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ARTICLE

Mycoparasitic species of *Sphaerellopsis*, and allied lichenicolous and other genera

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Abstract: Species of *Sphaerellopsis* (sexual morph *Eudarluca*) are well-known cosmopolitan mycoparasites occurring on a wide range of rusts. Although their potential role as biocontrol agents has received some attention, the molecular phylogeny of the genus has never been resolved. Based on morphology and DNA sequence data of the large subunit nuclear ribosomal RNA gene (LSU, 28S) and the internal transcribed spacers (ITS) and 5.8S rRNA gene of the nrDNA operon, the genus *Sphaerellopsis* is shown to belong to *Leptosphaeriaceae* in *Dothideomycetes*. *Sphaerellopsis* is circumscribed, and the sexually typified generic name *Eudarluca* treated as a synonym on the basis that *Sphaerellopsis* is more commonly used in literature, is the older generic name, and is the morph commonly encountered by plant pathologists in the field. A neotype is designated for *Sphaerellopsis filum*, and two new species are introduced, *S. macroconidialis* and *S. paraphysata* spp. nov. Species previously incorrectly placed in *Sphaerellopsis* are allocated to *Neosphaerellopsis* gen. nov. as *N. thailandica*, and to the genus *Acrocalymma,* as *A. fici*. The genus *Rhizopycnis* is nestled among species of *Acrocalymma*, and reduced to synonymy based on its morphology and DNA phylogeny, while *Acrocalymmaceae* is introduced as novel family to accommodate members of this genus in the *Dothideomycetes*. Furthermore, *Sphaerellopsis* proved to be phylogenetically closely allied to a lichenicolous complex of phoma-like taxa, for which the new genera *Diederichomyces* and *Xenophoma* are established. Several new combinations are introduced, namely *D. xanthomendozae, D. ficuzzae, D. caloplacae, D. cladoniicola, D. foliaceiphila*, and *X. puncteliae* combs. nov, while *Paraphaeosphaeria parmeliae* sp. nov. is newly described.

Key words:
Ascomycota
Dothideomycetes
Eudarluca
Fungiculous fungi
ITS
LSU
Pleosporales
Rust fungi
systematics

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INTRODUCTION

Sphaerellopsis filum (*Dothideomycetes, Pleosporales*, *Leptosphaeriaceae*) and its purported sexual morph *Eudarluca caricis* is a well-known cosmopolitan mycoparasite occurring on a wide range of rust species. The species has been commonly recorded in North and South America, Europe, and Asia. Most records are as *S. filum*, as *E. caricis* is not so commonly observed (Yuan *et al*. 1998). Given the wide host range, *S. filum* is thought to be a common rust mycoparasite, and its potential role as biocontrol agent has received some attention (Kuhlman *et al*. 1978, Whelan *et al.* 1997, Pei *et al*. 2003, Nischwitz *et al.* 2005). Little is known, however, about the ecology, and genetic diversity within the taxon.

Sphaeria filum was originally described from rust species on *Convolvulus sepium* and *Populus nigra* in Sicily (Bivona-Bernadi 1813–16). Fries (1823) transferred *S. filum* to *Phoma*, while Castagne (1851) established the genus *Darluca* based on *Darluca vagans*, treating *Sphaeria filum* as a synonym. Eriksson (1966) pointed out, however, that even though synonymous, the species epithet "*filum*" had priority over "*vagans*".

Spegazzini (1908) established the genus *Eudarluca* for an ascomycete associated with uredinia of a rust on *Canna* sp. in Brazil, assuming it to be the sexual morph of *Darluca*. Keener (1951) proved the connection between *Eudarluca* and *Darluca*, which was confirmed *via* culture studies (Yuan *et al*. 1998). Eriksson (1966) introduced the combination *Eudarluca caricis* for the sexual morph of *D. filum*, while Sutton (1977) relocated *D. filum* to the genus *Sphaerellopsis*. Since this time the application of the names have proven stable, with *Sphaerellopsis filum* being reported on close to 369 species and 30 genera of rusts in more than 50 countries (Kranz & Brandenburger 1981).

Eriksson (1967) observed *Eudarluca caricis* to occur as a mycoparasite on *Puccinia* spp. occurring on *Poaceae* in Sweden, and also to appear pathogenic to the grass species themselves, though this has not been tested experimentally. Eriksson (1966) provided an overview of the taxonomy, nomenclature, and distribution of *E. caricis*. He also located some material in Herb. Fries (UPS), consisting of three leaf fragments of a *Carex* sp., containing asci and ascospores matching that of *E. australis*, a synonym of *E. caricis*.

The abolishment of dual nomenclature in July 2011 (Hawksworth 2011, Hawksworth *et al*. 2011, McNeill *et al*. 2012, Wingfield *et al*. 2012), means that the generic name *Sphaerellopsis* 1883 has priority over *Eudarluca* 1908. *Sphaerellopsis* presently has two acknowledged species (Nag Raj 1993), while *Eudarluca* has eight, though the genus is in need of revision. Because *Sphaerellopsis* is more commonly used in literature, is the older generic name, and is the morph commonly encountered by plant pathologists in the field, we propose to retain only *Sphaerellopsis* on the list of protected generic names (Kirk *et al*. 2013), and reduce *Eudarluca* to synonymy. This listing will avoid the necessity of making a formal separate proposal to retain *Eudarluca* and awaiting its rejection before taking up *Sphaerellopsis* as required under the current Art. 57.6 of the ICN (McNeill *et al*. 2012), which is in any case to be proposed for deletion (Hawksworth 2014).

Liesebach & Zaspel (2004) compared 77 isolates of *S. filum* isolated from *Populus* spp., *Parthenium hysterophorus* and *Bellis perennis* in Europe. Based on ITS sequence data, they revealed two main clades, with each containing further subclades, suggesting as many as five species to be present in their samples, and also showing that different *Sphaerellopsis* species could occur on the same rust samples. They refrained from naming any new taxa, however, and referred all isolates to *S. filum*.

By conducting inoculation experiments with *S. filum* isolates obtained from various rust and host species, Nischwitz *et al*. (2005) were able to demonstrate a strong level of host specificity. Furthermore, in their phylogenetic analysis, isolates grouped in four separate clades, again suggesting several species to be present, with isolates from grass hosts clustering separately to those obtained from poplar. Based on the morphological continuum observed among isolates, however, Nischwitz *et al*. (2005) also refrained from naming any new species.

The aim of the present paper was thus to conduct a DNA phylogenetic study of the *S. filum* isolates available to us from the CBS-KNAW Fungal Biodiversity Centre (CBS) culture collection (Utrecht, The Netherlands), supplemented with fresh collections from Brazil, South Africa, Thailand, and The Netherlands. A further aim was also to delineate *Sphaerellopsis* from genera that are phylogenetically closely related, or morphologically similar.

MATERIALS AND METHODS

Isolates

Fresh collections were made from rust sori on diverse hosts. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA; Crous *et al*. 2009). Additional strains were obtained from the culture collection of the CBS. Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA) (Crous *et al*. 2009), MEA, and pine needle agar (PNA) (Smith *et al*. 1996), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Voucher strains were deposited in CBS.

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer's protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The primers ITS4 (White *et al*. 1990) and LSU1Fd (Crous *et al*. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. Part of the translation elongation factor 1-alpha (TEF-1α) was amplified and sequenced using primers EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell *et al*. 1998), while T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) were used for the beta-tubulin (TUB) gene region. Amplification conditions for ITS, LSU and TEF-1α followed Crous *et al*. (2013) and for TUB, Lee *et al*. (2004). Megablast searches (Altschul *et al*. 1997) using the ITS and LSU sequences were performed in NCBI's GenBank nucleotide sequence database to identify the closest matching sequences, which were added to the sequence alignment. The sequence alignment and subsequent phylogenetic analyses for all the above were carried out using the methods in Crous *et al*. (2006). Sequences derived in this study were lodged at GenBank, the alignments and trees in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al*. 2004).

Morphology

Observations were made with a Zeiss V20 Discovery stereomicroscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Measurements and photographs were made from structures mounted in clear lactic acid. The 95 % confidence intervals were derived from 30 observations (× 1000), with the extremes given in parentheses. Ranges of the dimensions of other characters are given. Colony characters and pigment production were noted after 2 wk of growth on different media incubated at 25 ºC. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Morphological descriptions were based on cultures sporulating on MEA.

RESULTS

Phylogeny

Four phylogenies were generated; the first is based on LSU sequences and was used to determine the familial

relationships of the studied species (Fig. 1), the second is based on an ITS alignment of stagonospora- and phoma-like isolates (Fig. 2), the third is based on an ITS alignment of *Acrocalymma* and related species (Fig. 3), and the final tree on an ITS alignment of *Sphaerellopsis* isolates (Fig. 4). The ITS alignments were split to facilitate more robust multiple alignments of the included sequences rather than having an ambiguous alignment containing all of the ITS sequences in a single analysis. The TEF-1α and TUB sequences (Table 1) confirmed the ITS results and were therefore not subjected to a separate phylogenetic analysis.

The first analysis (LSU) is based on 112 isolates (including the outgroup sequence) and the resulting dataset of 751 characters, including alignment gaps which are treated as fifth base, consisted of 546 constant characters, 64 variable parsimony-uninformative characters and 141 parsimony-informative characters. The maximum of 1000 equally most parsimonious trees were retained (TL = 610; CI = 0.430; RI = 0.848; RC = 0.364), the first of which is presented in Fig. 1. It was not possible to determine a more precise phylogenetic position of *Acrocalymma*, neither in the phylogeny nor by megablast searches of NCBI's GenBank nucleotide database (closest matches being *Pyrenochaeta quercina* and *Pyrenochaetopsis pratorum* with 97 % identity over approximately 1195 nucleotides), therefore a new family name is introduced below to accommodate it. *Sphaerellopsis* is shown to belong to *Leptosphaeriaceae* ('clade A' *sensu* de Gruyter *et al*. 2013), while the three newly recognised genera in this study, *Diederichomyces*, *Neosphaerellopsis* and *Xenophoma*, are allied to *Phaeosphaeriaceae*. The new species *Paraphaeosphaeria parmeliae* is placed in *Montagnulaceae*. The large number of nodes without support in this phylogeny shows that LSU alone does not have the resolution to resolve the complexity of many genera and families in *Pleosporales*.

The second analysis (ITS alignment focussed on stagonospora-like and phoma-like species) is based on 50 isolates (including the outgroup sequence) and the resulting dataset of 522 characters, including alignment gaps which are treated as fifth base, consisted of 240 constant characters, 62 variable parsimony-uninformative characters and 220 parsimony-informative characters. Sixty equally most parsimonious trees were retained (TL = 1090; CI = 0.517; RI = 0.707; RC = 0.365), the first of which is presented in Fig. 2. The phylogenetic placement of the three newly described genera, *Diederichomyces*, *Neosphaerellopsis* and *Xenophoma*, are shown as being sister to *Phaeosphaeriopsis*, *Parastagonospora* and the broader lineage "*Sclerostagonospora*" / *Neosphaerellopsis* / *Parastagonospora*, respectively. Five species of *Diederichomyces* are distinguished in the phylogeny.

The third analysis (ITS alignment focussed on *Acrocalymma* and related species) is based on 48 isolates (including the outgroup sequence) and the resulting dataset of 401 characters, including alignment gaps which are treated as fifth base, consisted of 218 constant characters, 37 variable parsimony-uninformative characters and 146 parsimony-informative characters. Two equally most parsimonious trees were retained (TL = 404; CI = 0.735; RI = 0.871; RC = 0.640), the first of which is presented in Fig. 3. Eight distinct lineages represent *Acrocalymma* in this phylogeny, including *A. fici,* which is described as a taxonomic novelty below. *Massarina walkeri* is nestled inside the broader *Acrocallyma* lineage, distinct from *M. eburnea*, the type species of the genus *Massarina,* and is therefore allocated to *Acrocalymma*. Likewise, *Rhizopycnis vagum* is included here as *A. vagum*.

The fourth analysis (ITS alignment focussed on *Sphaerellopsis* isolates) is based on 27 isolates (including the outgroup sequence) and the resulting dataset of 518 characters, including alignment gaps which are treated as fifth base, consisted of 326 constant characters, 50 variable parsimony-uninformative characters and 142 parsimony-informative characters. A total of 288 equally most parsimonious trees were retained (TL = 319; CI = 0.881; RI = 0.964; RC = 0.850), the first of which is presented in Fig. 4. Four distinct, well-supported clades are found, of which two are newly named below, as *S. paraphysata* and *S. macroconidialis*.

TAXONOMY

Although the present study focuses of *Sphaerellopsis*, several isolates deposited under this name turned out to be unrelated, and to belong to other genera, phylogenetically allied to a complex of phoma-like species. The type species of the genus *Sphaerellopsis* is neotypified below, and new generic names are introduced to accommodate other taxa in this complex.

Phoma-like genera

The morphology of the lichenicolous phoma-like species has been well studied in the past (Hawksworth 1981, Diederich *et al*. 2007, von Brackel 2008, Lawrey *et al*. 2012). These species are considered to be host-specific to varying extents, being confined to a single species, a single genus, or a few closely related genera (Diederich *et al.* 2007). However, based on recent cultural and DNA phylogenetic data, Lawrey *et al*. (2012) questioned the past practise of identifying lichenicolous *Phoma* species based on host preference, echoing the caution needed in naming lichenicolous fungi generally (Hawksworth 1977, 2003). Furthermore, as shown here (Fig. 1), the lichenicolous *Phoma* species are not congeneric with the genus *Phoma* (Aveskamp *et al*. 2010, de Gruyter *et al*. 2010, 2013), and thus need to be accommodated elsewhere.

Diederichomyces Crous & Trakunyingcharoen, **gen. nov.**
MycoBank MB810828

Etymology: Named after Paul Diederich, who contributed significantly to our present knowledge of lichenicolous fungi.

Diagnosis: *Conidiomata* globose, brown, uni- to multilocular, ostiolate, frequently with brown setae around ostiolar area. *Paraphyses* mostly absent. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, ampulliform to doliiform, mono- to polyphialidic, at times with percurrent proliferation. *Conidia* dimorphic, forming fusoid-

Table 1. Details of fungal strains included in the molecular and morphological analyses.

Species name in manuscript	Strain accession number[1]	Substrate of isolation	Origin	GenBank accession numbers[3]			
				ITS	LSU	TEF-1α	TUB
Acrocalymma fici	CBS 317.76 ex-type	Bark of *Ficus* sp.	India	KP170619	KP170712	KP170663	KP170687
Acrocalymma medicaginis	CPC 24340 = BRIP 5876a = IMI 165613 ex-type	*Medicago sativa*	Australia: QLD	KP170620	KP170713	—	—
	CPC 24341	*Medicago sativa*	Australia: QLD	KP170621	KP170714	—	—
	CPC 24342 = BRIP 14544a	*Medicago sativa*	Australia: QLD	KP170622	KP170715	—	—
	CPC 24343	*Medicago sativa*	Australia: QLD	KP170623	KP170716	—	—
	CPC 24344 = BRIP 15915a	*Medicago sativa*	Australia: QLD	KP170624	KP170717	—	—
	CPC 24345	*Medicago sativa*	Australia: QLD	KP170625	KP170718	—	—
	CPC 24346 = BRIP 16416a	*Medicago sativa*	Australia: SA	—	KP170719	—	—
	CPC 24347	*Medicago sativa*	Australia: SA	—	KP170720	—	—
Acrocalymma vagum	CPC 24216 = Rv-17	Water melon	Spain	KP170626	—	—	—
	CPC 24217 = Rv-25	*Cucumis melo*	Spain	KP170627	—	—	—
	CPC 24218 = Rv-43	*Cucumis melo*	Spain	KP170628	—	—	—
	CPC 24219 = Rv-55	*Cucumis melo*	Spain	KP170629	—	—	—
	CPC 24220 = Rv-77	*Vitis vinifera*	Spain	KP170630	—	—	—
	CPC 24221 = Rv-86	*Amaranthus* sp.	Spain	KP170631	—	—	—
	CPC 24222 = Rv-110	*Cucumis melo*	USA: Texas	KP170632	—	—	—
	CPC 24223 = Rv-0103	*Cucurbita* rootstock	Spain	KP170633	—	—	—
	CPC 24224 = Rv-0703	*Citrullus lanatus*	Spain	KP170634	—	—	—
	CPC 24225 = Rv-1403	*Cucumis sativus*	Spain	KP170635	—	—	—
	CPC 24226 = Rv-0504	*Cucurbita* rootstock	Spain	KP170636	—	—	—
	CPC 24227 = Rv-0106	*Eriobotrya japonica*	Spain	KP170637	—	—	—
Diederichomyces caloplacae	CBS 129140	*Caloplaca cerina*	Canada	KP170638	JQ238637	KP170664	KP170688
	CBS 129338	*Caloplaca cerina*	Canada	KP170639	JQ238643	KP170665	KP170689
Diederichomyces cladoniicola	CBS 128023	*Squamarina cartilaginea*	Belgium	KP170640	JQ238622	KP170666	KP170690
	CBS 128025	*Squamarina cartilaginea*	Belgium	KP170641	JQ238625	KP170667	KP170691
	CBS 128026	*Cladonia* sp.	Spain	KP170642	JQ238628	KP170668	KP170692
	CBS 128027	*Parmelina tiliacea*	Spain	KP170643	JQ238631	KP170669	KP170693
	CBS 131731	*Ramalina pollinaria*	France	KP170644	—	KP170670	KP170694
	CBS 131732	*Cladonia symphycarpa*	France	KP170645	—	KP170671	KP170695
	CBS 131733	*Cladonia rangiformis*	France	KP170646	—	KP170672	KP170696
Diederichomyces ficuzzae	CBS 128019	*Ramalina fastigiata*	France	KP170647	JQ238616	KP170673	KP170697
Diederichomyces foliaceiphila	CBS 129141	*Cladonia squamosa*	Belgium	KP170648	JQ238640	KP170674	KP170698

Table 1. (Continued).

Species name in manuscript	Strain accession number[1]	Substrate of isolation	Origin	GenBank accession numbers[3]			
				ITS	LSU	TEF-1α	TUB
	CBS 131729	*Cladonia*	Belgium	KP170649	—	KP170675	KP170699
	CBS 131730	*Parmelia sulcata*	Belgium	KP170650	—	KP170676	KP170700
Diederichomyces xanthomendozae	CBS 129666 ex-type	*Xanthomendoza hasseana*	Canada	KP170651	JQ238634	KP170677	KP170701
Neosphaerellopsis thailandica	CPC 21659 ex-type	*Bothriochloa bladhii*	Thailand	KP170652	KP170721	KP170678	KP170702
Neottiosporina paspali	CBS 331.37	*Paspalum notatum*	USA: Florida	KP170653	EU754172	—	—
Paraphaeosphaeria parmeliae	CBS 131728 ex-type	*Parmelia sulcata*	Belgium	KP170654	KP170722	KP170679	KP170703
Sphaerellopsis filum	CBS 234.51 = ATCC 22603	*Puccinia coronata* on *Lolium italicum*	Switzerland	KP170655	KP170723	KP170680	KP170704
	CBS 235.51 = ATCC 22604	*Puccinia hordei* on *Ornithogalum divergens*	Portugal	KP170656	KP170724	KP170681	KP170705
	CBS 317.68 ex-neotype	*Puccinia deschampsiae* uredinium, on *Deschampsia caespitosa*	Germany	KP170657	KP170725	KP170682	KP170706
Sphaerellopsis macroconidialis	CBS 233.51 = ATCC 11100 = VKM F-2880	*Uromyces caryophylli* on *Dianthus caryophyllus*	Italy	KP170658	KP170726	KP170683	KP170707
	CBS 658.78 ex-type	*Puccinia allii* sori, on *Allium schoenoprasum*	Netherlands	KP170659	KP170727	KP170684	KP170708
	CPC 21113	Rust on *Carex acutiformis*	Netherlands	KP170660	KP170728	—	KP170709
Sphaerellopsis paraphysata	CBS 137231 = CPC 23547	*Ravenelia macowania* on *Vachellia karroo*	South Africa	KP170661	—	—	—
	CPC 21841 ex-type	*Pennistum* sp.	Brazil	KP170662	KP170729	KP170685	KP170710
Xenophoma puncteliae	CBS 128022 ex-type	*Punctelia rudecta*	USA	JQ238617	JQ238619	KP170686	KP170711

[1] ATCC: American Type Culture Collection, Virginia, USA; BRIP: Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, UK; VKM: All-Russian Collection of Microorganisms, Russian Academy of Sciences, Institute of Biochemistry and Physiology of Microorganisms, 142292 Pushchino, Moscow Region, Russia.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: large subunit (28S) of the nrRNA gene operon; TEF-1α: partial translation elongation factor 1-alpha gene; TUB: partial beta-tubulin gene.

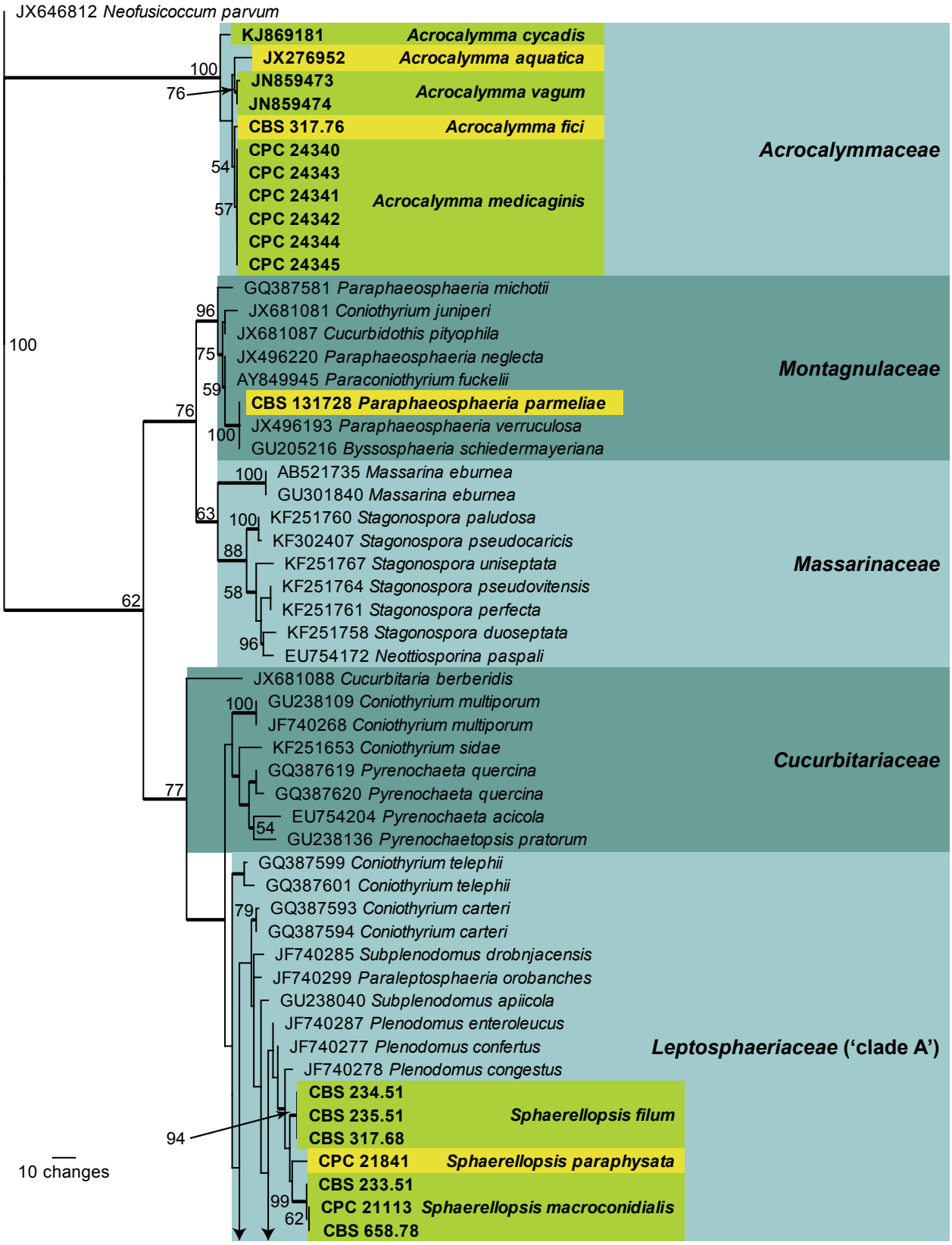

Fig. 1. The first of 1 000 equally most parsimonious trees (TL = 610; CI = 0.430; RI = 0.848; RC = 0.364) resulting from a parsimony analysis of the LSU sequence alignment. The bootstrap support values from 1 000 replicates are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Family names based on literature are indicated to the right of the tree in darker and lighter blocks. Species of interest are shown in **bold** text and are highlighted in the yellow and green blocks. The tree was rooted to *Neofusicoccum parvum* (GenBank JX646812).

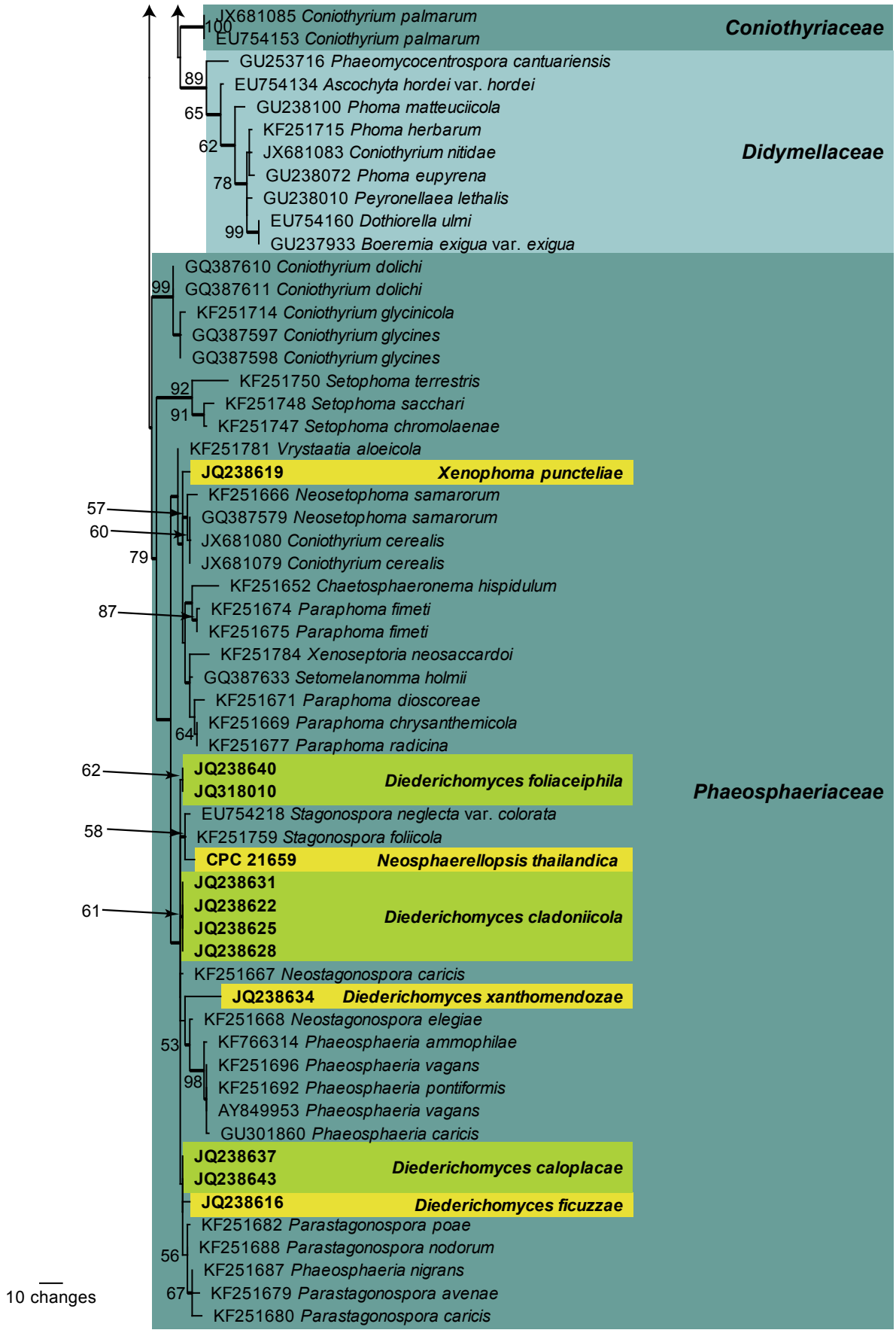

Fig. 1. (Continued).

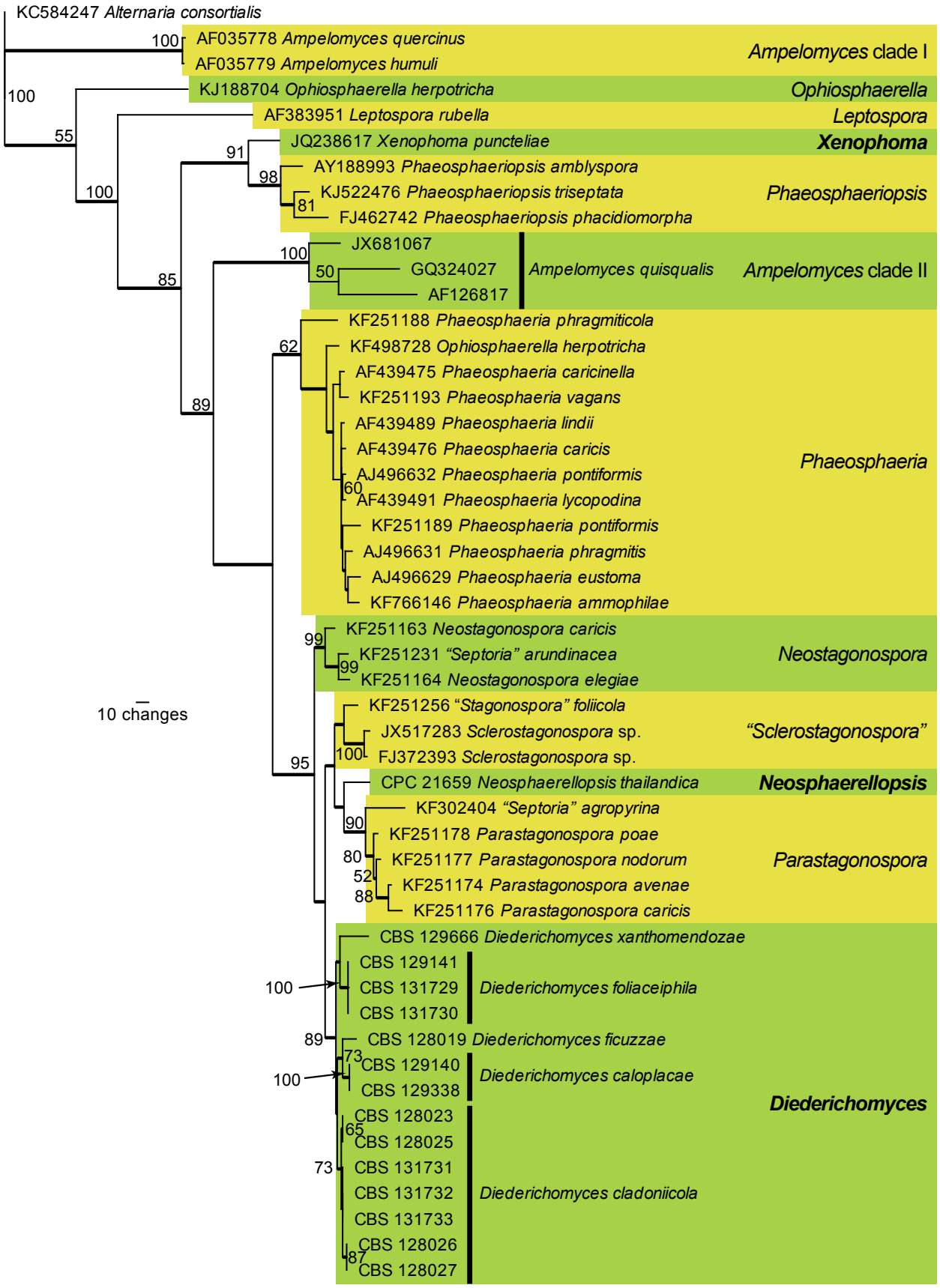

Fig. 2. The first of 60 equally most parsimonious trees (TL = 1090; CI = 0.517; RI = 0.707; RC = 0.365) resulting from a parsimony analysis of the ITS alignment representing stagonospora-like and phoma-like genera. The bootstrap support values from 1 000 replicates are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Different genera are highlighted in the yellow and green blocks, with the genera of interest shown in **bold** text. The tree was rooted to *Alternaria consortialis* (GenBank KC584247).

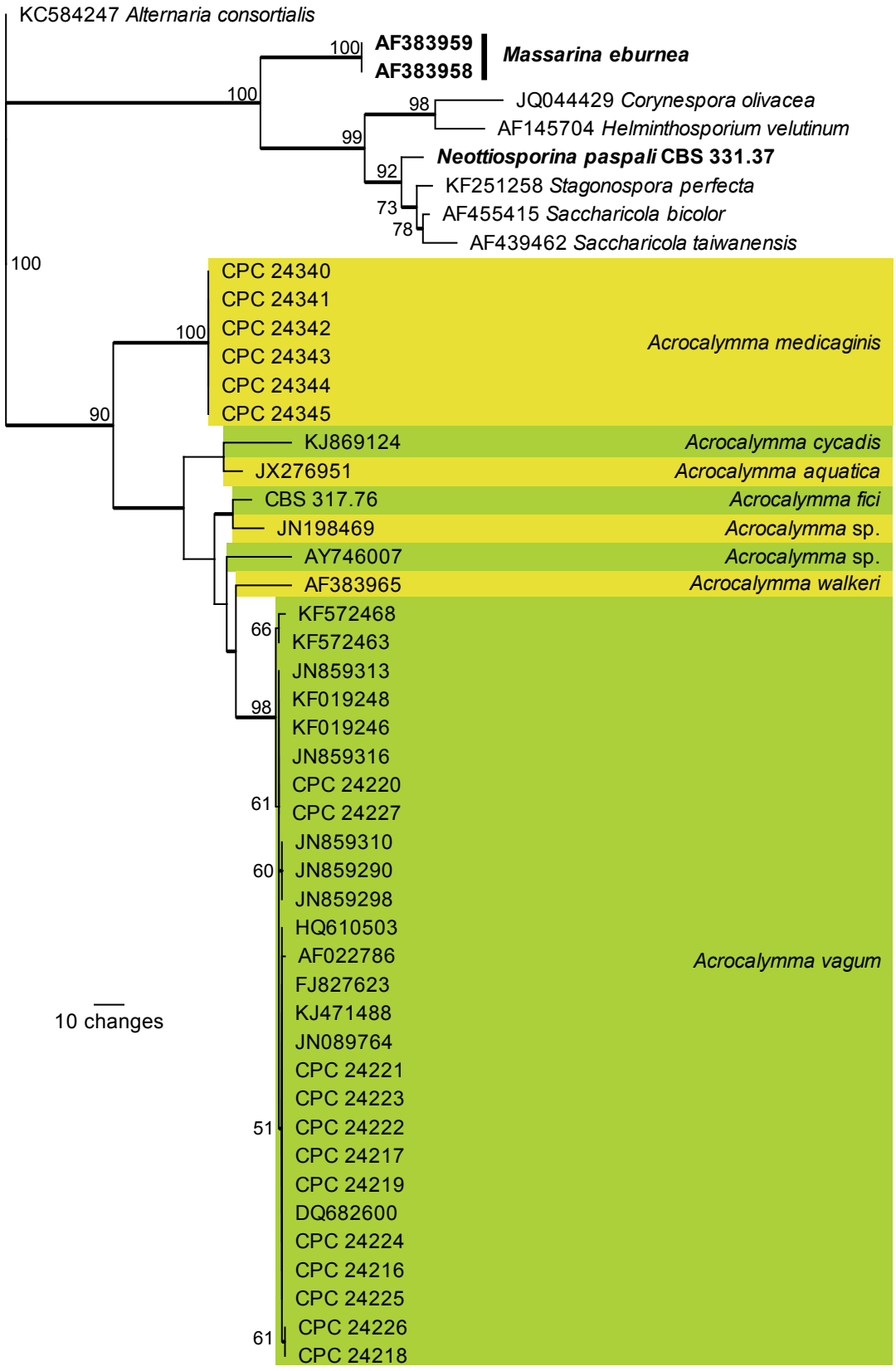

Fig. 3. The first of two equally most parsimonious trees (TL = 404; CI = 0.735; RI = 0.871; RC = 0.640) resulting from a parsimony analysis of the ITS alignment representing *Acrocalymma* and related genera. The bootstrap support values from 1 000 replicates are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Species of *Acrocalymma* are highlighted in the yellow and green blocks. Additional species names of interest to this study are shown in **bold** text. The tree was rooted to *Alternaria consortialis* (GenBank KC584247).

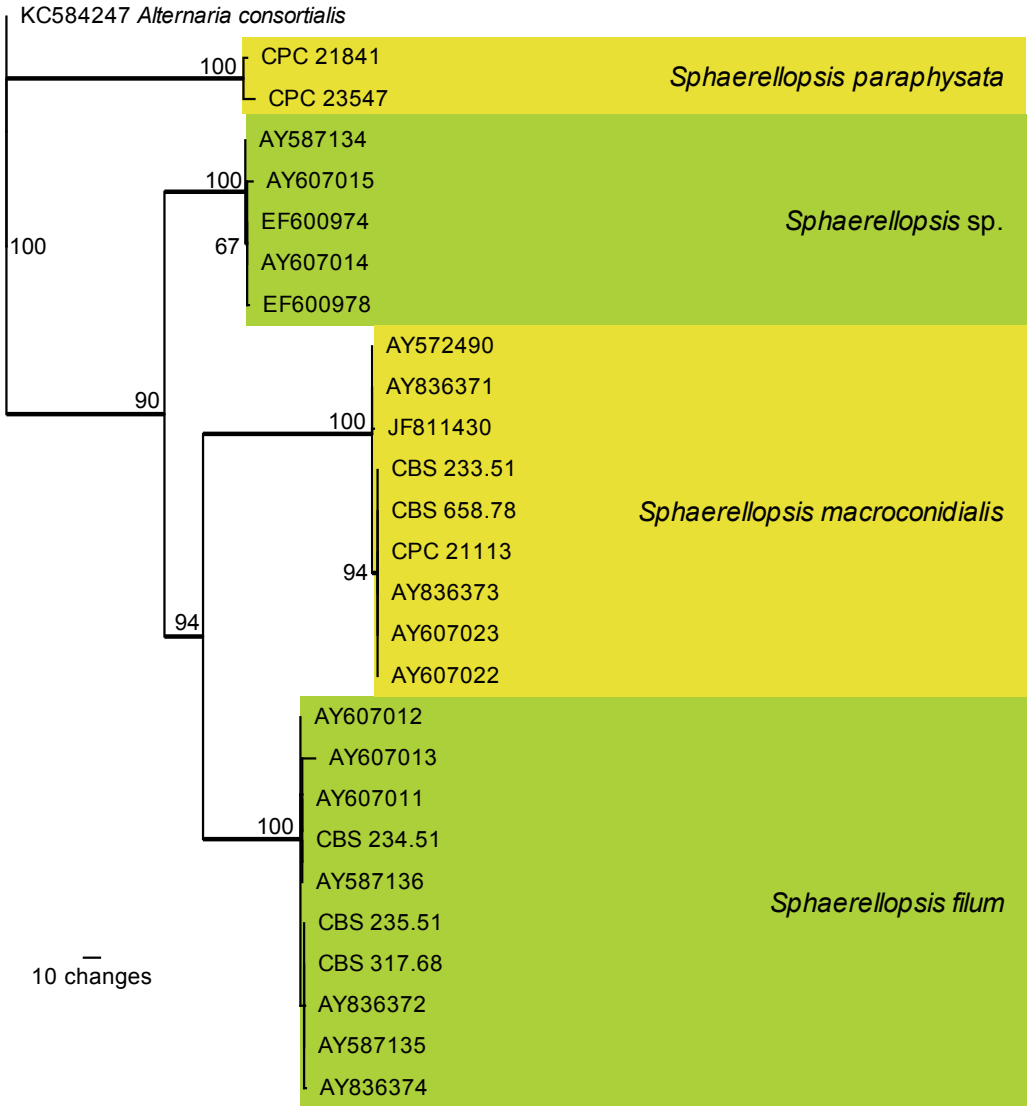

Fig. 4. The first of 288 equally most parsimonious trees (TL = 319; CI = 0.881; RI = 0.964; RC = 0.850) resulting from a parsimony analysis of the ITS alignment representing *Sphaerellopsis* isolates. The bootstrap support values from 1 000 replicates are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. The four species are highlighted in the yellow and green blocks. The tree was rooted to *Alternaria consortialis* (GenBank KC584247).

ellipsoid and globose conidia in same conidioma. Frequently forming orange crystals in the agar.

Type species: *Diederichomyces xanthomendozae* (Diederich & Freebury) Crous & Trakunyingcharoen 2014 (syn. *Phoma xanthomendozae* Diederich & Freebury 2013),

Description: *Conidiomata* globose, brown, superficial to immersed, solitary to aggregated, uni- to multilocular, ostiolate, frequently with brown setae around ostiolar area; wall layers of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Paraphyses* mostly absent, hyaline, cylindrical, 1–2-septate, with rounded ends. *Conidiophores* reduced to conidiogenous cells, or with a supporting cell. *Conidiogenous cells* hyaline, ampulliform to doliiform, mono- to polyphialidic, with prominent periclinal thickening, with outer collarette, and at times with percurrent proliferation. *Conidia* solitary, hyaline, smooth, thin-walled, 1–3 guttulate, dimorphic, forming fusoid-ellipsoid and globose conidia in same conidioma. Frequently forming orange crystals in the agar.

Notes: *Diederichomyces* is distinct from *Phoma* in that it has dimorphic conidia, and forms orange crystals in culture. Features that are expressed in some species in the genus include the presence of ostiolar setae, paraphyses, and polyphialidic conidiogenous cells with prominent collarettes, rendering it morphologically variable. Furthermore, based on the LSU phylogeny (Fig. 1) the genus appears to be paraphyletic, but more collections would be required to suitably delineate these taxa, and identify the synapomorphies associated with potential additional genera.

Diederichomyces caloplacae (D. Hawksw.) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810829
(Fig. 5)
Basionym: *Phoma caloplacae* D. Hawksw., *Bull. Brit. Mus. (Nat. Hist.), Bot.* **9**: 50 (1981).

Materials examined: **Canada**: *Saskatchewan*: lichenicolous on *Caloplaca cerina*, *C. Freebury* (CBS 129140, CBS 129338).

Note: The species was originally described from apothecia of *Caloplaca cerina* from the former Soviet Union (Hawksworth 1981).

Diederichomyces cladoniicola (Diederich *et al.*) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810830
(Fig. 6)
Basionym: *Phoma cladoniicola* Diederich *et al.*, *Lichenologist* **39**: 157 (2007).

Materials examined: **Belgium**: parasitic on lichen *Squamarina cartilaginea*, *D. Ertz* (CBS 128023, CBS 128025). – **Spain**: Mallorca, parasitic on *Cladonia* sp., *P. Diederich* (CBS 128026, CBS 128027). – **France**: Ardennes, Chooz, on thallus of *Ramalina pollinaria*, *D. Ertz* (CBS 131731); Ardennes, Chooz, on thallus of *Cladonia symphycarpa*, *D. Ertz* (CBS 131732); Ardennes, Chooz, on thallus of *Cladonia rangiformis*, *D. Ertz* (CBS 131733).

Notes: This species was originally described from the thallus of *Cladonia pyxidata*, collected in Minnesota, USA. Conidia are ellipsoid, biguttulate, (3.5–)4.5–6(–7.5) × (2–)2.5–3 µm, corresponding to those of the isolates studied here (see Materials examined).

Diederichomyces ficuzzae (Brackel) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810831
Basionym: *Phoma ficuzzae* Brackel, *Sauteria* **15**: 109 (2008).

Material examined: **France**: Boulonnais, parasitic on lichen *Ramalina fastigata*, *D. van den Broeck* (CBS 128019).

Notes: This species was originally described from *Ramalina fastigata* growing on the bark of *Pyrus amygdaliformis* in Sicily, Italy. It lacks an ex-type strain (von Brackel 2008), and ideally an isolate should be obtained and sequenced to fix the genetic application of the name.

Diederichomyces foliaceiphila (Diederich *et al.*) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810832
(Fig. 7)
Basionym: *Phoma foliaceiphila* Diederich *et al.*, *Lichenologist* **39**: 159 (2007).

Materials examined: **Belgium**: lichenicolous on *Cladonia squamosa*, *P. Diederich* (CBS 129141); Ardenne district, on thallus of *Cladonia* sp., *D. Ertz*, (CBS 131729); Ardenne district, on thallus of *Parmelia sulcata*, *D. Ertz* (CBS 131730).

Notes: This species was originally described from the thallus of *Cladonia foliacea* collected in the Czech Republic. No cultures were made from the type collection.

Diederichomyces xanthomendozae (Diederich & Freebury) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810833
Basionym: *Phoma xanthomendozae* Diederich & Freebury, Fungal Div. **55**: 208 (2013).

Material examined: **Canada**: *Quebec*: Les Collines-de-l'Outaouais RCM, Gatineau Park, near Wakefield, grassy ditch beside Route 05, 45°37.8′N, 75°56.4′W, on fallen *Salix*, on *Xanthomendoza hasseana*, 3 May 2010, *C. Freebury* (CANL – holotype; JL451-10, CBS 129666 – ex-type cultures).

Paraphaeosphaeria parmeliae Crous & Trakunyingcharoen, **sp. nov.**
MycoBank MB810834
(Fig. 8)

Etymology: Named after the lichen genus on which it occurs, *Parmelia.*

Diagnosis: *Conidiomata* globose, dark brown, unilocular. *Conidiophores* reduced to conidiogenous cells that are brown, ampulliform, enteroblastic, 4.5–6.5 × 3.5–7 µm. *Conidia* globose, brown, aseptate, thick-walled, smooth to rough, (3–)3.5–4(–4.5) × 3–4(–4.5) µm.

Type: **Belgium**: Ardenne district, on thallus of *Parmelia sulcata*, 2010, *D. Ertz* (CBS H-21850 – holotype; CBS 131728 – ex-type culture).

Description: *Conidiomata* globose, dark brown, semi-immersed to immersed, solitary to aggregated, unilocular, ostiolate, thin-walled, 120–175 × 150–220 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* brown, ampulliform, enteroblastic, proliferation at the same level with visible periclinal thickening, 4.5–6.5 × 3.5–7 µm. *Conidia* globose, brown, aseptate, thick-walled, smooth to rough, (3–)3.5–4(–4.5) × 3–4(–4.5) µm.

Culture characteristics: Colonies on OA with white fluffy and moderately dense mycelium, abundant black pycnidia forming semi-immersed into the culture media, exuding a dark brown-black conidial mass. Colony surface on OA pale olivaceous grey, reverse olivaceous grey. Colony surface on MEA dirty white to pale olivaceous grey, reverse sienna with patches of umber.

Notes: The isolate on which *Paraphaeosphaeria parmeliae* is based, was originally identified as *Phoma foliaceiphila.* It differs from this taxon by forming brown, thick-walled

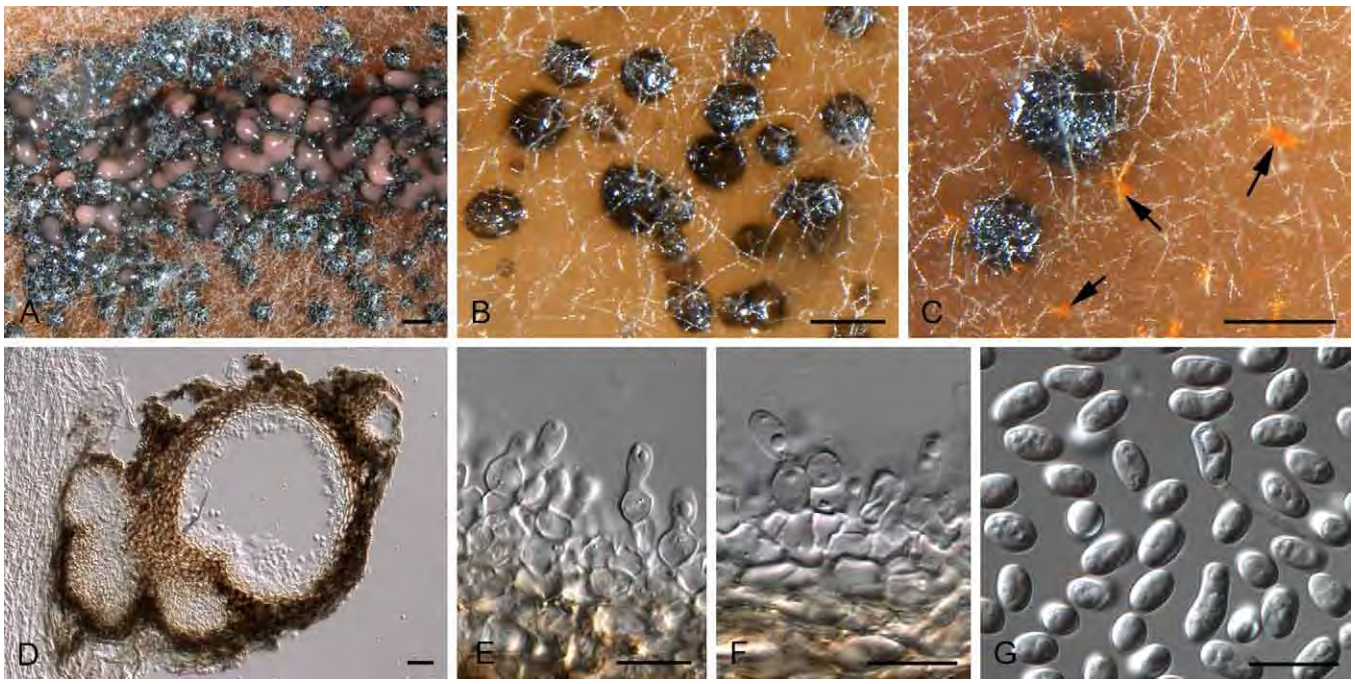

Fig. 5. *Diederichomyces caloplacae* (CBS 129140). **A–C.** Conidiomata on MEA (arrows indicate red crystals formed in agar). **D.** vertical section through conidioma. **E, F.** Conidiogenous cells. **G.** Conidia. Bars: A–D = 200 µm, E–G = 10 µm.

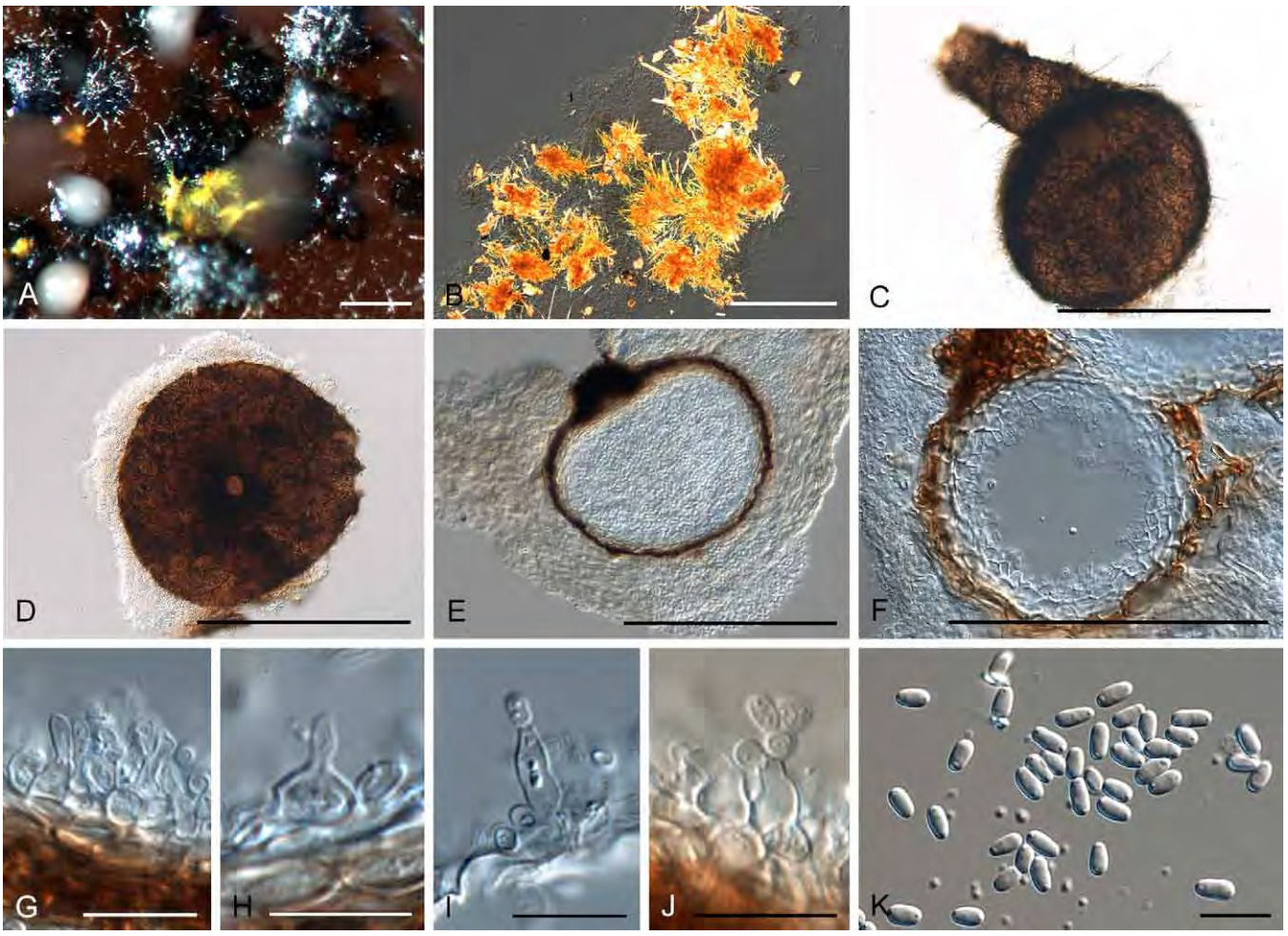

Fig. 6. *Diederichomyces cladoniicola* (CBS 128023). **A.** Conidioma on MEA. **B.** Red crystals formed in agar. **C, D.** Conidiomata. **E, F.** Sections through conidiomata. **G–J.** Conidiogenous cells. **K.** Conidia. Bars: A, C–F = 300 µm, B = 100 µm, G–K = 10 µm.

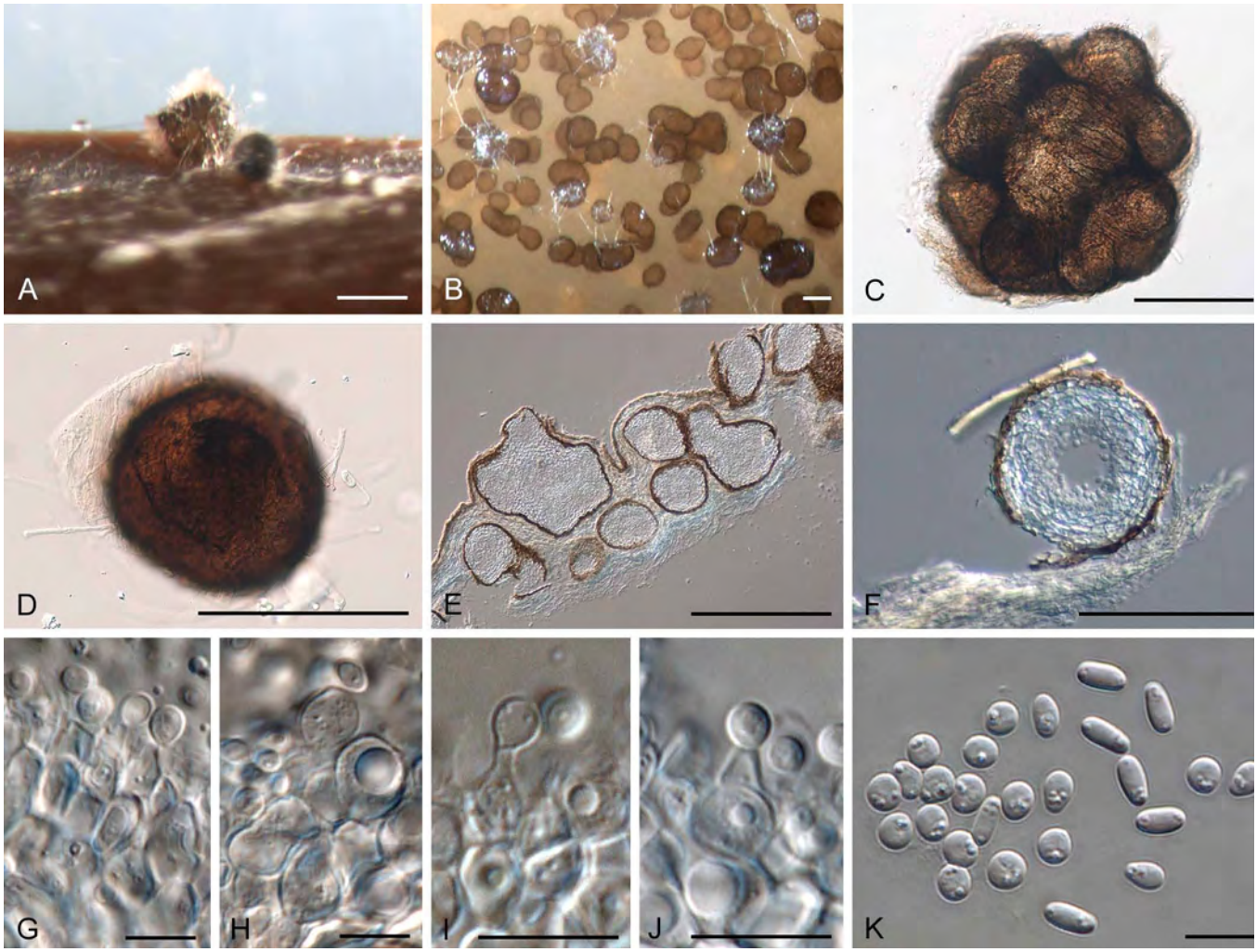

Fig. 7. *Diederichomyces foliaceiphila* (CBS 129141). **A–D.** Conidiomata in culture. **E, F.** Sections through conidiomata. **G–J.** Conidiogenous cells. **K.** Dimorphic conidia. Bars: A–E = 250 µm, F = 200 µm, G–K = 10 µm.

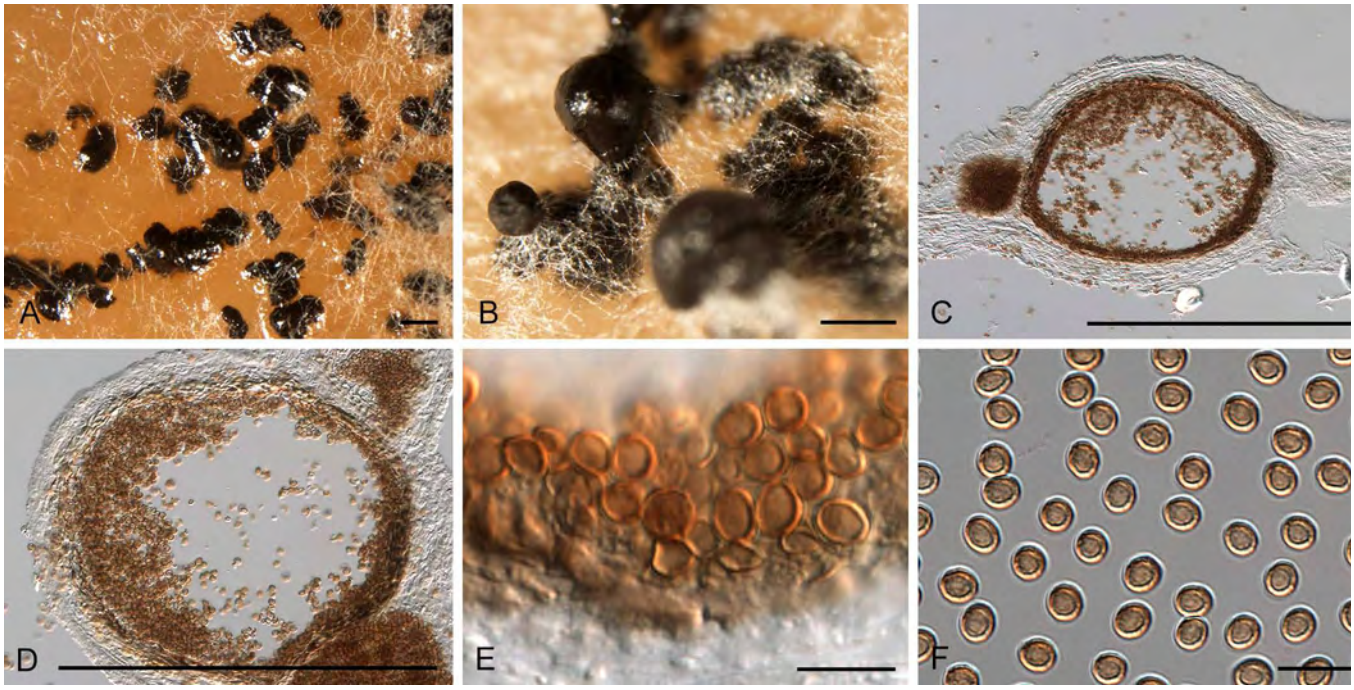

Fig. 8. *Paraphaeosphaeria parmeliae* (CBS 131728). **A, B.** Conidiomata in culture. **C, D.** Sections through conidiomata. **E.** Conidiogenous cells. **F.** Conidia. Bars: A–D = 200 µm, E, F = 10 µm.

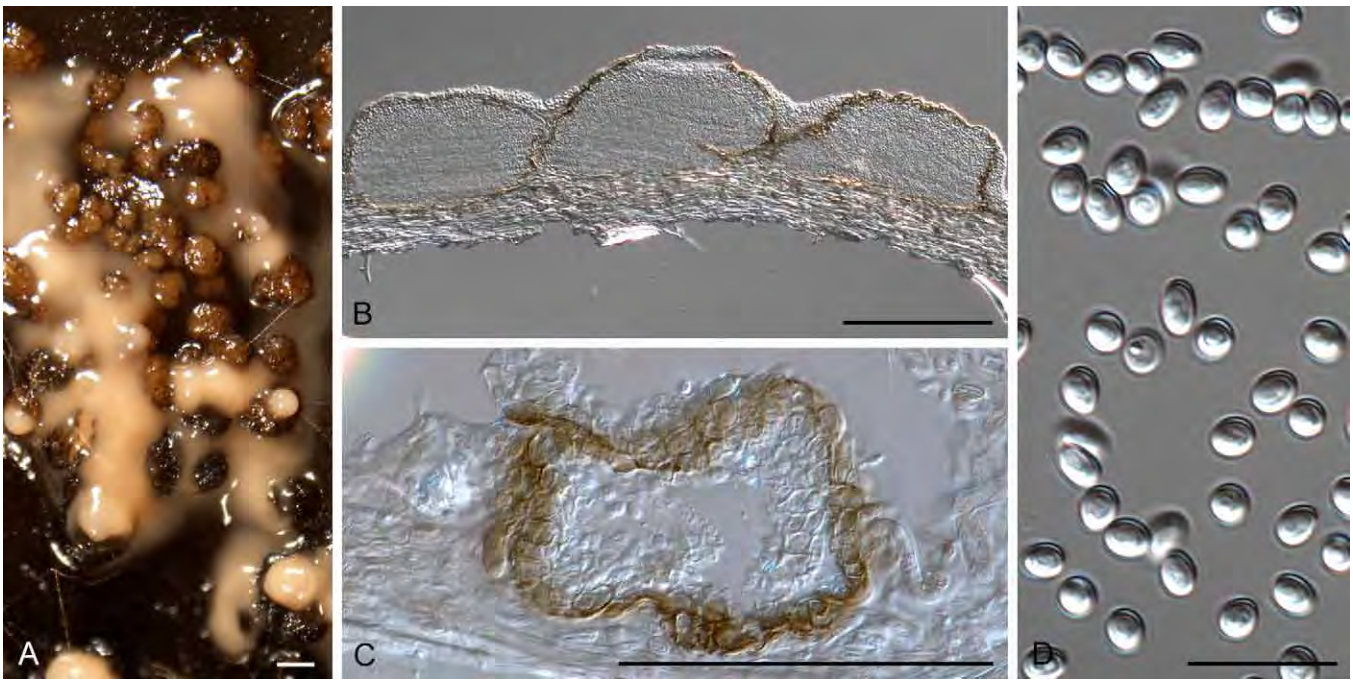

Fig. 9. *Xenophoma puncteliae* (CBS 128022). **A.** Conidiomata forming on MEA. **B, C.** Sections through conidiomata. **D.** Conidia. Bars: A–C = 50 µm, D = 10 µm.

conidia. The genus *Paraphaeosphaeria* was shown to not be congeneric with *Paraconiothyrium* (Verkley *et al*. 2013).

Xenophoma Crous & Trakunyingcharoen, **gen. nov.**
MycoBank MB810835

Etymology: Named after its morphological similarity to the genus *Phoma*.

Diagnosis: *Conidiomata* uni- to multilocular, irregular to cauliflower-shaped. *Paraphyses* absent. *Conidiophores* reduced to conidiogenous cells, hyaline, ampulliform, monophialidic. *Conidia* solitary, hyaline, smooth, thin-walled, guttulate, subspherical to ellipsoid.

Type species: *Xenophoma puncteliae* (Diederich & Lawrey) Crous & Trakunyingcharoen 2014 (syn. *Phoma puncteliae* Diederich & Lawrey 2013)

Description: Conidiomata globose to subglobose, uni- to multilocular, irregular to cauliflower-shaped, solitary to aggregated, mostly solitary, ostiolate; wall layers of 2–3 layers of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region. *Paraphyses* absent. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity, hyaline, ampulliform, monophialidic, with prominent periclinal thickening. *Conidia* in creamy white masses, solitary, hyaline, smooth, thin-walled, guttulate, subspherical to ellipsoid.

Notes: *Xenophoma* is morphologically similar to the genus *Phoma*, the only differences being the cauliflower-shaped, uni- to multilocular conidiomata, and subspherical to ellipsoid conidia.

Xenophoma puncteliae (Diederich & Lawrey) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810836
(Fig. 9)
Basionym: *Phoma puncteliae* Diederich & Lawrey, *Fungal Div.* **55**: 207 (2013).

Type: **USA**: Maryland: Frederick Co., Catoctin Mt. National Park, Hog Rock Trail, open oak-woodland, 39°38′55.1″N, 77°27′08.6″W, parasitic on *Punctelia rudecat* on *Quercus rubra*, 13 Oct. 2009, *J.D. Lawrey* (BR – holotype; CBS 128022 – ex-type culture).

Note: Based on the ITS phylogeny (Fig. 2), *Xenophoma puncteliae* clusters basal to species of *Phaeosphaeriopsis*.

Sphaerellopsis-like genera

As shown in Fig. 1, the genus *Acrocalymma* represents an undefined lineage of *Dothideomycetes* that have massarina-like sexual morphs. A new family name is herewith introduced to accommodate these species.

Acrocalymmaceae Crous & Trakunyingcharoen, **fam. nov.**
MycoBank MB810837

Etymology: Named after the genus *Acrocalymma*.

Diagnosis: *Ascomata* globose, opening by central beak with ostiole lined with periphyses; inner layer giving rise to hyaline pseudoparaphyses, septate, anastomosing. *Asci* cylindrical, sessile in rosette, 8-spored, bitunicate. *Ascospores* narrowly

fusoid, straight to slightly curved, initially hyaline, 1-septate, with a mucoid sheath, becoming transversely 3-septate after discharge, constricted or not, pale brown. *Conidiomata* pycnidial, papillate or rostrate, globose, with central ostiole. *Conidiophores* reduced to conidiogenous cells or a supporting cell. *Conidiogenous cells* ampulliform to doliiform or cylindrical, hyaline, smooth, proliferating inconspicuously percurrently at apex. *Conidia* hyaline, but becoming pigmented with age, smooth, 0–3-septate, not constricted at septa, with flaring mucoid apical and basal appendages.

Type genus: *Acrocalymma* Alcorn & J.A.G. Irwin 1987.

Description: *Ascomata* globose, immersed, becoming erumpent, covered with pale grey hyphae, opening by central beak with ostiole lined with periphyses; wall of *textura angularis*; inner layer giving rise to hyaline pseudoparaphyses, septate, anastomosing. *Asci* cylindrical, sessile in rosette, 8-spored, bitunicate, with biseriate ascospores. *Ascospores* narrowly fusoid, straight to slightly curved, initially hyaline, 1-septate, with a mucoid sheath, becoming transversely 3-septate after discharge, constricted or not, pale brown. *Conidiomata* pycnidial, papillate or rostrate, globose, erumpent, separate but aggregated in clusters, subhyaline to brown with central ostiole; wall of 3–6 layers of hyaline to brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells or a supporting cell. *Conidiogenous cells* ampulliform to doliiform or cylindrical, hyaline, smooth, proliferating inconspicuously percurrently at apex. *Conidia* hyaline, but becoming pigmented with age, smooth, guttulate, cylindrical to fusoid with subobtuse apex, acutely tapered at base to a small flattened central scar, 0–3-septate, not constricted at septa, with flaring mucoid apical and basal appendages.

Notes: Shoemaker *et al*. (1991) linked *Acrocalymma* to massarina-like sexual morphs in culture, establishing the asexual/sexual connection. Phylogenetically the genus *Acrocalymma* represents an undefined lineage in the *Dothideomycetes*, for which *Acrocalymmaceae* is herewith introduced.

Acrocalymma Alcorn & J.A.G. Irwin, *Trans. Brit. mycol. Soc.* **88**: 163 (1987).

Synonym: *Rhizopycnis* D.F. Farr, *Mycologia* **90**: 291 (1998).

Description: *Conidiomata* pycnidial, papillate or rostrate, globose, erumpent, separate but aggregated in clusters, subhyaline to brown with central ostiole; wall of 3–6 layers of hyaline to brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells or a supporting cell. *Conidiogenous cells* ampulliform to doliiform or cylindrical, hyaline, smooth, proliferating inconspicuously percurrently at apex. *Conidia* hyaline, but becoming pigmented with age, smooth, guttulate, cylindrical to fusoid with subobtuse apex, acutely tapered at base to a small flattened central scar, 0–3-septate, not constricted at septa, with flaring mucoid apical and basal appendages, originating from a sheath surrounding developing conidia.

Type species: *Acrocalymma medicaginis* Alcorn & J.A.G. Irwin 1987.

Acrocalymma fici Crous & Trakunyingcharoen, **sp. nov.**

MycoBank MB810838

(Fig. 10)

Etymology: Named after the host genus on which it occurs, *Ficus*.

Diagnosis: *Conidiomata* pycnidial, globose, up to 200 µm diam. *Conidiophores* reduced to conidiogenous cells, ampulliform to doliiform, hyaline, smooth, 5–12 × 3–5 µm; inconspicuous percurrent proliferation visible at apex. *Conidia* hyaline, smooth, guttulate, cylindrical with subobtuse apex, medianly 1-septate, not constricted at septum, (12–)13–15(–16) × 2.5(–3) µm, with flaring mucoid apical appendage.

Type: **India**: New Delhi: on *Ficus* sp., 23 Dec. 1975, *G. Malhotra* (CBS H-11698 – holotype; CBS 317.76 – ex-type culture).

Description: *Conidiomata* pycnidial, globose, up to 200 µm diam, erumpent, separate but aggregated in clusters, subhyaline with dark brown region around ostiole, 20–30 µm diam; wall of 3–6 layers of hyaline to subhyaline *textura angularis*. *Conidiophores* reduced to conidiogenous cells or a supporting cell. *Conidiogenous cells* ampulliform to doliiform, hyaline, smooth, 5–12 × 3–5 µm; inconspicuous percurrent proliferation visible at apex. *Conidia* hyaline, smooth, guttulate, cylindrical with subobtuse apex, acutely tapered at base to a small flattened central scar, medianly 1-septate, not constricted at septum, (12–)13–15(–16) × 2.5(–3) µm, with flaring mucoid apical appendage (3–5 µm diam), visible in water mounts.

Culture characteristics: Colonies on MEA flat, spreading, with moderate aerial mycelium, and smooth, even margins; surface smoke-grey in centre, pale olivaceous grey in outer region, smoke-grey in reverse.

Acrocalymma medicaginis Alcorn & J.A.G. Irwin, *Trans. Brit. mycol. Soc.* **88**: 163 (1987).

(Fig. 11)

Description: *Conidiomata* separate, immersed, globose, brown with central ostiole, up to 250 µm diam; wall of 3–8 layers of brown *textura angularis*, becoming hyaline towards the inner region. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity of conidioma, hyaline, smooth, ampulliform to doliiform, 5–10 × 6–7 µm, proliferating with visible periclinal thickening at apex. *Conidia* solitary, hyaline, smooth, guttulate, thin-walled, straight, subcylindrical, apex obtuse, tapering at base to truncate hilum, 1.5 µm diam, (11–)13–15(–16) × (3.5–)4 µm; ends with mucoid caps, conidia becoming pale olivaceous and 1-septate with age.

Materials examined: **Australia**: Queensland: Hermitage, on *Medicago sativa*, 10 Mar. 1972, *J.A.G. Irvin* (CPC 24340, CPC 24340, BRIP 5876a, IMI 165613 – ex-type cultures); Gatton, 14 Nov. 1984, *J.A.G. Irvin* (CPC 24342, CPC 24343, BRIP 14544a); Gatton,

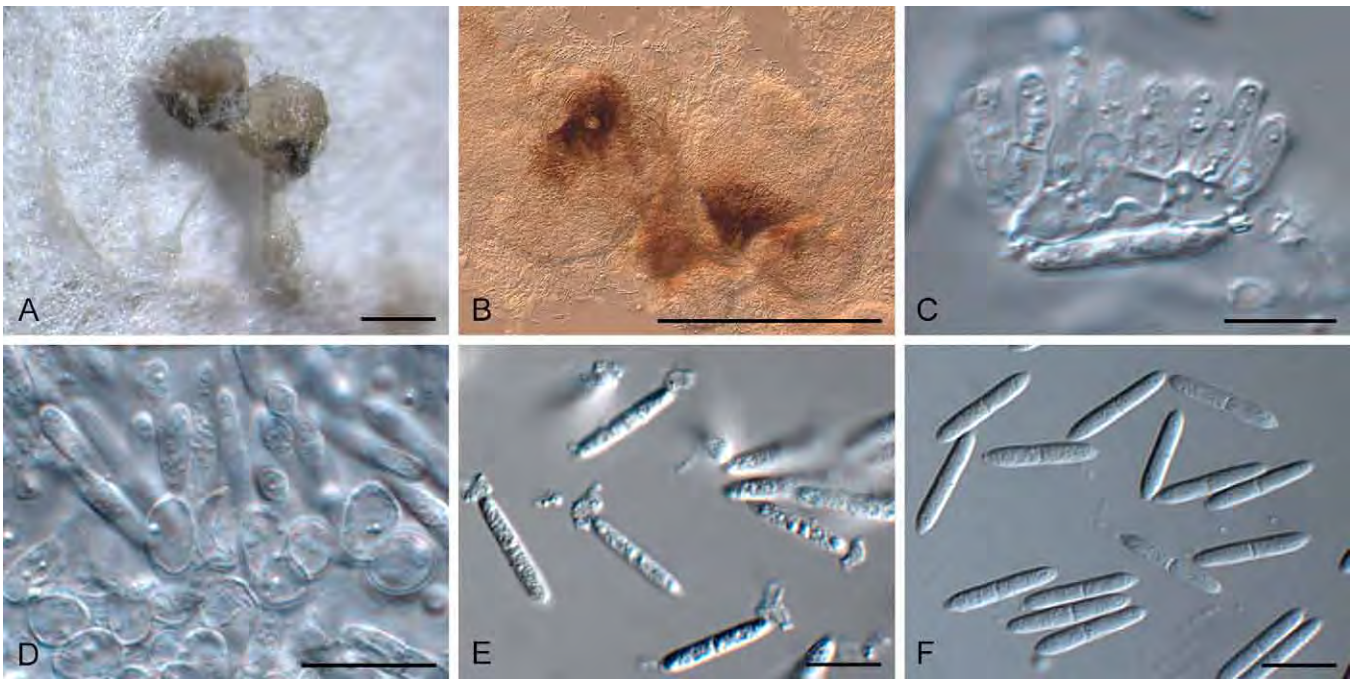

Fig. 10. *Acrocalymma fici* (CBS 317.76). **A, B.** Conidiomata forming in agar. **C, D.** Conidiogenous cells. **E, F.** Conidia. Bars: A, B = 200 µm, C–F = 10 µm.

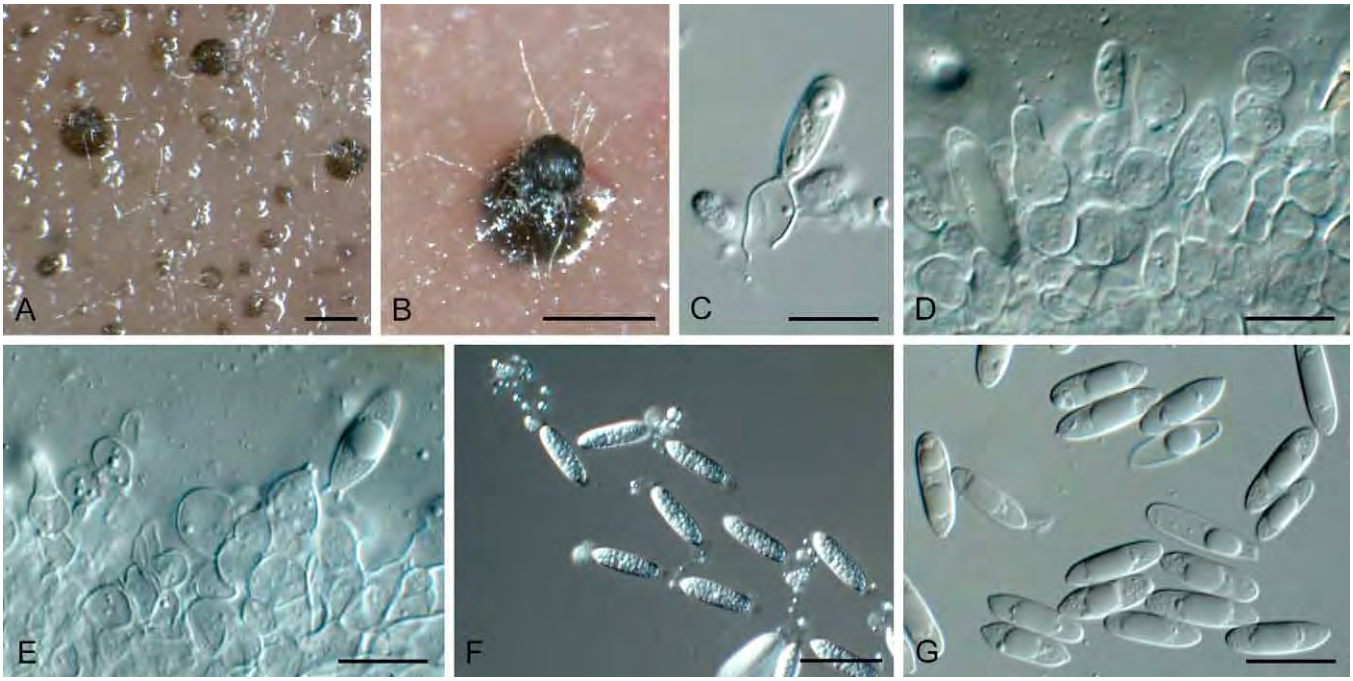

Fig. 11. *Acrocalymma medicaginis* (BRIP 5876a). **A, B.** Conidiomata forming in agar. **C–E.** Conidiogenous cells. **F, G.** Conidia. Bars: A, B = 250 µm, C–G = 10 µm.

DPI Research Station, 22 Jul. 1987, *J.A.G. Irvin* (CPC 24344, BRIP 15915a). Southern Australia: Langhorne Creek, 8 Oct. 1975, *A. Nikandrow* (CPC 24346, CPC 24347, BRIP 16416a).

Notes: The isolate we describe here as *A. fici* clusters with two genera, namely *Acrocalymma* (based on *A. medicaginis*) and *Rhizopycnis* (based on *R. vagum*). These two genera were established a few years apart to accommodate two root pathogens, namely *A. medicaginis* on *Medicago* in Australia, and *R. vagum* on *Cucumis* in Texas (Alcorn & Irwin 1987, Farr *et al.* 1998). Furthermore, *Acrocalymma medicaginis* has been linked to "*Massarina*" *walkeri* as sexual morph (Shoemaker *et al*. 1991). Morphologically *Acrocalymma* resembles *Rhizopycnis* (pycnidial conidiomata, phialidic conidiogenous cells, cylindrical to fusoid, 1–3-septate conidia, that turn brown with age). Reported differences between the two genera are that *R. vagum* lacks mucoid conidial caps, has phialidic conidiogenesis with periclinal thickening, and

conidia turn brown with age (Farr *et al.* 1998). However, when ex-type strains of *A. medicaginis* were studied in culture, they exhibited phialidic conidiogenesis, and conidia also become septate and pigmented with age, explaining why isolates of *R. vagum* clustered among those of *Acrocalymma*. *Rhizopycnis* is therefore reduced to synonymy under *Acrocalymma*, and a new combination introduced for *R. vagum*.

Recently, a second species of *Acrocalymma*, *A. aquatica*, was described from freshwater in Thailand (Zhang *et al.* 2012), while Crous *et al*. (2013) introduced *A. cycadis* from leaves of *Cycas calcicola* in Australia. *Acrocalymma aquatica* is similar to *A. fici*, except that it has slightly wider conidia (12–17 × 3–4 µm), while those of *A. cycadis* are larger (25–35 × 4–5 µm).

Acrocalymma vagum (D.F. Farr) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810839
Basionym: *Rhizopycnis vagum* D.F. Farr, *Mycologia* **90**: 291 (1998).

Specimens examined: **Spain**: Castellón, Almenara, on *Amaranthus* sp., CPC 24221 = Rv-86); on *Cucumis sativus*, CPC 24225 = Rv-1403; on *Cucurbita* rootstock, CPC 24223 = Rv-0103, CPC 24226 = Rv-0504; Ciudad Real, Daimiel, on *Vitis vinifera*, CPC 24220 = Rv-77; on Loquat, CPC 24227 = Rv-0106; Ciudad Real, Argamasilla de Alba, on *Cucumis melo*, CPC 24217 = Rv-25; Valencia, El Romaní, CPC 24218 = Rv-43; Murcia, La Palma, CPC 24219 = Rv-55; Valencia, Silla, on *Citrullus lanatus*, CPC 24216 = Rv-17, CPC 24224 = Rv-0703. – **USA**: Texas: on *Cucumis melo*, CPC 24222 = Rv-110.

Acrocalymma walkeri (Shoemaker *et al.*) Crous & Trakunyingcharoen, **comb. nov.**
MycoBank MB810840

Basionym: *Massarina walkeri* Shoemaker *et al.*, *Canad. J. Bot.* **69**: 569 (1991).

Type: **Australia**: *Queensland*: on *Medicago sativa* cv. Hunter River, 22 Jul. 1987, *J.A.G. Irwin* (DAOM 198791a – holotype).

Notes: *Acrocalymma medicaginis*, which is also known from *Medicago sativa* in Queensland, was originally assumed to represent the asexual morph of *Massarina walkeri*. The two species are, however, phylogenetically distinct, and *A. medicaginis* has somewhat smaller conidia than those of *A. walkeri*, which are 11–21 × 3.5–5 µm (Shoemaker *et al.* 1991).

Sphaerellopsis-like isolates associated with rust sori on *Bothriochloa bladhii* in Thailand proved to represent yet another genus, which is phylogenetically distinct from *Sphaerellopsis s. str.*

Neosphaerellopsis Crous & Trakunyingcharoen, **gen. nov.**
MycoBank MB810841

Etymology: Named after the genus *Sphaerellopsis*, which it superficially resembles.

Diagnosis: Morphologically similar to *Sphaerellopsis*, but phylogenetically distinct based on sequence data of the ITS and LSU regions. Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Phaeosphaeria avenaria* (GenBank FJ623271; Identities = 535/571 (94 %), Gaps = 11/571 (1 %)), *Parastagonospora poagena* (GenBank KJ869116; Identities = 528/564 (94 %), Gaps = 9/564 (1 %)), and *Phaeosphaeria avenaria* f. sp. *triticae* (GenBank AY196988; Identities = 534/571 (94 %), Gaps = 9/571 (1 %)). The LSU sequence of *Neosphaerellopsis thailandica* differs from *Stagonospora neglecta* var. *colorata* (GenBank EU754218) at positions 57 (C), 94 (A), 96 (T), 114 (C), 167 (T), and 168 (A); and from *Stagonospora foliicola* KF251759 at positions 94 (A), 114 (C), 167 (T), and 168 (A).

Type species: *Neosphaerellopsis thailandica* Crous & Trakunyingcharoen 2014.

Description: *Conidiomata* globose, superficial, solitary or aggregated, mostly unilocular, sometimes multilocular within the same stromata, with central ostiole; outer layers composed of pale to medium brown *textura angularis*, becoming thin-walled and hyaline toward the inner region, ostiolate. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, phialidic with visible periclinal thickening, at times with 1–2 minute apical percurrent proliferations, dolliform to ampulliform. *Conidia* hyaline, narrowly ellipsoidal, the base truncate, with central median septum, with flaring mucoid appendage at one end.

Notes: There is no clear morphological difference between *Sphaerellopsis* and *Neosphaerellopsis*, and these genera are chiefly distinguished based on DNA phylogeny. *Neosphaerellopsis* clusters (Fig. 1) between *Parastagono-spora* and *Sclerostagonospora* in *Phaeosphaeriaceae* (Quaedvlieg *et al*. 2013), but can be distinguished from them in that neither have mucoid conidial appendages. *Neosphaerellopsis* is also reminiscent of *Tiarospora*, except that the latter has deeply immersed, stromatic pycnidia, percurrent proliferating conidiogenous cells, and broadly ellipsoidal conidia that turn brown with age (Nag Raj 1993).

Neosphaerellopsis thailandica Crous & Trakunyingcharoen, **sp. nov.**
MycoBank MB810842
(Fig. 12)

Etymology: Named after the country where the fungus was first collected, Thailand.

Diagnosis: *Conidiomata* globose, sometimes multilocular. *Conidiophores* reduced to conidiogenous cells, hyaline, phialidic with visible periclinal thickening, dolliform–ampulliform, 3.5–7 × 2.5–4 µm. *Conidia* hyaline, narrowly ellipsoidal, the base truncate, with central median septum,

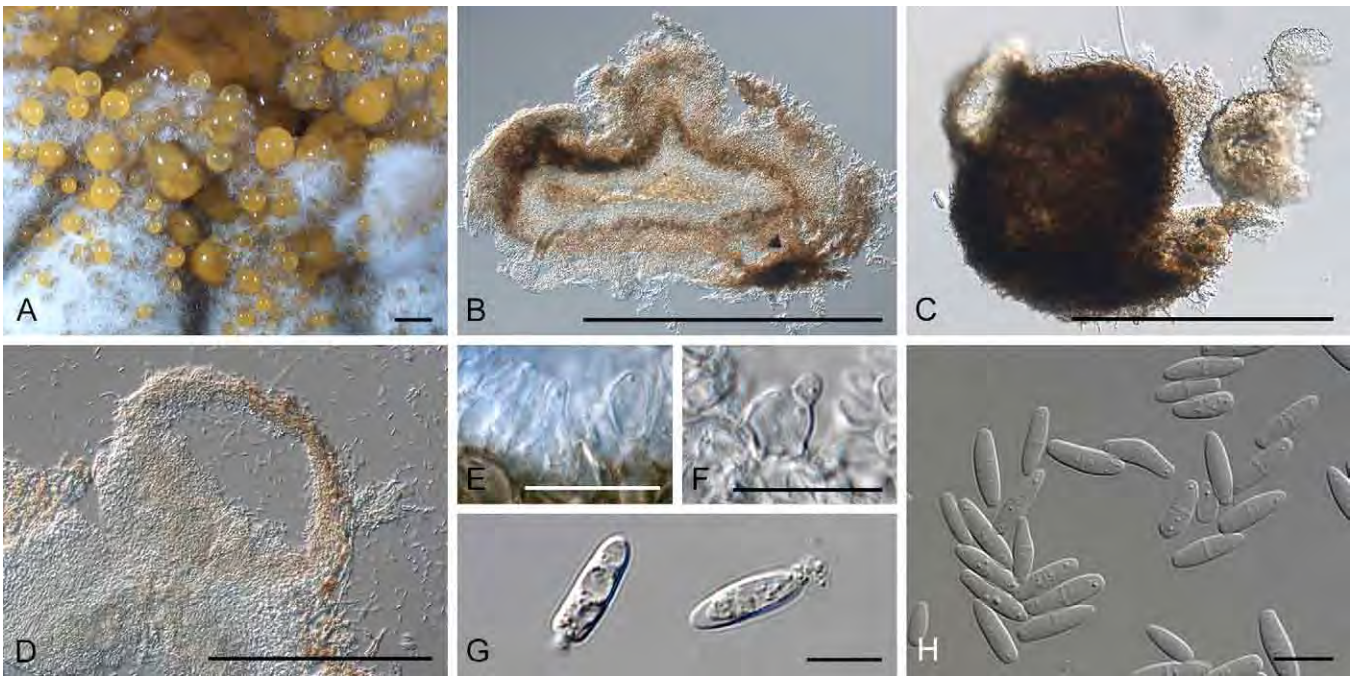

Fig. 12. *Neosphaerellopsis thailandica* (CBS 138578). **A, C.** Conidiomata forming in agar. **B, D.** Sections through conidiomata. **E, F.** Conidiogenous cells. **G, H.** Conidia. Bars: A–D = 300 µm, E–H = 10 µm.

(10.5–)11–13(–15) × (3–)3.5–4(–4.5) µm; with flaring mucoid appendage at one end.

Type: **Thailand**: Royal Project, N18º09'24.8" E98º23'19.6", on uredinio rust sori on leaves of *Bothriochloa bladhii* (*Poaceae*), 29 Oct. 2012, *P.W. Crous* (CBS H-21847 – holotype; CPC 21659, CBS 138578 – ex-type cultures).

Description: *Conidiomata* globose, superficial, solitary or aggregated, mostly unilocular, sometimes multilocular within the same stromata, with central ostiole; outer layers composed of pale to medium brown *textura angularis*, becoming thin-walled and hyaline toward the inner region, ostiolate, to 400 µm diam; conidiomata exude a yellow-orange conidial mass. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, phialidic with visible periclinal thickening, at times with 1–2 minute apical percurrent proliferations, dolliform–ampulliform, 3.5–7 × 2.5–4 µm. *Conidia* hyaline, narrowly ellipsoidal, the base truncate, with central median septum, (10.5–)11–13(–15) × (3–)3.5–4(–4.5) µm; with flaring mucoid appendage at each end, visible in water mounts.

Culture characteristics: Colonies on MEA with white moderate aerial mycelium, flat to low convex, surface folded towards the centre, orange-brown on surface and reverse. Colonies on PDA with grey-brown, moderate aerial mycelium, immersed mycelium greenish, margin fimbriate, forming dark brown pycnidia in media, exuding a creamy conidial mass; greenish grey in reverse. Colonies on OA with white-grey flat mycelium, surface folded towards the centre, smoky grey in reverse.

Sphaerellopsis Cooke, *Grevillea* **12** (6): 23 (1883).
Synonyms: *Darluca* Castagne, *Suppl. Cat. Pl. Marseille*: 53 (1851).
Eudarluca Speg., *Revta Mus. La Plata* **15**: 22 (1908).

Additional generic synonyms are listed in Sutton (1980) and Nag Raj (1993).

Description: *Mycelium* immersed, branched, septate, pale brown. *Conidiomata* eustromatic, pycnidioid, immersed, but becoming erumpent, locules often appearing as separate pycnidia, dark brown to black *in vivo*, pale brown to brown *in vitro*, uni- or multilocular, each locule with a separate simple ostiole; basal wall composed of pale brown *textura angularis*, locular wall of dark brown, thick-walled *textura angularis*. *Paraphyses* when present hyaline, filiform, septate, with end-round tip, sometimes branching. *Conidiophores* reduced to conidiogenous cells, occasionally with a supporting cell. *Conidiogenous cells* phialidic, indeterminate, cylindrical to doliiform, hyaline to pale brown, smooth, often with 1–3 percurrent proliferations, or determinate with visible periclinal thickening. *Conidia* hyaline, becoming pale brown and irregularly verruculose, 0–1(–3)-euseptate, constricted at septa, apex obtuse, base truncate, straight, fusoid-ellipsoidal, occasionally Y-shaped or digitate; ends with mucoid polar appendages (type H sensu Nag Raj 1993). *Microconidia* subcylindrical to ellipsoid or globose, aseptate, smooth, hyaline. *Stromata* developing in rust sori, brown in outer zone, hyaline in inner part; loci immersed, subglobose to ampulliform, with protruding papillate neck and ostiole; wall of a few layers of *textura prismatica*. *Pseudoparaphyses* cellular, septate, branched, hyaline. *Asci* numerous, 8-spored, bitunicate, fissitunicate, cylindrical-clavate, short stipitate. *Ascospores* irregularly biseriate, fusoid, hyaline to

Fig. 13. *Eudarluca caricis* (K(M)124143). **A.** Aggregated ascomata forming on an immersed stroma in leaf tissue, associated with rust pustules. **B, C.** Vertical section through ascomata. **D.** Ascomatal wall of textura angularis. **E–G.** Asci. **H.** Ascospores. Bars: A–C = 300 µm, D–H = 10 µm.

pale yellow, (1–)2(–3)-septate, constricted at the primary septum; with a mucoid cupula at each polar end.

Type species: *S. filum* (Biv.) B. Sutton 1980 (syn. *S. quercuum* Cooke 1883).

Notes: Although this study confirmed earlier observations (Keener 1951, Yuan *et al*. 1998) that *Sphaerellopsis* and *Eudarluca* (Fig. 13) are congeneric, we could not confirm which *Sphaerellopsis* species is conspecific with *Eudarluca caricis* (? syn. *E. australis*). Eriksson (1966) located what he considered to be "obviously an original collection" of *Sphaeria caricis* Fr., 1823 in UPS, consisting of three leaf fragments of a *Carex* sp. from Sweden infected with *Puccinia caricina*. The specimen has no indication of date, and whether it was collected before he came to Uppsala in 1835, is unknown, and in 1823 Fries evidently was aware of other material apart from his own, such as some of Kunze. The Fries material can confidently be termed "authentic" (i.e. named by the author of the taxon), but it cannot be considered to be a holotype; further, Eriksson did not make an explicit later typification. Although we have one isolate from *Carex* sp. collected in The Netherlands in the present study (*Sphaerellopsis macroconidialis* sp. nov. below), we cannot confirm or refute if this could be *E. caricis* as understood by Fries, as fresh collections of the sexual morph need to be made in Sweden. While there is no doubt about the generic placement of Fries's name, the application of the specific epithet (a sanctioned name) remains uncertain.

Sphaerellopsis filum (Biv.) B. Sutton, *Mycol. Pap.* **141**: 196 (1977).
(Fig. 14)
Basionym: *Sphaeria filum* Biv., *Stirp. Rar. Sic.* **3**: 12 (1815).
Synonyms: See Sutton (1980) and Nag Raj (1993)

Description: *Conidiomata* pycnidial, erumpent, aggregated, globose, up to 300 µm diam, dark brown with central ostiole, exuding copious amounts of creamy orange conidia; wall of 3–6 layers of dark brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* brown, smooth, thick-walled, ampulliform, to doliiform, 5–10 × 3–5 µm; with prominent periclinal thickening or several prominent, flaring, percurrent proliferations at apex. *Conidia* hyaline, smooth, guttulate, fusoid to fusoid-ellipsoid, 1(–2)-septate, usually constricted at septa, apex subobtuse, tapering at base to flattened scar, with funnel-shaped mucoid appendages at both ends, (11–)14–16(–18) × (3–)4(–5) µm.

Culture characteristics: Colonies on MEA erumpent, spreading, with sparse aerial mycelium and even, smooth margins; centre olivaceous grey (due to numerous aggregated conidiomata); outer region dirty white (due to mycelial growth in absence of conidiomata). Reverse olivaceous grey in centre, saffron in outer region.

Type: **Sicily**: on rust of *Populus nigra*, *A. Bivona-Bernadi* (holotype not traced and presumably lost). – **Germany**: Hollingstedt near Husum, on *Puccinia deschampsia* on *Deschampsia caespitosa*, Nov. 1966, *U.G. Schlösser* (CBS

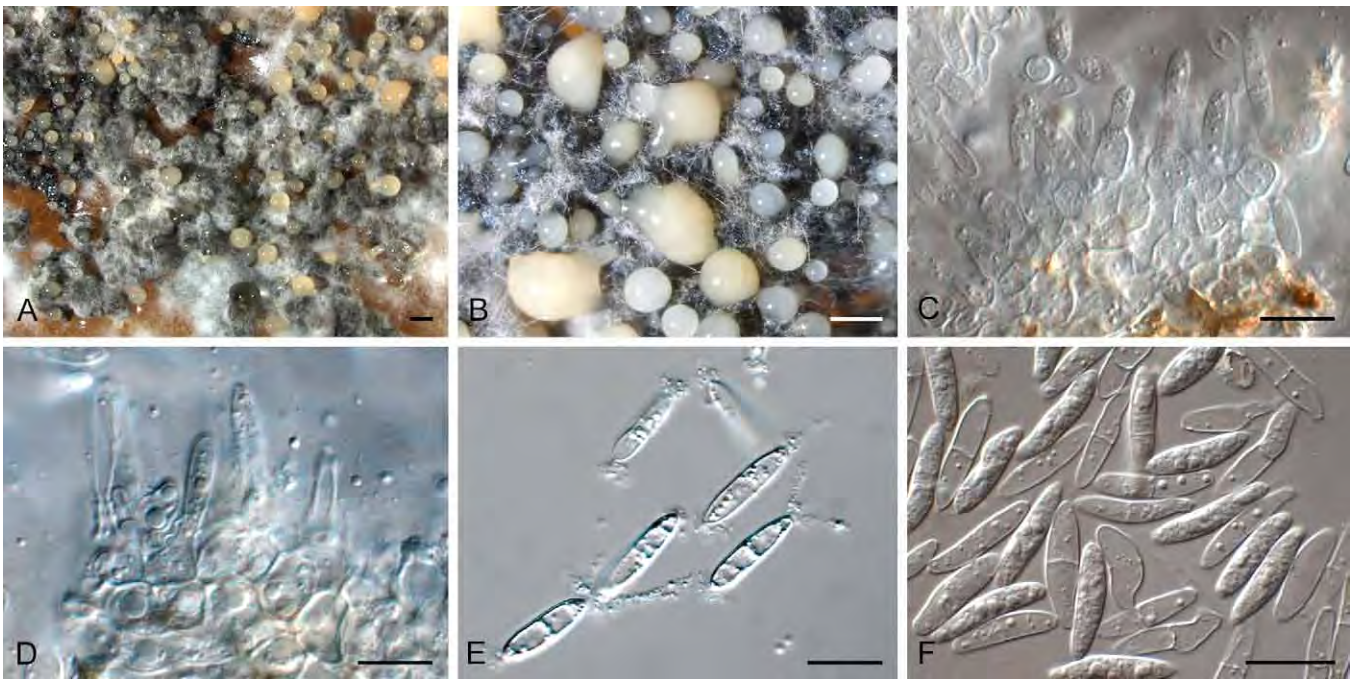

Fig. 14. *Sphaerellopsis filum* (CBS 317.68). **A, B.** Conidiomata sporulating on MEA. **C, D.** Conidiogenous cells. **E, F.** Conidia. Bars: A, B = 300 µm, C–F = 10 µm.

H-21851 – **neotype designated here** MBT200131; CBS 317.68 – ex-neotype culture).

Additional materials examined: **Portugal**: on *Puccinia hordei* on *Ornithogalum divergens*, May 1951, *B. d'Oliveira* (ATCC 22604, CBS 235.51). – **Switzerland**: Zürich-Oerlikon, on *Puccinia coronata* on *Lolium italicum*, 23 May 1951, *E. Müller* (ATCC 22603, CBS 234.51).

Notes: Saccardo (1884) stated that conidia of *S. filum* were 15–18 × 3–4 µm, 1-septate, constricted at the septum. Sutton (1980) examined numerous collections, and gave the conidia as 1-septate, 17–20 × 5.5–6.5 µm, while Nag Raj (1993) regarded conidia as being 1–3-septate, 10–20(–23) × 3–5 µm. The neotype chosen here, closely matches the original description and dimensions provided by Saccardo (1884).

Sphaerellopsis macroconidialis Crous & Trakunyingcharoen, **sp. nov.**
MycoBank MB810843
(Fig. 15)

Etymology: Named after the large conidial dimensions.

Diagnosis: *Conidiomata* globose, erumpent to superficial, up to 250 µm diam. *Conidiophores* reduced to conidiogenous cells, globose to ampulliform, pale brown, smooth, thick-walled with prominent periclinal thickening, or percurrent, 5–8 × 4–10 µm. *Conidia* fusoid to fusoid-ellipsoid, 1(–3)-septate, with funnel-shaped mucoid appendage at each end, (13–)17–20(–27) × (3.5–)4.5(–5) µm.

Type: **The Netherlands**: Nieuwendam, garden, on *Puccinia alii* sori on *Allium schoenoparsum*, 8 Oct. 1978, *G. van Zanen* (CBS H-11700 – holotype; CBS 658.78 – ex-type culture).

Description: *Conidiomata* globose, erumpent to superficial, also occurring in aerial mycelium, up to 250 µm diam, brown, exuding a creamy white conidial mass. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity, globose to ampulliform, pale brown, smooth, thick-walled with prominent periclinal thickening, or also with percurrent proliferation at apex (when ampulliform), 5–8 × 4–10 µm. *Conidia* fusoid to fusoid-ellipsoid, 1(–3)-septate, hyaline, smooth, guttulate, constricted at septa, base truncate, apex subobtuse to obtusely rounded, with funnel-shaped mucoid appendage at each end, (13–)17–20(–27) × (3.5–)4.5(–5) µm.

Culture characteristics: Colonies on MEA spreading, erumpent, with sparse aerial mycelium, and feathery margin; surface dirty white with patches of olivaceous grey due to profuse sporulation; reverse sienna to umber.

Additional materials examined: **Italy**: Bologna, on *Uromyces caryophylli* on *Dianthus caryophyllus*, June 1951, *G. Goidánich* (ATCC 11100, CBS 233.51); Veenendal, on rust on *Carex acutiformis*, 2013, *W. Quaedvlieg* (CPC 21114, CBS 138761).

Note: Conidia of *S. macroconidialis* are longer than those of *S. filum*, which measure (11–)14–16(–18) × (3–)4(–5) µm, and also have up to three septa, though the latter feature is not expressed in all isolates.

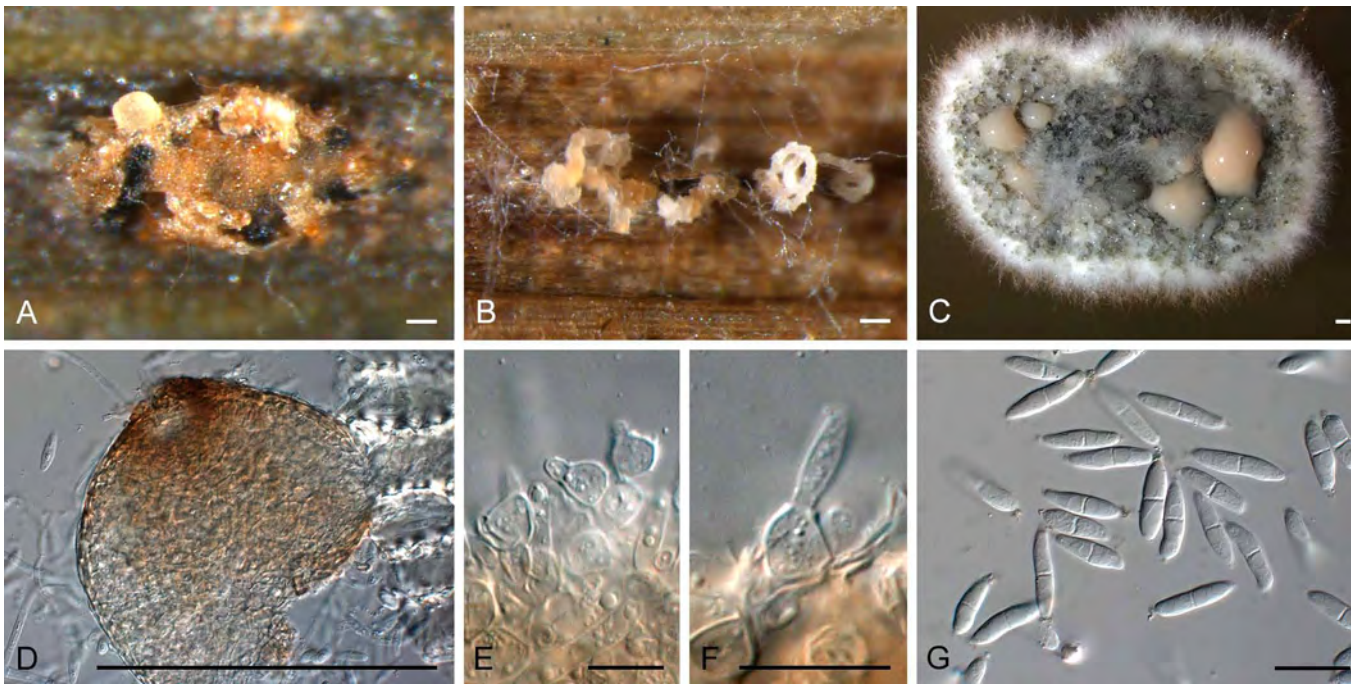

Fig. 15. *Sphaerellopsis macroconidialis* (CPC 21113). **A, B.** Conidiomata sporulating inside rust pustules. **C.** Colony sporulating on MEA. **D.** Conidioma formed on MEA. **E, F.** Conidiogenous cells. **G.** Conidia. Bars: A–D = 250 µm, E–G = 10 µm.

Sphaerellopsis paraphysata Crous & Alfenas, **sp. nov.**
MycoBank MB810844
(Fig. 16)

Etymology: Named after the presence of conidiomatal paraphysis-like structures.

Diagnosis: *Paraphyses* hyaline, filiform, 2–5 septate, with end-round tip, sometimes branching, 11.5–49 × 2–6 µm. *Conidiophores* reduced to conidiogenous cells, ampulliform to doliiform, 4.5–12.5 × 3–8 µm. *Conidia* fusoid-ellipsoid, 1(–2)-septate, with mucilaginous appendage at both ends, (14.5–)15–18(–20) × (4–)4.5–5.5(–6) µm.

Type: **Brazil**: Minas Gerais, Viçosa, Universidade Federal de Viçosa campus, on rust on *Pennisetum* sp., 18 Nov. 2012, *A.C. Alfenas* (CBS H-21848 – holotype; CPC 21841 = CBS 138579 – ex-type cultures).

Description: *Conidiomata* brown, superficial to semi-immersed, globose to subglobose, solitary to aggregated, ostiolate, papilate, unilocular, outer layers composed of dark brown *textura angularis*, becoming thin-walled and hyaline toward the inner region, up to 450 µm diam. *Paraphyses* hyaline, filiform, 2–5 septate, with end-round tip, sometimes branching, 11.5–49 × 2–6 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, ampulliform to doliiform, hyaline, smooth, monophialidic with inconspicuous percurrent proliferation, 4.5–12.5 × 3–8 µm. *Conidia* solitary, hyaline, fusoid-ellipsoid, widest in the middle, mostly with 1-median septum, rarely 2-septate, with mucilaginous appendage at both ends, (14.5–)15–18(–20) × (4–)4.5–5.5(–6) µm.

Culture characteristics: Colony on MEA with white cottony aerial mycelium, producing abundant conidiomata, which are covered with white aerial mycelium, sporulating in a yellow-cream conidial mass, crenated, dark brown-grey in reverse. Colony on PDA with grey fluffy aerial mycelium, olivaceous grey, with fimbriate margin, dark olivaceous grey in reverse. Colony on OA with white fluffy aerial mycelium, olivaceous grey on surface with undulate margin; olivaceous grey in reverse.

Additional material examined: **South Africa**: KwaZulu-Natal, Howick, Amber Valley, on *Ravenelia macowania* on *Vachellia karroo*, 4 Aug. 2013, *F. Rijkenberg* (CBS H-21849, CPC 23548, CPC 23547 = CBS 137231).

Note: *Sphaerellopsis paraphysata* has conidia that are slightly longer and wider than those of *S. filum*, and also has conidiomatal paraphyses, which are absent in *S. filum*.

DISCUSSION

The present study aimed to elucidate the taxonomy of *Sphaerellopsis filum* and its purported sexual morph *Eudarluca caricis* by generating a multigene DNA phylogeny of several fungal isolates tentatively identified under this name. In the process of determining the generic boundaries of *Sphaerellopsis*, several morphologically similar genera also had to be elucidated, namely *Acrocalymma*, and a closely related phoma-like complex of lichenicolous fungi.

Species of *Sphaerellopsis* were shown to be congeneric with *Eudarluca*, the latter name being treated as a synonym based on the grounds that it is the younger name, and less commonly used in literature, even though it represents the

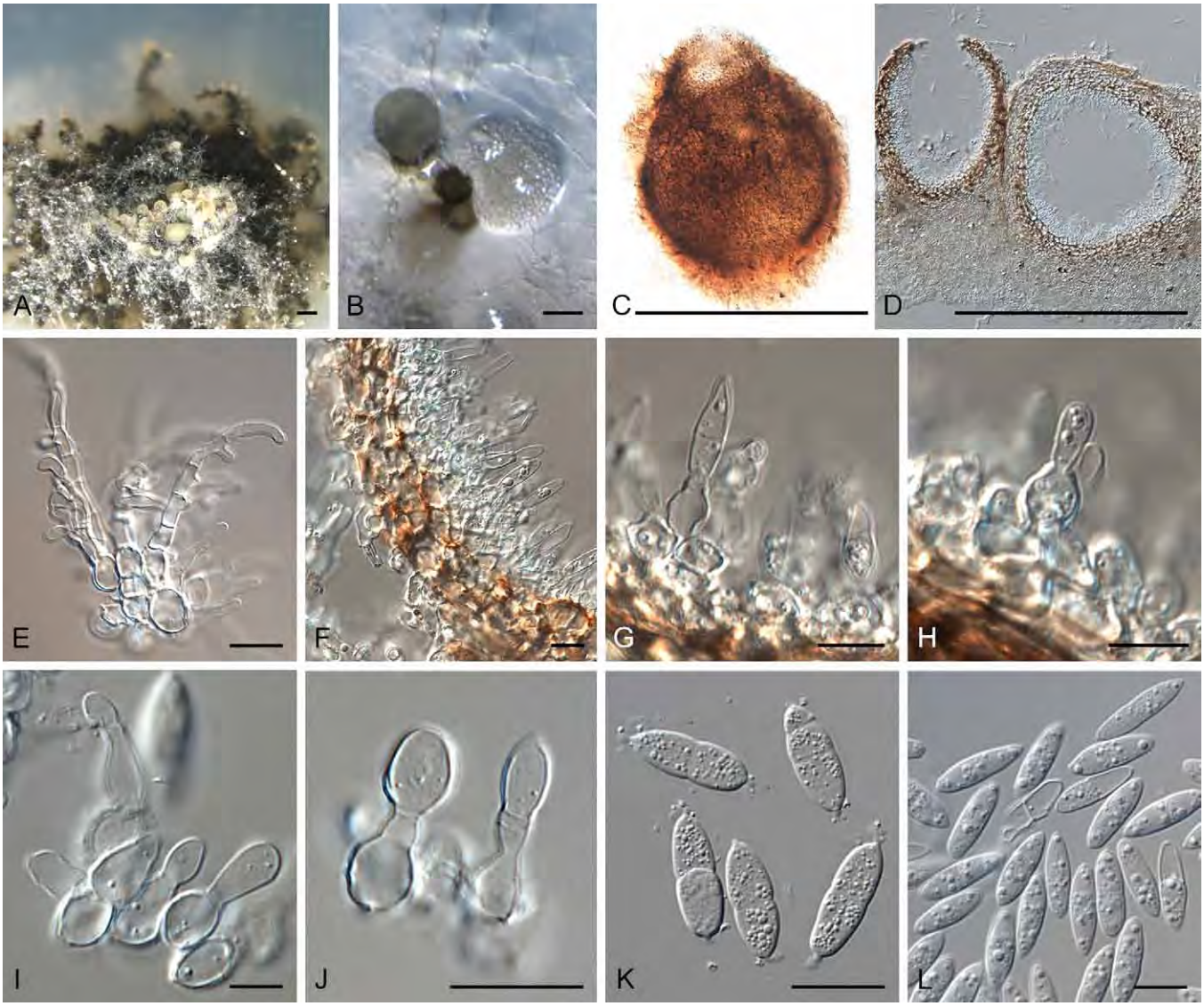

Fig. 16. *Sphaerellopsis paraphysata* (CBS 138579). **A.** Colony sporulating on SNA. **B, C.** Conidiomata formed in agar. **D.** Section through conidiomata. **E.** Paraphyses. **F–H.** Section through conidiomatal wall, showing conidiogenous cells. **I, J.** Conidiogenous cells. **K, L.** Conidia. Bars: A–D = 450 µm, E–L = 10 µm.

sexual morph. Furthermore, the application of the generic name is fixed in the sense that a neotype is designated for *S. filum*. As suspected in previous studies (Liesebach & Zaspel 2004, Nischwitz *et al*. 2005), *S. filum* was revealed to be a species complex, leading to the introduction of two new species names here, *S. paraphysata* (on a rust on *Pennisetum* sp. in Brazil, and on *Ravenelia macowania* on *Vachellia karroo* in South Africa), and *S. macroconidialis* (on *Uromyces caryophylli* on *Dianthus caryophyllus* in Italy, and on *Puccinia alii* on *Allium schoenoparsum*, and an unidentified rust on *Carex acutiformis* in The Netherlands). Furthermore, the genus *Neosphaerellopsis* was introduced to accommodate *N. thailandica* (occurring on a rust on *Bothriochloa bladhii* in Thailand), a species morphologically similar to *Sphaerellopsis s.str.*, but phylogenetically distinct.

The genus *Acrocalymma* (type species *A. medicaginis* on *Medicago* in Australia; Alcorn & Irwin 1987) proved to be morphologically similar to an isolate previously incorrectly identified as *S. filum* (CBS 317.76), which could subsequently be described as *A. fici* (on *Ficus* sp. from India). *Acrocalymma* was also shown to be phylogenetically closely related to the genus *Rhizopycnis* (type species *R. vagum*, described from *Cucumis* sp. in Texas; Farr *et al.* 1998), the only difference being that *R. vagum* lacks mucoid conidial caps, and that the conidia turn brown with age. Based on our molecular results and their morphology, these two generic names are congeneric, with *R. vagum* nestled between *A. medicaginis* and the recently described *A. aquatica* (Zhang *et al.* 2012), and therefore a new combination is proposed for *R. vagum* in *Acrocalymma*. Furthermore, species of *Acrocalymma* represented an undefined lineage in *Dothideomycetes*, for which the family name *Acrocalymmaceae* is introduced.

During the course of this study it became obvious that several DNA sequences deposited in GenBank as "*Phoma* sp." were unrelated to *Phoma s. str*. (Aveskamp *et al*. 2009, 2010, de Gruyter *et al*. 2009, 2010, 2013), but closely related to *Sphaerellopsis*. As no genera were available to accommodate these taxa, two new genera were introduced.

Diederichomyces is distinguished from *Phoma* in having dimorphic conidia, and forming orange crystals in culture, while *Xenophoma* is distinguished from *Phoma s. str.* in having cauliflower-shaped, uni- to multilocular conidiomata, and subspherical to ellipsoid conidia.

The genus *Neottiosporina* (type species *N. apoda*, on *Achyrocline saturejoides* from Argentina), is still poorly understood, with unknown phylogeny. *Neottiosporina apoda*, appears to be distinct from several other taxa presently accommodated in the genus, having pigmented, multi-septate conidia. The single species included in the present study and for which DNA data are available, *N. paspali* (CBS 331.37; from *Paspalum notatum*, Florida, USA), appears to be closely allied to *Stagonospora* in *Massarinaceae* (Fig. 1, also see Quaedvlieg *et al*. 2013). However, *S. paspali* (K(M) IMI 175641 ex herb. CUP) was allocated to *Neottiosporina* on the basis of the conidia being hyaline, 2-septate, and having apical, infundibuliform mucoid appendages (Sutton & Alcorn 1974). Unfortunately CBS 331.37 proved to be sterile, so this matter could not be resolved, but it appears likely that this strain was incorrectly identified.

What started out as a straightforward study to resolve the generic synonymy of *Sphaerellopsis* and *Eudarluca*, quickly snowballed into a wider study even including several new phoma-like genera. Although we have tried to designate clear morphological characters to separate these genera, it will be difficult if not impossible to separate *Sphaerellopsis* from *Neosphaerellopsis* without the aid of DNA data, and even more so to distinguish all the known, and as yet undescribed species of *Sphaerellopsis.* Further research on genera of coelomycetes and their sexual morphs is urgently required, merging morphology with DNA phylogenetic data, and fixing the application of these generic names *via* neo- and epitypification where appropriate.

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