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Article

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Taxonomic circumscription and phylogenetics of novel didymellaceous taxa with brown muriform spores

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Abstract

Sexual morph of didymellaceous taxa are characterized by their ascomata with relatively thin peridium, cylindric-clavate to clavate, short-pedicellate or apedicellate asci, hyaline to brown, 1-septate to muriform ascospores. Its asexual morphs are coelomycetous and comprising pycnidial or acervulus conidiomata, phialidic, hyaline conidiogenous cells and hyaline or pale brown, septate or aseptate conidia. The majority of these cosmopolitan species are plant associated fungi which can be pathogens on a wide range of hosts and some species are of particular relevance for quarantine measures. Recent studies have significantly improved the taxonomy and systematics of didymellaceous taxa based on molecular phylogenetics. In contrast to the accurate and detailed studies on the asexual morphs which are common obligate pathogens, information on their usually saprobic sexual morphs is still limited. Among these phenotypically diverse species, spore characteristics are quite unique as most have hyaline spores with 0–1 septum, while only *Neomicrosphaeropsis* and *Didymellocamarosporium* are reported as producing pigmented, muriform spores. These dematiaceous muriform spores are characteristic of a considerable number of species that may be quite divergent in other characters. During taxonomic investigations on the diversity of didymellaceous taxa, we have isolated species from *Alhagi pseudalhagi*, *Coronilla emerus*, *Cytisus* sp., *Elaeagnus angustifolia* and *Spartium junceum* in Italy, Russia and Uzbekistan. A comprehensive phylogeny, based on four loci (ITS, LSU, *rpb2* and *tub2*) is used to infer species relationships. Comprehensive morphological descriptions and in-depth phylogenetic investigations of five new species viz. *Ascochyta coronillae-emeri*, *Microsphaeropsis spartii-juncei*, *Neomicrosphaeropsis alhagi-pseudalhagi*, *N. cytisicola* and *N. elaeagni* are presented.

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Introduction

The family Didymellaceae was proposed by de Gruyter et al. (2009) to accommodate phoma-like taxa, *viz. Ascochyta, Didymella* and *Phoma*, which probably diverged in the Jurassic or earlier from an ancestor whose origin can be estimated about 63 mya (crown age) or 115 mya (stem age) ago (Liu et al. 2017). Didymellaceae is one of the most species-rich families in the fungal kingdom and includes 4956 and 4713 taxon epithets listed in MycoBank and Index Fungorum, respectively (2017). More than 50% from the total epithets are listed as *Phoma* and over 30% are recorded as *Ascochyta*. In a recent study, Chen et al. (2017) revised Didymellaceae and improved our understanding of their distribution and biodiversity. They have proposed 19 genera in the family and currently the family comprises 31 genera, including *Cumuliphoma*, *Didymellocamarosporium*, *Didysimulans*, *Ectophoma*, *Endocoryneum*, *Juxtiphoma*, *Neodidymella*, *Pseudoascochyta*, *Pseudohendersonia*, *Remotididymella*, *Similiphoma* and *Vacuiphoma* (Ariyawansa et al. 2015, Crous et al. 2016, Wijayawardene et al. 2016, 2018, Tibpromma et al. 2017, Valenzuela-Lopez et al. 2018). The majority of members in Didymellaceae are plant associated fungi which can be pathogens on a wide range of hosts, largely causing leaf and stem lesions, with some of particular relevance for quarantine measures (Aveskamp et al. 2008, 2010, Chen et al. 2015, 2017). Didymellaceae are cosmopolitan and able to adapt to extreme environmental conditions *i.e*. temperature, nutrients, moisture, absolute darkness and they can grow in exposed habitats such as air, soil, water, limestone from caves (Chen et al. 2017) and inorganic materials including asbestos, cement and paint (Aveskamp et al. 2008). Given their ubiquitous nature, additional taxonomic and ecological knowledge are prerequisites to understand their biology and their significance in the environment, especially in agriculture.

In contrast to the accurate and detailed studies on their asexual morphs, information is still limited on their sexual morphs, which usually grow as saprobes, in contrast to their pathogenic asexual counterparts (Chen et al. 2017). Determining the phylogenetic placement of sexual morphs is crucial to properly define the taxonomic boundaries within the polyphyletic and morphologically homogeneous genera (*i.e. Ascochyta*, *Didymella* and *Phoma*). Knowledge of the sexual-asexual relationships will considerably improve our understanding of many of the specific biological features. Of the 28 genera in this family, sexual morphs are known for 12 genera (Jayasiri et al. 2017) and their ascospores are mostly hyaline and 1-septate. There is only one sexual morph recorded in this family with pigmented muriform spores, *Neomicrosphaeropsis tamaricicola* (= *Phoma tamaricicola*), introduced by Crous et al. (2014). Pigmented muriform spores are characteristic for a considerable number of species being divergent in other characters. For asexual morphs, *Didymellocamarosporium tamaricis* (Wijayawardene et al. 2016) is the only asexual member recorded with pigmented muriform conidia in this family.

We are investigating the diversity of microfungi that produce brown, muriform spores with the aim of clarifying their taxonomy based on morphology coupled with multigene phylogeny (Wanasinghe et al. 2014a, b, 2015, 2016, 2017a, b, 2018). As part of this study, we have isolated taxa from *Alhagi pseudalhagi*, *Coronilla emerus*, *Cytisus* sp., *Elaeagnus angustifolia* and *Spartium junceum* species in Italy, Russia and Uzbekistan which belong to the family Didymellaceae. Here we present comprehensive morphological descriptions and in-depth phylogenetic investigation of those taxa.

Materials and Methods

Sampling, examination and isolation

The novel strains were isolated from *Alhagi pseudalhagi*, *Coronilla emerus*, *Cytisus* sp., *Elaeagnus angustifolia* and *Spartium junceum* in Italy and Russia. Uzbekistan specimens were loaned from Tashkent Mycological Herbarium (TASM) of the Institute of Botany, Academy of

Sciences of Uzbekistan, Tashkent. These collections were examined and isolated following the methods used by Wanasinghe et al. (2017a). Type and isotype specimens of new species in this study are deposited in the Mae Fah Luang University (MFLU) Herbarium. Living cultures are deposited at the Culture Collection of Mae Fah Luang University (MFLUCC) and duplicated in International Collection of Microorganisms from Plants (ICMP), Landcare Research, Auckland, New Zealand.

DNA isolation, amplification and phylogenetic analyses

Total genomic DNA was extracted from fresh mycelia using the protocol described by Wanasinghe et al. (2017a). When fungi failed to grow in culture, DNA was extracted directly from ascomycete fruiting bodies by following the protocol described by Wanasinghe et al. (2018). DNA to be used as template for PCR were stored at 4 °C for use in regular work and duplicated at -20 °C for long term storage. The primers ITS5 and ITS4 (White et al. 1990) were used to amplify part of rDNA 18S (3' end), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2), and part of the 28S rRNA (5' end); the primers LR0R (Rehner & Samuels 1994), LR5 (Vilgalys & Hester 1990) were used for LSU amplification; Btub2Fd and Btub4Rd (Woudenberg et al. 2009) for the partial β-tubulin (*tub2*) gene region, and RPB2-5F (Sung et al. 2007) and fRPB2-7cR (Liu et al. 1999) for the RNA polymerase II second largest subunit (*rpb2*). Amplicons for ITS and LSU locus were generated following the protocols listed in Wanasinghe et al. (2017a) and the protocols of Chen et al. (2015) were used to amplify *tub2* and *rpb2*.

Sequencing was conducted in both directions with the same primer pair used for amplification at BGI, Ltd., Shenzhen, P.R. China. Consensus sequences were assembled in BioEdit v. 7.0.5.2 (Hall 1999) and additional reference sequences were obtained from GenBank (Table 1). Subsequent alignments for each locus were generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Kuraku et al. 2013, Katoh et al. 2017), and manually corrected when necessary in BioEdit v7.0.9 (Hall 1999). Each locus and the concatenated aligned dataset were analysed separately using Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI). The best-fit models of evolution for the four loci tested (GTR+I+G for all gene regions) were estimated by MrModeltest v. 2.3 (Nylander 2004).

Parsimony analysis was carried out with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 with the following parameter settings: characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length was zero. Alignment gaps were treated as missing characters in the analysis of the combined data set, where they occurred in relatively conserved regions. Trees were inferred using the heuristic search option with 1000 random sequence additions, with maxtrees set at 5000. Descriptive tree statistics for parsimony; tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC) and homoplasy index (HI) were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Other details pertaining to analyses (e.g. consideration of TT ratios, comparison of tree topologies and selection of outgroups) are outlined in Jeewon et al. (2003a, b, 2004, 2013).

Bayesian (BI) analyses were performed on MrBayes v. 3.2.1 (Ronquist et al. 2012) based on the models selected by the MrModeltest. The Markov Chain Monte Carlo (MCMC) algorithm of six chains was initiated for 5 M generations in parallel from a random tree topology. The trees were sampled every 200th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer v. 1.5 (Rambaut & Drummond 2007). All sampled topologies beneath the asymptote (10 %) were discarded as part of a burn-in procedure; the remaining trees were used for calculating PP in the majority rule consensus tree. Posterior probabilities values of the BI analyses (BYPP) over 0.95 were considered significant.

The ML analyses were conducted with RAxML-HPC BlackBox (v. 8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using a GTR+I+G substitution model with 1 000 bootstrap replicates. The robustness of the analyses was evaluated by bootstrap support (MLBS).

Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2007) and Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA).

One hundred and twenty-six taxa are used (including our newly generated sequences) as ingroup taxa, *Leptosphaeria conoidea* (CBS 616.75) and *L. doliolum* (CBS 505.75) were selected as outgroup taxa. Sequences generated in this study were deposited in GenBank (Table 1), the final matrices and trees in TreeBASE (accession number: 22328), (Study Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S22328) and novel taxonomic descriptions and nomenclature in Faces of Fungi and Index Fungorum as outlined in Jayasiri et al. (2015), Index Fungorum (2018). New species were established based on recommendations outlined by Jeewon & Hyde (2016).

Table 1 Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold.

Species	Strain no[1]	Status[2]	GenBank Accession no[3]			
			LSU	ITS	RPB2	TUB
Allophoma minor	CBS 325.82	T	GU238107	GU237831	KT389553	GU237632
Allophoma nicaraguensis	CBS 506.91	T	GU238058	GU237876	KT389551	GU237596
Allophoma piperis	CBS 268.93	T	GU238129	GU237816	KT389554	GU237644
Allophoma tropica	CBS 436.75	T	GU238149	GU237864	KT389556	GU237663
Ascochyta boeremae	CBS 372.84	T	KT389697	KT389480		KT389774
Ascochyta boeremae	CBS 373.84		KT389698	KT389481	KT389560	KT389775
Ascochyta coronillae-emeri	**MFLUCC 13-0820**	**T**	**MH069661**	**MH069667**	**MH069679**	**MH069686**
Ascochyta herbicola	CBS 629.97	R	GU238083	GU237898	KP330421	GU237614
Ascochyta medicaginicola var. macrospora	CBS 112.53	T	GU238101	GU237749		GU237628
Ascochyta medicaginicola var. macrospora	BRIP 45051		KY742198	KY742044	KY742132	KY742286
Ascochyta medicaginicola var. medicaginicola	MFLUCC 16-0599		KX698025	KX698036	KX698033	KX698029
Ascochyta phacae	CBS 184.55	T	KT389692	KT389475		KT389769
Ascochyta pisi	CBS 122751		KP330444	KP330432	EU874867	KP330388
Ascochyta rabiei	CBS 206.30		KT389695	KT389478	KT389559	KT389772
Ascochyta rabiei	CBS 237.37	T	KT389696	KT389479		KT389773
Ascochyta rabiei	CBS 534.65		GU237970	GU237886	KP330405	GU237533
Boeremia exigua var. heteromorpha	CBS 443.94	T	GU237935	GU237866	KT389573	GU237497
Boeremia exigua var. opuli	CGMCC 3.18354	T	KY742199	KY742045	KY742133	KY742287
Boeremia hedericola	CBS 367.91	R	GU237949	GU237842	KT389579	GU237511
Boeremia hedericola	CBS 367.91	R	GU237949	GU237842	KT389579	GU237511
Briansuttonomyces eucalypti	CBS 114879	T	KU728519	KU728479		KU728595
Briansuttonomyces eucalypti	CBS 114887		KU728520	KU728480		KU728596
Calophoma aquilegiicola	CBS 107.96	R	GU238041	GU237735	KT389586	GU237581
Calophoma clematidina	CBS 102.66		FJ515630	FJ426988	KT389587	FJ427099
Calophoma clematidina	CBS 108.79	T	FJ515632	FJ426989	KT389588	FJ427100

Table 1 Continued.

Species	Strain no[1]	Status[2]	GenBank Accession no[3]			
			LSU	ITS	RPB2	TUB
Calophoma rosae	CGMCC 3.18347	T	KY742203	KY742049	KY742135	KY742291
Cumuliphoma indica	CBS 654.77	T	GU238122	FJ427043	LT623261	FJ427153
Cumuliphoma omnivirens	CBS 341.86	T	LT623214	FJ427042	LT623260	FJ427152
Cumuliphoma pneumoniae	CBS 142454	T	LN907392	LT592925	LT593063	LT592994
Didymella aquatica	CGMCC 3.18349	T	KY742209	KY742055	KY742140	KY742297
Didymella arachidicola	CBS 333.75	T	GU237996	GU237833	KT389598	GU237554
Didymella exigua	CBS 183.55	T	EU754155	GU237794	EU874850	GU237525
Didymella heteroderae	CBS 109.92	T	GU238002	FJ426983	KT389601	FJ427098
Didymella macrophylla	CGMCC 3.18357	T	KY742224	KY742070	KY742154	KY742312
Didymellocamarosporium tamaricis	MFLUCC 14-0241	T	KU848183			
Didysimulans italica	MFLUCC 15-0059	T	KY496730	KY496750	KY514408	
Didysimulans mezzanensis	MFLUCC 15-0067	T	KY496733	KY496753	KY514411	
Ectophoma multirostrata	CBS 274.60	T	GU238111	FJ427031	LT623265	FJ427141
Ectophoma multirostrata	CBS 368.65		GU238112	FJ427033	LT623266	FJ427143
Ectophoma pomi	CBS 267.92	T	GU238128	GU237814	LT623263	GU237643
Endocoryneum festucae	MFLUCC 14-0461	T	KU848203			
Epicoccum brasiliense	CBS 120105	T	GU238049	GU237760	KT389627	GU237588
Epicoccum camelliae	CGMCC 3.18343	T	KY742245	KY742091	KY742170	KY742333
Epicoccum huancayense	CBS 105.80	T	GU238084	GU237732	KT389630	GU237615
Epicoccum latusicollum	CGMCC 3.18346	T	KY742255	KY742101	KY742174	KY742343
Epicoccum nigrum	CBS 173.73	T	GU237975	FJ426996	KT389632	FJ427107
Heterophoma verbascicola	CGMCC 3.18364	T	KY742273	KY742119	KY742187	KY742361
Heterophoma verbascicola	LC 8164		KY742274	KY742120	KY742188	KY742362
Heterophoma adonidis	CBS 114309		KT389724	KT389506	KT389637	KT389803
Heterophoma dictamnicola	CBS 507.91		GU238065	GU237877	KT389638	GU237603
Juxtiphoma eupyrena	CBS 374.91		GU238072	FJ426999	LT623268	FJ427110
Juxtiphoma eupyrena	CBS 527.66		GU238073	FJ427000	LT623269	FJ427111
Leptosphaeria conoidea	CBS 616.75		JF740279	JF740201	KT389639	KT389804
Leptosphaeria doliolum	CBS 505.75	T	GQ387576	JF740205	KT389640	JF740144
Leptosphaerulina americana	CBS 213.55		GU237981	GU237799	KT389641	GU237539
Leptosphaerulina arachidicola	CBS 275.59		GU237983	GU237820		GU237543
Leptosphaerulina australis	CBS 317.83		EU754166	GU237829	GU371790	GU237540
Leptosphaerulina trifolii	CBS 235.58		GU237982	GU237806		GU237542
Macroventuria anomochaeta	CBS 502.72		GU237985	GU237873		GU237545
Macroventuria anomochaeta	CBS 525.71	T	GU237984	GU237881	GU456346	GU237544
Macroventuria wentii	CBS 526.71	T	GU237986	GU237884	KT389642	GU237546
Microsphaeropsis olivacea	CBS 442.83		EU754171	GU237865		GU237547
Microsphaeropsis olivacea	CBS 233.77		GU237988	GU237803	KT389643	GU237549

Table 1 Continued.

Species	Strain no[1]	Status[2]	GenBank Accession no[3]			
			LSU	ITS	RPB2	TUB
Microsphaeropsis olivacea	CBS 432.71		GU237987	GU237863		GU237548
Microsphaeropsis olivacea	MFLUCC 14-0507		KR025863	KR025859		
Microsphaeropsis proteae	CPC 1425		JN712563	JN712497		JN712650
Microsphaeropsis proteae	CPC 1424		JN712562	JN712496		JN712649
Microsphaeropsis proteae	CPC 1423		JN712561	JN712495		
Microsphaeropsis spartii-juncei	**MFLU 16-0100**	**T**	**MH069663**	**MH069669**	**MH069681**	**MH069688**
Microsphaeropsis spartii-juncei	**MFLU 16-0097**		**MH069662**	**MH069668**	**MH069680**	**MH069687**
Neoascochyta desmazieri	CBS 297.69	T	KT389726	KT389508	KT389644	KT389806
Neoascochyta europaea	CBS 820.84	T	KT389729	KT389511	KT389646	KT389809
Neoascochyta paspali	CBS 560.81	T	GU238124	FJ427048	KP330426	FJ427158
Neoascochyta triticicola	CBS 544.74	T	EU754134	GU237887	KT389652	GU237488
Neodidymella thailandicum	MFLUCC 11-0140	T	MG520976	MG520956		
Neodidymelliopsis achlydis	CBS 256.77	T	KT389749	KT389531		KT389829
Neodidymelliopsis cannabis	CBS 234.37		GU237961	GU237804	KP330403	GU237523
Neodidymelliopsis polemonii	CBS 109181	T	GU238133	GU237746	KP330427	GU237648
Neodidymelliopsis xanthina	CBS 383.68	T	GU238157	GU237855	KP330431	GU237668
Neomicrosphaeropsis alhagi-pseudalhagi	**MFLUCC 17-0825**	**T**	**MH069664**	**MH069670**	**MH069682**	**MH069689**
Neomicrosphaeropsis cytisi	MFLUCC 13–0396		KX572342	KX572337	KX572355	
Neomicrosphaeropsis cytisicola	**MFLU 16-0114**	**T**	**MH069665**	**MH069671**	**MH069683**	**MH069690**
Neomicrosphaeropsis cytisinus	MFLUCC 16-0790	T	KX611241			
Neomicrosphaeropsis elaeagni	**MFLUCC 17-0740**	**T**	**MH069666**	**MH069672**	**MH069684**	**MH069691**
Neomicrosphaeropsis italica	MFLUCC 15-0485	T	KU729854	KU900318	KU674820	
Neomicrosphaeropsis italica	MFLUCC 15-0484		KU729853	KU900319	KU695539	KX453298
Neomicrosphaeropsis italica	MFLUCC 16-0284		KU900296	KU900321		KX453299
Neomicrosphaeropsis minima	MFLUCC 13–0394		KX572341	KX572336		
Neomicrosphaeropsis novorossica	MFLUCC 14-0578	T	KX198710	KX198709		
Neomicrosphaeropsis rossica	MFLUCC 14-0586	T	KU729855	KU752192		
Neomicrosphaeropsis tamaricicola	MFLUCC 14-0443		KU729851	KU900322		
Neomicrosphaeropsis tamaricicola	MFLUCC 14-0439		KU729858	KU900323		
Neomicrosphaeropsis tamaricicola	MFLUCC 14-0602	T	KM408754	KM408753	**MH069684**	**MH069691**
Nothophoma anigozanthi	CBS 381.91	T	GU238039	GU237852	KT389655	GU237580

Table 1 Continued.

Species	Strain no[1]	Status[2]	GenBank Accession no[3]			
			LSU	ITS	RPB2	TUB
Nothophoma arachidis-hypogaeae	CBS 125.93	R	GU238043	GU237771	KT389656	GU237583
Nothophoma gossypiicola	CBS 377.67		GU238079	GU237845	KT389658	GU237611
Nothophoma infossa	CBS 123395	T	GU238089	FJ427025	KT389659	FJ427135
Nothophoma quercina	CBS 633.92		EU754127	GU237900	KT389657	GU237609
Paraboeremia adianticola	CBS 187.83		GU238035	GU237796	KP330401	GU237576
Paraboeremia camellae	CGMCC 3.18106	T	KX829042	KX829034	KX829050	KX829058
Paraboeremia litseae	CGMCC 3.18109	T	KX829037	KX829029	KX829045	KX829053
Paraboeremia oligotrophica	CGMCC 3.18111	T	KX829039	KX829031	KX829047	KX829055
Paraboeremia selaginellae	CBS 122.93	T	GU238142	GU237762		GU237656
Phoma herbarum	CBS 134.96		KT389753	KT389535	KT389661	KT389834
Phoma herbarum	CBS 274.37		KT389754	KT389537	KT389662	KT389835
Phoma herbarum	CBS 377.92		KT389756	KT389536	KT389663	KT389837
Phoma herbarum	CBS 502.91		GU238082	GU237874	KP330419	GU237613
Phoma herbarum	CBS 615.75	R	EU754186	FJ427022	KP330420	FJ427133
Phomatodes aubrietiae	CBS 383.67	R	GU238044	GU237854		GU237584
Phomatodes aubrietiae	CBS 627.97	T	GU238045	GU237895	KT389665	GU237585
Phomatodes nebulosa	CBS 117.93		GU238114	GU237757	KP330425	GU237633
Phomatodes nebulosa	CBS 740.96		KT389758	KT389540	KT389667	KT389839
Phomatodes nebulosa	CBS 100191		KP330446	KP330434	KT389666	KP330390
Pseudoascochyta novae-zelandiae	CBS 141689		LT592893	LT592892	LT592895	LT592894
Pseudohendersonia galiorum	MFLUCC 14 – 0452	T	KU848207			
Remotididymella anthropophila	CBS 142462	T	LN907421	LT592936	LT593075	LT593005
Remotididymella destructiva	CBS 133.93		GU238064	GU237779	LT623257	GU237602
Remotididymella destructiva	CBS 378.73	T	GU238063	GU237849	LT623258	GU237601
Similiphoma crystallifera	CBS 193.82	T	GU238060	GU237797	LT623267	GU237598
Stagonosporopsis actaeae	CBS 106.96	T	GU238166	GU237734	KT389672	GU237671
Stagonosporopsis crystalliniformis	CBS 713.85	T	GU238178	GU237903	KT389675	GU237683
Stagonosporopsis dennisii	CBS 631.68	T	GU238182	GU237899	KT389677	GU237687
Stagonosporopsis helianthi	CBS 200.87	T	KT389761	KT389545	KT389683	KT389848
Vacuiphoma bulgarica	CBS 357.84	T	GU238050	GU237837	LT623256	GU237589
Vacuiphoma oculihominis	UTHSC DI16-308	T	LN907451	LT592954	LT593093	LT593023
Xenodidymella applanata	CBS 195.36	T	KT389764	KT389548		KT389852
Xenodidymella applanata	CBS 115577		KT389762	KT389546	KT389688	KT389850
Xenodidymella catariae	CBS 102635		GU237962	GU237727	KP330404	GU237524

[1] BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Westerdijk Fungal Biodiversity Institute (formerly CBSKNAW), Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection, Beijing, China; CPC: Culture collection of Pedro Crous, housed at CBS; LC: Corresponding author's personal collection deposited in laboratory, housed at CAS, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA.

[2] T: ex-type strain; R: representative strain.
[3] ITS: internal transcibed spacer regions 1 & 2 including 5.8S nrDNA gene; LSU: 28S large subunit of the nrRNA gene; *rpb2*: RNA polymerase II second subunit; *tub2*: ß-tubulin.

Results and Discussion

Phylogenetic analyses

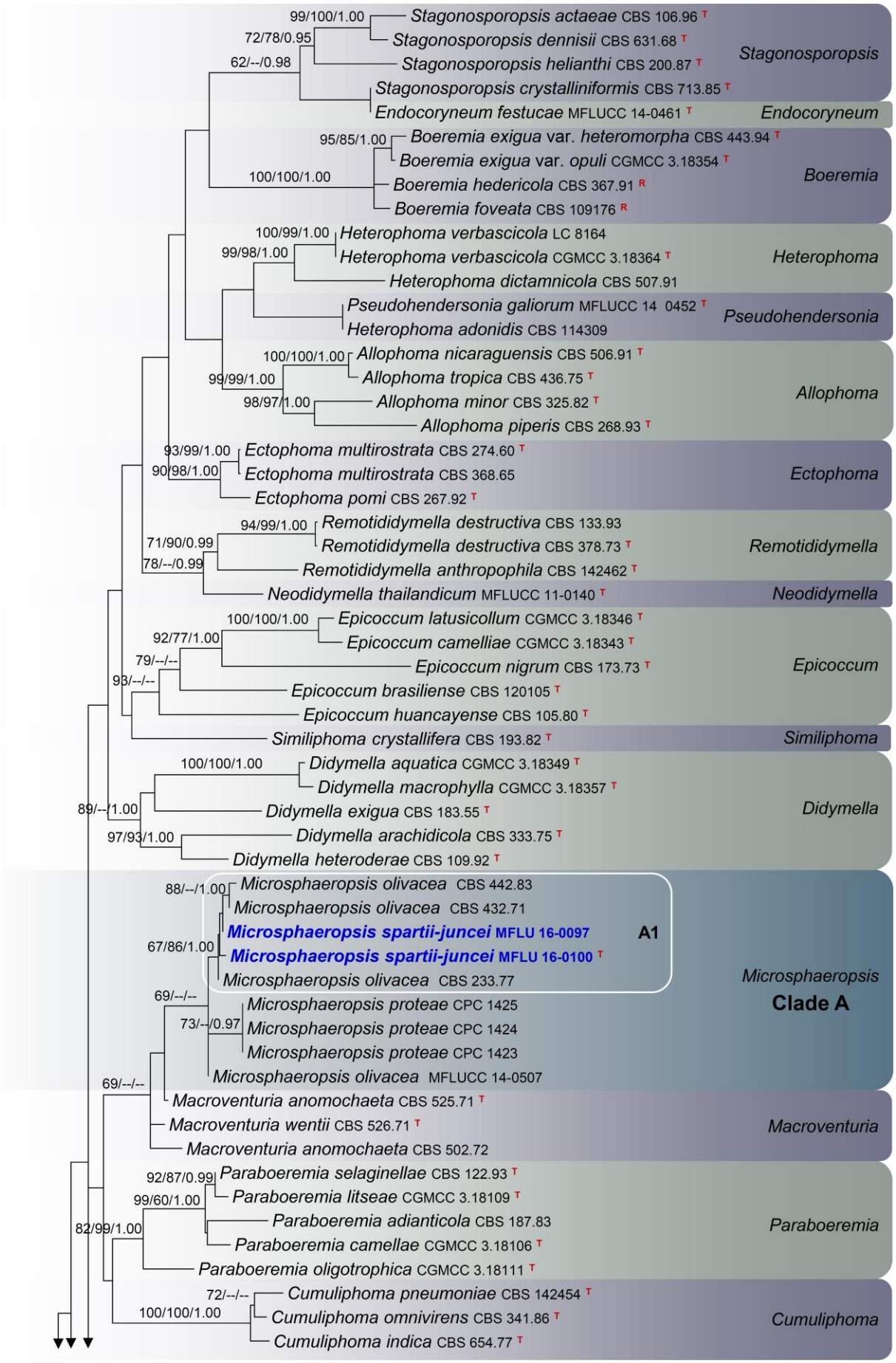
Topologies of trees (under ML, MP and BI criteria) recovered for each gene dataset were visually compared and the overall tree topology was congruent to those obtained from the combined dataset.

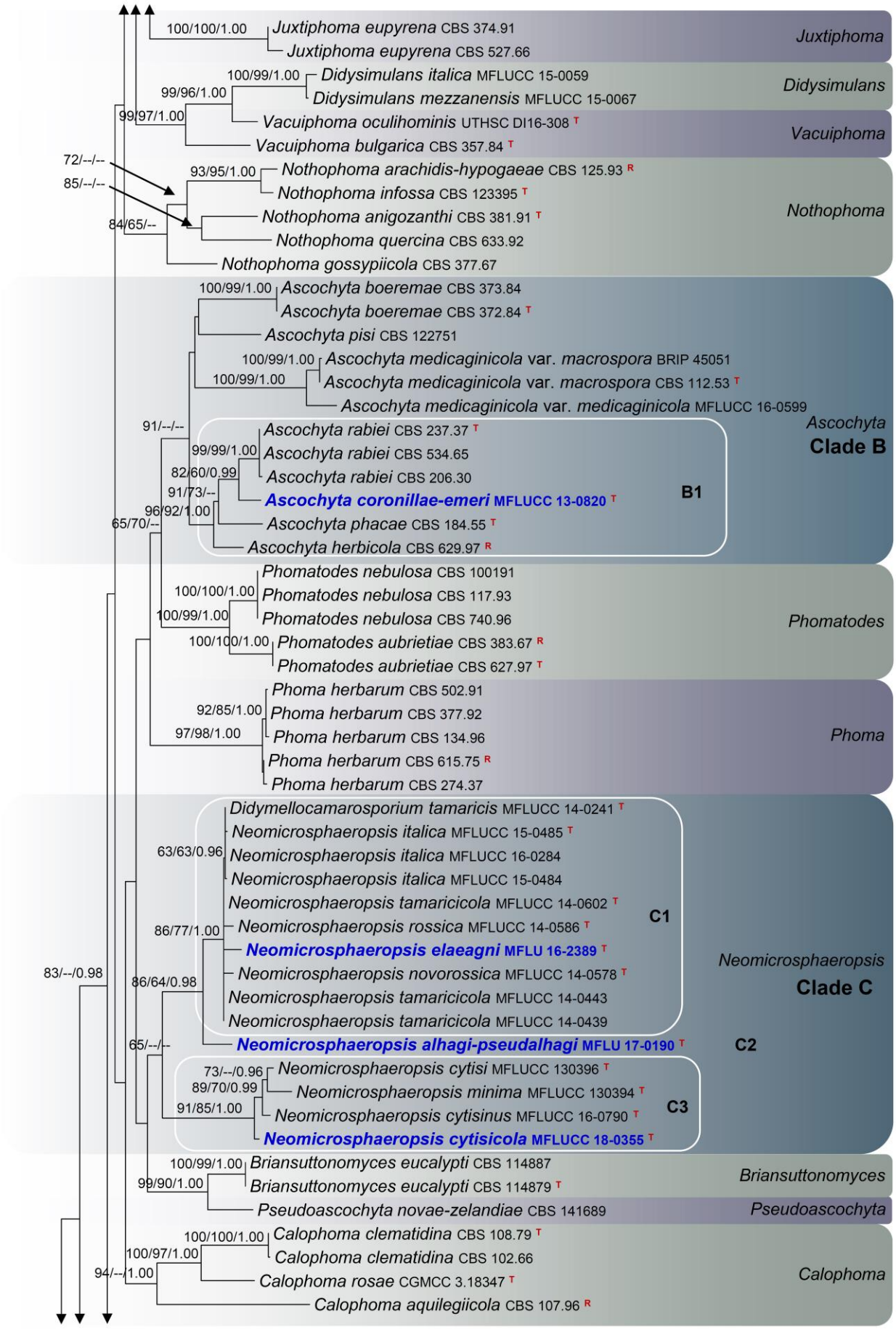
The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -23881.01104. The matrix had 734 distinct alignment patterns, with 8.95 % proportion of gaps and completely undetermined characters in this alignment. Parameters for the GTR + I + G model of the combined LSU, ITS, *rpb2* and *tub2* were as follows: Estimated base frequencies were as follows: A = 0.238058, C = 0.241410, G = 0.27525, T = 0.245283; substitution rates AC = 1.943648, AG = 6.96474, AT = 2.220889, CG = 0.925886, CT = 14.019529, GT = 1.000; proportion of invariable sites I = 0.63074; gamma distribution shape parameter α = 0.584276. The maximum parsimonious dataset for the combined gene sequences consisted of 2231 characters, of which 1560 were constant, 615 (27.6 %) parsimony-informative and 56 parsimony-uninformative. The parsimony analysis of the data matrix resulted in the maximum of 2325 equally most parsimonious trees with a length of 4662 steps (CI = 0.238, RI = 0.636, RC = 0.151, HI = 0.762) in the first tree. The Bayesian analysis resulted in 25001 trees after 5 M generations with 0.009735 as the average standard deviation of split frequency. Therefore, the first 2500 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 22501 trees were used or calculating posterior probabilities in the majority rule consensus tree.

Newly generated sequences from two *Microsphaeropsis* isolates (MFLU 16-0100 and MFLU 16-0097) grouped with isolates currently circumscribed as *Microsphaeropsis olivacea* and *M. proteae* (de Gruyter et al. 2009, Aveskamp et al. 2010, Crous et al. 2011, Verkley et al. 2014, Chen et al. 2015). These taxa formed an isolated clade (Clade A, Fig 1) within Didymellaceae, but poorly supported in multi-gene analyses (69% in ML, <60 % in MP and <0.95 in BI). Within Clade A (Fig 1), our novel isolates are closely related and monophyletic with *Microsphaeropsis olivacea* (CBS 442.83, CBS 432.71, CBS 233.77) and retrieved 67% (ML), 86% (MP), 1.00 (BI) bootstrap support for this lineage (Subclade A1).

Ascochyta coronillae-emeri (MFLUCC 13-0820), showed a close phylogenetic affinity to *A. rabiei* (CBS 206.30, CBS 237.37, CBS 534.65), *A. phacae* (CBS 184.55) and *A. herbicola* (CBS 629.97) in the combined phylogeny (Subclade B1) and this relationship retrieved 96% ML, 92% MP and 1.00 BI support.

Three newly generated sequences, *Neomicrosphaeropsis alhagi-pseudalhagi* (MFLUCC 17-0825), *N. cytisicola* (MFLU 16-0114) and *N. elaeagni* (MFLUCC 17-0740), grouped with *Didymellocamarosporium tamaricis* and eleven *Neomicrosphaeropsis* isolates. These taxa form a monophyletic clade (Clade C) in Didymellaceae with poor statistical support (65% in ML, <60 % in MP and <0.95 in BI). *Didymellocamarosporium tamaricis*, *Neomicrosphaeropsis elaeagni* sp. nov., *N. italica*, *N. novorossica*, *N. rossica* and *N. tamaricicola* forms a subclade (Subclade C1) in the combined phylogeny with 86% ML 77% MP and 1.00 BI support. *Neomicrosphaeropsis cytisi*, *N. cytisicola* sp. nov., *N. cytisinus* and *N. minima* forms a separate cluster (Subclade C3) within Clade C with high statistical support (91% ML, 84% MP and 1.00 BI). *Neomicrosphaeropsis alhagi-pseudalhagi* sp. nov. nested in between subclades C1 and C3.


99/100/1.00
Stagonosporopsis actaeae CBS 106.96 T
72/78/0.95
Stagonosporopsis dennisii CBS 631.68 T
62/--/0.98
Stagonosporopsis helianthi CBS 200.87 T
Stagonosporopsis crystalliniformis CBS 713.85 T
Stagonosporopsis
Endocoryneum festucae MFLUCC 14-0461 T
Endocoryneum
95/85/1.00
Boeremia exigua var. heteromorpha CBS 443.94 T
Boeremia exigua var. opuli CGMCC 3.18354 T
100/100/1.00
Boeremia hedericola CBS 367.91 R
Boeremia foveata CBS 109176 R
Boeremia
100/99/1.00
Heterophoma verbascicola LC 8164
99/98/1.00
Heterophoma verbascicola CGMCC 3.18364 T
Heterophoma dictamnicola CBS 507.91
Heterophoma
Pseudohendersonia galiorum MFLUCC 14 0452 T
Heterophoma adonidis CBS 114309
Pseudohendersonia
100/100/1.00
Allophoma nicaraguensis CBS 506.91 T
99/99/1.00
Allophoma tropica CBS 436.75 T
98/97/1.00
Allophoma minor CBS 325.82 T
Allophoma piperis CBS 268.93 T
Allophoma
93/99/1.00
Ectophoma multirostrata CBS 274.60 T
90/98/1.00
Ectophoma multirostrata CBS 368.65
Ectophoma pomi CBS 267.92 T
Ectophoma
94/99/1.00
Remotididymella destructiva CBS 133.93
71/90/0.99
Remotididymella destructiva CBS 378.73 T
78/--/0.99
Remotididymella anthropophila CBS 142462 T
Remotididymella
Neodidymella thailandicum MFLUCC 11-0140 T
Neodidymella
100/100/1.00
Epicoccum latusicollum CGMCC 3.18346 T
92/77/1.00
Epicoccum camelliae CGMCC 3.18343 T
79/--/--
Epicoccum nigrum CBS 173.73 T
93/--/--
Epicoccum brasiliense CBS 120105 T
Epicoccum huancayense CBS 105.80 T
Epicoccum
Similiphoma crystallifera CBS 193.82 T
Similiphoma
100/100/1.00
Didymella aquatica CGMCC 3.18349 T
Didymella macrophylla CGMCC 3.18357 T
89/--/1.00
Didymella exigua CBS 183.55 T
97/93/1.00
Didymella arachidicola CBS 333.75 T
Didymella heteroderae CBS 109.92 T
Didymella
88/--/1.00
Microsphaeropsis olivacea CBS 442.83
Microsphaeropsis olivacea CBS 432.71
Microsphaeropsis spartii-juncei MFLU 16-0097
A1
67/86/1.00
Microsphaeropsis spartii-juncei MFLU 16-0100 T
Microsphaeropsis olivacea CBS 233.77
69/--/--
Microsphaeropsis proteae CPC 1425
73/--/0.97
Microsphaeropsis proteae CPC 1424
Microsphaeropsis proteae CPC 1423
Microsphaeropsis olivacea MFLUCC 14-0507
Microsphaeropsis
Clade A
69/--/--
Macroventuria anomochaeta CBS 525.71 T
Macroventuria wentii CBS 526.71 T
Macroventuria anomochaeta CBS 502.72
Macroventuria
92/87/0.99
Paraboeremia selaginellae CBS 122.93 T
99/60/1.00
Paraboeremia litseae CGMCC 3.18109 T
82/99/1.00
Paraboeremia adianticola CBS 187.83
Paraboeremia camellae CGMCC 3.18106 T
Paraboeremia oligotrophica CGMCC 3.18111 T
Paraboeremia
72/--/--
Cumuliphoma pneumoniae CBS 142454 T
100/100/1.00
Cumuliphoma omnivirens CBS 341.86 T
Cumuliphoma indica CBS 654.77 T
Cumuliphoma


100/100/1.00
Juxtiphoma eupyrena CBS 374.91
Juxtiphoma eupyrena CBS 527.66
Juxtiphoma
100/99/1.00
Didysimulans italica MFLUCC 15-0059
99/96/1.00
Didysimulans mezzanensis MFLUCC 15-0067
Didysimulans
99/97/1.00
Vacuiphoma oculihominis UTHSC DI16-308 T
Vacuiphoma bulgarica CBS 357.84 T
Vacuiphoma
72/--/--
85/--/--
93/95/1.00
Nothophoma arachidis-hypogaeae CBS 125.93 R
Nothophoma infossa CBS 123395 T
Nothophoma anigozanthi CBS 381.91 T
84/65/--
Nothophoma quercina CBS 633.92
Nothophoma gossypiicola CBS 377.67
Nothophoma
100/99/1.00
Ascochyta boeremae CBS 373.84
Ascochyta boeremae CBS 372.84 T
Ascochyta pisi CBS 122751
100/99/1.00
Ascochyta medicaginicola var. macrospora BRIP 45051
100/99/1.00
Ascochyta medicaginicola var. macrospora CBS 112.53 T
Ascochyta medicaginicola var. medicaginicola MFLUCC 16-0599
91/--/--
Ascochyta rabiei CBS 237.37 T
99/99/1.00
Ascochyta rabiei CBS 534.65
82/60/0.99
Ascochyta rabiei CBS 206.30
91/73/--
Ascochyta coronillae-emeri MFLUCC 13-0820 T
B1
96/92/1.00
65/70/--
Ascochyta phacae CBS 184.55 T
Ascochyta herbicola CBS 629.97 R
Ascochyta
Clade B
Phomatodes nebulosa CBS 100191
100/100/1.00
Phomatodes nebulosa CBS 117.93
100/99/1.00
Phomatodes nebulosa CBS 740.96
100/100/1.00
Phomatodes aubrietiae CBS 383.67 R
Phomatodes aubrietiae CBS 627.97 T
Phomatodes
Phoma herbarum CBS 502.91
92/85/1.00
Phoma herbarum CBS 377.92
97/98/1.00
Phoma herbarum CBS 134.96
Phoma herbarum CBS 615.75 R
Phoma herbarum CBS 274.37
Phoma
Didymellocamarosporium tamaricis MFLUCC 14-0241 T
Neomicrosphaeropsis italica MFLUCC 15-0485 T
63/63/0.96
Neomicrosphaeropsis italica MFLUCC 16-0284
Neomicrosphaeropsis italica MFLUCC 15-0484
Neomicrosphaeropsis tamaricicola MFLUCC 14-0602 T
C1
86/77/1.00
Neomicrosphaeropsis rossica MFLUCC 14-0586 T
Neomicrosphaeropsis elaeagni MFLU 16-2389 T
83/--/0.98
Neomicrosphaeropsis novorossica MFLUCC 14-0578 T
86/64/0.98
Neomicrosphaeropsis tamaricicola MFLUCC 14-0443
Neomicrosphaeropsis tamaricicola MFLUCC 14-0439
Neomicrosphaeropsis
Clade C
65/--/--
Neomicrosphaeropsis alhagi-pseudalhagi MFLU 17-0190 T
C2
73/--/0.96
Neomicrosphaeropsis cytisi MFLUCC 130396 T
89/70/0.99
Neomicrosphaeropsis minima MFLUCC 130394 T
91/85/1.00
Neomicrosphaeropsis cytisinus MFLUCC 16-0790 T
C3
Neomicrosphaeropsis cytisicola MFLUCC 18-0355 T
100/99/1.00
Briansuttonomyces eucalypti CBS 114887
99/90/1.00
Briansuttonomyces eucalypti CBS 114879 T
Briansuttonomyces
Pseudoascochyta novae-zelandiae CBS 141689
Pseudoascochyta
100/100/1.00
Calophoma clematidina CBS 108.79 T
100/97/1.00
Calophoma clematidina CBS 102.66
94/--/1.00
Calophoma rosae CGMCC 3.18347 T
Calophoma aquilegiicola CBS 107.96 R
Calophoma

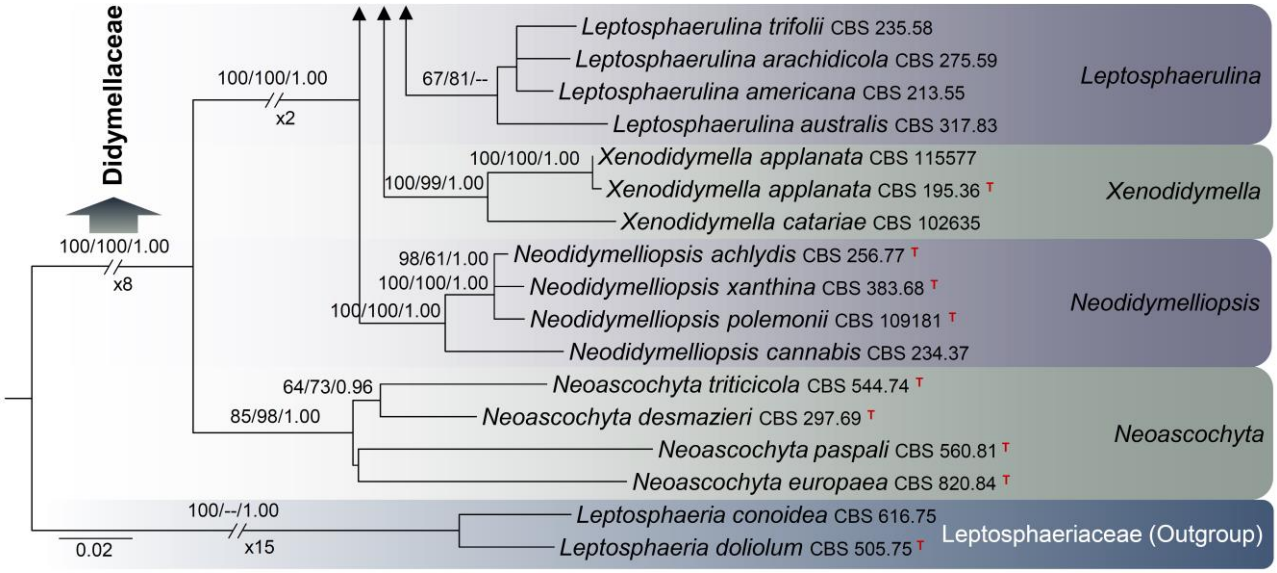


Fig. 1 – RAxML tree based on analysis of a combined dataset of LSU, ITS, *rpb2* and *tub2* partial sequence data. Bootstrap support values for ML and MP equal to or greater than 60 %, Bayesian posterior probabilities (PP) equal to or greater than 0.95 are defined as ML/MP/PP above the nodes. Genera, where known, and selected regions are indicated with coloured blocks. The new isolates are in blue. The ex-type strains are noted with superscripted T and representative strains are noted with superscripted R. The scale bar represents the expected number of nucleotide substitutions per site.

Taxonomy

Based on the results of the combined multi-gene phylogenies (Fig. 1), morphological observations, five novel species are described.

Ascochyta coronillae-emeri Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, sp. nov. Fig. 3

Index Fungorum number: IF554394; Facesoffungi number: FoF 04466

Etymology – Name reflects the host species *Coronilla emerus*, from which the species was isolated.

Holotype – MFLU 16-0163.

Saprobic on *Coronilla emerus* L. Sexual morph: *Ascomata* 120–150 µm high, 150–220 µm diam. ($\bar{x}$ = 133.1 × 186.1 µm, n = 5), immersed to semi-erumpent, globose or subglobose, dark brown to black, coriaceous. *Peridium* 10–15 µm wide at the base, 15–20 µm wide at the sides, comprising reddish to dark brown cells of *textura angularis*. *Hamathecium* comprising numerous, 2–3 µm wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 90–110 × 25–35 µm ($\bar{x}$ = 100.3 × 29.1 µm, n = 20), 8-spored, bitunicate, fissitunicate, clavate, pedicellate, thick-walled at the apex, with minute ocular chamber. *Ascospores* 36–35 × 13–15 µm ($\bar{x}$ = 32.7 × 13.8 µm, n = 30), overlapping biseriate, mostly ellipsoidal, muriform, 4−6-transversely septate, with 1 vertical septum, slightly constricted at the septa, initially hyaline to pale yellow, becoming brown to dark brown at maturity, upper part wider than the lower part, rounded at both end, surrounded by a thick mucilaginous sheath (20–30 µm wide). Asexual morph: coelomycetous. *Conidiomata* superficial or immersed in the agar, pale brown to dark brown, 0.5–1 mm diam, simple, or complex with several merging cavities. *Conidiomatal wall* composed of *textura angularis* cells. *Conidiogenous* cells discrete, assembled into protruding masses of cells, or integrated in very compact conidiophores. *Conidia* 6–7 × 1.9–2.4 µm ($\bar{x}$ = 6.3 × 2.1 µm, n = 30), ellipsoidal or short-cylindrical, hyaline, straight or slightly curved, rounded at both ends, 1-celled, with 1–2 small, guttules.

Known distribution – On *Coronilla emerus*, Italy.

Fig. 2 – Habitats. a. Italy (Bagno di Cetica). b-d Russia (c, d *Elaeagnus angustifolia* L.). e Uzbekistan. Photos by Erio Camporesi, Timur Bulgakov and Yusufjon Gafforov.

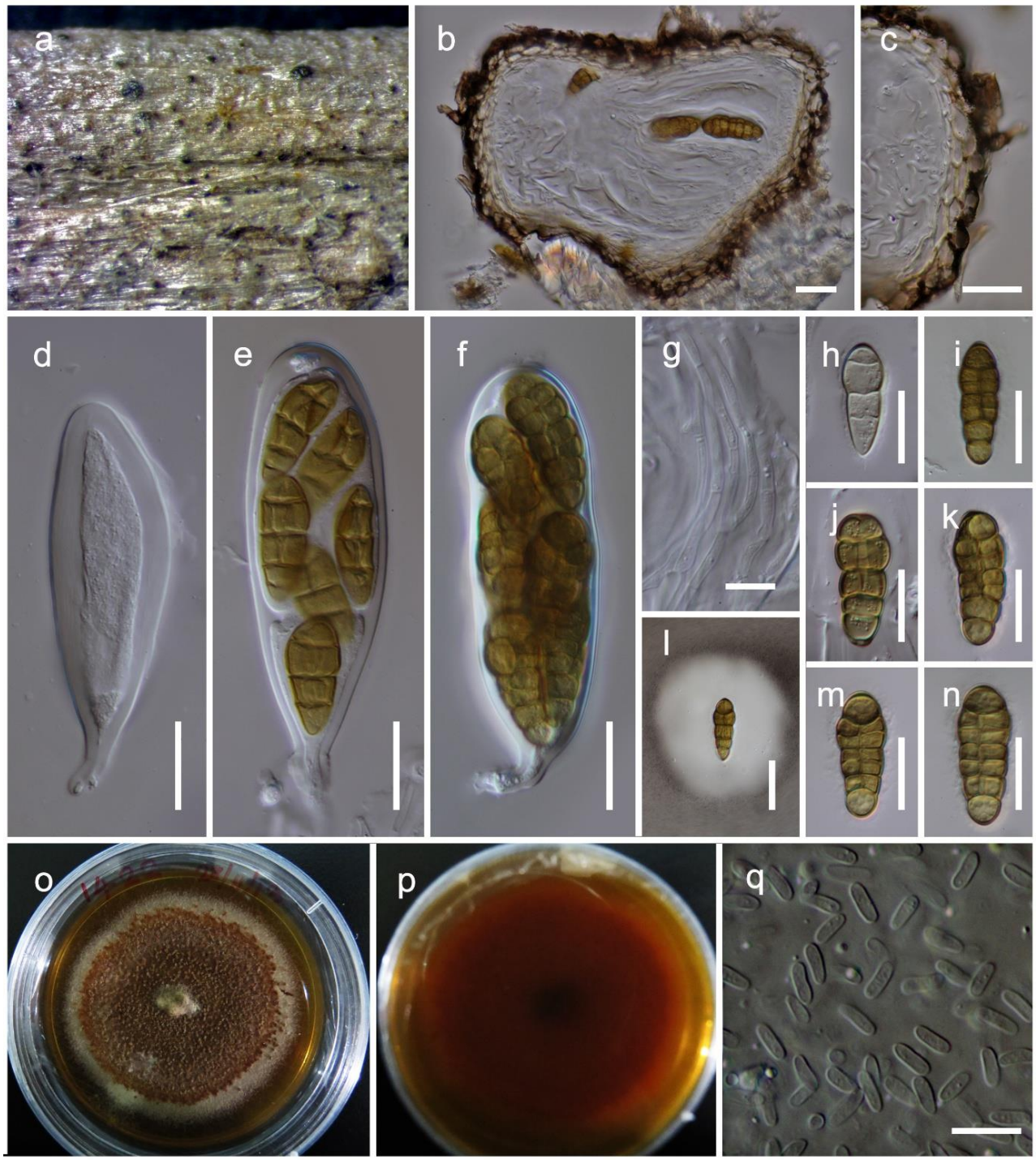


Fig. 3 – *Ascochyta coronillae-emeri* (MFLU 16-0163, holotype). a Appearance of ascomata on host substrate. b Section of ascoma. c Peridium. d-f Asci. g Pseudoparaphyses. i-n Ascospores (Note the ascospore stained with Indian Ink in l). o, p Culture on PDA (note p reverse). q Conidia. Scale bars: b–f, h–n = 20 µm, g, q = 10 µm.

Material examined – ITALY, Forlì-Cesena Province, Bagno di Romagna, Valbonella, on dead aerial branch of *Coronilla emerus* (Fabaceae) 23 August 2013, E. Camporesi IT 1422 (MFLU 16-0163, holotype); ex-type living culture, MFLUCC 13-0820.

Notes – Muriform ascospores are reported here for the first time in this genus. The new fungus was collected from *Coronilla emerus* in Italy and it morphologically resembles most of the Pleosporaceae taxa (e.g. *Alternaria*, *Comoclathris*, *Pleospora*) by its clavate, pedicellate asci with thick-walled at the apex and mostly ellipsoidal, muriform, brown ascospores. However,

phylogenetically it has a close affinity to *Ascochyta herbicola*, *A. phacae* and *A. rabiei* in Didymellaceae (subclade B1, Fig. 1). Among them, the sexual morph is known only for *Ascochyta phacae*, which differs from our new isolate in having cylindrical to subclavate asci and hyaline, uniseptate ascospores. Though the ascospore characters are different of our new isolate from all other *Ascochyta* species, its thin peridium and asexual morph characteristics (ellipsoidal or short-cylindrical, hyaline conidia) are in agreement with its phylogenetic placement within *Ascochyta*.

Microsphaeropsis spartii-juncei Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, sp. nov. Fig. 4

Index Fungorum number: IF554395; Facesoffungi number: FoF 04467

Etymology – Name reflects the host species *Spartium junceum*, from which the species was isolated.

Holotype – MFLU 16-0100.

Saprobic on *Spartium junceum* L. Sexual morph: *Ascomata* 180–250 µm high, 180–220 µm diam. ($\overline{x}$ = 219.7 × 206.9 µm, n = 5), immersed to semi-erumpent, globose or subglobose, dark brown to black, coriaceous. *Peridium* 10–15 µm wide at the base, 15–30 µm wide at the sides, comprising reddish to dark brown cells of *textura angularis*. *Hamathecium* comprising numerous, 2–3 µm wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 120–140 × 28–35 µm ($\overline{x}$ = 133.4 × 31.3 µm, n = 20), 8-spored, bitunicate, fissitunicate, clavate, pedicellate, thick-walled at the apex, with minute ocular chamber. *Ascospores* 32–36 × 13–15 µm ($\overline{x}$ = 34.7 × 13.7 µm, n = 30), overlapping biseriate, mostly ellipsoidal, muriform, 6−7-transversely septate, with 1−2 vertical septa, slightly constricted at the septa, initially hyaline to pale yellow, becoming brown to dark brown at maturity, rounded at both end, surrounded by a thick mucilaginous sheath (15–20 µm wide). Asexual morph: coelomycetous. *Conidiomata* superficial or immersed in the agar, pale brown to dark brown, 0.5–1 mm diam, simple, or complex with several merging cavities. *Conidiomatal wall* composed of *textura angularis* cells. *Conidiogenous* cells discrete, assembled into protruding masses of cells, or integrated in very compact conidiophores. *Conidia* 4.5–5.5 × 2.5–3.5 µm ($\overline{x}$ = 4.8 × 3.2 µm, n = 30), ellipsoidal or globose, straight or slightly curved, rounded at both ends, 1-celled, with 1–2 small, guttules, and with thin and smooth walls that are hyaline at secession, becoming light brown and rough-walled.

Known distribution – On *Spartium junceum*, Italy.

Material examined – ITALY, Arezzo Province, Pieve Santo Stefano, Valsavignone, on dead aerial twigs of *Spartium junceum* (Fabaceae), 27 May 2012, E. Camporesi IT 384 (MFLU 16-0100, holotype); ITALY, Forlì-Cesena Province, Premilcuore, Fiumicello, on dead aerial branch of *Spartium junceum* (Fabaceae), 1 April 2012, E. Camporesi IT 208 (MFLU 16-0097).

Notes – *Microsphaeropsis* is one of the oldest genera in Didymellaceae which was introduced by von Höhnel (1917). The exact familial placement of this genus was uncertain and it has been considered as an asexual morph of Phaeosphaeriaceae (Barr 1987) and Didymosphaeriaceae (Zhang et al. 2012, Thambugala et al. 2017). However, with further morpho-phylo debates, *Microsphaeropsis* has been referred as a member of Didymellaceae (De Gruyter et al. 2013, Hyde et al. 2013). In a recent study, Chen et al. (2015) reported *Microsphaeropsis* as a distinct lineage basal to Didymellaceae and the family Microsphaeropsidaceae was introduced. Taxa in *Microsphaeropsis* produce 'pale greenish brown, finely roughened conidia' (Chen et al. 2015), which differ from most other taxa in Didymellaceae which have mainly hyaline, smooth conidia (phoma-like). Nevertheless, many species of *Microsphaeropsis* are still unknown from culture or DNA sequence data and Chen et al. (2015), while introducing Microsphaeropsidaceae, recommended that further studies are needed to clarify its precise taxonomic identity and species boundaries.

During our investigation on the diversity of microfungi in Italy, two isolates (MFLU 16-0100, MFLU 16-0097) were recovered from *Spartium junceum* in Arezzo and Forli-Cesena Provinces. These new isolates share similarities to other Pleosporaceae taxa in their asci and ascospore characteristics, but they share a close phylogenetic affinity to *Microsphaeropsis* species in our sequence data analyses (Clade A, Fig. 1). However, in this study, *Microsphaeropsis* species could

not be segregated from Didymellaceae, in contrast to the results of Chen et al. (2015). Larger datasets of each gene region (ITS, *rpb2*, *tub2*) basically yielded the same major clades as those derived from the concatenated dataset (Fig. 1). Among them, LSU did not provide a better resolution at the generic level and the taxa of *Calophoma*, *Didysimulans*, *Macroventuria*, *Microsphaeropsis*, *Neomicrosphaeropsis*, *Paraboeremia*, *Phomatodes* and *Pseudoascochyta* grouped together in an unsupported clade. Although we analysed larger datasets incorporating other family members, we could not find support for segregating *Microsphaeropsis* from Didymellaceae neither from individual ITS, *rpb2* and *tub2* data, nor from concatenated multi-gene analyses. Among the various genes analysed, we noted that *rpb2* and *tub2* DNA sequence data yielded rather well-resolved topologies to support intergeneric relationships within Didymellaceae and especially in connection with *Microsphaeropsis* (data not shown).

Even though the asci and ascospore characters of our new isolates are different from all other *Microsphaeropsis* species, its asexual morph characteristics are in agreement with the phylogenetic placement, as it has conidia similar to *Microsphaeropsis*. In concatenated data analyses, our new strains resemble *Microsphaeropsis olivacea* strains (CBS 233.77, CBS 432.71, CBS 442.83). These strains are however unrelated to any type material and therefore we introduce our new isolates as *Microsphaeropsis spartii-juncei* sp. nov. Unfortunately, we could not manage to maintain a living culture as subsequent attempts to subculture failed, and hence a living culture is unavailable.

We admit that our phylogeny generated herein does not exactly translate into an appropriate scenario to really demarcate our species but we still recognize it as a different single species occupying a totally different ecological niche. As stated in our paper, there are some degrees of morphological differences in the ascospore characters (despite similarities in conidial characters), which support our new species. However, neither *Microsphaeropsis olivacea* nor *M. proteae* have sexual characteristics to compare with *M. spartii-juncei*. Under circumstances where compelling evidence are not available, we follow Jeewon & Hyde et al. (2016) herein to justify our new species. We note 100% and 99% similarity for LSU and ITS in *Microsphaeropsis* species. There was a 17/334 (5.1 %) difference in the TUB region. There are no RPB2 sequences for *Microsphaeropsis olivacea* and *M. proteae*. We suspect herein that the genes analysed and the taxon sampling used generating phylogenies could have had an impact and fail to resolve that clade. It is beyond the scope of the study to resolve these. It might also not be a surprise if future discoveries of more species within *Microsphaeropsis* split the clade and there is a need to segregate one species into several. We have recently witnessed such a phenomenon with *Dematiopleospora* (Huang et al. 2017). Unless we do some extensive taxonomic reassessment, we would not be tempted to synonymise any extant taxa here.

Neomicrosphaeropsis alhagi-pseudalhagi Wanas., Gafforov & K.D. Hyde, sp. nov. Fig. 5

Index Fungorum number: IF554396; Facesoffungi number: FoF 04468

Etymology – Name reflects the host species *Alhagi pseudoalhagi*, from which the species was isolated.

Holotype – TASM 6134.

Saprobic on *Alhagi pseudalhagi* (M. Bieb.) Fisch. Sexual morph: Undetermined. Asexual morph: coelomycetous. *Conidiomata* 150–220 μm high × 40–70 μm diam. ($\bar{x}$ = 187 × 52 μm, n = 6), acervuli, hemispherical to spherical, composed of brown to reddish-brown, pseudoparenchymatous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 7–12 × 8–10 μm ($\bar{x}$ = 10.8 × 9.1 μm, n = 20), holoblastic, phialidic, ampulliform to cylindrical, unbranched, pale brwon, smooth. *Conidia* 30–45 × 18–22 μm ($\bar{x}$ = 37.2 × 20.7 μm, n = 30), variable and irregular, mostly ellipsoidal, terminal, solitary, muriform, 3−5-transversely septate, with 1−3 vertical septa, deeply constricted at the middle septum, slightly constricted at remaining septa, initially pale brown, becoming dark brown at maturity, upper part wider than lower part, rounded at upper end, with flat lower end.

Known distribution – On *Alhagi pseudalhagi*, Uzbekistan.

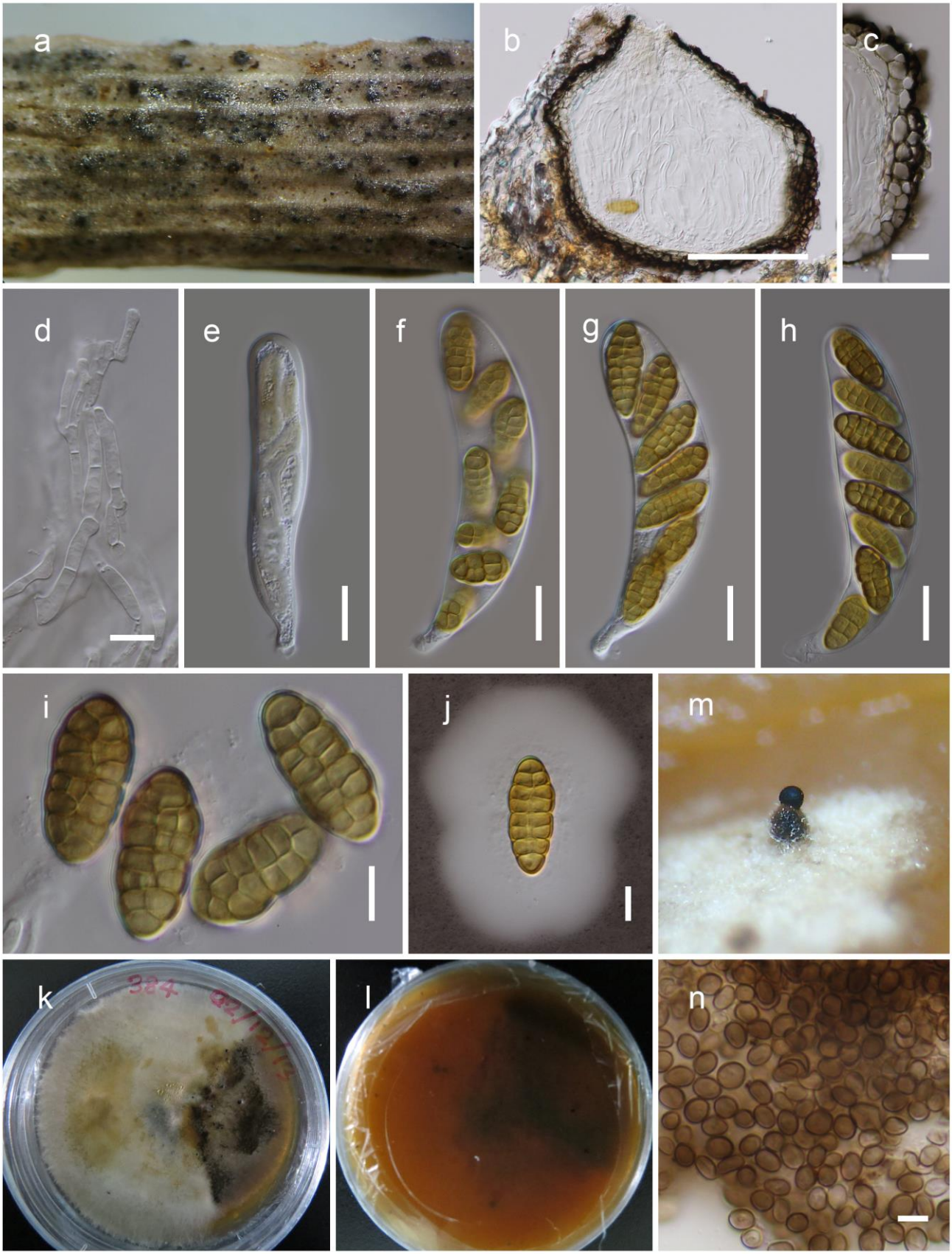


Fig. 4 – *Microsphaeropsis spartii-juncei* (MFLU 16-0100, holotype). a Appearance of ascomata on host substrate. b Section of ascoma. c Peridium. d Pseudoparaphyses. e-h Asci. i, j Ascospores (Note the ascospore stained with Indian Ink in j). k, l Culture on PDA (note l reverse). m Conidiama on PDA. n Conidia. Scale bars: b = 100 µm, c, e–h = 20 µm, d, i, j = 10 µm, n = 5 µm.

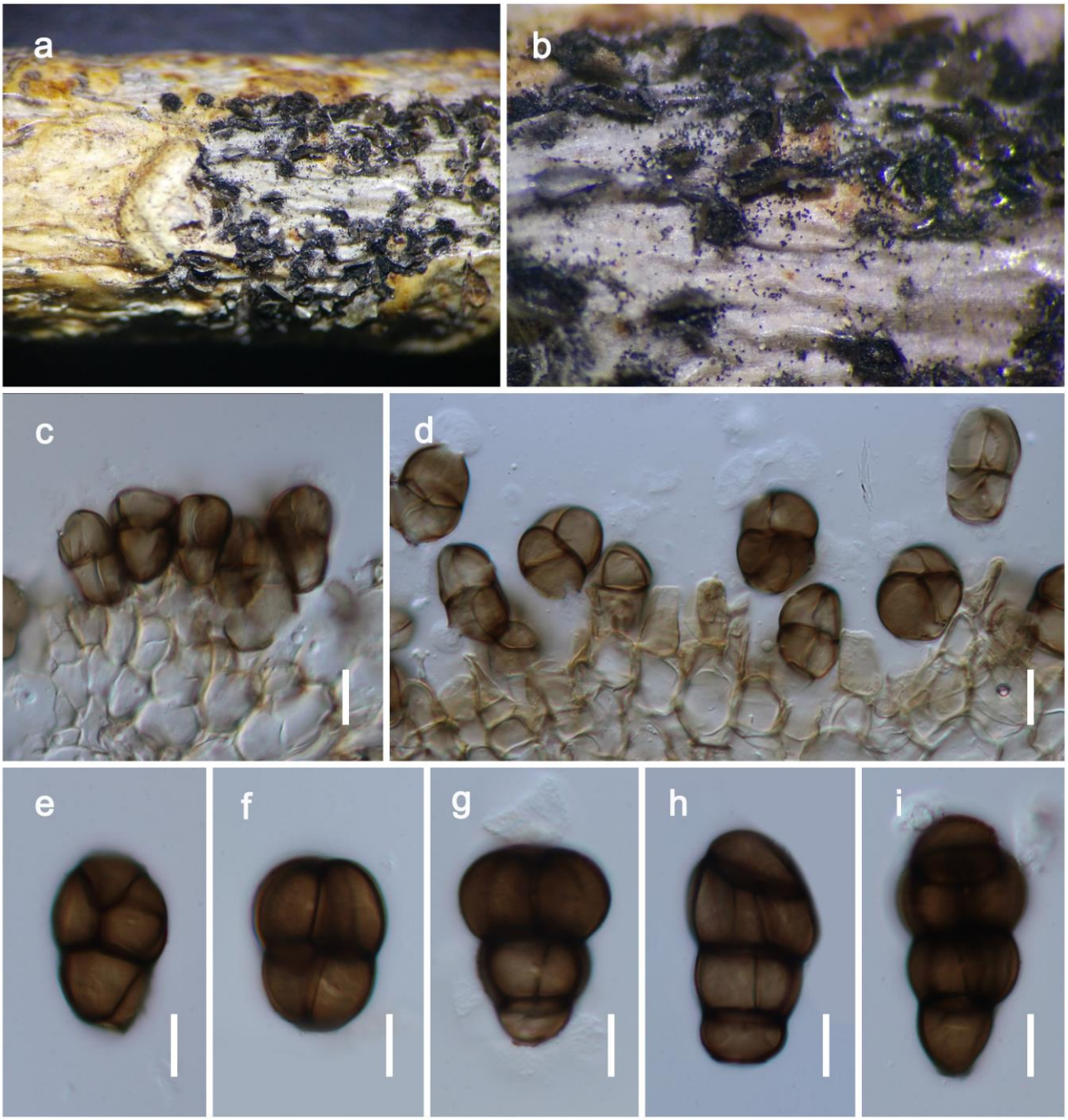


Fig. 5 – *Neomicrosphaeropsis alhagi-pseudalhagi* (TASM 6134, holotype). a, b Appearance of conidiomata on host substrate. c, d Conidia and conidiogenous cells. e-i Conidia. Scale bars: c–i = 10 µm.

Material examined – UZBEKISTAN, Surxondaryo Province, Boysun District, Omonxona Village, South-Western Hissar Mountains, on branches of *Alhagi pseudalhagi* (Fabaceae), 13 May 2016, Yusufjon Gafforov YG-S24-2 (TASM 6134, holotype; MFLU 17-0190, isotype).

Notes – *Neomicrosphaeropsis alhagi-pseudalhagi*, collected from *Alhagi pseudalhagi* in Uzbekistan, is in an independent lineage with good support and phylogenetically distinct from other extant species of *Neomicrosphaeropsis* (subclade C1, Fig. 1). This new species differs from other taxa in *Neomicrosphaeropsis* in having acervulus type conidiomata and conidia with 1−3 vertical septa and a deep constriction at the middle septum, whereas other species have pycnidial conidiomata, conidia with 1−2 vertical septa and slight constrictions at their septa.

Neomicrosphaeropsis cytisicola Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, sp. nov. Fig. 6

Index Fungorum number: IF554397; Facesoffungi number: FoF 04469

Etymology – Name reflects the host genus *Cytisus*, from which the species was isolated.

Holotype – MFLU 16-16-0114.

Saprobic on *Cytisus* sp. Sexual morph: *Ascomata* 180–250 µm high, 180–220 µm diam. ($\overline{x}$ = 319.6 × 265.7 µm, n = 5), immersed to semi-erumpent, globose or subglobose, dark brown to black, coriaceous, ostiolate. *Ostioles* 60–80 long, 100–120 µm wide, apapillate, central, filled with hyaline to brown cells. *Peridium* 10–15 µm wide at the base, 15–20 µm wide at the sides, comprising reddish to dark brown cells of *textura angularis*. *Hamathecium* comprising numerous, 2–2.5 µm wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 140–160 × 30–40 µm ($\overline{x}$ = 146.6 × 35.6 µm, n = 20), 8-spored, bitunicate, fissitunicate, clavate, pedicellate, thick-walled at the apex, with minute ocular chamber. *Ascospores* 32–38 × 13–18 µm ($\overline{x}$ = 35.8 × 15.4 µm, n = 30), overlapping biseriate, mostly ellipsoidal, muriform, 6−7-transversely septate, with 2−3 vertical septa, slightly constricted at the septa, initially hyaline to pale yellow, becoming brown to dark brown at maturity, narrowly rounded at upper end and rounded at lower end, guttulate, surrounded by a thick mucilaginous sheath (20–30 µm wide). Asexual morph: coelomycetous. *Conidiomata* superficial or immersed in the agar, pale brown to dark brown, 0.5–1 mm diam, simple, or complex with several merging cavities. *Conidiomatal wall* composed of *textura angularis* cells. *Conidiophores* occasionally present, hyaline, doliiform to ampulliform, arising from inner layers of the pycnidial wall. *Conidiogenous cells* enteroblastic, phialidic, doliiform or cylindrical to ampulliform, with a periclinal wall thickening at the tip, hyaline, smooth. *Conidia* 4–7 × 2.5–3.5 µm ($\overline{x}$ = 5.1 × 3.1 µm, n = 30), ellipsoidal, straight or slightly curved, rounded at both ends, 1-celled, with 1–2 small guttules, and with thin and smooth walls that are hyaline at secession, becoming light brown.

Known distribution – On *Cytisus* sp., Italy.

Material examined – ITALY, Arezzo Province, Bagno di Cetica, on dead aerial branches of *Cytisus* sp. (Fabaceae), 1 October 2012, E. Camporesi IT 762 (MFLU 16-0114, holotype); ex-type living culture, MFLUCC 18-0355.

Notes – *Neomicrosphaeropsis cytisicola* also a novel taxon in this study, which has muriform ascospores, but resembles *Laburnicola* species in Didymosphaeriaceae more closely than Pleosporaceae taxa in its ascospore characteristics. This novel taxon has closer phylogenetic affinities to *Neomicrosphaeropsis cytisi*, *N. cytisinus* and *N. minima* (subclade C3, Fig. 1). All these mentioned species were isolated from *Cytisus* and *Verbascum* species in Italy. Our new species is the first record of sexual morph of taxa in Subclade C3 (Fig. 1) and it differs from the other remaining sexual morph (*Neomicrosphaeropsis tamaricicola*) in having comparatively larger ascospores (32–38 × 13–18 µm) with more septa (6−7 transverse septa, with 2−3 vertical septa), while *N. tamaricicola* has smaller ascospores (15–20 × 7–10 µm) with less septa (4–6 transverse septa, with 1 vertical septum). All taxa in *Neomicrosphaeropsis* produce aseptate brown conidia similar to taxa in *Microsphaeropsis* including the sexual morph we observed from *N. cytisicola* sp. nov.

While our two new taxa, *Neomicrosphaeropsis alhagi-pseudalhagi* and *N. cytisicola* are well-supported and resolved, we noted within clade C, where *Neomicrosphaeropsis* is interspersed, interspecies relationships are obscure. Even our multigene phylogeny fails to properly delineate species in this clade and all species cluster together despite bearing morphological differences. There is a need to redefine species delimitation among these species, possibly using a different approach. In addition, it is also noteworthy to point out taxa in subclade C1 and C3 can also be considered as different genera, but until more samples are collected, analysed and typification reevaluated, we refrain from revising the current taxonomic concept.

Neomicrosphaeropsis elaeagni Wanas., Bulgakov, E.B.G. Jones & K.D. Hyde, sp. nov. Figs 2, 7

Index Fungorum number: IF554398; Facesoffungi number: FoF 04470

Etymology – Name reflects the host genus *Elaeagnus*, from which the species was isolated.

Holotype – MFLU 16-2389.

Necrotrophic/saprobic on dying branches of *Elaeagnus angustifolia* L. Sexual morph: Undetermined. Asexual morph: coelomycetous. *Conidiomata* pycnidial, 350−400 µm high, 450−550 µm diam ($\bar{x}$ = 378.7 × 500.1 µm, n = 10), black, superficial to semi-immersed,

Fig. 6 – *Neomicrosphaeropsis cytisicola* (MFLU 16-0114, holotype). a Appearance of ascomata on host substrate. b Section of ascomata. c Peridium. d Pseudoparaphyses. e-h Asci. i-m Ascospores (Note the ascospore stained with Indian Ink in m). n, o Culture on PDA (note o reverse). p Conidia. Scale bars: b = 100 µm, c, e–h = 20 µm, d, i–m = 10 µm, p = 5 µm.

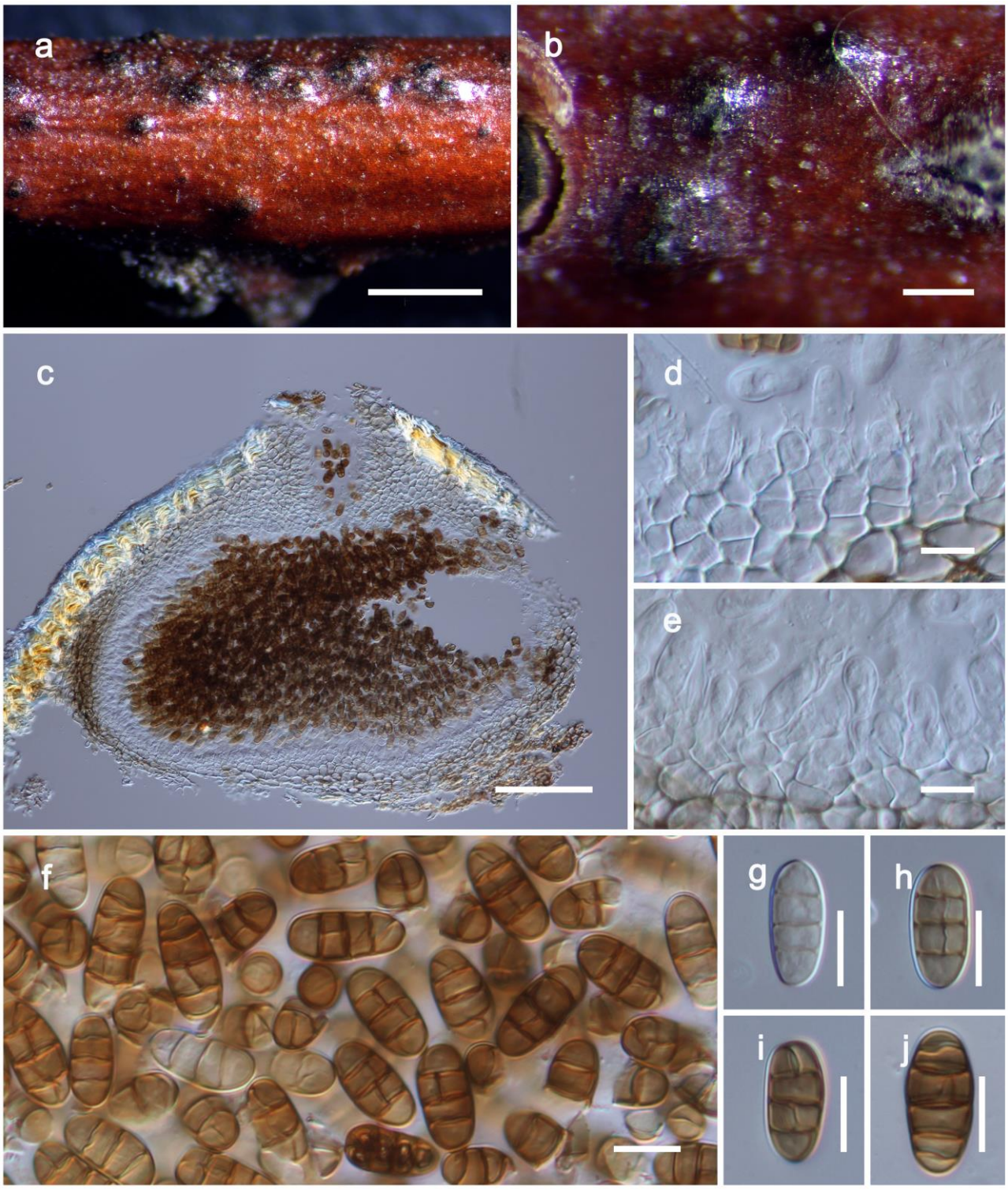


Fig. 7 – *Neomicrosphaeropsis elaeagni* (MFLU 16-2389, holotype). a Conidiomata on host surface. b Vertical section through conidioma. c Conidiomata wall. d, e. Conidiogenous cells producing conidia. f–j Conidia. Scale bars: a = 1 mm; b = 200 µm; c = 100 µm; d–j = 10 µm.

confluent, gregarious, sometimes scattered beneath the host periderm or on decorticated wood, fully or partly erumpent, globose, ostiolate. *Ostiole* central, 100−130 µm long, 50−80 µm diam ($\bar{x}$ = 117.1 × 62.7 µm, n = 10), central, long, smooth, sometimes ostiolar canal filled with hyaline or pale brown cells. *Pycnidial wall* multi-layered, 20–30 µm wide at the base, 30–40 µm wide in sides, thick, comprising two layers, outer layer heavily pigmented, thick-walled, comprising blackish to dark reddish-brown cells of *textura angularis*, cells towards the inside lighter, inner layer composed

of hyaline, thin-walled cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, annellidic, doliiform, integrated, solitary, hyaline, smooth-walled, and formed from the inner layer of pycnidium wall. *Conidia* 16−20 × 7−9 μm ($\overline{x}$ = 17.5 × 7.7 μm; n = 50), oblong, straight, rounded at both ends, sometimes narrowly rounded ends, 3–5-transversely septate, one longitudinal septum, smooth-walled, initially hyaline, becoming brown to dark brown at maturity.

Known distribution – On *Elaeagnus angustifolia*, European Russia (Krasnodar region).

Material examined – RUSSIA, Krasnodar region, Novorossiysk, trees near Sudzhuk lagoon (N 44.68114°, E 37.79712°), on twigs of *Elaeagnus angustifolia* L. (Elaeagnaceae), 14 June 2016, Timur S. Bulgakov NK-081 (MFLU 16-2389, holotype).

Notes – *Neomicrosphaeropsis elaeagni* is a novel species which was recovered from *Elaeagnus angustifolia* in Russia. It was identified as a camarosporium-like taxon by its morphology and further sequence analyses indicate a strong affinity to taxa related to *Neomicrosphaeropsis* (subclade C1, Fig. 1). *Didymellocamarosporium tamaricis* also clusters in this clade as another camarosporium-like species. Wijayawardene et al. (2016) proposed *Didymellocamarosporium* as a monotypic genus based on rDNA sequence data available from GenBank for the type, *D. tamaricis*. Both *Neomicrosphaeropsis elaeagni* and *Didymellocamarosporium tamaricis* are morphologically similar in their conidiomata, conidiogenous cells and conidial characteristics. However, taxa in this subclade C1 are heterogenous and we could not demarcate *Didymellocamarosporium* and *Neomicrosphaeropsis* into two separate genera from our multi-gene phylogenetic analyses. It is therefore necessary to collect more fungi similar to *Didymellocamarosporium* and *Neomicrosphaeropsis* in different geographic regions, isolate them into culture, describe their morphology, analyse their DNA sequences and investigate their phylogenetic relationships to better identify and classify them.

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