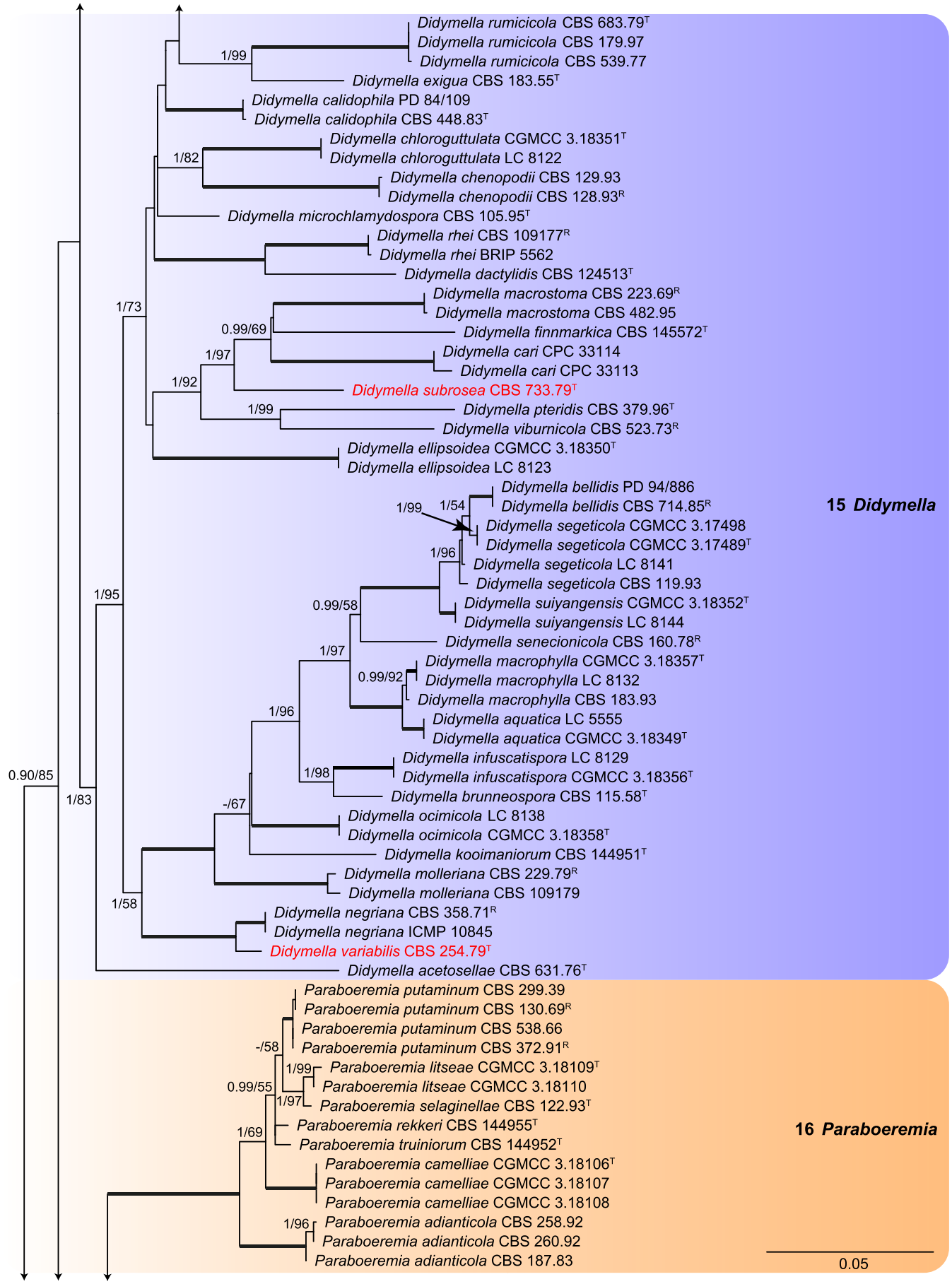

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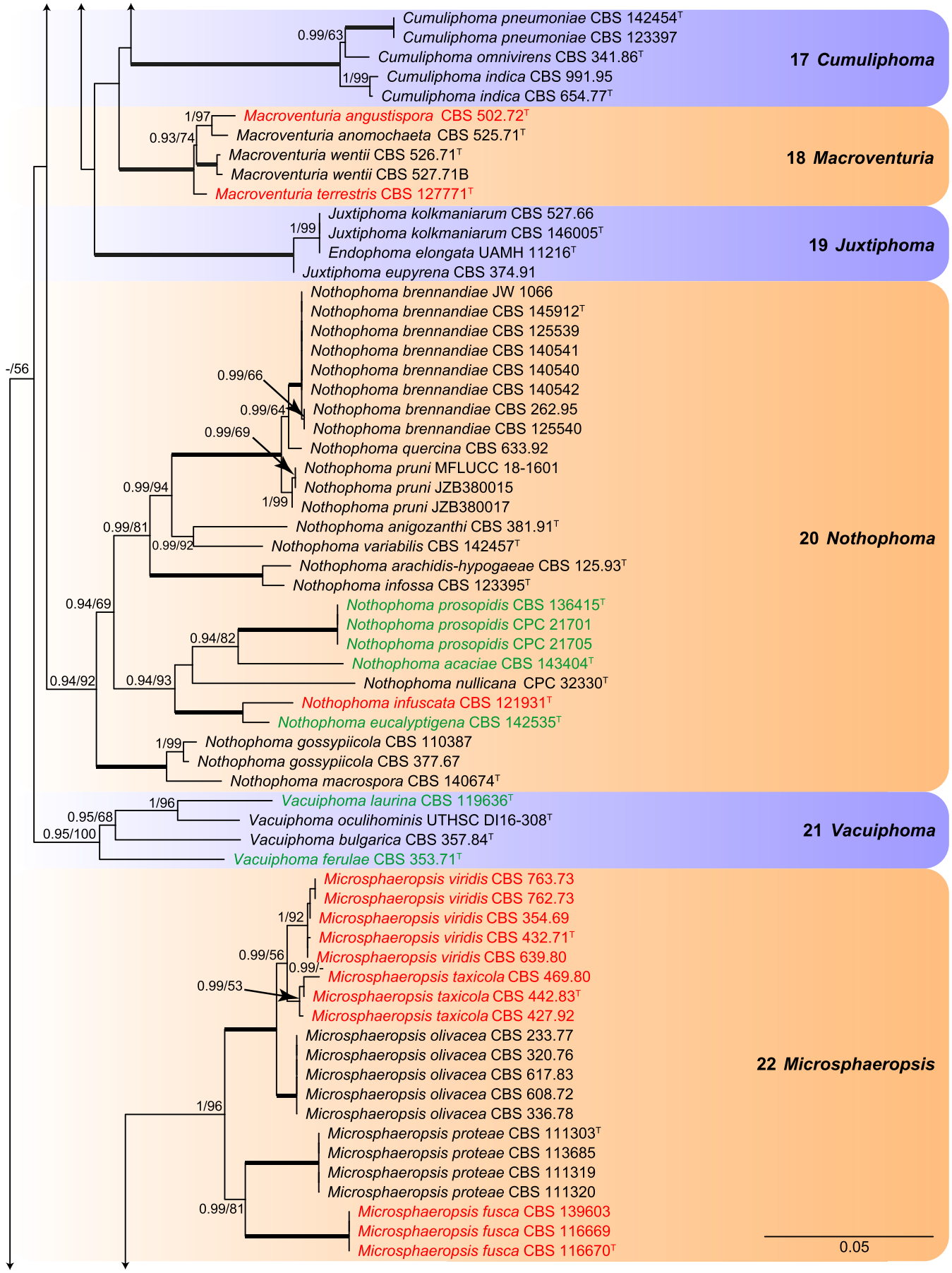

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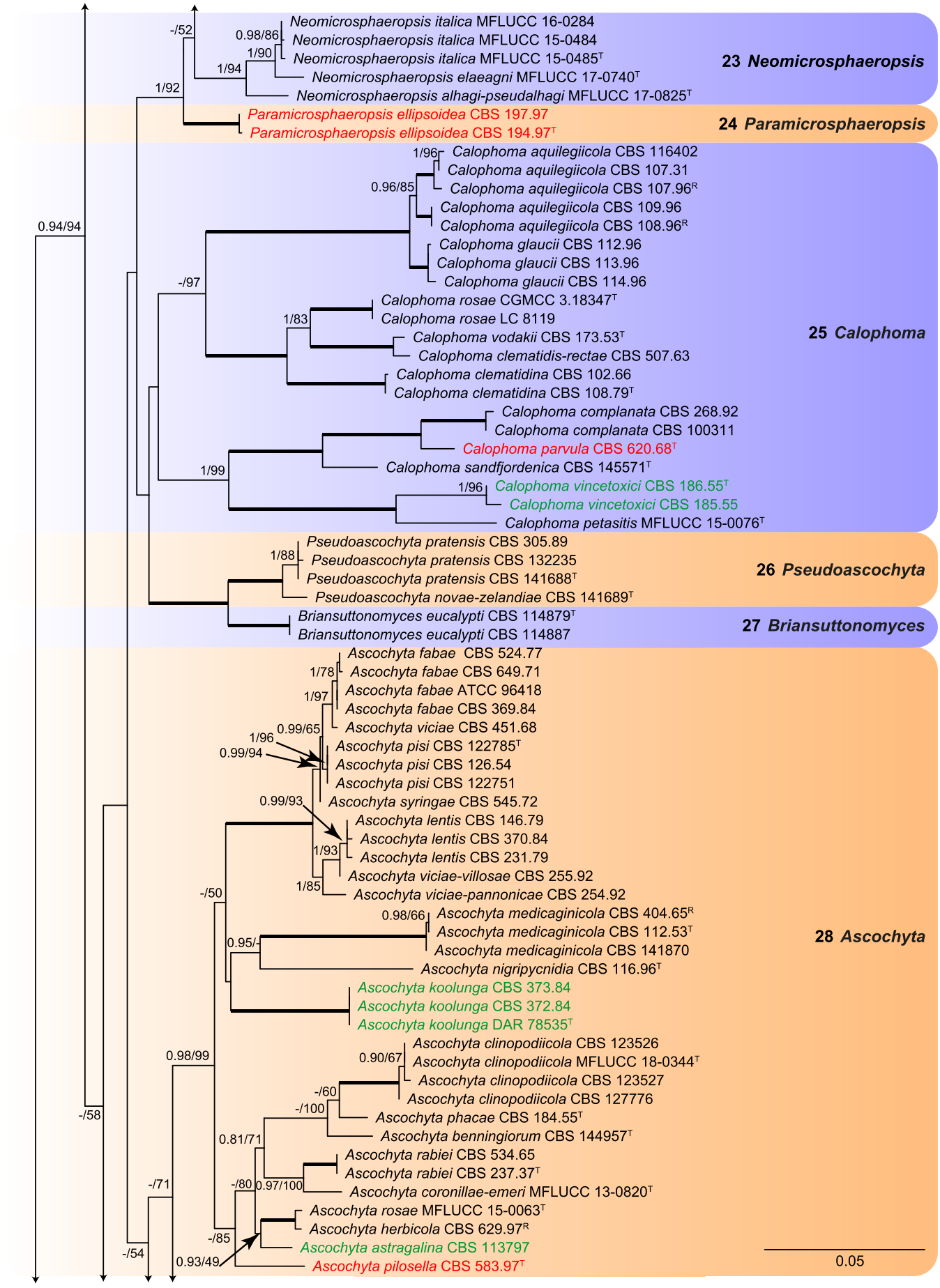

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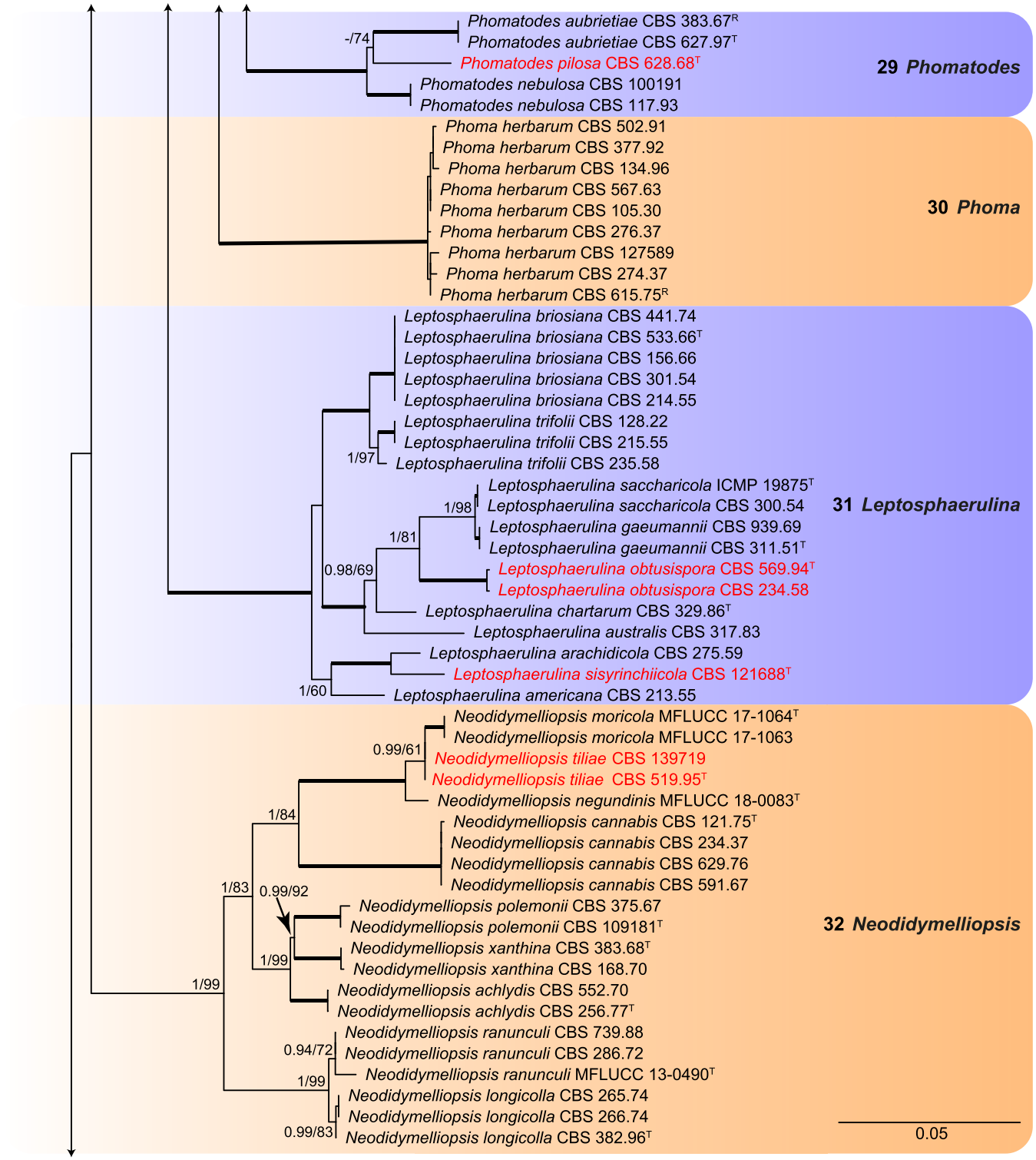

Fig. 1. (Continued).

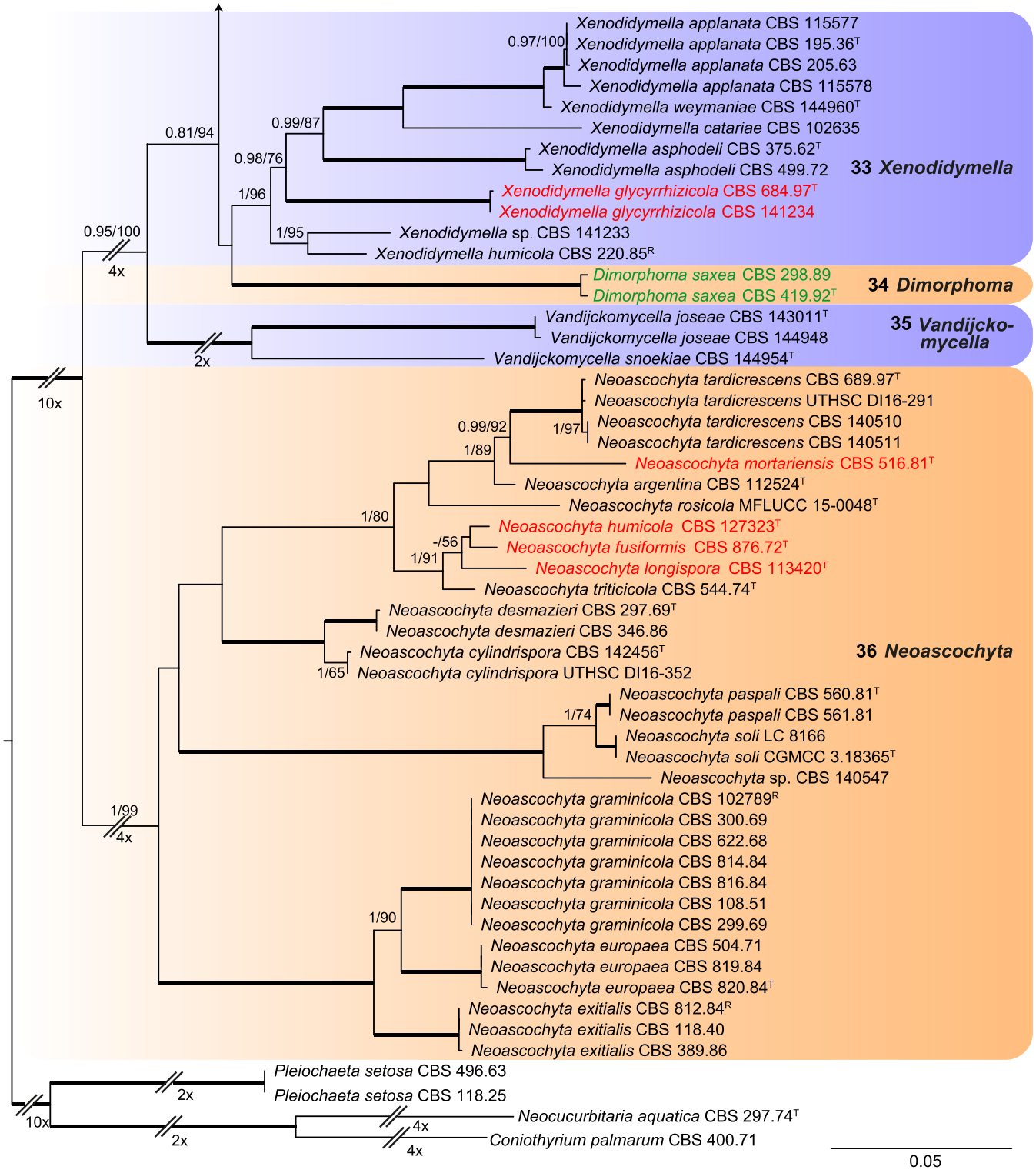

Fig. 1. (Continued).

tree with combined loci with a reduced set of isolates representing the different species (Fig. 1), the phylogenetic tree with combined loci with all isolates belonging to *Didymellaceae* included in this study (Supplementary Fig. S1), and a phylogenetic tree based only on the *rpb2* locus with a reduced set of isolates representing the different species (Supplementary Fig. S2).

Evaluation of loci for species resolution

Species names were applied to the trees based on the phylogenetic analyses performed in the present study but were also based on prior studies where, in some cases, more loci were employed for species recognition than the number of loci used here. Using loci to distinguish species was evaluated as

described by Gomes *et al.* (2013) and Alvarez *et al.* (2016). RAxML trees with the GTR model were generated for each locus of the corresponding alignments. The trees were then manually evaluated as means to resolve species from one another. A species was counted only if it was distinct from its closest relatives and if the species clade contained all strains of that species included in the analysis.

RESULTS

Phylogenetic analyses

Among the 1 124 isolates, 177 isolates were identified as not belonging to genera in *Didymellaceae*, but were distributed in 13 other families in *Pleosporales* (Table S2), which will be treated elsewhere. The phylogenetic tree based on the concatenated DNA sequence dataset (ITS, LSU, *rpb2* and *tub2*) for all 947 *Didymellaceae* isolates examined in this study is showed in Fig. S1.

Among all the *Didymellaceae* isolates, 470 isolates with the complete dataset of genes together with 127 references isolates were selected to be included in the overview phylogenetic analyses (Fig. 1;Table S1 and the strain numbers showed in bold). The final concatenated DNA sequence dataset (ITS, LSU, *rpb2* and *tub2*) used to infer delimitation at the family and genus levels comprised 593 isolates representing 301 ingroup *Didymellaceae* taxa, with *Coniothyrium palmarum* CBS 400.71, *Neocucurbitaria aquatica* CBS 297.74 and *Pleiochaeta setosa* CBS 496.63 and CBS 118.25 serving as outgroup. The concatenated aligned dataset consisted of 2 418 characters, including alignment gaps (gene boundaries ITS: 1–477, 477 bp; LSU: 483–1 444, 962 bp; *rpb2*: 1 450–2 048, 599 bp; *tub2*: 2 054–2 418, 365 bp). Among those, 1 581 characters are conserved sites and 811 are variable sites, including 95 characters that are parsimony-uninformative and 716 characters that are parsimony-informative. Based on the result of jModeltest, for BI, a TIM2ef+I+G model was selected for ITS, the GTR+I+G model for LSU and *rpb2*, and a TIM3+I+G model for *tub2*. The sequence dataset did not show conflict in the tree topologies for the 70 % reciprocal bootstrap trees, which allowed us to combine the four genes for the multi-locus analysis. The ML tree and the Bayesian consensus tree confirmed the congruent topologies. A total of 22 502 trees were generated after the BI analysis reached the stop value of 0.01, with the first 25 % trees discarded as the burn-in phase, and posterior probabilities (PP) were calculated from the remaining 16 877 trees. The topology of the BI tree was confirmed by ML analysis for the distinction of 36 well-supported monophyletic clades; therefore, only the ML tree based on the combined dataset was presented here with the bootstrap support values of the ML analysis (MLBS) and relevant Bayesian posterior probabilities (BPP) shown at the nodes (MLBS > 50 %, BPP > 0.80; Fig. 1).

A total of 593 ingroup isolates formed a fully-supported clade (BPP = 1; MLBS = 100 %) representing *Didymellaceae*, which included 36 monophyletic generic clades. Twenty-two genera were previously recognised, including *Allophoma* (Clade 2; BPP = 1; MLBS = 88 %), *Ascochyta* (Clade 28; BPP = 0.98; MLBS = 99 %), *Boeremia* (Clade 4; BPP = 1; MLBS = 100 %), *Briansuttonomyces* (Clade 27; BPP = 1; MLBS = 100 %), *Calophoma* (Clade 25; BPP = 0.97; MLBS = 68 %), *Chaetasbolisia* (Clade 5; BPP = 1; MLBS = 100 %), *Didymella* (Clade 15; BPP = 1; MLBS = 83 %), *Epicoccum* (Clade 12; BPP = 1; MLBS = 99 %), *Heterophoma* (Clade 3; BPP = 1; MLBS = 97 %), *Leptosphaerulina* (Clade 31; BPP = 1; MLBS = 100 %), *Macroventuria* (Clade 18; BPP = 1; MLBS = 100 %), *Microsphaeropsis* (Clade 22; BPP = 1; MLBS = 96 %), *Neoascochyta* (Clade 36; BPP = 1; MLBS = 99 %), *Neodidymelliopsis* (Clade 32; BPP = 1; MLBS = 99 %), *Neomicrosphaeropsis* (Clade 23; BPP = 1; MLBS = 94 %), *Nothophoma* (Clade 20; BPP = 0.94; MLBS = 92 %), *Paraboeremia* (Clade 16; BPP = 1; MLBS = 100 %), *Phoma* (Clade 30; BPP = 1; MLBS = 100 %), *Phomatodes* (Clade 29; BPP = 1; MLBS = 100 %), *Pseudoascochyta* (Clade 26; BPP = 1; MLBS = 100 %), *Stagonosporopsis* (Clade 1; BPP = 1; MLBS = 94 %), and *Xenodidymella* (Clade 33; BPP = 1; MLBS = 96 %). Several genera were recently added to this family, namely, *Cumuliphoma* (Clade 17; BPP = 1; MLBS = 100 %), *Ectophoma* (Clade 7; BPP = 1; MLBS = 100 %), *Juxtiphoma* (Clade 19; BPP = 1; MLBS = 100 %), *Remotididymella* (Clade 8; BPP = 1; MLBS = 100 %), *Similiphoma* (Clade 13; single strain lineage), *Vacuiphoma* (Clade 21; BPP = 0.95; MLBS = 100 %), and *Vandijckomycella* (Clade 35; BPP = 1; MLBS = 100 %). Seven new genera are recognised and described in the present study, namely *Dimorphoma* (Clade 34; BPP = 1; MLBS = 100 %), *Ectodidymella* (Clade 11; BPP = 1; MLBS = 100 %), *Longididymella* (Clade 10; BPP = 1; MLBS = 100 %), *Macroascochyta* (Clade 6; single strain lineage), *Paramicrosphaeropsis* (Clade 24; BPP = 1; MLBS = 100 %), *Pseudopeyronellaea* (Clade 9; BPP = 1; MLBS = 100 %) and *Sclerotiophoma* (Clade 14; BPP = 1; MLBS = 100 %), which were fully supported as independent clades.

Evaluation of loci for species resolution

Of the 947 *Didymellaceae* examined in this study, 194 isolates already had *rpb2* sequences in GenBank and were downloaded as reference sequences, 715 isolates were successfully amplified and sequenced for *rpb2*, while the remaining 38 failed; the success rate for amplification of *rpb2* is 94.95 %. Therefore, for the complete dataset in this study, 93.53 % of isolates have available *rpb2* sequences. The RAxML overview *rpb2* phylogenetic tree (Supplementary Fig. S2), including 573 selected representative ingroup isolates belonging to 286 taxa, had a topology highly similar to that of the four-gene tree (Fig. 1), and was able to discriminate 35 well-supported genera. Species of the genus *Calophoma* did not cluster in monophyletic lineages but formed two separate clades, although the discrimination for all species in this genus was successful. Among the 286 species, 279 species with *rpb2* sequences could be distinguished in the *rpb2* phylogeny, but failed for the following species: *Boeremia heteromorpha*, *Boeremia populi*, *Didymella sinensis*, *Didymella aeria*, *Neomicrosphaeropsis elaeagni*, *Neomicrosphaeropsis alhagi-pseudalhagi* and *Neomicrosphaeropsis italica*; while 15 other species represented by 24 isolates that were recognised in the four-gene tree lacked *rpb2* sequence data. For the genus *Boeremia*, *rpb2* did not satisfactorily resolve the species boundaries on its own; therefore, species recognition requires the use of additional loci, such as *tub2* and ITS.

For the *tub2* gene, a total of 366 isolates had *tub2* sequences available in GenBank. For the rest of the isolates used, 565 isolates were successfully amplified and sequenced but failed for the remaining 16 isolates; the success rate was 97.25 %. Therefore, 96 % of isolates in the dataset have *tub2* sequences.

The phylogenetic analysis of *tub2* comprised 582 selected isolates representing 291 taxa recognised in the four-gene tree. This locus could distinguish most genera except four genera: *Calophoma* separated into two clades, *Epicoccum* clustered in three different clades, *Remotididymella* clustered together with one part of *Calophoma* and *Allophoma* clustered in three lineages. Compared to the 301 species recognised in the four-gene tree, 15 isolates representing 10 species lacked *tub2* sequence data. At species level, the single *tub2* tree could recognise 285 species, but failed to distinguish six species, namely *Stagonosporopsis papillata*, *St. bomiensis*, *Neodidymelliopsis longicolla*, *Neodidymelliopsis ranunculi*, *Ascochyta lentis* and *As. viciae-villosae*.

The LSU phylogenetic tree was able to distinguish the *Boeremia*, *Dimorphoma*, *Ectophoma*, *Juxtiphoma*, *Leptosphaerulina*, *Longididymella*, *Macroventuria*, *Neoascochyta*, *Neodidymelliopsis*, *Neomicrosphaeropsis*, *Paramicrosphaeropsis*, and *Pseudopeyronellaea* clades and 32.48 % of the species.

The ITS phylogenetic tree failed to recognise 13 genera, namely, *Allophoma*, *Ascochyta*, *Chaetasbolisia*, *Didymella*, *Ectophoma*, *Epicoccum*, *Heterophoma*, *Macroventuria*, *Microsphaeropsis*, *Nothophoma*, *Stagonosporopsis*, *Vacuiphoma*, and *Xenodidymella*, while 61.92 % of the species could be recognised.

Taxonomy

The multi-locus sequence analysis of 597 strains delineated 36 clades, representing 301 taxa belonging to the *Didymellaceae*. After morphological examination, seven new genera and 40 new species were described. A further 21 new combinations, six epitypifications and six neotypifications were proposed, seven species were reduced to synonymy, and four *Epicoccum* species were resurrected. Three new species that proved to be sterile were described based on DNA sequence data, following the approach of Gomes *et al.* (2013) and Lombard *et al.* (2016). Taxa

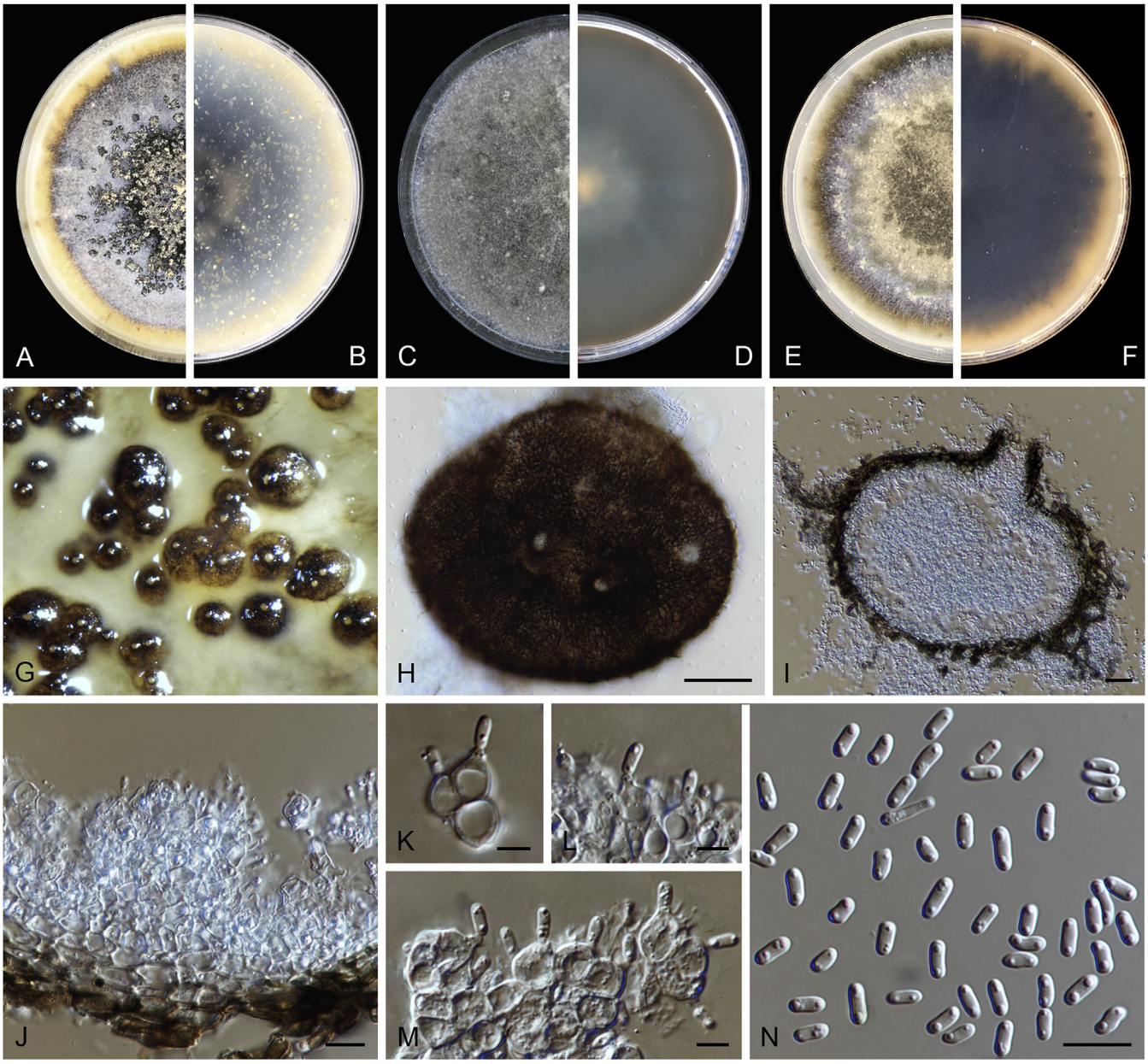

Fig. 2. *Stagonosporopsis ailanthicola* (CBS 140556). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 25 µm; J, N = 10 µm; K–M = 5 µm.

are arranged according to their position on the phylogenetic tree (Fig. 1).

Clade 1: *Stagonosporopsis* Died. emend. Aveskamp *et al.*, Stud. Mycol. 65: 44. 2010.

Type species: *Stagonosporopsis hortensis* (Sacc. & Malbr.) Petr.

Stagonosporopsis ailanthicola Manawas. *et al.*, Fungal Diversity 83: 82. 2017. Fig. 2.

Description from culture CBS 140556: *Conidiomata* pycnidial, produced partly in the agar, solitary or aggregated, sometimes 2–4 confluent, scattered, mostly globose, subglobose to flask-shaped, pale brown to brown, thin-walled, glabrous, ostiolate, 520–830 × 420–550 µm. *Ostioles* 1–6, slightly papillate, increasing to eight when pycnidia confluent. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–5 layers, 15–40(–69) µm thick, outer two cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or lageniform, 5–10 × 4–9 µm. *Conidia* oblong, with both ends rounded, smooth and thin-walled, aseptate, 4–7 × 2–2.5 µm, 2–3-guttulate. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA, 65–70 mm diam after 7 d at 25 °C, margin regular, sparse aerial mycelium, grey olivaceous, pale luteous toward the periphery, abundant production of pycnidia; reverse concolourous. Colonies on MEA, 65–70 mm diam after 7 d, margin regular, aerial mycelium floccose, pale olivaceous grey; reverse concolourous. Colonies on PDA, 60–65 mm diam after 7 d, margin regular, covered by sparse felty aerial mycelium, concentric circles of different colours, centre hazel, buff, grey, olivaceous toward periphery; reverse olivaceous grey, salmon toward periphery. NaOH spot test: a pale greenish discolouration on OA.

Typus: **Italy**, Forlì Cesena (FC) Province, Via Pietro Nenni-Forlì, on dead branch of *Ailanthus altissima* (*Simaroubaceae*), 2 May 2015, E. Camporesi (**holotype** MFLU 16-2678, ex-type living culture MFLUCC 16-1439).

Materials examined: **Australia**, Tasmania, Antill Street, South Hobart, Hobart 7004, house dust, 31 Jan. 2009, B. Horton, culture CBS 140553. **New Zealand**, Wellington, Wyndham Rd., Silverstream, house dust, 3 May 2009, T. Atkinson, culture CBS 140556. **USA**, California, Koshland Hall, University of California Berkeley, Berkeley 94705, Alameda, house dust, 31 Mar. 2005, A. Amend, culture CBS 140554.

Notes: Four isolates from house dust in Australia, New Zealand and the USA were found to be genetically identical to *St. ailanthicola*, which was originally described from a dead branch of *Ailanthus altissima* in Italy (Tibpromma *et al.* 2017), suggesting *St. ailanthicola* to be highly cosmopolitan and plurivorous.

Stagonosporopsis cucumeris L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833477. Fig. 3.

Etymology: Name refers to the host genus from which it was isolated, *Cucumis*.

Description: *Ascomata* pseudothecial, superficial or semi-immersed, mostly solitary, scattered, globose to lageniform, dark brown, up to 350 µm diam, papillate, sometimes elongated with a short neck. *Pseudothecial wall* pseudoparenchymatous 24–41.5 µm thick, outer wall consisting of 2–4 layers of cells of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* bitunicate, clavate to cylindrical, with stipe short or absent, 45–78 × 8.5–11.5 µm. *Ascospores* biseriate, sparsely uniseriately arranged, ellipsoidal, straight to slightly curved, 11.5–15 × 4.5–6.5 µm, hyaline, smooth, apex obtuse, base broadly obtuse to subobtuse, medianly 1-septate, upper cell often wider than lower cell, constricted at the septum. *Conidiomata* pycnidial, mostly solitary, sometimes confluent, scattered, globose and subglobose to flask-shaped, pale to dark brown, thin-walled, glabrous, semi-immersed, sometimes immersed or superficial on agar, ostiolate, 110–320 × 100–320 µm. *Ostioles* 1–3, slightly papillate, sometimes elongated as a short neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–5 layers, 11–29(–50.5) µm thick, outer 3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose to ampulliform, 5–8.5 × 4.5–6 µm. *Conidia* oblong or ovoid, smooth- and thin-walled, hyaline, aseptate, 4–7.5 × 3–4.5 µm, with 2–3 large guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 80–85 mm diam after 7 d 25 °C, margin regular, aerial mycelium flat, grey olivaceous, abundant production of buff pycnidia and ascomata; reverse concolourous. Colonies on MEA reaching 80–85 mm diam after 7 d, margin regular, aerial mycelium flat, pale olivaceous grey, olivaceous grey near centre; reverse concolourous. Colonies on PDA reaching 80–85 mm diam after 7 d, margin regular, covered by felty aerial mycelium, pale olivaceous grey; reverse olivaceous grey. NaOH spot test negative on OA.

Typus: **The Netherlands**, from greenhouse in Groningen, canker on *Cucumis sativus* (*Cucurbitaceae*), Dec. 1965, H.A. van Kesteren (**holotype** CBS H-23694, ex-type living culture CBS 386.65).

Notes: The isolates CBS 386.65 and CBS 214.65 were received as "*Stagonosporopsis cucurbitacearum*" (basionym: *Sphaeria cucurbitacearum*), and were originally isolated from *Cucumis sativus*, which represents a different host from that of *St. cucurbitacearum* (*Cucurbitae* and *Momordicae*; Fries 1823). Isolate CBS 214.65 was genetically identical to *St. citrulli* (holotype FLAS F-58996) that was morphologically similar with *St. cucurbitacearum*, based on ITS, LSU and *tub2* sequences (Stewart *et al.* 2015). Therefore, CBS 214.65 was re-identified as *St. citrulli*. Besides, based on the multi-locus phylogenetic analyses, isolate CBS 386.65 formed a distinct lineage sister to *St. citrulli* and distant from the representative strain of *St. cucurbitacearum* (CBS 133.96). Morphologically, CBS 386.65 could be differentiated from *St. cucurbitacearum* by producing wider and aseptate conidia (aseptate, 4–7.5 × 3–4.5 µm *vs.* 0–1-septate, 4–8 × 2–3 µm) (Boerema *et al.* 2004). Although *St. citrulli* was originally introduced based on a phylogenetic analysis that showed it differs from the known *Stagonosporopsis* species, CBS 386.65 is distinct from *St. citrulli* (CBS 214.65) in 11 bp of *rpb2* sequence and 6 bp of *tub2* sequence (Stewart *et al.* 2015). We therefore introduced a new species *St. cucumeris* based on CBS 386.65.

Stagonosporopsis nemophilae (Neerg.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833494.

Basionym: *Phoma nemophilae* Neerg., Bot. Tidsskr. 44(3): 361. 1938.

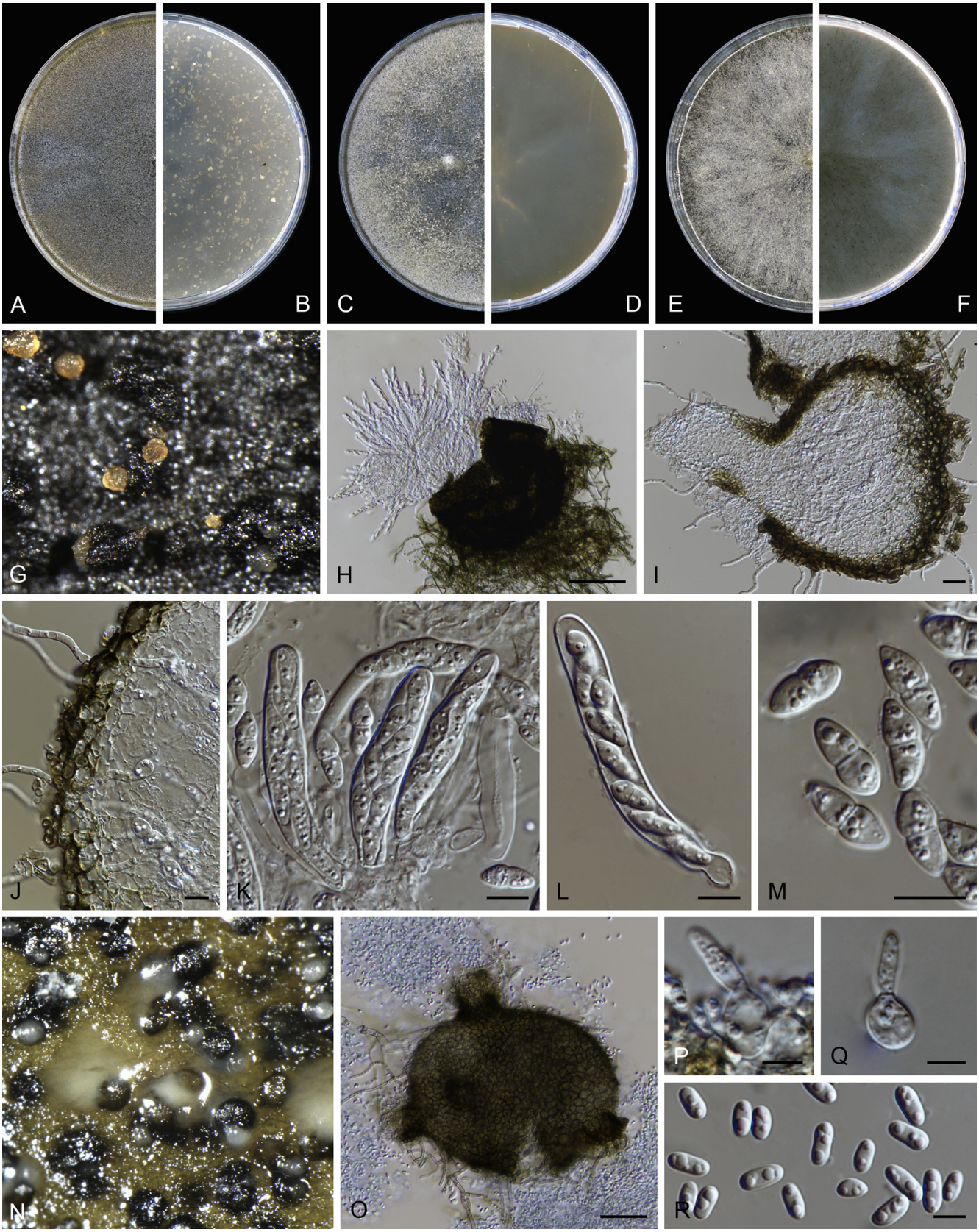

Fig. 3. *Stagonosporopsis cucumeris* (CBS 386.65). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pseudothecia forming on OA. **H.** Pseudothecium. **I.** Section through pseudothecium. **J.** Section of pseudothecial wall. **K–L.** Asci. **M.** Ascospores. **N.** Pycnidia forming on OA. **O.** Pycnidium. **P–Q.** Conidiogenous cells. **R.** Conidia. Scale bars: H = 100 µm; I = 20 µm; J–M = 10 µm; O = 50 µm; P–R = 5 µm.

Description: De Gruyter *et al.* (2002).

Typus: **Denmark**, from seed of *Nemophila insignis* (*Hydrophyllaceae*), Oct. 1938, Rijksproefstation v. Zaadcontrole, culture CBS 249.38 (presumed ex-type, but sterile). **The Netherlands**, from seed of *Nemophila insignis* (*Hydrophyllaceae*), Nov. 1985, isolated by G.H. Boerema (**neotype designated here** CBS H-24316, MBT389690, ex-neotype living culture CBS 715.85 = PD 74/364).

Notes: *Phoma nemophilae* was described from seed of *Nemophila insignis* collected in Denmark, with cylindrical conidia measuring 4.5–9 × 1–2 µm (Neergaard 1938). However, the

type specimen of *Phoma nemophilae* was not indicated in the original description and could not be traced in any fungarium after many efforts, and was therefore considered lost. In this study, CBS 249.38 was recognised as possible ex-type culture of *Phoma nemophilae* based on the information deposited in the CBS culture collection by the original collector and depositor of this species (Rijksproefstation v. Zaadcontrole). Unfortunately, this culture proved to be sterile. De Gruyter *et al.* (2002) described a representative culture of *Phoma nemophilae* (CBS 715.85), having morphological congruence with the original description of this species. In the present study, CBS 715.85 is genetically identical to the possible ex-type culture CBS 249.38, forming a well-supported distinct lineage in *Stagonosporopsis* (Fig. 1), and was therefore chosen as ex-neotype culture. Presently it is the only phoma-like species in *Didymellaceae* recorded on *Nemophila insignis*.

Stagonosporopsis sambucella L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833495.

Etymology: Name refers to the host genus *Sambucus*, from which this species was isolated.

Description: Culture sterile. *Stagonosporopsis sambucella* differs from its closest phylogenetic species *St. astragali* CBS 178.25 based on alignment of the concatenated four loci deposited in TreeBASE (S25826): ITS position 43(C), 50(A), 56(C), 293(C), 328(T), 347(C), 353(A), 423(A), deletion in position 444; LSU position 657(C), 879(C), 900(A); *rpb2* positions: 1 469(T), 1 487(A), 1 514(T), 1 547(C), 1 559(C), 1 571(A), 1 610(T), 1 616(T), 1 634(T), 1 664(T), 1 700(G), 1 703(T), 1 712(C), 1 724(A), 1 745(A), 1 775(C), 1 829(T), 1 838(T), 1 841(G), 1 853(G), 1 878(T), 1 898(G), 1 913(C), 1 937(C), 1 940(G), 1 946(T), 1 949(T), 2 021(C), 2 033(T); *tub2* positions: 2 062(C), 2 079(C), 2 124(C), 2 143(T), 2 167(T), 2 170(T), 2 209(T),

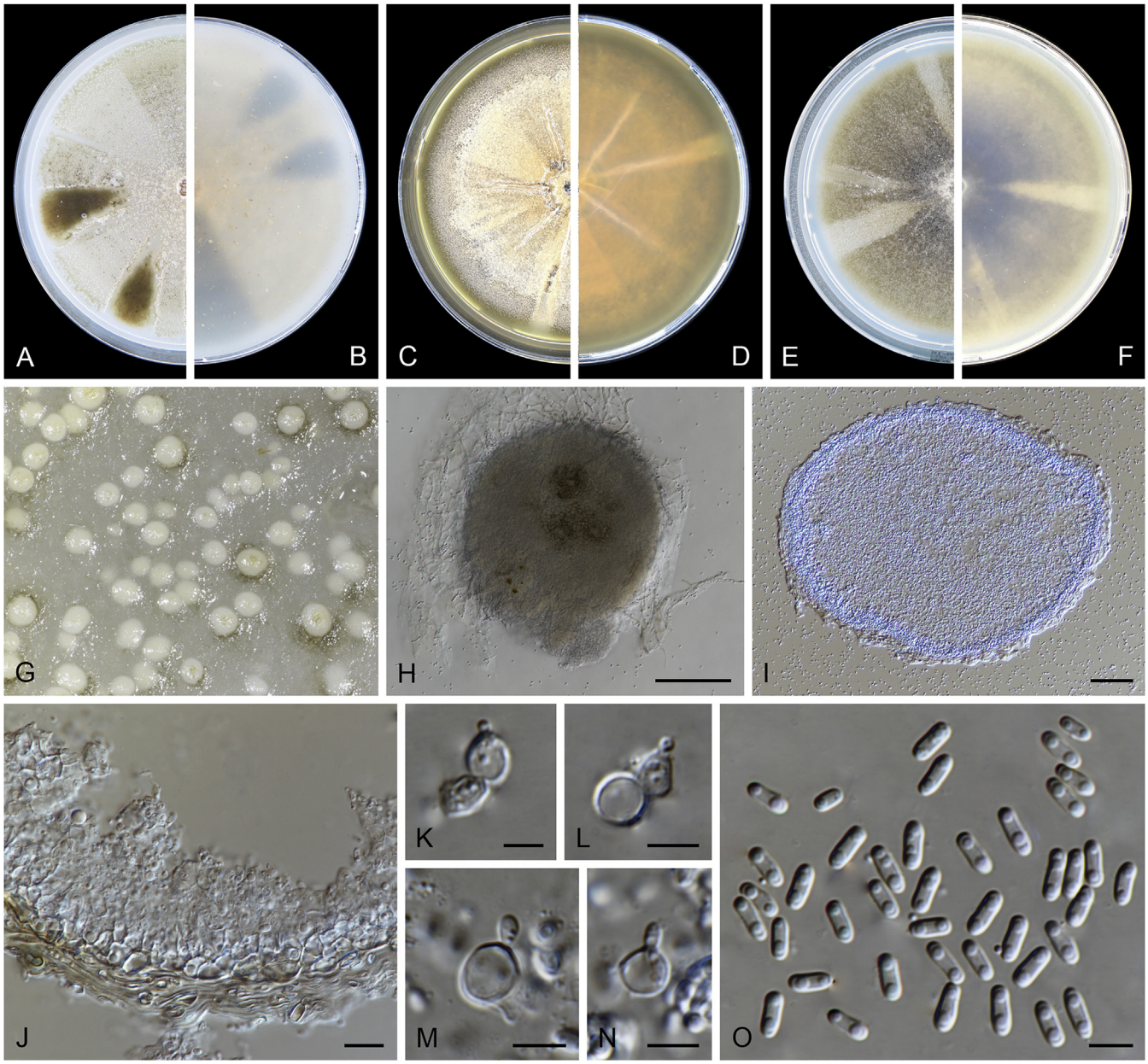

Fig. 4. *Allophoma alba* (CBS 120422). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H = 100 µm; I = 50 µm; J = 10 µm; K–O = 5 µm.

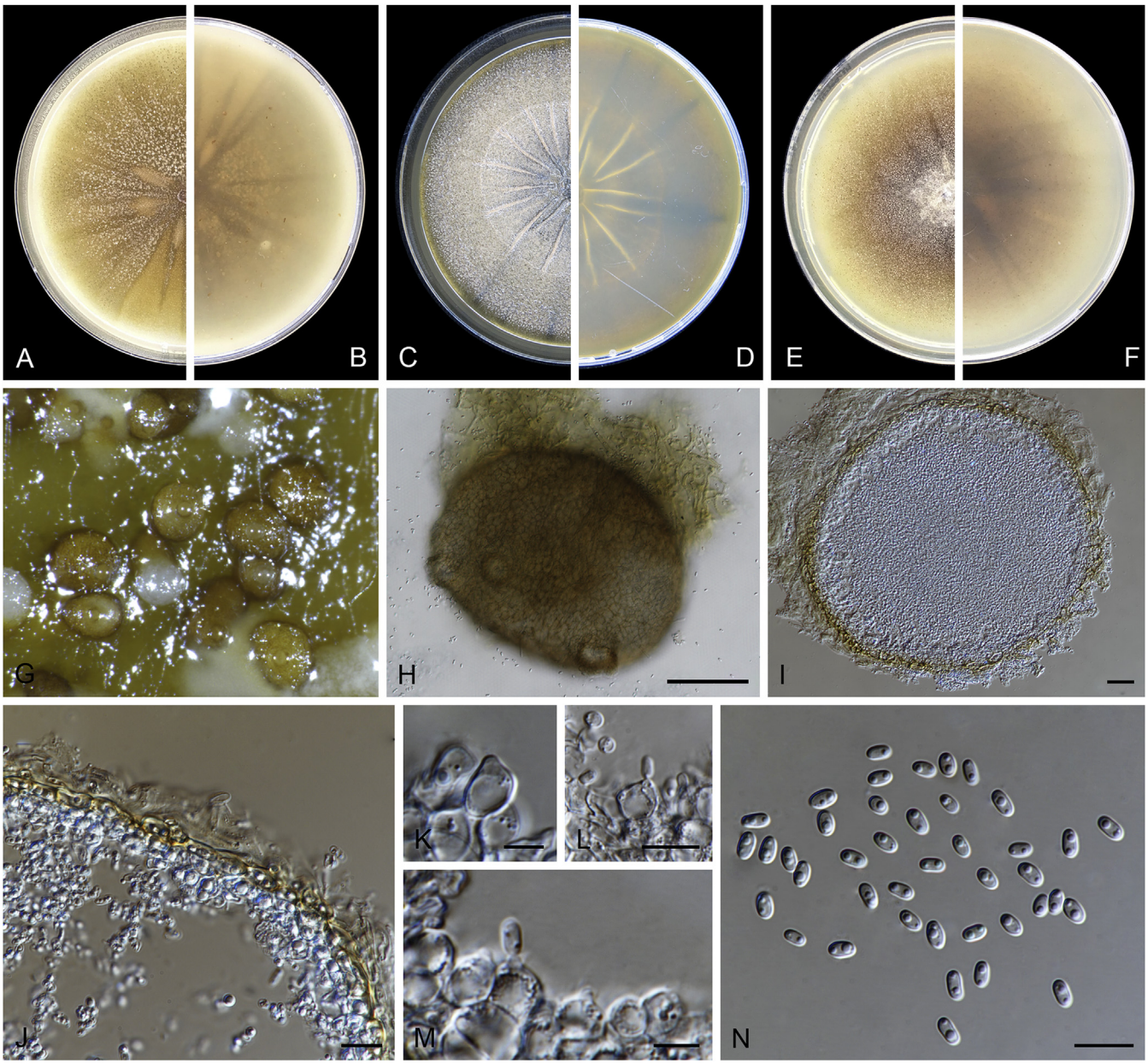

Fig. 5. *Allophoma anatii* (CBS 124673). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 20 µm; J, L, N = 10 µm; K, M = 5 µm

2 212(C), 2 269(T), 2 272(A), 2 275(T), 2 276(T), 2 277(T), 2 291(A), 2 400(C), 2 415(C).

Typus: **Austria**, Styria, *Sambucus nigra* (*Caprifoliaceae*), date unknown, A. Holler & D. Horvat (**holotype** CBS H-24317, ex-type living culture CBS 130003).

Notes: Isolate CBS 130003 formed a distinct lineage in the genus *Stagonosporopsis* (Fig. 1), but remained sterile in all culture media tested in this study. Considering that it is phylogenetically distinct, a new species, *St. sambucella*, was introduced here.

Clade 2: *Allophoma* Qian Chen & L. Cai, Stud. Mycol. 82: 162. 2015.

Type species: *Allophoma tropica* (R. Schneid. & Boerema) Qian Chen & L. Cai

Allophoma alba L.W. Hou, Pfenning, L. Cai & Crous, ***sp. nov.*** MycoBank MB833479. Fig. 4.

Etymology: Latin, *alba* = white, referring to the whitish pycnidia.

Description: *Conidiomata* pycnidial, (semi-)immersed in the agar, mostly solitary, scattered towards periphery of colony, some arranged in concentric zones, (sub-)globose to ellipsoidal, whitish at onset, slightly brown around the ostioles with age, thin-walled, glabrous, ostiolate, (100–)205–635 × (100–)195–510 µm. *Ostioles* 1–3, non-papillate or slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–4 layers, 19–45 µm thick, without pigmented layers or the outer 1 cell layer slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose to ampulliform, 3.5–6.5(–8) × 4.5–9 µm. *Conidia* oblong, with both ends rounded, hyaline, smooth- and thin-walled, aseptate, 3–4.5 × 1.5–2.3 µm, with two large, polar guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 50–52 mm diam after 7 d at 25 °C, margin regular, aerial mycelium sparse,

whitish to buff, with some olivaceous sterile sections, abundantly produced large pycnidia, conidial matrix visible; reverse concolourous. Colonies on MEA reaching 45–52 mm diam after 7 d, margin regular, aerial mycelium sparse, yellow to buff, pale olivaceous toward the periphery; reverse cinnamon, pale olivaceous near the margin. Colonies on PDA reaching 50–55 mm diam after 7 d, margin regular, covered by sparse floccose aerial mycelium, pale olivaceous, darker olivaceous near the centre; reverse concolourous. NaOH spot test negative on OA.

Typus: **Brazil**, Lavras, from the stem of *Bambusa vulgaris* (*Poaceae*), Oct. 2005, L.H. Pfenning (**holotype** CBS H-23661, ex-type living culture CBS 120422 = CML 660).

Notes: Isolate CBS 120422 was initially received as "*Allophoma tropica*", but it formed an independent branch, being distant from the rest of the isolates including the ex-type strain of *Al. tropica* (CBS 436.75; Fig. 1). Morphologically, CBS 120422 produces larger conidiogenous cells [3.5–6.5(–8) × 4.5–9 µm *vs.* 2–6 × 3–6 µm] and shorter conidia than *Al. tropica* (3–4.5 × 1.5–2.3 µm *vs.* 3–5.9 × 1.2–2.1 µm). Furthermore, it produces white pycnidia, which is rare in this family. Thus *Al. alba* is introduced as a new species, based on isolate CBS 120422.

Allophoma anatii L.W. Hou & O. Yarden, ***sp. nov.*** MycoBank MB833478. Fig. 5.

Etymology: The name was chosen to honour Prof. Anat Yarden for her contributions to integrate experiments using fungi as part of the high school curriculum in biology.

Description: *Conidiomata* pycnidial, produced on the agar surface, scattered, mostly solitary, globose, or subglobose to ellipsoidal, sometimes the confluent pycnidia merge with age, relatively large, pale brown, thin-walled, glabrous, (90–)130–400(–460) × (75–)120–370 µm. *Ostioles* 1–3, papillate or elongated with a short cylindrical neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–4 layers, 9.5–28.5 µm thick, outer two layers composed of pale brown cells. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform or lageniform, slightly papillate, 5–7 × 5.5–9 µm. *Conidia* oblong with both ends rounded or ovoid, smooth- and thin-walled, hyaline, aseptate, 3.5–5.5 × 2–3 µm, with 1–2 medium, polar guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d 25 °C, margin regular, aerial mycelium sparse, pale cinnamon, abundant production of pycnidia, conidial matrix visible; reverse concolourous. Colonies on MEA reaching 45–55 mm diam after 7 d, margin regular, aerial mycelium sparse, vinaceous buff, with some radially furrowed zones near the centre; reverse brown, with white radial line near the centre, honey towards periphery. Colonies on PDA reaching 42–52 mm diam after 7 d, margin regular, aerial mycelium sparse, umber near the centre, buff toward periphery; reverse concolourous. NaOH spot test negative on OA.

Typus: **Australia**, Great Barrier Reef, from a brown band on *Acropora formosa* (*Acroporidae*; coral), Jul. 2006, O. Yarden (**holotype** CBS H-23688, ex-type living culture CBS 124673 = OY6506).

Notes: In the phylogenetic tree *Al. anatii*, which was isolated from *Acropora formosa* (*Acroporidae*; coral), formed an independent lineage basal to the genus *Allophoma*, and clearly separate from other species (Fig. 1). Morphologically, *Al. anatii* is characterised by larger pycnidia with thin and resilient pycnidial wall.

Allophoma anatii is phylogenetically distant from species in *Didymellaceae* that were recorded growing in marine habitats, such as *Calophoma sandfjordenica*, *Didymella finnmarkica*, *Phoma antarctica*, *Phoma capitulum*, *Phoma hibernica*, *Phoma ostiolata*, and *Phoma multipora* (World Register of Marine Species, WoRMS; Crous *et al.* 2019a). Most of these have been treated as synonyms of species in other families and were phylogenetically distant from *Al. anatii*. For example, *Phoma ostiolata* was treated as synonym of *Westerdykella capitulum* in *Sporormiaceae*, while *Phoma multipora* and *Phoma capitulum* were transferred to *Coniothyriaceae* and *Sporormiaceae* respectively (Pawar *et al.* 1967, De Gruyter *et al.* 2013). On the other hand, *Allophoma anatii* differed morphologically from *Ca. sandfjordenica* and *Did. finnmarkica* in its aseptate and smaller conidia [3.5–5.5 × 2–3 µm *vs.* (8–)10–14(–18) × (2–)3 µm in *Ca. sandfjordenica* and (6–)7–16(–18) × (2–)2.5(–4) µm in *Did. finnmarkica*, Crous *et al.* 2019a].

Allophoma oligotrophica Qian Chen *et al.*, Stud. Mycol. 87: 119. 2017.

Typus: **China**, Guizhou, Shuanghe Cave National Geopark, from air, 8 May 2015, Z.F. Zhang (**holotype** HMAS 247035, ex-type living culture CGMCC 3.18114 = LC 6245).

Additional materials examined: **China**, Guizhou, Shuanghe Cave National Geopark, from air, 8 May 2015, Z.F. Zhang, culture CGMCC 3.18115 = LC 6246; *ibid.* culture CGMCC 3.18116 = LC 6247. **The Netherlands**, Wageningen, on *Oncidium* (*Orchidaceae*) in glasshouse, Jul. 1975, G.H. Boerema, specimen CBS H-16594, culture CBS 353.75. **USA**, South Carolina, Clemson, on *Ipomoea batatas* (*Convolvulaceae*), 7 Jun. 2005, A. Rossman, culture CBS 120112.

Notes: *Allophoma oligotrophica* was the first species isolated from air using carbon-free silica gel medium in a Chinese Cast cave (Jiang *et al.* 2017). In this study, two cultures isolated from different plants in the Netherlands and the USA grouped together with the ex-type strain of *Al. oligotrophica* (CGMCC 3.18114). This species was confirmed to be widely distributed and associated with diverse substrates. Two strains (CBS 353.75 and CBS 120112) clustered slightly apart from the other strains of *Al. oligotrophica* based on seven changes over the four genes; presently we refrain from introducing an additional species pending collection of more isolates.

Clade 3: *Heterophoma* Qian Chen & L. Cai, Stud. Mycol. 82: 165. 2015.

Type species: *Heterophoma sylvatica* (Sacc.) Qian Chen & L. Cai

Heterophoma nobilis (Kabát & Bubák) Qian Chen & L. Cai, Stud. Mycol. 82: 165. 2015. Fig. 6.
Basionym: *Ascochyta nobilis* Kabát & Bubák, Oesterr. Bot. Z. 54: 3. 1904.
Synonym: *Phoma dictamnicola* Boerema *et al.*, Persoonia 15: 90. 1992.

Description from ex-neotype (CBS 507.91): *Conidiomata* pycnidial, (semi-)immersed in the agar, under thick mycelial layers, mostly solitary, sometimes confluent, scattered, (sub-)globose to ellipsoidal, pale brown at onset, dark brown with age, thick-walled, glabrous, 170–415 × 170–350 µm. *Ostioles* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 5–9 layers, 47–74 µm thick, the outer 5 cell layers slightly

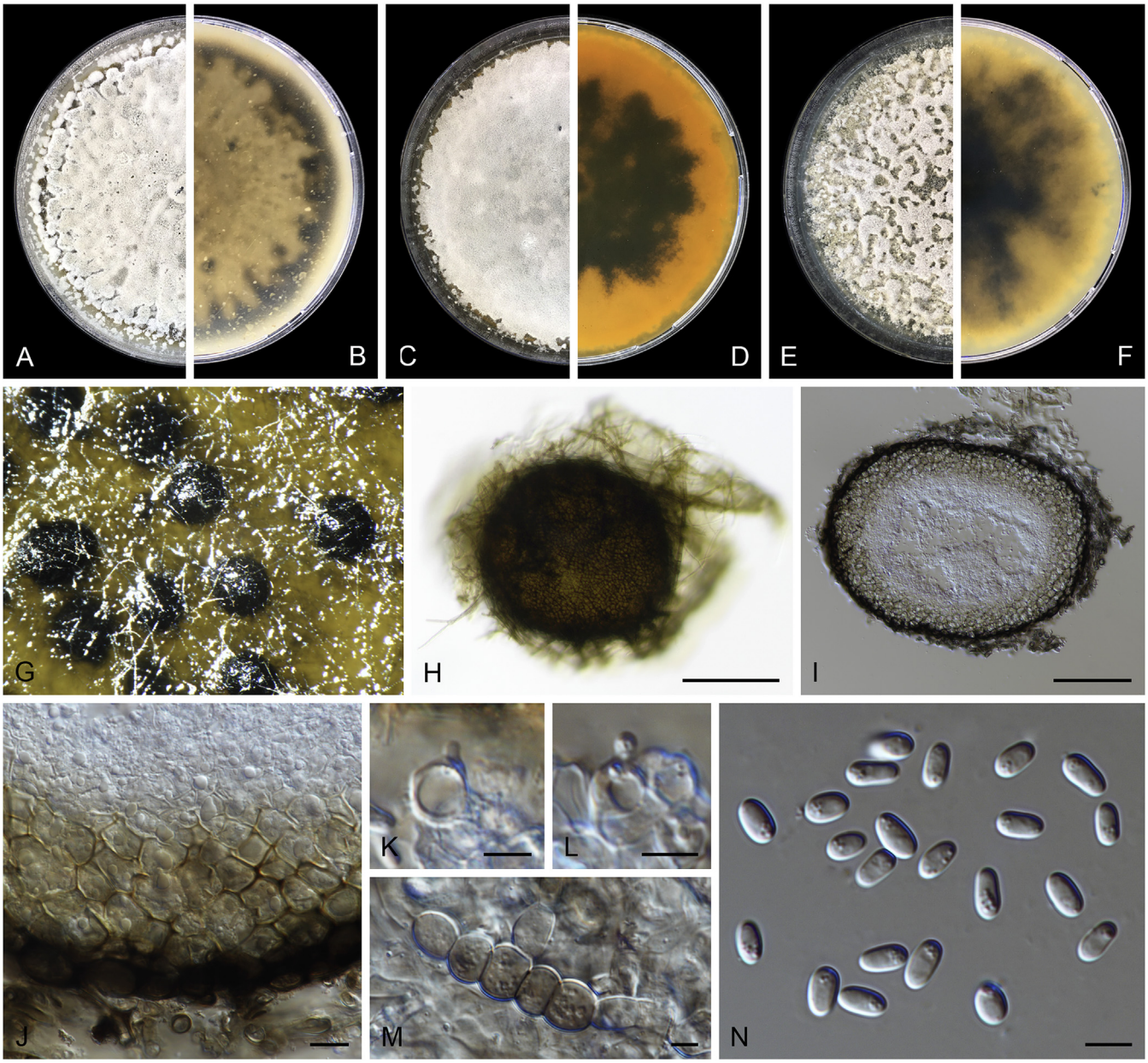

Fig. 6. *Heterophoma nobilis* (CBS 507.91). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–L.** Conidiogenous cells. **M.** Chlamydospores. **N.** Conidia. Scale bars: H–I = 100 µm; J = 10 µm; K–N = 5 µm.

pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, lageniform to ampulliform, 6–8 × 5–7.5 µm. *Conidia* ellipsoidal to oblong with both ends rounded, hyaline, smooth- and thin-walled, aseptate, 3–4.5 × 1.5–2.5 µm, without guttules or with 2–5 minute guttules.

Culture characteristics: Colonies on OA reaching 65–70 mm diam after 7 d at 25 °C, margin regular, aerial mycelium woolly, pale grey; reverse pale olivaceous to olivaceous. Colonies on MEA reaching 65–70 mm diam after 7 d, margin regular, aerial mycelium woolly, pale grey; reverse orange to dark brown. Colonies on PDA reaching 65–70 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, pale grey; reverse buff to dark brown. NaOH spot test negative on OA.

Typus: **The Netherlands**, Gelderland Province, Arnhem, from a stem of *Dictamnus albus* (*Rutaceae*), Sep. 1991, J. de Gruyter (**neotype designated here** MBT389692, CBS H-24313, ex-neotype living culture CBS 507.91 = PD 74/148).

Notes: *Heterophoma nobilis* was originally described from dying leaves of *Dictamnus fraxinella* in Anlagern Turnau, Czechia (conidia aseptate, later becoming medially 1-septate). However, Bubák & Kabät (1904) did not designate a holotype specimen. Later a representative culture of *H. nobilis* (CBS 507.91) was described by Boerema *et al.* (2004), which was isolated from same host in Europe. The morphology of CBS 507.91 agreed with the original description of *H. nobilis* (De Gruyter & Noordeloos 1992). Since no holotype specimen could be located in this study, we designated CBS 507.91 as ex-neotype culture.

Heterophoma verbasci-densiflori L.W. Hou, L. Cai & Crous, ***nom. nov.*** MB834017. Fig. 7.
Basionym: *Phyllosticta verbascicola* Ellis & Kellerm., Bull. Torrey Bot. Club 11: 115. 1884.
Synonyms: *Phoma poolensis* var. *verbascicola* (Ellis & Kellerm.) Aa & Boerema, Persoonia 15: 385. 1993.
Phoma novae-verbascicola Aveskamp *et al.*, Stud. Mycol. 65: 41. 2010.

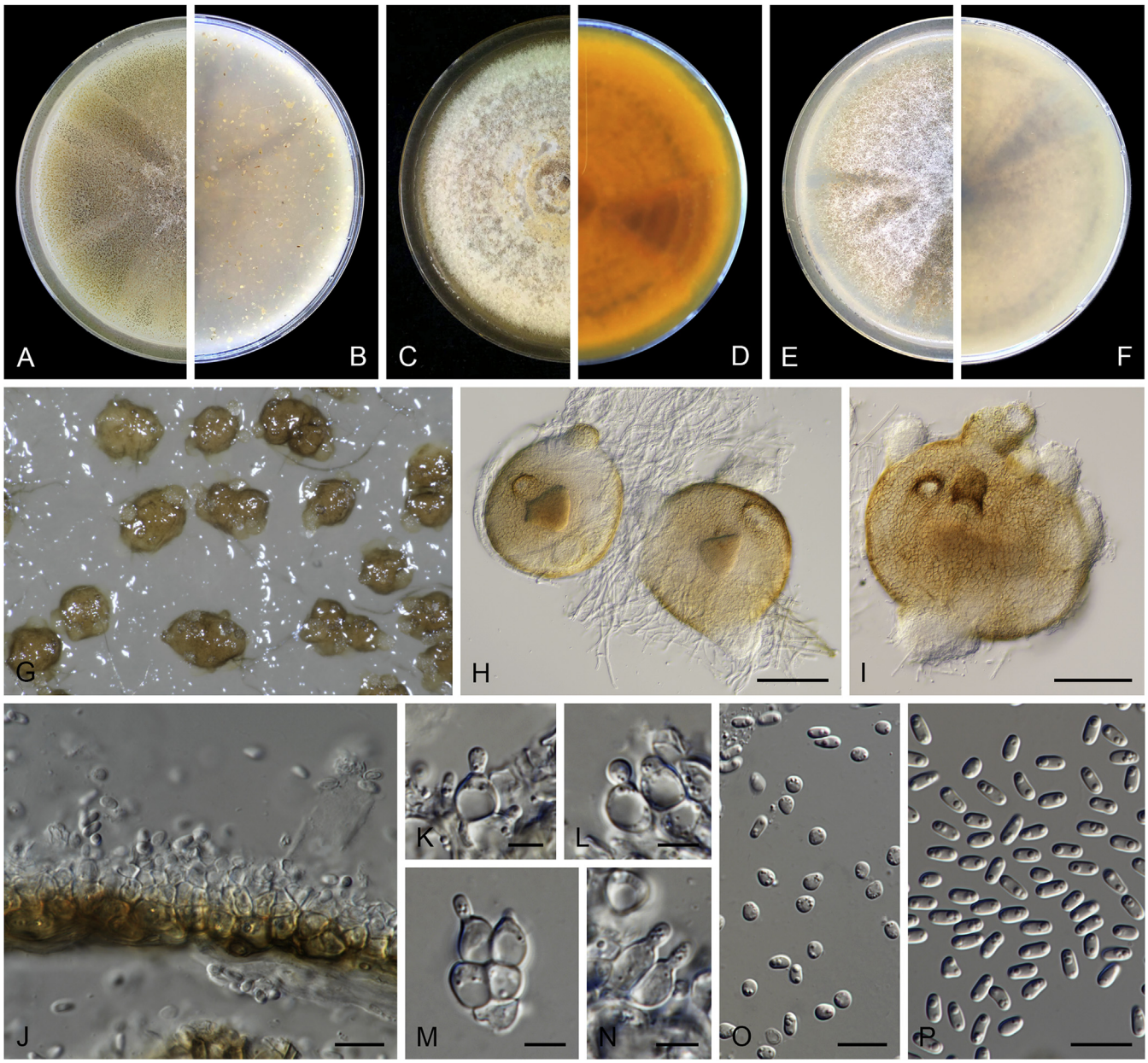

Fig. 7. *Heterophoma verbasci-densiflori* (CBS 127.93). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H–I.** Pycnidia. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O–P.** Conidia. Scale bars: H, I = 100 µm; J, O, P = 10 µm; K–N = 5 µm.

Heterophoma novae-verbascicola (Aveskamp *et al.*) Qian Chen *et al.*, Stud. Mycol. 82: 165. 2015. *Nom. illegit.*, Art. 52.1.

Description from ex-epitype (CBS 127.93): *Conidiomata* pycnidial, semi-immersed in the agar, mostly solitary, sometimes confluent, scattered, (sub-)globose, whitish to buff at beginning, irregular-shaped and pale brownish with age, ostiolate, 160–390 × 155–345 µm. *Ostioles* 1–6, up to 12 with age, papillate or elongated into a short neck. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, 10–17 µm thick, the outer two cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, lageniform to ampulliform, or doliiform, 5–7.5(–10.5) × 3.5–8 µm. *Conidia* two types, both originating from the same pycnidium. Conidia of type 1: oblong, smooth- and thin-walled, hyaline, aseptate, 3.5–5.5 × 2–3 µm, two polar guttulate, medium. Conidia of type 2: globose to sub-globose, smooth- and thin-walled, aseptate, 3–4 × 2.5–3.5 µm, guttulate. *Chlamydospores* not observed.

Culture characteristics: Colonies on OA reaching 60–65 mm diam after 7 d at 25 °C, margin regular, aerial mycelium sparsely, buff to pale brown; reverse rosy buff to pale brown. Colonies on MEA reaching 60–65 mm diam after 7 d, margin regular, aerial mycelium woolly, whitish to buff; reverse orange to pale brown. Colonies on PDA reaching 65–70 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, whitish to buff; reverse buff to pale brown. NaOH spot test negative on OA.

Typus: **The Netherlands**, Utrecht Province, Zeist, Abburg nursery, from *Verbascum* sp. (*Scrophulariaceae*) (**holotype** L 9893.00.134); North Holland Province, Haarlem, from dead stem of *Verbascum densiflorum*, Jan. 1993, J. de Gruyter (**epitype designated here** MBT389693, CBS H-24314, ex-epitype living culture CBS 127.93 = PD 92/347).

Additional material examined: **The Netherlands**, Baarn, from dead stem of *Verbascum* sp., 29 Jun. 1981, H.A. van der Aa, culture CBS 449.81.

Notes: Isolates CBS 127.93 and CBS 114.93 were originally received as "*Phoma poolensis* var. *verbascicola*" (De Gruyter *et al.*

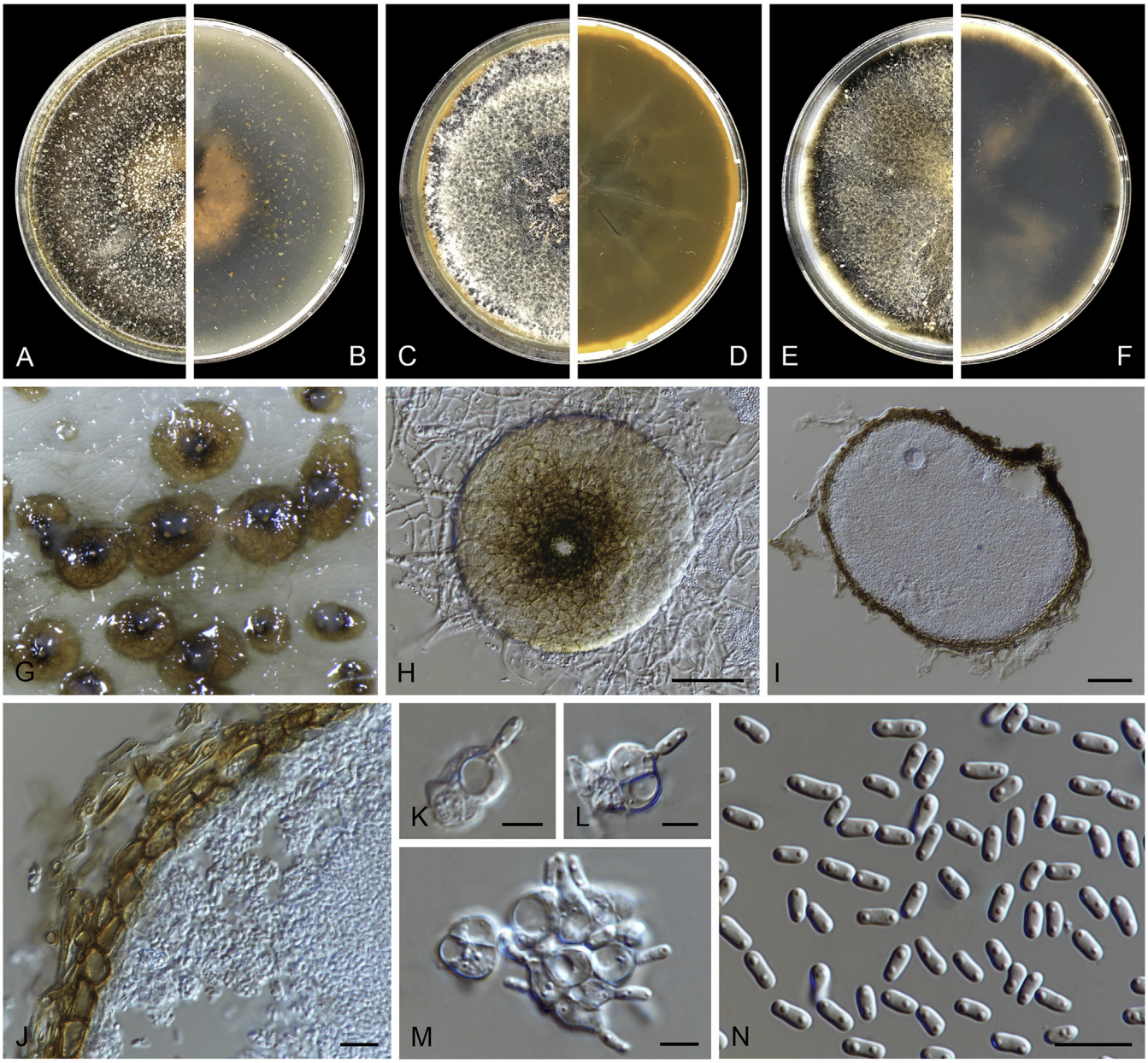

Fig. 8. *Chaetasbolisia argentina* (CBS 148.94). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 50 µm; J, N = 10 µm; K–M = 5 µm.

1993). Later, Boerema *et al.* (2004) described CBS 127.93 as a representative culture of *Phoma poolensis* var. *verbascicola*, as it agreed well with the original description with regards to morphology, host and location. Subsequently, a new name, *Phoma novae-verbascicola*, was proposed for this species as it is distinguishable from *Phoma poolensis* and could not be elevated to species level as *Phoma verbascicola* has already occupied. When Chen *et al.* (2015) allocated this name to *Heterophoma*, they should have given preference to the older epithet of *Phyllosticta verbascicola*, thus rendering their new combination illegitimate. The name is herewith validated with the introduction of a new name, *H. verbasci-densiflori*, as *H. verbascicola* is already occupied. In the present study, we followed Boerema's suggestion and designated CBS 127.93 as ex-epitype of this species.

Phylogenetically, isolate CBS 114.93 formed an intermediate clade between *H. verbasci-densiflori* and *H. verbascicola* (described from leaves of *Verbascum thapsus* in China) and appeared to represent an undescribed species (Fig. 1).

Clade 4: *Boeremia* Aveskamp *et al.*, Stud. Mycol. 65: 36. 2010.

Type species: *Boeremia exigua* (Desm.) Aveskamp *et al.*

Boeremia foveata (Foister) Aveskamp *et al.*, Stud. Mycol. 65: 40. 2010.
Basionym: *Phoma foveata* Foister, Trans. & Proc. Bot. Soc. Edinburgh 33: 66. 1940.

Typus: **UK**, from a tuber canker on *Solanum tuberosum* (*Solanaceae*), unknown date, C.E. Foister, ex-isotype culture CBS 200.37.

Additional material examined: **Bulgaria**, from a tuber of *Solanum tuberosum* (*Solanaceae*), Jan. 2001, H. de Gruyter, culture CBS 109176 = CECT 2828 = PD 94/1394.

Note: In this paper, the ex-isotype strain of *Boeremia foveata* (CBS 200.37) was examined, LSU and *rpb2* sequences were added, and a more complete *tub2* sequence was provided.

Clade 5: *Chaetasbolisia* Speg., Physis Rev. Soc. Arg. Cienc. Nat. 4: 293. 1918.

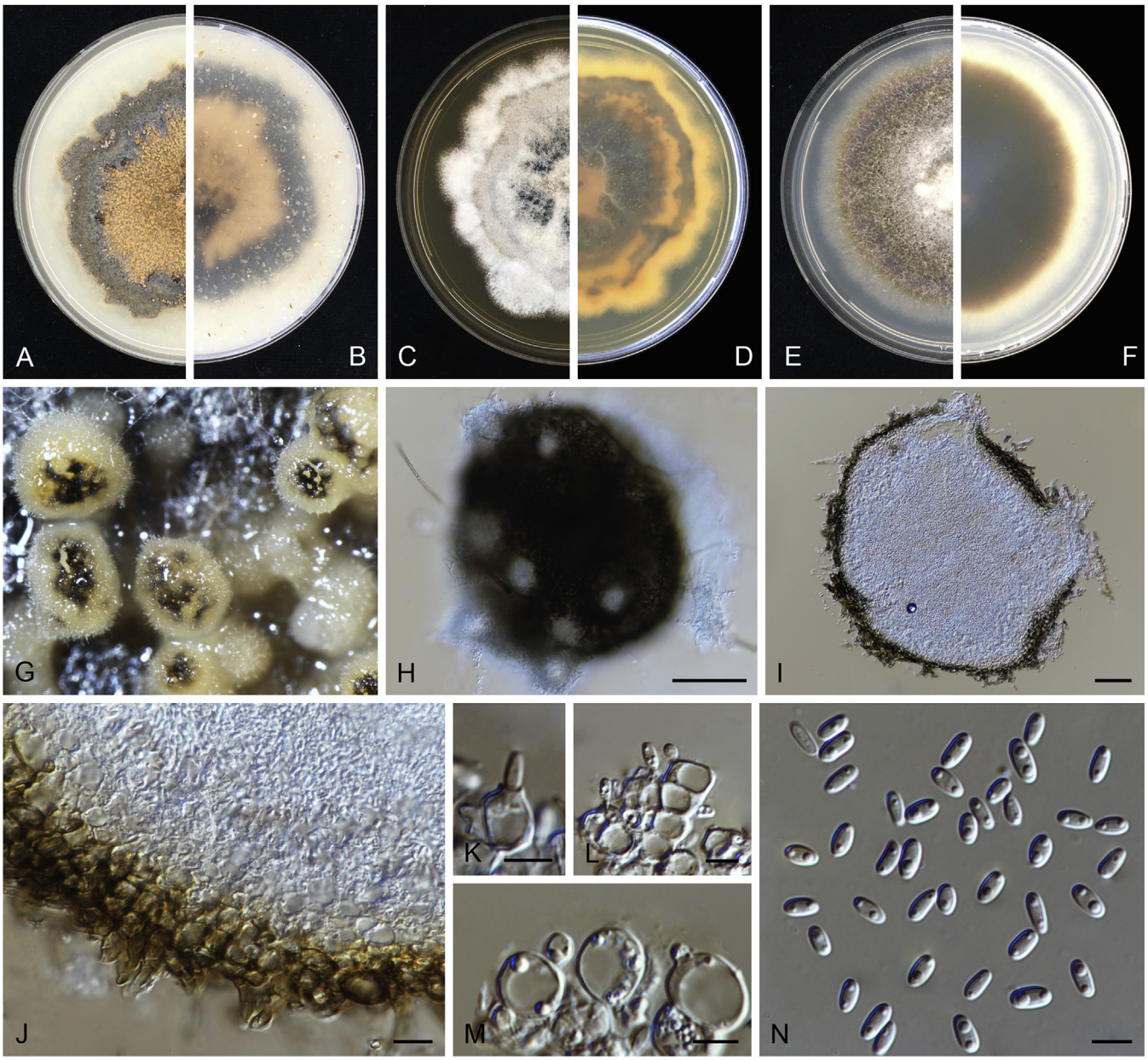

Fig. 9. *Chaetasbolisia eupatorii* (CBS 123.93). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 50 µm; J = 10 µm; K–N = 5 µm

Conidiomata pycnidial, solitary, globose to subglobose, superficial, black, ostiolate. *Pycnidial wall* textura angularis, with long branched septate fuliginous hyphal outgrowths or glabrous. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform to lageniform. *Conidia* globose, broad ovoid, cylindrical or oblong, hyaline, smooth- and thin-walled, aseptate, eguttulate or guttulate. (Griffon & Maublanc 1909, Spegazzini 1918).

Type species: *Chaetasbolisia erysiphoides* (Griffon & Maubl.) Griffon & Maubl.

Notes: *Chaetasbolisia* was introduced by Spegazzini in 1918 to accommodate *Chaetophoma erysiphoides* with a brief generic description (Spegazzini 1918). Subsequently, additional species were introduced in this genus, but most lacking molecular data. In the present study, *Chaetasbolisia* is represented by two species and forms a well-supported separate clade from other genera of *Didymellaceae*. The generic circumscription of *Chaetasbolisia* is emended to incorporate the morphological features of these newly added species, such as glabrous pycnidial conidiomata and cylindrical or oblong shaped conidia. However, the taxonomy of the genus *Chaetasbolisia* remains unresolved.

Chaetasbolisia argentina L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833551. Fig. 8.

Etymology: Epithet derived from the country of origin, Argentina.

Description: *Conidiomata* pycnidial, (semi-)immersed, solitary, sometimes 2–4 confluent, scattered or aggregated, mostly (sub-) globose or flask-shaped, pale brown, darker brown around the ostioles, thin-walled, glabrous, ostiolate, 110–460 × 110–310 µm. *Ostioles* mostly 1–2, increasing to 8 when confluent, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–4 layers, 10–28.5 µm thick, outer 2 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, lageniform, ampulliform or doliiform, 5–8.5 × 4.5–6.5 µm. *Conidia* cylindrical or oblong, hyaline, smooth- and thin-walled, aseptate, 4–7.5 × 2–2.5 µm, with two small, polar guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 80–85 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, grey olivaceous, pale brown near the centre, abundant production of pycnidia; reverse olivaceous grey, orange near the centre. Colonies on MEA reaching 80–85 mm diam after 7 d, margin regular, aerial mycelium felty, pale olivaceous grey to olivaceous grey; reverse olivaceous, orange near the margin. Colonies on PDA reaching 80–85 mm diam after 7 d, margin regular, covered by felty aerial mycelium, pale olivaceous grey, olivaceous black towards the periphery; reverse olivaceous grey. NaOH spot test negative on OA.

Typus: **Argentina**, from soil, Apr. 1994, A. Arambarri (**holotype** CBS H-24310, ex-type living culture CBS 148.94).

Notes: *Chaetophoma erysiphoides* was originally described from *Quercus ilicis* in Cadillac, Gironde, France (Griffon & Maublanc 1909). Later a new genus, *Chaetasbolisia*, was proposed to accommodate this species (Spegazzini 1918). In the present study, isolate CBS 148.94 which was initially identified as *Chaeta. erysiphoides* from soil in Argentina was examined. It differed from the original description of *Chaeta. erysiphoides* by its larger guttulate conidia (4–7.5 × 2–2.5 µm, 2–3 guttules *vs*. 3–5.5 × 2.5–4 µm, eguttulate), and was therefore described as a new species.

Chaetasbolisia eupatorii (Died.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833496. Fig. 9.
Basionym: *Phoma eupatorii* Died., Ann. Mycol. 10: 447. 1912.

Description from ex-epitype (CBS 123.93): *Conidiomata* pycnidial, superficial on the agar or (semi-)immersed in the agar, solitary, sometimes confluent, scattered or aggregated, mostly (sub-) globose to flask-shaped, whitish to buff at beginning, brownish with age, with white short hyphal outgrowths, ostiolate, 250–400 × 250–350 µm. *Ostioles* 1–6(–10), non-papillate or slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–5 layers, 16–42.5(–52) µm thick, without pigmented layers or outer 1–3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform, lageniform to doliiform, with periclinal thickening, 4.5–10.5 × 4–9.5 µm. *Conidia* cylindrical, oblong or ovoid, hyaline, smooth- and thin-walled, aseptate, 4–6 × 2–2.5 µm, with two medium, polar guttules. *Conidial matrix* buff.

Culture characteristics: Colonies on OA reaching 55–60 mm diam after 7 d at 25 °C, margin irregular, aerial mycelium flat, abundant production of pycnidia, scattered near the centre, in the beginning whitish, but turning saffron because of conidial mass exuding from pycnidia, umber towards the periphery; reverse concolourous. Colonies on MEA reaching 60–65 mm diam after 7 d, margin irregular, covered by floccose aerial mycelium, buff, whitish towards the periphery, dark brown conidiomata formed in centre; reverse concentric circles of different colours, centre buff, olivaceous black, yellow towards periphery, a dark brown circle formed in the middle. Colonies on PDA reaching 50–55 mm diam after 7 d, margin regular, aerial mycelium floccose, brown, whitish near the centre and buff towards periphery; reverse dark brown, whitish towards periphery. NaOH spot test negative on OA.

Typus: **Germany**, Sperenberg bei Zossen, from stems of *Eupatorium cannabinum* (*Asteraceae*), 16 May 1912, H. Sydow (**lectotype designated here** MBT389670, F56137). **The Netherlands**, Gelderland Province, Arnhem, Openlucht museum, from dead stem of *Eupatorium cannabinum* (*Asteraceae*), Jan. 1993, J. de Gruyter (**epitype designated here**, CBS H-23676, MBT389671, ex-epitype living culture CBS 123.93 = PD 77/1148).

Notes: This species was initially described from dead stems of *Eupatorium cannabinum* in Germany, with conidia being oblong-elliptical, usually with two small oil droplets, 4–5.5 × 1.5–2 µm (Sydow & Sydow 1912). However, no holotype specimen was cited in the original description. In the present study, we located two type specimens in the Swedish Museum of Natural History (S), and both properly matched the information in the protologue, including one (F49345) from Sydow's own herbarium. Since the holotype specimen was not cited in the original paper, we designated F49345 as lectotype. Furthermore, in this study, the isolate (CBS 123.93) from *Eu. cannabinum* in the Netherlands agreed well in morphology with the protologue, as conidia were aseptate, measuring 4–6 × 2–2.5 µm, with two small-sized polar guttules. Thus CBS 123.93 was designated as ex-epitype culture and a new combination *Chaetasbolisia eupatorii* was therefore introduced. *Chaetasbolisia eupatorii* differed from other species in this family by producing whitish pycnidia covered in whitish mycelium.

Clade 6: *Macroascochyta* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833497.

Etymology: Morphologically resembling the genus *Ascochyta*, but distinct in having larger conidia.

Conidiomata pycnidial, solitary or confluent, globose to subglobose, semi-immersed or immersed in the agar, ostiolate. *Pycnidial wall* pseudoparenchymatous, multi-layered, outer layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform to lageniform. *Conidia* cylindrical, bacilliform to fusiform, sometimes curved, hyaline, smooth- and thin-walled, aseptate, guttulate. *Chlamydospores* hyaline or pale brown, intercalary, unicellular, globose to subglobose, solitary, eguttulate. *Sexual morph* unknown.

Notes: A new asexual species clustered in *Didymellaceae* as a distinct monophyletic clade (Fig. 1) in both ML and Bayesian analyses. This species was collected from *Tradescantia* sp. in post entry quarantine in New Zealand. Since fungi collected herein clearly form an independent lineage and are phylogenetically segregated from other genera, we introduce *Macroascochyta* as a new genus to accommodate this species.

Type species: *Macroascochyta grandis* L.W. Hou, L. Cai & Crous

Macroascochyta grandis L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833498. Fig. 10.

Etymology: Name reflects the large (= *grandis* in Latin) conidia produced by this species.

Description: *Conidiomata* pycnidial, (semi-)immersed in the agar, solitary, scattered or aggregated, mostly (sub-)globose to flask-shaped, pale to dark brown, with hyphal outgrowths, ostiolate, 175–400 × 160–375 µm. *Ostioles* single, central, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–6 layers, 18.5–35.5 µm thick, without pigmented layers or outer 1–4 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, (sub-) globose, ampulliform to lageniform, with minute periclinal thickening, 7–13.5 × 6.5–12.5 µm. *Conidia* cylindrical, bacilliform to fusiform, hyaline, smooth- and thin-walled, aseptate, 12.5–21 × 4.5–7 µm, with 6–10 small guttules each end. *Chlamydospores* hyaline or pale brown, intercalary, unicellular,

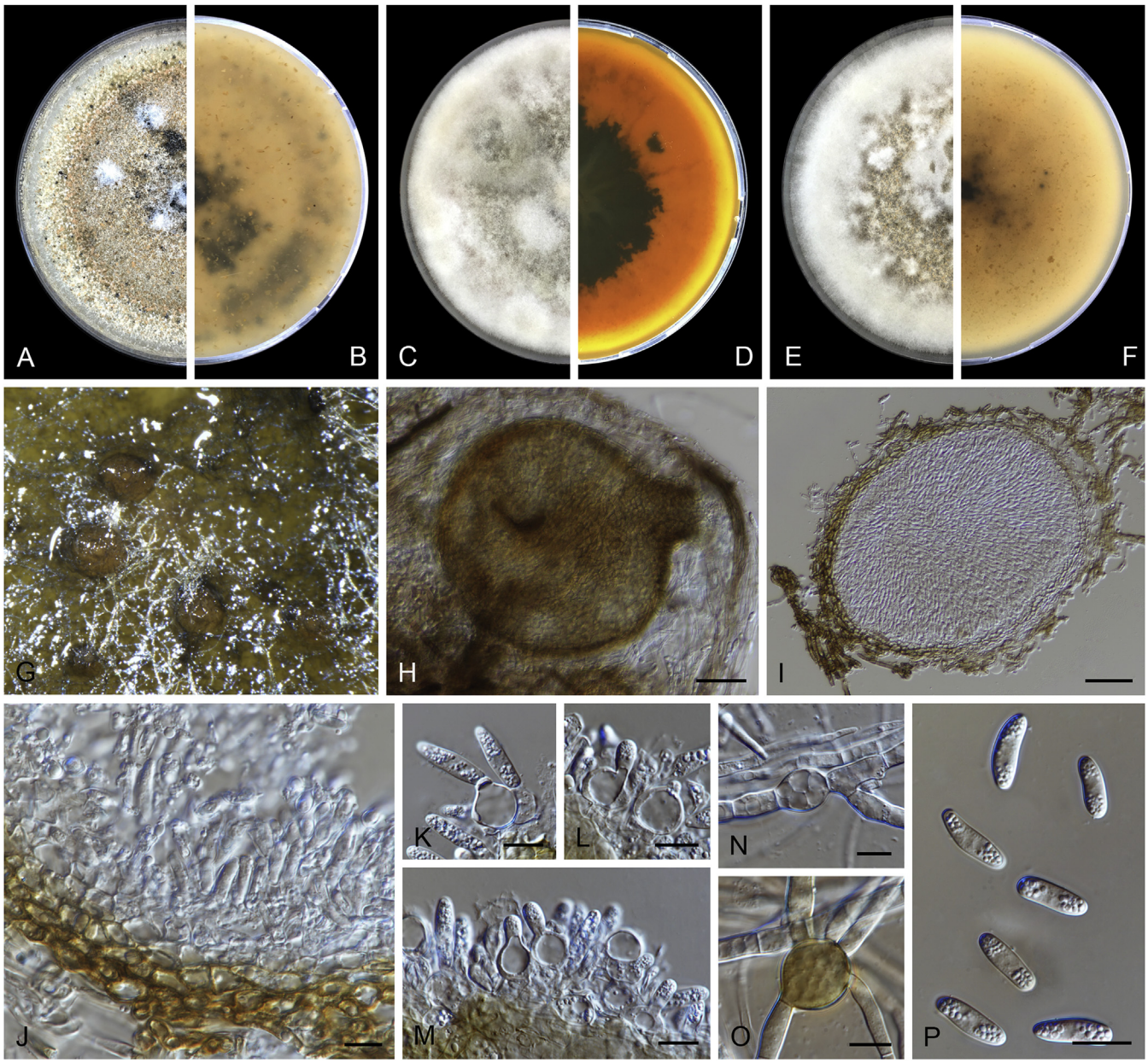

Fig. 10. *Macroascochyta grandis* (CBS 100409). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N–O.** Chlamydospores. **P.** Conidia. Scale bars: H–I = 50 µm; J–P = 10 µm.

(sub-)globose, solitary, eguttulate, thin-walled. *Conidial matrix* orange.

Culture characteristics: Colonies on OA reaching 70–75 mm diam after 7 d at 25 °C, margin irregular, aerial mycelium flat, buff to vinaceous buff, abundant production of pycnidia, orange conidial mass exuding from pycnidia; reverse pale cinnamon. Colonies on MEA reaching 75–80 mm diam after 7 d, margin irregular, covered by woolly aerial mycelium, whitish to pale grey; reverse orange to leaden black, yellow towards periphery. Colonies on PDA reaching 75–80 mm diam after 7 d, margin regular, aerial mycelium woolly, whitish to buff; reverse buff to pale brown. NaOH spot test negative on OA.

Typus: **New Zealand**, South Auckland, Alfriston, from *Tradescantia* sp. (*Commelinaceae*; in post entry quarantine), date unknown, coll. K. Ramsay, isol. C.F. Hill (**holotype** CBS H-23675, ex-type living culture CBS 100409).

Notes: *Phoma commelinicola* (basionym: *Phyllosticta commelinicola*) was originally reported from *Commelina nudiflora* in Puerto Rico, with conidia measuring 9.6–14.4 × 4.8–7.2 µm (Young 1915). The isolate CBS 100409 was collected from *Tradescantia* sp., the same host family as *Phoma commelinicola* (*Commelinaceae*), and therefore it was initially identified as "*Phoma commelinicola*" (De Gruyter 2002). However, its conidia [(10.5–)13–17(–21) × (4–)5–6.5 µm] were found to differ from the original description of *Phoma commelinicola*. Phylogenetically, CBS 100409 formed a distinct lineage, separated from all genera previously described in *Didymellaceae*. Thus, a new genus and species, *Macroascochyta grandis*, was introduced to accommodate this isolate.

Clade 7: *Ectophoma* Valenz.-Lopez *et al.*, Stud. Mycol. 90: 34. 2017 (2018).

Type species: *Ectophoma multirostrata* (P.N. Mathur *et al.*) Valenz.-Lopez *et al.*

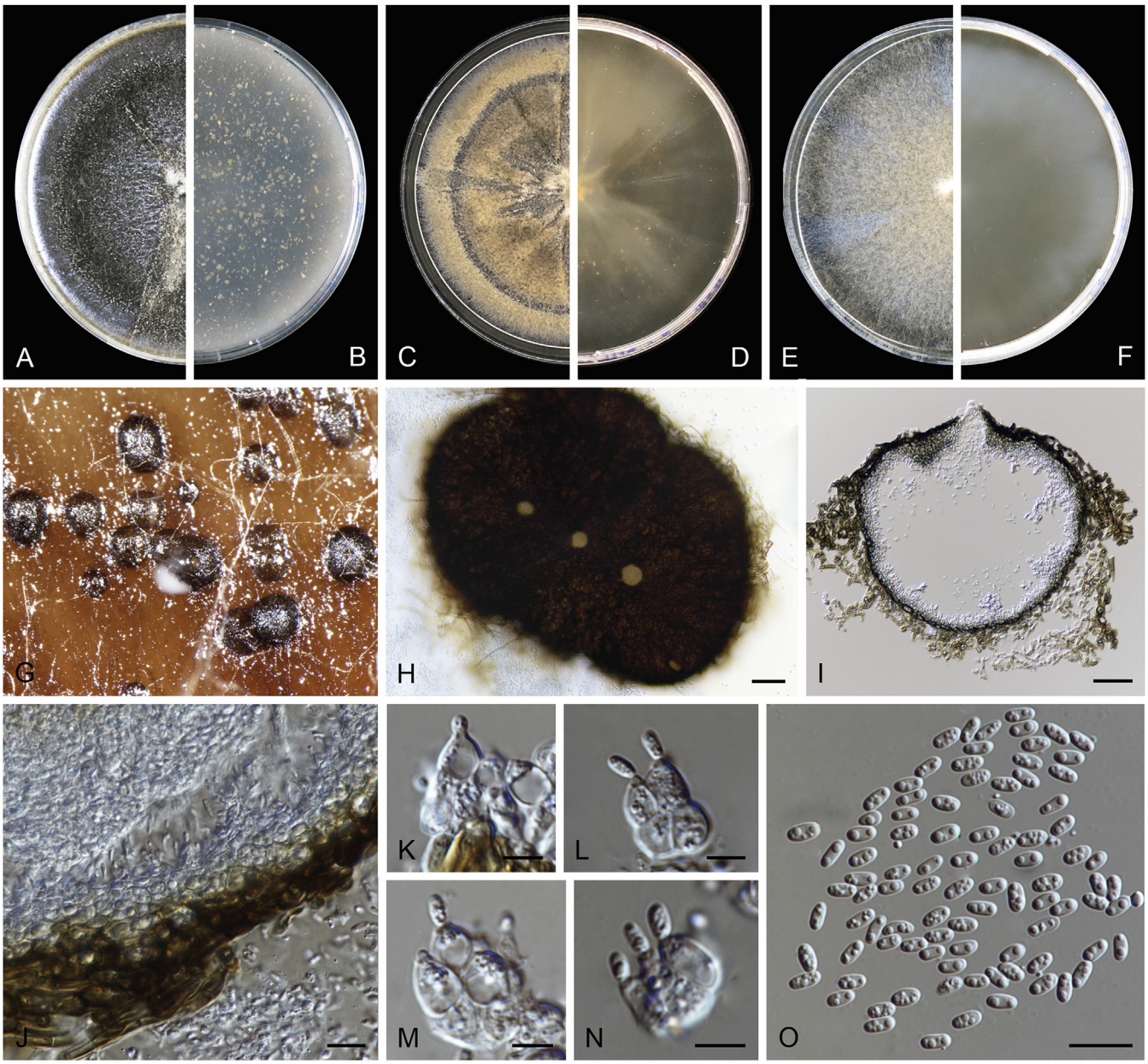

Fig. 11. *Ectophoma insulana* (CBS 252.92). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H–I = 50 µm; J, O = 10 µm; K–N = 5 µm.

Ectophoma insulana (Mont.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833480. Fig. 11

Basionym: *Phyllosticta insulana* Mont., Ann. Sci. Nat., Bot. IV 5: 343. 1856.

Synonym: *Phoma insulana* (Mont.) Boerema & Malathr., Versl. Meded. Plziektenk. Dienst Wageningen (Jaarb. 1981) 158: 28. 1982.

Description from ex-neotype (CBS 252.92): *Conidiomata* pycnidial, (semi-)immersed in the agar, solitary, or 2–5 confluent, scattered or aggregated, (sub-)globose, at the beginning buff, thin-walled, becoming dark brown with age, glabrous, ostiolate, 100–300(–510) × 100–270(–370) µm. *Ostioles* 1–4, increasing to 2–6(–12) when confluent, non-papillate or distinctly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–7 layers, 13–50 µm thick, outer 2–3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, lageniform or doliiform, 5–8 × 4–6.5 µm. *Conidia* ellipsoidal or oblong with both ends rounded, hyaline, smooth- and thin-walled, aseptate, 4–6.5 × 2–3 µm, with two large, polar guttules or several small scattered guttules. *Conidial matrix* whitish to buff.

Culture characteristics: Colonies on OA reaching 70–75 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, olivaceous black; reverse concolourous. Colonies on MEA reaching 65–70 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, with some radially furrowed zones, concentric circles of different colours, centre olivaceous, continued by a black circle formed by abundant pycnidia, buff towards periphery; reverse vinaceous buff to hazel. Colonies on PDA reaching 75–80 mm diam after 7 d, margin regular, covered by sparsely felty aerial mycelium, vinaceous buff; reverse olivaceous greenish. NaOH spot test negative on OA.

Typus: **Greece**, from fruit of *Olea europaea* (*Oleaceae*), deposited in May 1992, J. de Gruyter (**neotype designated here** CBS H-23679, MBT389673, ex-neotype living culture CBS 252.92 = PD 80/1144).

Additional material examined: **South Africa**, Western Cape Province, Kuilsrivier, Tarentaal Street, from house dust, 24 Jul. 2009, K. Jacobs, culture CBS 140548.

Notes: The holotype of *Phoma insulana* (basionym: *Phyllosticta insulana*), which was originally described from leaves of *Olea europaea* in Montaud-lès-Miramas, France, could not be located in PC (Muséum d'histoire naturelle; Montagne 1856), and is considered lost. In this study, isolate CBS 252.92 was collected from the same host in the Netherlands, and was described as a representative culture of *Phoma insulana* (De Gruyter *et al.* 1993). Here we designated CBS 252.92 as ex-neotype strain, as its morphological characters (conidial size) agreed well with the original description of *Phyllosticta insulana* (4–6.5 × 2–3 µm *vs*. 6 µm in length, Montagne 1856). Based on our phylogenetic result, culture CBS 252.92 clustered within the *Ectophoma* clade, forming a distinct lineage. Hence, we introduced a new combination for this taxon, *Ectophoma insulana*.

Ectophoma multirostrata (P.N. Mathur *et al.*) Valenz.-Lopez *et al.*, Stud. Mycol. 90: 34. 2017 (2018).
Basionym: *Sphaeronaema multirostratum* P.N. Mathur *et al.*, Sydowia 13: 146. 1959. (as "*Sphaeronema*")
Synonyms: *Phoma multirostrata* (P.N. Mathur *et al.*) Dorenb. & Boerema, Mycopathol. Mycol. Appl. 50: 256. 1973, *emend*. Aveskamp *et al.* Mycologia 101: 375. 2009.
Phoma liliana S. Chandra & Tandon, Curr. Sci. 34: 566. 1965.
Phoma ehretiae S. Chandra & Tandon, Mycopathol. Mycol. Appl. 29 (3–4): 276. 1966.
Phoma terrestris Saksena *et al.*, Mycopathol. Mycol. Appl. 29: 86. 1966 (as "*P. terrestre*"); non *P. terrestris* Hansen.

Description: Boerema *et al.* (2004).

Typus: **India**, Maharashtra, Poona, Talegaon, from poultry farm soil, Mar. 1959, M.J. Thirumalachar (**isotype** CBS H-7616, ex-isotype living culture CBS 274.60 = IMI 081598 = FMR 15335).

Additional materials examined : **India**, Maharashtra, Poona, Talegaon, from soil, Mar. 1959, M.J. Thirumalachar, culture CBS 368.65 = PD 92/1757 = FMR 15336; host unknown, M.J. Thirumalachar, culture CBS 383.65 (ex-isotype living culture of "*Labyrinthophoma pimprina* P.N. Mathur & Thirumalachar, *ined.*"); University of Allahabad, Botanical Garden, from leaves of *Lilium* sp. (*Liliaceae*), Nov. 1963, S. Chandra & R.N. Tandon, culture CBS 380.67 = IMI 105607 (ex-isotype living culture of *Phoma liliana*); host unknown, Jun. 1965, M.J. Thirumalachar, culture CBS 340.65 (ex-isotype living culture of "*Sphaeronaema roseum* P.N. Mathur & Thirumalachar, *ined.*"). **The Netherlands**, Hoorn, greenhouse, from the stem of *Cucumis sativus* (*Cucurbitaceae*), Aug. 1967, G.H. Boerema, culture CBS 110.79 = PD 65/8875 = FMR 15342.

Notes: CBS 380.67 and CBS 340.65 were received as ex-isotype strains of "*Phoma liliana*" and "*Sphaeronaema roseum*" respectively. *Phoma liliana* was originally described from leaves of *Lilium* sp. in India (Chandra & Tandon 1965). A subsequent study treated both species as synonyms of *Phoma multirostrata* (currently *Ectophoma multirostrata*) based on morphological characters (Dorenbosch & Boerema 1973). On the other hand, isolate CBS 383.65 was deposited in the CBS collection in 1965 as ex-isotype of "*Labyrinthophoma pimprina*". However, together with *Sp. roseum*, these two names were never published in a resource indexed in Index Fungorum or MycoBank. In the present study, the ex-isotype strains of *Phoma liliana*, *Sp. roseum* and *La. pimprina* were revealed to be genetically identical to *Ec. multirostrata* in all sequenced loci, which verified the treatment of Dorenbosch & Boerema (1973).

Clade 8: *Remotididymella* Valenz.-Lopez *et al.*, Stud. Mycol. 90: 35. 2017 (2018).

Conidiomata pycnidial, brown to dark brown, mostly confluent; *pycnidial wall* of textura angularis, mostly glabrous, globose or irregularly-shaped, with a single ostiole. *Conidiogenous cells* phialidic, hyaline, smooth-walled, globose or ampulliform. *Conidia* aseptate, hyaline, smooth- and thin-walled, allantoid or cylindrical, guttulate. (Valenzuela-Lopez *et al.* 2018). *Chlamydospores* intercalary or terminal, solitary, subhyaline to dark brown, variable, irregular, verruculose or incidentally tuberculate, 1–2 cells, smooth. *Ascomata* immersed or superficial, globose, conical globose to lenticular, scattered or clustered, papillate or apapillate, ostiolate. *Peridium* composed of several layers of brown to hyaline cells of *textura angularis*, fusing at the outside with the host tissue. *Hamathecium* dense, filamentous, septate, branching and hyaline, cellular pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, clavate to cylindrical, short-pedicellate. *Ascospores* overlapping 2–3-seriate, hyaline, fusiform, 1–3-septate, constricted at middle septum (Jayasiri *et al.* 2019).

Type species: *Remotididymella destructiva* (Plowr.) Valenz.-Lopez *et al.*

Notes: *Remotididymella* was typified by *R. destructiva* (basionym *Phoma destructiva*) that is notorious by infecting tomato crops and causing light brown necroses (Boerema *et al.* 2004, Valenzuela-Lopez *et al.* 2018). Later, several new species were introduced in this genus, including *R. bauhiniae* that is known from its sexual morph. *Remotididymella bauhiniae* is the first record of a sexual morph for this genus, and therefore the generic description of *Remotididymella* is emended with the addition of sexual morph characters.

Remotididymella brunnea L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833555. Fig. 12.

Etymology: Name reflects the brown (= *brunus* in Latin) pycnidia produced by this species.

Description: *Conidiomata* pycnidial, immersed or semi-immersed, solitary, sometimes confluent, scatter or aggregated, (sub-)globose, dark brown at beginning, thin-walled, with mycelial outgrowths, ostiolate, become pale brown with age, 90–240 × 100–190 µm. *Ostiole* 0–1, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 2–3 layers, 6.5–26 µm thick, outer 1–2 layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform or lageniform, 5.5–8.5 × 4.5–7.5 µm. *Conidia* cylindrical to oblong with both ends rounded, hyaline, smooth- and thin-walled, aseptate, sometimes constricted at the middle part, 4.5–6.5 × 2–3 µm, with 2–3 large guttules. *Conidial matrix* white.

Culture characteristics: Colonies on OA reaching 65 mm diam after 7 d at 25 °C, margin regular, the section produced pycnidia without aerial mycelium, olivaceous, and the sterile sector covered by flat and grey to darker grey mycelium; reverse concolourous. Colonies on MEA reaching 50–55 mm diam after 7 d, margin regular, aerial mycelium floccose, buff; reverse dark brown with orange edge. Colonies on PDA reaching 65–70 mm diam after 7 d, margin regular, densely covered by felty buff

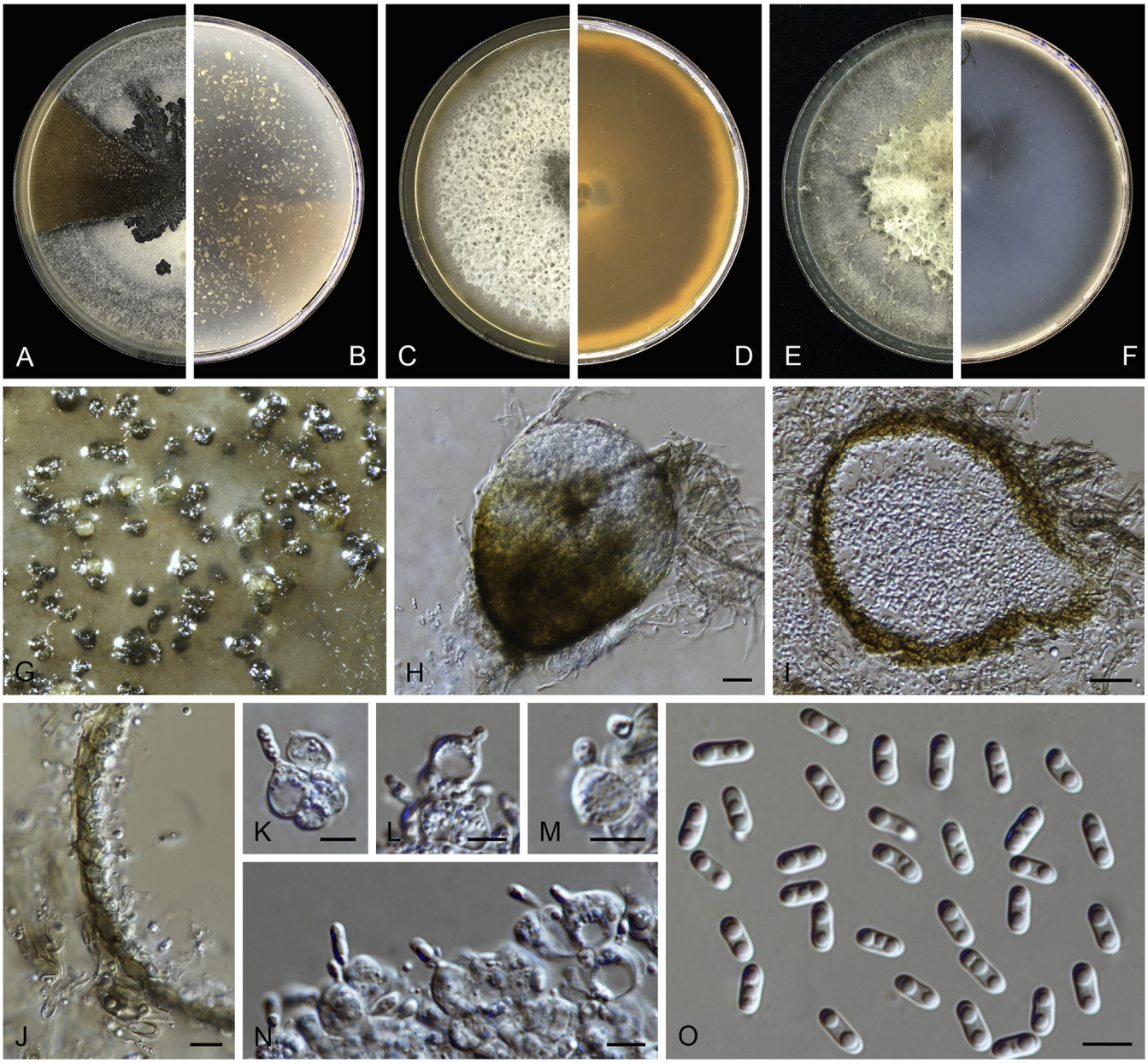

Fig. 12. *Remotididymella brunnea*. (CBS 993.95). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H, I = 20 µm; J = 10 µm; K–O = 5 µm.

aerial mycelium near the center, flat and pale olivaceous grey towards the periphery; reverse pale greenish grey. NaOH spot test negative on OA.

Typus: **Papua New Guinea**, Madang Provence, Brahman, from soil in tropical forest, Nov. 1995, A. Aptroot (**holotype** CBS H-23685, ex-type living culture CBS 993.95).

Notes: Phylogenetically, *Remotididymella brunnea* forms a sister clade to *R. bauhiniae* with moderate support. *Remotididymella brunnea* differs in 9 bp in ITS, 33 bp in *rpb2* from *R. bauhiniae* (*tub2* is missing in *R. bauhiniae*). However, the morphological comparison is difficult as *R. bauhiniae* was only described based on a sexual morph with production of chlamydospores (Jayasiri *et al.* 2019), while we only observed the asexual morph of *R. brunnea* in culture, lacking chlamydospores. Besides, morphological comparisons revealed that *R. brunnea* was similar to *R. humicola*, another novel species collected from soil and described in the present study, in the shape of its conidia and conidiogenous cells, but was clearly distinguished from the latter by producing shorter conidia [4.5–6.5 × 2–3 µm *vs.* 6–8.5(–11.5) × 2–3 µm].

Remotididymella capsici (Bond.-Mont.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB834117.
Basionym: *Ascochyta capsici* Bond.-Mont., Monitor Phytopath. Sect. Chief Bot. Gard. R.S.F.S.R. 12: 72. 1923.

Description: Culture sterile.

Culture characteristics: Colonies on OA reaching 70–75 mm diam after 7 d at 25 °C, margin regular, pale brown without aerial mycelium near the centre, buff with flat aerial mycelium toward periphery; reverse buff to honey. Colonies on MEA reaching 65–70 mm diam after 7 d, margin regular, aerial mycelium floccose, whitish with some black zones; reverse pale brown near the centre and brown toward periphery. Colonies on PDA reaching 80–85 mm diam after 7 d, margin regular, covered by

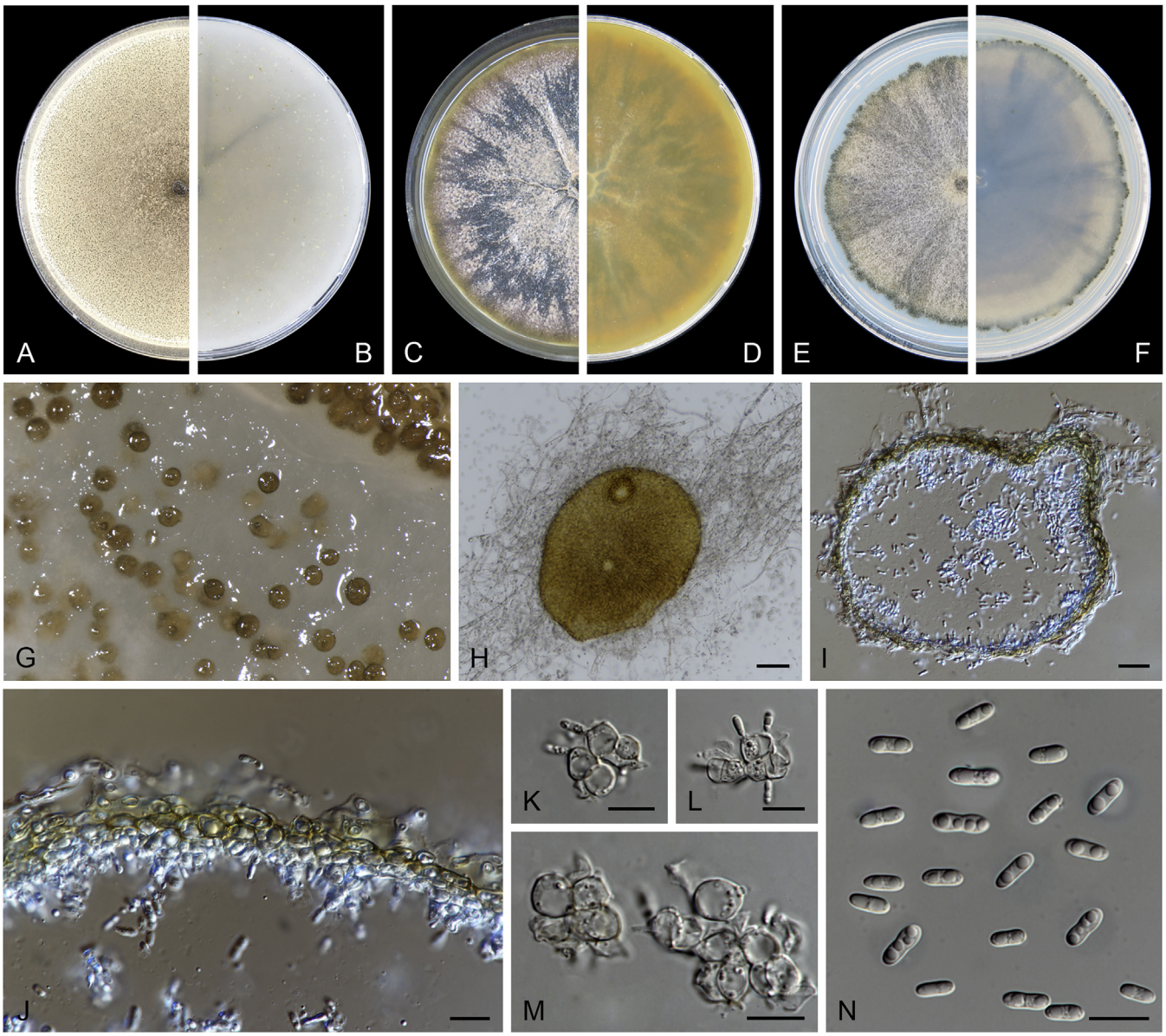

Fig. 13. *Remotididymella humicola* (CBS 120117). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 50 µm; I = 20 µm; J–N = 10 µm.

felty aerial mycelium, whitish, with olivaceous grey edge; reverse iron-black with buff edge. NaOH spot test a pale olivaceous discolouration on OA.

Material examined: **The Republic of Fiji**, from leaf spot on *Capsicum annuum* (*Solanaceae*), 9 Jul. 1977, isol. J.D. Baird, dep. G.F. Laundon, specimen CBS H-24340, culture CBS 679.77 = LEV 11926b.

Notes: In this study, isolate CBS 679.77 was originally received as *Ascochyta capsici*, isolated from the same host as *As. capsici* (from the living leaves of *Capsicum annuum* in Russia) in Fiji. Phylogenetically, CBS 679.77 clustered in *Remotididymella*, forming an independent lineage which was distinct from other species. Although we introduce a new combination for this taxon in *Remotididymella*, future collections from *Capsicum annuum* in Russia may well show CBS 679.77 to represent a new species.

Remotididymella humicola L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833499. Fig. 13.

Etymology: Name derived from the substrate this species was collected from, soil.

Description: *Conidiomata* pycnidial, (semi-)immersed, solitary, scattered on the agar, (sub-)globose, pale brown, thin-walled, glabrous, ostiolate, 110–410 × 110–400 µm. *Ostioles* 1–5, papillate, sometimes elongated with a long neck, up to 100 µm. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–4 layers, 6–21(–30) µm thick, outer cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose to ampulliform, 5.5–9.5 × 5–8 µm. *Conidia* cylindrical or oblong, with both ends rounded, hyaline, smooth- and thin-walled, aseptate, sometimes constricted in the middle, 6–8.5(–11.5) × 2–3 µm, with 2–4 large, polar guttules. *Conidial matrix* whitish to buff.

Culture characteristics: Colonies on OA reaching 70 mm diam after 7 d at 25 °C, margin regular, without aerial mycelium, buff, abundant production of pycnidia; reverse concolourous. Colonies on MEA reaching 70–75 mm diam after 7 d, margin regular, without aerial mycelium, pinkish with some black zones, brown towards periphery, becoming flesh with age because of the conidial matrix exuding from pycnidia; reverse brownish.

Colonies on PDA reaching 70–75 mm diam after 7 d, margin regular, covered by sparsely felty aerial mycelium, greyish brown with olivaceous margin; reverse concolourous. NaOH spot test negative on OA.

Typus: **Puerto Rico**, from soil in onion field, 1 Jul. 2004, A.R. Rossman (**holotype** CBS H-23687, ex-type living culture CBS 120117).

Notes: The genus *Remotididymella* was originally introduced to accommodate *R. anthropophila* and *R. destructiva* (Valenzuela-Lopez *et al.* 2018). *Remotididymella humicola* was represented by an isolate from soil collected in Puerto Rico, which formed a distinct lineage clearly different from other species in *Remotididymella*. Morphologically, *R. humicola* was distinguished from its closest neighbour *R. anthropophila* by having larger conidia [6–8.5(–11.5) × 2–3 µm *vs*. 5.5–7.5 × 1.5–2.5 µm] and conidiogenous cells (5.5–9.5 × 5–8 µm *vs*. 5–6 µm; Valenzuela-Lopez *et al.* 2018). Moreover, *R. humicola* and *R. anthropophila* also differed in the colour of their pycnidia and number of ostioles: *R. anthropophila* produced apricot to pale brown pycnidia with single ostioles, while pycnidia of *R. humicola* were brown with 1–6 ostioles.

Clade 9: *Pseudopeyronellaea* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833500.

Etymology: Name refers to the morphological similarity with the genus *Peyronellaea*.

Ascomata pseudothecial, globose, pyriform, superficial, with central papillate ostiole, lacking setae; *wall* of multi-layered. *Pseudoparaphyses* absent. *Asci* bitunicate, narrowly ellipsoid to subcylindrical, 8-spored. *Ascospores* bi- to triseriate, hyaline, ovate to fusoid, prominently guttulate with mucoid sheath, widest just above septum, ends subobtusely rounded. *Asexual morph* unknown.

Notes: Species of *Peyronellaea* are characterised by producing pycnidial conidiomata with phialidic conidiogenous cells as well as dictyochlamydospores, having both transverse and longitudinal septa (Aveskamp *et al.* 2009a). Later, *Peyronellaea* was synonymised with *Didymella* (Chen *et al.* 2015) after phylogenetic analysis. In the present study, *Peyronellaea eucalypti* that was collected from leaves of *Eucalyptus pellita* (*Myrtaceae*), with only a sexual morph observed in culture, is phylogenetically distant from the *Didymella* clade and other known genera of *Didymellaceae*. Therefore, a new genus *Pseudopeyronellaea* is introduced to accommodate *Peyronellaea eucalypti*. Morphologically, *Pseudopeyronellaea* differs from *Didymella* by producing bi- to triseriate, ovate to fusoid and prominently guttulate ascospores with mucoid sheaths, while ascospores of *Didymella* species are biseriate, ellipsoidal to cymbiform (Chen *et al.* 2015).

Type species: *Pseudopeyronellaea eucalypti* (Crous & M.J. Wingf.) L.W. Hou *et al.*

Pseudopeyronellaea eucalypti (Crous & M.J. Wingf.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833501.
Basionym: *Peyronellaea eucalypti* Crous & M.J. Wingf., Persoonia 38: 277. 2017.

Description: Crous *et al.* (2017a).

Typus: **Malaysia**, Sabah, from leaves of *Eucalyptus pellita* (*Myrtaceae*), May 2015, M.J. Wingfield (**holotype** CBS H-23085, ex-type living culture CBS 142522 = CPC 27678); *ibid*., culture CPC 27682.

Notes: The holotype of *Peyronellaea eucalypti* was described from leaves of *Eucalyptus pellita* collected in Sabah, Malaysia (Crous *et al.* 2017a). Based on the phylogenetic analysis of the ITS gene, two isolates of *Pe. eucalypti* (CBS 142522 and CPC 27682) were phylogenetically related to *Didymella glomerata* (Crous *et al.* 2017a). However, in the multigene analysis these isolates formed an independent lineage distant from *Peyronellaea* (currently: *Didymella*). Consequently, *Pseudopeyronellaea* was introduced as a new genus to accommodate this taxon.

Clade 10: *Longididymella* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833502.

Etymology: Name reflects the longer (= *Longi*, in Latin) conidia characteristic of these fungi.

Ascomata pseudothecial, globose, subglobose to pyriform, superficial, solitary or clustered, ostiolate, with prominent elongated neck, lacking setae; *wall* black, *textura globulosa*, multi-layered. *Pseudoparaphyses* absent. *Asci* bitunicate, cylindrical with club-shaped base, 8-spored. *Ascospores* hyaline, septate, ovate to obpyriform, smooth. *Ascospore mass* white. *Conidiomata* pycnidial, superficial or semi-immersed, also immersed in the agar or on aerial mycelium, (sub-)globose, flask-shaped or irregular, glabrous, scattered, solitary or confluent, ostiolate. *Ostioles* on an elongated neck. *Pycnidial wall* pseudoparenchymatous, 2–5 layers, outer layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, variable in shape and size, globose, flask-shaped, ampulliform or doliiform, with minute periclinal thickening. *Conidia* ellipsoidal, elongated, allantoid, smooth- and thin-walled, 0–1-septate, mostly guttulate, incidentally smaller, aseptate conidia present.

Notes: *Longididymella* was proposed to accommodate two species collected from leaves of *Clematis* spp. Both species were originally received as *Phoma clematidina*. However, their sexual morphs were observed to be similar to *Didymella* (Woudenberg *et al.* 2009). By means of DNA sequence comparisons, they were described as members of *Didymella* (Woudenberg *et al.* 2009). In the present study, *Didymella clematidis* and *Did. vitalbina* formed a fully supported clade that is distant from *Didymella* and distinct from other known genera in family *Didymellaceae*. Considering that both species produced relatively long conidia, a new genus *Longididymella* is introduced.

Type species: *Longididymella vitalbae* (Crous & A.R. Wood) L.W. Hou *et al.*

Longididymella clematidis (Woudenb. *et al.*) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833503.
Basionym: *Didymella clematidis* Woudenb. *et al.*, Persoonia 22: 60. 2009.

Description: Woudenberg *et al.* (2009).

Typus: **USA**, Washington State, Toppenish, on leaves of *Clematis ligusticifolia* (*Ranunculaceae*), 7 Sep. 1991, A.G. Spiers (**holotype** PDD69379, ex-type living culture ICMP 13664 = AGS 30/32 = CBS 123705 = PD 97/13460-1).

Notes: This species was originally described by Woudenberg *et al.* (2009) as *Didymella clematidis*, infecting leaves of *Clematis ligusticifolia* in Toppenish, Washington State, USA.

However, in our phylogenetic analysis of more loci, the ex-type strain of *Did. clematidis* (CBS 123705) clustered within the new genus *Longididymella* introduced in the present study. Both species of *Longididymella* could produce sexual and asexual morphs *in vitro*.

Longididymella vitalbae (Briard & Har.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833505.
Basionym: *Ascochyta vitalbae* Briard & Har., Rev. Mycol. (Toulouse) 13: 17. 1891.
Synonyms: *Diplodina vitalbae* (Briard & Har.) Allesch., Rabenh. Krypt.-Fl., ed. 2. Pilze 6 (Lief. 69): 683. 1900 (vol. dated1901).
Diplodina clematidina Fautrey & Roum., Rev. Mycol. (Toulouse) 14: 105. 1892.
Didymella vitalbina Petr., Ann. Mycol. 38: 348. 1940.

Description: Woudenberg *et al.* (2009).

Typus: **Switzerland**, Gampel-Steg, on leaves of *C. vitalba*, 10 Oct. 1991, A.G. Spiers, **epitype** of *Didymella vitalbina* PDD69378, ex-epitype living culture ICMP 13663 = AGS 9 = PD 97/13460-2 = CBS 123707.

Additional materials examined: **France**, Var, Jouques, on leaves of *Clematis vitalba* (*Ranunculaceae*), Dec. 1964, E. Müller, specimen CBS H-11972, culture ETH 2672 = CBS 454.64. **Germany**, Alf a. d. Mosel, on leaves of *C. vitalba*, 3 Oct. 1987, H.A. van der Aa, specimen CBS H-16192, culture CBS 911.87. **The Netherlands**, Renkum, Jufferswaard, on leaves of *C. vitalba*, 9 Apr. 2008, J. de Gruyter, culture CBS 123706.

Notes: The holotype of *Didymella vitalbina* (basionym: *Ascochyta vitalbae*) was originally isolated from *Clematidis vitalbae* in Gaisberg, Austria (Petrak 1940). Woudenberg *et al.* (2009) designated CBS 123707 as ex-epitype for this species based on its similar morphological characters. In this study, *Did. vitalbina* was represented by five strains including the ex-epitype (CBS 123707), which together with the ex-type strain of *Did. clematidis* (CBS 123705), formed a fully supported independent lineage that was distinct from its closest genus *Ectophoma*. Therefore, *Longididymella* was proposed as a new genus to accommodate these two species.

Clade 11: *Ectodidymella* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833506.

Etymology: From the Greek εκτος, outside, because it is phylogenetically distant from *Didymella*.

Conidiomata pycnidial, scattered, globose, depressed globose, black, large, usually with a conspicuous neck. *Pycnidial wall* thick, pseudoparenchymatous, outer layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform, with minute periclinal thickening. *Conidia* oblong to subcylindrical, eguttulate or with a small guttule at each end. *Ascomata* pseudothecial, only recorded in one species in vivo, erumpent, globose to irregular-shaped, solitary or confluent, scattered or aggregated, ostiolate, with a small papilla-shaped ostioles, lacking setae. *Pseudothecial wall* rough, multi-layered, composed of pseudoparenchymatous cells, outer layers pigmented, black-brown. *Asci* narrow, cylindrical to clavate, 4–8-spored, coarse and thick-walled. *Pseudoparaphyses* scanty and atypical, coarse, branched. *Ascospores* hyaline, biseriate, elongated clavate to fusiform, uniseptate, slightly or barely constricted at the septum, upper cell slightly wider than the lower cell (Petrak 1928).

Notes: *Ectodidymella* is typified by *Phoma nigrificans*. The sexual morph of *Phoma nigrificans* (*Didymella macropodii*) is morphologically similar with species of *Didymella*. However, phylogenetically it forms a distinct clade that is distant from *Didymella* and separated from all genera previously described in *Didymellaceae*. Morphologically, *Ectodidymella* differs from *Didymella* by occasionally producing four ascospores in a single ascus, which is rare in *Didymellaceae*.

Type species: *Ectodidymella nigrificans* (P. Karst.) L.W. Hou *et al.*

Ectodidymella nigrificans (P. Karst.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833507. Fig. 14.
Basionym: *Sphaeronaema nigrificans* P. Karst., Meddeland. Soc. Fauna Fl. Fenn. 16: 17. 1888. (as "*Sphaeronema*").
Synonyms: *Phoma nigrificans* (P. Karst.) Boerema *et al.*, J. Phytopathol. 115(3): 270. 1986.
Didymella macropodii Petr., Hedwigia 68: 219. 1928.
Plenodomus macropodii Petr., Hedwigia 68: 237. 1928.

Description: Boerema & de Gruyter (1998) and Petrak 1928.

Materials examined: **Germany**, from *Brassica napus* (*Brassicaceae*), 1982, G.H. Boerema, culture CBS 100190 = PD 82/736. **The Netherlands**, from an unidentified crucifer (*Cruciferae* sp., *Brassicaceae*), 1984, G.H. Boerema, culture PD 84/512.

Notes: *Sphaeronaema nigrificans* was described from *Armoraciae rusticanae* in Finland, with conidia measuring 6–9 × 2–3 µm (Karsten 1888). This species was later recombined into *Phoma* as *Phoma nigrificans*, while its connection with *Didymella macropodii* was established by Boerema *et al.* (1986). Later Boerema *et al.* (2004) described CBS 100190 and CBS 100191 as representative cultures of *Phoma nigrificans* based on morphology. However, CBS 100191 clustered with *Phomatodes nebulosa* (Chen *et al.* 2015), distinct from CBS 100190, which was also examined in the present study (Fig. 1). Isolate CBS 100190, together with PD 84/512 formed an independent clade which was distant from other genera in *Didymellaceae*. Morphologically CBS 100190 agreed well with the original description of *Sp. nigrificans* by producing comparably sized conidia (6–9 × 2–3 µm *vs.* 4.5–8 × 2–3.5 µm). Hence, a new genus *Ectodidymella* was introduced to accommodate this species.

Clade 12: *Epicoccum* Link, Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 7: 32. 1815, *emend*. Q. Chen & L. Cai, Stud. Mycol. 82: 171. 2015.

Type species: *Epicoccum nigrum* Link

Epicoccum brahmansense L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833508. Fig. 15.

Etymology: Epithet derived from the location of origin, Brahman, Madang Province in Papua New Guinea.

Description: *Conidiomata* pycnidial, (semi-)immersed in agar, solitary or confluent, scattered, mostly (sub-)globose to flask-shaped, pale brown, thin-walled, with hyphal outgrowths, ostiolate, 150–350 × 135–265 µm. *Ostioles* 1–4(–8), non-papillate or slightly papillate and forming a collarette. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–6 layers, (8.5–)12.5–29 µm thick, outer 1–3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose to ampulliform, 5–9 × 3.5–8 µm. *Conidia* ovoid to ellipsoidal,

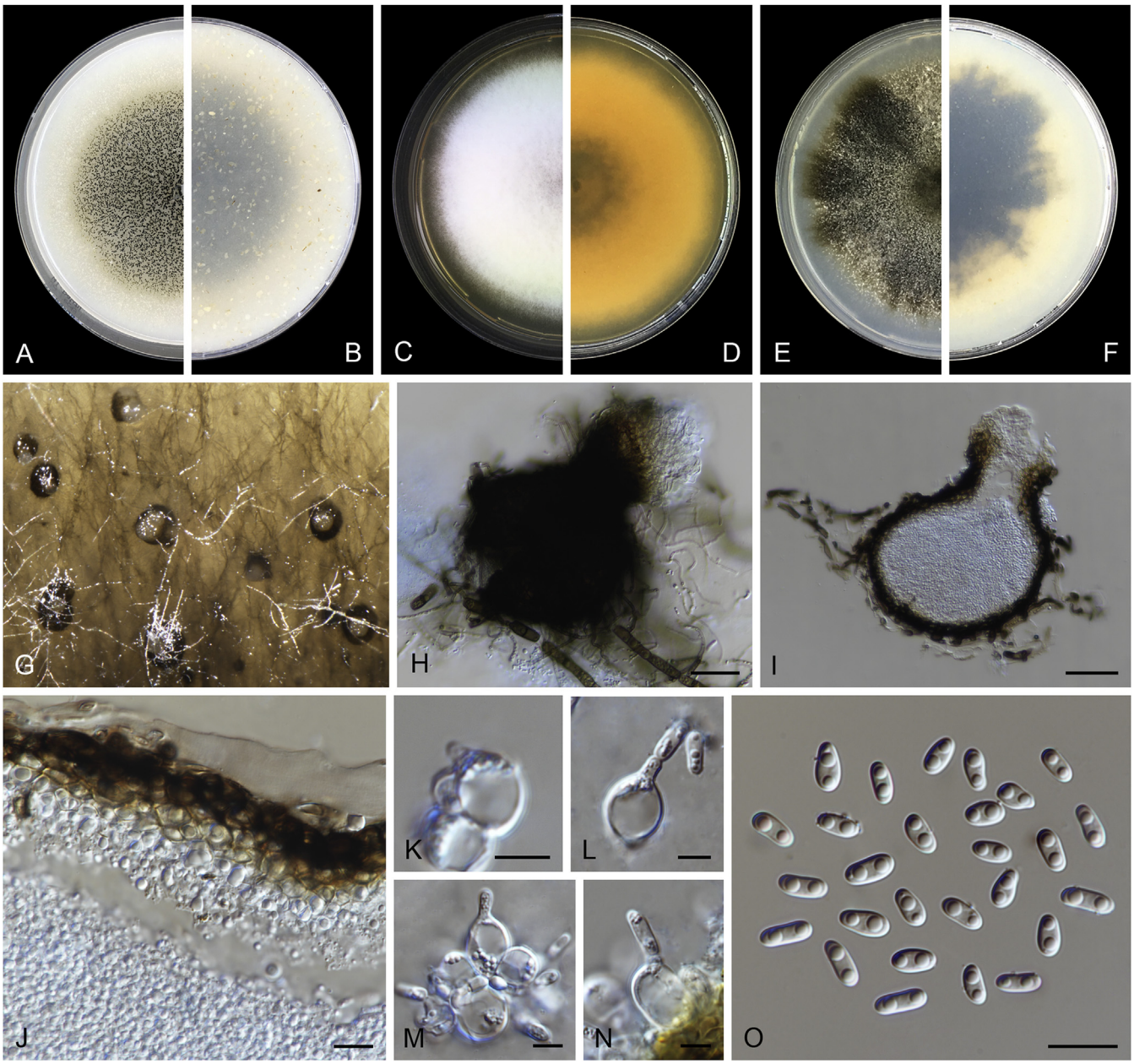

Fig. 14. *Ectodidymella nigrificans* (CBS 100190). **A–B**. Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H–I = 50 µm; J, O = 10 µm; K–N = 5 µm.

hyaline, smooth- and thin-walled, aseptate, 3.5–6 × 2.5–3.5 µm, with two medium, polar guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 50–55 mm diam after 7 d at 25 °C, margin regular, aerial mycelium felty, whitish to pink; reverse olivaceous to dark brick. Colonies on MEA reaching 50–55 mm diam after 7 d, margin regular, aerial mycelium abundant, woolly, buff, mycelium flat and dark brown near the centre; reverse brown with orange edge. Colonies on PDA reaching 40–45 mm diam after 7 d, margin regular, covered by felty aerial mycelium, floccose near the centre, buff to pink, smoke grey toward periphery; reverse olivaceous with brownish centre, abundant dark brown secondary metabolite visible. NaOH spot test: olivaceous with red margin on OA.

Typus: **Papua New Guinea**, Madang Province, Brahman, from soil in tropical forest, Nov. 1995, A. Aptroot (**holotype** CBS H-23667, ex-type living culture TA10NZ-306 = CBS 990.95).

Additional material examined: **Papua New Guinea**, Madang Province, Brahman, from soil in tropical forest, Nov. 1995, A. Aptroot, culture CBS 985.95.

Notes: In the phylogenetic tree, *Epicoccum brahmansense* was represented by two isolates (CBS 990.95 and CBS 985.95; from soil in Papua New Guinea) which clustered in a distinct clade clearly distinguished from other species in this genus. Morphologically, *Ep. brahmansense* could be differentiated from its closest neighbours *Ep. draconis* and *Ep. multiceps* in its conidial size and the numbers of ostioles: *Ep. brahmansense* differed from *Ep. draconis* in its larger pycnidia (150–350 × 135–265 µm *vs*. 90–220 µm) and smaller conidia (3.5–6 × 2.5–3.5 µm *vs*. 4–8.5 × 2–4 µm). Although *Ep. brahmansense* is similar to *Ep. multiceps* in conidial size, the latter usually produces larger pycnidia with more ostioles [150–350 × 135–265 µm, 1–4(–8) ostioles *vs*. 135–540 × 128–465 µm, 3–13 ostioles].

Epicoccum dickmanii L.W. Hou & O. Yarden, ***sp. nov.*** MycoBank MB834682. Fig. 16.

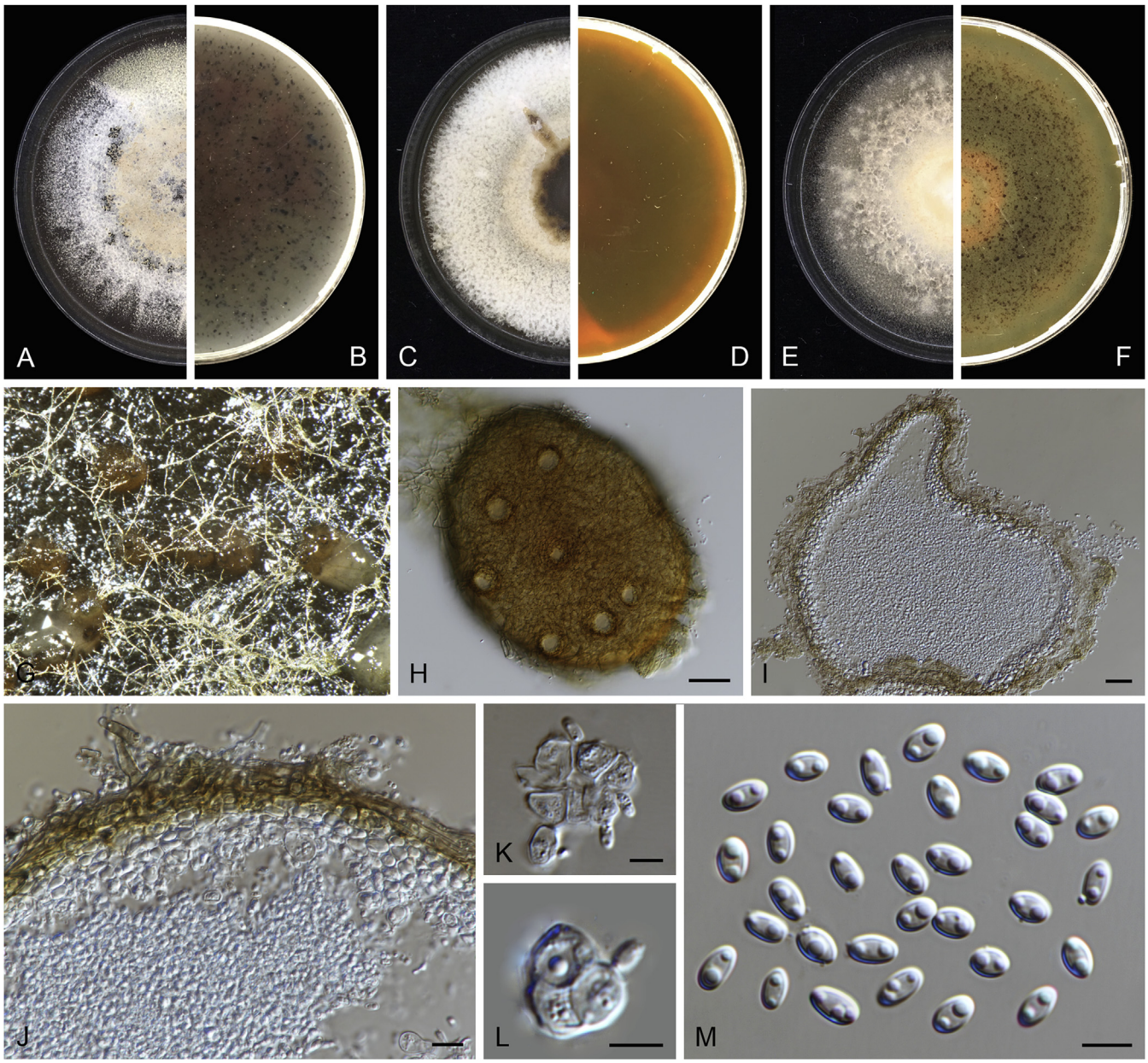

Fig. 15. *Epicoccum brahmansense* (CBS 990.95). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–L.** Conidiogenous cells. **M.** Conidia. Scale bars: H = 50 µm; I = 20 µm; J = 10 µm; K–M = 5 µm.

Etymology: Name in memory of Prof. Martin B. Dickman, a leader in the study of fungal biology and fungal-host interactions.

Description: *Conidiomata* pycnidial, immersed in agar, scattered, mostly solitary, (sub-)globose, or ellipsoidal, rosy buff to buff, thin-walled, with hyphal outgrowths, 100–310 × 95–260 µm. *Ostioles* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–4 layers, 8–10 µm thick, without pigmented layers. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform or lageniform, 7.5–12 × 7.5–10 µm. *Conidia* variable in shape and size, oblong, ovoid or broad ellipsoidal, smooth- and thin-walled, aseptate, 4.5–10.5 × 3–4.5 µm, with 2–3 minute guttules. *Conidial matrix* rosy buff.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d 25 °C, margin regular, aerial mycelium floccose, whitish, aerial mycelium flat and buff towards periphery; reverse buff to pale olivaceous. Colonies on MEA reaching 45–55 mm diam after 7 d, margin regular, aerial mycelium woolly, rosy buff; reverse orange, buff towards periphery. Colonies on PDA reaching 50–55 mm diam after 7 d, margin regular, aerial mycelium floccose, buff near the centre, aerial mycelium sparse, pale brown to whitish toward periphery, abundant production of pycnidia; reverse buff, pale brown to brown. NaOH spot test negative on OA.

Typus: **Australia**, Great Barrier Reef, from a brown band on *Acropora formosa* (*Acroporidae*; coral), Jul. 2006, O. Yarden (**holotype** CBS H-24342, ex-type living culture CBS 124671 = OY8406).

Additional material examined: **Australia**, Great Barrier Reef, from a brown band on *Acropora formosa*, Jul. 2006, O. Yarden, culture CBS 124672.

Notes: Two strains of *Epicoccum dickmanii* that were isolated from *Acropora formosa* formed a distinct branch on the multi-locus tree (Fig. 1). *Epicoccum dickmanii* is phylogenetically close to *Ep. duchesneae*, a species reported from leaves of *Duchesnea indica* in China. However, it differs from the latter in

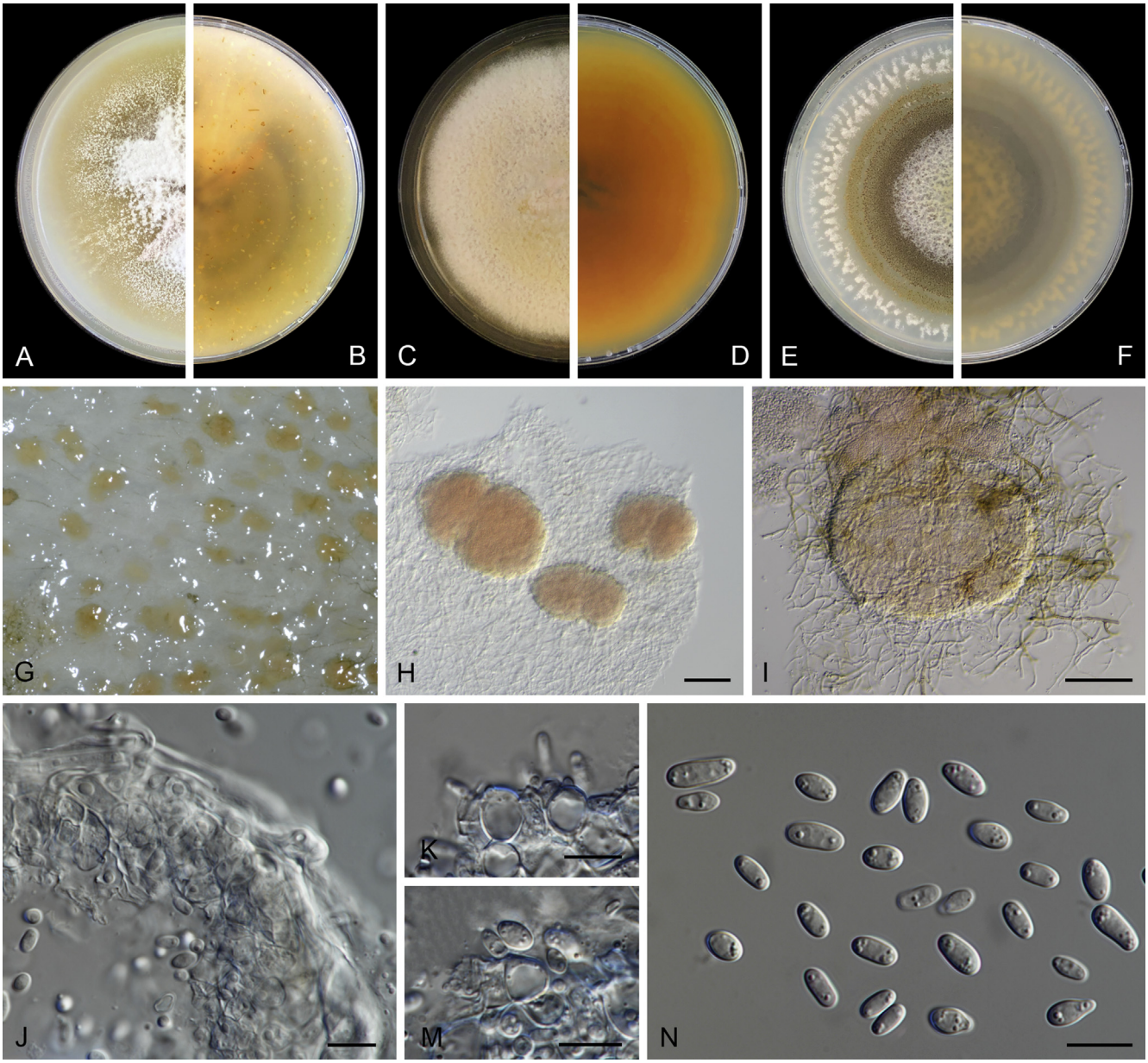

Fig. 16. *Epicoccum dickmanii* (CBS 124671). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H–I.** Pycnidia. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 100 µm; J–N = 10 µm.

producing pycnidia without conspicuous ostioles while *Ep. duchesneae* produces pycnidia with single, sometimes elongated ostioles. Besides, the conidia and conidiogenous cells of *Epicoccum dickmanii* (conidiogenous cells 7.5–12 × 7.5–10 µm, and conidia 4.5–10.5 × 3–4.5 µm) are larger than those of *Ep. duchesneae* (conidiogenous cells 4.5–9.5 × 3.5–7 µm, and conidia 2.5–3.5 × 1.5–2 µm).

Epicoccum longiostiolatum L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833510. Fig. 17.

Etymology: Name refers to the long ostiolate of conidiomata.

Description: *Conidiomata* pycnidial, produced superficially on agar, as well as on aerial mycelium, solitary, sometimes 2–3 confluent, scattered or aggregated, mostly (sub-)globose or lageniform, with some mycelium outgrowths, pale brown, thin-walled, ostiolate, 165–435 × 140–325 µm. *Ostioles* 1–2, up to four when confluent, papillate, sometimes elongated to a short neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–5 layers, 10–34 µm thick, outer three cell layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to lageniform, or irregular-shaped, 6.5–11.5 × 4–9 µm. *Conidia* variable in shape and size, oblong, ovoid or ellipsoidal, smooth- and thin-walled, aseptate, 5–9(–12) × 2–4 µm, with two small, polar guttules; globose conidia smooth- and thin-walled, aseptate, 3.5–4 × 3–3.5 µm. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 70–75 mm diam after 7 d at 25 °C, margin regular, covered by woolly aerial mycelium, buff with pale olivaceous margin; reverse buff with pale olivaceous zones. Colonies on MEA reaching 60–65 mm diam after 7 d, margin irregular, covered by woolly aerial mycelium, with feathered edge, slightly pinkish and buff near the centre, with whitish edge; reverse orange. Colonies on PDA reaching 40–45 mm diam after 7 d, margin irregular, densely covered by felty aerial mycelium, whitish with olivaceous buff zones; reverse gradually changed from brown to yellow, buff towards periphery. NaOH test on OA changes from buff to herbage green with red margin.

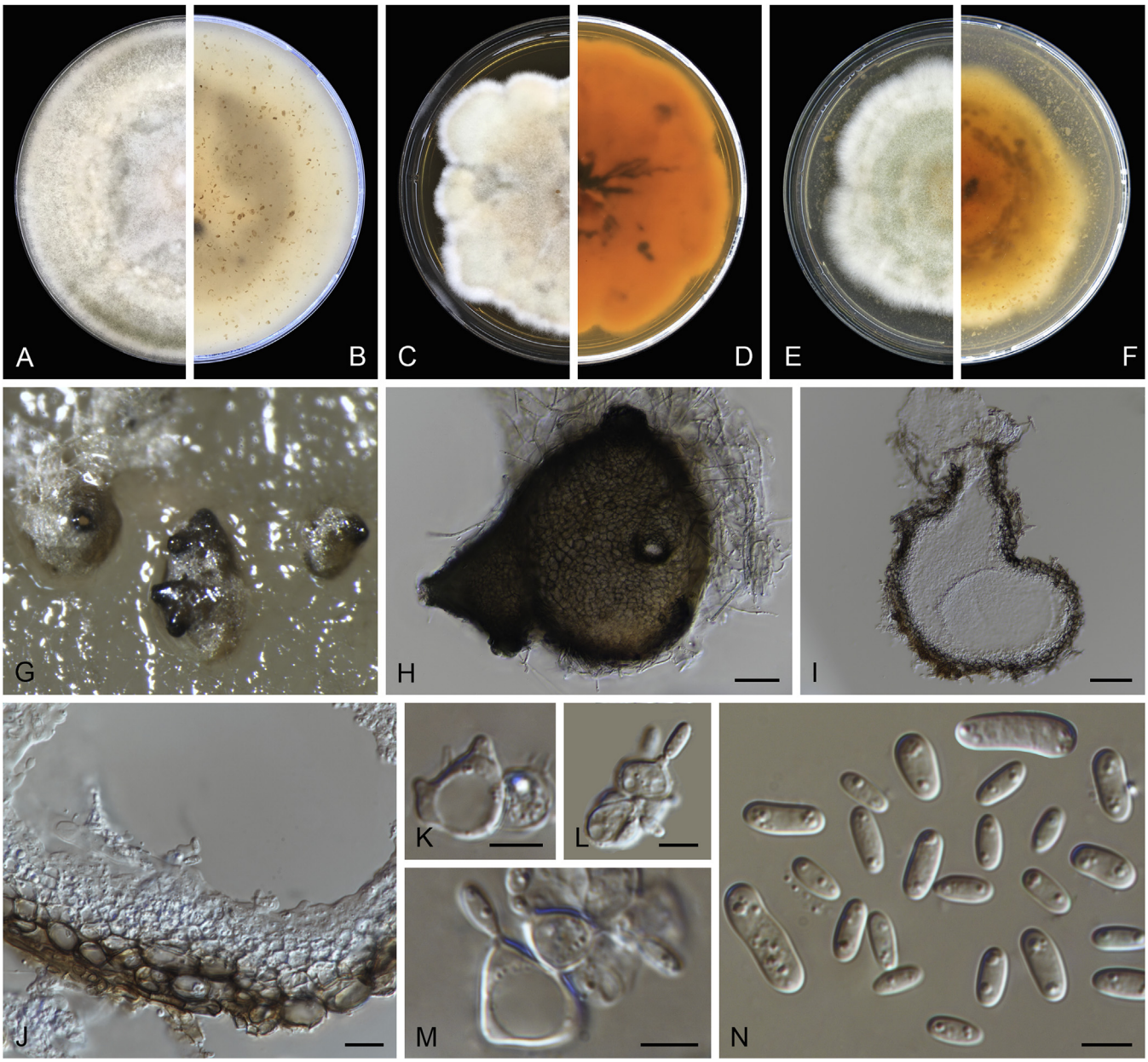

Fig. 17. *Epicoccum longiostiolatum* (CBS 886.95). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 50 µm; J = 10 µm; K–N = 5 µm.

Typus: **Papua New Guinea**, Northern Province, Myola, from *Stellaria* sp. (*Caryophyllaceae*), Oct. 1995, A. Aptroot (**holotype** CBS H-23666, ex-type living culture CBS 886.95).

Additional material examined: **Papua New Guinea**, Northern Province, Myola, Owen Stanley Range, near guesthouse, alt. 2 100 m, from *Cyperus* sp. (*Cyperaceae*), 19 Oct. 1995, A. Aptroot, specimen CBS H-6369, culture CBS 902.96.

Notes: The isolates CBS 886.95 and CBS 902.96 were originally identified as *Phoma sorghina*, but formed an obviously distinct clade from other strains of this species (Aveskamp *et al.* 2009a). *Phoma sorghina* was subsequently transferred to the genus *Epicoccum* after phylogenetic analyses, excluding the isolates CBS 886.95 and CBS 905.96 (Aveskamp *et al.* 2010). In our phylogenetic study, these two isolates formed a well-supported clade distant from *Phoma sorghina* (currently *Ep. sorghinum*). Thus *Ep. longiostiolatum* was introduced to accommodate these isolates. Morphologically, *Ep. longiostiolatum* differs from its most closely related species, *Ep. polychromum*, by producing larger conidia [5–9(–12) × 2–4 µm *vs.* 4.5–8 × 2–4 µm] and having larger conidiogenous cells (6.5–11.5 × 4–9 µm *vs.* 4.5–8.5 × 3.5–7 µm).

Epicoccum multiceps L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833557. Fig. 18.

Etymology: Name derived from the characteristic that many ostioles are produced on the pycnidia by this species.

Description: *Conidiomata* pycnidial, produced partly or fully immersed in the agar, mostly solitary, sometimes two or more aggregated, variable in shape and size, (sub-)globose, flask-shaped, irregular-shaped with age, pale brown, dark brown around the ostioles, thin-walled, glabrous, ostiolate, 135–540 × 128–465 µm. *Ostioles* 3–11(–13), slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–4 layers, 11–25 µm thick, outer two cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform to lageniform, 3.5–7.5 × 3.5–6 µm. *Conidia* ellipsoidal, oblong or ovoid,

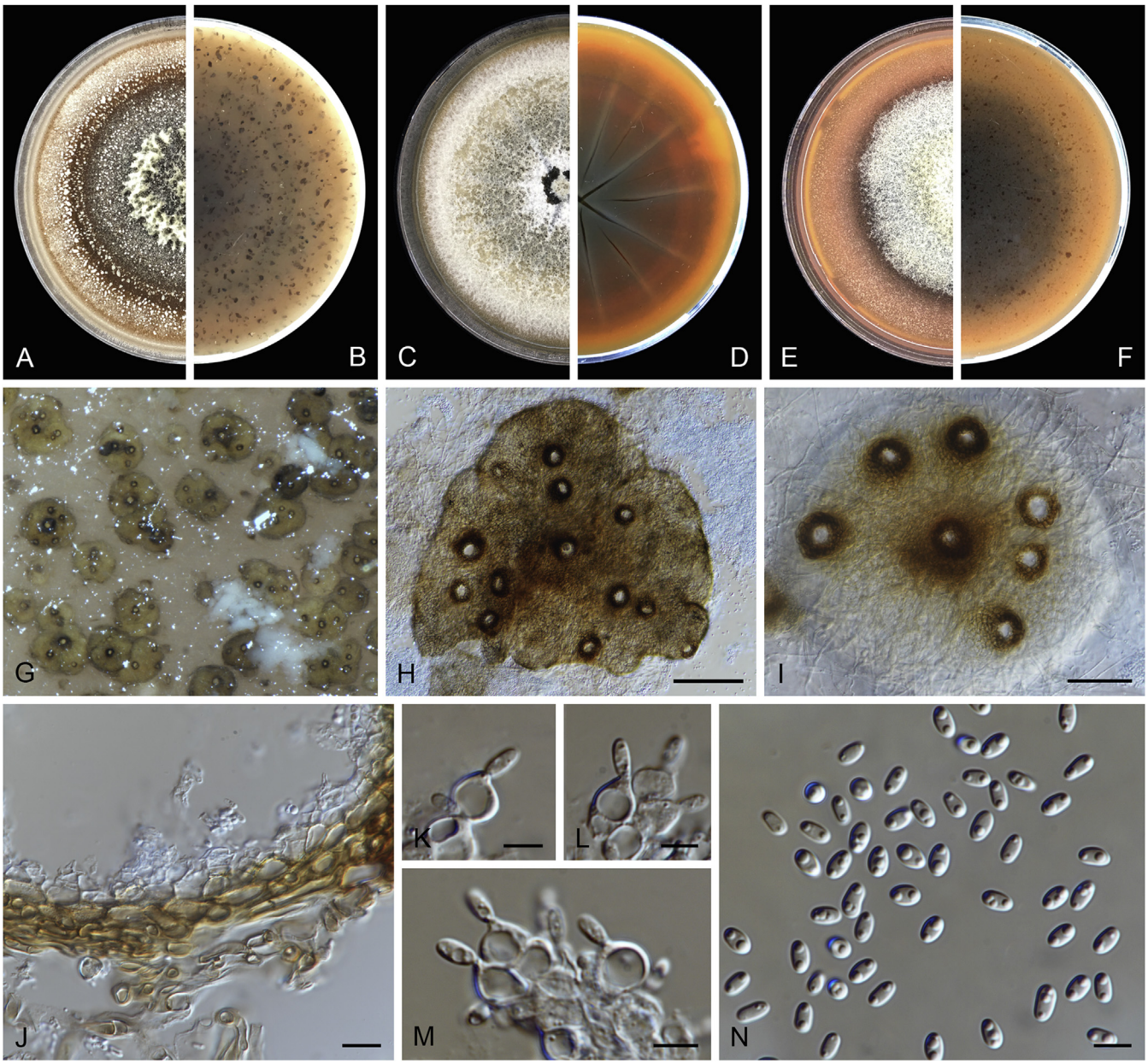

Fig. 18. *Epicoccum multiceps* (CBS 119734). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H–I.** Pycnidia. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 50 µm; J = 10 µm; K–N = 5 µm.

smooth- and thin-walled, aseptate, 3.5–5.5 × 2–3 µm, with 2–3 medium, polar guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 60–65 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, concentric circles of different colour, centre buff and olivaceous, pale olivaceous grey, dark brick, buff towards periphery; reverse brown to darker brown. Colonies on MEA reaching 60–65 mm diam after 7 d, margin regular, aerial mycelium felty, pale olivaceous grey, whitish near the centre and buff towards periphery; reverse concentric circles of different colour, centre black, dark brown, orange to yellow. Colonies on PDA reaching 60–65 mm diam after 7 d, margin regular, covered by sparse felty aerial mycelium, buff to pale honey, flat and pale brick coloured towards periphery; reverse olivaceous black, pale brick towards periphery. NaOH spot test negative on OA.

Typus: **Brazil**, São Paulo, Araraquara, from leaf of *Cupania vernalis* (*Sapindaceae*), Jun. 2003, L.H. Pfenning (**holotype** CBS H-23678, ex-type living culture CBS 119734 = CML 289).

Notes: Phylogenetically, *Epicoccum multiceps* clustered with the representative strain of *Ep. draconis* (CBS 186.83; Fig. 1), but could be differentiated from the latter in producing larger pycnidia (135–540 × 128–465 µm *vs.* 90–220 µm diam) with more ostioles [3–11(–13) *vs.* 1–2] and smaller conidia (3.5–5.5 × 2–3 µm *vs.* 4–8.5 × 2–4 µm). In addition, the test of metabolite E production was negative for *Ep. multiceps* while a positive reaction as a greenish, then red (E+ reaction) discolouration was observed in *Ep. draconis* (De Gruyter *et al.* 1998). This is the first and only report of a *Didymellaceae* species infecting a member of *Cupania* (*Sapindaceae*).

Epicoccum mezzettii Goid., Boll. Staz. Patol. Veg. Roma, N.S. 17: 452. 1937.

Description: *Mycelium* hyaline at beginning, 1.8 µm in diam, later become brownish, regular thickened at septa, branched, 2.5–5.2 in diam. *Colony* dull yellow to brown. *Sporodochia* black, stromata formed from the irregularly rounded rectangular cells. *Conidiophores* short, unicellular, bearing conidia at the apex. *Conidia* spherical, obscure, septate, thickened with age, red-brown, with or without few but large incrustations, conidia size constant, 9–11 µm (Goidànich 1937).

Typus: **Italy**, from *Populus* sp. (*Salicaceae*) wood pulp in paper factory, Apr. 1938, isol. G. Goidánich, culture CBS 173.38 (ex-type living culture of *Epicoccum mezzettii*).

Additional materials examined: **Germany**, former West-Germany, *Solanum tuberosum* (*Solanaceae*), Dec. 1922, H.W. Wollenweber, culture CBS 120.22. **India**, Madhya Pradesh, Balaghat, from leaf of *Artocarpus heterophylla* (*Moraceae*), Jul. 1972, S.M. Singh, culture CBS 873.72 = IFO 32913. **Unknown**, from leaf of *Malus sylvestris* (*Rosaceae*), Nov. 1959, D. Mulder, culture CBS 238.59 = IMI 079498; from *Triticum aestivum*, unknown date and collector, culture CBS 169.33.

Notes: *Epicoccum mezzettii* was originally isolated from wood pulp for paper making in Italy (Goidànich 1937). However, together with several other *Epicoccum* species, it was incorrectly treated as synonym of *Ep. nigrum* by Schol-Schwarz (1959). In this study, CBS 173.38 (ex-type living culture of *Ep. mezzettii*), together with four other plant-associated isolates, formed a distinct clade that was phylogenetically distant from the ex-type strain of *Ep. nigrum* (CBS 173.73). Morphologically, *Ep. mezzettii* is characterised by producing consistently smaller conidia compared with *Ep. nigrum* (9–11 µm *vs.* 13–31 µm) and less verrucose on conidia (Goidànich 1937, Punithalingam *et al.* 1972). Besides, conidiophores of *Ep. mezzettii* are unicellular, while that of *Ep. nigrum* are usually 2-septate. Therefore, *Ep. mezzettii* was resurrected as a separate species.

Epicoccum oryzae S. Ito & Iwadare, Rep. Hokkaido Prefect. Agric. Exp. Sta. 31: 1. 1934.

Description: Culture sterile.

Culture characteristics: Colonies on OA reaching 65–70 mm diam after 7 d 25 °C, margin regular, aerial mycelium felty, rosy buff, greyish near the centre; reverse brick to dark brown. Colonies on MEA 60–65 mm diam after 7 d, margin regular, densely covered by felty aerial mycelium, rosy buff, with some brick or pinkish zones; reverse brick. Colonies on PDA, 65–70 mm diam after 7 d, margin regular, covered by felty aerial mycelium, rosy buff to brick with pale olivaceous margin; reverse brick near the centre, coral towards periphery. NaOH spot test negative on OA.

Typus: **Japan**, from *Oryza sativa* (*Poaceae*), Jan. 1934, deposited by S. Ito (**neotype designated here** CBS H-24312, MBT390301, ex-neotype living cultures CBS 173.34 = ATCC 24428 = IMI 164070); *ibid*., culture CBS 174.34.

Notes: *Epicoccum oryzae* was initially described from harvested rice grains causing "red blotch" disease in Hokkaido, Japan (Ito & Iwadare 1934), which is characterised by "olivaceous hyphae, globose or subglobose sporodochia, and globose, subglobose, or pyriform, granular, verrucose, olivaceous conidia, 9.9–23.1 × 6.6–16.5 µm, consisting of one to five cells". However, *Ep. oryzae* was synonymised as *Ep. nigrum* (Schol-Schwarz 1959). The isolates CBS 173.34 and CBS 174.34 were recorded as *Ep. oryzae*, which were deposited in CBS collection by the original author of the species (S. Ito). Both isolates agreed well with the original description of *Ep. oryzae* with regard to the date, host and location. Archived correspondence revealed that they could be ex-isotype strains of *Ep. oryzae.* Since no holotype specimen is known, we proposed CBS 173.34 as ex-neotype of *Ep. oryzae*. Unfortunately, both isolates proved to be sterile, but still produced the red pigment on OA plates as reported in the original description. Phylogenetically, *Epicoccum oryzae* clustered in a sister clade to *Ep. mackenziei*, distant from the ex-type strain of *Ep. nigrum* (CBS 173.73). Therefore, *Ep. oryzae* was resurrected as a separate species.

Epicoccum polychromum L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833509. Fig. 19.

Etymology: Epithet derived from the colony colour.

Description: *Conidiomata* pycnidial, produced on the agar surface, semi-immersed as well as produced on aerial mycelium, solitary, scattered or aggregated, (sub-)globose or flask-shaped, irregular-shaped with age, brown to dark brown, thin-walled, with hyphal outgrowths, ostiolate, 125–695 × 90–535 µm. *Ostioles* 1–4(–8), slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3 layers, 10–41 µm thick, outer 1–2 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose to ampulliform, 4.5–8.5 × 3.5–7 µm. *Conidia* cylindrical or oblong with both ends obtusely rounded, smooth and thin-walled, aseptate, 4.5–8 × 2–4 µm, with 0–2 min guttules. *Chlamydospores* aseptate, brown, in chains or singly, (sub-)globose, 5.5–10 × 4.5–8.5 µm. *Conidial matrix* buff.

Culture characteristics: Colonies on OA reaching 50–55 mm diam after 7 d at 25 °C, margin regular, densely covered by felty aerial mycelium, concentric circles of different colour, centre buff, flesh, whitish, pale olivaceous grey towards periphery; reverse olivaceous grey with pinkish circles. Colonies on MEA reaching 45–50 mm diam after 7 d, margin regular, densely covered by felty aerial mycelium, rosy buff to whitish, pale grey near the centre, some radially furrowed zones near the centre; reverse dark brown, brick or orange towards the periphery, with some radial lines near the centre. Colonies on PDA reaching 50–55 mm diam after 7 d, margin regular, covered by felty aerial mycelium, buff, with flat mycelium towards the periphery and in blood colour; reverse orange, dark brown near the centre. NaOH spot test results in a dark greenish discolouration on OA.

Typus: **France**, La Réunion island, from leaves of *Paspalum dilinateum* (*Poaceae*), 7 Mar. 2015, P.W. Crous (**holotype** CBS H-23668, ex-type living culture CPC 26197 = CBS 141502).

Notes: Isolate CBS 141502 was isolated from leaves of *Paspalum dilinateum* in La Réunion island, France, and identified as *Epicoccum sorghinum* (Hernández-Restrepo *et al.* 2016) based on its ITS sequence data. Currently, five species are known to occur on *Paspalum* spp., namely, *Ascochyta paspali*, *Ep. andropogonis*, *Ep. nigrum*, *Ep. sorghinum* and *Phoma paspali*. However, CBS 141502 formed a phylogenetic lineage distinct from the ex-type strain of *Ep. nigrum* (CBS 173.73) and the representative strain of *Ep. sorghinum* (CBS 179.80). Although molecular data of *As. paspali*, *Ep. andropogonis* and *Phoma paspali* were unavailable, CBS 141502 could be differentiated from *Ep. andropogonis* by only producing aseptate conidia, while *Ep. andropogonis* only produced sporodochia and multi-septate conidia; from *Phoma paspali* in its larger

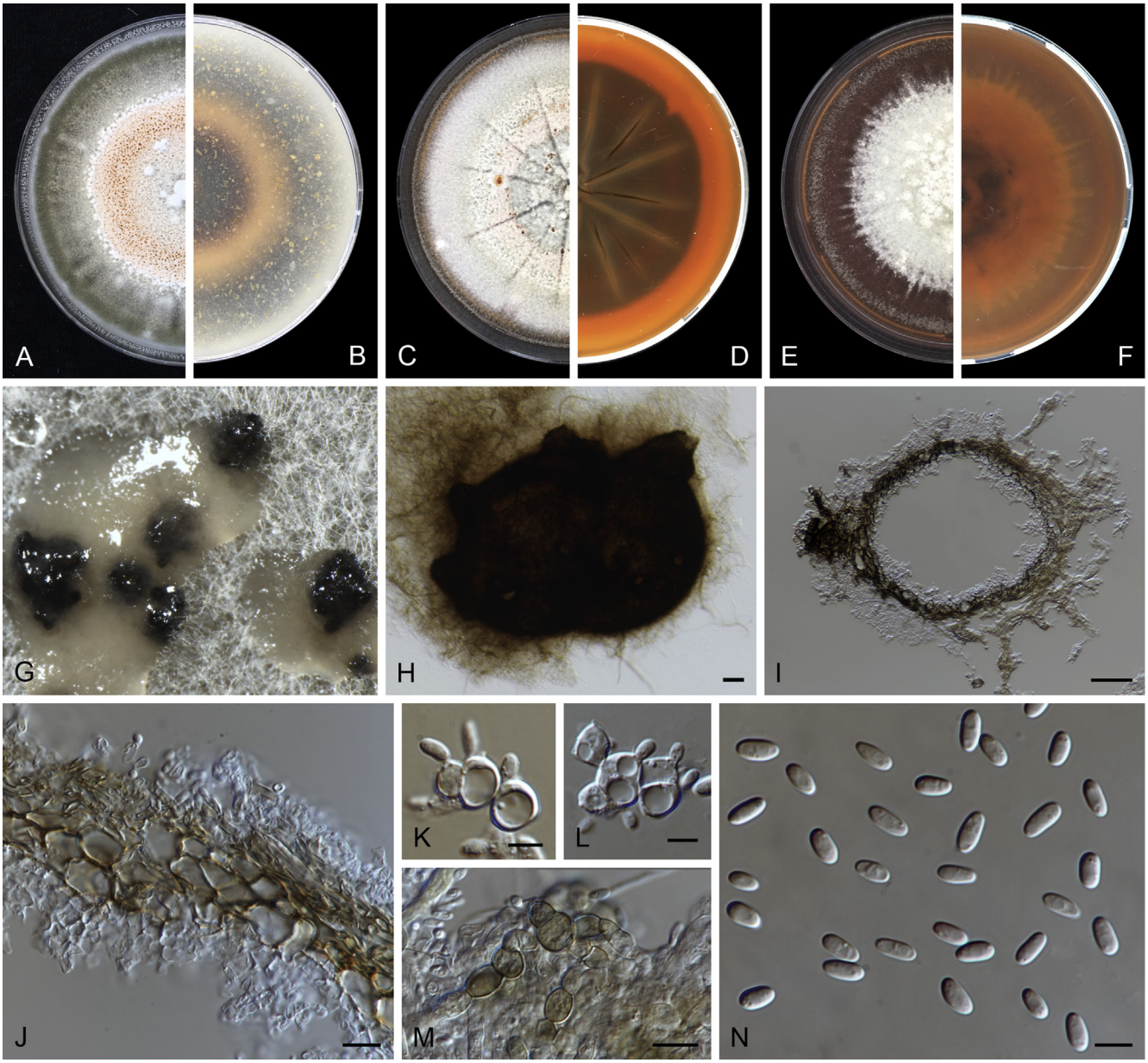

Fig. 19. *Epicoccum polychromum* (CBS 141502). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–L.** Conidiogenous cells. **M.** Chlamydospores. **N.** Conidia. Scale bars: H–I = 50 µm; J, M = 10 µm; K–L, N = 5 µm.

pycnidia with more ostioles [125–695 × 90–535 µm, 1–4(–8) ostioles *vs.* 120–250 µm, single ostiole] and smaller conidia [4.5–8 × 2–4 µm *vs.* 6.5–9(–11) × 3–4 µm]; from *As. paspali* in its larger pycnidia (125–695 × 90–535 µm *vs.* 100–200 µm) with aseptate, smaller and hyaline conidia (4.5–8 × 2–4 µm), while *As. paspali* produced conidia that were 0–3-septate, pale brown, measuring 13–33 × 4–7.5 µm.

Epicoccum purpurascens Ehrenb. ex Schltdl., Fl. Berol. (Berlin) 2: 136. 1824. Fig. 20.

Description from reference culture (CBS 128906): *Conidiomata* sporodochial, abundant, scattered or aggregated, superficial on the agar, pale brown initially, turning black with age. *Vegetative hyphae* septate, frequently branched. *Conidiophores* macronematous or semi-macronematous, unbranched, sometimes elongated and covered in mycelial hairs, yellow to pale brown. *Conidia* solitary, acrogenous, globose, aseptate and smooth when young, later becoming multicellular-phragmosporous, verrucose, subglobose to pyriform, brown, 9.5–24 µm diam.

Culture characteristics: Colonies on OA, 55–60 mm diam after 7 d 25 °C, margin regular, without aerial mycelium, olivaceous to brown, conidiophores and conidia abundant; reverse vinaceous buff, sienna near the centre. Colonies on MEA, 45–50 mm diam after 7 d, margin irregular, aerial mycelium flat, conidiophores and conidia abundant, brick with red edge, reverse fuscous black with yellow edge. Colonies on PDA, 65–75 mm diam after 7 d, margin regular, aerial mycelium flat, with feathery margin, dark brown, with buff to cinnamon elongated conidiophores on the surface; reverse olivaceous to olivaceous black. NaOH spot test: a slight olivaceous discolouration on OA.

Materials examined: **China**, from *Poa annua* (*Poaceae*), unknown date and collector, culture LC 8159. **Denmark**, from human toenail, date unknown, D.M. Saunte, culture CBS 124435. **Unknown**, unknown host, 1932, G.D. Ruehle, culture CBS 166.32. **USA**, Dubois, Wyoming, soil, 1997, M. Christensen, culture CBS 128906.

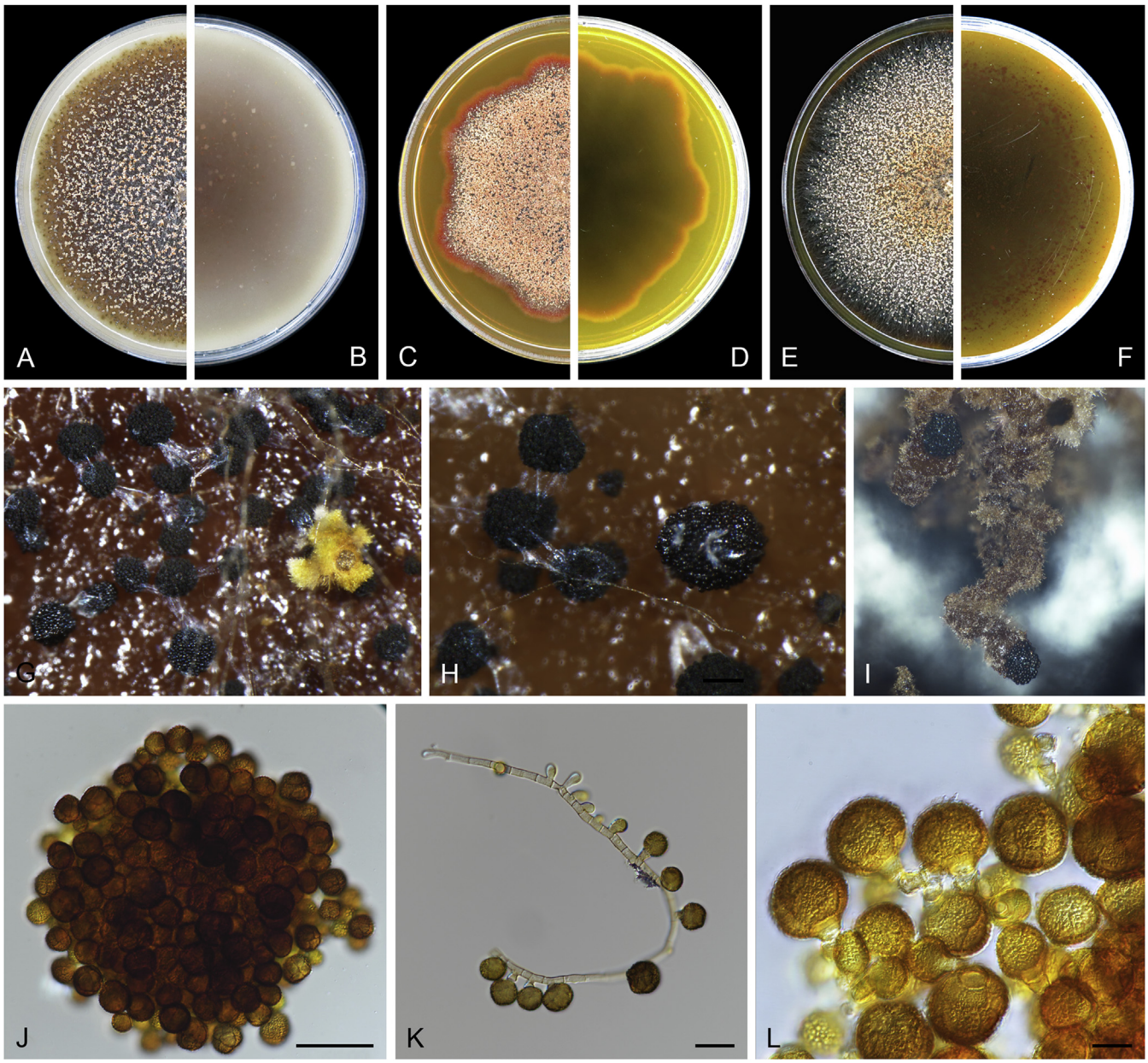

Fig. 20. *Epicoccum purpurascens* (CBS 128906). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G–H.** Sporodochia on OA. **I–J.** Sporodochium. **K.** Conidiogenous cells. **L.** Conidia. Scale bars: J = 50 µm; K = 20 µm; L = 10 µm.

Notes: *Epicoccum purpurascens* was originally introduced by Von Schlechtendal (1824). According to Saccardo's records, this species has a diverse host range and is mainly recorded in Europe. However, this species, together with several other species in *Epicoccum*, were formerly regarded as synonyms of *Ep. nigrum* (Schol-Schwarz 1959). Isolate CBS 128906 was originally received as *Ep. purpurascens*, with which it agreed well morphologically, and therefore we regarded CBS 128906 to represent *Ep. purpurascens*. In the present study, four isolates of *Ep. purpurascens* formed a well-supported clade, which was clearly distant from the ex-epitype strain of *Ep. nigrum* (CBS 173.73).

Epicoccum thailandicum Goonas. *et al.*, Mycosphere 8: 713. 2017.
Synonym: *Epicoccum pneumoniae* Valenz.-Lopez *et al.* Stud. Mycol. 90: 32. 2017 (2018).

Description: Thambugala *et al.* (2017).

Typus: **Thailand**, Chiang Mai Province, Mae Taeng, Mushroom Research Centre, on stems of grass litter (*Poaceae*), 24 Mar. 2016, I.D. Goonasekara (**holotype** MFLU 16-2855, ex-type living culture MFLUCC 16-0892 = KUMCC 17-0026).

Material examined: **USA**, from human sputum sample, 2008, D.A. Sutton (**holotype** of *Ep. pneumoniae* FMR H-13747, ex-type living culture UTHSC DI16-257).

Notes: *Epicoccum thailandicum* (holotype: MFLU 16-2855) was introduced by Thambugala *et al.* based on phylogenetic analyses of ITS and LSU and was reported on the stems of grass litter (*Poaceae*) in Thailand (Thambugala *et al.* 2017; published 30 Apr. 2017), while *Ep. pneumoniae* (UTHSC DI16-257) was isolated from a human sputum sample in the USA (Valenzuela-Lopez *et al.* 2018; published 21 Nov. 2017). However, the ITS and LSU sequence data of *Ep. pneumoniae* were identical to those of *Ep. thailandicum* that was earlier published. Unfortunately, *Epicoccum pneumoniae* was sterile. Further loci should be sequenced of *Ep. thailandicum* to confirm the synonymy.

Epicoccum tobaicum (Szilv.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB834115.

Basionym: *Toruloidea tobaica* Szilv., Arch. Hydrobiol. Suppl. 14: 519. 1936.
Synonym: *Epicoccum layuense* Qian Chen *et al.*, Stud. Mycol. 87: 145. 2017.

Description: Von Szilvinyi (1936).

Typus: **Indonesia**, Sumatra, Toba Heath, heath soil, Jun. 1936, A. von Szilvinyi, specimen CBS H-24336, ex-type living culture CBS 384.36 = MUCL 9832.

Additional materials examined: **China**, Tibet, Lulang, on leaves of *Perilla* sp. (*Lamiaceae*), 15 Jun. 2015, Q. Chen (**holotype** of *Epicoccum layuense* HMAS 247165, ex-type living culture CGMCC 3.18362 = LC 8155); *ibid*., culture LC 8156. **France**, Loire, host unknown, 1955, J. Nicot, culture CBS 235.59. **USA**, Ohio, Cincinnati, air, date unknown, W.B. Cooke, culture CBS 232.59; *ibid*., culture CBS 233.59 = IMI 079496.

Notes: In this study the ex-type isolate of *Toruloidea tobaica* (CBS 384.36) was examined. This species was originally described from heath soil in Sumatra (Von Szilvinyi 1936), but later synonymised with *Epicoccum nigrum*. In our phylogenetic study, the ex-type strain of *T. tobaica* formed a distinct clade in the genus *Epicoccum*, distant from *Ep. nigrum* (CBS 173.73). The ex-type strain of the more recently described *Ep. layuense* (CGMCC 3.18362; Chen *et al.* 2017) was found to be genetically identical to that of *Ep. tobaicum* (CBS 384.36) and was therefore reduced to synonymy.

Epicoccum variabile L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833511. Fig. 21.

Etymology: From Latin *variabilis*, due to the variable shape of the conidia.

Description: *Conidiomata* pycnidial, produced superficially on agar and covered by sparse aerial mycelium, scattered or aggregated, solitary or confluent, brown, thin-walled, mostly (sub-)globose, glabrous or with some hyphal outgrowths, 110–450 × 110–350 µm. *Ostioles* inconspicuous. *Pycnidial wall* pseudoparenchymatous, thin, composed of

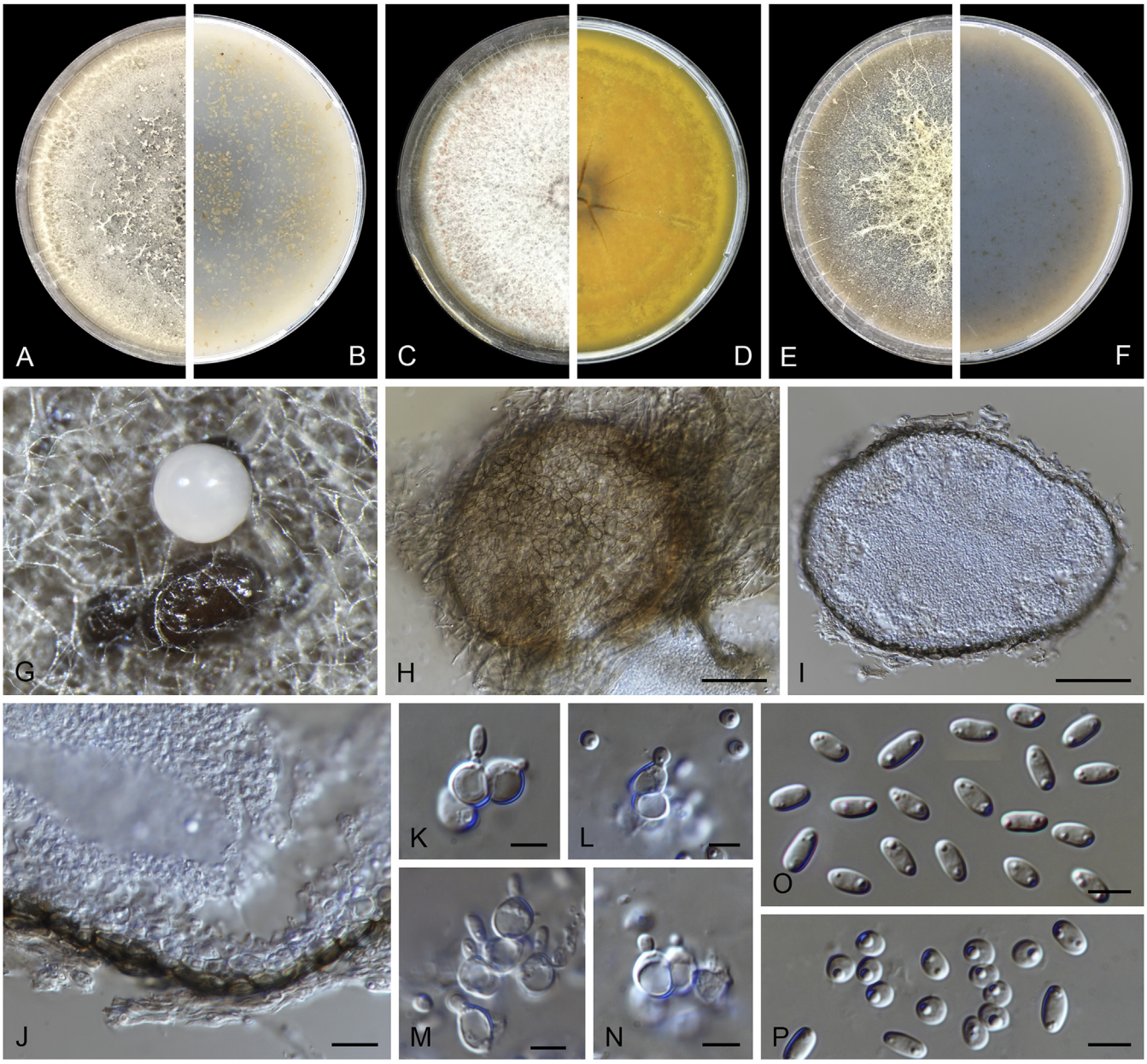

Fig. 21. *Epicoccum variabile* (CBS 119733). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. **P.** Globose conidia. Scale bars: H–I = 50 µm; J = 10 µm; K–P = 5 µm.

oblong to isodiametric cells, 9–21.5 µm thick, 2–3 layers, outer cell layer pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform or doliiform, 5–8 × 4–6.5 µm. *Conidia* variable in shape and size, ovoid or oblong, hyaline, smooth- and thin-walled, aseptate, with 2 minute, polar guttules, 4.5–6 × 2.5–3.5 µm; globose conidia present, smooth- and thin-walled, aseptate, 3–4 × 2.5–3.5 µm, with one medium-sized guttule. *Conidial matrix* pinkish.

Culture characteristics: Colonies on OA reaching 75–80 mm diam after 7 d at 25 °C, margin regular, densely covered by felty aerial mycelium, pale smoke grey, buff towards periphery; reverse concolourous. Colonies on MEA reaching 75–80 mm diam after 7 d, margin regular, densely covered by floccose aerial mycelium, whitish to buff, pink conidial matrix visible; reverse yellow, pale brown near the centre. Colonies on PDA reaching 70–75 mm diam after 7 d, margin regular, covered by flat aerial mycelium, floccose near the centre, grey to buff near the centre, vinaceous buff towards periphery; reverse dark brown, vinaceous buff towards periphery. NaOH spot test negative on OA.

Typus: **Brazil**, Minas Gerais, Capelinha, from leaf of *Coffea arabica* (*Rubiaceae*), Sep. 1999, L.H. Pfenning (**holotype** CBS H-23677, ex-type living culture CBS 119733 = CML 190).

Notes: Phylogenetically, *Epicoccum variabile* grouped in a distinct clade closely related to *Ep. camelliae* and *Ep. viticis*. Morphologically, *Epicoccum variabile* could be clearly differentiated from *Ep. viticis* in its larger pycnidia [110–450 × 110–350 µm *vs.* 120–200 × 100–175 µm] and shorter conidiogenous cells (5–8 × 4–6.5 µm *vs.* 5.5–9 × 3–6 µm). Moreover, *Ep. variabile* differed from *Ep. viticis* by producing pycnidia with inconspicuous ostioles and globose conidia (Chen et al. 2017). Although the ex-type culture of *Ep. camelliae* was sterile, they differed genetically in their *rpb2* and *tub2* sequences.

Clade 13: *Similiphoma* Valenz.-Lopez *et al.*, Stud. Mycol. 90: 37. 2017 (2018).

Type species: *Similiphoma crystallifera* (de Gruyter *et al.*) Valenz.-Lopez *et al.*

Clade 14: *Sclerotiophoma* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833512.

Etymology: Name refers to the producing of pycnosclerotia.

Conidiomata pycnidial, globose, subglobose, scattered, solitary or confluent, semi-immersed or immersed in agar, without distinct ostioles, occasionally with a pore; pycnidial wall pseudoparenchymatous, multi-layered, outer layers slightly pigmented. *Pycnosclerotia* present. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform or bottle-shaped, with minute periclinal thickening. *Conidia* ellipsoidal to ovoid, smooth- and thin-walled, aseptate, with or without several guttules. *Chlamydospores* not observed. *Sexual morph* unknown.

Notes: According to Boerema *et al.* (2004), most species of *Phoma* section *Sclerophomella* have thick-walled pseudoparenchymatous pycnidia *in vivo*. The type species *Phoma complanata* of this section has been recombined into *Calophoma*, and most other species have also been transferred to different genera based on the current phylogenetic analysis, such as

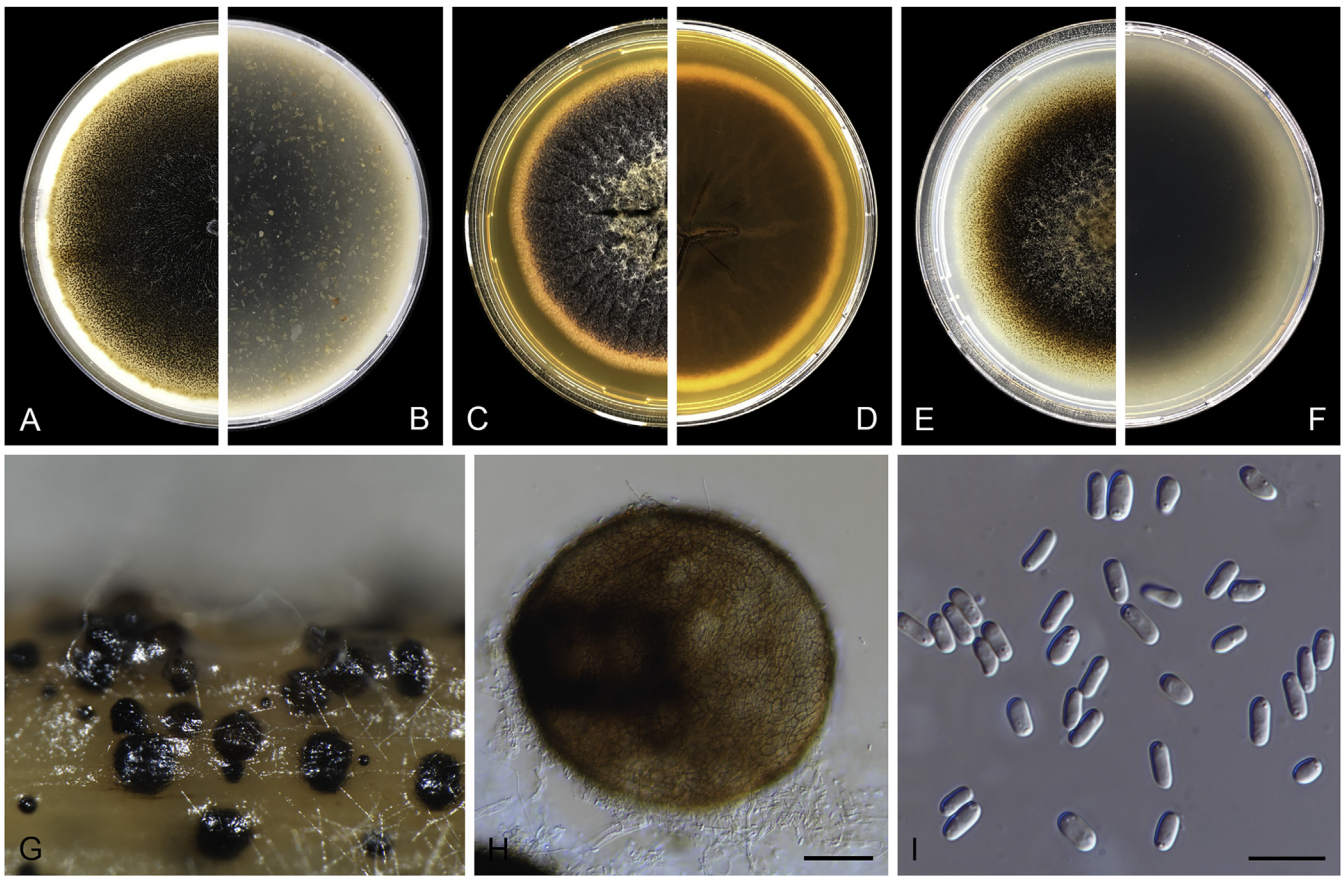

Fig. 22. *Sclerotiophoma versabilis* (CBS 876.97). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Conidia. Scale bars: H = 50 µm; I = 10 µm.

Phoma dictamnicola to *Heterophoma*, and *Phoma polemonii* to *Neodidymelliopsis* (Chen *et al.* 2015). Among the species included in *Phoma* section *Sclerophomella*, *Phoma versabilis* is characterised by the production of pycnidia showing a retarded development of the pycnidial cavity (pycnosclerotia), containing a compact mass of cells that afterwards disintegrates (Boerema *et al.* 2004). In the present study, phylogenetic analysis showed that this species formed a separate lineage distant from all known genera in *Didymellaceae*. Therefore a new genus *Sclerotiophoma* is introduced.

Type species: *Sclerotiophoma versabilis* (Boerema *et al.*) L.W. Hou, L. Cai & Crous

Sclerotiophoma versabilis (Boerema *et al.*) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833530. Fig. 22

Basionym: *Phoma versabilis* Boerema *et al.*, Persoonia 16: 154. 1996.

Synonym: *Ascochyta versabilis* (Boerema *et al.*) Qian Chen & L. Cai, Stud. Mycol. 82: 190. 2015.

Description: Boerema *et al.* (1996).

Typus: **Germany**, Westfalen, Oberdresselendorf, on stems of *Cardamine impatiens* (*Brassicaceae*), Oct. 1925, A. Ludwig (**holotype** L 995.229.369).

Materials examined: **Denmark**, from human toenail, date unknown, D.M. Saunte, culture CBS 124689. **The Netherlands**, Wageningen, from a stem of *Silene* sp. (*Caryophyllaceae*), Jun. 1997, specimen CBS H-24329, culture CBS 876.97 = PD 82/1008.

Notes: This species was introduced by Boerema *et al.* (1996), on stems of *Cardamine impatiens* collected in Germany (Boerema *et al.* 1996). Later, Boerema *et al.* (2004) described a representative strain for *Phoma versabilis* (CBS 876.97) which morphologically agreed well with the original description.

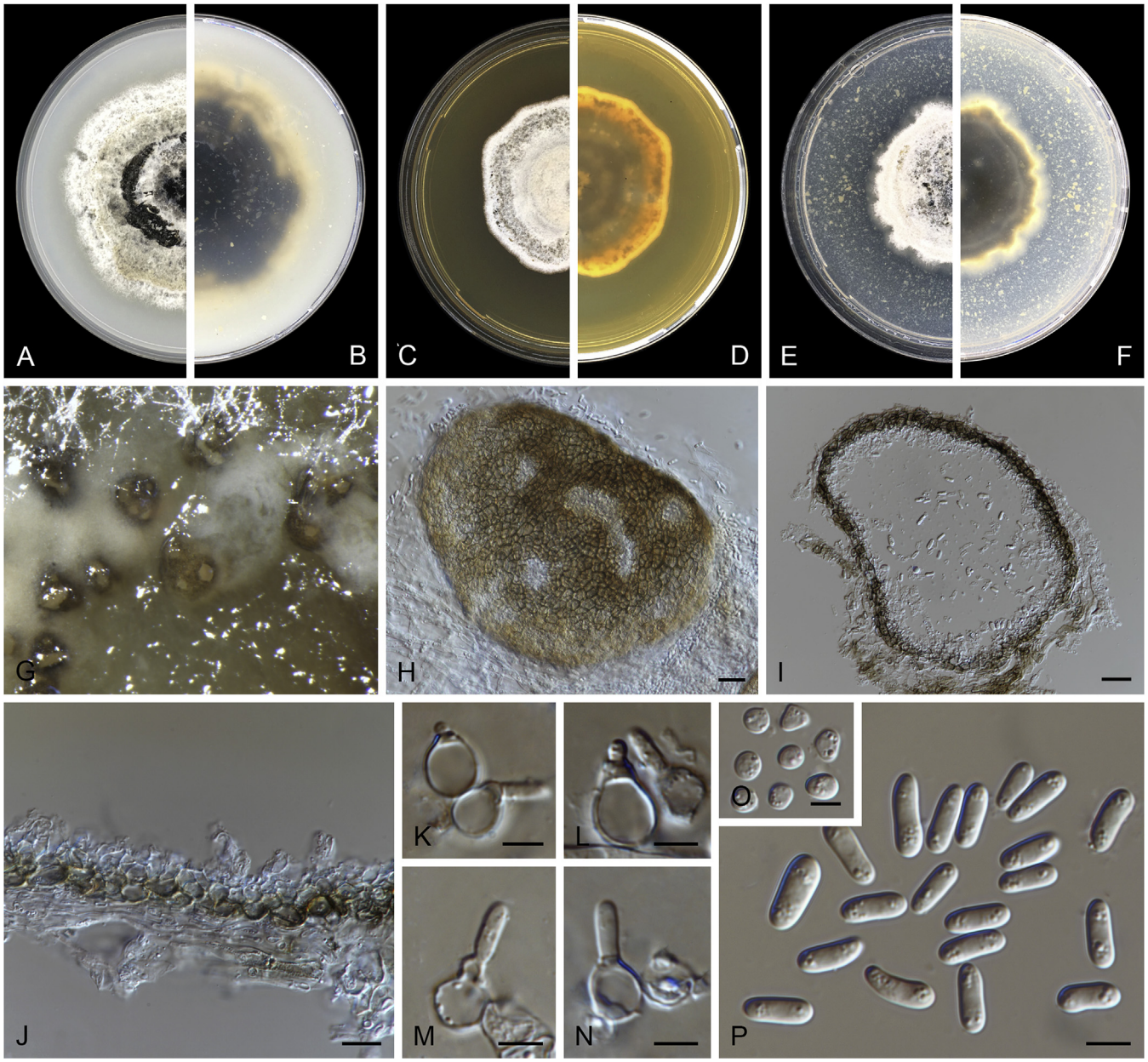

Fig. 23. *Didymella acetosellae* (CBS 631.76). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Globose conidia. **P.** Conidia. Scale bars: H–I = 20 µm, J = 10 µm; K–P = 5 µm.

In the present study, *Phoma versabilis* was re-examined based on two isolates (CBS 876.97 and CBS 124689). In our phylogenetic analysis, these two isolates formed an independent lineage which was distant from other genera in *Didymellaceae*, closely related to the genus *Similiphoma* and *Epicoccum* (Fig. 1). Thus, *Sclerotiophoma* was introduced to accommodate this species. The type species of this genus is characterised by producing pycnosclerotia, which gradually develop into poroid pycnidia.

Clade 15: *Didymella* Sacc., Michelia 2(no. 6): 57. 1880, *emend*. Chen *et al*., Stud. Mycol. 82: 173. 2015.

Synonym: *Peyronellaea* Goid. ex Togliani, Ann. Sperim. Agrar. II 6: 93. 1952.

Type species: *Didymella exigua* (Niessl) Sacc.

Didymella acetosellae (A.L. Sm. & Ramsb.) Qian Chen & L. Cai, Stud. Mycol. 82: 173. 2015. Fig. 23.
Basionym: *Phyllosticta acetosellae* A.L. Sm. & Ramsb., Trans. Brit. Mycol. Soc. 4: 173. 1913.
Synonym: *Phoma acetosellae* (A.L. Sm. & Ramsb.) Aa & Boerema, Persoonia 18: 16. 2002.

Description from ex-epitype (CBS 631.76): *Conidiomata* pycnidial, semi-immersed or immersed, scattered, mostly solitary, sometimes 2–3 confluent; cover by whitish mycelium, (sub-)globose, pale brown, thin-walled, with hyphal outgrowths, ostiolate, 170–345 × 145–300 µm. *Ostioles* 1–3(–7), non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–4 layers, 5.5–26 µm thick, outer cell layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, lageniform, ampulliform, slightly papillate, 3.5–11.5 × 3.5–10.5 µm. *Conidia* variable in shape and size, always two types: cylindrical, ovoid or globose, smooth- and thin-walled, aseptate, the cylindrical conidia 5–9.5(–11) × 2.5–3.5 µm; globose conidia 3.6–5.5 × 3–5, mostly with 2–6 minute, polar guttules. *Conidial matrix* milky to buff.

Culture characteristics: Colonies on OA reaching 30–35 mm diam after 7 d, margin irregular, aerial mycelium floccose, flat mycelium in the middle, whitish to mouse grey; reverse buff to dark brown. Colonies on MEA reaching 25–30 mm diam after 7 d, margin irregular, covered with felty aerial mycelium, whitish to pale grey; reverse brown to darker brown, with yellow edge. Colonies on PDA reaching 20–25 mm diam after 7 d, margin irregular, covered by felty aerial mycelium, whitish, pale grey near the centre; reverse buff to olivaceous grey. NaOH spot test negative on OA.

Typus: **UK**, Glangonner, Lanarkshire, on *Rumex acetosella* (*Polygonaceae*), 29 Jun. 1912, D.A. Boyd (**holotype** BM). **France**, Corrèze, Monteil sur Bois, from leaf spot on *Rumex acetosella* (*Polygonaceae*), 21 Jul. 1976, H.A. van der Aa (**epitype designated here** CBS H-16138, MBT389708, ex-epitype living culture CBS 631.76).

Notes: *Phyllosticta acetosellae* was originally collected from leaves of *Rumex acetosella* in the UK, having ellipsoidal to cylindrical conidia measuring 8–10 × 3–4 µm (Smith & Ramsbottom 1913). Based on morphology, De Gruyter *et al.* (2002) regarded isolate CBS 631.76 to represent *Phy. acetosellae*, and recombined it into the genus *Phoma*. Subsequently this species was transferred to *Didymella* after phylogenetic analysis (Chen *et al.* 2015). According to De Gruyter *et al.* (2002), CBS 631.76, isolated from the leaf spot of *Rumex acetosella* from France, was a good representative of this species as it morphologically agreed well with the original description. We followed this suggestion and designated CBS 631.76 as ex-epitype culture of *Phyllosticta acetosellae.* In the present study, CBS 631.76 was found to be phylogenetically distant from CBS 179.97 (also received as "*Phoma acetosellae*"), the latter being re-identified as *Did. rumicicola*.

Didymella aloeicola L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833514. Fig. 24.

Etymology: Name reflects the host genus *Aloe*, from which this species was isolated.

Description: *Conidiomata* pycnidial, produced on the agar surface or (semi-)immersed, scattered, mostly solitary, sometimes 2–3 confluent, mostly globose, subglobose or lageniform, brown or darker brown, thin-walled, with hyphal outgrowths, ostiolate, 60–200 × 55–175 µm. *Ostioles* 1–2, mostly central, not to slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–6 layers, 16–19 µm thick, outer cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, doliiform to lageniform, 3.5–5.5 × 3.5–5 µm. *Conidia* ovoid or oblong, hyaline, smooth- and thin-walled, aseptate, 3.5–4 × 1.5–2 µm, mostly with 1–2 minute, polar guttules. *Conidial matrix* whitish to buff.

Culture characteristics: Colonies on OA reaching 60–65 mm diam after 7 d at 25 °C, margin regular, aerial mycelium sparse, pale olivaceous grey near the centre, saffron towards periphery; reverse concolourous. Colonies on MEA reaching 60–65 mm diam after 7 d, margin regular, covered with flat aerial mycelium, pale mouse grey to mouse grey; reverse dark brown. Colonies on PDA reaching 75–80 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, vinaceous; reverse concolourous. NaOH spot test negative on OA.

Typus: **Italy**, Sicily, *Aloe* sp. (*Asphodelaceae*), date unknown, F. Seigle-Murandi (**holotype** CBS H-23683, ex-type living culture CBS 562.88).

Notes: Phylogenetically, *Didymella aloeicola* formed a distinct lineage from other species in the genus. Morphologically, *Did. aloeicola* was easily distinguished from its closest neighbour *Did. pedeiae* in the NaOH reactions (pale reddish discolouration on *Did. aloeicola*, and no effect on *Did. pedeiae*) (Aveskamp *et al.* 2010).

Phoma aloes was originally isolated from dead bark of *Aloe dichotoma*, the same host genus as for *Did. aloeicola*, in South Africa (Crous et al. 2013b). However, it clearly differed from *Did. aloeicola* phylogenetically (Fig. S1). Morphologically, *Did. aloeicola* produced smaller conidiogenous cells [3.5–5.5 × 3.5–5 µm *vs.* (7–)8(–9) × (3–)4 µm] and conidia (3.5–4 × 1.5–2 µm *vs.* 4–7 × 2–2.5 µm) than *Phoma aloes*.

Didymella anserina (Marchal) Qian Chen & L. Cai, Stud. Mycol. 82: 173. 2015.
Basionym: *Phoma anserina* Marchal, Champignon Copr. Belg. 6: 11. 1891.
Synonyms: *Peyronellaea anserina* (Marchal) Aveskamp *et al.*, Stud. Mycol. 65: 31. 2010.
Phoma radicis-callunae R.W. Rayner, Bot. Gaz. 73: 231. 1922.
Phoma suecica J.F.H. Beyma, Antonie van Leeuwenhoek 8: 110. 1942.

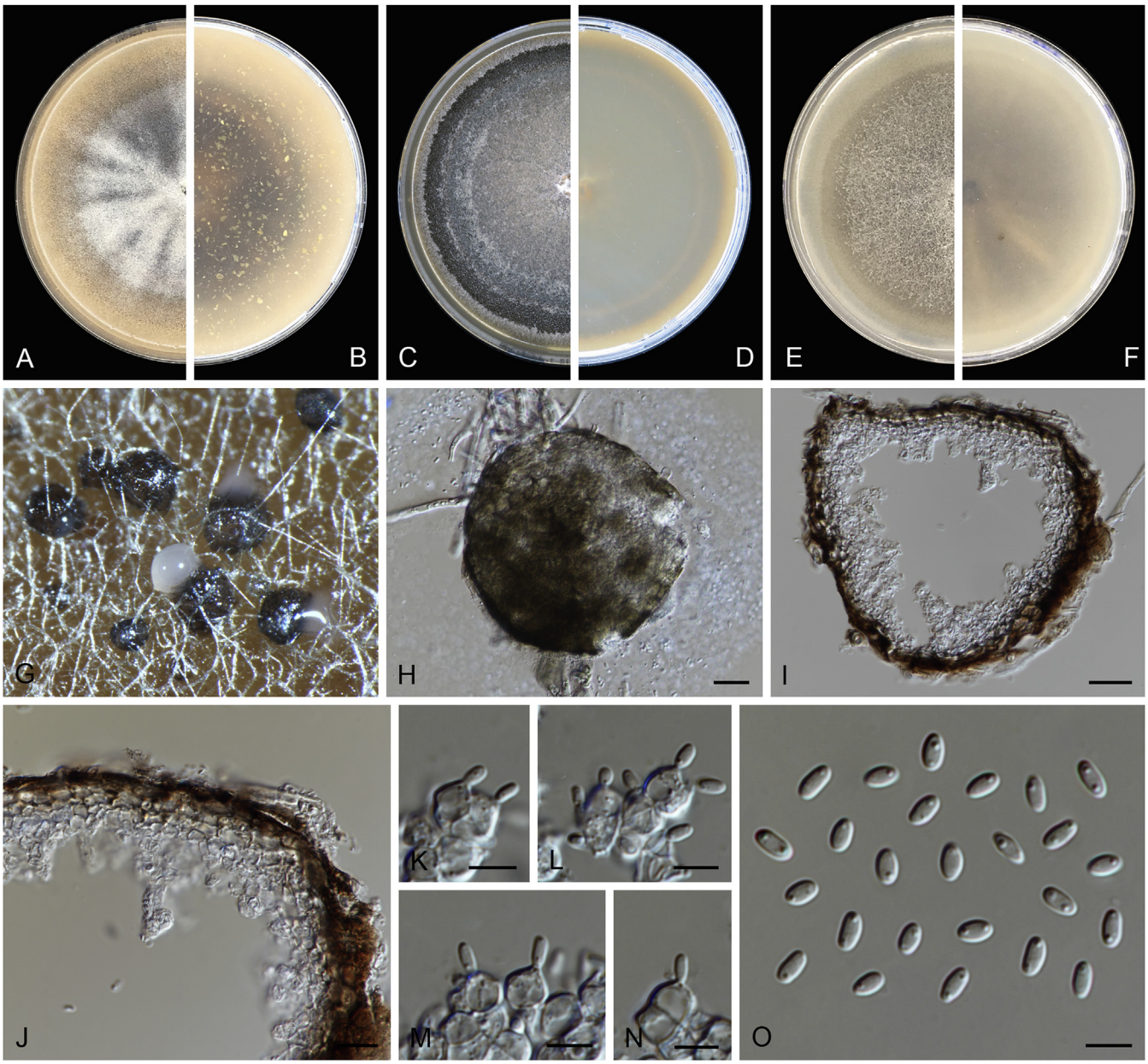

Fig. 24. *Didymella aloeicola* (CBS 562.88). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H–I = 20 µm; J = 10 µm; K–O = 5 µm.

Description: De Gruyter & Noordeloos (1992).

Materials examined: **Germany**, Giessen, Dec. 1979, R. Hadlok, specimen CBS H-16562, culture CBS 253.80; former West-Germany, from plastic, Dec. 1965, H. Kühlwein, culture CBS 397.65; Pfalz, *Robinia pseudoacacia* (*Fabaceae*), unknown date and collector, culture CBS 300.36. **The Netherlands**, Ter Apel, from potato flour, 1983, collector unknown, culture CBS 360.84. **UK**, from *Calluna* sp. (*Ericaceae*), Nov. 1929, R.W. Rayner (ex-type living culture of *Phoma radicis-callunae* CBS 285.29). **Unknown**, wood pulp, Jan. 1940, E. Rennerfelt (ex-type living culture of *Phoma suecica* CBS 167.42).

Notes: *Phoma suecica* was treated as synonym of *Didymella anserina* based on a reference isolate CBS 397.65 (Chen *et al.* 2015). In this study, the ex-type culture of *Phoma suecica* (CBS 167.42) was examined. In our phylogenetic analyses, it clustered with the representative strain of *Did. anserina* (CBS 360.84), which agreed with the findings of Chen *et al.* (2015).

Didymella combreti (Crous) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833516.

Basionym: *Peyronellaea combreti* Crous, Persoonia 32: 223. 2014.

Description: Crous *et al.* (2014).

Typus: **Zambia**, S16°55.766', E27°75.914', on *Combretum mossambicensis* (*Combretaceae*), 28 Feb. 2013, M. van der Bank (**holotype** CBS H-21694, ex-type living culture CPC 22587 = CBS 137982).

Notes: *Peyronellaea combreti* was originally introduced based on a phylogenetic study of ITS and LSU sequence data (Crous *et al.* 2014). A more extensive phylogenetic analysis on DNA sequences from four loci ITS, LSU, *rpb2* and *tub2* in this study revealed that the ex-type isolate (CBS 137982) grouped in the genus *Didymella* and was distinct from other species (Fig. 1).

Didymella guttulata L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833521. Fig. 25.

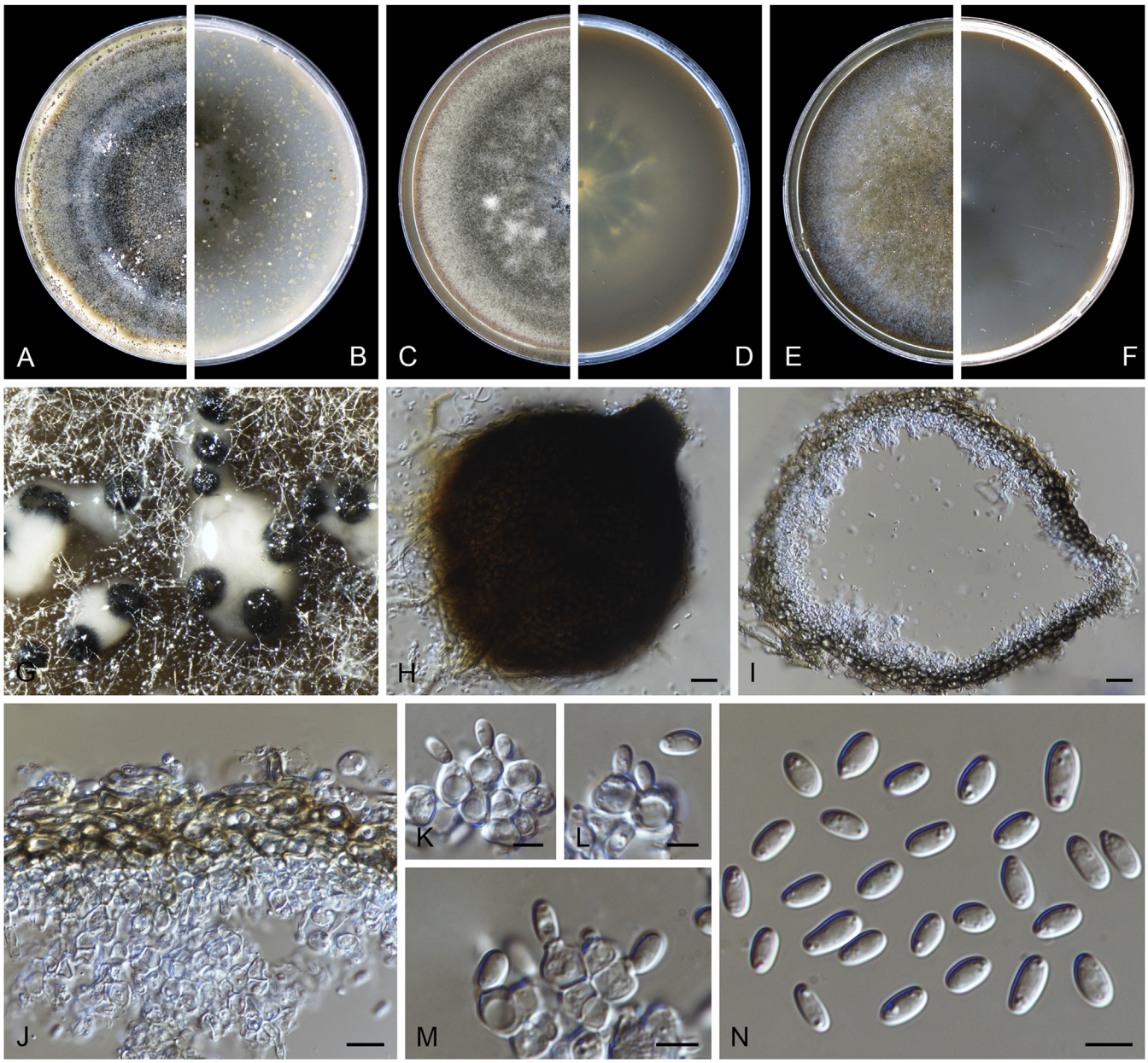

Fig. 25. *Didymella guttulata* (CBS 127976). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 20 µm; J = 10 µm; K–N = 5 µm.

Etymology: Name derived from the guttulate conidia produced by this species.

Description: *Conidiomata* pycnidial, produced superficially on the agar as well as in the mycelium, solitary, scattered or aggregated, mostly globose, subglobose or flask-shaped, darker brown, with hyphal outgrowths, ostiolate, 120–360 × 105–280 µm. *Ostioles* single, centric. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, 23.5–51.5 µm thick, outer 1–3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, doliiform or lageniform, 4.5–9 × 4–7.5 µm. *Conidia* oblong, ovoid or ellipsoidal, smooth- and thin-walled, aseptate, (3.5–)4.5–7(–8.5) × 2.5–4 µm, mostly with two minute, polar guttules, sometimes 3–4 minute guttules. *Conidial matrix* pale pink.

Culture characteristics: Colonies on OA reaching 60–65 mm diam after 7 d at 25 °C, margin regular, aerial mycelium felty, dark mouse grey to pale mouse grey, margin brownish, abundant production of pycnidia; reverse olivaceous. Colonies on MEA reaching 65–70 mm diam after 7 d, margin regular, covered with moderate aerial mycelium, felty, olivaceous grey, pale grey towards the periphery; reverse olivaceous grey with brown edge. Colonies on PDA reaching 68–72 mm diam after 7 d, margin regular, covered by felty aerial mycelium, olivaceous, mouse grey towards periphery; reverse dark mouse grey. NaOH spot test negative on OA.

Typus: **Zimbabwe**, near Umtali, from soil in deciduous forest, date and collector unknown (**holotype** CBS H-23691, ex-type living culture CBS 127976).

Notes: *Didymella guttulata* was phylogenetically allied to *Did. combreti* and *Did. eucalyptica*. Morphologically, it differed from *Did. combreti* in having larger pycnidia (120–360 × 105–280 µm *vs.* up to 250 µm diam), smaller conidia [(3.5–)

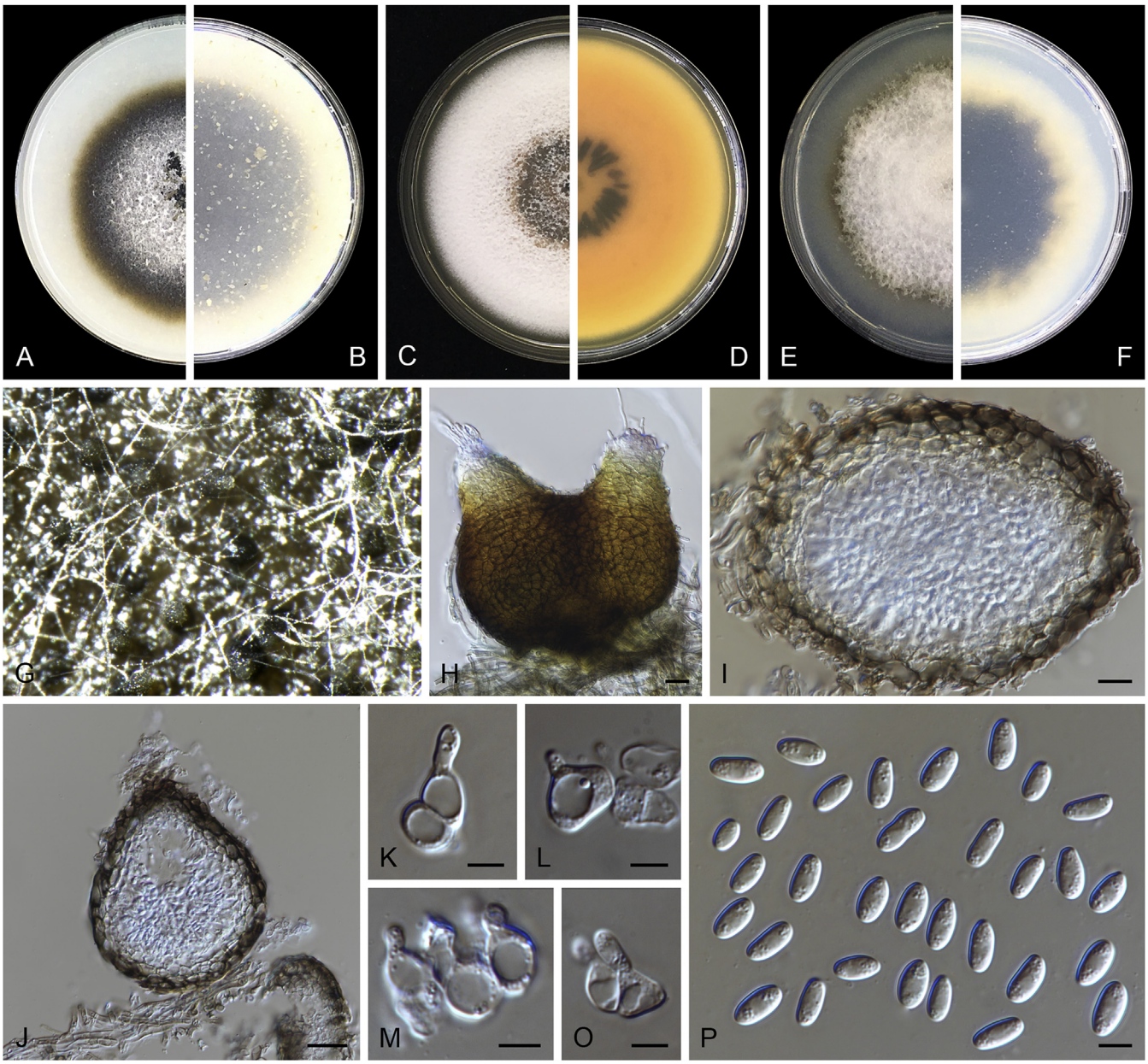

Fig. 26. *Didymella indica* (CBS 653.77). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidia. **I–J.** Section through pycnidium. **K–O.** Conidiogenous cells. **P.** Conidia. Scale bars: H = 20 µm; I–J = 10 µm; K–P = 5 µm.

4.5–7(–8.5) × 2.5–4 µm *vs.* (5–)8–10(–12) × 3.5(–4) µm] and absence of chlamydospores (Crous *et al.* 2014). *Didymella guttulata* could be distinguished from *Did. eucalyptica* by producing larger pycnidia (120–360 × 105–280 µm *vs.* 120–250 × 80–200 µm) and larger, guttulate conidia [(3.5–) 4.5–7(–8.5) × 2.5–4 µm *vs.* 2.8–4.2 × 1–2 µm] (De Gruyter & Noordeloos 1992).

Didymella indica L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833517. Fig. 26.

Etymology: Named after the country where this species was collected, India.

Description: *Conidiomata* pycnidial, produced on the agar surface or semi-immersed, scattered and solitary, obpyriform or subglobose, brown or darker brown, thin-walled, with hyphal outgrowths, ostiolate, 145–220 × 85–160 µm. *Ostioles* single, central, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–4 layers, 8–22 µm thick, outer three cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to lageniform, 6.5–9 × 6–8 µm. *Conidia* oblong to ellipsoidal, hyaline, smooth- and thin-walled, aseptate, 5–9 × 3.5–4.5 µm, mostly with minute guttules. *Conidial matrix* buff.

Culture characteristics: Colonies on OA, 65–70 mm diam after 7 d at 25 °C, margin regular, aerial mycelium felty, whitish, olivaceous towards periphery; reverse pale olivaceous. Colonies on MEA 70–75 mm diam after 7 d, margin regular, covered with medium aerial mycelium, felty, whitish, olivaceous near the centre; reverse buff to pale brownish. Colonies on PDA, 70–75 mm diam after 7 d, margin regular, covered by felty aerial mycelium, buff, but aerial mycelium near margin flat, pale olivaceous; reverse olivaceous black, with buff edge. NaOH spot test negative on OA.

Typus: **India**, Kurukshetra, host and date unknown, K.R. Aneja (**holotype** CBS H-24311, ex-type living culture CBS 653.77).

Notes: *Didymella indica* formed a well-supported and distinct clade on the four-locus phylogenetic tree (Fig. 1), closely related

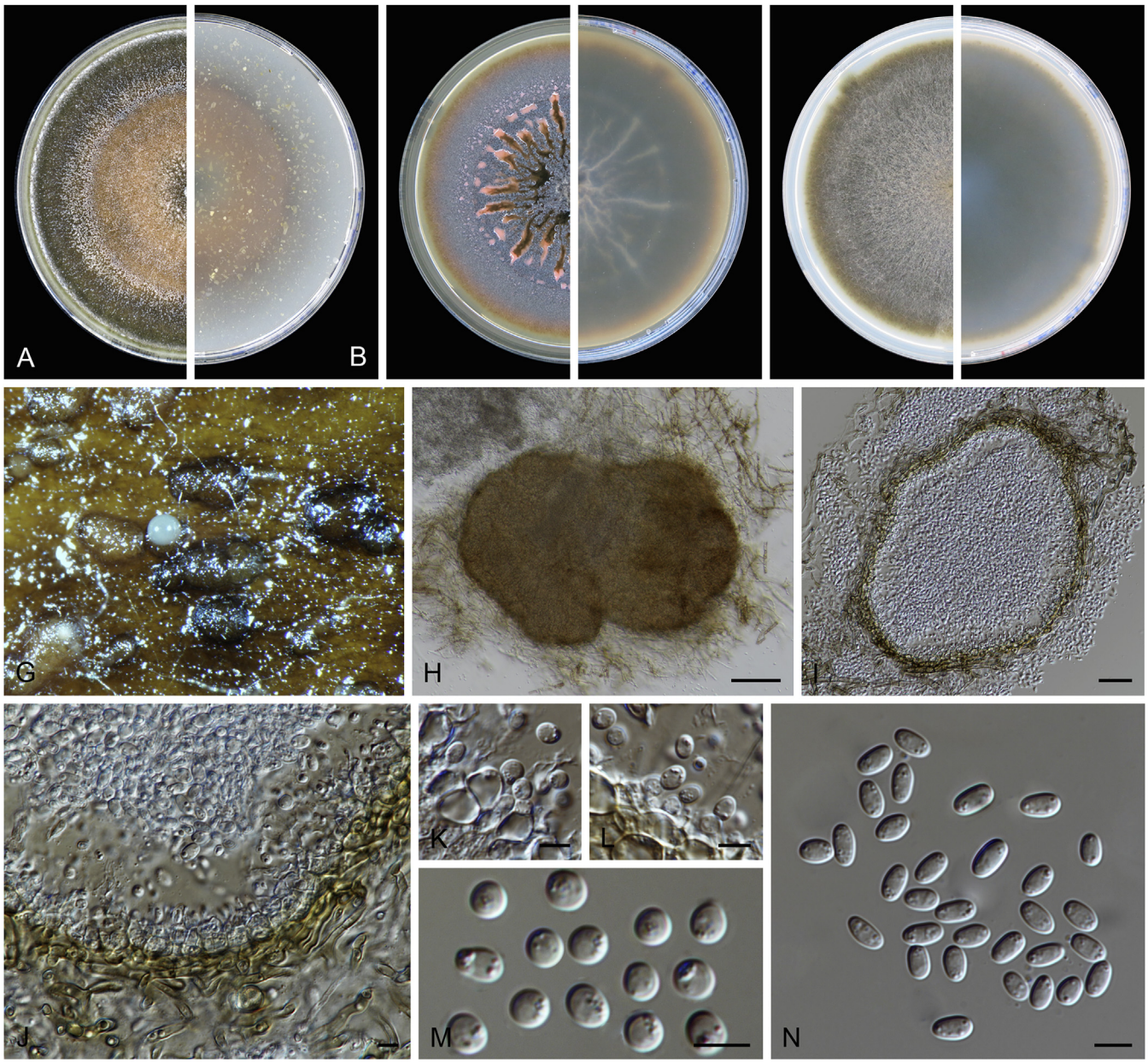

Fig. 27. *Didymella mitis* (CBS 443.72). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–L.** Conidiogenous cells. **M**. Globose conidia. **N.** Conidia. Scale bars: H = 100 µm; I = 50 µm; J–N = 5 µm.

to *Did. boeremae* and *Did. dimorpha*. *Didymella indica* differs genetically from *Did. boeremae* in 17 bp of the ITS nucleotide sequence, 2 bp of LSU, 10 bp of *tub2* and 35 bp of *rpb2*; and it differs from *Did. dimorpha* in 6 bp of the ITS nucleotide sequence, 10 bp of *tub2* and 36 bp of *rpb2*, based on alignment of the concatenated four loci deposited in TreeBASE (S25826). Morphologically, *Did. indica* can be distinguished from *Did. dimorpha* by producing wider conidia [3.5–4.5 µm *vs*. (2–) 2.5–3(–3.5) µm] *in vitro*; from *Did. boeremae* by producing shorter conidia (5–9 µm *vs*. 8–15 µm). Besides, *Did. indica* is clearly different from the latter two species by producing obpyriform pycnidia while *Did. boeremae* and *Did. dimorpha* produce (sub-)globose to irregular pycnidia.

Didymella mitis L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833519. Fig. 27.

Etymology: Name derived from the soft pycnidia wall produced by this species, *mitis* = soft.

Description: *Conidiomata* pycnidial, produced partly in the agar, solitary, sometimes 2–5 confluent, scattered or aggregated, mostly (sub-)globose, always irregular-shaped with age, buff to pale brown at the beginning, darker brown with age, thin-walled, glabrous, 270–600 × 180–400 µm. *Ostioles* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–4 layers, 13–25 µm thick, outer cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, (sub-) globose, ampulliform, 5.5–6.5 × 5.5–6 µm. *Conidia* oblong, ovoid or ellipsoidal, hyaline, smooth- and thin-walled, aseptate, (3.5–)4.5–6.5 × 2.5–3.5 µm, with two minute guttules or eguttulate; globose conidia exist, smooth- and thin-walled, aseptate, 3–4 × 2.5–4 µm. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 60–65 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, salmon near the centre and olivaceous towards periphery, abundant production of pycnidia; reverse concolourous. Colonies on MEA reaching 55–60 mm diam after 7 d, margin regular, aerial mycelium flat, sparse, grey with brown margin, abundant production of pycnidia, pinkish conidial matrix visible; reverse dark brown with pale brown margin. Colonies on PDA reaching 55–60 mm diam after 7 d, margin regular, covered by sparse floccose aerial mycelium, pale olivaceous; reverse olivaceous black. NaOH spot test negative on OA.

Typus: **South Africa**, near Otavi, from soil, Oct. 1971, G. Franz (**holotype** CBS H-16400, ex-type living culture CBS 443.72).

Additional material examined: **Namibia**, about 18 km west of Mariental, 90 km east of Maltahohe, from surface soil, desert, clay hardpan, under *Boscia albitrunca* (*Capparaceae*), date unknown, M. Christensen, culture CBS 126162.

Notes: Isolate CBS 443.72 was initially deposited as "*Didymella musae*". In this study, CBS 443.72 together with CBS 126162 clustered in a well-supported clade in the genus *Didymella*, distant from the representative culture of *Did. musae* (CBS 463.69; Fig. 1). Phylogenetically, *Didymella mitis* was closely related to *Did. sancta*, *Did. keratinophila* and *Did. coffeae-arabicae*, but distinguished from the latter three species by producing inconspicuously ostiolate pycnidia. Moreover, *Didymella mitis* differed from *Did. sancta* and *Did. coffeae-arabicae* by producing smaller conidia [(3.5–) 4.5–6.5 × 2.5–3.5 µm in *Did. mitis*, 5–7(–7.5) × 2.5–4(–4.5) µm in *Did. sancta*, and (4–)4.5–6(–7) × (2.5–)3–4(–4.5) µm in *Did. coffeae-arabicae*] (Aveskamp *et al.* 2009a).

Didymella pinodella (L.K. Jones) Qian Chen & L. Cai, Stud. Mycol. 82: 178. 2015.
Basionym: *Ascochyta pinodella* L.K. Jones, Bull. New York Agric. Exp. Sta., Geneva 547: 10. 1927.
Synonyms: *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema, Netherlands J. Pl. Pathol. 71: 88. 1965.
Ascochyta sojicola Abramov, Bolezni i Vrediteli Soievykh Bobov na Dal'nem Vostoke 68. 1931.
Phoma trifolii E.M. Johnson & Valleau, Bull. Kentucky Agric. Exp. Sta. 339: 57. 1933.
Phomatosphaeropsis pinicola Ribaldi, Ann. Sperim. Agrar. 7(3): 849. 1953.
Phoma pinodella (L.K. Jones) Morgan-Jones & K.B. Burch, Mycotaxon 29: 485. 1987.
Phoma sojicola (Abramov) Kövics, Gruyter & Aa, Mycol. Res. 103(8): 1066. 1999.
Peyronellaea pinodella (L.K. Jones) Aveskamp *et al.*, Stud. Mycol. 65: 33. 2010.

Description: De Gruyter *et al.* (2002).

Materials examined: **Hungary**, from seed of *Glycine max* (*Fabaceae*), 2000, Plant Protection Service Wageningen, culture CBS 100580 = PD D/ 054; from *G. max*, 1997, G. Kövics, culture CBS 567.97 = PD 97/2160. **Italy**, Torino, from bark and wood of *Pinus nigra* var. *austriaca* (*Pinaceae*), 1953, M. Ribaldi, culture CBS 300.53 (ex-type living culture of *Phomatosphaeropsis pinicola*). **The Netherlands**, from a stem of *Pisum sativum* (*Fabaceae*), Jul. 1990, M.E. Noordeloos, culture CBS 318.90 = PD 81/729. **USA**, Minnesota, from *Trifolium pratense* (*Fabaceae*), Sep. 1966, culture CBS 531.66; from *Trifolium* sp. (*Fabaceae*), May 1934, E.M. Johnson & W.D. Valleau, culture CBS 351.34 = MUCL 18217 = MUCL 9927 (authentic strain of *Phoma trifolii*).

Notes: *Phoma trifolii* was originally described by Johnson & Valleau (1933) from *Trifolium* spp. in America and later treated as a variety *Phoma medicaginis* var. *pinodella* based on morphology (Boerema *et al.* 1965). However, *Phoma medicaginis* var. *pinodella* was recently elevated to species status as *Didymella pinodella* (Chen *et al.* 2015). Another species, *Phomatosphaeropsis pinicola*, was originally isolated from *Pinus nigra* var. *austriaca* in Italy. In this study, based on the multi-locus phylogenetic analyses, the ex-type strain of *Phomatos. pinicola* (CBS 300.53) and the authentic strain of *Phoma trifolii* (CBS 351.34), clustered with the reference culture of *Did. pinodella*. Therefore, we regarded *Phomatos. pinicola* and *Phoma trifolii* as synonyms of *Did. pinodella*.

Phoma sojicola was first described from *Glycine max* in Russia. De Gruyter *et al.* (2002) described CBS 100580 as a representative strain of *Phoma sojicola* as it was morphologically similar to the original description. Together with CBS 100580, another two isolates CBS 567.97 and CBS 113.53 were also originally identified as *Phoma sojicola*. However, CBS 100580 and CBS 567.97 (from *Glycine max* in Hungary), clustered with *Did. pinodella* in the phylogenetic tree (Fig.1). Therefore, *Phoma sojicola* was reduced to synonymy with *Did. pinodella*. Isolate CBS 113.53 clustered in the genus *Allophoma*, and was re-identified as *Al. zantedeschiae*.

Didymella pomorum (Thüm.) Qian Chen & L. Cai, Stud. Mycol. 82: 179. 2015.
Basionym: *Phoma pomorum* Thüm., Fungi Pomicoli: 105. 1879.
Synonyms: *Phoma triticina* E. Müll., Phytopathol. Z. 19: 413. 1952.
Peyronellaea nigricans Kusnezowa, Novoste Sist. Nizsh. Rast. 8: 191. 1971.
Peyronellaea circinata Kusnezowa, Novoste Sist. Nizsh. Rast. 8: 189. 1971.
Phoma jolyana var. *circinata* (Kusnezowa) Boerema & Kesteren, Kew Bull. 31: 535. 1977.
Phoma pomorum var. *circinata* (Kusnezowa) Aveskamp *et al.*, Mycologia 101: 377. 2009.
Peyronellaea pomorum var. *circinata* (Kusnezowa) Aveskamp *et al.*, Stud. Mycol. 65: 33. 2010.
Phoma cyanea Jooste & Papendorf, Mycotaxon 12: 444. 1981.
Phoma pomorum var. *cyanea* (Jooste & Papendorf) Aveskamp *et al.*, Mycologia 101: 377. 2009.
Peyronellaea pomorum var. *cyanea* (Jooste & Papendorf) Aveskamp *et al.*, Stud. Mycol. 65: 32. 2010.
Peyronellaea pomorum var. *pomorum* (Thüm.) Aveskamp *et al.*, Stud. Mycol. 65: 33. 2010.

Description: Boerema (1993).

Materials examined: **Russia**, Siberia, Novosibirsk, Hortus Botanicus, from a leaf of *Allium nutans* (*Liliaceae*), 1963, T.T. Kuznetsova (**holotype** of *Peyronellaea nigricans* CBS H-16399, ex-type living culture CBS 286.76 = ATCC 26242 = IMI 176743 = VKM F-1844). **The Netherlands**, Wageningen, from *Polygonum tataricum* (*Polygonaceae*), Sep. 1966, M.M.J. Dorenbosch, specimen CBS H-16540, culture CBS 539.66 = ATCC 16791 = IMI 122266 = PD 64/914.

Notes: *Peyronellaea nigricans* was originally isolated from a leaf of *Allium nutans* in Siberia (Kuznetsova 1971). However, the ex-type culture of *Pe. nigricans* (CBS 286.76) clustered with the representative strain of *Didymella pomorum* (CBS 539.66) based on phylogenetic data (Aveskamp *et al.* 2010). In the present study, this species was re-examined and *rpb2* sequences were provided.

Didymella prolaticolla L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833518. Fig. 28.

Etymology: Name refers to the elongated ostiolar necks, *prolatus* (= extended), *collum* = neck.

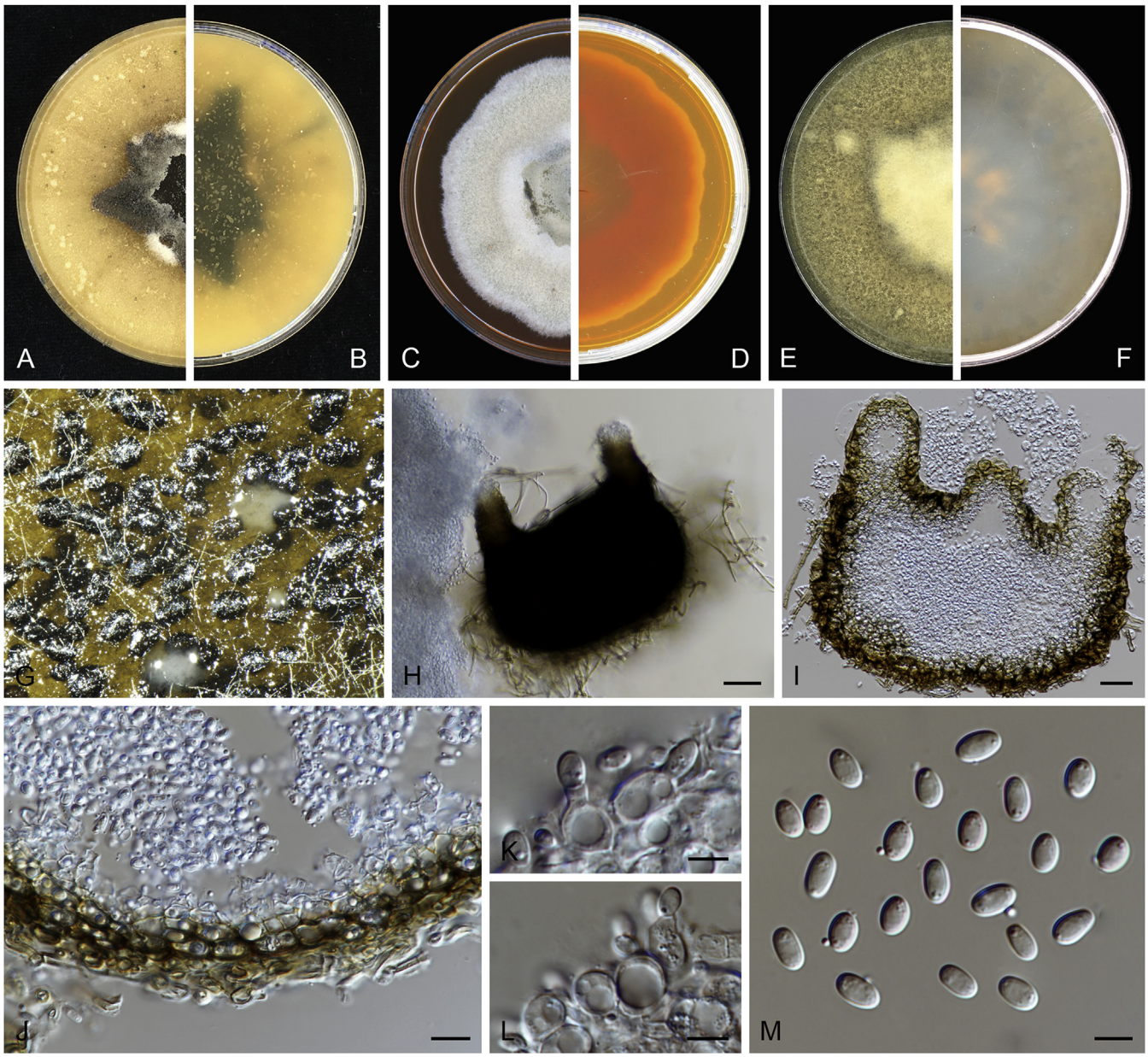

Fig. 28. *Didymella prolaticolla* (CBS 126182). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K, L.** Conidiogenous cells. **M.** Conidia. Scale bars: H = 50 µm; I = 20 µm, J = 10 µm; K–M = 5 µm.

Description: *Conidiomata* pycnidial, semi-immersed, scattered or aggregated, mostly solitary, sometimes confluent, mostly (sub-) globose or lageniform, darker brown, with hyphal outgrowths, ostiolate, (100–)190–350(–470) × (90–)175–275 µm. *Ostioles* 1–3, mostly 1–2, developing into elongated necks. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–6 layers, 16–51 µm thick, outer three cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform to lageniform, sometimes with a papilla or a column at top, 5–10 × 4.5–8 µm. *Conidia* ovoid or ellipsoidal, hyaline, smooth- and thin-walled, aseptate, 5–7 × 2.5–4 µm, eguttulate or with 1–2 minute, polar guttules. *Conidial matrix* whitish, turning buff or pale brown with age.

Culture characteristics: Colonies on OA reaching 65–70 mm diam after 7 d at 25 °C, margin regular, aerial mycelium sparsely, flat, straw but mouse grey near the central zone; reverse concolourous. Colonies on MEA reaching 55–60 mm diam after 7 d, margin regular, covered with medium aerial mycelium, felty, whitish to buff; reverse orange to brick. Colonies on PDA reaching 80–85 mm diam after 7 d, margin regular, covered by felty aerial mycelium, pale olivaceous, but aerial mycelium near centre floccose, buff; reverse olivaceous. NaOH spot test results in a pale reddish discolouration near the margin of mycelium on OA.

Typus: **Namibia**, about 18 km west of Mariental, 90 km east of Maltahohe, from surface soil, clay hardpan, under *Boscia albitrunca* in desert, date unknown, M. Christensen (**holotype** CBS H-23664, ex-type living culture CBS 126182).

Notes: Phylogenetically, isolate CBS 126182 formed a distinct lineage sister to *Didymella gardeniae*. Morphologically, CBS 126182 clearly differed from *Did. gardeniae* by producing larger pycnidia with more ostioles and elongated necks, [(100–)190–350(–470) × (90–)175–275 µm, 1–3 ostioles *vs.* 50–180 µm diam, single ostiole]. In addition, CBS 126182 produced shorter conidia than *Did. gardeniae* [5–7 × 2.5–4 µm *vs.*

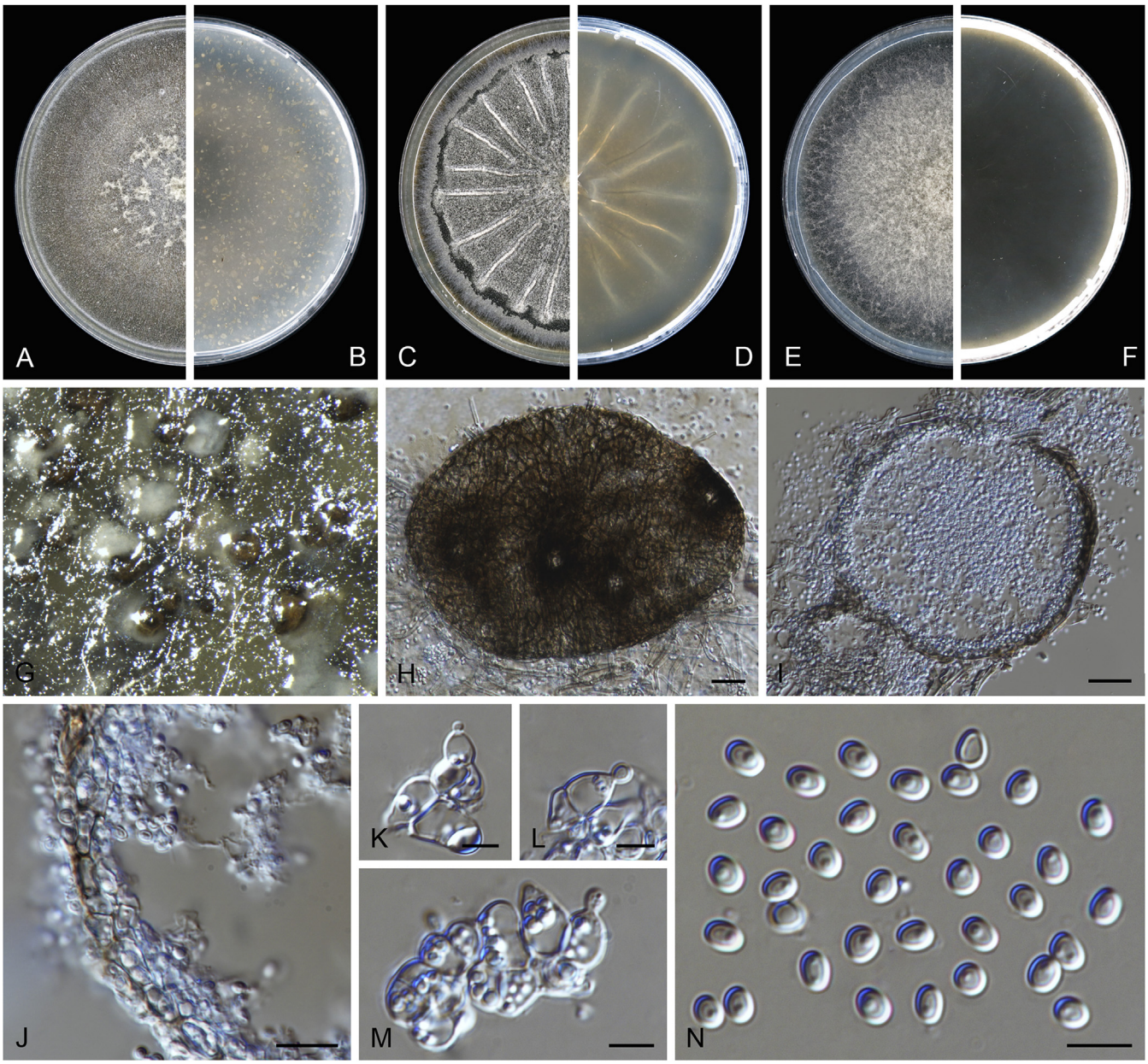

Fig. 29. *Didymella subglobispora* (CBS 364.91). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 20 µm; J = 10 µm; K–N = 5 µm.

(3.5–)5–8.5(–10.5) × (1.5–)2–3.5(–4.5) µm]. Therefore, CBS 126182 was introduced as a new species, *Did. prolaticolla*.

Didymella prosopidis (Crous & A.R. Wood) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833520.
Basionym: *Peyronellaea prosopidis* Crous & A.R. Wood, Persoonia 31: 207. 2013.

Description: Crous *et al.* (2013b).

Typus: **South Africa**, Western Cape Province, Calvinia, associated with a stem disease of *Prosopis* sp. (*Fabaceae*), Sep. 2012, A. Wood (**holotype** CBS H-21425, ex-type living culture CPC 21698 = CBS 136414).

Additional material examined: **South Africa**, Western Cape Province, Calvinia, associated with a stem disease of *Prosopis* sp. (*Fabaceae*), Sep. 2012, A. Wood, culture CPC 21704 = CBS 136550.

Notes: The holotype of *Peyronellaea prosopidis* was originally isolated from stems of a *Prosopis* sp. in South Africa (Crous *et al.* 2013b). Later, the genus *Peyronellaea* was treated as synonym of *Didymella* (Chen *et al.* 2015). Based on the combined four-gene phylogenetic analysis in the present study, *Pe. prosopidis* clustered in a distinct and well-supported lineage in *Didymella*. Therefore, a new combination was proposed as *Did. prosopidis*.

Didymella subglobispora L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833515. Fig. 29.

Etymology: Name refers to the subglobose conidia produced by this species.

Description: *Conidiomata* pycnidial, immersed or semi-immersed, scattered or aggregated, solitary, mostly globose, flask-shaped, brown to darker brown, thin-walled, glabrous,

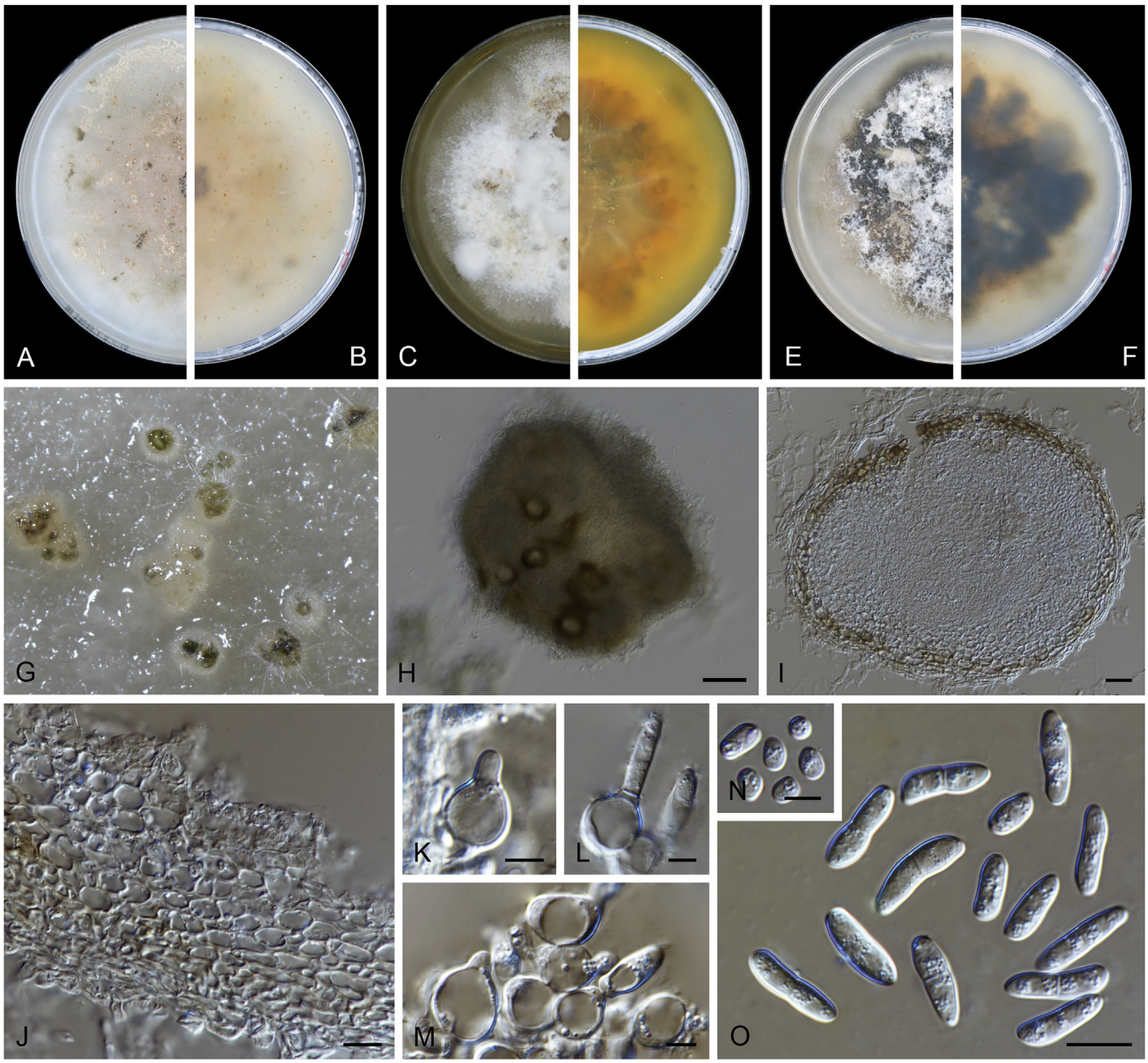

Fig. 30. *Didymella subrosea* (CBS 733.79). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N**. Globose conidia. **O.** Conidia. Scale bars: H = 100 µm; I = 20 µm, J, O = 10 µm; K–N = 5 µm.

ostiolate, 100–230 × 95–190 µm. *Ostiole* single and centric, up to four with age. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 2–4 layers, 7.5–18.5 µm thick, outer cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or lageniform, 5–9 × 4–7 µm. *Conidia* globose, subglobose or broad ellipsoidal, smooth- and thin-walled, aseptate, 2.5–3.5 × 2–3 µm, mostly with one minute-sized guttule. *Conidial matrix* buff.

Culture characteristics: Colonies on OA reaching 70–75 mm diam after 7 d at 25 °C, margin regular, covered by medium aerial mycelium, flat, pale mouse grey; reverse concolourous. Colonies on MEA reaching 65–70 mm diam after 7 d, margin regular, covered with medium aerial mycelium, flat, mouse grey, some radially furrowed zones near the centre; reverse olivaceous, olivaceous black towards periphery. Colonies on PDA reaching 75–80 mm diam after 7 d, margin regular, covered by felty aerial mycelium, pale mouse grey, dark mouse grey towards periphery; reverse dark mouse grey. NaOH spot test negative on OA.

Typus: **Unknown**, from fruit of *Ananas sativus* (*Bromeliaceae*), Jun. 1991, Plant Protection Service, Wageningen (**holotype** CBS H-23663, ex-type living culture CBS 364.91 = PD 81/290).

Notes: The cultures CBS 364.91, CBS 365.91 and CBS 363.91 were originally received as *Phoma anserina*, a species that was described from goose manure in Werbomont, Belgium (Saccardo 1892). Later Boerema *et al.* (2004) proposed CBS 365.91 and CBS 363.91 as representative cultures for *Phoma anserina*, and they were subsequently transferred to the genus *Didymella* as *Did. anserina* (Chen *et al.* 2015). In this study, CBS 364.91 was phylogenetically closely related to other isolates of *Did. anserina*, but formed a separate lineage with 1 bp difference in the LSU nucleotide sequence, 4 bp of *tub2* and 11 bp of *rpb2*, based on

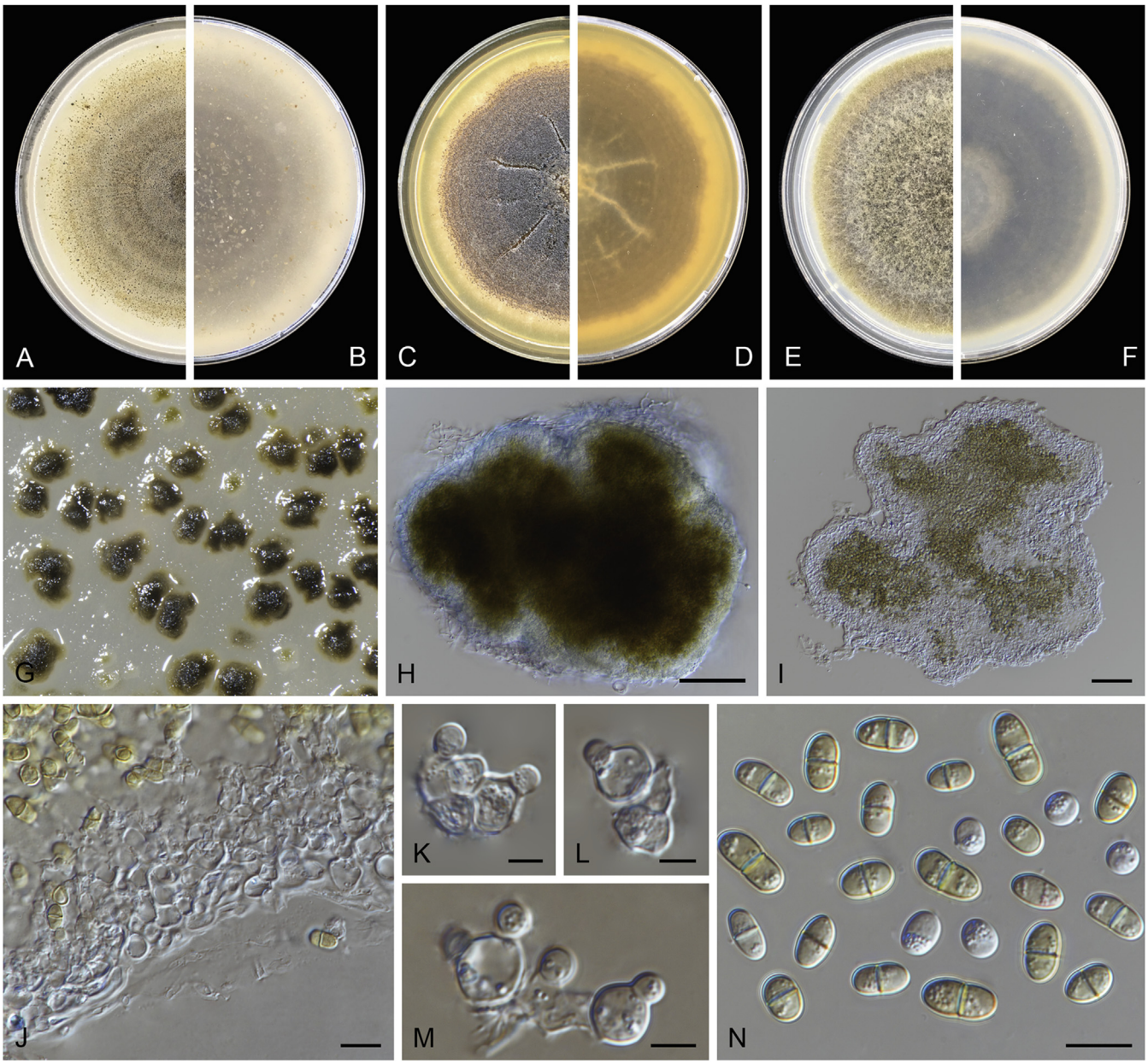

Fig. 31. *Didymella variabilis* (CBS 254.79). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 50 µm; J, N = 10 µm; K–M = 5 µm.

alignment of the four sequenced loci. Morphologically, CBS 364.91 was characterised by having globose to subglobose conidia, while *Did. anserina* had broadly ellipsoidal conidia. Besides, CBS 364.91 was recorded as different from the other two isolates in having less intensely pigmented colonies (De Gruyter & Noordeloos 1992). Therefore, we treated CBS 364.91 as a distinct species.

Didymella subrosea L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833513. Fig. 30.

Etymology: Name reflects the rosy colour of the colony on OA medium.

Description: *Conidiomata* pycnidial, produced on the surface of agar, mostly aggregated and confluent, seldom solitary, mostly globose or subglobose, whitish at beginning, turning pale brown and irregular shaped with age, especially around the ostioles (darker brown), thick-walled, with white hyphal outgrowths, ostiolate, variable in size, 160–560(–1 080) × 120–425(–630) µm. *Ostioles* 3–5, sometimes up to 12, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric or oblong cells, 4–6 layers, outer cell layers slightly pigmented, 17–50 µm thick. *Conidiogenous cells* phialidic, hyaline, smooth, (sub-)globose, lageniform or ampulliform, 8.5–15 × 7–12.5 µm. *Conidia* variable in shape and size, ovoid, fusoid to cylindrical or globose to ellipsoidal, hyaline, smooth- and thin-walled, 0–1-septate, 13–22.5 × 3.5–6 µm when 1-septate, 5–20 × 3–6 µm when aseptate, 3–6 × 2.5–4 µm for globose conidia, mostly with numerous minute guttules scattered or aggregated. *Conidial matrix* whitish to pink.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d 25 °C, margin irregular, aerial mycelium sparse, whitish to rosy buff because of the pink conidial matrix, abundant production of pale brown pycnidia; reverse buff. Colonies on MEA reaching 35–40 mm diam after 7 d, margin irregular, covered with medium aerial mycelium, felty, whitish; reverse

brownish, yellow towards periphery. Colonies on PDA reaching 45–50 mm diam after 7 d, margin irregular, covered by flat aerial mycelium, pale olivaceous grey to whitish, with some olivaceous black zones; reverse pale brown to dark brown, margin buff. NaOH spot test negative on OA.

Typus: **France**, Lyon, from *Abies alba* litter (*Pinaceae*), date unknown, F. Gourbière (**holotype** CBS H-9103, ex-type living culture CBS 733.79).

Notes: *Didymella subrosea* was proposed to accommodate isolate CBS 733.79 collected from litter of *Abies alba* in France. In the phylogenetic tree, *Did. subrosea* formed a distinct lineage separate from other species in this genus and most closely related to *Did. macrostoma* (Fig. 1). Morphologically, *Did. subrosea* was well distinguished from *Did. macrostoma* by producing larger pycnidia with more ostioles [160–560(–1080) × 120–425(–630) µm, numbers of ostioles up to 12 *vs.* 80 × 300 µm, 1–2 ostioles] and larger conidia (13–22.5 × 3.5–6 µm *vs.* 8.5–14 × 2.5–4 µm; Boerema *et al.* 2004). *Didymella subrosea* differed from *Phoma abietis-albae* (also on *Abies*) by producing larger conidia (13–22.5 × 3.5–6 µm *vs.* 5–7 × 1.5–3.5 µm; Allescher 1898).

Didymella variabilis L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833522. Fig. 31.

Etymology: From Latin *variabilis*, due to the variable shape of the pycnidia.

Description: *Conidiomata* pycnidial, immersed or semi-immersed, scattered, mostly solitary, sometimes 2–3 confluent, variable in shape and size, mostly irregularly shaped, glabrous, brown, thin-walled, pale brown gelatinous outside layer, 230–875 × 180–740 µm. *Ostiole* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of oblong, globose or isodiametric cells, 2–4 layers, 17.5–54.5 µm thick, no pigmented layers or outer cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform, 5–9.5 × 5–9 µm. *Conidia* smooth- and thin-walled, variable in shape and size, 1-septate or aseptate, round-ellipsoidal or oblong with both ends rounded, hyaline and aseptate at the beginning, 5.5–9 × 4–6 µm; turning pale brown and 1-septate with age, 7–12(–14) × 4.5–7 µm, mostly with minute polar guttules; globose conidia present, 3.5–6.5 × 3.5–5.5 µm. *Conidial matrix* not observed.

Culture characteristics: Colonies on OA reaching 65–70 mm diam after 7 d at 25 °C, margin regular, aerial mycelium sparse, buff but pale olivaceous grey near the central zone; reverse concolourous. Colonies on MEA reaching 55–60 mm diam after 7 d, margin regular, covered with sparse aerial mycelium, brown to dark brown, buff towards periphery; reverse concolourous. Colonies on PDA, 80–85 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, buff to honey; reverse concolourous. NaOH spot test results in a pale reddish discolouration near the margin of mycelium on OA.

Typus: **Italy**, Bari, from *Vitis vinifera* (*Vitaceae*), date unknown, A. Ciccarone (**holotype** CBS H-23693, ex-type living culture CBS 254.79).

Notes: Phylogenetically *Did. variabilis* clustered in the genus *Didymella*, and formed a distinct lineage sister to *Did. negriana* by having 21 bp differences, mainly in *rpb2* and *tub2* sequences. Morphologically, *Did. variabilis* differed from *Did. negriana* by producing larger pycnidia with inconspicuous ostioles [230–875 × 180–740 µm, ostioles inconspicuous *vs.* 70–220 um, 1–2(–4) papillate ostioles] and larger conidia [7–12(–14) × 4.5–7 µm *vs.* 4.5–8.5 (–10.5) × 2–4 µm]. These species could also be distinguished based on their conidial colour and septation: conidia brown and 0–1-septate in *Did. variabilis*, hyaline and aseptate in *Did. negriana*.

Multiple phoma-like species were found in association with *Vitis vinifera*, *e.g. Phoma flaccida*, *Phoma vitis* and *Phoma viniferae*. *Phoma flaccida* had been reallocated to *Macrophoma*. The new species *Didymella variabilis* clearly differed from *Phoma vitis* and *Phoma viniferae* by its larger conidia (3.5–14 × 3.5–7 µm *vs.* 3–3.5 × 1–2 µm in *Phoma vitis* and 7 × 4 µm in *Phoma viniferae*) (Bonorden 1864, Saccardo 1884, Cooke 1888).

Clade 16: *Paraboeremia* Qian Chen & L. Cai, Stud. Mycol. 82: 183. 2015.

Type species: *Paraboeremia selaginellae* (Sacc.) Qian Chen & L. Cai

Paraboeremia putaminum (Speg.) Qian Chen & L. Cai, Stud. Mycol. 82: 184. 2015.
Basionym: *Phoma putaminum* Speg., Atti Soc. Crittog. Ital. 3: 66. 1881.
Synonyms: *Aposphaeria putaminum* (Speg.) Sacc., Syll. Fung. 3: 177. 1884.
Coniothyrium putaminum (Speg.) Kuntze, Revis. Gen. Pl. 3 (3): 459. 1898.
Phoma dunorum Ten Houten, Kiemplziekt. Conif. [Thesis Univ. Utrecht]: 88. 1939. *Nom. inval*.

Description: De Gruyter & Noordeloos (1992).

Materials examined: **Denmark**, from the rhizosphere of *Malus sylvestris* (*Rosaceae*), Feb. 1969, E. Sønderhousen, culture CBS 130.69 = CECT 20054 = IMI 331916. **The Netherlands**, from a branch of *Ulmus* sp. (*Ulmaceae*), Jun. 1991, G.H. Boerema, culture CBS 372.91 = PD 75/960; Gelderland Province, Wageningen, from *Zygocactus* sp. (*Cactaceae*), unknown date, M.M.J. Dorenbosch, culture CBS 538.66 = PD 62/93; North Holland Province, Bakkum, *Pinus nigra* var. *austriaca* (*Pinaceae*) seedling root, on dune soil, unknown date, J.G. ten Houten, culture CBS 299.39 (ex-type living culture of *Phoma dunorum*).

Notes: *Phoma dunorum* was originally described from *Pinus nigra* var. *austriaca* (Ten Houten 1939) and later treated as facultative or heterotypic synonym of *Phoma putaminum* (currently: *Paraboeremia putaminum*) based on morphological characteristics (De Gruyter & Noordeloos 1992). According to the phylogenetic analysis in this study (Fig. 1), the ex-type strain of *Phoma dunorum* (CBS 299.39) clustered with the representative strains of *Parab. putaminum* (CBS 372.91 and CBS 373.91), confirming the treatment of De Gruyter & Noordeloos (1992). *Didymella aeria* is genetically similar with *Parab. putaminum* based on ITS, LSU, tub sequences, taxonomy of which await to be confirmed with more genes (Fig. S1).

Clade 17: *Cumuliphoma* Valenz.-Lopez *et al.*, Stud. Mycol. 90: 38. 2017(2018).

Type species: *Cumuliphoma omnivirens* (Aveskamp *et al.*) Valenz.-Lopez *et al.*

Clade 18: *Macroventuria* Aa, Persoonia 6: 359. 1971.

Type species: *Macroventuria anomochaeta* Aa

Macroventuria angustispora L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833523. Fig. 32.

Etymology: Name reflects the narrowing cell of the ascospores produced by this species.

Description: *Ascomata* pseudothecial, superficial on the agar or immersed, solitary and scattered, uniloculate, (sub-) globose, with a prominent papilla, membranous, dark brown, 95–180 × 85–165 µm. *Ostioles* inconspicuous. *Pseudothecial wall* black, 2–4 layers, 15.5–45 µm, outer wall comprising two layers of dark brown, thick-walled cells, *textura angularis*. *Setae* apical on pseudothecia, pale brown, with sub-hyaline tips. *Pseudoparaphyses* absent. *Asci* 59.5–91 × 18–30.5 µm, 8-spored, biseriate, bitunicate, fissitunicate, saccate, cylindrical or obpyriform. *Ascospores* 2-celled, often constricted at the septum, the upper one slightly larger than the lower one, often with one cell narrowing at the middle, 23–28 × 10.5–13 µm, hyaline, smooth-walled, surrounded by distinct mucilaginous sheath. *Chlamydospores* not observed.

Culture characteristics: Colonies on OA, 40–45 mm diam after 7 d 25 °C, margin entire, regular, aerial mycelium sparse, flat, olivaceous black; reverse leaden grey. Colonies on MEA 30–35 mm diam after 7 d, margin irregular, aerial mycelium sparse, flat, whitish near the centre, lavender grey with an orange edge, some radially furrowed zones near the centre; reverse concolourous. Colonies on PDA, 30–35 mm diam after 7 d, margin irregular, covered by flat mycelium, olivaceous black to olivaceous, with a buff edge; reverse concolourous. NaOH spot test negative on OA.

Typus: **South Africa**, Cape Province, from trunks of *Medicago sativa* (*Fabaceae*), Jun. 1972, W.F.O. Marasas (**holotype** CBS H-14193, ex-type living culture CBS 502.72).

Notes: CBS 502.72 was initially received as *Macroventuria anomochaeta.* However, this isolate formed a distinct lineage in the genus *Macroventuria* and can be distinguished from the ex-type strain of *Ma. anomochaeta* (CBS 525.71) by producing much larger asci (59.5–91 × 18–30.5 µm *vs.* 60–75 × 16–21 µm) and wider ascospores (23–28 × 10.5–13 µm *vs.* 20–27 × 9–11 µm). Therefore, *Ma. angustispora* was introduced as a new species, based on isolate CBS 502.72.

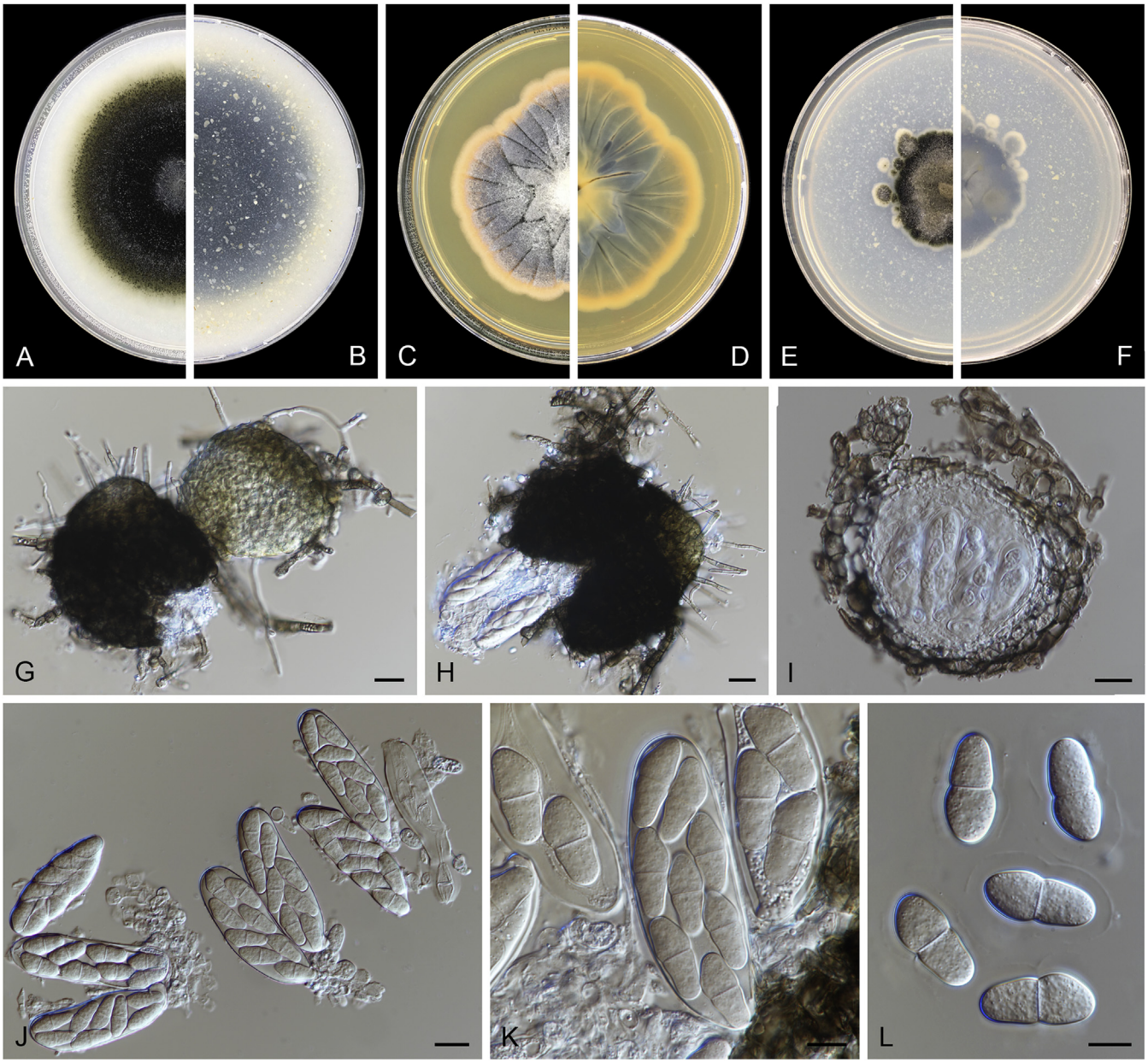

Fig. 32. *Macroventuria angustispora* (CBS 502.72). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G–H.** Pseudothecia. **I.** Section through pseudothecia. **J–K.** Asci. **L.** Ascospores. Scale bars: G–J = 20 µm; K–L = 10 µm.

Macroventuria terrestris L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833524. Fig. 33.

Etymology: The species name refers to soil, the substrate from which this fungus was isolated.

Description: *Pseudothecia* superficial on or semi-immersed in the agar, solitary and scattered, uniloculate, subglobose to ellipsoidal, membranous, dark brown to black, 135–295 × 95–285 µm. *Ostioles* inconspicuous, central. *Setae* pale brown/olivaceous, with sub-hyaline tip, cylindrical or tapering to the rounded or somewhat pointed tip. *Pseudothecial wall* black, 17.5–43.5 µm, 4–5 layers, outer wall comprising 1–3 layers of pigmented, thin-walled cells, of *textura angularis*. *Pseudoparaphyses* absent. *Asci* 102–134 × 20.5–29 µm, 8-spored, uniseriate, bitunicate, fissitunicate, saccate, cylindrical to club-shaped. *Ascospores* 2-celled, often constricted at the septum, the upper cell slightly larger than the lower one, 20–29.5 × 11.5–13 µm, hyaline, smooth- and thin-walled, surrounded by distinct mucilaginous sheath. *Chlamydospores* not observed.

Culture characteristics: Colonies on OA, 25–35 mm diam after 7 d, margin irregular, covered by flat aerial mycelium, buff to darker olivaceous; reverse concolourous. Colonies on MEA 55–60 mm diam after 7 d, margin regular, aerial mycelium flat, pale olivaceous grey, with orange margin; reverse yellow to brown. Colonies on PDA, 15–20 mm diam after 7 d, margin irregular, covered by felty, olivaceous aerial mycelium, with thin buff margin; reverse vinaceous buff to olivaceous. NaOH spot test results in a pale brown discolouration on OA.

Typus: **USA**, Utah, Canyonlands National Park, from soil, 1997, M. Christensen (**holotype** CBS H-23670, ex-type living culture CBS 127771).

Notes: Isolate CBS 127771 was received as *Macroventuria wentii*. However, it was phylogenetically distinct from the ex-type strain of *Ma. wentii* (CBS 526.71). Isolate CBS 127771 also differed morphologically from *Ma. wentii* by its ellipsoidal pseudothecia (globose or piriform in *Ma. wentii*). Furthermore, CBS 127771 produced larger pseudothecia than *Ma. wentii* (134–295 × 95–285 µm *vs.* 135–180 × 105–160 µm).

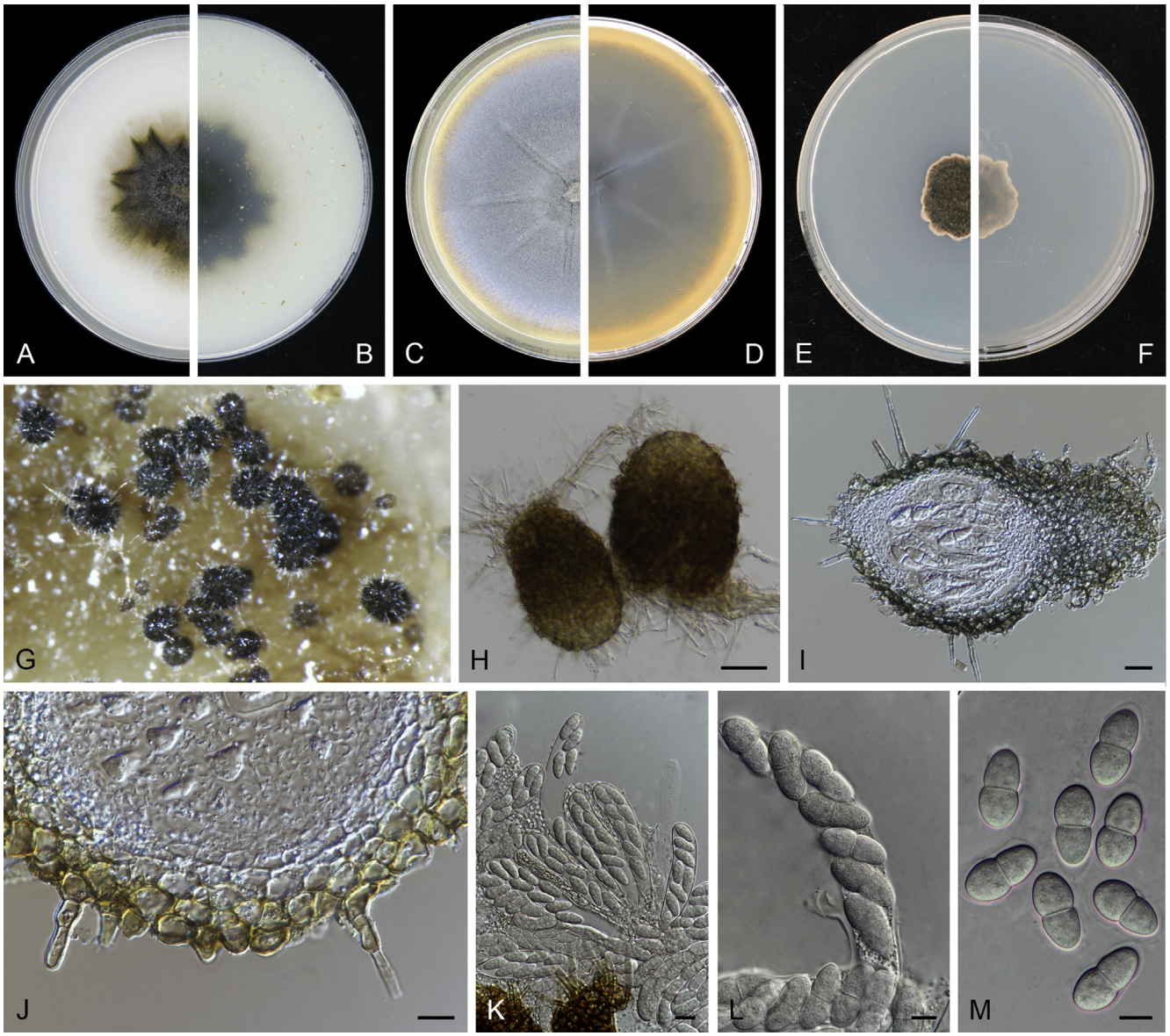

Fig. 33. *Macroventuria terrestris* (CBS 127771). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Perithecia forming on OA. **H.** Pseudothecia. **I.** Section through pseudothecium. **J.** Section of pseudothecial wall. **K–L.** Asci. **M.** Ascospores. Scale bars: H = 50 µm; I, K = 20 µm, J, L–M = 10 µm.

Clade 19: *Juxtiphoma* Valenz.-Lopez *et al.*, Stud. Mycol. 90: 40. 2017(2018).

Notes: *Juxtiphoma* was introduced by Valenzuela-Lopez *et al.* (2018) to accommodate *Phoma eupyrena* reported on stems of *Solanum tuberosum* (geographic origin not cited; Boerema *et al.*2004). It formed a well-supported monophyletic clade, and is separated from the other genera of *Didymellaceae* in the phylogenetic tree (Valenzuela-Lopez *et al.* 2018). However, when this genus was introduced, *Endophoma elongata* that was isolated from soil of a bat cave in Canada, was not included in the phylogenetic analysis. In the present study, *Endophoma elongata* clusters within the *Juxtiphoma* clade. However, they are clearly different morphologically: *E. elongata* is characterised by producing two kinds of pycnidia, spherical conidiomata and branched, non-ostiolate, cylindrical conidiomata, both of which always produced conidia endogenously. In contrast, species of *Juxtiphoma* only produce globose pycnidia with normal phoma-like conidia. Therefore, these two genera appear to be distinct.

Type species: *Juxtiphoma eupyrena* (Sacc.) Valenz.-Lopez *et al.*

Juxtiphoma kolkmaniarum Hern.-Restr. *et al.*, MycoKeys 65: 76. 2020.

Description: Hou *et al.* (2020).

Typus: **The Netherlands**, Ophemert, isolated from garden soil, Mar. 2017, L. & M. Kolkman (holotype CBS H-24214, ex-type culture CBS 146005 = JW 185006).

Additional materials examined: **Canada**, Alberta, Whitehorse Wildland Provincial Park, Boulder Run in Cadomin Cave, soil in bat cave, 13 Jun. 2010, A. Hudgins, (**holotype** of *Endophoma elongata* UAMH 11216). **Germany**, Kiel-Kitzeberg, from wheat field soil, unknown collector and collect date, specimen CBS H-16216, culture CBS 527.66.

Notes: *Juxtiphoma kolkmaniarum* was isolated from Dutch garden soil and was named after the sample collectors (Hou *et al.* 2020). *Endophoma elongata* was isolated from soil of a bat cave in Canada, but the DNA sequences proved to be identical to *J. kolkmaniarum* (based on ITS, LSU and *tub2* sequences; Tsuneda *et al.* 2011). However, *E. elongata* lacks *rpb2* sequence data for analysis. Although the sequence data show that they might be synonyms, their morphological characters are very different.

Clade 20: *Nothophoma* Qian Chen & L. Cai, Stud. Mycol. 82: 212. 2015.

Conidiomata pycnidial, globose to elongated, or irregular, superficial on or immersed into the agar, solitary or confluent, ostiolate, sometimes with a short neck. *Pycnidial wall* pseudoparenchymatous, 2–9-layered, outer wall pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, doliiform or globose, sometimes flask-shaped. *Conidia* variable in shape, hyaline but incidentally brown, smooth- and thin-walled, (0–)1(–2)-septate, *i.e.* ovoid, oblong to ellipsoidal, eguttulate or guttulate (Chen *et al.* 2015).

Type species: *Nothophoma infossa* (Ellis & Everh.) Qian Chen & L. Cai

Notes: Two newly added species of *Nothophoma*, *N. infuscata* and *N. acaciae* produce 0–1-septate conidia, and *N. eucalyptigena* produces conidia with (0–)1(–2)-septate, therefore, the generic circumscription of *Nothophoma* was emended according to the morphological features of the new member introduced in this genus.

Nothophoma acaciae (Crous) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833533.
Basionym: *Didymella acaciae* Crous, Persoonia 39: 407. 2017.

Description: Crous *et al.* (2017b).

Typus: **Australia**, New South Wales, Merimbula, on leaves of *Acacia melanoxylon* (*Fabaceae*), 28 Nov. 2016, P.W. Crous (**holotype** CBS H-23295, ex-type living culture CPC 32504 = CBS 143404).

Notes: This species, originally published as *Didymella acaciae* (Crous *et al.* 2017b), was mentioned as being phylogenetically closely related to several genera, including *Didymella*, *Paraboeremia*, *Peyronellaea* and *Verrucoconiothyrium*, containing several species which clustered in the genus *Nothophoma* in the present study. Phylogenetically, *Didymella acaciae* grouped in a sister lineage to *No. prosopidis* (Fig. 1). Therefore, a new combination was introduced as *No. acaciae*. Morphologically, *No. acaciae* is characterised by producing 1-septate conidia, which is rare in the genus *Nothophoma*.

Nothophoma arachidis-hypogaeae (V.G. Rao) Qian Chen & L. Cai, Stud. Mycol. 82: 213. 2015. Fig. 34.
Basionym: *Phyllosticta arachidis-hypogaeae* V.G. Rao, Sydowia 16: 275. 1962.
Synonyms: *Phoma arachidis-hypogaeae* (V.G. Rao) Aa & Boerema, Persoonia 15: 388. 1993.

Description from ex-epitype (CBS 125.93): *Conidiomata* pycnidial, semi-immersed or superficial on the agar surface, mostly aggregated, solitary or confluent, mostly (sub-)globose or flask-shaped, pale brown, dark brown near the ostioles, glabrous, with hypha around the ostioles, 95–260 × 80–190 µm. *Ostioles* single, central, papillate or elongated to a neck, up to 70 µm. *Pycnidial wall* pseudoparenchymatous, composed of oblong, isodiametric or globose cells, 3–5 layers, 18–23 µm thick, outer two cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, (sub-)globose, ampulliform or lageniform, 5.5–8.5 × 4.5–8 µm. *Conidia* oblong, smooth- and thin-walled, hyaline, aseptate, 4–7 × 2–3 µm, with two minute, polar guttules. *Conidial matrix* whitish to buff.

Culture characteristics: Colonies on OA, 65–70 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, buff to dark brown; reverse pale brown. Colonies on MEA 60–65 mm diam after 7 d, margin regular, densely covered with floccose aerial mycelium, buff; reverse luteous to dark brown. Colonies on PDA, 70–75 mm diam after 7 d, margin regular, densely covered by floccose aerial mycelium, buff, rosy buff towards periphery; reverse dark brown. NaOH spot test negative on OA.

Typus: **India**, Poona, from leaves of *Arachis hypogaea* (*Fabaceae*), Sep. 1962, V. Rao (**holotype** M.A.C.S. No. 134). **India**, Madras, from a leaf of *Arachis hypogaea* (*Fabaceae*), dep. Jan. 1993, J. de Gruyter (**epitype designated here** CBS H-24315, MBT389731, ex-epitype living culture CBS 125.93 = PD 77/1029).

Notes: *Phyllosticta arachidis-hypogaeae* was originally described from leaves of *Arachis hypogaea* in Poona, India (Rao 1962), with conidia measuring 5.25–6.9 × 2.2–3.57 µm. The

morphology of the specimen selected in this study, isolated from the same host and country as the holotype, agreed well with the original description (conidia 4–7 × 2–3 µm), and thus CBS H-24315 was chosen as epitype, with CBS 125.93 as ex-epitype culture.

Nothophoma brennandiae Hern.-Restr. *et al.*, Mycokeys 65: 77. 2020. Fig. 35.

Description from culture CBS 125539: *Conidiomata* pycnidial, semi-immersed or superficial on the agar surface, mostly aggregated and five or more confluent, some scattered and solitary, mostly cylindrical, club-shaped, flask-shaped or sub-globose, dark brown, thick-walled, with hyphal outgrowths, especially around the central ostioles, 210–400 × 150–300 µm. *Ostioles* 1–2, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong, isodiametric or globose cells, 4–5 layers, 11.5–38.5 µm thick, outer three cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, lageniform or doliiform, 3.5–8.5 × 3–6.5 µm. *Conidia* oblong to ovoid, smooth- and thin-walled, hyaline, turning brown with age, aseptate, 4.5–8.5 × 3–3.5 µm, with small polar guttules. *Conidial matrix* dark brown or black.

Culture characteristics: Colonies on OA, 55–60 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, pale mouse grey; reverse olivaceous. Colonies on MEA 45–50 mm diam after 7 d, margin regular, covered with sparse aerial mycelium, flat, mouse grey with brown edge, black in the centre because of the black conidial exudate, with pale mouse grey sterile section; reverse concolourous. Colonies on PDA, 55–60 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, olivaceous to pale olivaceous grey, with some pale brown area; reverse olivaceous black with olivaceous edge. NaOH spot test results in a brown discolouration near the margin of mycelium on OA.

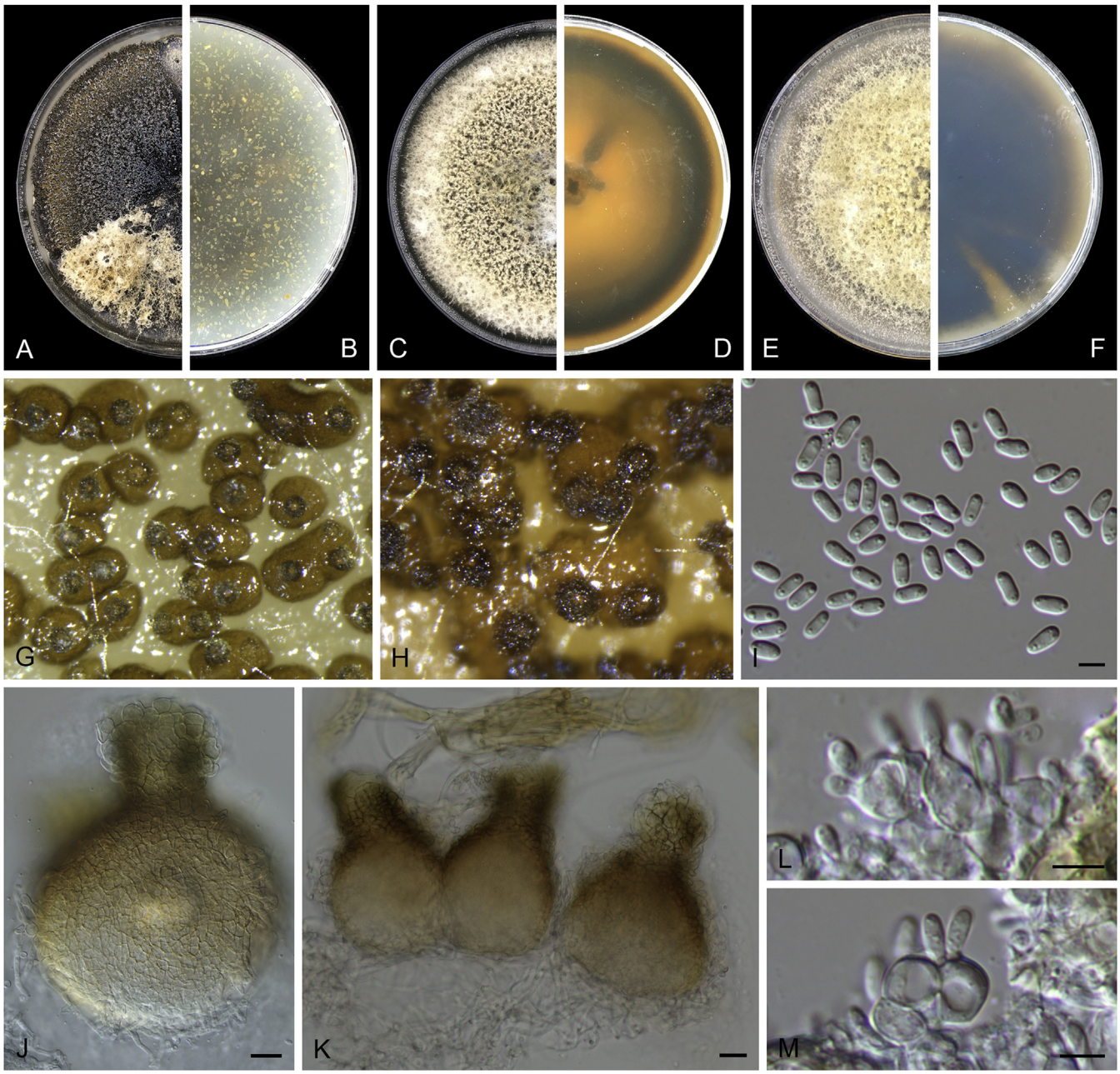

Fig. 34. *Nothophoma arachidis-hypogaeae* (CBS 125.93). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G–H.** Pycnidia forming on OA. **I.** Conidia. **J–K.** Pycnidia. **L–M.** Conidiogenous cells. Scale bars: I, L–M = 5 µm; J–K = 20 µm.

Typus: **The Netherlands**, Limburg Province, Ell, isolated from garden soil, Mar. 2017, K. Brennand (**holotype** CBS H-24103, ex-type living culture CBS 145912 = JW 53011).

Additional materials examined: **Canada**, Regina, Municipality of Sherwood, Saskatchewan, house dust, unknown date and collector, culture CBS 140540; *ibid*., cultures CBS 140541, CBS 140542. **Greece**, Northern Greece, Prefecture of Imathia, from shoot of *Amygdalus* sp. (*Rosaceae*; canker), 6 Dec. 2008, T. Thomidis, culture CBS 125539. **Italy**, Apulia, from *Olea europaea* (*Oleaceae*), 6 Dec. 2008, S. Frisullo, culture CBS 262.95; from branches of *Ostrya carpinifolia* (*Betulaceae*; hop hornbeam), unknown date and collector, culture CBS 125540. **The Netherlands,** North Holland province, Amsterdam, garden soil, Mar. 2017, J. van Dijk, JW 1066.

Notes: Three isolates from plant material in two southern European countries clustered with the ex-type strain of *Nothophoma brennandiae* (CBS 145912; Fig. 1), which was isolated from Dutch garden soil (Hou *et al.* 2020), revealing the widespread distribution of *No. brennandiae*.

Nothophoma eucalyptigena (Crous) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833534.

Basionym: *Verrucoconiothyrium eucalyptigenum* Crous, Persoonia 38: 299. 2017.

Description: Crous *et al.* (2017a).

Typus: **Australia**, Western Australia, Perth, King's Park Botanic Gardens, on leaf litter of *Eucalyptus* sp. (*Myrtaceae*), 27 Sep. 2015, P.W. Crous (**holotype** CBS H-23098, ex-type living culture CPC 29000 = CBS 142535).

Notes: This species was introduced as *Verrucoconiothyrium eucalyptigenum* based on the megablast result of ITS and LSU sequences (Crous *et al.* 2017a). The multi-locus phylogenetic analyses indicated, however, that this species grouped within the genus *Nothophoma*, and formed a distinct lineage closely related to *No. infuscata* (Fig. 1), the novel species collected from dying leaves of *Acacia longifolia* (*Fabaceae*) and described in the present study. Morphologically, both species produced brown and septate conidia (see *No. infuscata* for discussion).

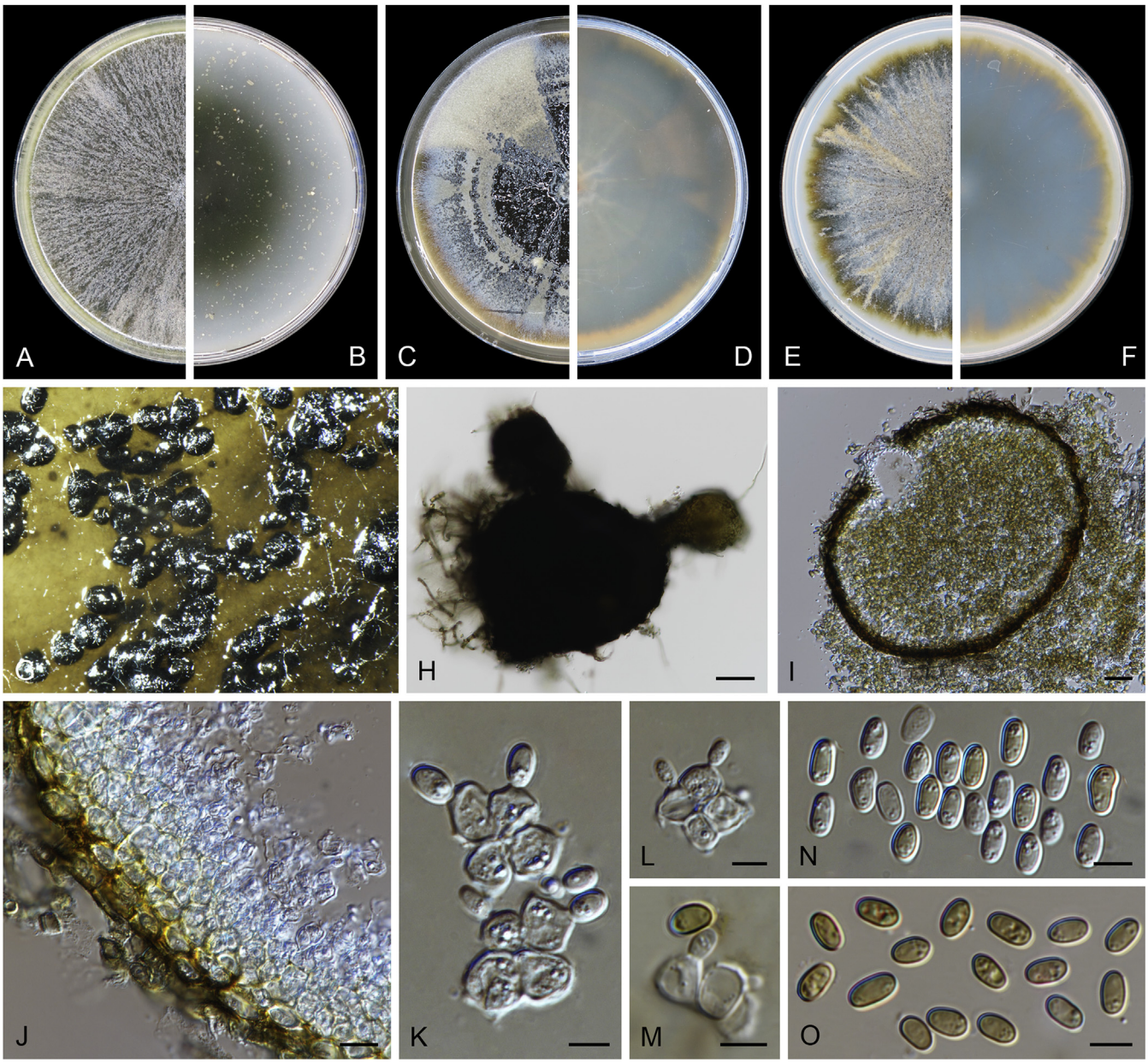

Fig. 35. *Nothophoma brennandiae* (CBS 125539). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N–O.** Conidia. Scale bars: H = 50 µm; I = 20 µm, J = 10 µm; K–O = 5 µm.

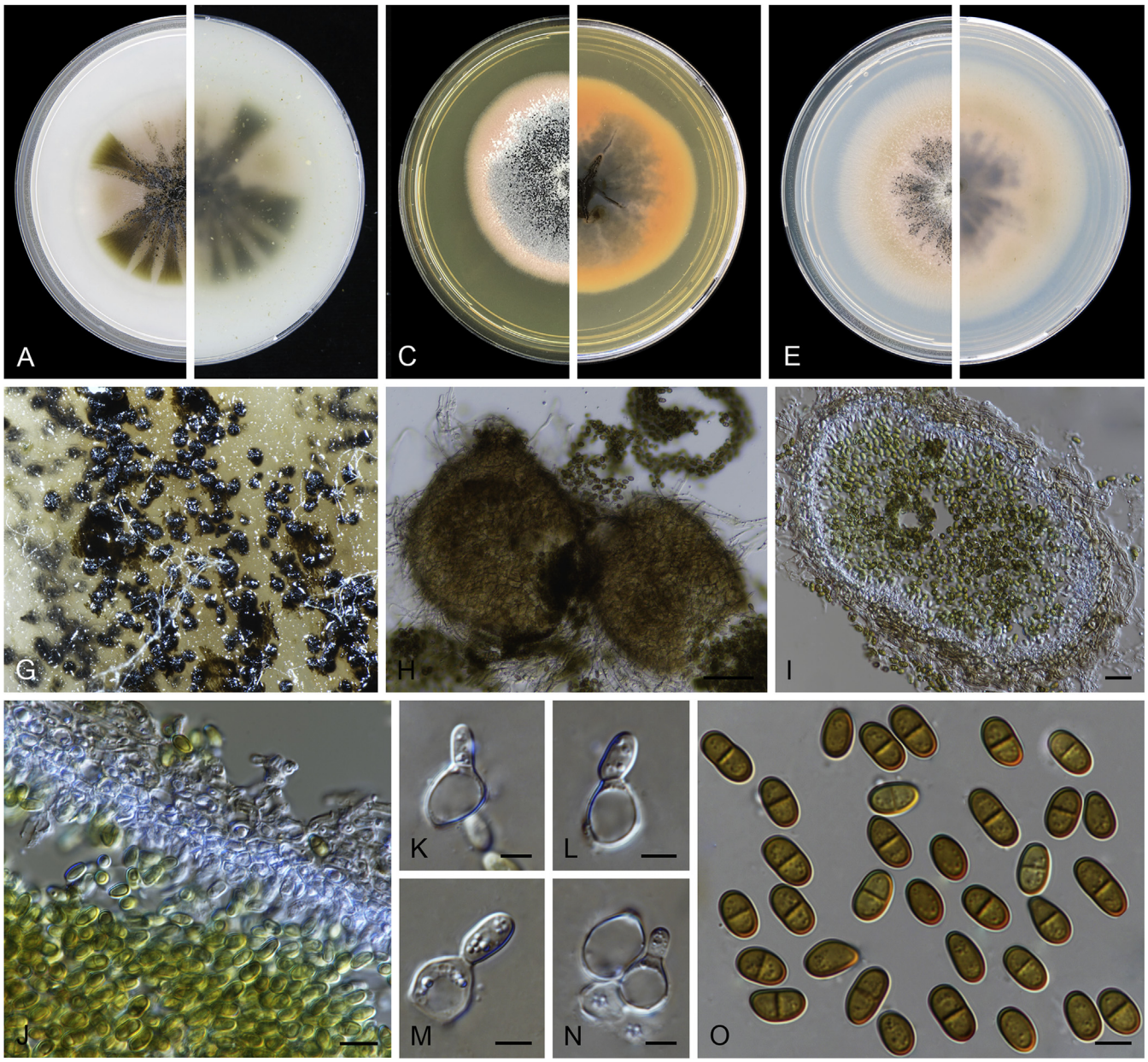

Fig. 36. *Nothophoma infuscata* (CBS 121931). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidia. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H = 50 µm; I = 20 µm; J = 10 µm; K–O = 5 µm.

Nothophoma infuscata L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833535. Fig. 36.

Etymology: Epithet derived from the brown conidial matrix produced by this species.

Description: Conidiomata pycnidial, mostly immersed in the agar, sometimes semi-immersed, scattered, and arranged in radiate lines, solitary or 2–3 confluent, (sub-)globose, brown to dark brown, thin-walled, with abundant hyphal outgrowths, especially around the ostioles, 115–335 × 95–280 µm. *Ostioles* single, mostly in the centre, papillate or not papillate, sometimes elongated into a short neck, up to 50 µm. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–6 layers, 10.5–32 µm thick, outer 1–2 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or lageniform, 7–11 × 5.5–10.5 µm. *Conidia* ovoid, ellipsoidal, oblong with round end or cylindrical, hyaline, smooth- and thin-walled, brown, 0–1-septate, (4.5–)6–11 × 3.5–5.5 µm, mostly with 5–6 minute scattered guttules. *Conidial matrix* dark brown.

Culture characteristics: Colonies on OA reaching 30–35 mm diam after 7 d at 25 °C, margin regular, without aerial mycelium, olivaceous but with some rosy buff sterile sections, production of pycnidia abundant; reverse concolourous. Colonies on MEA reaching 30–35 mm diam after 7 d, margin regular, covered with medium aerial mycelium, felty, pale olivaceous grey, salmon towards periphery, abundant production of pycnidia; reverse dark brown with orange edge. Colonies on PDA reaching 35–40 mm diam after 7 d, margin regular, covered by flat aerial mycelium, sparse, rosy buff to buff, production of pycnidia abundant; reverse concolourous. NaOH spot test results in a pale reddish discolouration near the margin of mycelium.

Typus: **New Zealand**, Auckland, Waterview, Traherne Island, from dying leaves of *Acacia longifolia* (*Fabaceae*), 16 May 2007,

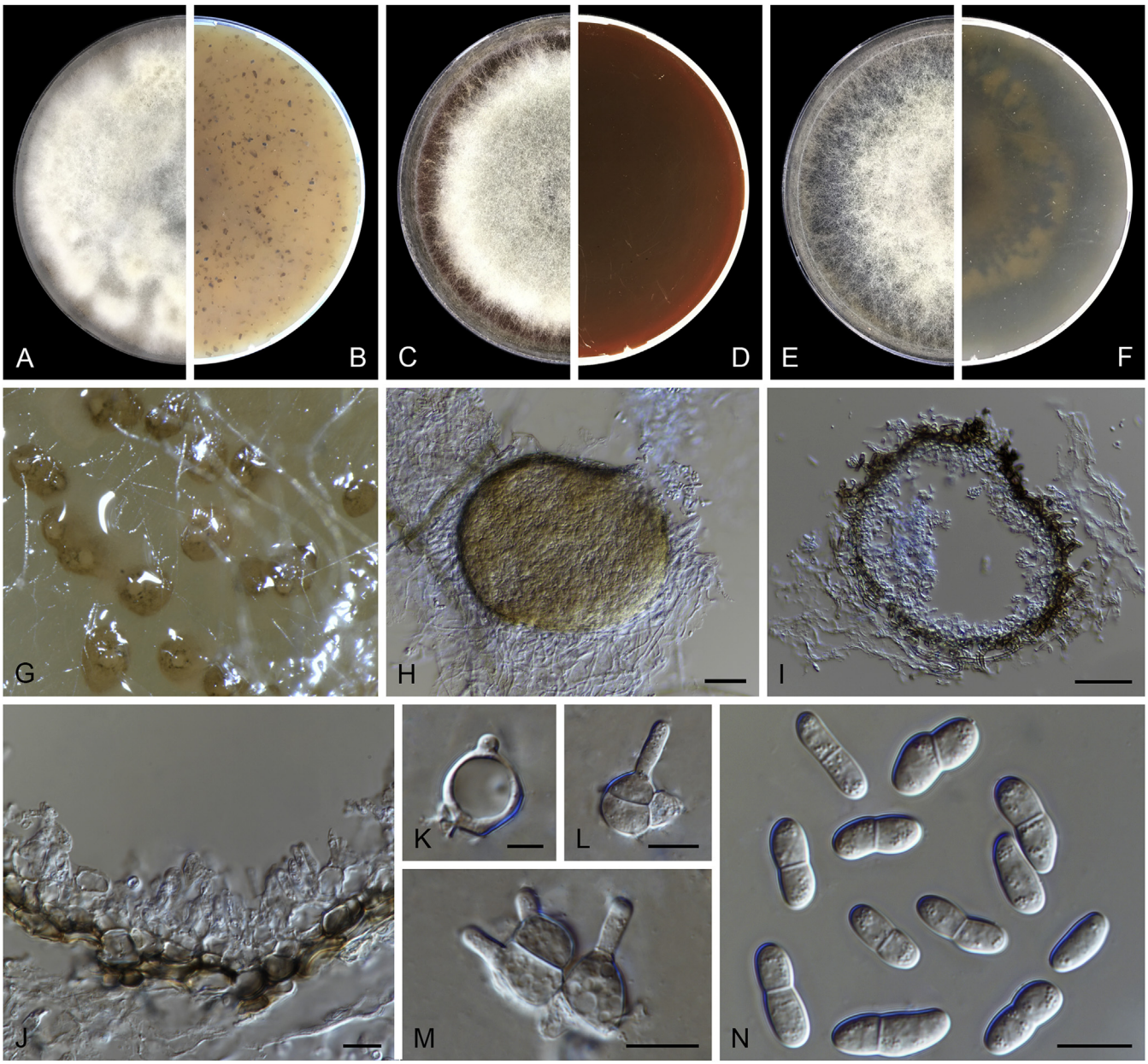

Fig. 37. *Vacuiphoma ferulae* (CBS 353.71). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 50 µm; J, L–N = 10 µm; K = 5 µm.

H. Pearson (**holotype** CBS H-23674, ex-type living culture CBS 121931).

Notes: *Nothophoma infuscata* was isolated from the same host as *Phoma acaciae* (*Acacia longifolia*, Italy). However, its conidia differ from the original description of *Phoma acaciae* [(4.5–)6–11 × 3.5–5.5 µm *vs.* 7–8 × 2–3 µm; Saccardo 1884]. It is genetically distinct from other species in the genus *Nothophoma*, forming a sister lineage to *No. eucalyptigena* (Fig. 1). Morphologically, *No. eucalyptigena* has smaller conidiogenous cells (6–8 × 5–7 µm *vs.* 7–11 × 5.5–10.5 µm) and larger conidia than *No. infuscata* [(8–)9–13(–15) × (4–)5(–6) µm *vs.* (4.5–)6–11 × 3.5–5.5 µm; Crous *et al.* 2017a].

Nothophoma nullicana L.W. Hou, L. Cai & Crous, ***nom. nov.*** MycoBank MB834021.
Basionym: *Verrucoconiothyrium acaciae* Crous, Persoonia 38: 403. 2017.

Etymology: Name refers to Nullica State Forest, Australia, where this fungus was collected.

Description: Crous *et al.* (2017b).

Typus: **Australia**, New South Wales, Nullica State Forest, on leaves of *Acacia falciformis* (*Fabaceae*), 29 Nov. 2016, P.W. Crous (**holotype** CBS H-23292, culture ex-type CPC 32330 = CBS 143448).

Notes: *Verrucoconiothyrium acaciae* was described from leaves of *Acacia falciformis* (*Fabaceae*), the same host genus with *Didymella acaciae*, which was treated as a new combination, *Nothophoma acaciae*, in the present study. Phylogenetically, the ex-type strain of *Verrucoconiothyrium acaciae* (CBS 143448) also clusters within *Nothophoma* and shares a close phylogenetic affinity with *N. acaciae* and *N. prosopidis*, but forms a clearly separate lineage that is distinct from the latter two species. However, the epithet is already occupied, and therefore, a

new name is proposed for this species as *Nothophoma nullicana*. *Nothophoma nullicana* can be easily distinguished from *N. acaciae* in producing brown, aseptate and shorter conidia measuring (6.5–)8–9(–10) × (3–)3.5(–4) µm, while *N. acaciae* produces hyaline, 1-septate conidia measuring (16–)18–21(–25) × (3–)3.5(–4) µm. *Nothophoma nullicana* differs from *N. prosopidis* by producing conidiomata with smaller ostioles (10–15 µm diam. *vs*. 80 µm diam.) and narrower conidia [(6.5–)8–9(–10) × (3–)3.5(–4) µm, fusoid-ellipsoid *vs.* (7–)8–9(–10) × (4–)5–6(–7) µm, ellipsoid to globose] (Crous *et al.* 2013b).

Nothophoma prosopidis (Crous & A.R. Wood) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833536.
Basionym: *Coniothyrium prosopidis* Crous & A.R. Wood, Persoonia 31: 207. 2013.
Synonym: *Verrucoconiothyrium prosopidis* (Crous & A.R. Wood) Crous, Persoonia 38: 299. 2017.

Description: Crous *et al.* (2013b).

Typus: **South Africa**, Northern Cape Province, Calvinia, associated with a bark disease on *Prosopis* sp. (*Fabaceae*), Sep. 2012, A. Wood (**holotype** CBS H-21424, ex-type living culture CPC 21699 = CBS 136415).

Additional material examined: **South Africa**, Northern Cape Province, Calvinia, associated with a bark disease on *Prosopis* sp. (*Fabaceae*), Sep. 2012, A. Wood, culture CPC 21701; *ibid*., culture CPC 21705.

Notes: This species was initially introduced as *Coniothyrium prosopidis* (Crous *et al.* 2013b) and later recombined in *Verrucoconiothyrium* as *Ve. prosopidis* (Crous *et al.* 2017a). In the present study, *Ve. prosopidis* grouped in the genus *Nothophoma* and formed a distinct lineage closely related to *No. acaciae*, thus a new combination, *No. prosopidis*, was introduced here.

Clade 21: *Vacuiphoma* Valenz.-Lopez *et al.*, Stud. Mycol. 90: 40. 2017 (2018).

Type species: *Vacuiphoma bulgarica* (Aveskamp *et al.*) Valenz.-Lopez *et al.*

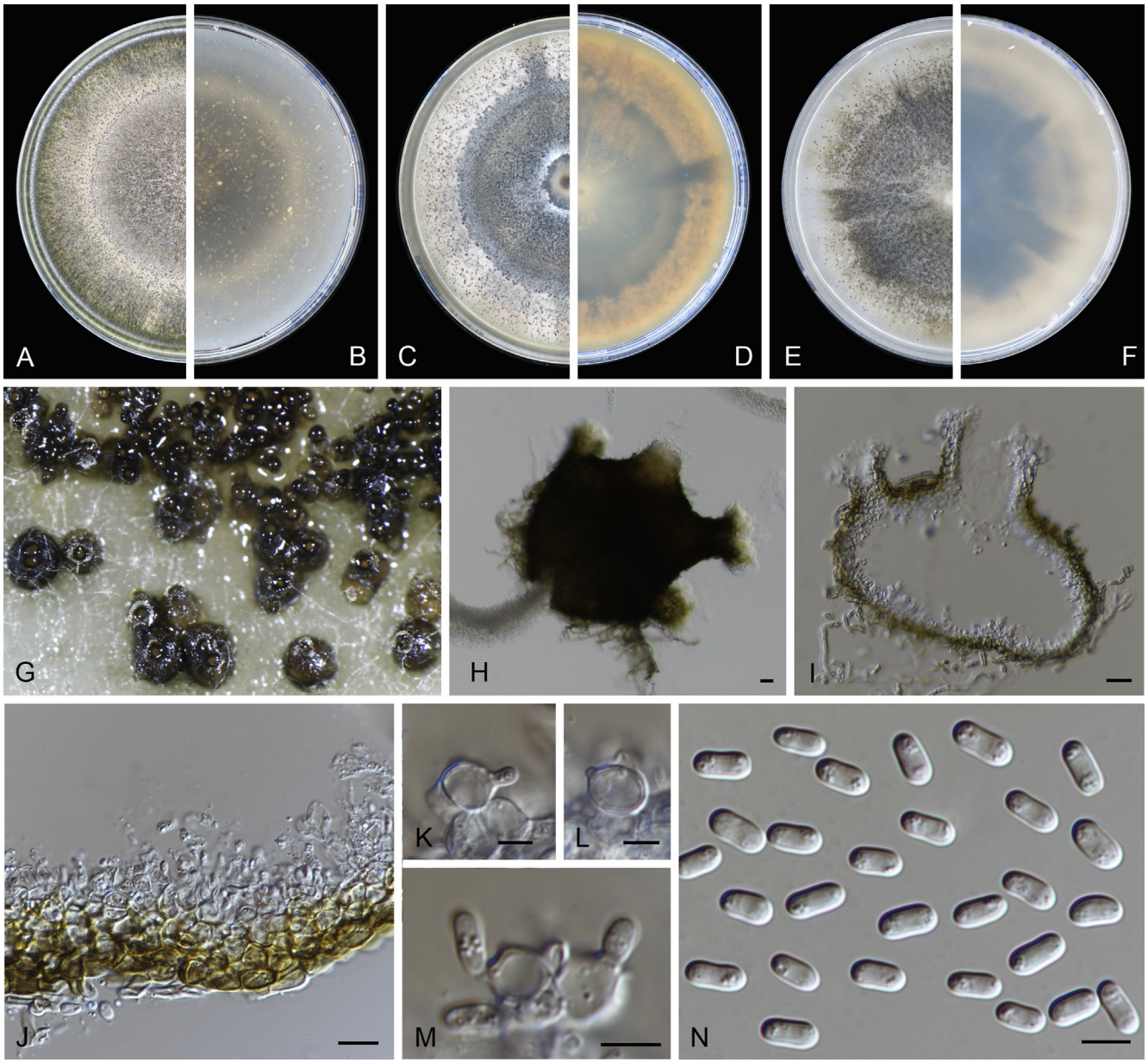

Fig. 38. *Vacuiphoma laurina* (CBS 119636). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 20 µm; J = 10 µm; K–N = 5 µm.

Vacuiphoma ferulae (Pat.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833531. Fig. 37.
Basionym: *Ascochyta ferulae* Pat., Énum. Champ. Tunisie (Paris) 17. 1892.

Description from ex-neotype (CBS 353.71): *Conidiomata* pycnidial, semi-immersed, immersed or produced on aerial mycelium, mostly scattered, solitary, sometimes 2–3 confluent, (sub-)globose or flask-shaped, pale brown, glabrous, ostiolate, 130–380 × 90–245 µm. *Ostioles* single, central, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 2–5 layers, 9–26 µm thick, outer 1–3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, lageniform, ampulliform to doliiform, 7.5–16.5 × 5.5–11.5 µm. *Conidia* oblong with rounded apices, smooth- and thin-walled, hyaline, 0–1-septate and constricted at the septum, 6.5–17 × 3.5–6.5 µm, eguttulate or with minute scattered guttules. *Conidial matrix* slightly pink.

Culture characteristics: Colonies on OA, 75–80 mm diam after 7 d 25 °C, margin irregular, aerial mycelium woolly, whitish; reverse buff. Colonies on MEA 75–80 mm diam after 7 d, margin regular, aerial mycelium woolly, whitish to pale grey; reverse dark brick. Colonies on PDA, 75–80 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, buff to olivaceous grey; reverse olivaceous grey to olivaceous. NaOH spot test: a pale red discolouration on OA.

Typus: **Italy**, Sardegna, Monte Toddeito, from dead stem of *Ferula communis* (*Apiaceae*), 24 Aug. 1970, W. Gams (**neotype designated here** CBS H-23660, MBT389727, ex-neotype living culture CBS 353.71).

Notes: *Ascochyta ferulae* was originally described from a *Ferulae* sp. in Monastir, Tunisia (Patouillard 1892). However, no type material could be located (FH, PC, B), and it is thus considered lost. Culture CBS 353.71 was received as *As. ferulae*, the morphological characters of which agrees well with the description of *As. ferulae* from Saccardo & Sydow (1899). Isolate CBS 353.71 was therefore designated as ex-neotype culture for *As. ferulae*.

Vacuiphoma laurina (Thüm.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833532. Fig. 38.
Basionym: *Phoma laurina* Thüm., Boll. Soc. Adriat. Sci. Nat. Trieste. 3 (2): 452. 1877.

Description from ex-neotype (CBS 119636): *Conidiomata* pycnidial, abundant, semi-immersed or immersed, mostly aggregated, solitary, sometimes 2–4 confluent, (sub-)globose or flask-shaped, brown, with sparse hyphal outgrowths, ostiolate, 105–435(–505) × 90–300(–425) µm. *Ostioles* 1–4(–5), up to 10 with age, slightly papillate or elongated into a short collared neck. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–5 layers, 12–32 µm thick, outer 2–3 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, doliiform or lageniform, 5–8 × 4.5–7.5 µm. *Conidia* oblong with rounded apices, smooth- and thin-walled, hyaline, aseptate, 4.5–7.5 × 2–3 µm, eguttulate or with 2–4 minute, polar guttules. *Conidial matrix* white, hyaline.

Culture characteristics: Colonies on OA reaching 55–60 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, pale mouse grey near the centre, with a buff circle, pale olivaceous towards periphery; reverse concolourous. Colonies on MEA reaching 55–60 mm diam after 7 d, margin regular, aerial mycelium flat, concentric circles of different colours, mouse grey near the centre, greyish in the middle, salmon towards periphery; reverse dark brown, orange towards periphery. Colonies on PDA reaching 60–65 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, pale olivaceous; reverse olivaceous black with buff edge. NaOH spot test results in a pale reddish discolouration, with pale olivaceous near the centre on OA.

Typus: **Turkey**, Western Anatolia, from *Laurus nobilis* (*Lauraceae*), date unknown, M.E. Göre (**neotype designated here** CBS H-23686, MBT389728, ex-neotype living culture CBS 119636).

Notes: *Phoma laurina* was originally described from *Laurus nobilis* in Gorizia, Italy, with ellipsoidal conidia measuring 4–6 × 1.5–2 µm (Bolle & von Thümen 1877, Saccardo 1884). However, the type specimen of *Phoma laurina* could not be traced. In the present study, isolate CBS 119636 (*Laurus nobilis*, Turkey) morphologically agrees well with the original description of *Phoma laurina*, having cylindrical to oblong conidia measuring 4.5–7.5 × 2–3 µm. We therefore designated CBS 119636 as ex-neotype culture of *Phoma laurina*. Phylogenetically, CBS 119636 grouped in the genus *Vacuiphoma*, and thus a new combination was introduced.

Clade 22: *Microsphaeropsis* Höhn., Hedwigia 59: 267. 1917.

Type species: *Microsphaeropsis olivacea* (Bonord.) Höhn.

Notes: The genus *Microsphaeropsis* was introduced by Von Höhnel (1917) and was originally placed in *Montagnulaceae* to accommodate species with pigmented, phoma-like conidia. Later a new family *Microsphaeropsidaceae* was introduced to accommodate this genus (Chen *et al.* 2015). However, in the present study, all remaining isolates accommodated as "*Microsphaeropsis* spp." in the CBS culture collection were sequenced. The phylogenetic analysis based on the increased number of isolates and expanded dataset revealed that *Microsphaeropsis* species reside inside the *Didymellaceae*, being closely related to *Neomicrosphaeropsis* and *Paramicrosphaeropsis*. Moreover, all three genera are morphologically comparable in initially producing hyaline conidia that turn brownish with age. Therefore, the family *Microsphaeropsidaceae* was reduced to synonymy, and the genus *Microsphaeropsis* was again placed in *Didymellaceae*.

Microsphaeropsis fusca L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833537. Fig. 39.

Etymology: Name refers to the dark brown pycnidia produced by this species.

Conidiomata pycnidial, semi-immersed in the agar, solitary, scattered, sometimes confluent, (sub-)globose or irregular-shaped, dark brown to black, with hyphal outgrowths, 210–335 × 190–260 µm. *Ostioles* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–4 layers, with outer 3 layers pigmented, 16.5–30 µm thick. *Conidiogenous cell* phialidic, hyaline, smooth, ampulliform, lageniform or doliiform, 7–14 × 4.5–10.5 µm. *Conidia* variable in shape and size, (sub-)globose, oblong, ellipsoidal or oval,

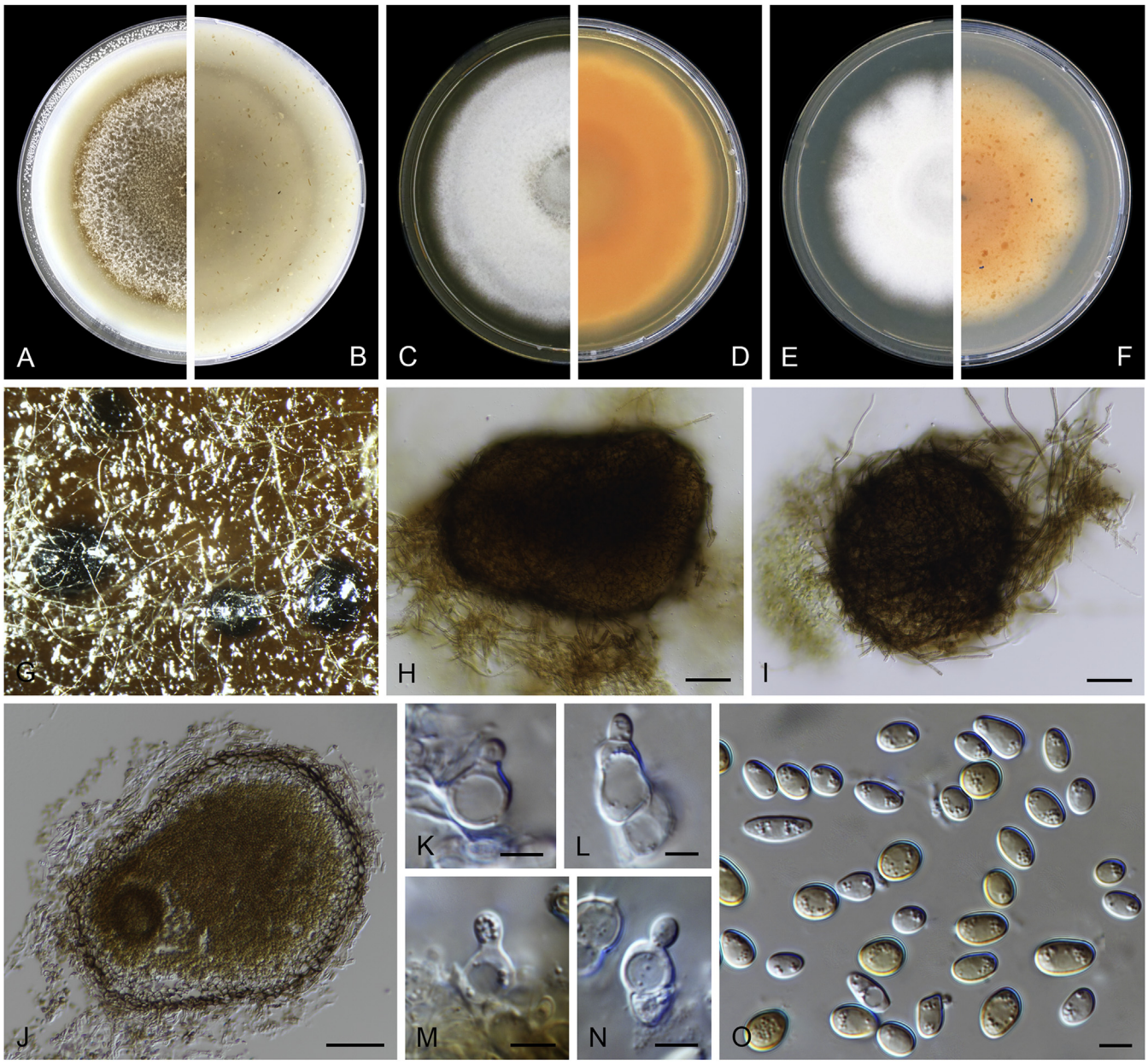

Fig. 39. *Microsphaeropsis fusca* (CBS 116670). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H–I.** Pycnidia. **J.** Section through pycnidium. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H–J = 50 µm; K–O = 5 µm.

hyaline at beginning, pale brown with age, smooth- and thin-walled, aseptate, 5–10.5 × 3.5–6.5 µm, guttulate.

Culture characteristics: Colonies on OA, 40–45 mm diam after 7 d at 25 °C, margin regular, covered by sparsely floccose aerial mycelium, buff to cinnamon; reverse concolourous. Colonies on MEA 35–40 mm diam after 7 d, margin regular, covered by woolly aerial mycelium, white to cinnamon; reverse orange to sienna. Colonies on PDA, 35–40 mm diam after 7 d, margin regular, covered by woolly aerial mycelium, whitish, buff toward periphery; reverse buff, pale orange near the centre. NaOH spot test negative on OA.

Typus: **The Netherlands**, from twig lesions of *Sarothamnus scoparius* (*Fabaceae*), 23 Aug. 2004, G. Verkley & M. Starink (**holotype** CBS-H 24339, ex-type living culture CBS 116670).

Additional materials examined: **The Netherlands**, from twig lesions of *Sarothamnus scoparius* (*Fabaceae*), 23 Aug. 2004, G. Verkley & M. Starink, culture CBS 116669. **Germany**, from *Sarothamnus scoparius* (*Fabaceae*), 21 Dec. 2013, R.K. Schumacher, culture CBS 139603.

Notes: *Microsphaeropsis fusca* was isolated from twig lesions of *Sarothamnus scoparius* in the Netherlands, and formed a well-supported sister clade with *Mi. proteae* (Fig. 1). It differed phenotypically from *Mi. proteae* by its larger conidiogenous cell (7–14 × 4.5–10.5 µm *vs.* 3–8 × 3–4 µm) and larger conidia (5–10.5 × 3.5–6.5 µm *vs.* 3–4 × 2–2.5 µm *in vitro*; Swart *et al.* 1998, Crous *et al.* 2011).

Microsphaeropsis olivacea (Bonord.) Höhn., Hedwigia 59: 267. 1917.
Basionym: *Coniothyrium olivaceum* Bonord., Jahrb. Nassauischen Vereins Naturk. 23–24: 377. 1869.

Description: Chen *et al.* (2015).

Typus: Austria, on stem of *Hedera helix* (**holotype** BPI 797151, ex herb. Fuckel, ex herb. Boiss).

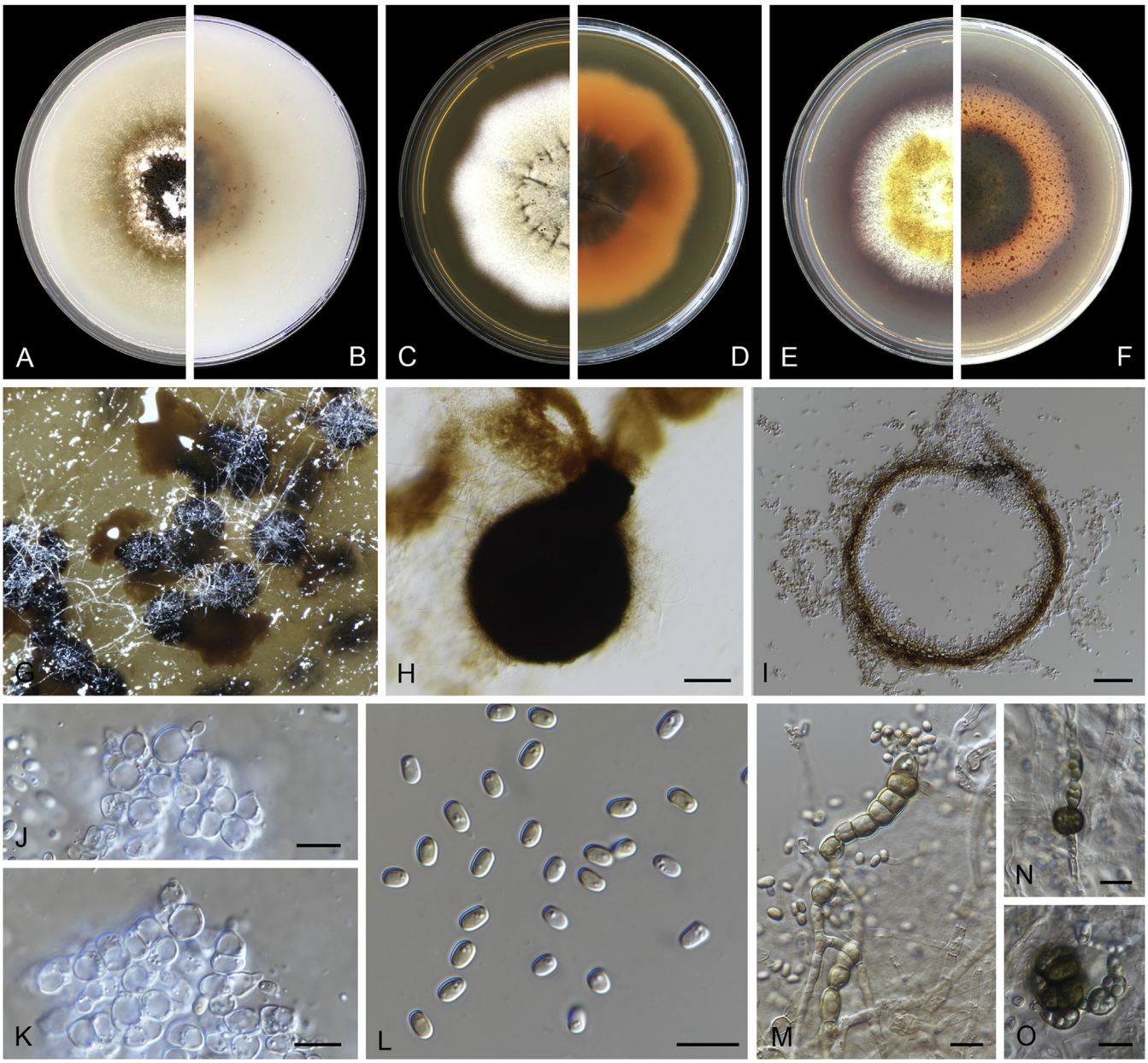

Fig. 40. *Microsphaeropsis taxicola* (CBS 442.83). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J–K.** Conidiogenous cells. **L.** Conidia. **M–O.** Chlamydospores. Scale bars: H = 100 µm; I = 50 µm; J–O = 10 µm.

Materials examined: **France**, Lorraine, *Cronartium ribicola* (*Cronartiaceae*), May 1976, M. Morelet, CBS 320.76; Nancy, from needle of *Pinus laricio* (*Pinaceae*), unknown date, M. Morelet, CBS 233.77; **Germany**, Baden-Württemberg, from wood of *Picea abies* (*Pinaceae*), unknown date, S. Schönhar, CBS 336.78. **The Netherlands**, Baarn, Groeneveld, *Tremella foliacea* (*Tremellaceae*), 2 Mar. 1972, J. Stalpers, CBS 608.72. **Switzerland**, Egerkingen, *Pinus sylvestris* (*Pinaceae*), unknown date and collector, CBS 617.83.

Notes: *Microsphaeropsis olivacea* was originally collected from stems of *Hedera helix* in Austria. In this study, additional isolates cluster with the reference strain of *Microsphaeropsis olivacea* (CBS 233.77), but were collected from different hosts. Therefore, further studies are needed to resolve its typification and to clarify its phylogeny.

Microsphaeropsis taxicola L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB835482. Fig. 40.

Etymology: Named after the host genus *Taxus*, from which this species was isolated.

Description: *Conidiomata* pycnidial, (semi-)immersed in the agar, solitary and aggregate, (sub-)globose, dark brown or black, with white hyphal outgrowths, especially around the ostioles, ostiolate, 275–545 × 245–435 µm. *Ostioles* 1–3, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–4 layers, with outer 3 layers pigmented, 10–18 µm thick. *Conidiogenous cell* phialidic, hyaline, smooth, globose, ampulliform, lageniform, 5.5–10.5 × 4–8 µm. *Conidia* oblong, hyaline at beginning, pale brown with age, smooth- and thin-walled, aseptate, 3.5–6 × 2.5–3.5 µm, 1–2 guttulate, minute. *Chlamydospores* uni- or multicellular, unicellular intercalary, guttulate, thick-walled, pale brown to brown, 6–10 µm diam, multicellular irregular dictyo/phragmosporous, somewhat botryoid and in combination with unicellular chlamydospores, dark greenbrown, 16–32 × 15–25 µm. *Conidial matrix* brown.

Culture characteristics: Colonies on OA, 30–35 mm diam after 7 d at 25 °C, margin regular, covered by sparsely floccose aerial

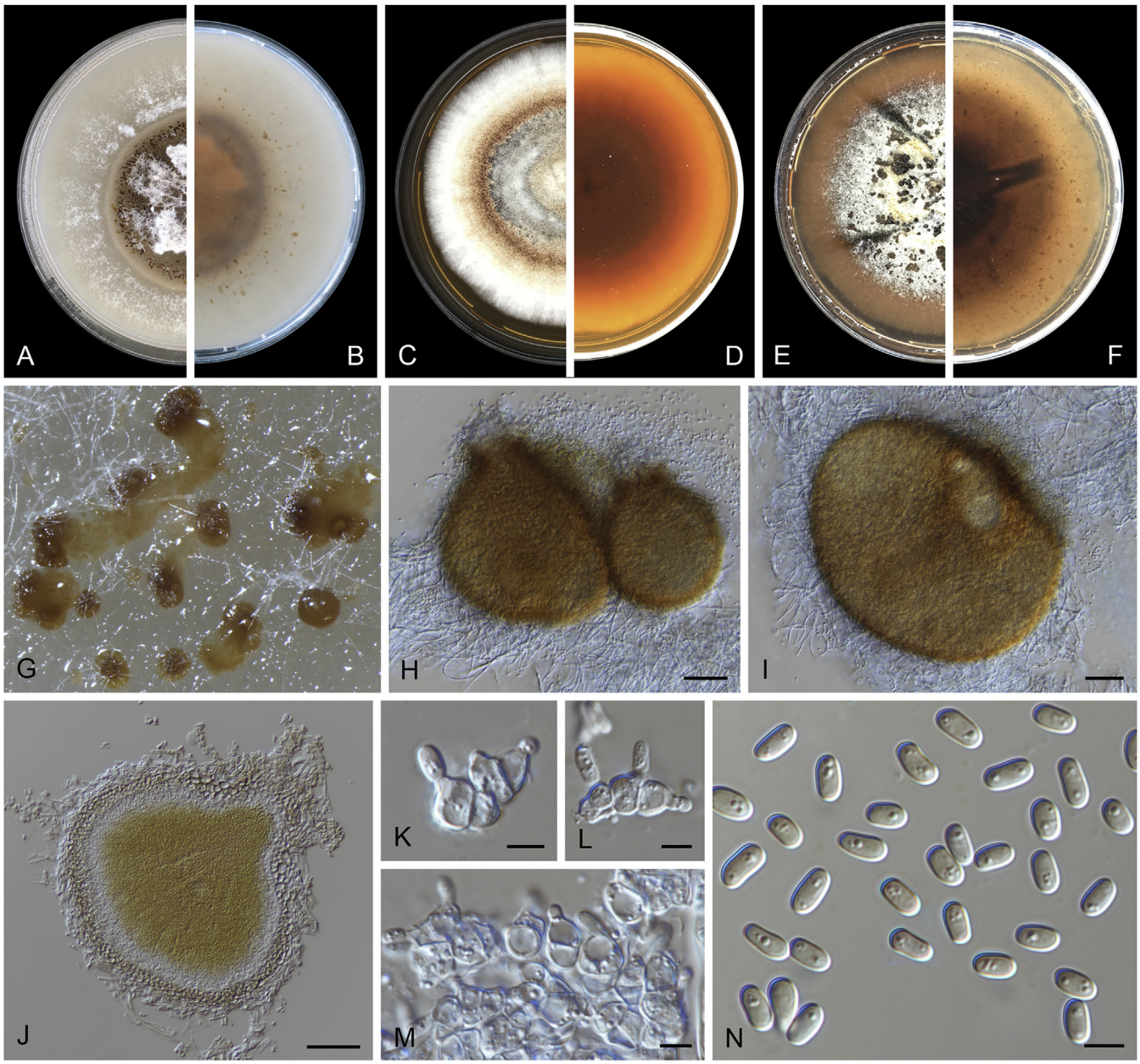

Fig. 41. *Microsphaeropsis viridis* (CBS 432.71). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H–I.** Pycnidia. **J.** Section through pycnidium. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–J = 50 µm; K–N = 5 µm.

mycelium, with sepia, white, cinnamon and buff concentric rings; reverse concolourous. Colonies on MEA 25–30 mm diam after 7 d, margin irregular, covered by felty aerial mycelium, buff at centre, whitish at periphery; reverse dark brick, orange towards periphery. Colonies on PDA, 30–35 mm diam after 7 d, margin regular, covered by felty aerial mycelium, pale luteous, luteous to whitish towards periphery, with vinaceous pigment on the agar; reverse brown vinaceous, saffron towards periphery. NaOH spot test negative on OA.

Typus: **The Netherlands**, Baarn, *Taxus baccata* (*Taxaceae*), unknown date and collector, (**holotype** CBS H-24379, ex-type living culture CBS 442.83).

Additional materials examined: **The Netherlands**, Baaer, from wood of *Rhus typhina* (*Anacardiaceae*), Jul. 1980, W. Gams, culture CBS 469.80; Kortenhoef, *Opuntia cladodes* (*Cactaceae*, cultivated, several years ago imported from Spain), May 1992, J. Daams, culture CBS 427.92.

Notes: Three isolates collected from different hosts in the Netherlands and formed an isolated clade with moderate support values, showing a close phylogenetic affinity to *Microsphaeropsis olivacea* and *Mi. viridis*. A new species is therefore proposed here as *Mi. taxicola*. The morphological differences between *Mi. taxicola* and *Mi. viridis* are discussed under *Mi. viridis*. *Microsphaeropsis taxicola* can be clearly distinguished from *Mi. olivacea* by producing aseptate conidia, while the latter produces 0–1-septate conidia.

Microsphaeropsis viridis L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB835483. Fig. 41.

Etymology: Name refers to the dark green discolouration on the agar by application of NaOH.

Description: *Conidiomata* pycnidial, (semi-)immersed in the agar, solitary, aggregate, (sub-)globose or irregular-shaped, pale brown, with hyphal outgrowths, especially around the ostioles, ostiolate, 150–395 × 135–285 µm. *Ostioles* 0–2, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–4 layers, with outer 2–3 layers

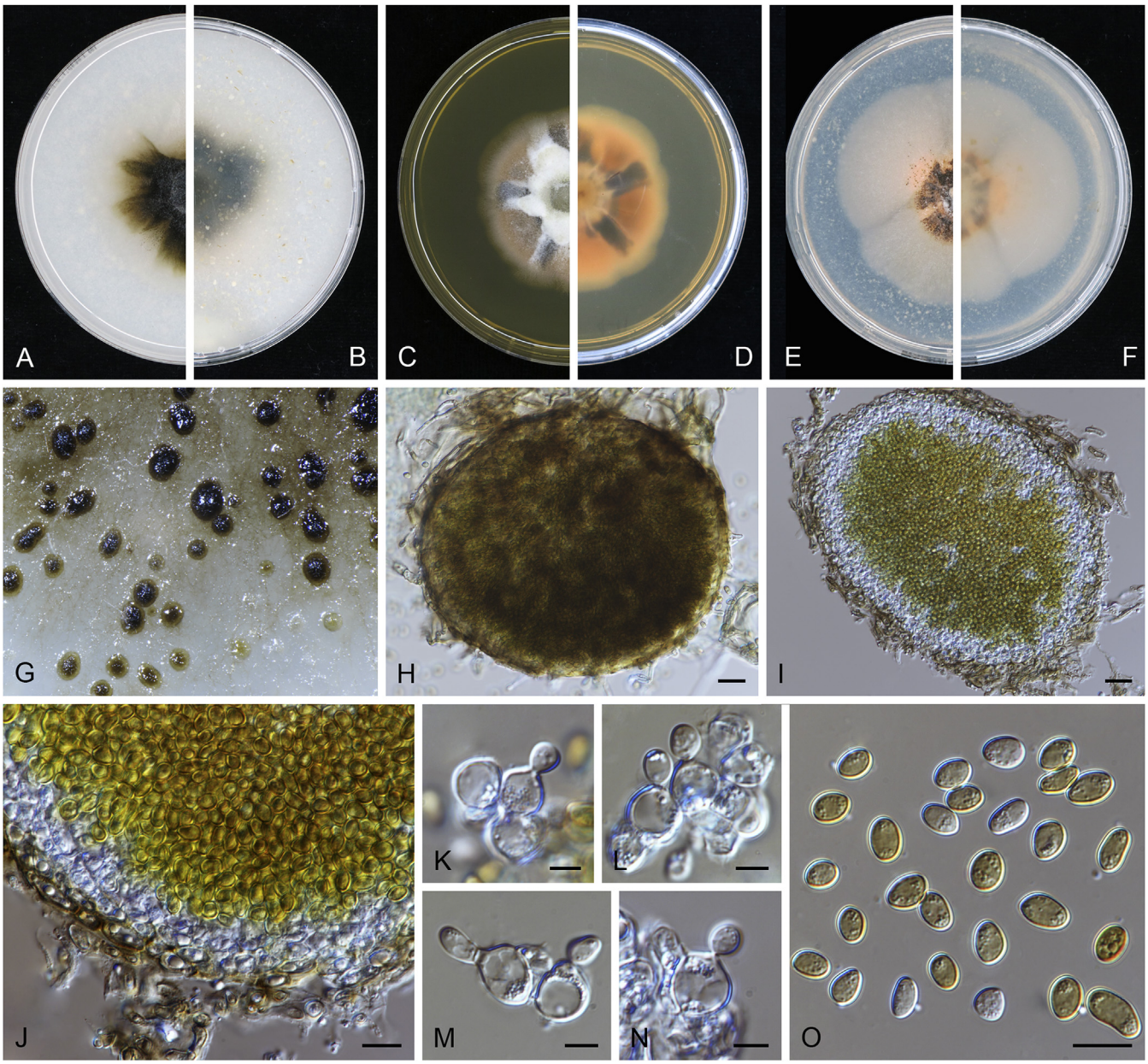

Fig. 42. *Paramicrosphaeropsis ellipsoidea* (CBS 194.97). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–N.** Conidiogenous cells. **O.** Conidia. Scale bars: H–I = 20 µm; J, O = 10 µm; K–N = 5 µm.

pigmented, 17–40 µm thick. *Conidiogenous cell* phialidic, hyaline, smooth, ampulliform, lageniform or doliiform, 5–8 × 4.5–7 µm. *Conidia* variable in shape and size, oblong, ellipsoidal, hyaline at beginning, pale brown with age, smooth- and thin-walled, aseptate, 5.5–8.5 × 2.5–3.5 µm, guttulate. *Conidial matrix* hyaline to pale brown.

Culture characteristics: Colonies on OA, 35–40 mm diam after 7 d at 25 °C, margin regular, covered by sparsely floccose aerial mycelium, with white, pale brown and buff concentric rings, pycnidia aggregate and formed in the middle rings; reverse concolourous. Colonies on MEA 30–35 mm diam after 7 d, margin regular, covered by felty aerial mycelium, with buff, pale grey, brick and whitish concentric rings; reverse with umber, orange, ochreous rings. Colonies on PDA, 30–35 mm diam after 7 d, margin regular, covered by felty aerial mycelium, whitish, with isabelline areas, cinnamon at periphery; reverse with sepia, fawn, and saffron rings. Application of NaOH results in a dark green discolouration of the agar.

Typus: **The Netherlands**, Valkenswaard, from dead twig and pod of *Sarothamnus* sp. (*Fabaceae*), 21 Feb. 1971, H.A. van der Aa (**holotype** CBS H-10870, ex-type living culture CBS 432.71).

Additional materials examined: **France**, Meurthe et Moselle, from leaf of *Populus tremula* (*Salicaceae*), M. Morelet, 17 Aug. 1972, culture CBS 763.73; Haute Vienne, *Pseudotsuga menziesii* (*Pinaceae*), M. Morelet, 21 Mar. 1972, culture CBS 762.73. **Germany**, University of Freiburg, Nov. 1980, G. Schüler, *Abies alba* (*Pinaceae*), culture CBS 639.80. **The Netherlands**, Baarn, Valkenswaard, garden Peperstraat 6, from leaf spots of *Berberis* sp. (*Berberidaceae*), cult. H.A. van der Aa, 23 Mar. 1969, culture CBS 354.69.

Notes: *Microsphaeropsis viridis* is represented by five isolates from different hosts. It forms a well-supported clade and is closely related with *Mi. olivacea* and *Mi. taxicola* (Fig. 1). In our phylogenetic study, *Mi. viridis* is distinct from the references

strain of *Mi. olivacea* (CBS 233.77) in 17 bp of *rpb2* and 3 bp of *tub2* based on alignment of the concatenated four loci deposited in TreeBASE (S25826); from *Mi. taxicola* differing in 2 bp of ITS and 13 bp of *rpb2*. This new species differs from *Mi. taxicola* by its longer conidia (5.5–8.5 × 2.5–3.5 µm *vs.* 3.5–6 × 2.5–3.5 µm); from *Mi. olivacea* by its aseptate conidia (Chen *et al.* 2015). *Microsphaeropsis viridis* could also be distinguished from the other two species by producing pycnidia with 0–2 ostioles, while pycnidia of *Mi. olivacea* has single ostiole and *Mi. taxicola* has 1–3 ostioles. Besides, the application on NaOH results in a dark green discolouration of the agar of *Mi. viridis*, with *Mi. taxicola* having a negative reaction in NaOH, and that of *Mi. olivacea* is not recorded.

Clade 23: *Neomicrosphaeropsis* Thambug. *et al.*, Fungal Diversity 82: 261. 2016.

Type species: *Neomicrosphaeropsis italica* Thambug. *et al.*, Fungal Diversity 82: 264. 2016.

Clade 24: *Paramicrosphaeropsis* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833538.

Etymology: From Greek παρα-, beside, referring to the close morphological and phylogenetic relationship with the genus *Microsphaeropsis*.

Conidiomata pycnidial, mostly semi-immersed, abundant, scattered or aggregated, mostly solitary, sometimes confluent, globose to subglobose, pale brown and the pycnidial wall hyaline at first, dark brown with age. *Pycnidial wall* pseudoparenchymatous, thin, multi-layered. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform to lageniform. *Conidia* variable in shape, broad ellipsoidal, oblong or ovoid, thin-walled, smooth, hyaline at beginning, brown with age, aseptate, mostly guttulate. *Sexual morph* unknown.

Notes: *Paramicrosphaeropsis* is introduced as a new genus belonging to *Didymellaceae* based on the multi-locus phylogenetic analysis and morphological characters. This genus is phylogenetically close to *Neomicrosphaeropsis* and *Microsphaeropsis*, and distant from all other known genera in *Didymellaceae* with high statistical support (100 % MLBS/1.0 BPP, Fig. 1). Morphologically, *Paramicrosphaeropsis* could be distinguished from other genera in this family by producing pycnidia with an extremely thin and hyaline pycnidial wall (almost hyaline when the conidia have exuded).

Type species: *Paramicrosphaeropsis ellipsoidea* L.W. Hou, L. Cai & Crous

Paramicrosphaeropsis ellipsoidea L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833539. Fig. 42.

Etymology: Name refers to the ellipsoidal conidia of this species.

Description: *Conidiomata* pycnidial, semi-immersed, abundant, scattered or aggregated, mostly solitary, sometimes 2–5 confluent, globose to subglobose, pale brown to dark brown, with hyphal outgrowths, (100–)150–490 × (80–)110–440 µm. *Ostiole* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, thin, hyaline at beginning, dark brown with age, 4–5 layers, 10–30 µm thick, outer 1–2 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or doliiform, 7–11 × 5.5–10 µm. *Conidia* variable in shape, mostly broad ellipsoidal, some oblong or ovoid, straight or slightly curved, smooth- and thin-walled, hyaline, later becoming brown with age, aseptate, 5–11 × 3.5–6.5 µm, with numerous minute guttules. *Conidial matrix* brownish.

Culture characteristics: Colonies on OA reaching 10–15 mm diam after 7 d at 25 °C, margin irregular, aerial mycelium flat, olivaceous, some zone formed pycnidia without aerial mycelium and in buff colour; reverse dark olivaceous with buff edge. Colonies on MEA 10–15 mm diam after 7 d, margin regular, aerial mycelium flat to floccose, pink to white, with some iron grey zone; reverse pale brown with buff edge, with some iron grey zone. Colonies on PDA reaching 10–15 mm diam after 7 d, margin regular, covered by flat aerial mycelium, rosy buff to pink, some brown pycnidia visible near the centre; reverse concolourous. NaOH spot test: a pale red-brown discolouration on OA.

Typus: **Spain**, from *Quercus ilex* (*Fagaceae*), 1996, R.F. Castañeda (**holotype** CBS H-23671, ex-type living culture CBS 194.97).

Additional material examined: **Spain**, from decayed twig of *Quercus ilex* (*Fagaceae*), 20 Jul. 1996, R.F. Castañeda, culture CBS 197.97.

Notes: Isolates CBS 194.97 and CBS 197.97 were previously identified as "*Microsphaeropsis* sp.", and characterised by producing brown conidia. However, based on the phylogenetic analysis (Fig. 1), these two isolates formed a distinct clade basal to *Neomicrosphaeropsis* and *Microsphaeropsis*. Therefore, we accommodated CBS 197.97 and CBS 194.97 as a new species in a novel genus, *Paramicrosphaeropsis*. Morphologically, *Paramicrosphaeropsis* differs from *Neomicrosphaeropsis* in its subglobose, ampulliform or doliiform conidiogenous cells, while *Neomicrosphaeropsis* produces subcylindrical conidiogenous cells.

Clade 25: *Calophoma* Qian Chen & L. Cai, Stud. Mycol. 82: 191. 2015.

Type species: *Calophoma clematidina* (Thüm.) Qian Chen & L. Cai

Calophoma parvula L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833540. Fig. 43.

Etymology: Latin *parvula* (= very small), refers to the size of the pycnidia produced by this species.

Description: *Conidiomata* pycnidial, immersed, scattered and solitary, globose to subglobose, with slightly papilla at the top, pale brown and thin-walled, glabrous, ostiolate, 65–155 × 60–140 µm. *Ostiole* single. *Pycnidial wall* pseudoparenchymatous, composed of oblong or isodiametric cells, 2–3 layers, 8–37 µm thick, outer 1–2 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or lageniform, 5.5–9.5 × 3.5–8 µm. *Conidia* oblong with both ends rounded or broad ellipsoidal, smooth- and thin-walled, hyaline, aseptate, 4–7 × 2–3.5 µm, with two medium-sized polar guttules. *Chlamydospores* multi-celled, dark brown, smooth- and thick-walled, singly, globose to ellipsoidal, 18–30 × 18–25 µm.

Culture characteristics: Colonies on OA reaching 60–65 mm diam after 7 d at 25 °C, margin irregular, aerial mycelium flat, pale olivaceous to olivaceous black, saffron near the centre; reverse dark olivaceous. Colonies on MEA 55–60 mm diam after 7 d, margin regular, aerial mycelium woolly, white to buff; reverse pale brown with buff edge, some with a black zone. Colonies on PDA reaching 55–60 mm diam after 7 d, margin regular, covered

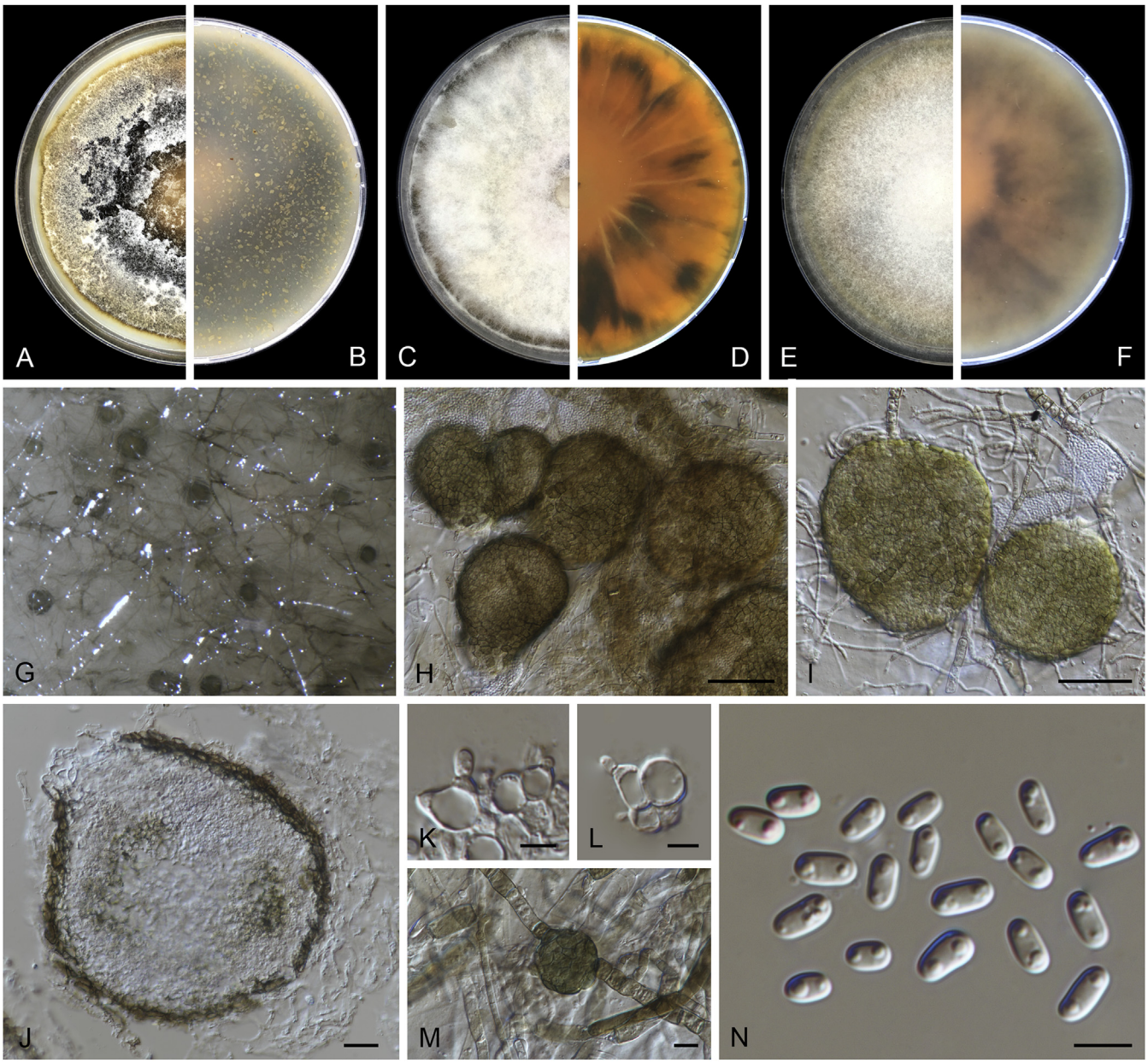

Fig. 43. *Calophoma parvula* (CBS 620.68). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H–I.** Pycnidia. **J.** Section through pycnidium. **K–L.** Conidiogenous cells. **M.** Chlamydospore. **N.** Conidia. Scale bars: H–I = 50 µm; J = 20 µm; K–L, N = 5 µm; M = 10 µm.

by felty aerial mycelium, whitish, pale olivaceous towards periphery; reverse saffron to pale brown, buff near the centre. NaOH spot test: a pale red-brown discolouration on OA.

Typus: **The Netherlands**, Baarn, Buitenzorg, from leaf spots of *Aegopodium podagraria* (*Umbelliferae*), 7 Jul. 1968, H.A. van der Aa (**holotype** CBS H-16196, ex-type living culture CBS 620.68).

Notes: Isolate CBS 620.68 was originally identified as "*Calophoma complanata*", but is distinct from *Ca. complanata* (CBS 268.92) both in morphology and phylogeny (Fig. 1). Isolate CBS 620.68 was characterised by smaller pycnidia (65–155 × 62–140 µm), while *Ca. complanata* produced larger pycnidia (300–400 µm) (Boerema & de Gruyter 1998). Moreover, CBS 620.68 was well distinguished from *Ca. complanata* in producing smaller and aseptate conidia (aseptate, 4–7 × 2–3.5 µm *vs.* 0–1-septate, 5–9 × 2–3.5 µm; Boerema & de Gruyter 1998). We consequently described CBS 620.68 as a new species, *Ca. parvula*.

Calophoma vincetoxici (De Not.) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833541. Fig. 44.

Basionym: *Amphisphaeria vincetoxici* De Not., Sf. Ital. 72. 1863.

Synonyms: *Didymella vincetoxici* (De Not.) Sacc., Syll. Fung. (Abellini) 1: 552. 1882.

Cercidospora vincetoxici (De Not.) Kuntze, Revis. Gen. Pl. 3(3): 454. 1898.

Description from ex-epitype culture (CBS 186.55): *Conidiomata* pycnidial, semi-immersed or immersed in the agar, mostly solitary, sometimes aggregated, globose, subglobose or flask-shaped, glabrous or with some hyphal outgrowths, ostiolate, (70–)220–380 × (70–)190–380 µm. *Ostiole* single, non-papillate or slightly papillate, sometimes elongated to a short neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–4 layers, 10.5–30 µm thick, outer wall 1–3-layered, pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, (sub-)globose, ampulliform or flask-shaped,

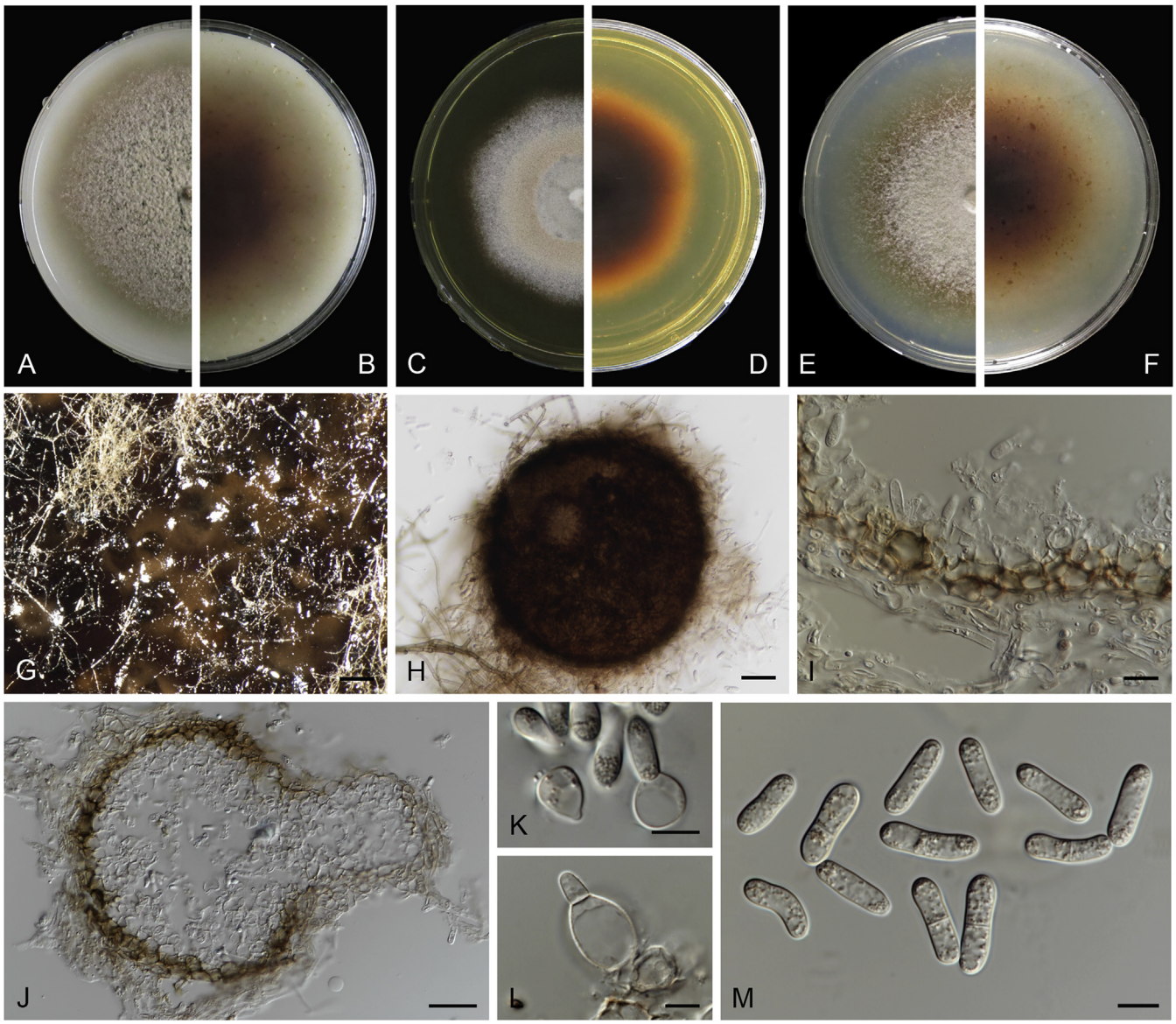

Fig. 44. *Calophoma vincetoxici* (CBS 186.55). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section of pycnidial wall. **J.** Section through pycnidium. **K–L.** Conidiogenous cells. **M.** Conidia. Scale bars: H = 50 µm; J = 20 µm; I, K–M = 10 µm.

(7.5–)9.5–17.5 × (6.5–)8.5–13.5 µm. *Conidia* variable in shape and size, subcylindrical with obtuse ends, oblong or allantoid, smooth- and thin-walled, hyaline, 0–1-septate, (9–)14–22.5 × 4.5–7 µm, with small guttules per cell. *Conidial matrix* pale salmon.

Culture characteristics: Colonies on OA, 25–35 mm diam after 7 d at 25 °C, margin entire, regular, densely covered by olivaceous buff and floccose aerial mycelium, greyish pink or greyish brown near the margin; reverse olivaceous, reddish brown, greyish brown near the margin. Colonies on MEA 25–30 mm diam after 7 d, margin regular, aerial mycelium floccose, white, with a buff concentric ring; reverse orange in outer ring, darkening towards the centre of the colony, brick to darker brown. Colonies on PDA, 35–40 mm diam after 7 d, margin regular, covered by floccose, white mycelium, with flat and pale reddish brown near the margin; reverse buff in outer ring, darkening towards the centre of the colony, honey, brick to darker brown. NaOH spot test: a pale greenish discolouration on OA.

Typus: **Japan** (**lectotype** designated here of *Amphisphaeria vincetoxici*, Sferiacei Italici, Centuria 1–Fascicolo 1: illustration on page 72, 1863, G. De Notaris, MBT389739). **Switzerland**, Gabi am Simplon, from *Vincetoxicum officinale* (*Apocynaceae*), 30 Oct. 1953, E. Müller (**epitype** designated here CBS H-24309, MBT389812, ex-epitype living culture CBS 186.55).

Additional material examined: **Switzerland**, St. Gallen, Speergebiet, Durchschlägi, from *Ononis* sp. (*Fabaceae*), 30 Oct. 1953, E. Müller, culture CBS 185.55.

Notes: *Didymella vincetoxici* (basionym: *Amphisphaeria vincetoxici*) was originally isolated from *Cynanchi vincetoxici* (currently: *Vincetoxicum officinale*) in Italy (De Notaris 1863, Saccardo 1882). However, the original description did not refer to a holotype specimen, nor could we locate one in the present study. Isolate CBS 186.55 was originally received as *Did. vincetoxici* and was isolated from the same host plant (*Vin. officinale*) in Europe. Thus, we proposed CBS 186.55 as ex-epitype culture. The morphology of the epitype (CBS H-24309), which we designated here, agrees with the description of *Did. vincetoxici*

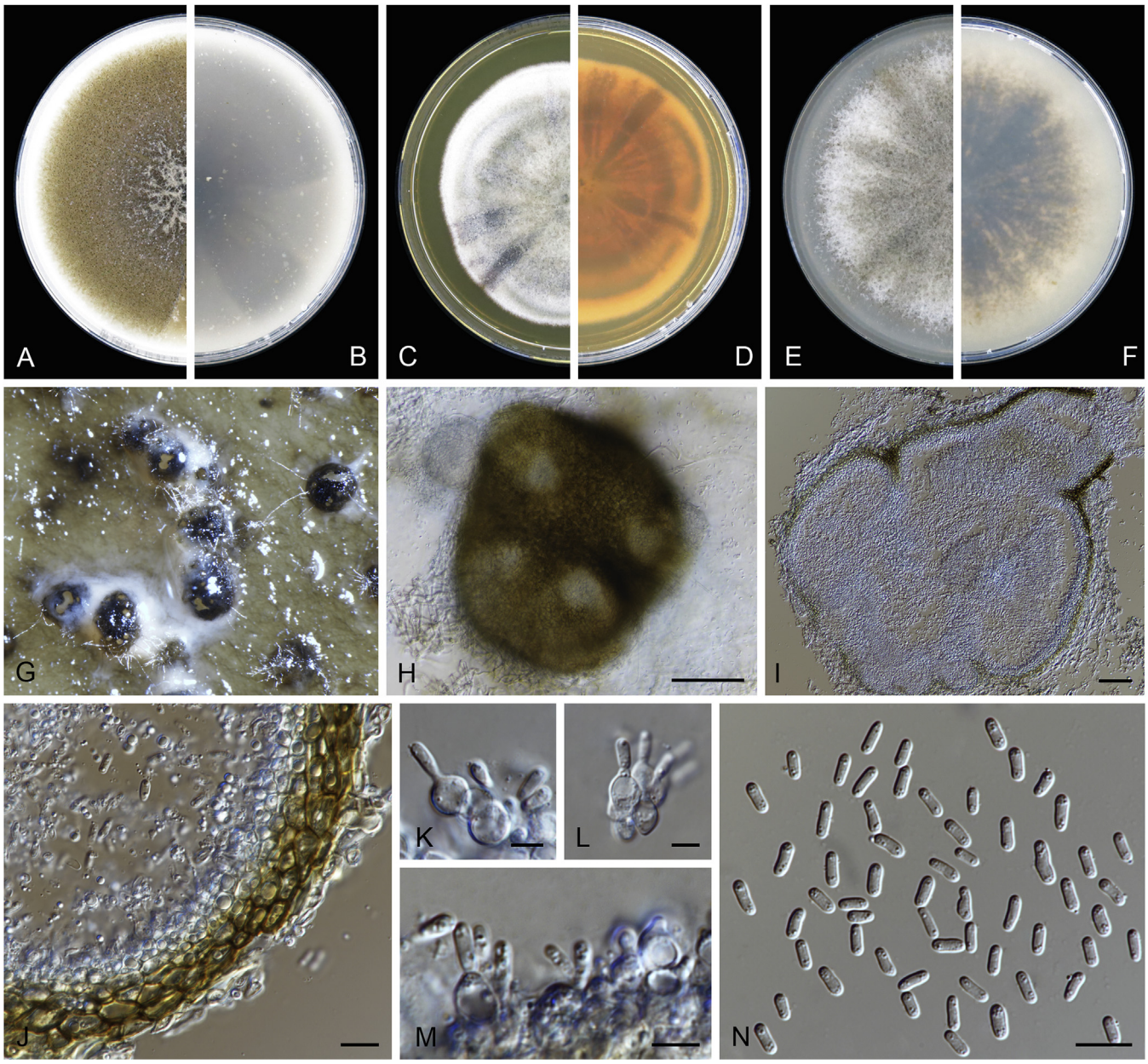

Fig. 45. *Ascochyta clinopodiicola* (CBS 123526). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 50 µm; J, N = 10 µm; K–M = 5 µm.

by Saccardo (1882). In our study, these isolates clustered in the genus *Calophoma* and resided in an independent clade, forming a distinct sister group with *Ca. complanata* (Fig. 1).

Clade 26: *Pseudoascochyta* Valenz.-Lopez *et al.*, Persoonia 37: 255. 2016.

Type species: *Pseudoascochyta pratensis* Valenz.-Lopez *et al*.

Clade 27: *Briansuttonomyces* Crous, Fungal Biology 120: 1412. 2016.

Type species: *Briansuttonomyces eucalypti* Crous

Clade 28: *Ascochyta* Lib. emend. Qian Chen & L. Cai, Stud. Mycol. 82: 185. 2015.

Type species: *Ascochyta pisi* Lib.

Ascochyta astragalina (Rehm ex Sacc.) L.W. Hou, L. Cai & Crous, ***stat. et. comb. nov.*** MycoBank MB834116.

Basionym: *Didymella bryoniae* var. *astragalina* Rehm ex Sacc., Syll. fung. (Abellini) 1: 557. 1882.
Synonym: *Didymella astragalina* (Rehm ex Sacc.) Corbaz, Phytopath. Z. 28: 395. 1957.

Description: Corbaz (1957).

Material examined: **Sweden**, Uppland, on *Lathyrus vernus* (*Fabaceae*), May 1987, K. & L. Holm, specimen CBS H-24335, culture CBS 113797 = UPSC 2222.

Notes: Isolate CBS 113797 was originally identified as *Didymella astragalina*, and was from the same host family (*Fabaceae*) in Europe (Sweden) as the original description (*Astragalus* sp. in Germany; Saccardo 1882, Corbaz 1957). Phylogenetically, it clustered in the genus *Ascochyta* in the present study. However, because the type material could not be traced, and isolate CBS 113797 remains sterile, further studies are needed to resolve its typification.

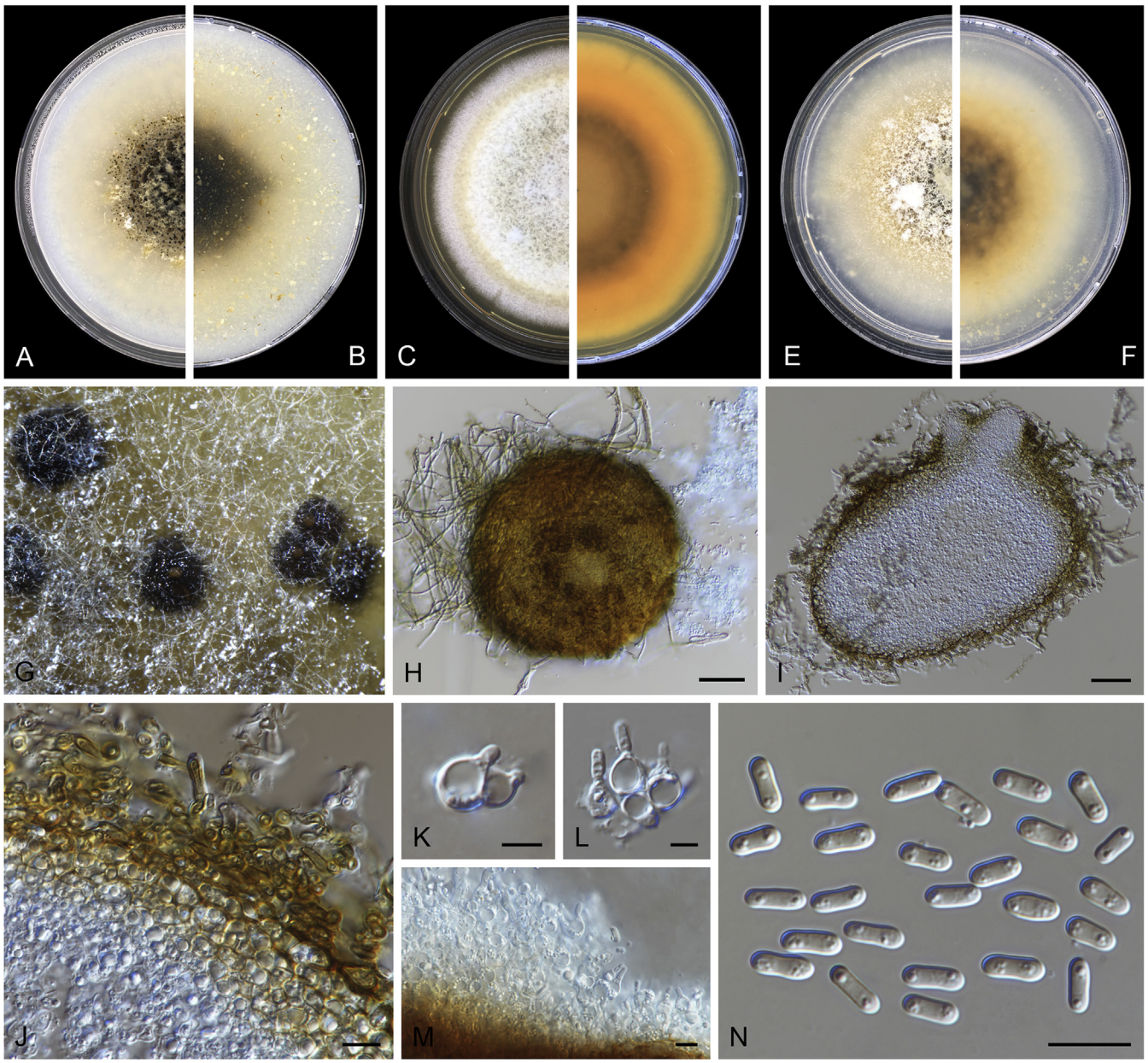

Fig. 46. *Ascochyta pilosella* (CBS 583.97). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 50 µm; J, M–N = 10 µm; K–L = 5 µm.

Ascochyta clinopodiicola D. Pem *et al.*, Mycosphere 9: 288. 2018. Fig. 45.

Description from culture CBS 123526: *Conidiomata* pycnidial, (semi-)immersed, abundant, mostly scattered, few aggregated, solitary, becoming 2–3 confluent with age, globose to subglobose, dark brown, with hyphal outgrowths, especially around the ostioles, 180–475 × 175–410 µm. *Ostioles* 2–8, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong, globose or isodiametric cells, 4–7-layered, 14–36.5 µm thick, outer 1–2(–4) cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, globose, ampulliform to doliiform, slightly papillate, 5.5–8 × 4–7.5 µm. *Conidia* oblong with rounded apices, smooth- and thin-walled, hyaline, straight or slightly curved, aseptate, 4.5–7.5 × 2–2.5 µm, with 2–6 small guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d at 25 °C, margin regular, aerial mycelium flat, hazel with honey sterile zones; reverse concolourous. Colonies on MEA reaching 50–55 mm diam after 7 d, margin regular, aerial mycelium floccose, whitish with some grey section; reverse buff to orange, with some brown sections. Colonies on PDA reaching 50–55 mm diam after 7 d, margin regular, covered by felty aerial mycelium, whitish to pale olivaceous grey; reverse buff to dark brown. NaOH spot test: a brownish discolouration on mycelium of OA.

Typus: **Italy**, Province of Forlì-Cesena, Spinello-Santa Sofia, on dead aerial stem of *Clinopodium nepeta* (*Lamiaceae*), 18 May 2017, E. Camporesi, (**holotype** MFLU 17-1034, ex-type living culture MFLUCC 18-0344).

Additional materials examined: **USA**, Washington, from *Pisum sativum* (*Fabaceae*), 2008, M.I. Chilvers, culture CBS 123526; *ibid.*, culture CBS 123527; Utah,

Canyonlands National Park, from soil, 1997, M. Christensen, culture CBS 127776.

Notes: *Ascochyta clinopodiicola* was originally described from a sexual morph collected on dead stems of *Clinopodium nepeta* in Italy (Hyde *et al.* 2018). In this study, three isolates from *Pisum sativum* were revealed to be genetically identical to the ex-type isolate of *As. clinopodiicola* (MFLUCC 18-0344) and the morphological characters of the asexual morph were also described (based on CBS 123526).

Ascochyta koolunga (J.A. Davidson *et al.*) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833543.
Basionym: *Phoma koolunga* J.A. Davidson *et al.*, Mycologia 101: 124. 2009.
Synonym: *Ascochyta boeremae* L.W. Hou *et al.*, Stud. Mycol. 87: 126. 2017.

Description: Davidson *et al.* (2009).

Typus: **Australia**, Minnipa, Meridionalis, Nova Hollandia, on *Pisum sativum* (*Fabaceae*), 26 Oct. 2004, J. Davidson (**holotype** DAR 78535).

Materials examined: **Australia**, from a leaf of *P. sativum*, dep. Sep. 1984, G.H. Boerema (**holotype** of *A. boeremae*, CBS H-23017, ex-type living culture CBS 372.84 = PD 80/1246); from a leaf of *P. sativum*, dep. Sep. 1984, G.H. Boerema, specimen CBS H-9078, culture CBS 373.84 = PD 80/1247.

Notes: *Phoma koolunga* was isolated from field peas (*Pisum sativum*) causing Ascochyta blight in South Australia (Davidson *et al.* 2009). This species was identified based on morphological characters and sequences of the ITS region compared with those of the accepted pathogens causing Ascochyta blight of field peas. In our phylogenetic analyses, the holotype of *Phoma koolunga* (DAR 78535) clustered in a well-supported clade with the ex-type strain of *As. boeremae* (CBS 372.84; Chen *et al.* 2017) which was isolated from same host species (*Pisum sativum*) without any difference in the ITS and *rpb2* sequences. Therefore, *As. boeremae* was synonymised under *Phoma koolunga*. A new combination was also proposed for this species as *As. koolunga.*

Ascochyta pilosella L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833542. Fig. 46.

Etymology: Name reflects the pycnidia bearing hairs (= *pilus* in Latin) produced by this species.

Description: *Conidiomata* pycnidial, semi-immersed or immersed, abundant, mostly scattered and solitary, sometimes 2–3 aggregated, (sub-)globose or flask-shaped, thick-walled, pale brown, becoming dark brown with age, with hyphal outgrowths, ostiolate, (185–)300–650(–800) × (180–)250–550(–700) µm. *Ostioles* 1–6(–12), non-papillate or slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–12 layers, 35–65 µm thick, outer 2–5 cell layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or lageniform, 5–10.5 × 4–8.5 µm. *Conidia* cylindrical or oblong with rounded apices, smooth- and thin-walled, hyaline, aseptate, 5.5–9 × 2.5–3.5 µm, mostly with two polar guttules, sometimes 2–3 minute guttules at both end. *Conidial matrix* brownish.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d at 25 °C, margin regular, aerial mycelium floccose, dark brown near the centre, and flat, buff towards the periphery; reverse concolourous. Colonies on MEA reaching 50–55 mm diam after 7 d, margin regular, aerial mycelium woolly, white with a buff circle, pinkish towards the periphery; reverse pale brown to orange. Colonies on PDA, 50–55 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, buff, aerial mycelium flat towards the periphery; reverse brown to yellow, buff towards periphery. NaOH spot test results in a brownish discolouration on OA.

Typus: **Canada**, British Columbia, south of Whitler, North-side of Cheakamus River, from leaf spot on *Clintonia uniflora* (*Liliaceae*), 18 Aug. 1994, V. Mel'nik (**holotype** CBS H-23672, ex-type living culture CBS 583.97).

Notes: Isolate CBS 583.97 formed a basal lineage in *Ascochyta* which was phylogenetically closely related to *As. astragalina*, *As. herbicola* and *As. rosae* (Fig. 1). Although *As. astragalina* is sterile, isolate CBS 583.97 is well separated from it based on their sequence differences: 1 bp of ITS sequences, 30 bp in *rpb2* and 13 bp in *tub2* . Besides, isolate CBS 583.97 could be well distinguished from *As. herbicola* in the number of pycnidial ostioles (1–12 ostioles, non-papillate or slightly papillate *vs.* 1–2 ostioles, with long elongated neck; De Gruyter *et al.* 1998); and from *As. rosae* in the size of its conidia (5.5–9 × 2.5–3.5 µm *vs.* 3.2–4.4 × 1.2–1.8 µm; Tibpromma *et al.* 2017). Thus, a new species is proposed here as *As. pilosella* based on CBS 583.97.

Ascochyta viciae-villosae Ondřej, Biológia (Bratislava) 23: 815. 1968.

Description: Ondřej (1968).

Material examined: **Czechoslovakia**, on leaf of *Vicia villosa* (*Fabaceae*), unknown date and collector, culture CBS 255.92 = CCM F-244.

Note: Isolate CBS 255.92 was from the same host and location with the original description of *Ascochyta viciae-villosae* (on leaves of *Vicia villosa*, former Czechoslovakia; Ondřej 1968). However, CBS 255.92 remained sterile in all culture media tested in this study. Therefore, further studies and more suitable cultures are needed to resolve its typification and clarify its phylogeny.

Clade 29: *Phomatodes* Qian Chen & L. Cai, Stud. Mycol. 82: 191. 2015.

Type species: *Phomatodes aubrietiae* (Moesz) Qian Chen & L. Cai

Phomatodes nebulosa (Pers.) Qian Chen & L. Cai, Stud. Mycol. 82: 191. 2015.
Basionym: *Sphaeria nebulosa* Pers., Observ. Disp. Mycol. 2: 69. 1800.
Synonym: *Phoma nebulosa* (Pers.) Berk., Outl. Brit. Fung. (London): 314. 1860.

Materials examined: **Poland**, near Gryfice, on *Thlaspi arvense* (*Cruciferae*), Dec. 1997, J. Marcinkowska, culture CBS 100191. **The Netherlands**, from a stem of *Mercurialis perennis* (*Euphorbiaceae*), Jan. 1993, J. de Gruyter, culture CBS 117.93 = PD 83/90.

Phomatodes pilosa L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833544. Fig. 47.

Etymology: Name refers to the piliferous ostioles of the pycnidia produced by this species.

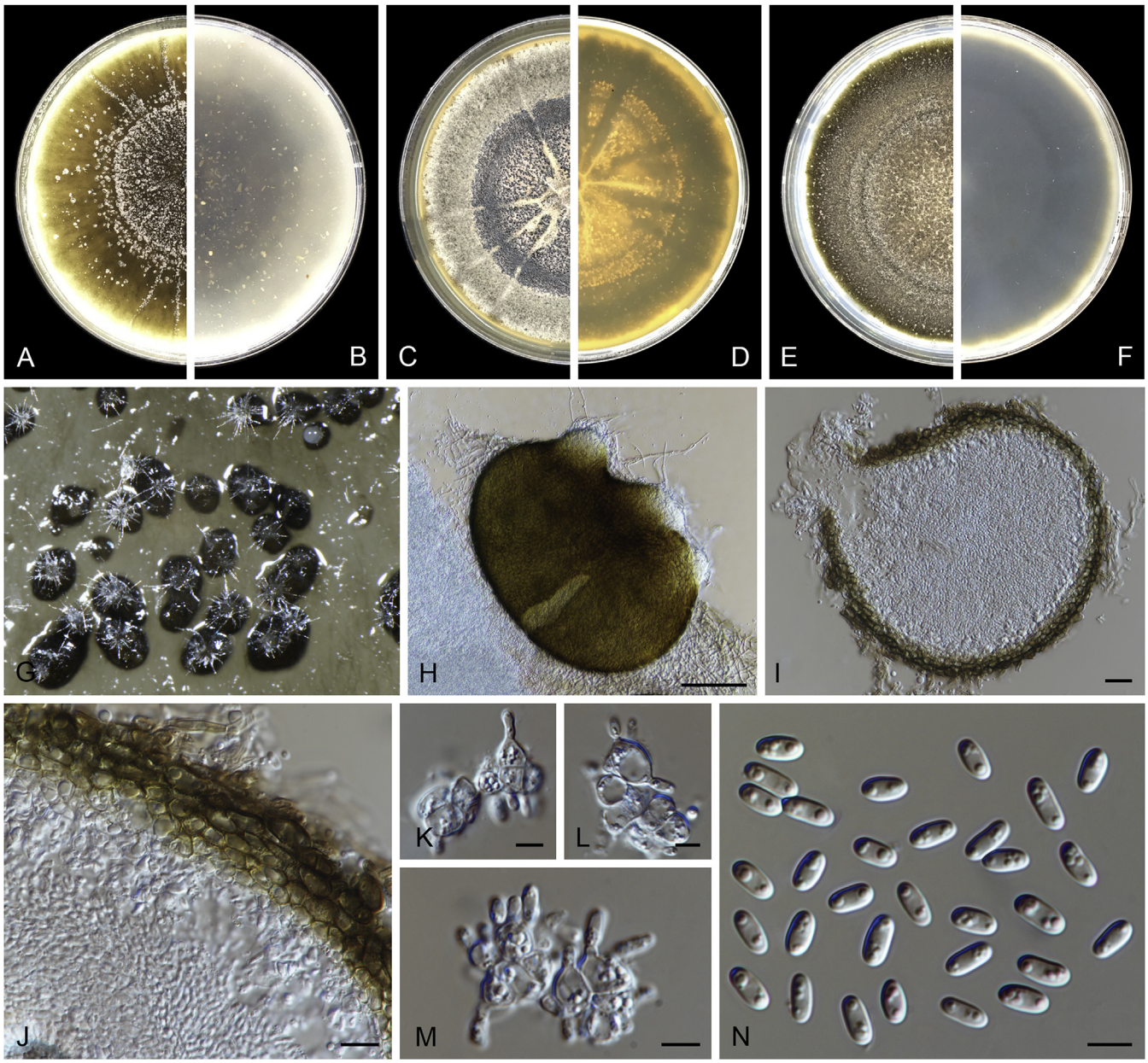

Fig. 47. *Phomatodes pilosa* (CBS 628.68). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 20 µm; J = 10 µm; K–N = 5 µm.

Description: *Conidiomata* pycnidial, mostly semi-immersed or immersed, abundant, scattered or aggregated, solitary or confluent, (sub-)globose, brown to dark brown, glabrous, with hypha around the ostioles, 210–530 × 200–470 µm. *Ostioles* mostly single, central, up to 15 with age, slightly papillate or elongated into to a short neck. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric or oblong cells, 4–8 layers, 14.5–33.5 µm thick, outer 3–4 cell layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, lageniform or ampulliform, 5–7.5 × 3.5–7.5 µm. *Conidia* oblong with rounded apices, smooth- and thin-walled, hyaline, aseptate, 4.5–7 × 2–3.5 µm, mostly with two polar guttules, sometimes 1–4 minute-sized guttules. *Conidial matrix* whitish.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d at 25 °C, margin regular, without aerial mycelium, olivaceous, production of pycnidia abundant, formed in the centre as well as in several radial lines near the margin; reverse dark olivaceous with a grey margin. Colonies on MEA reaching 45–50 mm diam after 7 d, margin regular, aerial mycelium flat to floccose, black at the centre and pale olivaceous grey towards the periphery, some radially furrowed zones from the centre to the edge; reverse pale brown to dark brown. Colonies on PDA reaching 45–50 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, olivaceous grey, with olivaceous edge; reverse olivaceous black. NaOH test results in a brownish discolouration on OA.

Typus: **The Netherlands**, Wageningen, from stem of *Humulus lupulus* (*Cannabaceae*), Sep. 1968, Plant Protection Service, Wageningen (**holotype** CBS H-23680, ex-type living culture CBS 628.68 = PD 67/884).

Notes: Phylogenetically, *Phomatodes pilosa* was allied to the ex-type strain of *Phomat. aubrietiae* (CBS 627.97; Fig. 1). However, it differed from the latter in the number of pycnidial ostioles and in conidial size. *Phomatodes aubrietiae* produced smaller pycnidia measuring 110–255(–290) × 90–215(–245) µm with a single

ostiole, and longer conidia measuring 6–8.5 × 2.5–3 µm (Chen *et al.* 2015). *Phomatodes pilosa* produced larger pycnidia, 210–530 × 200–470 µm, with more ostioles (up to 15), but smaller conidia, 4.5–7 × 2–3.5 µm. Furthermore, conidia produced by *Phoma humuli-japonici* (isolated from *Humulus japonicus*) were much larger than those of *Phomat. pilosa* (10–13 × 3.5–4 µm *vs.* 4.5–7 × 2–3.5 µm; Roumeguère 1891).

Clade 30: *Phoma* Sacc., Michelia 2: 4. 1880. *emend*. Q. Chen & L. Cai, Stud. Mycol. 82: 194. 2015.

Type species: *Phoma herbarum* Westend.

Phoma herbarum Westend., Bull. Acad. Roy. Sci. Belgique, Cl. Sci. 19: 118. 1852, *emend*. Qian Chen & L. Cai, Stud. Mycol. 82: 195. 2015.
Synonyms: *Phoma lignicola* Rennerf., Svensk Skogsvårdsförening Tidskr. 35(1): 60. 1937.
Phoma cruris-hominis Punith., Nova Hedwigia 31: 135. 1979.
Atradidymella muscivora M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.
Phoma muscivora M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.

Description: Chen *et al.* (2015).

Materials examined: **Sweden**, from wood pulp, unknown date, E. Rennerfelt (ex-type living culture of *Phoma lignicola*, culture CBS 276.37 = MUCL 9920). **The Netherlands**, Flevoland Province, Emmeloord, from the stem of *Rosa multiflora* var. *cathayensis* (*Rosaceae*), Dec. 1975, G.H. Boerema, culture CBS 615.75 = PD 73/665 = IMI 199779; South Holland Province, Naaldwijk, from a stem base of *Nerium* sp. (*Apocynaceae*), Sep. 1991, J. de Gruyter, culture CBS 502.91 = PD 82/276; Emmeloord, from a leaf of *Delphinium sp.*, deposited in CBS Feb. 1996, CBS 134.96 = PD 84/676. **UK**, from a leg of woman, Apr. 1977, Y.M. Clayton (**holotype** of *Phoma cruris-hominis* IMI 213845, ex-type living culture CBS 377.92 = IMI 213845); near Dumfries, from die-back of *Picea excelsa* (*Pinaceae*), Oct. 1937, T.R. Peace, culture CBS 274.37. **USA**, Michigan, Wolf Lake, from gametophytes of *Polytrichum juniperinum* (*Polytrichaceae*) growing on the base of an uprooted *Picea mariana* (*Pinacea*) tree, 2008, M.L. Davey (ex-type living culture of *Atradidymella muscivora* UAMH 10909 = CBS 127589 = Pj8-D); Maryland, from fruit *Malus sylvestris*, unknown date, M.A. Smith, specimen CBS H-7608, culture CBS 567.63 = ATCC 15053 = MUCL 9889. **USSR**, unknown substrates, unknown date and collector, culture CBS 105.30.

Notes: *Phoma lignicola* was originally isolated from wood pulp in Sweden (Rennerfelt 1937). In our phylogenetic tree, the ex-type strain of *Phoma lignicola* (CBS 276.37) clustered with the representative strain of *Phoma herbarum* (CBS 615.75; Fig. 1), and therefore *Phoma lignicola* is reduced to synonymy here.

Clade 31: *Leptosphaerulina* McAlpine, Fungus diseases of stone-fruit trees in Australia: 103. 1902.

Type species: *Leptosphaerulina australis* McAlpine

Leptosphaerulina briosiana (Pollacci) J.H. Graham & Luttr., Phytopathology 51: 685. 1961.
Basionym: *Pleosphaerulina briosiana* Pollacci, Atti Ist. Bot. R. Univ. Pavia, 2 Sér. 7: 51. 1902.
Synonym: *Pseudoplea briosiana* (Pollacci) Höhn., Ann. Mycol. 16 (1–2): 162. 1918.

Description: Graham & Luttrell (1961).

Typus: **Italy,** Utinum, from leaves of *Medicaginis sativae* (*Fabaceae*), **lectotype** designated here of *Pleosphaerulina briosiana*, Sopra una nuova malattia dell'erba medica (*Pleosphaerulina briosiana* Pollacci). Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia: illustration on page 51, 1902, Pollacci G, MBT390299). **The Netherlands**, from leaf *Medicago sativa* (*Fabaceae*), date unknown, Plant Protection Service, Wageningen (**epitype designated here** CBS H-24328, MBT390093, ex-epitype living culture CBS 533.66).

Additional materials examined: **USA**, Minnesota, St. Paul, from leaf *Medicago sativa* (*Fabaceae*), Feb. 1966, L. Sundheim, culture CBS 156.66; Minnesota, University of farm, St. Paul, from leaf *M. sativa*, Feb. 1954, R.R. Nelson, culture CBS 301.54; Georgia, from *M. sativa*, 23 Mar. 1954, E.S. Luttrell, culture CBS 214.55; host unknown, Jul. 1974, K.L. O'Donnell, culture CBS 441.74.

Notes: According to the original literature, *Pleosphaerulina briosiana* was described from *Medicago sativa* collected in Italy (Pollacci 1902), and later recombined into *Leptosphaerulina* as *Le. briosiana* based on morphological characters (Graham & Luttrell 1961). Unfortunately, no type was indicated. In this study, four cultures received as *Le. briosiana*, which were isolated from the same host as *Le. briosiana* in the USA were examined. Based on the phylogenetic analysis, these four isolates clustered together with isolate CBS 533.66, which was received as *Ascochyta medicaginicola* but was phylogenetically distant from the ex-type strain of *As. medicaginicola* (CBS 112.53; Fig. 1). Because CBS 533.66 was collected from the same host and continent as the holotype of *Le. briosiana*, it was designated as ex-epitype culture.

Leptosphaerulina chartarum Cec. Roux, Trans. Brit. Mycol. Soc. 86: 320. 1986.
[not sexual morph of *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis, Mycol. Pap. 76: 13 (1960)]

Typus: **South Africa**, Cape Province, Middelburg, from *Galenia procumbens* (*Aizoaceae*), 1981, coll. A. Barnhoorn, dep. C. Roux (**holotype** PREM 47900, ex-type living culture CBS 329.86).

Notes: *Leptosphaerulina chartarum* was originally described as the sexual morph of *Pithomyces chartarum* (Roux 1986). However, phylogenetic analyses revealed that the ex-type strain (CBS 329.86) of *Le. chartarum* did not belong to the *Pi. chartarum* clade, nor was it related to any of the *Pithomyces* lineages within *Montagnulaceae* (Da Cunha *et al.* 2014). In the present study, *Le. chartarum* grouped with other *Leptosphaerulina* species and together clustered within *Didymellaceae* (Fig. 1), which further revealed that *Le. chartarum* is not the sexual morph of *Pi. chartarum*.

Leptosphaerulina gaeumannii (E. Müll.) Wehm., World Monograph of the Genus Pleospora and its Segregates: 322. 1961.
Basionym: *Pleospora gaeumannii* E. Müll., Ber. Schweiz. Bot. Ges. 61: 165. 1951.
Synonym: *Pseudoplea gaeumannii* (E. Müll.) Wehm., Mycologia 47(2): 164. 1955.

Typus: **Switzerland**, Zürich, lawn, Aug. 1950, E. Müller, specimen CBS H-24338, ex-type living culture of *Pleospora gaeumannii* CBS 311.51.

Additional material examined: **The Netherlands,** Baarn, Pekingtuin, soil, J.A. Stalpers, Sep 1969, culture CBS 939.69.

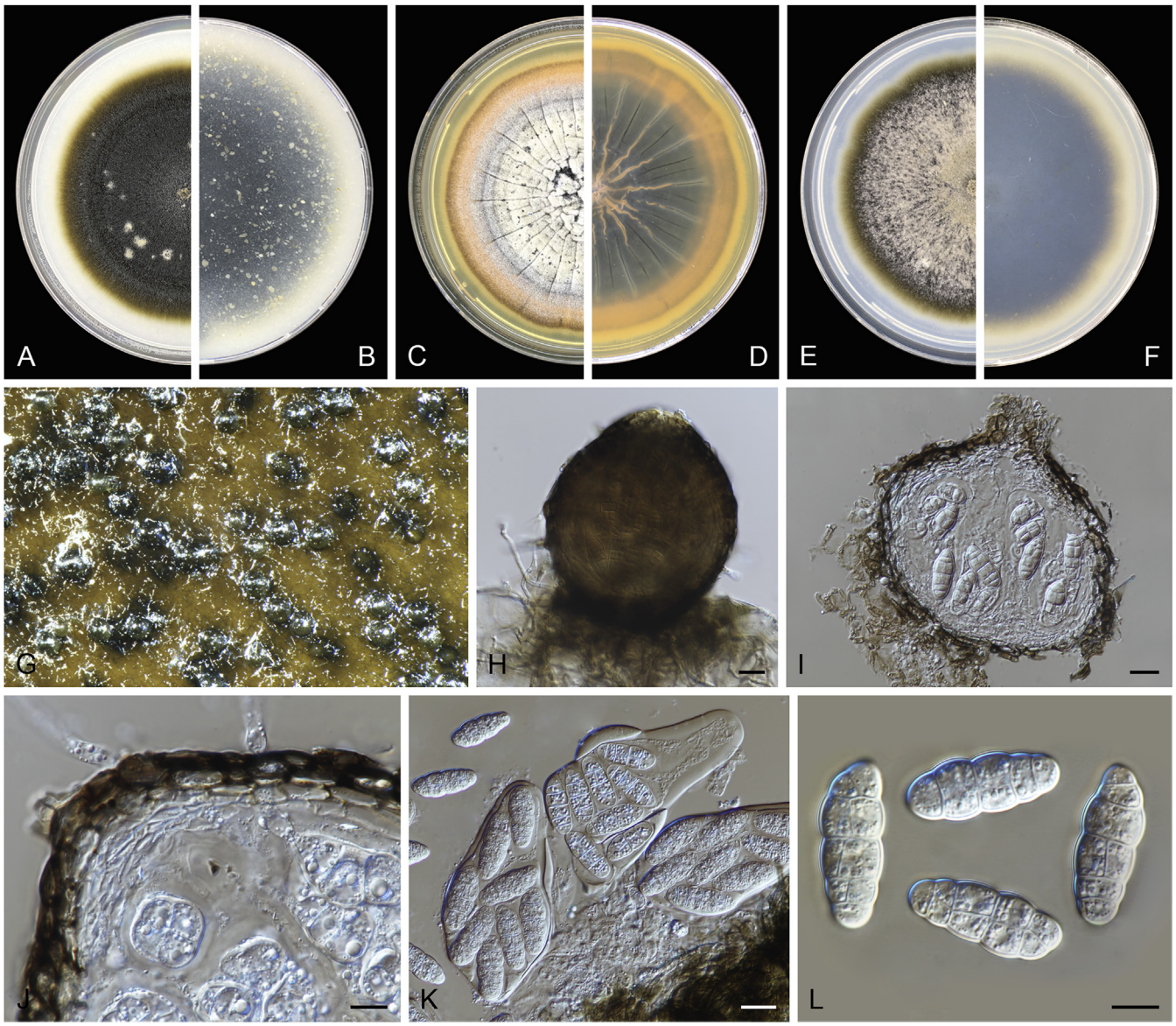

Fig. 48. *Leptosphaerulina obtusispora* (CBS 569.94). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pseudothecia forming on OA. **H.** Pseudothecium. **I.** Section through pseudothecium. **J.** Section of pseudothecial wall. **K.** Asci and ascospores. **L.** Ascospores. Scale bars: H–I, K = 20 µm; J, L = 10 µm.

Notes: *Pleospora gaeumannii* was originally described from dead stems of *Poaceae* in Switzerland (Müller 1951), and later placed in *Leptosphaerulina* based on morphology (Wehmeyer 1961). In the present study, the ex-type strain of *Le. gaeumannii* (CBS 311.51) formed a distinct lineage within *Leptosphaerulina*. Unfortunately, this isolate remained sterile in all culture media tested in this study.

Leptosphaerulina obtusispora L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833545. Fig. 48.

Etymology: Name refers to the ascospores with obtuse apices.

Description: *Ascomata* pseudothecial, superficial, or semi-immersed, solitary or clustered, scattered, uniloculate, globose, subglobose or pyriform, membranous, brown to dark brown, 100–310 × 80–285 µm. *Ostioles* circular, central, papillate. *Pseudothecial wall* black, 3–4 layers, 18–42 µm thick, outer wall comprising 1–2 layers of brown to dark brown cells, of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 84–130.5 × 41.5–64 µm, 8-spored, bitunicate, fissitunicate, saccate, obpyriform or ovoid. *Ascospores* 31–43.5 × 13–16.5 µm, irregularly tri-seriate, cylindrical or ellipsoidal, hyaline, muriform, with 4–5 transverse septa, and 3–4 longitudinal septa, apex obtuse, base broadly obtuse to subobtuse, slightly constricted at the septum, smooth-walled, surrounded by distinct mucilaginous sheath. *Chlamydospores* not observed.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d at 25 °C, margin entire, regular, covered by flat aerial mycelium, olivaceous to olivaceous black; reverse buff to black. Colonies on MEA reaching 45–50 mm diam after 7 d, margin regular, covered by flat aerial mycelium, some radially furrowed zones near the centre, concentric circles of different colours, centre buff, greyish, orange, pale olivaceous towards the periphery; reverse dark mouse grey, brick to orange edge, with pale brown radial lines from centre. Colonies on PDA reaching 30–35 mm diam after 7 d, margin regular, covered by flat aerial mycelium, pale olivaceous grey, buff to olivaceous toward periphery; reverse black with buff edge. NaOH spot test negative on OA.

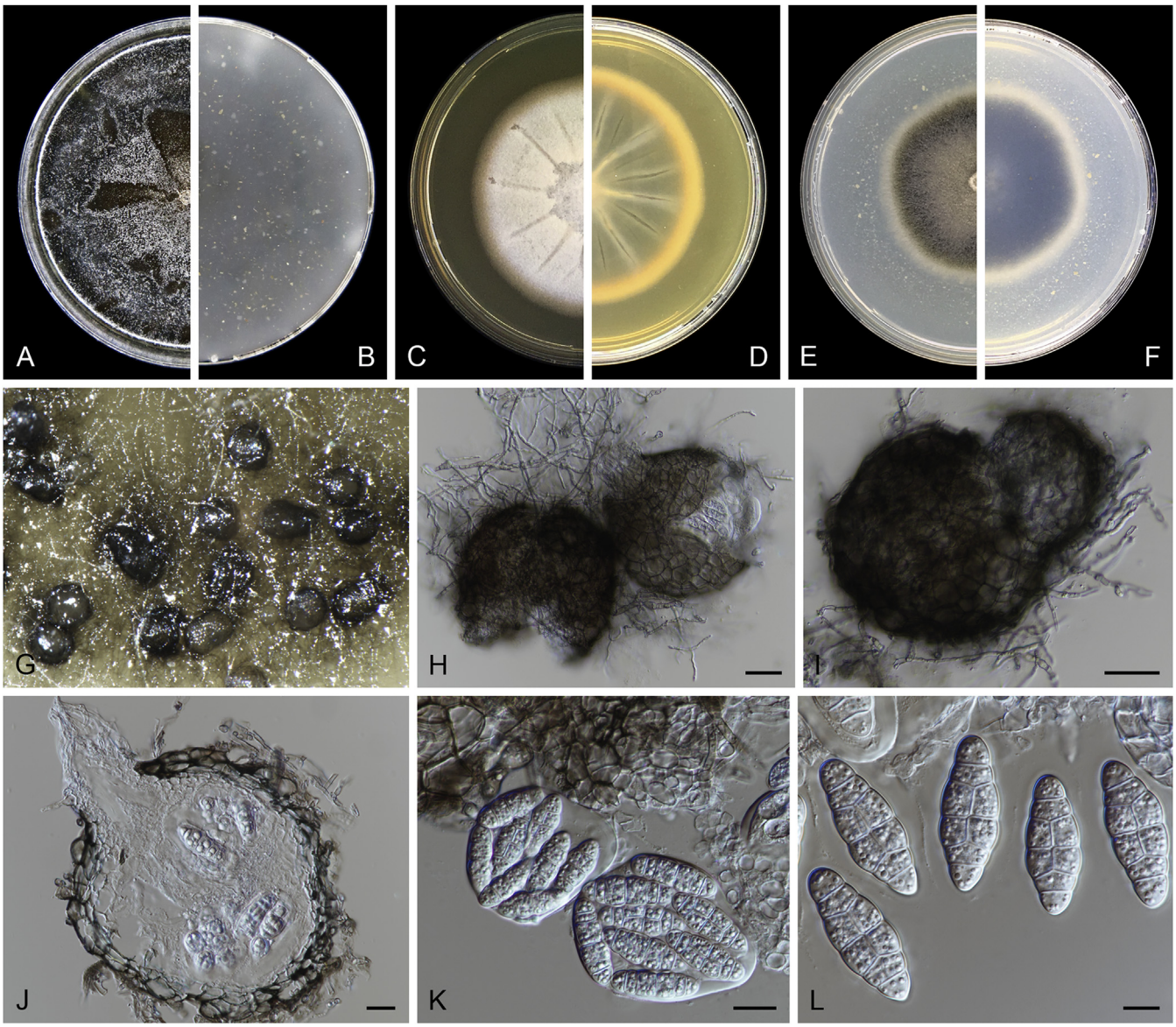

Fig. 49. *Leptosphaerulina sisyrinchiicola* (CBS 121688). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pseudothecia forming on OA. **H–I.** Pseudothecia. **J.** Section through pseudothecium. **K.** Asci. **L.** Ascospores. Scale bars: H–I = 50 µm; J, K = 20 µm; L = 10 µm.

Typus: **The Netherlands**, South Holland Province, Boskoop, tree nursery, from leaf spot of *Lonicera periclymenum* (*Caprifoliaceae*), 5 Oct. 1994, Plant Protection Service, Wageningen (**holotype** CBS H-23669, ex-type living culture CBS 569.94).

Additional material examined: **The Netherlands**, Utrecht Province, Baarn, CBS garden, lawn, Apr. 1958, J.A. von Arx, culture CBS 234.58.

Notes: *Leptosphaerulina argentinensis* was originally described from *Datura stramonium* (*Solanaceae*) collected in Argentina, with ascospores measuring 30–35 × 12–16 µm (Spegazzini 1909, Graham & Luttrell 1961). Isolate CBS 569.94 was originally received as *Le. argentinensis*, but differed morphologically by producing larger pseudothecia, asci and ascospores (pseudothecia: 100–310 × 80–285 µm *vs.* 100–120 µm, asci: 84–130.5 × 41.5–64 µm *vs.* 80–90 × 40–45 µm, ascospores: 31–43.5 × 13–16.5 µm *vs.* 30–35 × 12–16 µm). Besides, CBS 569.94 could be easily differentiated from its most closely related species *Le. saccharicola* in ascospore morphology. Isolate CBS 569.94 produced ascospores with 4–5 transverse septa, and 3–4 longitudinal septa, while *Le. saccharicola* produced ascospores with 4 transverse septa, and 0–2 longitudinal septa. Furthermore, CBS 569.94 could be distinguished from the latter by producing larger ascomata (100–310 × 80–285 µm *vs.* 70–110 µm × 100–140 µm) and larger ascospores [31–43.5 × 13–16.5 µm *vs.* (25–)27–32(–35.5) × (9–)10–11.5(–12) µm]. Thus, we proposed a new species to accommodate this isolate.

Leptosphaerulina sisyrinchiicola L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833837. Fig. 49.

Etymology: Named after the host genus *Sisyrinchium*, from which this species was isolated.

Description: *Ascomata* pseudothecial, superficial or semi-immersed in agar, solitary or clustered, scattered, uniloculate, globose or subglobose, brown to dark brown, 175–325 × 175–260 µm. *Ostioles* central, papillate, with mycelium around the ostioles. *Pseudothecial wall* black, 4–5 layers, 14.5–43 µm, outer wall comprising 2–3 layers of dark brown cells, of *textura angularis*. *Pseudoparaphyses* not observed. *Asci* 70–113.5 × 56–73 µm, 8-spored, bitunicate, fissitunicate, saccate, subglobose, obpyriform or ovoid.

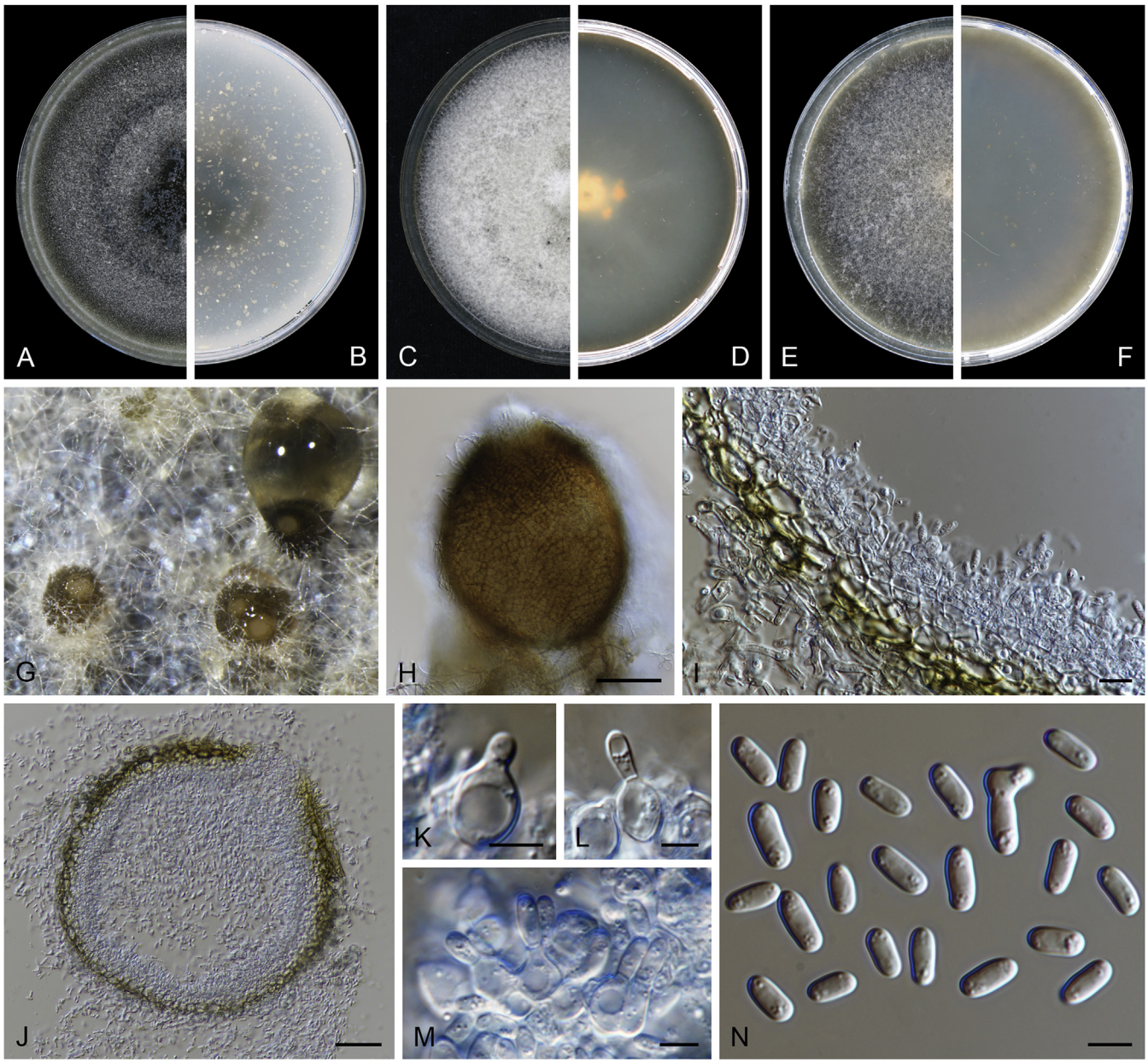

Fig. 50. *Neodidymelliopsis tiliae* (CBS 519.95). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section of pycnidial wall. **J.** Section through pycnidium. **K–M** Conidiogenous cells. **N.** Conidia. Scale bars: H = 100 µm; I = 10 µm; J = 50 µm; K–N = 5 µm.

Ascospores (28–)33–50.5 × (10.75–)11.5–18 µm, irregularly triseriate, oval to cylindrical or ellipsoidal, hyaline, muriform, with 4–5 transverse septa, and 2 longitudinal septa, apex obtuse, base broadly obtuse to subobtuse, usually widest at the third cell, slightly constricted at the septum, smooth-walled, surrounded by distinct mucilaginous sheath. *Chlamydospores* not observed.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d at 25 °C, margin entire, regular, covered by flat aerial mycelium, olivaceous to olivaceous black; reverse concolourous. Colonies on MEA reaching 55–60 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, some radially furrowed zones near the centre, vinaceous buff to buff; reverse isabelline, yellow toward periphery. Colonies on PDA reaching 45–50 mm diam after 7 d, margin regular, covered by flat aerial mycelium, vinaceous buff to hazel, buff toward periphery; reverse black with buff edge. NaOH spot test negative on OA.

Typus: **New Zealand**, Auckland, Mt. Albert, Harbutt Street, Phyllis Reserve, from leaf spot on *Sisyrinchium* sp. (*Iridaceae*), 10 Dec. 2006, C.F. Hill (**holotype** CBS H-24330, ex-type culture CBS 121688).

Notes: Isolate CBS 121688 was received as *Leptosphaerulina argentinensis*, but collected from a different host and locality than the holotype of *Le. argentinensis* (Graham & Luttrell 1961). Morphologically, CBS 121688 clearly differed from *Le. argentinensis* by producing larger ascospores (28–50.5 × 11–18 µm *vs.* 30–35 × 12–16 µm). Based on our phylogenetic analysis, CBS 121688 formed a separate lineage sister to *Le. arachidicola* (Fig. 1), but differed from the latter by producing larger asci and ascospores (asci: 70–113.5 × 56–73 µm *vs.* 56–71.75 × 29.75–31.5 µm, ascospores: 28–50.5 × 11–18 µm *vs.* 25–33.25 × 11.25–16.25 µm). Consequently, we proposed isolate CBS 121688 as a new species, *Le. sisyrinchiicola.*

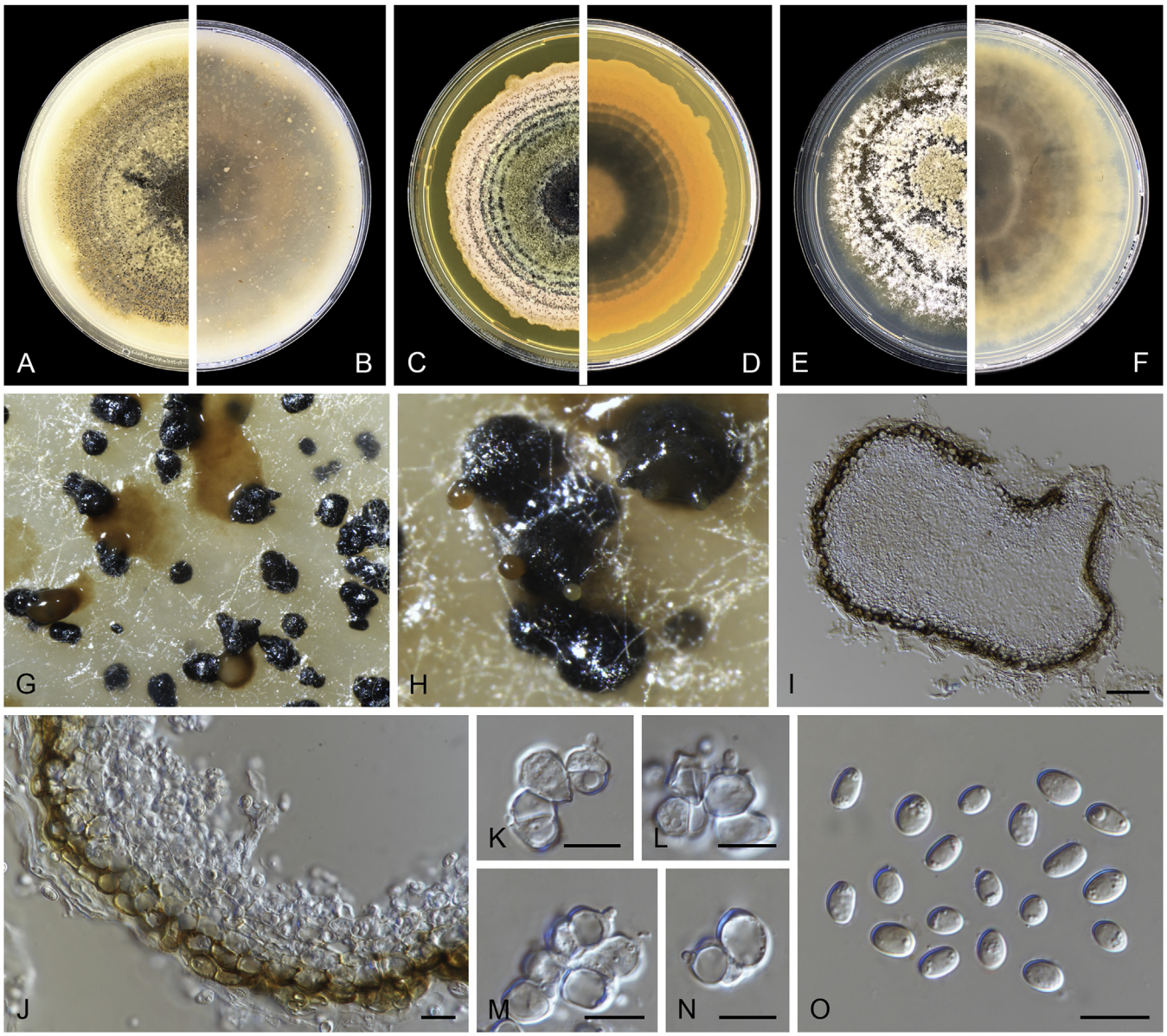

Fig. 51. *Xenodidymella glycyrrhizicola* (CBS 684.97). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G–H**. Pycnidia forming on OA. **I**. Section through pycnidium. **J**. Section of pycnidial wall. **K–N**. Conidiogenous cells. **O**. Conidia. Scale bars: I = 50 µm; J–O = 10 µm.

Clade 32: *Neodidymelliopsis* Qian Chen & L. Cai, Stud. Mycol. 82: 207. 2015.

Type species: *Neodidymelliopsis cannabis* (G. Winter) Qian Chen & L. Cai

Neodidymelliopsis tiliae L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB834685. Fig. 50.

Etymology: Name reflects the host genus *Tilia*, from which this species was isolated.

Description: *Conidiomata* pycnidial, superficial on or semi-immersed in the agar, solitary and aggregated, sometimes confluent, (sub-)globose, brown to dark brown, with white hyphal outgrowths, especially at young age, ostiolate, 290–420 × 190–325 µm. *Ostioles* 1–3, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 5–6 layers, 23.5–55.5 µm thick, with outer 2–3-layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or doliiform, (5–)6–9.5 × 3.5–8 µm. *Conidia* oblong, smooth- and thin-walled, hyaline, aseptate, 5–9 × 2.5–3.5 µm, guttulate. *Conidial matrix* pale salmon.

Culture characteristics: Colonies on OA, 45–50 mm diam after 7 d at 25 °C, margin regular, covered by floccose aerial mycelium, dark grey with olivaceous black cycles near the middle and margin; reverse grey to olivaceous. Colonies on MEA 45–50 mm diam after 7 d, margin regular, covered by woolly aerial mycelium, grey; reverse olivaceous black. Colonies on PDA, 50–55 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, greyish olivaceous to olivaceous; reverse olivaceous black to olivaceous. NaOH spot test negative on OA.

Typus: **Italy**, Perugia, from branch of *Tilia* sp. (*Tiliaceae*), date unknown, T. Schiappa, (**holotype** CBS H-23684, ex-type culture CBS 519.95).

Additional material examined: **Italy**, Viterbo, Ocriculum, from leaf of *Celtis australis* (*Cannabaceae*) with sooty mould, 26 Oct. 2014, W. Gams, culture CBS 139719.

Notes: Based on the phylogenetic analyses in this study, *Neod. tiliae* clustered with *Neod. moricola*, which was described by Hyde *et al.* (2017) from a dead branch of *Morus alba* in Russia, with conidia measuring 3.5–6 × 1–3.5 µm (Fig. 1). *Neodidymelliopsis tiliae* were morphologically similar to *Neod. moricola* in conidial shape, but differed in producing larger conidia (5–9 × 2.5–3.5 µm *vs.* 3.5–6 × 1–3.5 µm) and smaller conidiogenous cells [(5–)6–9.5 × 3.5–8 µm *vs.* 7.5–11.5 × 2.5–5 µm].

Clade 33: *Xenodidymella* Qian Chen & L. Cai, Stud. Mycol. 82: 205. 2015.

Type species: *Xenodidymella applanata* (Niessl) Qian Chen & L. Cai

Xenodidymella glycyrrhizicola L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833546. Fig. 51.

Etymology: Named after the host genus *Glycyrrhiza*, from which this species was isolated.

Description: *Conidiomata* pycnidial, superficial on or immersed in the agar, scattered or aggregated, solitary, sometimes 2 or 3 confluent, globose or subglobose, irregular-shaped with age, brown, becoming dark brown or black with age, thick-walled, glabrous or with some hyphal outgrowths, ostiolate, 270–465 × 215–340 µm. *Ostioles* 2–3, papillate or elongated to a short neck. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–6 layers, 20.5–73 µm thick, with outer 2–4 layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, subglobose, ampulliform or doliiform, 6.5–10.5 × 5.5–10.5 µm. *Conidia* variable in shape and size, oblong, broadly ellipsoidal or subglobose, smooth- and thin-walled, hyaline at beginning, pale brown with age, aseptate, 4.5–9.5 × 3.5–5.5 µm, guttulate. *Conidial matrix* brown.

Culture characteristics: Colonies on OA reaching 45–50 mm diam after 7 d at 25 °C, margin regular, covered by flat aerial mycelium, felty, pale olivaceous or saffron, abundant production of black pycnidia in circles, especially near the centre; reverse olivaceous, reddish brown, greyish brown near the margin. Colonies on MEA reaching 35–45 mm diam after 7 d, margin irregular, aerial mycelium felty, saffron to olivaceous, black in the middle because of the abundant pycnidia aggregated in several circles, especially near the centre; reverse orange to dark brown, orange near the centre. Colonies on PDA reaching 40–45 mm diam after 7 d, margin irregular, covered by floccose and felty aerial mycelium, pale olivaceous to whitish, aerial mycelium in buff colour near the centre; reverse buff to pale olivaceous, with some black area. NaOH spot test negative on OA.

Typus: **New Zealand**, Hamilton, from leaf spot on *Glycyrrhiza lepidota* (*Fabaceae*), date unknown, J. Follet (**holotype** CBS H-23673, ex-type living culture CBS 684.97).

Additional material examined: **Iran**, Hamedan, Aran village, from *Glycyrrhiza* sp. (*Fabaceae*), 28 May 2009, P. Razaghi, culture CBS 141234.

Notes: *Xenodidymella glycyrrhizicola* was represented by two cultures isolated from *Glycyrrhiza* spp. in New Zealand and Iran, which formed a distinct clade, clearly separated from other species (Fig. 1). Morphologically, it is characterised by producing pale brown conidia, while conidia of other species in *Xenodidymella* were hyaline. This is the first report of a *Didymellaceae* species infecting *Glycyrrhiza* plants.

Clade 34: *Dimorphoma* L.W. Hou, L. Cai & Crous, ***gen. nov.*** MycoBank MB833547.

Etymology: The genus name refers to the dimorphic conidia of the type species.

Conidiomata pycnidial, solitary, (sub-)globose, glabrous or covered with hyphal outgrowths. *Ostioles* single, papillate. *Pycnidial wall* pseudoparenchymatous, multi-layered. *Conidiogenous cells* phialidic, hyaline, simple, smooth, variable in appearance, flask-shaped, oblong or isodiametric. *Conidia* two types, both originating from the same pycnidium. Conidia of type 1: (sub-)globose, smooth- and thin-walled, hyaline, aseptate, guttulate. Conidia of type 2: cylindrical to ellipsoidal, thin-walled, smooth, hyaline, aseptate, mainly eguttulate. *Chlamydospores* ubiquitously present in the agar, unicellular, globose, in long chains, greenish pigmented. *Sexual morph* unknown.

Notes: Although phoma-like species are known from various substrates, the number of rock-inhabiting isolates is relatively low. The type species of *Dimorphoma* was described from stone and characterised by producing pycnidia with an extremely thin pycnidial wall, being almost hyaline when the conidia have exuded (Aveskamp *et al.* 2010). Besides, conidia of the type species are of two types (globose conidia and cylindrical to ellipsoidal conidia), both originating from the same pycnidia. Phylogenetically, this species is also clearly separate from all known genera of *Didymellaceae* (Fig. 1), and therefore a new genus is introduced as *Dimorphoma*.

Type species: *Dimorphoma saxea* (Aveskamp *et al.*) L.W. Hou, L. Cai & Crous

Dimorphoma saxea (Aveskamp *et al.*) L.W. Hou, L. Cai & Crous, ***comb. nov.*** MycoBank MB833548.
Basionym: *Phoma saxea* Aveskamp *et al.*, Stud. Mycol. 65: 23. 2010.
Synonym: *Xenodidymella saxea* (Aveskamp *et al.*) Valenz.-Lopez *et al.*, Stud. Mycol. 90: 44. 2017 (2018).

Description: Aveskamp *et al.* (2010).

Typus: **Germany**, Oldenburg, from corroded Mediterranean marble, Jun. 1992, J. Kuroczkin (**holotype** CBS H-20240, ex-type living culture CBS 419.92).

Additional material examined: **Germany**, Oldenburg, from limestone, 1987, J. Kuroczkin, culture CBS 298.89.

Notes: The description of *Phoma saxea* was based on morphology and molecular phylogenetic analyses of CBS 419.92 and CBS 298.89 isolated from stone (Aveskamp *et al.* 2010). Later Valenzuela-Lopez *et al.* (2018) recombined this species into *Xenodidymella*. However, in our phylogenetic analyses, this species formed an independent lineage clearly distinct from *Xenodidymella* and other genera in *Didymellaceae* (Fig. 1). This result was in consensus with that of Aveskamp *et al.* (2010), and also agreed well with the statement of Valenzuela-Lopez *et al.* (2018) that this species could represent a distinct genus. Morphologically, this species produced two types conidia, hence the name, *Dimorphoma*.

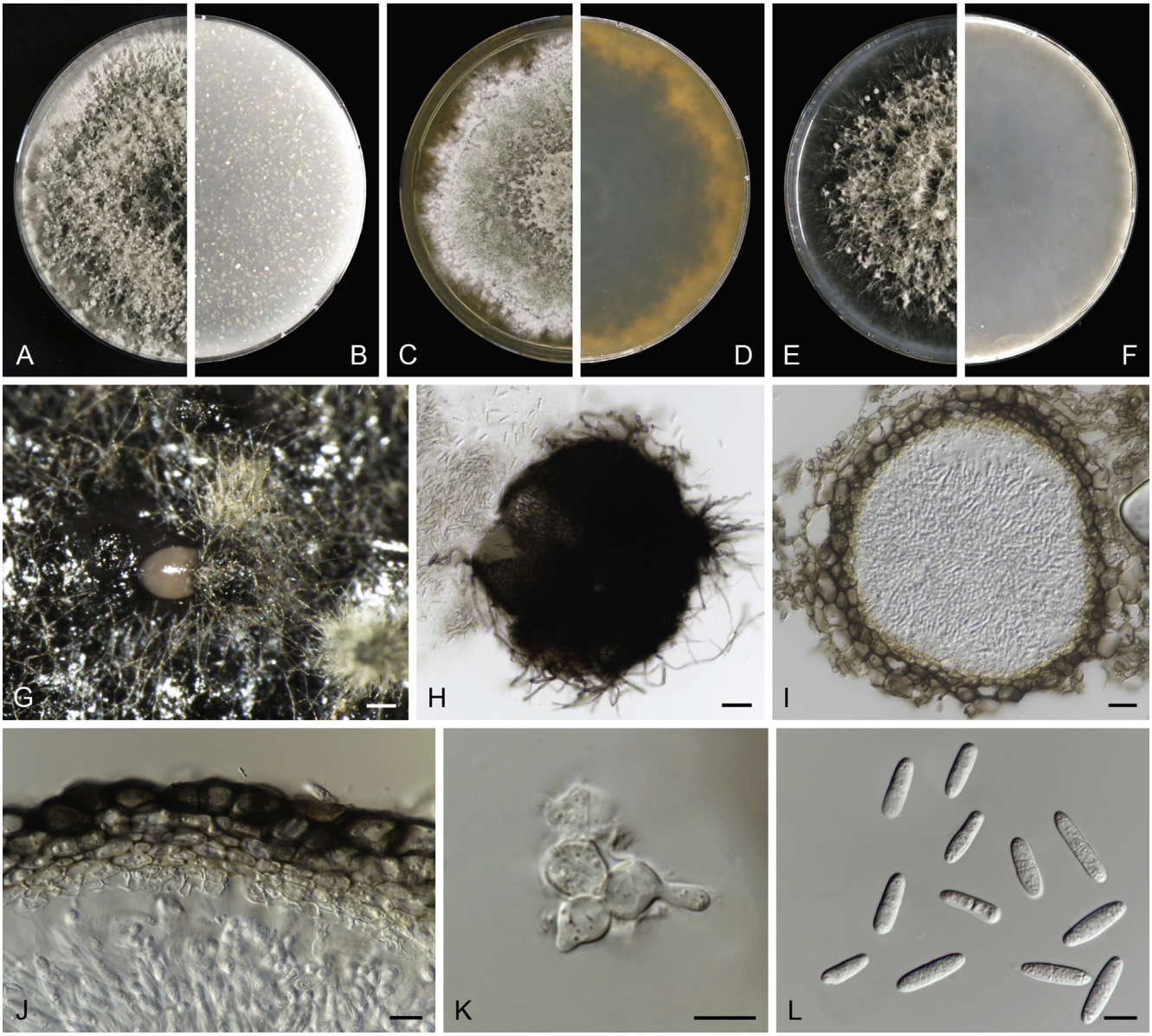

Fig. 52. *Neoascochyta fusiformis* (CBS 876.72). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K.** Conidiogenous cells. **L.** Conidia. Scale bars: H = 50 µm; I = 20 µm; J–L = 10 µm.

Clade 35: *Neoascochyta* Qian Chen & L. Cai, Stud. Mycol. 82: 198. 2015.

Type species: *Neoascochyta exitialis* (Morini) Qian Chen & L. Cai

Neoascochyta humicola L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB834683.

Etymology: Name derived from the substrate this species was collected from, soil.

Description: Culture sterile. *Neoascochyta humicola* differs from its closest phylogenetic neighbour *Neoa*. *fusiformis* (type strain: CBS 876.72) by unique fixed alleles in three loci based on alignments of the concatenated four loci deposited in TreeBASE (S25826): ITS position: 445(T, insertion); *rpb2* positions: 1 460(T), 1 466(C), 1 577(G), 1 598(A), 1 691(G), 1 727(C), 1 742(T), 1 820(C), 1 823(T), 1 835(T), 1 925(G); *tub2* positions: 2 138(G), 2 143(T), 2 152(C).

Typus: **USA**, Kansas, near Manhattan, from soil, unknown date and collector (**holotype** CBS H-24343, ex-type living culture CBS 127323).

Notes: Phylogenetically, isolate CBS 127323 formed a distinct lineage sister to *Neoa*. *fusiformis*, another new species introduced in the present study (Fig. 1), but remained sterile in culture. Considering that it is phylogenetically distinct, a new species, *Neoa. humicola*, was introduced here to accommodate it.

Neoascochyta fusiformis L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833549. Fig. 52.

Etymology: "*fusiformis*" referring to the fusiform ascospores of this fungus.

Description: *Conidiomata* pycnidial, superficial on or semi-immersed in the agar, solitary and aggregated, sometimes confluent, globose, dark brown, later becoming irregular and black with age, thick-walled, with dense greyish green hyphal

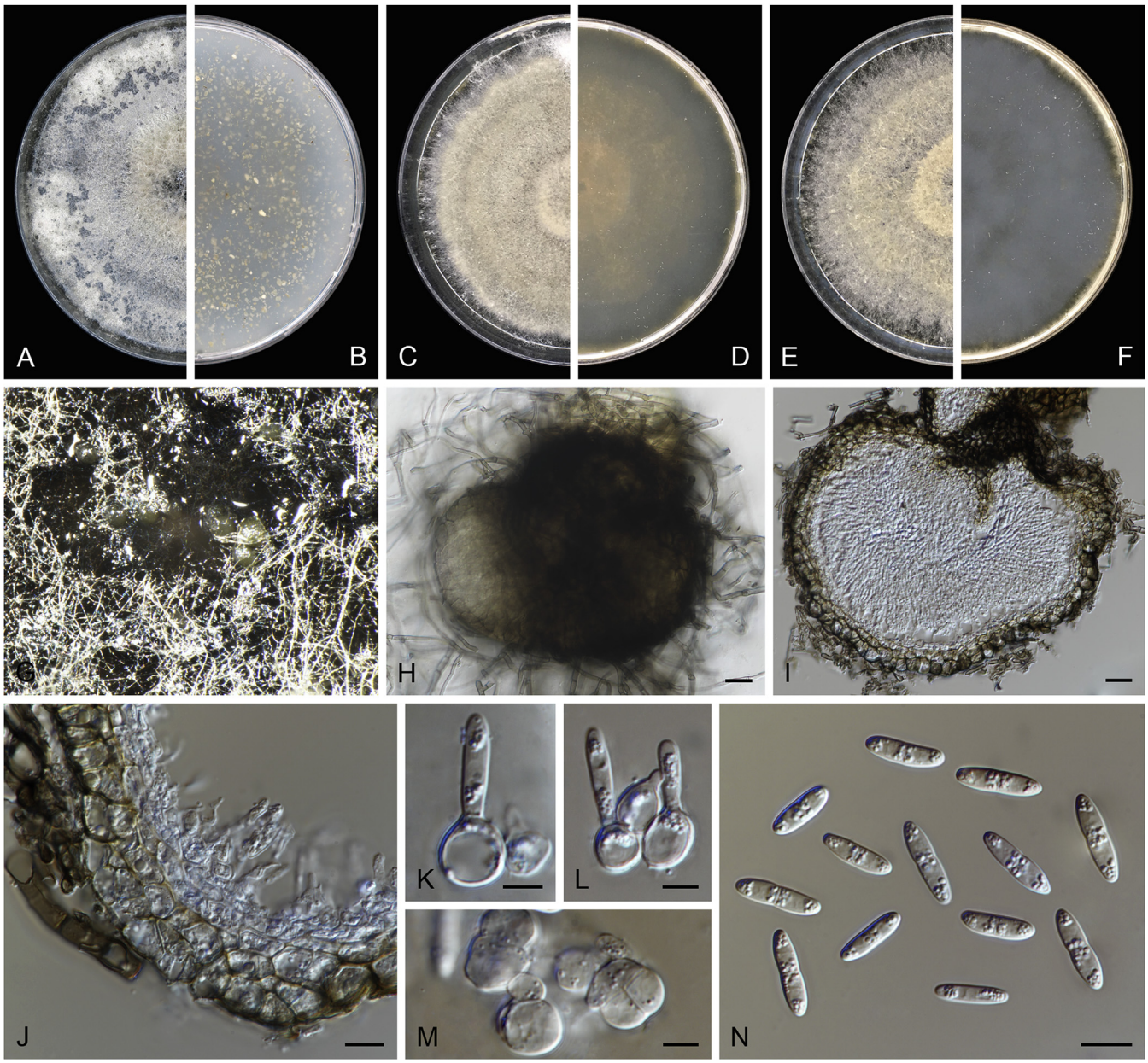

Fig. 53. *Neoascochyta longispora* (CBS 113420). **A–B.** Colony on OA (front and reverse). **C–D.** Colony on MEA (front and reverse). **E–F.** Colony on PDA (front and reverse). **G.** Pycnidia forming on OA. **H.** Pycnidium. **I.** Section through pycnidium. **J.** Section of pycnidial wall. **K–M.** Conidiogenous cells. **N.** Conidia. Scale bars: H–I = 20 µm; J, N = 10 µm; K–M = 5 µm.

outgrowths, 230–600 × 220–440 µm. *Ostioles* single, slightly papillate or elongated to a long neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–6 layers, with outer 2–3 layers pigmented, 21.5–45 µm thick. *Conidiogenous cell* phialidic, hyaline, smooth, ampulliform or lageniform, 7.5–11 × 6–10 µm. *Conidia* variable in shape and size, oblong, fusiform or bacilliform, hyaline, smooth- and thin-walled, 1-septate or aseptate, (16–)17.5–24(–25.5) × 4.5–6.5 µm, guttulate. *Conidial matrix* whitish cream or pale salmon.

Culture characteristics: Colonies on OA, 70–75 mm diam after 7 d 25 °C, margin regular, densely covered by floccose aerial mycelium, greyish olivaceous; reverse concolourous. Colonies on MEA 50–52 mm diam after 7 d, margin irregular, dendritic, covered by floccose aerial mycelium, white in the outer ring and changing to grey olivaceous to fawn; reverse greenish black with brick margin. Colonies on PDA, 70–75 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, greyish olivaceous, with flat and greenish black flat mycelium near the margin; reverse black olivaceous. NaOH spot test: a dull green discolouration on OA.

Typus: **South Africa**, North West Province, Potchefstroom, from wheat straw (*Poaceae*), Oct. 1972, M.C. Papendorf (**holotype** CBS H-8974, ex-type living culture CBS 876.72).

Notes: *Diplodina brachypodii* was described from a decaying leaf of *Brachypodium silvaticum* (*Poaceae*) collected in Germany (conidiomata 100–150 µm diam, conidia 1-septate, 2–3 guttulate, 16–22 × 5–6 µm). Although isolate CBS 876.72 was originally identified as *Dip. brachypodii*, it produced much larger conidiomata and larger conidia [conidiomata: 230–600 × 220–440 µm, conidia: (16–)17.5–24(–25.5) × 4.5–6.5 µm]. Furthermore, CBS 876.72 was isolated from wheat straw in South Africa. We therefore regarded CBS 876.72 as distinct, and described it as a new species.

Neoascochyta longispora L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB834684. Fig. 53.

Etymology: Name reflects the longer (= *Longi*, in Latin) conidia characteristic of this species.

Description: *Conidiomata* pycnidial, semi-immersed, solitary or aggregated, globose or subglobose, dark brown to black, buff to pale brown on the upper part on the agar, later becoming confluent and irregular-shaped, thick-walled, glabrous, 125–295 × 110–245 µm. *Ostioles* inconspicuous. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, with outer 2–3 layers pigmented, 15–20 µm thick. *Conidiogenous cell* phialidic, hyaline, smooth, ampulliform or lageniform, 6.5–9.5 × 6.5–9 µm. *Conidia* variable in size, oblong or bacilliform, hyaline, smooth- and thin-walled, 1-septate or aseptate, slightly constricted at the septum, 12.5–19 × 3.5–5 µm, guttulate. *Conidial matrix* unobserved.

Culture characteristics: Colonies on OA, 70–75 mm diam after 7 d 25 °C, margin regular, covered by flat aerial mycelium, vinaceous buff to pale mouse grey; reverse concolourous. Colonies on MEA 50–55 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, buff to rosy buff; reverse rosy buff to greyish sepia. Colonies on PDA, 70–75 mm diam after 7 d, margin regular, covered by floccose aerial mycelium, buff to vinaceous buff; reverse mouse grey, with olivaceous margin. NaOH spot test negative on OA.

Typus: **The Netherlands**, Zuid-Holland, De Zilk, from *Cerastium semidecandrum* (*Caryophyllaceae*), 4 Jun. 2003, H.V.D. Goes (**holotype** CBS H-24344, ex-type living culture CBS 113420).

Notes: *Neoascochyta longispora* was isolated from *Cerastium semidecandrum*, and forms a distinct lineage close to *Neoa. fusiformis* and *Neoa. humicola*. However, *Neoa. longispora* differs from *Neoa. fusiformis* in the size and shape of pycnidia: pycnidia of *Neoa. longispora* are globose or subglobose, measuring 125–295 × 110–245 µm, while those of *Neoa. fusiformis* are mostly globose, measuring 230–600 × 220–440 µm. Furthermore, *Neoa. longispora* is also distinguishable from the latter in having smaller conidiogenous cells and conidia: *Neoa. longispora* produces conidiogenous cell measuring 6.5–9.5 × 6.5–9 µm and conidia measuring 12.5–19 × 3.5–5 µm, while *Neoa. fusiformis* produces conidiogenous cells measuring 7.5–11 × 6–10 µm and conidia measuring (16–)17.5–24(–25.5) × 4.5–6.5 µm. Although *Neoa. humicola* is sterile, *Neoa. longispora* can be clearly distinguished based on sequence differences in four genes.

Neoascochyta mortariensis L.W. Hou, L. Cai & Crous, ***sp. nov.*** MycoBank MB833550.

Etymology: Epithet derived from the location where it was collected, Mortara, Italy.

Description: Culture sterile. *Neoascochyta mortariensis* differs from its closest phylogenetic neighbour *Neoa. tardicrescens* (type strain: CBS 689.97) by unique fixed alleles in three loci based on alignments of the concatenated four loci deposited in TreeBASE (S25826): ITS position: 68(T), 330(C), 351(A), 408(A), 410(C), 437(C); *rpb2* positions: 1 471(G), 1 537(C), 1 549(A), 1 564(C), 1 591(T), 1 594(C), 1 603(T), 1 612(C), 1 616(A), 1 621(C), 1 657(C), 1 663(G), 1 723(G), 1 744(C), 1 765(A), 1 777(T), 1 780(T), 1 786(C), 1 795(G), 1 816(C), 1 837(G), 1 840(G), 1 846(C), 1 879(G), 1 882(G), 1 894(A), 1 906(C), 1 915(C), 1 916(A), 1 930(G), 1 942(G), 1 951(C), 1 954(A), 1 966(T), 1 969(T), 1 975(T), 1 993(G), 1 996(T), 1 999(C), 2 002(G), 2 008(G), 2 014(C); *tub2* positions: 2 060(C), 2 069(T), 2 083(A), 2 084(C), 2 086(G), 2 087(A), 2 106(A), 2 112(C), 2 122(A), 2 145(T), 2 229(T), 2 282(G), 2 420(T).

Culture characteristics: Colonies on OA reaching 55–60 mm diam after 7 d 25 °C, margin irregular, aerial mycelium felty, pale mouse grey, brown to dark brown towards periphery; reverse dark mouse grey. Colonies on MEA reaching 75–80 mm diam after 7 d, margin regular, aerial mycelium woolly, whitish pale grey near the centre; reverse dark brown with orange edge. Colonies on PDA reaching 80 mm diam after 7 d, margin regular, covered by dense felty aerial mycelium, pale grey; reverse dark mouse grey. NaOH spot test a pale red-brown discolouration on OA.

Typus: **Italy**, Centro Richerche sul riso, Mortara, from *Oryza sativa* (*Poaceae*), Aug. 1981, E.N.R. (**holotype** CBS H-11964, ex-type living culture CBS 516.81).

Notes: Isolate CBS 516.81 was received as *Didymella graminicola*. However, it was phylogenetically distant from the authentic culture of *Did. graminicola* (currently: *Neoascochyta graminicola*). In the present study, this isolate formed a distinct lineage in *Neoascochyta* with 64 bp differences in four loci from its closest neighbour, *Neoa. tardicrescens* (Fig. 1). Thus, we described it here as a new species, *Neoa. mortariensis*.

DISCUSSION

The taxonomy of *Didymellaceae* has undergone significant changes in recent years, especially with extensive application of molecular data to resolve the phylogenetic relationships for taxa with similar morphological characteristics (Aveskamp *et al.* 2010, Chen *et al.* 2015, Valenzuela-Lopez *et al.* 2018). The currently recognised phylogenetic relationships of *Didymellaceae* were mainly based on the ex-type strains and specimens examined by Boerema and his co-workers. However, hitherto only around 300 of the approximately 5 000 described phoma-like species have been studied using multi-locus molecular data (Aveskamp *et al.* 2010, Chen *et al.* 2015, 2017). A large number of species await to be re-examined, including numerous phoma-like species accepted by Boerema *et al.* (2004) and Aveskamp *et al.* (2010) such as *Phoma nemophilae*, *Phoma eupatorii*, *Phoma commelinicola*, *Didymella macropodii*, *Ascochyta ferulae*, and species introduced based on limited sequence data before the establishment of an extended multi-locus backbone phylogeny of *Didymellaceae*. Although many species may end up being reduced to synonymy, the total number of *Phoma* taxa remains largely unknown. The present study provides an extended phylogenetic backbone and natural classification system of *Didymellaceae* based on the hitherto largest number of cultures with a broad distribution, diverse substrates and geographic origins.

Generic assessment

The number of genera in *Didymellaceae* has significantly increased in recent years (Aveskamp *et al.* 2010, Chen *et al.* 2015). Several additional genera have been introduced (Chen *et al.* 2017), such as *Briansuttonomyces*, *Neomicrosphaeropsis*

and *Pseudoascochyta* from plant materials (Crous & Groenewald 2016, Crous *et al.* 2016, Thambugala *et al.* 2016). Six new genera, namely *Cumuliphoma*, *Ectophoma*, *Juxtiphoma*, *Remotididymella*, *Similiphoma* and *Vacuiphoma*, established 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). Some genera were reduced to synonymy with others in *Didymellaceae*, *e.g. Heracleicola*, or not accepted due to lack of genetic and morphological divergence, *e.g. Neodidymella*, *Didymellocamarosporium*, *Endocoryneum*, and *Pseudohendersonia* (Ariyawansa *et al.* 2015a, Wijayawardene *et al.* 2016, Chen *et al.* 2017). Furthermore, the taxonomic placement of *Didysimulans* (Tibpromma *et al.* 2017) remains unconfirmed, as it clustered together with the genus *Vacuiphoma* based on the multi-locus phylogenetic analysis in the current study (Supplementary Fig. S1). Species of *Didysimulans* are similar to those of *Didymellaceae* by producing a peridium and cylindrical asci with 1-septate ascospores; however, it is characterised by its chlamydospores with hyaline to yellow-brown mycelia, arranged in spiral chains. Currently, species of *Didysimulans* are only known from their sexual morph, while *Vacuiphoma* is only known for its asexual morph. Despite *Didysimulans* being phylogenetically closely related to *Vacuiphoma*, its relatively low phylogenetic support renders it questionable. Thus, more loci of taxa belonging to this genus need to be sequenced to resolve their taxonomic placement within the *Didymellaceae*. However, if *Didysimulans* and *Vacuiphoma* are shown to be congeneric, then *Didysimulans* would have preference as it was published on 4 May 2017 and *Vacuiphoma* on 21 November 2017.

The genus *Platychora* was established by Petrak (1925) and is considered as a member of *Venturiaceae.* Recently, a representative strain of the type species *Platychora ulmi* (CBS 361.52) was sequenced for SSU and LSU, revealing a close relationship with *Didymella cucurbitacearum*, the taxonomic status of which awaits to be confirmed (Winton *et al.* 2007). *Platychora* was therefore relocated to the *Didymellaceae* (Zhang *et al.* 2009, Chen *et al.* 2015, Shen *et al.* 2020). In the present study, CBS 361.52 clustered with the representative strain of *Phoma herbarum* (CBS 615.75) (Fig. S1), having identical SSU and LSU sequences. Therefore, these two genera appear to be synonyms. Another genus *Endophoma* was established to accommodate an endoconidial taxon which was isolated from bat-cave soil. The phylogenetic analysis revealed that it clustered with *Phoma eupyrena* (currently *Juxtiphoma eupyrena*; Tsuneda *et al.* 2011, Valenzuela-Lopez *et al.* 2018). However, *Endophoma* differs from the latter in having dimorphic conidiomata in culture, *i.e.* spherical, ostiolate pycnidium-like conidiomata, and cylindrical, setose and closed conidiomata, both of which produced conidia exclusively endogenously. In the present study, sequences of the ex-type strain (UAMH 11216) of *Endophoma elongata* were revealed to be identical to *Juxtiphoma kolkmaniarum* (CBS 146005) (CBS 527.66) (Fig. 1 and Supplementary Fig. S1). However, considering the limited sampling of these two genera and the incomplete sequence datasets, the taxonomic placement of *Platychora* and *Endophoma* remains unresolved and will be treated once more loci have been sequenced.

Didymellaceous taxa with a hyphomycetous synasexual morph

Epicoccum is the only genus producing a hyphomycetous synasexual morph in *Didymellaceae*, which is characterised by darkly pigmented multi-septate conidia (dictyochlamydospores) formed on sporodochia. The taxonomy of *Epicoccum* has long been a topic of intense debate. The present study revealed a highly-supported monophyletic lineage, based on which a natural classification system of *Epicoccum* is presented. The multi-locus phylogenetic tree indicated that species clustering in the basal part of *Epicoccum* were mostly plant-associated, producing hyaline, single-celled conidia in pycnidia (coelomycetous asexual morph, except for *Epi. sorghinum*). However, those taxa that diverged later were mostly isolated from soil or other substrates (Schol-Schwarz 1959, Punithalingam *et al.* 1972, Pieckenstain *et al.* 2001, Hashem & Ali 2004, De Cal *et al.* 2009) and tend to produce pigmented, verruculose dictyoconidia (hyphomycetous synasexual morph). The currently available data strongly suggest that the general evolutionary direction in the genus *Epicoccum* is from producing a coelomycetous asexual morph to those with a hyphomycetous synasexual morph. This evolutionary trend is understandable as production of epicoccoid conidia from mycelia and sporodochia are probably more nutrient/energy efficient. Besides, the thick-walled and multicellular conidia were reported to successfully survive under extreme temperatures, drought, and UV radiation (Ellis 1971, 1976, Ellis & Ellis 1997, Grishkan & Nevo 2010), making it more resistant to the complex soil environment. Further genome and comparative analyses might explain this interesting phenomenon.

In accordance with previous studies, the present study supports 36 well-defined genera in *Didymellaceae* based on comprehensive morphological and phylogenetic data, including seven new genera introduced in the present study. However, four additional genera, *Didysimulans*, *Endophoma*, *Platychora* and *Vacuiphoma*, as well as four genera mentioned in Chen *et al.* (2017), *Neodidymella*, *Didymellocamarosporium*, *Endocoryneum*, and *Pseudohendersonia* remain unresolved.

Species diversity in *Didymellaceae*

The 947 isolates belonging to 301 taxa investigated in this study represent the largest sample of *Didymellaceae* studied to date in a single *Didymellaceae* phylogenetic study. The majority of these isolates occurred on plants from 287 genera in 121 plant families, either as pathogens, endophytes or saprobes. However, in contrast to the accurate and detailed studies of species on plant material, information is still limited on the distribution and species diversity from other substrates. Several studies have revealed that *Didymellaceae* species are able to adapt to extreme environmental conditions and grow in exposed habitats such as air, soil, water, limestone in caves (Chen *et al.* 2017), and inorganic materials including asbestos, cement and paint (Aveskamp *et al.* 2008), or saline-alkali soils, deep-sea sediments and oligotrophic environments. In the present study, 230 isolates from different substrates other than plant materials were also examined. In total

78 isolates originated from the diverse soil environments including desert soil, saline soil, and soil near a glacier, representing 46 species in 20 genera. Among them, seven new species were described, namely *Didymella mitis*, *D. prolaticolla*, *D. guttulata*, *Epicoccum brahmansense*, *Macroventuria terrestris*, *Remotididymella brunnea* and *R. humicola*. Besides, species from *Allophoma*, *Cumuliphoma*, *Ectophoma*, *Leptosphaerulina*, *Neoascochyta* and *Neodidymelliopsis* were reported from soil for the first time. Similarly, 12 new species and one new genus were recently described from Dutch garden soils by Hou *et al.* (2020), which revealed that species from many genera in *Didymellaceae* could be found in soil environments, representing a species-rich and largely unexplored habitat for *Didymellaceae*. In addition, a total of 152 isolates representing 72 species in 20 genera, mostly *Didymella* and *Epicoccum*, were isolated from other substrates such as house dust (21 strains), air (20 isolates), humans (21 isolates), rocks (five isolates), water (five isolates) and others (decayed canvas, juice, paint, plastic, potato flour, *etc*.; 80 isolates). Among them two new species were described from coral, *i.e. Allophoma anatii* and *Epicoccum dickmanii*, demonstrating their cosmopolitan and wide distribution. This study indicates that diverse undetected natural and artificial substrates/habitats harbour large numbers of novel *Didymellaceae*. Given their ubiquitous nature, the potential applications in industry, and the economic importance, additional taxonomic and ecological investigations of this group would be useful in understanding their biology and environmental significance, as well as their role in agriculture.

Our study examined isolates originating from seven continents and 92 countries. Although this is the hitherto broadest sampling of *Didymellaceae* investigated to date, most isolates were from Europe, South Africa and America. Continents such as Africa, Asia, Oceania and Antarctica therefore remain largely unexplored. This fact stresses the need to enhance global community-wide efforts to investigate species diversity of *Didymellaceae*.

Loci for species and genus resolution in *Didymellaceae*

Different combinations of genomic loci have been used to resolve the phylogenetic relationships of species in *Didymellaceae*, and to solve problems related to rapid identification of quarantine species during inspections. The combination of ITS-LSU or ITS-LSU-*tub2* has been consistently used in many studies of *Didymellaceae*, and these combinations are also widely used in other families of *Dothideomycetes* (Phookamsak *et al.* 2014, Ertz *et al.* 2015, Ariyawansa *et al.* 2015b). However, both combinations failed to provide sufficiently clear species boundaries in most genera, as many genera were found to be polyphyletic. Chen *et al.* (2015) were the first to utilise the *rpb2* locus in phylogenetic analyses of phoma-like taxa, revealing a largely improved phylogeny of *Didymellaceae*. In the present study we followed Chen *et al.* (2015) and Valenzuela-Lopez *et al.* (2018) and used ITS, LSU, *tub2* and *rpb2* to investigate the unidentified strains in the CBS collection and to complement the backbone phylogenetic tree provided by Chen *et al.* (2015) and Valenzuela-Lopez *et al.* (2018). A combination of *tub2* and *rpb2* loci (TreeBASE S25826) and even the *rpb2* locus alone (Supplementary Fig. S2) provided a phylogenetic tree similar to those obtained with all four loci. This combination also proved to be highly suitable for the delineation of genera and species. *Boeremia* is the only genus for which none of the loci performed well on their own; species in this genus are therefore best resolved based on a combined phylogeny rather than that from any single locus. According to the study of Berner *et al.* (2015), the combination of five loci, namely actin, beta-tubulin, calmodulin, translation elongation factor 1-alpha, and ITS, is recommended to identify species in *Boeremia*.

Several markers have previously been proposed and applied as standard loci for DNA barcodes in *Didymellaceae*, including ITS (Druzhinina *et al.* 2005), actin (*ACT*, Aveskamp *et al.* 2009b), and cytochrome c oxidase subunit I (*COI*, Seifert 2008). ITS is known to be too conserved to distinguish species or generic level relationships in *Didymellaceae*. *ACT*, in contrast, was too diverse to be aligned (Aveskamp *et al.* 2010). Furthermore, the *COI* analysis did not reveal taxon-specific conserved SNPs and was not robust enough (Aveskamp *et al.* 2009b, 2010), therefore all three loci were abandoned for use as barcodes. The partial beta-tubulin gene (*tub2*) was selected as DNA barcode for some groups of fungi in *Dothideomycetes*, such as *Septoria* and allied genera (Verkley *et al.* 2013). Based on the phylogenetic analysis in the present study, *tub2* was also an option as a secondary barcode for *Didymellaceae*. In *Didymellaceae*, the *tub2* are easily to amplify, therefore the success rate of PCR amplification is always high. In the present study, 97.25 % of isolates have available *tub2* sequences. Besides, the reference sequence dataset of *tub2* is relatively complete (96 %) in GenBank and Q-bank, enabling BLAST identifications. In addition, most species in *Didymellaceae* could be distinguished. However, the *tub2* locus does have problems associated with it, as the phylogeny using *tub2* did not satisfactorily delimitate taxa at generic level, especially in *Allophoma*, *Calophoma*, *Epicoccum*, and *Remotididymella*. Furthermore, considering that the *tub2* sequence is rather short (300–350 bp), the number of informative sites for distinguishing closely related taxa are limited. Therefore, for *Didymellaceae*, *tub2* is more suitable for a quick identification at species level. Compared to *tub2*, the *rpb2* gene proved more advantageous as a DNA barcode. In the present study, *rpb2* was confirmed to reliably distinguish 35 genera in *Didymellaceae*, and to provide a good resolution at both species and generic level, distinguishing 279 species. However, it failed for seven species with *rpb2* sequences and 24 isolates belonging to 15 species failed to amplify for this gene. In contrast to *tub2*, *rpb2* works well for generic identification, and it only failed to resolve the genus *Calophoma* in the present study. The topology of the *rpb2* tree is highly similar to that from the combined four-locus tree (Chen *et al.* 2015; present study). However, in previous studies the success rate for the amplification of *rpb2* was unsatisfactory, as only 62 % of strains were successfully amplified for *rpb2* in Chen *et al.* (2015). In the present study, a modified PCR protocol with BSA increased the success rate to 95 %, thus solving the final hurdle of using *rpb2* as the secondary barcode in *Didymellaceae*, especially at generic level. However, *tub2* could be used as an alternative barcode for species identification, especially for isolates where PCR amplification of *rpb2* prove to be problematic.

The utilisation of the *rpb2* barcode for *Didymellaceae* has high value for rapid, accurate and precise identification for species in a family encompassing numerous plant pathogenic species. In addition, the *rpb2* phylogeny has the advantage of being highly congruent with the four-locus tree (95 %), recognising 35 among the 36 genera in this family; at the species level, it distinguished the 279 species studied, and was easily amplified with the improved protocol. Sequences were also easily aligned throughout the family.

CONCLUSIONS

In the present study we provided a relatively complete sequence dataset of *Didymellaceae* consisting of four loci (ITS, LSU, *rpb2*, *tub2*; Table S1), aiming to resolve the delimitation of novelties at the species, genus and family levels. Our results resolved 36 well-supported monophyletic clades, representing 36 genera of which seven are described as new. Furthermore, a total of 40 new species and 21 new combinations were also introduced. Six epitypifications and six neotypifications were performed, and four basionyms, namely *Epicoccum mezzettii* (Goidànich 1937), *Epi. oryzae* (Ito & Iwadare 1934), *Epi. purpurascens* and *Toruloidea tobaica* (Von Szilvinyi 1936) were resurrected based on morphological and phylogenetic evidence of the ex-type or reference strains. Although the genera *Didysimulans*, *Endophoma*, *Platychora* and *Vacuiphoma* were revealed to belong to *Didymellaceae*, their taxonomic status remains unresolved.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.simyco.2020.05.001.

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