## Didymellaceae revisited

### Q. Chen<sup>1</sup>, L.W. Hou<sup>1,2</sup>, W.J. Duan<sup>3</sup>, P.W. Crous<sup>4,5\*</sup>, and L. Cai<sup>1,2\*</sup>

<sup>1</sup>State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China; <sup>2</sup>College of Life Science, University of Chinese Academy of Sciences, Beijing 100049, China; <sup>3</sup>Ningbo Academy of Inspection and Quarantine, Ningbo 315012, China; <sup>4</sup>Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; <sup>5</sup>Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CT Utrecht, The Netherlands;

\*Correspondence: P.W. Crous, p.crous@westerdijkinstitute.nl; L. Cai, cail@im.ac.cn

Abstract: The *Didymellaceae* is one of the most species-rich families in the fungal kingdom, and includes species that inhabit a wide range of ecosystems. The taxonomy of *Didymellaceae* has recently been revised on the basis of multi-locus DNA sequence data. In the present study, we investigated 108 *Didymellaceae* isolates newly obtained from 40 host plant species in 27 plant families, and various substrates from caves, including air, water and carbonatite, originating from Argentina, Australia, Canada, China, Hungary, Israel, Italy, Japan, South Africa, the Netherlands, the USA and former Yugoslavia. Among these, 68 isolates representing 32 new taxa are recognised based on the multi-locus phylogeny using sequences of LSU, ITS, *rpb2* and *tub2*, and morphological differences. Within the *Didymellaceae*, five genera appeared to be limited to specific host families, with other genera having broader host ranges. In total 19 genera are recognised in the family, with *Heracleicola* being reduced to synonymy under *Ascochyta*. This study has significantly improved our understanding on the distribution and biodiversity of *Didymellaceae*, although the placement of several genera still need to be clarified.

#### Key words: Host-associated, Karst caves, Multi-locus phylogeny, Phoma, Taxonomy.

Taxonomic novelties: New species: Allophoma oligotrophica Q. Chen, Crous & L. Cai, Ascochyta boeremae L.W. Hou, Crous & L. Cai, Calophoma rosae Q. Chen, Crous & L. Cai, Didymella aeria Q. Chen, Crous & L. Cai, D. aquatica Q. Chen, Crous & L. Cai, D. chloroguttulata Q. Chen, Crous & L. Cai, D. ellipsoidea Q. Chen, Crous & L. Cai, D. ilicicola Q. Chen, Crous & L. Cai, D. infuscatispora Q. Chen, Crous & L. Cai, D. macrophylla Q. Chen, Crous & L. Cai, D. ocimicola Q. Chen, Crous & L. Cai, D. pteridis L.W. Hou, Crous & L. Cai, D. sinensis Q. Chen, Crous & L. Cai, D. suiyangensis Q. Chen, Crous & L. Cai, E. dendrobii Q. Chen, Crous & L. Cai, E. duchesneae Q. Chen, Crous & L. Cai, E. hordei Q. Chen, Crous & L. Cai, E. italicum Q. Chen, Crous & L. Cai, E. latusicollum Q. Chen, Crous & L. Cai, E. layuense Q. Chen, Crous & L. Cai, E. poae Q. Chen, Crous & L. Cai, E. viticis Q. Chen, Crous & L. Cai, Heterophoma verbascicola Q. Chen, Crous & L. Cai, Neoascochyta argentina L.W. Hou, Crous & L. Cai, Neoa. soli Q. Chen, Crous & L. Cai, Neoa. triticicola L.W. Hou, Crous & L. Cai, S. papillata Q. Chen, Crous & L. Cai; New variety: Boeremia exigua var. opuli Q. Chen, Crous & L. Cai; New combinations: Ascochyta premilcurensis (Tibpromma et al.) Q. Chen, Crous & L. Cai, Didymella segeticola (Q. Chen) Q. Chen, Crous & L. Cai.

Available online 9 June 2017; http://dx.doi.org/10.1016/j.simyco.2017.06.002.

### INTRODUCTION

The Didymellaceae is the largest family in the Pleosporales (Ascomycota, Pezizomycotina, Dothideomycetes), with more than 5 400 taxon names listed in MycoBank (Crous et al. 2004). The family *Didymellaceae* was established by de Gruyter et al. (2009) to encompass three main genera, viz. Ascochyta, Didymella and Phoma, and other allied phoma-like genera which grouped in the Didymellaceae. Aveskamp et al. (2010) circumscribed the boundaries of Didymellaceae, redefined the genera Epicoccum, Peyronellaea and Stagonosporopsis, and established the genus Boeremia. He also acknowledged two sexual genera in the family, namely Leptosphaerulina and Macroventuria. In spite of these studies, the polyphyly of Ascochyta, Didymella and Phoma remained unresolved. A revision of the Didymellaceae has recently been published, comprising 17 wellsupported monophyletic clades which were treated as individual genera (Chen et al. 2015a). Moreover, the generic delimitations of Ascochyta, Didymella, Epicoccum and Phoma were further emended to reveal more natural evolutionary relationships (Chen et al. 2015a). Subsequent to this revision, several additional genera were added, namely Briansuttonomyces (Crous & Groenewald 2016), Neomicrosphaeropsis (Thambugala et al. 2017), Didymellocamarosporium (Wijayawardene et al. 2016), Heracleicola and Neodidymella (Ariyawansa et al. 2015).

Species of *Didymellaceae* are cosmopolitan and distributed throughout a broad range of environments. Most of the members in this family are plant pathogens of a wide range of hosts, mainly causing leaf and stem lesions; some are of quarantine significance (Aveskamp *et al.* 2008, 2010, Chen *et al.* 2015a, b). Several species belonging to *Ascochyta* and *Nothophoma* have been reported to be host-specific to a single plant genus or family (Aveskamp *et al.* 2010, Chen *et al.* 2015a). Nevertheless, host specificity in genera of *Didymellaceae* has not been specifically addressed.

Correct species identification in this family has always proven difficult, chiefly relying on morphology and plant host association (Aveskamp *et al.* 2010, Chen *et al.* 2015a). However, a robust backbone tree based on internal transcribed spacer regions and intervening 5.8S nrDNA (ITS), partial 28S large subunit nrDNA (LSU) sequences, and partial regions of RNA polymerase II second largest subunit (*rpb2*) and  $\beta$ -tubulin (*tub2*) genes provide a relatively robust phylogenetic backbone for taxon determination (Chen *et al.* 2015a).

The present study reports on a collection of 108 *Didymellaceae* isolates obtained from 40 host plant species in 27 plant families in China, as well as several other countries. Of these, 68

Peer review under responsibility of Westerdijk Fungal Biodiversity Institute.

<sup>© 2017</sup> Westerdijk Fungal Biodiversity Institute. Production and hosting by ELSEVIER B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

isolates representing 32 new taxa are described by employing a polyphasic approach using morphological characteristics and multi-locus phylogenetics.

### MATERIALS AND METHODS

### Sampling and isolation

The majority of *Didymellaceae* strains were isolated from diseased plants in seven provinces of China (Gansu, Guizhou, Inner Mongolia, Jiangxi, Qianghai, Shandong and Tibet), as well as Australia, Italy, Japan and the USA. Some strains isolated from air, soil, water and faeces were collected from the Mingyong Glacier in Yunnan Province and inside the Karst caves in Guizhou Province in China. The air, soil and water samples were collected from inside the cave following the methods used by Zhang *et al.* (2017). Several strains were obtained from the Herbarium BRIP (Dutton Park, Queensland, Australia), the International Collection of Microorganisms from Plants (ICMP, Landcare Research, Auckland, New Zealand), and the Westerdijk Fungal Biodiversity Institute (CBS, Utrecht, the Netherlands), as listed in Table 1.

Plant-associated isolates were obtained from symptomatic tissue with sporocarps using the single spore isolation protocols of Choi et al. (1999) and Zhang et al. (2013), and from tissue according to the techniques outlined by Cai et al. (2009). Isolates from other substrates were obtained following the methods described by Zhang et al. (2017) and further screened with carbon-free silica gel medium to select the oligotrophic strains (Wainwright & Al-Talhi 1999). All the Didymellaceae isolates were primarily identified based on morphology and ITS sequence data, which distinguished them from other groups of fungi. Type specimens of new species in this study were deposited in the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), with the ex-type living cultures deposited in China General Microbiological Culture Collection Center (CGMCC), or the other Biological Resource Centres cited above.

### Morphology

Isolates were incubated on oatmeal agar (OA), malt extract agar (MEA) and potato dextrose agar (PDA) (Crous et al. 2009) at 25 °C, and under near-ultraviolet (UV) light (12 h light/12 h dark) or on pine needle agar (PNA) (Smith et al. 1996) to induce sporulation. Colony diameters were measured after 7 d of incubation, and the culture characters were determined after 14 d (Boerema et al. 2004). Colony colours were rated according to the colour charts of Rayner (1970). Preparations were mounted in distilled water to study the micromorphological structures of mature ascomata/conidiomata, ascospores/conidia and conidiogenous cells from OA cultures (Aveskamp et al. 2010, Chen et al. 2015a, b). Observations were conducted with a Leica M125 dissecting microscope and a Nikon Eclipse 80i compound microscope under differential interference contrast (DIC) illumination. To study the pseudothecial/pycnidial wall, sections of mature pseudothecia/pycnidia were made by a Leica CM1950 freezing microtome (Aveskamp et al. 2010, Chen et al. 2015a, b). The NaOH spot test was carried out by a drop of 1N NaOH to determine the secretion of metabolite E on MEA cultures (Boerema et al. 2004).

# DNA isolation, amplification and phylogenetic analyses

Total genomic DNA was extracted from fresh mycelia using the MP Fastprep-24 sample preparation system, according to the protocol described by Cubero *et al.* (1999). The primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990) were used to amplify part of the nuclear rDNA operon (ITS) spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2), and the first 100 bp of the 5' end of the 28S rRNA gene (LSU); the primers LR0R (Rehner & Samuels 1994), LR7 and LR5 (Vilgalys & Hester 1990) were used for LSU amplification; Btub2Fd and Btub4Rd (Woudenberg *et al.* 2009) for the partial  $\beta$ -tubulin (*tub2*) gene region, and RPB2-5F2 (Sung *et al.* 2007) and fRPB2-7cR (Liu *et al.* 1999) for the RNA polymerase II second largest subunit (*rpb2*). Amplicons for each locus were generated following the protocols listed in Chen *et al.* (2015a).

Sequencing was conducted in both directions with the same primer pair used for amplification at the Omega Genetics Company (Beijing, China). Consensus sequences were assembled in MEGA v. 6.0 (Tamura *et al.* 2013) 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; Katoh & Standley 2013), and manually corrected when necessary. The concatenated aligned dataset and each locus were analysed separately using Maximum Likelihood (ML) and Bayesian Inference (BI). The best-fit models of evolution for the four loci tested (SYM+I+G for ITS and GTR+I+G for LSU, *rpb2* and *tub2*) were estimated by MrModeltest v. 2.3 (Nylander 2004).

The ML analyses were conducted with RAxML v. 7.2.6 (Stamatakis & Alachiotis 2010) using a GTRGAMMA substitution model with 1 000 bootstrap replicates. The robustness of the analyses was evaluated by bootstrap support (MLBS). Bayesian (BI) analyses were performed on MrBayes v. 3.2.1 (Ronguist et al. 2012) based on the models selected by the MrModeltest. The Markov Chain Monte Carlo (MCMC) algorithm of four chains was initiated in parallel from a random tree topology. The analyses lasted until the average standard deviation of split frequencies was below 0.01 with trees saved each 1000 generations. The first 25 % of trees were removed as burn-in phase and the remaining trees were used to calculate posterior probabilities. Posterior probabilities values of the BI analyses (BPP) over 0.95 were considered significant. Leptosphaeria conoidea (CBS 616.75) and L. doliolum (CBS 505.75) were selected as outgroup. Sequences generated in this study were deposited in GenBank (Table 1), the final matrices and trees in TreeBASE (www.treebase.org; accession number: S20724), and novel taxonomic descriptions and nomenclature in MycoBank (www.MycoBank.org; Crous et al. 2004).

Unique fixed nucleotide positions are used to describe a sterile species (see Taxonomy below), and the closest phylogenetic neighbour was selected and subjected to single nucleotide polymorphism (SNP) analyses using MEGA v. 6.0 (Tamura *et al.* 2013).

### Statistical analysis

A heatmap showing the host distribution of each genus of *Didymellaceae* was generated with R v. 3.3.1 heatmap.2 (https://www.r-project.org/).

Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	ank acces	ssion num	on numbers <sup>3</sup>	
						LSU	ITS	RPB2	TUB	
Allophoma labilis	CBS 124.93; PD 87/269		Lycopersicon esculentum	Solanaceae	Netherlands	GU238091	GU237765	KT389552	GU23761	
Al. minor	CBS 325.82	т	Syzygium aromaticum	Myrtaceae	Indonesia	GU238107	GU237831	KT389553	GU23763	
Al. nicaraguensis	CBS 506.91; PD 91/876; IMI 215229	т	Coffea arabica	Rubiaceae	Nicaragua	GU238058	GU237876	KT389551	GU23759	
Al. oligotrophica	CGMCC 3.18114; LC 6245	т	Air		China	KY742194	KY742040	KY742128	KY742282	
	CGMCC 3.18115; LC 6246		Air		China	KY742195	KY742041	KY742129	KY742283	
	CGMCC 3.18116; LC 6247		Air		China	KY742196	KY742042	KY742130	KY742284	
Al. piperis	CBS 268.93; CBS 108.93; PD 88/720	т	Peperomia pereskiifolia	Piperaceae	Netherlands	GU238129	GU237816	KT389554	GU23764	
	CBS 108.93; PD 90/2011		Peperomia sp.	Piperaceae	Netherlands	GU238130	GU237921	KT389555	GU23764	
Al. tropica	CBS 436.75; DSM 63365	т	Saintpaulia ionantha	Gesneriaceae	Germany	GU238149	GU237864	KT389556	GU23766	
Al. zantedeschiae	CBS 131.93; PD 69/140		Calla sp.	Araceae	Netherlands	GU238159	FJ427084	KT389557	FJ427188	
	CBS 229.32		Cicer arietinum	Fabeceae	Romania	KT389690	KT389473	KT389558	KT389767	
	ICMP 16850		Lycopersicon esculentum	Solanaceae	Hungary	KY742197	KY742043	KY742131	KY74228	
Ascochyta boeremae	CBS 372.84; PD 80/1246	т	Pisum sativum	Fabeceae	Australia	KT389697	KT389480	_	KT389774	
As. boeremae	CBS 373.84; PD 80/1247		Pisum sativum	Fabeceae	Australia	KT389698	KT389481	KT389560	KT389775	
As. fabae	CBS 524.77		Phaseolus vulgaris	Fabeceae	Belgium	GU237963	GU237880	_	GU23752	
	CBS 649.71		Vicia faba	Fabeceae	Netherlands	GU237964	GU237902	_	GU23752	
	PD 83/492		Phaseolus vulgaris	Fabeceae	Netherlands	GU237965	GU237917	_	GU23752	
As. herbicola	CBS 629.97; PD 76/1017	R	Water		USA	GU238083	GU237898	KP330421	GU237614	
As. lentis	CBS 370.84; PD 81/783		Lens culinaris	Fabeceae	_	KT389691	KT389474	_	KT389768	
As. medicaginicola var. macrospora	BRIP 45051; LC 5258		Medicago sativa	Fabeceae	Australia	KY742198	KY742044	KY742132	KY742286	
	CBS 112.53	т	Medicago sativa	Fabeceae	USA	GU238101	GU237749	_	GU23762	
	CBS 404.65; IMI 116999	R	Medicago sativa	Fabeceae	Canada	GU238102	GU237859	KP330423	GU23762	
As. medicaginicola var. medicaginicola	CBS 316.90		Medicago sativa	Fabeceae	Czech Republic	GU238103	GU237828	_	GU23763	
As. nigripycnidia	CBS 116.96; PD 95/7930	т	Vicia cracca	Fabeceae	Russia	GU238118	GU237756	_	GU23763	
As. phacae	CBS 184.55	т	Phaca alpina	Fabeceae	Switzerland	KT389692	KT389475	_	KT389769	
As. pisi	CBS 122750; ATCC 201619		Pisum sativum	Fabeceae	USA	KT389694	KT389477	_	KT389771	
	CBS 122751; ATCC 201620		Pisum sativum	Fabeceae	Canada	KP330444	KP330432	EU874867	KP330388	
	CBS 122785; PD 78/517	т	Pisum sativum	Fabeceae	Netherlands	GU237969	GU237763	_	GU23753	
	CBS 126.54		Pisum sativum	Fabeceae	Netherlands	EU754137	GU237772	DQ677967	GU23753	
	CBS 108.49		Juglans regia	Juglandaceae	Netherlands	KT389693	KT389476	_	KT389770	
As. premilcurensis	MFLUCC 14-0518	т	Heracleum sphondylium	Apiaceae	Italy	KT326695	KT326694	_	_	

Table 1. (Continued).										
Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	Bank acce	ssion num	sion numbers <sup>3</sup>	
						LSU	ITS	RPB2	TUB	
As. rabiei	CBS 206.30		_	_	_	KT389695	KT389478	KT389559	KT389772	
	CBS 237.37	Т	Cicer arietinum	Fabeceae	Bulgaria	KT389696	KT389479	_	KT389773	
	CBS 534.65		Cicer arietinum	Fabeceae	India	GU237970	GU237886	KP330405	GU237533	
As. syringae	CBS 545.72		Syringa vulgaris	Oleaceae	Netherlands	KT389700	KT389483	_	KT389777	
As. versabilis	CBS 876.97; PD 82/1008	R	Silene sp.	Caryophyllaceae	Netherlands	GU238152	GU237909	KT389561	GU237664	
As. viciae	CBS 451.68		Vicia sepium	Fabeceae	Netherlands	KT389701	KT389484	KT389562	KT389778	
As. viciae-pannonicae	CBS 254.92		Vicia pannonica	Fabeceae	Czech Republic	KT389702	KT389485	_	KT389779	
Boeremia crinicola	CBS 109.79; PD 77/747	R	Crinum powellii	Amaryllidaceae	Netherlands	GU237927	GU237737	KT389563	GU237489	
B. diversispora	CBS 102.80; IMI 331907; PD 79/61		Phaseolus vulgaris	Fabeceae	Kenya	GU237930	GU237725	KT389565	GU237492	
	CBS 101194; PD 79/687; IMI 373349		Phaseolus vulgaris	Fabeceae	Netherlands	GU237929	GU237716	KT389564	GU237491	
B. exigua var. coffeae	CBS 119730		Coffea arabica	Rubiaceae	Brazil	GU237942	GU237759	KT389567	GU237504	
	CBS 109183; PD 2000/10506; IMI 300060	R	Coffea arabica	Rubiaceae	Cameroon	GU237943	GU237748	KT389566	GU237505	
	CBS 431.74; PD 74/2447	R	Solanum tuberosum	Solanaceae	Netherlands	EU754183	FJ427001	KT389569	FJ427112	
B. exigua var. forsythiae	CBS 101197; PD 95/721		Forsythia sp.	Oleaceae	Netherlands	GU237931	GU237718	KT389570	GU237493	
	CBS 101213; PD 92/959	R	Forsythia sp.	Oleaceae	Netherlands	GU237932	GU237723	KT389571	GU237494	
B. exigua var. gilvescens	CBS 101150; PD 79/118		Cichorium intybus	Asteraceae	Netherlands	EU754182	GU237715	KT389568	GU237495	
B. exigua var. heteromorpha	CBS 443.94	Т	Nerium oleander	Apocynaceae	Italy	GU237935	GU237866	KT389573	GU237497	
	CBS 101196; PD 79/176		Nerium oleander	Apocynaceae	France	GU237934	GU237717	KT389572	GU237496	
B. exigua var. linicola	CBS 114.28		Linum usitatissimum	Linaceae	Netherlands	GU237937	GU237752	_	GU237499	
	CBS 116.76; ATCC 32332; IMI 197074; PD 75/544	R	Linum usitatissimum	Linaceae	Netherlands	GU237938	GU237754	KT389574	GU237500	
	CBS 248.38		Nemophila insignis	Hydrophyllaceae	Netherlands	KT389703	KT389486	KT389575	KT389780	
B. exigua var. opuli	CGMCC 3.18354; LC 8117	Т	Viburnum opulus	Caprifoliaceae	USA	KY742199	KY742045	KY742133	KY742287	
	LC 8118		Viburnum opulus	Caprifoliaceae	USA	KY742200	KY742046	KY742134	KY742288	
B. exigua var. populi	CBS 100167; PD 93/217	Т	Populus (×) euramericana	Salicaceae	Netherlands	GU237939	GU237707	_	GU237501	
B. exigua var. pseudolilacis	CBS 423.67		Lathyrus sp.	Fabeceae	Netherlands	KT389704	KT389487	KT389576	KT389781	
	CBS 462.67		Lamium maculatum	Lamiaceae	Netherlands	KT389705	KT389488	_	KT389782	
	CBS 101207; PD 94/614	т	Syringa vulgaris	Oleaceae	Netherlands	GU237941	GU237721	_	GU237503	
B. exigua var. viburni	CBS 100354; PD 83/448	R	Viburnum opulus	Caprifoliaceae	Netherlands	GU237944	GU237711	KT389577	GU237506	
B. foveata	CBS 109176; PD 94/1394	R	Solanum tuberosum	Solanaceae	Bulgaria	GU237946	GU237742	KT389578	GU237508	
B. hedericola	CBS 367.91; PD 87/229	R	Hedera helix	Araliaceae	Netherlands	GU237949	GU237842	KT389579	GU237511	
B. lilacis	CBS 569.79; PD 72/741; IMI 331909	R	Syringa vulgaris	Oleaceae	Netherlands	GU237936	GU237892	_	GU237498	
	CBS 588.67		Philadelphus sp.	Saxifragaceae	Netherlands	KT389709	KT389492	_	KT389786	

CHEN ET AL.

Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	Bank acce	ssion num	nbers <sup>3</sup>
				,	<b>,</b>	LSU	ITS	RPB2	TUB
	LC 5178		Lonicera japonica	Caprifoliaceae	China	KY742201	KY742047	_	KY742289
	LC 8116		Ocimum sp.	Lamiaceae	China	KY742202	KY742048	_	KY742290
B. lycopersici	CBS 378.67; PD 67/276	R	Lycopersicon esculentum	Solanaceae	Netherlands	GU237950	GU237848	KT389580	GU237512
B. noackiana	CBS 100353; PD 87/718	R	Phaseolus vulgaris	Fabeceae	Guatemala	GU237952	GU237710	_	GU237514
	CBS 101203; PD 79/1114		Phaseolus vulgaris	Fabeceae	Colombia	GU237953	GU237720	KT389581	GU237515
B. sambuci-nigrae	CBS 629.68; CECT 20048; IMI 331913; PD 67/753	Т	Sambucus nigra	Caprifoliaceae	Netherlands	GU237955	GU237897	_	GU237517
B. strasseri	CBS 126.93; PD 73/642		Mentha sp.	Lamiaceae	Netherlands	GU237956	GU237773	KT389584	GU237518
B. telephii	CBS 760.73; PD 71/1616	R	Sedum telephium	Crassulaceae	Netherlands	GU237959	GU237905	_	GU237521
	CBS 109175; PD 79/524	R	Sedum telephium	Crassulaceae	Netherlands	GU237958	GU237741	KT389585	GU237520
B. trachelospermi	CGMCC 3.18222; LC 8105	Т	Trachelospermum jasminoides	Apocynaceae	USA	KY064032	KY064028	KY064033	KY064051
Briansuttonomyces eucalypti	CBS 114879; CPC 362	Т	<i>Eucalyptus</i> sp.	Myrtaceae	South Africa	KU728519	KU728479	_	KU728595
	CBS 114887; CPC 363		<i>Eucalyptus</i> sp.	Myrtaceae	South Africa	KU728520	KU728480	_	KU728596
Calophoma aquilegiicola	CBS 107.31		Aquilegia sp.	Ranunculaceae	_	KT389710	KT389493	_	KT389787
C. aquilegiicola	CBS 107.96; PD 73/598	R	Aconitum pyramidale	Ranunculaceae	Netherlands	GU238041	GU237735	KT389586	GU237581
	CBS 108.96; PD 79/611	R	Aquilegia sp.	Ranunculaceae	Netherlands	GU238042	GU237736	_	GU237582
	CBS 109.96; PD 83/832		Aquilegia sp.	Ranunculaceae	Netherlands	KT389711	KT389494	_	KT389788
	CBS 116402		Thalictrum dipterocarpum	Ranunculaceae	New Zealand	KT389712	KT389495	_	KT389789
C. clematidina	CBS 102.66		Clematis sp.	Ranunculaceae	UK	FJ515630	FJ426988	KT389587	FJ427099
	CBS 108.79; PD 78/522	Т	Clematis sp.	Ranunculaceae	Netherlands	FJ515632	FJ426989	KT389588	FJ427100
C. clematidis-rectae	CBS 507.63; PD 07/03486747; MUCL 9574		Clematis sp.	Ranunculaceae	Netherlands	FJ515647	FJ515606	KT389589	FJ515624
C. complanata	CBS 268.92; PD 75/3		Angelica sylvestris	Umbelliferae	Netherlands	EU754180	FJ515608	GU371778	FJ515626
	CBS 100311		Heracleum sphondylium	Umbelliferae	Netherlands	EU754181	GU237709	KT389590	GU237594
C. glaucii	CBS 112.96; PD 79/765		Dicentra sp.	Papaveraceae	Netherlands	GU238077	GU237750	_	GU237610
	CBS 114.96; PD 94/888		Chelidonium majus	Papaveraceae	Netherlands	FJ515649	FJ515609	_	FJ515627
C. rosae	CGMCC 3.18347; LC 5169	Т	Rosa sp.	Rosaceae	China	KY742203	KY742049	KY742135	KY742291
	LC 8119		<i>Rosa</i> sp.	Rosaceae	China	KY742204	KY742050	KY742136	KY742292
C. vodakii	CBS 173.53	т	Hepatica triloba	Ranunculaceae	Switzerland	KT389714	KT389497	_	KT389791
Didymella acetosellae	CBS 179.97		Rumex hydrolapathum	Polygonaceae	Netherlands	GU238034	GU237793	KP330415	GU237575
D. aeria	CGMCC 3.18353; LC 7441	т	Air		China	KY742205	KY742051	KY742137	KY742293
	LC 8120		Air		China	KY742206	KY742052	KY742138	KY742294
D. aliena	CBS 379.93; PD 82/945		Berberis sp.	Berberidaceae	Netherlands	GU238037	GU237851	KP330416	GU237578

Didymellaceae revisited

109

Table 1. (Continued).									
Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	Bank acce	ession numbers <sup>3</sup>	
					_	LSU	ITS	RPB2	TUB
	LC 8121		Pyrus calleryana	Rosaceae	Italy	KY742207	KY742053	_	KY742295
D. americana	CBS 185.85; PD 80/1191	R	Zea mays	Poaceae	USA	GU237990	FJ426972	KT389594	FJ427088
	CBS 568.97; ATCC 44494; PD 94/1544		Glycine max	Fabeceae	USA	GU237991	FJ426974	_	FJ427090
	LC 5157		Sorghum bicolor	Poaceae	China	KY742208	KY742054	KY742139	KY742296
D. anserina	CBS 253.80		_	_	Germany	KT389715	KT389498	KT389595	KT389795
	CBS 285.29		Calluna sp.	Ericaceae	UK	KT389716	KT389499	_	KT389796
	CBS 360.84	R	Potato flour		Netherlands	GU237993	GU237839	KT389596	GU237551
	CBS 397.65		Plastic		Germany	KT389717	KT389500	KT389597	KT389797
D. aquatica	CGMCC 3.18349; LC 5556	Т	Water		China	KY742209	KY742055	KY742140	KY742297
	LC 5555		Water		China	KY742210	KY742056	KY742141	KY742298
D. arachidicola	CBS 333.75; ATCC 28333; IMI 386092; PREM 44889	Т	Arachis hypogaea	Fabeceae	South Africa	GU237996	GU237833	KT389598	GU237554
D. aurea	CBS 269.93; PD 78/1087	Т	Medicago polymorpha	Fabeceae	New Zealand	GU237999	GU237818	KT389599	GU237557
D. bellidis	CBS 714.85; PD 74/265	R	Bellis perennis	Asteraceae	Netherlands	GU238046	GU237904	KP330417	GU237586
	PD 94/886		<i>Bellis</i> sp.	Asteraceae	Netherlands	GU238047	GU237923	_	GU237587
D. boeremae	CBS 109942; PD 84/402	Т	Medicago littoralis cv. Harbinger	Fabeceae	Australia	GU238048	FJ426982	KT389600	FJ427097
D. calidophila	CBS 448.83	Т	Soil		Egypt	GU238052	FJ427059	_	FJ427168
	PD 84/109		Cucumis sativus	Cucurbitaceae	Netherlands	GU238053	FJ427060	_	FJ427169
D. chenopodii	CBS 128.93; PD 79/140	R	Chenopodium quinoa cv. Sajana	Chenopodiaceae	Peru	GU238055	GU237775	KT389602	GU237591
D. chloroguttulata	CGMCC 3.18351; LC 7435	Т	Air		China	KY742211	KY742057	KY742142	KY742299
	LC 8122		Air		China	KY742212	KY742058	KY742143	KY742300
D. coffeae-arabicae	CBS 123380; PD 84/1013	Т	Coffea arabica	Rubiaceae	Ethiopia	GU238005	FJ426993	KT389603	FJ427104
	LC 8975		Lagerstroemia indica	Lythraceae	Italy	KY742213	KY742059	KY742144	KY742301
D. curtisii	CBS 251.92; PD 86/1145	R	Nerine sp.	Amaryllidaceae	Netherlands	GU238013	FJ427038	_	FJ427148
	PD 92/1460		Sprekelia sp.	Amaryllidaceae	Netherlands	GU238012	FJ427041	KT389604	FJ427151
D. dactylidis	CBS 124513; PD 73/1414	Т	Dactylis glomerata	Poaceae	USA	GU238061	GU237766	_	GU237599
D. dimorpha	CBS 346.82	Т	Opuntia sp	Cactaceae	Spain	GU238068	GU237835	_	GU237606
D. ellipsoidea	CGMCC 3.18350; LC 7434	Т	Air		China	KY742214	KY742060	KY742145	KY742302
	LC 8123		Air		China	KY742215	KY742061	KY742146	KY742303
D. eucalyptica	CBS 377.91; PD 79/210	R	<i>Eucalyptus</i> sp.	Myrtaceae	Australia	GU238007	GU237846	KT389605	GU237562
D. exigua	CBS 183.55	Т	Rumex arifolius	Polygonaceae	France	EU754155	GU237794	EU874850	GU237525
D. gardeniae	CBS 626.68; IMI 108771	Т	Gardenia jasminoides	Rubiaceae	India	GQ387595	FJ427003	KT389606	FJ427114
D. glomerata	CBS 133.72		Fresco in church		Romania	KT389718	FJ427004	_	FJ427115

CHEN ET AL.

Table 1. (Continued).Species	Strain number <sup>1</sup>	Statue <sup>2</sup>	Host, substrate	Host family	Country	GonE	ank acco	ssion num	bore <sup>3</sup>
Species	Strain number	Status	nosi, substrate	nost lailing	Country			RPB2	TUB
	CBS 528.66; PD 63/590	R	Chrysanthemum sp.	Asteraceae	Netherlands	EU754184		GU371781	
	LC 4963	IX.	Leymus chinensis	Poaceae	China		KY742062	KY742147	
	LC 8124		Faeces	1 Daceae	China		KY742063	KY742147	
D. heteroderae	CBS 109.92; PD 73/1405	т	Undefined food material		Netherlands	GU238002		KT389601	
D. Helefouerde	LC 8125	I	Hydrangea macrophylla	Saxifragaceae	China		KY7420905	KY742149	
D. ilicicola	CGMCC 3.18355; LC 8126; LC 8127	т	llex chinensis	Aquifoliaceae	Italy		KY742065	KY742149	
D. IIICICOIA	LC 8127	I	llex chinensis	Aquifoliaceae	Italy	KY742219		KY742150	
D infuscationary		т		,	China		KY742066	KY742151 KY742152	
D. infuscatispora	CGMCC 3.18356; LC 8128	I	Chrysanthemum indicum	Asteraceae		KY742221			KY742
	LC 8129		Chrysanthemum indicum	Asteraceae	China		KY742068	— KT000007	
D. lethalis	CBS 103.25		—	<u> </u>	<u> </u>		GU237729		GU237
	LC 8130	-	Liquidambar styraciflua		Italy	KY742223		KY742153	
D. longicolla	CBS 124514; PD 80/1189	T -	<i>Opuntia</i> sp.	Cactaceae	Spain	GU238095		_	GU237
D. macrophylla	CGMCC 3.18357; LC 8131	Т	Hydrangea macrophylla	Saxifragaceae	Italy		KY742070	KY742154	
	LC 8132	_	Hydrangea macrophylla	Saxifragaceae	Italy		KY742071	KY742155	
D. mascrostoma	CBS 223.69	R	Acer pseudoplatanus	Aceraceae	Switzerland	GU238096			
	CBS 247.38		Pinus nigra var. astriaca	Pinaceae	—	KT389719	KT389501	—	KT389
	CBS 482.95		Larix decidua	Pinaceae	Germany	GU238099	GU237869	KT389609	GU237
	CBS 529.66; PD 66/521	R	Malus sylvestris	Rosaceae	Netherlands	GU238098	GU237885	—	GU237
	LC 5203		Soil		China	KY742226	KY742072	KY742156	KY742
D. maydis	CBS 588.69	Т	Zea mays	Poaceae	USA	EU754192	FJ427086	GU371782	FJ427
D. microchlamydospora	CBS 105.95	Т	<i>Eucalyptus</i> sp.	Myrtaceae	UK	GU238104	FJ427028	KP330424	FJ427
D. molleriana	CBS 229.79; LEV 7660	R	Digitalis purpurea	Scrophulariaceae	New Zealand	GU238067	GU237802	KP330418	GU237
	CBS 109179; PD 90/835-1		<i>Digitalis</i> sp.	Scrophulariaceae	Netherlands	GU238066	GU237744	—	GU237
D. musae	CBS 463.69	R	Mangifera indica	Anacardiaceae	India	GU238011	FJ427026	_	FJ427
D. negriana	CBS 358.71	R	Vitis vinifera	Vitaceae	Germany	GU238116	GU237838	KT389610	GU237
	ICMP 10845; LC 5249		Vitis vinifera	Vitaceae	former Yugoslavia	KY742227	KY742073	_	KY742
D. nigricans	CBS 444.81; PDDCC 6546	Т	Actinidia chinensis	Actinidiaceae	New Zealand	GU238000	GU237867	—	GU237
	LC 8133		Robinia pseudoacacia f. decaisneana	Fabeceae	Italy	KY742228	KY742074	KY742157	KY742
	LC 8134		Acer palmatum	Aceraceae	Japan	KY742229	KY742075	KY742158	KY742
	LC 8135		Acer palmatum	Aceraceae	Japan	KY742230	KY742076	KY742159	KY742
	LC 8136		Acer palmatum	Aceraceae	Japan	KY742231	KY742077	KY742160	KY742
								(continued or	n next p

Didymellaceae revisited

Table 1. (Continued).									
Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	Bank acce	ssion num	nbers <sup>3</sup>
						LSU	ITS	RPB2	TUB
	PD 77/919		Actinidia chinensis	Actinidiaceae	New Zealand	GU238001	GU237915	KT389611	GU237559
D. ocimicola	CGMCC 3.18358; LC 8137	Т	Ocimum sp.	Lamiaceae	China	KY742232	KY742078	_	KY742320
	LC 8138		Ocimum sp.	Lamiaceae	China	KY742233	KY742079	_	KY742321
D. pedeiae	CBS 124517; PD 92/612A	Т	Schefflera elegantissima	Araliaceae	Netherlands	GU238127	GU237770	KT389612	GU237642
D. pinodella	CBS 318.90; PD 81/729		Pisum sativum	Fabeceae	Netherlands	GU238016	FJ427051	_	FJ427161
	CBS 531.66		Trifolium pretense	Fabeceae	USA	GU238017	FJ427052	KT389613	FJ427162
	LC 8139		Acer palmatum	Aceraceae	Japan	KY742234	KY742080	KY742161	KY742322
D. pinodes	CBS 525.77	Т	Pisum sativum	Fabeceae	Belgium	GU238023	GU237883	KT389614	GU237572
D. pomorum	CBS 285.76; ATCC 26241; IMI 176742; VKM F-1843		Heracleum dissectum	Umbelliferae	Russia	GU238025	FJ427053	KT389615	FJ427163
	CBS 354.52		Triticum spelta	Poaceae	Switzerland	KT389720	KT389502	KT389616	KT389799
	CBS 388.80		Triticum sp.	Poaceae	South Africa	GU238027	FJ427055	KT389617	FJ427165
	CBS 539.66; ATCC 16791; IMI 122266; PD 64/914	R	Polygonum tataricum	Polygonaceae	Netherlands	GU238028	FJ427056	KT389618	FJ427166
	LC 5185		Gentiana straminea	Gentianaceae	China	KY742235	KY742081	KY742162	KY742323
	LC 8140		Dendrobium fimbriatum	Orchidaceae	China	KY742236	KY742082	_	KY742324
D. protuberans	CBS 132.96; PD 93/853		Rhinanthus major	Scrophulariaceae	Netherlands	GU237989	GU237778	_	GU237550
	CBS 377.93; PD 80/976		Daucus carota	Umbelliferae	Netherlands	GU238014	GU237847	KT389619	GU237565
	CBS 381.96; PD 71/706	т	Lycium halifolium	Solanaceae	Netherlands	GU238029	GU237853	KT389620	GU237574
	CBS 391.93; PD 80/87		Spinacia oleracea	Chenopodiaceae	Netherlands	GU238015	GU237858	KT389621	GU237566
D. pteridis	CBS 379.96	т	<i>Pteri</i> s sp.	Pteridaceae	Netherlands	KT389722	KT389504	KT389624	KT389801
D. rhei	BRIP 5562; LC 5251		Rheum rhaponticum	Polygonaceae	Australia	KY742237	KY742083	KY742163	KY742325
	CBS 109177; LEV 15165; PD 2000/9941	R	Rheum rhaponticum	Polygonaceae	New Zealand	GU238139	GU237743	KP330428	GU237653
D. rumicicola	CBS 683.79; LEV 15094	т	Rumex obtusifolius	Polygonaceae	New Zealand	KT389721	KT389503	KT389622	KT389800
D. sancta	CBS 281.83	т	Ailanthus altissima	Simaroubaceae	South Africa	GU238030	FJ427063	KT389623	FJ427170
D. segeticola	CGMCC 3.17489; LC 1636	т	Cirsium segetum	Asteraceae	China	KP330455	KP330443	KP330414	KP330399
	CGMCC 3.17498; LC 1635		Cirsium segetum	Asteraceae	China	KP330454	KP330442	KP330413	KP330398
	LC 1633		Cirsium segetum	Asteraceae	China	KP330452	KP330440	KP330411	KP330396
	LC 1634		Cirsium segetum	Asteraceae	China	KP330453	KP330441	KP330412	KP330397
	LC 8141		Camellia sasanqua	Theaceae	Japan	KY742238	KY742084	KY742164	KY742326
D. senecionicola	CBS 160.78; LEV 11451	R	Senecio jacobaea	Asteraceae	New Zealand	GU238143	GU237787	_	GU237657
D. sinensis	CGMCC 3.18348; LC 5210	т	Cerasus pseudocerasus	Rosaceae	China	KY742239	KY742085	_	KY742327
	LC 5246		Urticaceae	Urticaceae	China	KY742240	KY742086	KY742165	KY742328
	LC 8142		Dendrobium officinale	Orchidaceae	China	KY742241	KY742087	KY742166	KY742329

CHEN ET AL.

Table 1. (Continued).									
Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	ank acces	ssion num	bers <sup>3</sup>
						LSU	ITS	RPB2	TUB
	LC 8143		Dendrobium officinale	Orchidaceae	China	KY742242	KY742088	KY742167	KY74233
D. subglomerata	CBS 110.92; PD 76/1010	R	Triticum sp.	Poaceae	USA	GU238032	FJ427080	KT389626	FJ42718
D. subherbarum	CBS 249.92; PD 78/1088		Solanum sp.	Solanaceae	Peru	GU238144	GU237808	_	GU2376
	CBS 250.92; DAOM 171914; PD 92/371	Т	Zea mays	Poaceae	Canada	GU238145	GU237809	_	GU2376
D. suiyangensis	CGMCC 3.18352; LC 7439	Т	Air		China	KY742243	KY742089	KY742168	KY7423
	LC 8144		Air		China	KY742244	KY742090	KY742169	KY7423
D. viburnicola	CBS 523.73; PD 69/800	R	Viburnum cassioides	Caprifoliaceae	Netherlands	GU238155	GU237879	KP330430	GU2376
Didymellocamarosporium tamaricis	MFLUCC 14-0241	Т	<i>Tamarix</i> sp.	Tamaricaceae	Italy	KU848183	_	_	_
Endocoryneum festucae	MFLUCC 14-0461	Т	Festuca sp.	Poaceae	Italy	KU848203	_	_	_
Epicoccum brasiliense	CBS 120105	Т	Amaranthus sp.	Amaranthaceae	Brazil	GU238049	GU237760	KT389627	GU2375
E. camelliae	CGMCC 3.18343; LC 4858	Т	Camellia sinensis	Theaceae	China	KY742245	KY742091	KY742170	KY7423
	LC 4862		Camellia sinensis	Theaceae	China	KY742246	KY742092	KY742171	KY7423
E. dendrobii	CGMCC 3.18359; LC 8145	Т	Dendrobium fimbriatum	Orchidaceae	China	KY742247	KY742093	_	KY7423
	LC 8146		Dendrobium fimbriatum	Orchidaceae	China	KY742248	KY74209	_	KY7423
E. draconis	CBS 186.83; PD 82/47	R	Dracaena sp.	Agavaceae	Rwanda	GU238070	GU237795	KT389628	GU2376
E. duchesneae	CGMCC 3.18345; LC 5139	Т	Duchesnea indica	Rosaceae	China	KY742249	KY742095	_	KY7423
	LC 8147		Duchesnea indica	Rosaceae	China	KY742250	KY742096	_	KY7423
E. henningsii	CBS 104.80; PD 74/1017	R	Acacia mearnsii	Fabeceae	Kenya	GU238081	GU237731	KT389629	GU2376
E. hordei	CGMCC 3.18360; LC 8148	Т	Hordeum vulgare	Poaceae	Australia	KY742251	KY742097	_	KY7423
	LC 8149		Hordeum vulgare	Poaceae	Australia	KY742252	KY742098	_	KY7423
E. huancayense	CBS 105.80; PD 75/908	Т	Solanum sp.	Solanaceae	Peru	GU238084	GU237732	KT389630	GU2376
E. italicum	CGMCC 3.18361; LC 8150	Т	Acca sellowiana	Myrtaceae	Italy	KY742253	KY742099	KY742172	KY7423
	LC 8151		Acca sellowiana	Myrtaceae	Italy	KY74225	KY742100	KY742173	KY7423
E. latusicollum	CGMCC 3.18346; LC 5158	Т	Sorghum bicolor	Poaceae	China	KY742255	KY742101	KY742174	KY7423
	LC 4859		Camellia sinensis	Theaceae	China	KY742256	KY742102	KY742175	KY7423
	LC 5124		Vitex negundo	Verbenaceae	China	KY742257	KY742103	_	KY7423
	LC 8152		Podocarpus macrophyllus	Podocarpaceae	Japan	KY742258	KY742104	KY742176	KY7423
	LC 8153		Podocarpus macrophyllus	Podocarpaceae	Japan	KY742259	KY742105	KY742177	KY7423
	LC 8154		Acer palmatum	Aceraceae	Japan	KY742260	KY742106	_	KY7423
E. layuense	CGMCC 3.18362; LC 8155	Т	Perilla sp.	Lamiaceae	China	KY742261	KY742107	_	KY7423
	LC 8156		Perilla sp.	Lamiaceae	China	KY742262	KY742108	_	KY7423

Table 1. (Continued).									
Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	Bank acces	ssion num	nbers <sup>3</sup>
		_				LSU	ITS	RPB2	TUB
E. nigrum	CBS 125.82; IMI 331914; CECT 20044		Human toenail		Netherlands	GU237974	FJ426995	KT389631	FJ427106
	CBS 173.73; ATCC 24428; IMI 164070	Т	Dactylis glomerata	Poaceae	USA	GU237975	FJ426996	KT389632	FJ427107
	LC 5180		Lonicera japonica	Caprifoliaceae	China	KY742263	KY742109	KY742178	KY742351
	LC 8157		Ocimum sp.	Lamiaceae	China	KY742264	KY742110	KY742179	KY742352
	LC 8158		Poa annua	Poaceae	USA	KY742265	KY742111	KY742180	KY742353
	LC 8159		Poa annua	Poaceae	USA	KY742266	KY742112	KY742181	KY742354
E. pimprinum	CBS 246.60; ATCC 22237; ATCC 16652; IMI 81601	Т	Soil		India	GU237976	FJ427049	_	FJ427159
	PD 77/1028		Soil		India	GU237977	FJ427050	KT389633	FJ427160
E. plurivorum	CBS 558.81; PDDCC 6873	Т	Setaria sp.	Poaceae	New Zealand	GU238132	GU237888	KT389634	GU237647
E. poae	CGMCC 3.18363; LC 8160	Т	Poa annua	Poaceae	USA	KY742267	KY742113	KY742182	KY742355
	LC 8161		Poa annua	Poaceae	USA	KY742268	KY742114	KY742183	KY742356
	LC 8162		Poa annua	Poaceae	USA	KY742269	KY742115	KY742184	KY742357
E. sorghinum	CBS 179.80; PD 76/1018		Sorghum vulgare	Poaceae	Puerto Rico	GU237978	FJ427067	KT389635	FJ427173
	CBS 627.68; PD 66/926		<i>Citrus</i> sp.	Rutaceae	France	GU237979	FJ427072	KT389636	FJ427178
	LC 4860		Camellia sinensis	Theaceae	China	KY742270	KY742116	KY742185	KY742358
E. viticis	BRIP 29294; LC 5257		Andropogon gayanus	Poaceae	Australia	KY742271	KY742117	_	KY742359
	CGMCC 3.18344; LC 5126	Т	Vitex negundo	Verbenaceae	China	KY742272	KY742118	KY742186	KY742360
Heterophoma adonidis	CBS 114309; UPSC 2982		Adonis vernalis	Ranunculaceae	Sweden	KT389724	KT389506	KT389637	KT389803
H. dictamnicola	CBS 507.91; PD 74/148		Dictamnus albus	Rutaceae	Netherlands	GU238065	GU237877	KT389638	GU237603
H. novae-verbascicola	CBS 127.93; PD 92/347		Verbascum densiflorum	Scrophulariaceae	Netherlands	GU238120	GU237774	_	GU237639
H. poolensis	CBS 113.20; PD 92/774		_	_	_	GU238119	GU237751	_	GU237638
	CBS 116.93; PD 71/884		Antirrhinum majus	Scrophulariaceae	Netherlands	GU238134	GU237755	_	GU237649
H. sylvatica	CBS 874.97; PD 93/764		Melampyrum pratense	Scrophulariaceae	Netherlands	GU238148	GU237907	_	GU237662
H. verbascicola	CGMCC 3.18364; LC 8163	Т	Verbascum thapsus	Scrophulariaceae	China	KY742273	KY742119	KY742187	KY742361
	LC 8164		Verbascum thapsus	Scrophulariaceae	China	KY742274	KY742120	KY742188	KY742362
Leptosphaeria conoidea	CBS 616.75; ATCC 32813; IMI 199777; PD 74/56		Lunaria annua	Cruciferae	Netherlands	JF740279	JF740201	KT389639	KT389804
Leptosphaeria doliolum	CBS 505.75	Т	Urtica dioica	Urticaceae	Netherlands	GQ387576	JF740205	KT389640	JF740144
Leptosphaerulina americana	CBS 213.55		Trifolium pratense	Fabeceae	USA	GU237981	GU237799	KT389641	GU237539
L. arachidicola	CBS 275.59; ATCC 13446		Arachis hypogaea	Fabeceae	Taiwan, China	GU237983	GU237820	_	GU237543
L. australis	CBS 317.83		Eugenia aromatica	Myrtaceae	Indonesia	EU754166	GU237829	GU371790	GU237540
L. trifolii	CBS 235.58		<i>Trifolium</i> sp.	Fabeceae	Netherlands	GU237982	GU237806	_	GU237542
Macroventuria anomochaeta	CBS 502.72		Medicago sativa	Fabeceae	South Africa	GU237985	GU237873	_	GU237545

Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenBank accession numbers <sup>3</sup>				
•				,	2	LSU	ITS	RPB2	TUB	
	CBS 525.71	Т	Decayed canvas		South Africa	GU237984	GU237881	GU456346	GU23754	
M. wentii	CBS 526.71	Т	Plant litter		USA	GU237986	GU237884	KT389642	GU23754	
Neoascochyta argentina	CBS 112524	Т	Triticum aestivum	Poaceae	Argentina	KT389742	KT389524	_	KT38982	
Neoa. desmazieri	CBS 247.79		Poaceae	Poaceae	Austria	KT389725	KT389507	_	KT38980	
	CBS 297.69	Т	Lolium perenne	Poaceae	Germany	KT389726	KT389508	KT389644	KT38980	
	CBS 758.97		Hay		Norway	KT389727	KT389509	_	KT38980	
Neoa. europaea	CBS 819.84		Hordeum vulgare	Poaceae	Germany	KT389728	KT389510	KT389645	KT38980	
	CBS 820.84	Т	Hordeum vulgare	Poaceae	Germany	KT389729	KT389511	KT389646	KT38980	
Neoa. exitialis	CBS 118.40		_	_	_	KT389732	KT389514	KT389647	KT38981	
	CBS 389.86		Triticum aestivum	Poaceae	Switzerland	KT389733	KT389515	KT389648	KT38981	
	CBS 811.84		Secale cereale	Poaceae	Germany	KT389734	KT389516	_	KT38981	
	CBS 812.84		Hordeum vulgare	Poaceae	Germany	KT389735	KT389517	_	KT38981	
	CBS 110124		Triticum sp.	Poaceae	Netherlands	KT389730	KT389512	_	KT38981	
	CBS 113693; UPSC 1929		Allium sp.	Liliaceae	Sweden	KT389731	KT389513	_	KT38981	
Neoa. graminicola	CBS 301.69		Lolium multiflorum	Poaceae	Germany	KT389737	KT389519	KT389650	KT38981	
	CBS 447.82		Triticum aestivum	Poaceae	Germany	KT389738	KT389520	_	KT38981	
	CBS 586.79		Hordeum vulgare	Poaceae	Belgium	KT389739	KT389521	_	KT3898 <sup>-</sup>	
	CBS 815.84		Hordeum vulgare	Poaceae	Germany	KT389740	KT389522	_	KT38982	
	CBS 816.84		Hordeum vulgare	Poaceae	Germany	KT389741	KT389523	KT389651	KT38982	
	CBS 102789	R	Lolium perenne	Poaceae	New Zealand	KT389736	KT389518	KT389649	KT38981	
Neoa. paspali	CBS 560.81; PD 92/1569	Т	Paspalum dilatatum	Poaceae	New Zealand	GU238124	FJ427048	KP330426	FJ42715	
Neoa. soli	CGMCC 3.18365; LC 8165	Т	Soil		China	KY742275	KY742121	_	KY74236	
	LC 8166		Soil		China	KY742276	KY742122	_	KY7423	
Neoa. triticicola	CBS 544.74	Т	Triticum aestivum	Poaceae	South Africa	EU754134	GU237887	KT389652	GU2374	
Neodidymelliopsis achlydis	CBS 256.77	Т	Achlys triphylla	Berberidaceae	Canada	KT389749	KT389531	_	KT38982	
Neod. cannabis	CBS 121.75; ATCC 32164; IMI 194767; PD 73/584	Т	Urtica dioica	Urticaceae	Netherlands	GU237972	GU237761	_	GU2375	
	CBS 234.37		Cannabis sativa	Moraceae	_	GU237961	GU237804	KP330403	GU2375	
	CBS 591.67		Urtica dioica	Urticaceae	Netherlands	KT389746	KT389528	_	KT38982	
	CBS 629.76		Packing material		Netherlands	KT389747	KT389529	_	KT38982	
Neod. longicolla	CBS 382.96	Т	Soil in desert		Israel	KT389750	KT389532	_	KT3898	
Neod. polemonii	CBS 375.67		Polemonium caeruleum	Polemoniaceae	Netherlands	KT389748	KT389530	_	KT38982	

Table 1. (Continued).									
Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	Bank acce	ssion num	nbers <sup>3</sup>
						LSU	ITS	RPB2	TUB
	CBS 109181; PD 83/757	Т	Polemonium caeruleum	Polemoniaceae	Netherlands	GU238133	GU237746	KP330427	GU237648
Neod. xanthina	CBS 168.70		Delphinium sp.	Ranunculaceae	Netherlands	KT389751	KT389533	_	KT389831
	CBS 383.68	Т	Delphinium sp.	Ranunculaceae	Netherlands	GU238157	GU237855	KP330431	GU237668
Neomicrosphaeropsis italica	MFLUCC 15-0485; ICMP 21253	Т	<i>Tamarix</i> sp.	Tamaricaceae	Italy	KU729854	KU900318	KU674820	_
	MFLUCC 15-0484		<i>Tamarix</i> sp.	Tamaricaceae	Italy	KU729853	KU900319	KU695539	KX453298
	MFLUCC 16-0284		<i>Tamarix</i> sp.	Tamaricaceae	Italy	KU900296	KU900321	KU714604	KX453299
Neom. novorossica	MFLUCC 14-0578; ICMP 20751	Т	Tamarix ramosissima	Tamaricaceae	Russia	KX198710	KX198709	_	_
Neom. rossica	MFLUCC 14-0586; ICMP 20753	Т	Tamarix ramosissima	Tamaricaceae	Russia	KU729855	KU752192	_	_
Neom. tamaricicola	MFLUCC 14-0443; ICMP 20708		Tamarix gallica	Tamaricaceae	Italy	KU729851	KU900322	_	_
	MFLUCC 14-0439; ICMP 20743		Tamarix gallica	Tamaricaceae	Italy	KU729858	KU900323	_	_
Nothophoma anigozanthi	CBS 381.91; PD 79/1110	Т	Anigozanthus maugleisii	Haemodoraceae	Netherlands	GU238039	GU237852	KT389655	GU237580
No. arachidis-hypogaeae	CBS 125.93; PD 77/1029	R	Arachis hypogaea	Fabeceae	India	GU238043	GU237771	KT389656	GU237583
No. gossypiicola	CBS 377.67		Gossypium sp.	Malvaceae	USA	GU238079	GU237845	KT389658	GU237611
No. infossa	CBS 123395	Т	Fraxinus pennsylvanica	Oleaceae	Argentina	GU238089	FJ427025	KT389659	FJ427135
No. quercina	CBS 633.92; ATCC 36786; VKM MF-325		Microsphaera alphitoides from Quercus sp.		Ukraine	EU754127	GU237900	KT389657	GU237609
Paraboeremia adianticola	CBS 187.83; PD 82/128		Polystichum adiantiforme	Dryopteridaceae	USA	GU238035	GU237796	KP330401	GU237576
	CBS 260.92; PD 86/1103		Pteris ensiformis	Pteridaceae	_	KT389752	KT389534	_	KT389832
Pa. camellae	CGMCC 3.18106; LC 4852	Т	<i>Camellia</i> sp.	Theaceae	China	KX829042	KX829034	KX829050	KX829058
	CGMCC 3.18107; LC 6253		<i>Camellia</i> sp.	Theaceae	China	KX829043	KX829035	KX829051	KX829059
	CGMCC 3.18108; LC 6254		<i>Camellia</i> sp.	Theaceae	China	KX829044	KX829036	KX829052	KX829060
Pa. litseae	CGMCC 3.18109; LC 5028	Т	<i>Litsea</i> sp.	Lauraceae	China	KX829037	KX829029	KX829045	KX829053
	CGMCC 3.18110; LC 5030		<i>Litsea</i> sp.	Lauraceae	China	KX829038	KX829030	KX829046	KX829054
Pa. oligotrophica	CGMCC 3.18111; LC 6250	Т	Carbonatite		China	KX829039	KX829031	KX829047	KX829055
	CGMCC 3.18112; LC 6251		Carbonatite		China	KX829040	KX829032	KX829048	KX829056
	CGMCC 3.18113; LC 6252		Carbonatite		China	KX829041	KX829033	KX829049	KX829057
Pa. putaminum	CBS 130.69; CECT 20054; IMI 331916	R	Malus sylvestris	Rosaceae	Denmark	GU238138	GU237777	_	GU237652
	CBS 372.91; PD 75/960	R	<i>Ulmus</i> sp.	Ulmaceae	Netherlands	GU238137	GU237843	_	GU237651
Pa. selaginellae	CBS 122.93; PD 77/1049	Т	Selaginella sp.	Selaginellaceae	Netherlands	GU238142	GU237762	_	GU237656
Phoma herbarum	CBS 134.96; PD 84/676		Delphinium sp.	Ranunculaceae	Netherlands	KT389753	KT389535	KT389661	KT389834
	CBS 274.37		Picea excelsa	Pinaceae	UK	KT389754	KT389537	KT389662	KT389835
	CBS 304.51		Achillea millefolium	Asteraceae	Switzerland	KT389755	KT389538	_	KT389836
	CBS 377.92; IMI 213845		Human leg		Netherlands	KT389756	KT389536	KT389663	KT389837

CHEN ET AL.

Table 1. (Continued).Species	Strain number <sup>1</sup>	Statue <sup>2</sup>	Host, substrate	Host family	Country	Gon	Sank acco	ssion num	hore <sup>3</sup>
opecies	Strain humber	Status		nost ianny	Country	LSU	ITS	RPB2	TUB
	CBS 502.91; PD 82/276		Nerium sp.	 Apocynaceae	Netherlands	GU238082	GU237874	KP330419	GU23761
	CBS 615.75; PD 73/665; IMI 199779	R	Rosa multiflora cv. Cathayensis	Rosaceae	Netherlands	EU754186	FJ427022	KP330420	FJ427133
	CBS 127589; UAMH 10909		Polytrichum juniperinum	Polytrichaceae	USA	KT389757	KT389539	KT389664	KT38983
Phomatodes aubrietiae	CBS 383.67; PD 65/223	R	Aubrietia hybrida cv. Superbissima	Cruciferae	Netherlands	GU238044	GU237854	_	GU23758
Phomat. aubrietiae	CBS 627.97; PD 70/714	т	Aubrietia sp.	Cruciferae	Netherlands	GU238045	GU237895	KT389665	GU23758
Phomat. nebulosa	CBS 117.93; PD 83/90		Mercurialis perennis	Euphorbiaceae	Netherlands	GU238114	GU237757	KP330425	GU23763
	CBS 740.96		Armoracia rusticana	Cruciferae	Netherlands	KT389758	KT389540	KT389667	KT38983
	CBS 100191		Thlaspi arvense	Cruciferae	Poland	KP330446	KP330434	KT389666	KP33039
Pseudohendersonia galiorum	MFLUCC 14-0452	т	<i>Galium</i> sp.	Rubiaceae	Italy	KU848207	_	_	_
Stagonosporopsis actaeae	CBS 106.96; PD 94/1318	т	Actaea spicata	Ranunculaceae	Netherlands	GU238166	GU237734	KT389672	GU23767
	CBS 114303; UPSC 2962		Actaea spicata	Ranunculaceae	Sweden	KT389760	KT389544	_	KT38984
S. ajacis	CBS 177.93; PD 90/115	т	Delphinium sp.	Ranunculaceae	Kenya	GU238168	GU237791	KT389673	GU23767
S. andigena	CBS 101.80; PD 75/909; IMI 386090	R	Solanum sp.	Solanaceae	Peru	GU238169	GU237714	_	GU2376
	CBS 269.80; PD 75/914		Solanum sp.	Solanaceae	Peru	GU238170	GU237817	_	GU23767
S. artemisiicola	CBS 102636; PD 73/1409	R	Artemisia dracunculus	Asteraceae	France	GU238171	GU237728	KT389674	GU2376
S. astragali	CBS 178.25; MUCL 9915	R	Astragalus sp.	Fabeceae	_	GU238172	GU237792	_	GU2376
S. bomiensis	CGMCC 3.18366; LC 8167	т	Boraginaceae	Boraginaceae	China	KY742277	KY742123	KY742189	KY74236
	LC 8168		Boraginaceae	Boraginaceae	China	KY742278	KY742124	KY742190	KY74236
S. caricae	CBS 248.90		Carica papaya	Caricaceae	Chile	GU238175	GU237807	_	GU23768
	CBS 282.76		Brassica sp.	Cruciferae	Indonesia	GU238177	GU237821	_	GU23768
S. chrysanthemi	CBS 500.63; MUCL 8090	R	Chrysanthemum indicum	Asteraceae	Germany	GU238190	GU237871	_	GU23769
	CBS 137.96; PD 84/75	R	Chrysanthemum indicum	Asteraceae	Netherlands	GU238191	GU237783	_	GU23769
S. crystalliniformis	CBS 713.85; ATCC 76027; PD 83/826	т	Lycopersicon esculentum	Solanaceae	Colombia	GU238178	GU237903	KT389675	GU23768
S. cucurbitacearum	CBS 133.96; PD 79/127		Cucumis sp.	Cucurbitaceae	New Zealand	GU238181	GU237780	KT389676	GU23768
S. dennisii	CBS 631.68; PD 68/147	Т	Solidago floribunda	Asteraceae	Netherlands	GU238182	GU237899	KT389677	GU23768
S. dorenboschii	CBS 426.90; IMI 386093; PD 86/551	Т	Physostegia virginiana	Lamiaceaee	Netherlands	GU238185	GU237862	KT389678	GU23769
S. helianthi	CBS 200.87	Т	Helianthus annuus	Asteraceae	Italy	KT389761	KT389545	KT389683	KT38984
S. heliopsidis	CBS 109182; PD 74/231	R	Heliopsis patula	Asteraceae	Netherlands	GU238186	GU237747	KT389679	GU23769
S. hortensis	CBS 104.42	R	_		Netherlands	GU238198	GU237730	KT389680	GU23770
	CBS 572.85; PD 79/269	R	Phaseolus vulgaris	Fabeceae	Netherlands	GU238199	GU237893	KT389681	GU2377
S. inoxydabilis	CBS 425.90; PD 81/520	т	Chrysanthemum parthenii	Asteraceae	Netherlands	GU238188	GU237861	KT389682	GU23769

Didymellaceae revisited

Species	Strain number <sup>1</sup>	Status <sup>2</sup>	Host, substrate	Host family	Country	GenE	ank acces	ssion num	ıbers <sup>3</sup>
				-	-	LSU	ITS	RPB2	TUB
S. loticola	CBS 562.81; PDDCC 6884	т	Lotus pedunculatus	Fabeceae	New Zealand	GU238192	GU237890	KT389684	GU237697
S. lupini	CBS 101494; PD 98/5247	Т	Lupinus albus	Fabeceae	UK	GU238194	GU237724	KT389685	GU237699
S. oculo-hominis	CBS 634.92; IMI 193307	Т	Human corneal ulcer		USA	GU238196	GU237901	KT389686	GU237701
S. papillatus	CGMCC 3.18367; LC 8169	Т	Rumex nepalensis	Polygonaceae	China	KY742279	KY742125	KY742191	KY742367
	LC 8170		Rumex nepalensis	Polygonaceae	China	KY742280	KY742126	KY742192	KY742368
	LC 8171		Boraginaceae	Boraginaceae	China	KY742281	KY742127	KY742193	KY742369
S. rudbeckiae	CBS 109180; PD 79/175	R	Rudbeckia bicolor	Asteraceae	Netherlands	GU238197	GU237745	_	GU237702
S. tanaceti	CBS 131484	Т	Tanacetum cinerariifolium	Asteraceae	Australia	JQ897461	NR_111724	_	JQ897496
S. trachelii	CBS 379.91; PD 77/675	R	Campanula isophylla	Campanulaceae	Netherlands	GU238173	GU237850	KT389687	GU237678
	CBS 384.68	R	Campanula isophylla	Campanulaceae	Sweden	GU238174	GU237856	_	GU237679
S. valerianellae	CBS 273.92; PD 82/43		Valerianella locusta	Caprifoliaceae	Netherlands	GU238200	GU237819	_	GU237705
	CBS 329.67; PD 66/302	Т	Valerianella locusta var. oleracea	Caprifoliaceae	Netherlands	GU238201	GU237832	_	GU237706
Xenodidymella applanata	CBS 195.36	Т	Rubus idaeus	Rosaceae	Netherlands	KT389764	KT389548	_	KT389852
X. applanata	CBS 205.63		Rubus idaeus	Rosaceae	Netherlands	GU237998	GU237798	KP330402	GU237556
	CBS 115577		Rubus idaeus	Rosaceae	Sweden	KT389762	KT389546	KT389688	KT389850
	CBS 115578		Rubus arcticus nothossp. stellarcticus	Rosaceae	Sweden	KT389763	KT389547	_	KT389851
X. asphodeli	CBS 375.62	Т	Asphodelus albus	Asphodelaceae	France	KT389765	KT389549	KT389689	_
	CBS 499.72		Asphodelus ramosus	Asphodelaceae	Italy	KT389766	KT389550	_	KT389853
X. catariae X. humicola	CBS 102635; PD 77/1131 CBS 220.85; PD 71/1030	R	Nepeta cataria Franseria sp.	Lamiaceaee Asteraceae	Netherlands USA	GU237962 GU238086	GU237727 GU237800	KP330404 KP330422	GU237524 GU237617

<sup>1</sup> ATCC: American Type Culture Collection, Virginia, U.S.A.; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Westerdijk Fungal Biodiversity Institute (formerly CBS-KNAW), Utrecht, The Netherlands; CECT: Colección Española de Cultivos Tipo, Valencia University, Spain; CGMCC: China General Microbiological Culture Collection, Beijing, China; CPC: Culture collection of Pedro Crous, housed at CBS; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; FMR, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; LC: Corresponding author's personal collection deposited in laboratory, housed at CAS, China; LEV: Plant Health and Diagnostic Station, Auckland, New Zealand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL: Mycotheque de l'Universite catholique de Louvain, Louvain-la-Neuve, Belgium; PD: Plant Protection Service, Wageningen, the Netherlands; PDDCC: Plant Diseases Division Culture Collection, Auckland, New Zealand; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA; VKM: All-Russian Collection of Microorganisms, Pushchino, Russia.

<sup>2</sup> T: ex-type strain; R: representative strain.

<sup>3</sup> 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; TUB: ß-tubulin.

118

CHEN ET AL.

## RESULTS

## Phylogeny

A multi-locus phylogeny, based on four loci, was used to infer the relationships among species in *Didvmellaceae* (Fig. 1). The resulting concatenated aligned dataset comprised 360 ingroup isolates belonging to 194 taxa and consisted of 2 460 characters (964 for LSU, 531 for ITS, 599 for rpb2 and 354 for tub2, including alignment gaps), of which 265 are conserved and 901 are phylogenetically informative (173 for LSU, 230 for ITS, 310 for rpb2 and 188 for tub2). The trees generated from ML and Bayesian analyses of the individual loci (data not shown) and the combined dataset showed essentially congruent topologies. The ML tree based on the combined dataset was presented, with bootstrap support values (MLBS) and Bayesian posterior probabilities (BPP) indicated for well-supported clades in Fig. 1. The LSU sequences were the least successful in resolving species with only 59 out of 194 taxa resolved (30 %), followed by ITS with 104 out of 194 taxa (54 %), and tub2 (90 %) and rpb2 (92 %) which proved to be more suitable for the resolution of species.

A total of 194 ingroup taxa formed a clade (BPP = 1; MLBS = 100 %) representing the Didymellaceae, which include 19 monophyletic generic clades. Seventeen genera previously recognised, namely Allophoma (BPP = 1; MLBS = 100 %), Ascochyta (BPP = 1; MLBS = 87 %), Boeremia (BPP = 1; MLBS = 100 %), Calophoma (BPP = 1; MLBS = 90 %), Didymella (BPP = 0.97; MLBS = 60 %), Epicoccum (BPP = 1; MLBS = 99 %), Heterophoma (BPP = 1; MLBS = 99 %), Leptosphaerulina (BPP = 1; MLBS = 100 %), Macroventuria (BPP = 1; MLBS = 100 %), Neoascochyta (BPP = 1; MLBS = 80 %), Neodidymelliopsis (BPP = 1; MLBS = 100 %), Nothophoma (BPP = 1; MLBS = 76 %), Paraboeremia (BPP = 1; MLBS = 77 %), Phoma (BPP = 1; MLBS = 100 %), Phomatodes (BPP = 1; MLBS = 100 %), Stagonosporopsis (BPP = 1; MLBS = 93 %) and Xenodidymella (BPP = 1; MLBS = 96 %), and two genera recently added in this family, namely Briansuttonomyces (BPP = 1; MLBS = 100 %) and Neomicrosphaeropsis (BPP = 1; MLBS = 97 %) were highly supported as independent groups.

### Host specificity analysis

The heatmap was plotted to reveal the distribution of *Didy-mellaceae* species in various host families. The colour-coding columns indicate the number of species in each fungal genus that are associated with a particular host family. A darker colour indicates more fungal species related to the host family. In the present study, all the plant-associated species are linked to 70 different host families in total, of which *Asteraceae*, *Fabaceae*, *Poaceae*, *Ranunculaceae*, *Rosaceae* and *Solanaceae* are the most common hosts for *Didymellaceae*. Most of the *Didymellaceae* genera have a wide host range, while *Ascochyta*, *Neoascochyta* and *Neomicrosphaeropsis* showed relatively high host specificity within *Fabaceae*, *Poaceae* and *Tamaricaceae*, respectively (Fig. 2).

### Taxonomy

As a result of morphological comparisons and multi-locus sequence analysis of 360 strains, including 108 strains studied

in the present paper and 252 reference strains, 194 taxa are recognised in 19 different genera of *Didymellaceae*. Recognised clades of novel taxa are described and illustrated, and two new combinations are proposed below. One species proved to be sterile in culture, and therefore is described based on DNA sequence data, following the approach of Gomes *et al.* (2013) and Lombard *et al.* (2016). Novel taxa are arranged in alphabetical order by genus and species.

Allophoma Q. Chen & L. Cai, Stud. Mycol. 82: 162. 2015.

*Allophoma oligotrophica* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818956. Fig. 3.

*Etymology*: *Oligotrophica*, referring to the oligotrophic substrate of the fungus.

Conidiomata pycnidial, solitary, globose to subglobose, brown, glabrous, semi-immersed or immersed,  $150-440(-590) \times 145-420 \ \mu\text{m}$ . Ostioles single, slightly papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 3-5 layers,  $11-19.5 \ \mu\text{m}$  thick. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform,  $4.5-7 \times 3.5-6.5 \ \mu\text{m}$ . Conidia oblong to cylindrical, smooth- and thin-walled, hyaline, aseptate,  $3-4.5 \times 1.5-2.5 \ \mu\text{m}$ , with 2 distinct pale green polar guttules. Conidia matrix whitish.

*Culture characteristics*: Colonies on OA, 45–50 mm diam after 7 d, margin regular, covered by white floccose aerial mycelia, white to pale olivaceous; reverse buff, with pale olivaceous concentric rings near the centre. Colonies on MEA 50–55 mm diam after 7 d, margin regular, aerial mycelia sparse, olivaceous, white near the centre; reverse olivaceous. Colonies on PDA, 50–55 mm diam after 7 d, margin regular, covered by dense white felty aerial mycelia, white, olivaceous near the centre; reverse buff, olivaceous near the centre; reverse buff, olivaceous near the centre. NaOH test negative.

Specimens examined: China, Guizhou, Shuanghe Cave National Geopark, from air, 8 May 2015, Z.F. Zhang (holotype HMAS 247035, dried culture, ex-holotype living culture CGMCC 3.18114 = LC 6245); *ibid.* CGMCC 3.18115 = LC 6246; *ibid.* CGMCC 3.18116 = LC 6247.

Notes: Species of Allophoma were hitherto all known as plant pathogens, while Al. oligotrophica is the first species which was isolated from air using carbon-free silica gel medium (Jiang et al. 2017). Allophoma oligotrophica is closely related to Al. nicaraguensis (1 bp difference in ITS, 14 in rpb2 and 3 in tub2) and Al. tropica (1 bp difference in ITS, 15 in rpb2 and 2 in tub2) (Fig. 1). Morphologically, Al. oligotrophica produces larger pycnidia (150-440 × 145-420 µm vs. 30-150 × 28-120 µm) and longer conidiogenous cells (4.5–7 × 3.5–6.5 µm vs. 3–4.5 × 3.5–4.5 µm) than Al. nicaraguensis (Chen et al. 2015a), and differs from Al. tropica in its slightly larger conidiogenous cells  $(4.5-7 \times 3.5-6.5 \mu m vs. 2-6 \times 3-6 \mu m)$  and oblong to cylindrical conidia (de Gruyter & Noordeloos 1992).

Ascochyta Lib. emend. Q. Chen & L. Cai. Stud. Mycol. 82: 185. 2015.

*Synonym: Heracleicola* Tibpromma *et al.*, Fungal Divers. 75: 58. 2015.

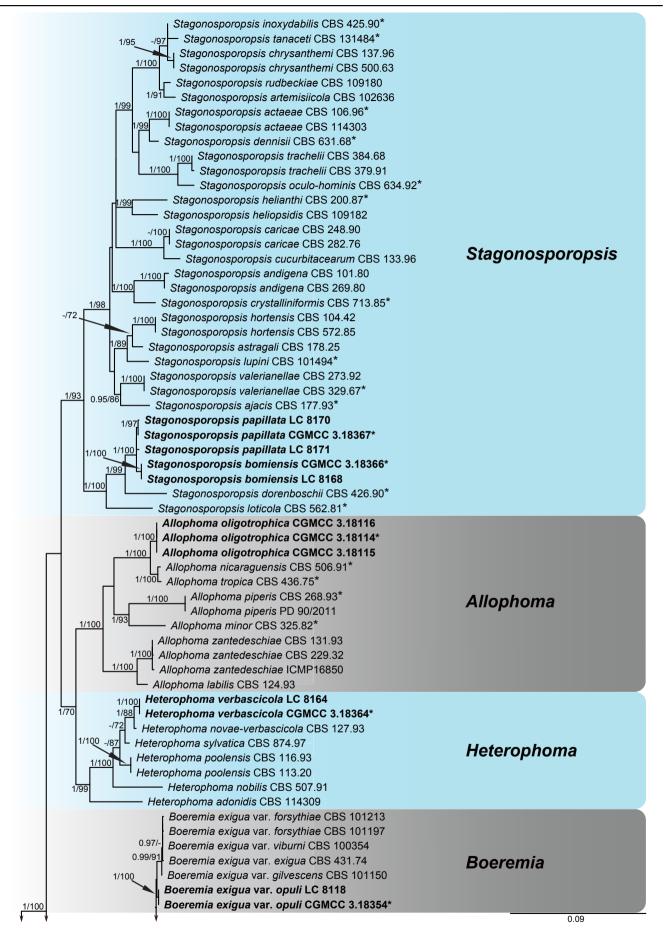


Fig. 1. Phylogenetic tree inferred from a Maximum likelihood analysis based on a concatenated alignment of LSU, ITS, *rpb2* and *tub2* sequences of 360 strains representing species in *Didymellaceae*. The RAxML bootstrap support values (MLBS) and Bayesian posterior probabilities (BPP) are given at the nodes (BPP/MLBS). Some branches were shortened to fit them to the page – these are indicated by two diagonal lines with the number of times a branch was shortened indicated next to the lines. New taxa and new combination introduced in this study are formatted in bold. Ex-type strains are marked by an asterisk (\*). The tree was rooted to *Leptosphaeria conoidea* (CBS 616.75) and *L. doliolum* (CBS 505.75).

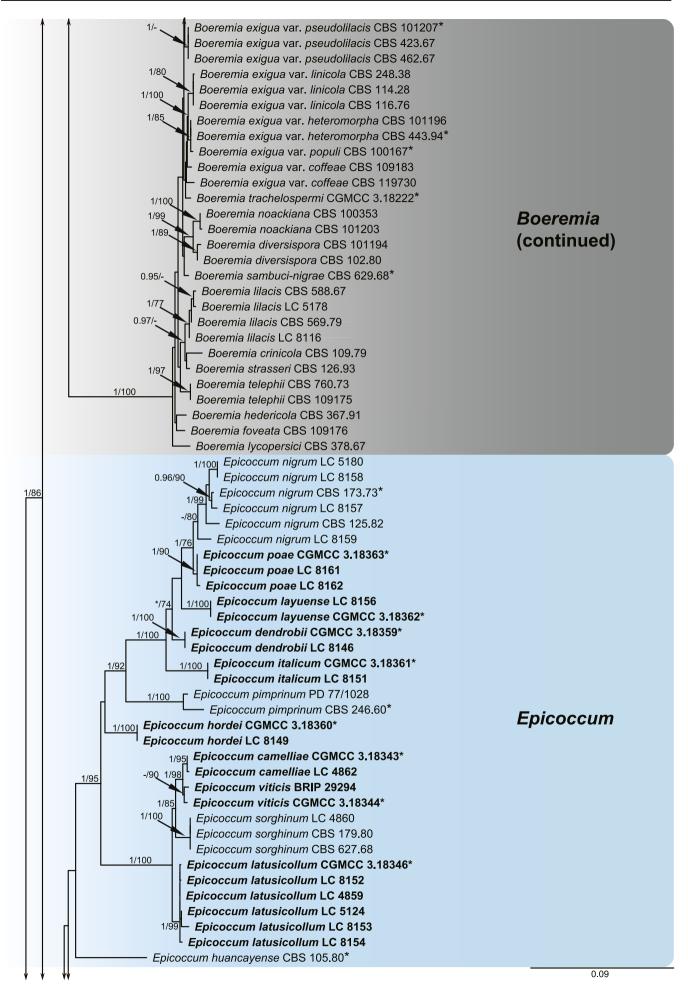


Fig. 1. (Continued).

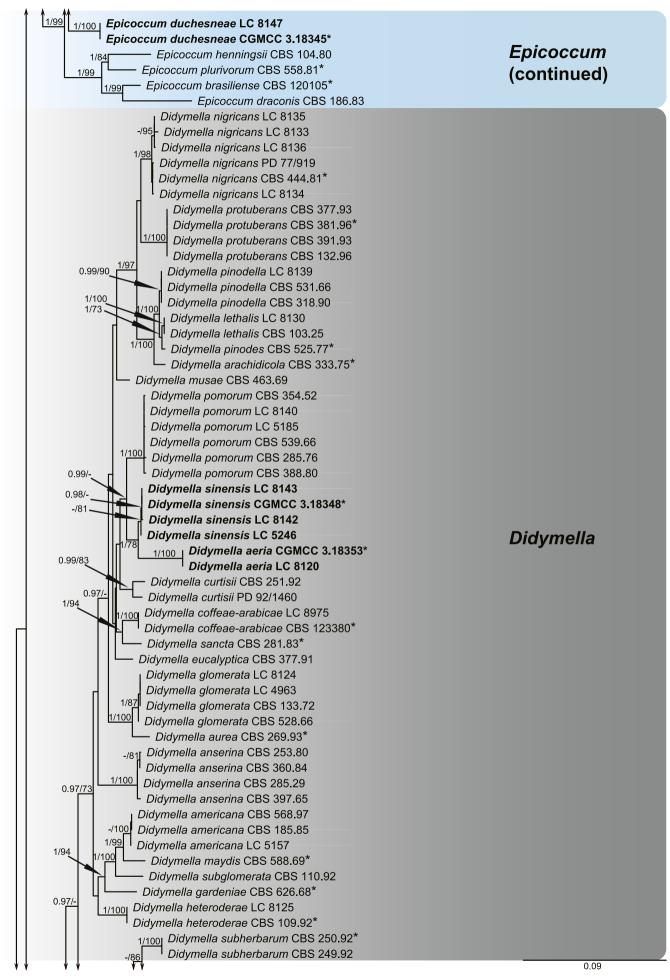


Fig. 1. (Continued).

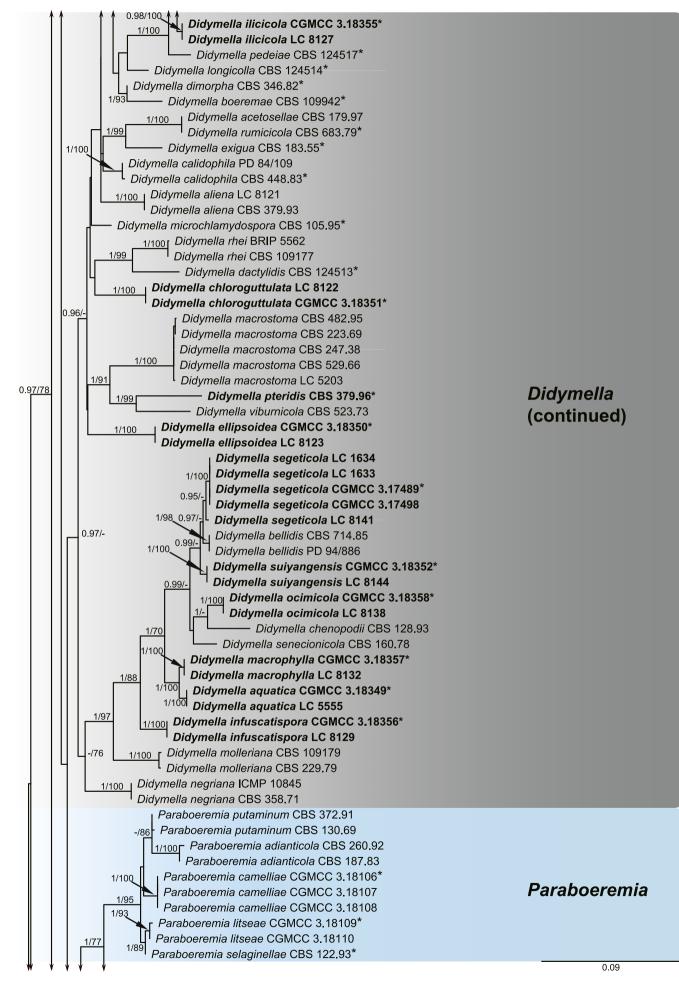


Fig. 1. (Continued).

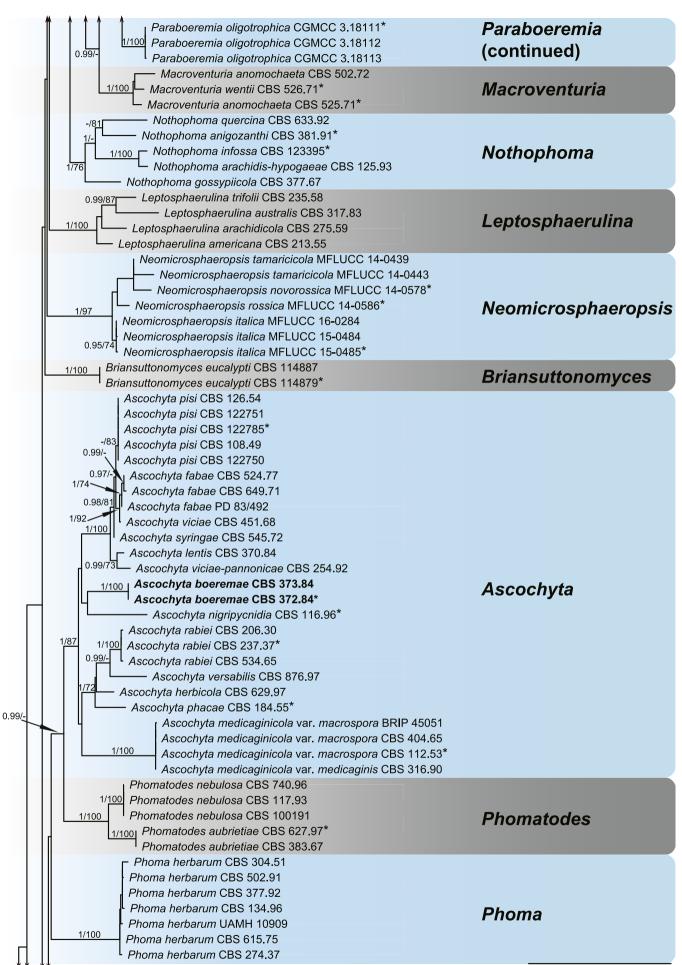


Fig. 1. (Continued).

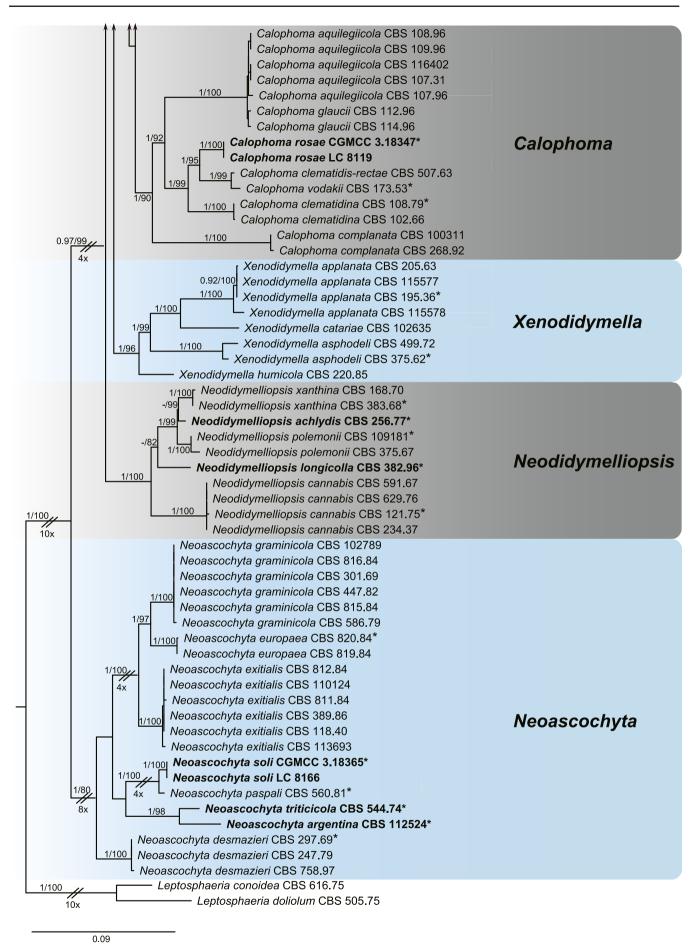


Fig. 1. (Continued).

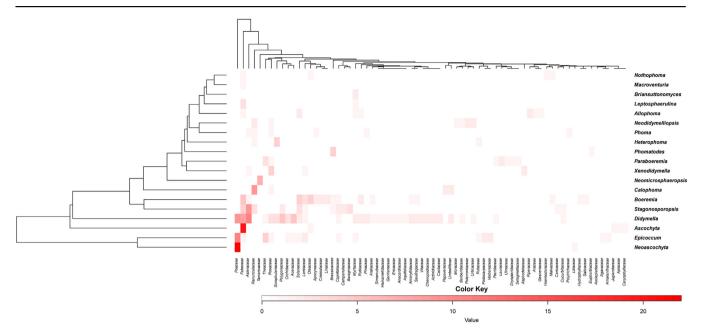


Fig. 2. Heatmap of relative abundances of different host plant families in each genus of *Didymellaceae*. The colour-coding for columns indicate the number of species in each fungal genus that are associated with a particular host family.

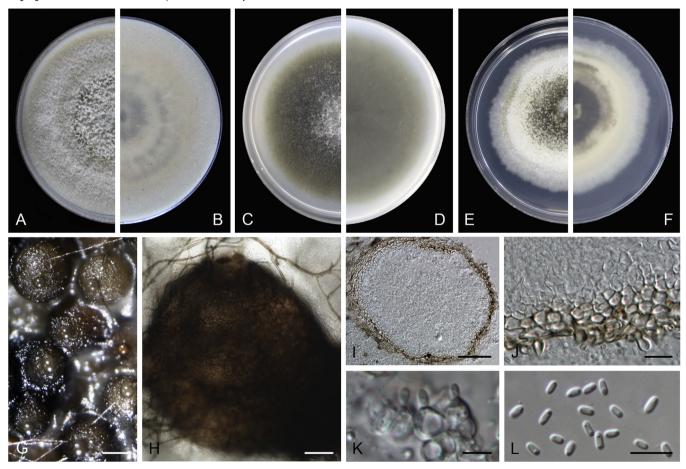


Fig. 3. Allophoma oligotrophica (CGMCC 3.18114). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia producing on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K. Conidiogenous cells. L. Conidia. Scale bars: G = 100 μm; H–I = 50 μm; J, L = 10 μm; K = 5 μm.

Ascochyta boeremae L.W. Hou, Crous & L. Cai, sp. nov. MycoBank MB820000. Fig. 4.

*Etymology*: Named after Gerhard H. Boerema, who collected the holotype of this species.

Conidiomata pycnidial, mostly solitary, sometimes confluent, (sub-) globose or flask-shaped, glabrous, semi-immersed in or superficial

on the agar, ostiolate,  $170-550(-650) \times 140-400(-650) \mu m$ . Ostiole single, slightly papillate, sometimes elongated as a short neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3-6-layers, with outer 2-3-layers pigmented,  $25-50 \mu m$  thick. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform or doliiform,  $9.5-14.5 \times 8.5-13 \mu m$ . *Conidia* greatly variable in shape and size, large conidia mostly oblong to bacilliform, or fusiform, mainly aseptate but sometimes uniseptate; small

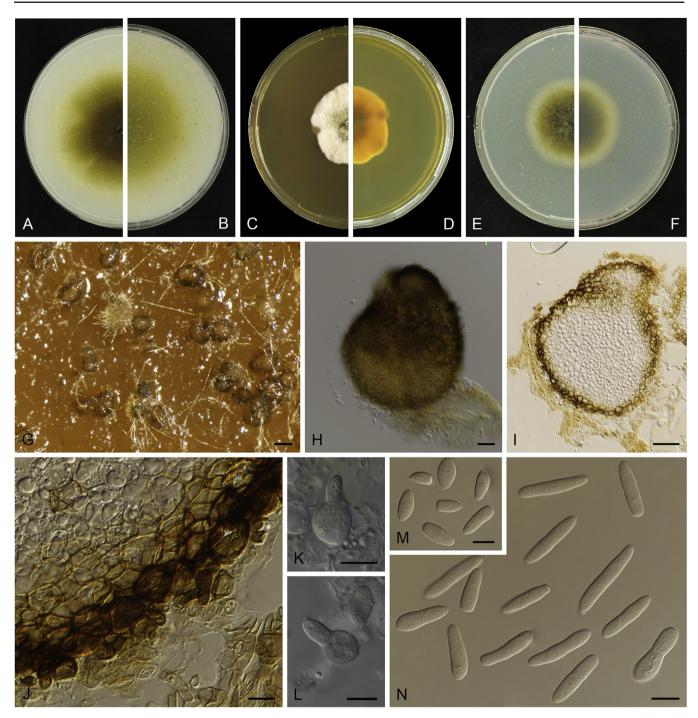


Fig. 4. Ascochyta boeremae (CBS 372.84). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K–L. Conidiogenous cells. M–N. Conidia. Scale bars: G = 300 µm; H–I = 50 µm; J–N = 10 µm.

conidia ellipsoidal to oval, broadly ovoid, smooth- and thin-walled, hyaline, aseptate,  $(14-)16.5-26(-32) \times 4.5-7.5(-8.5) \mu m$ , eguttulate or sometimes with 1–2 guttules per cell. *Conidial matrix* whitish cream.

*Culture characteristics*: Colonies on OA, 25–30 mm diam after 7 d, margin regular, covered by sparsely flat aerial mycelia, yellowish olivaceous; reverse concolourous. Colonies on MEA 20–25 mm diam after 7 d, margin regular, covered with floccose aerial mycelia, white, grey near the centre; reverse sienna to pale brown. Colonies on PDA, 15–20 mm diam after 7 d, margin regular, covered by woolly aerial mycelia, greenish olivaceous, buff near the margin; reverse concolourous. NaOH spot test: a dark reddish brown discolouration on MEA.

Specimens examined: Australia, from a leaf of *Pisum sativum*, deposited in CBS Sep. 1984, G.H. Boerema (holotype CBS H-23017, dried culture, ex-holotype living culture CBS 372.84 = PD 80/1246); from a leaf of *Pisum sativum*, deposited in CBS Sep. 1984, G.H. Boerema, CBS H-9078, culture CBS 373.84 = PD 80/1247.

Notes: CBS 372.84 and CBS 373.84 were originally deposited as "Ascochyta fabae", but are distinct from the authentic cultures of As. fabae (CBS 524.77, CBS 649.71 and PD 83/492) in the phylogenetic tree. Morphologically, these two strains produce aseptate conidia differing from the uniseptate conidia of As. fabae (Saccardo 1902). Therefore, we describe it as a new species, As. boeremae. Ascochyta boeremae is genetically closely related to As. nigripycnidia (Fig. 1), but differs morphologically from the latter by producing larger conidia (14–32 × 4.5–8.5 µm vs.  $5.5-15 \times 1.5-4$  µm; Boerema *et al.* 2004).

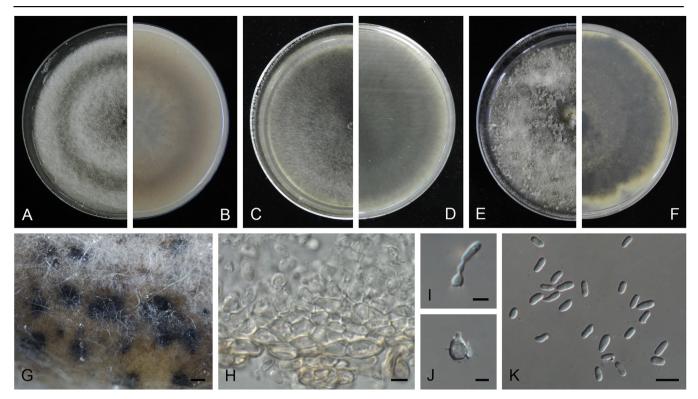


Fig. 5. Boeremia exigua var. opuli (CGMCC 3.18354). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Section of pycnidial wall. I–J. Conidiogenous cells. K. Conidia. Scale bars: G = 400 μm; H–J = 5 μm; K = 10 μm.

Ascochyta premilcurensis (Tibpromma et al.) Q. Chen, Crous & L. Cai, comb. nov. MycoBank MB820001.

*Basionym: Heracleicola premilcurensis* Tibpromma *et al.*, Fungal Divers. 75: 59. 2015.

### Description: Ariyawansa et al. (2015).

Specimen examined: **Italy**, Premilcuore, Province of Forli-Cesena, Valbura, on dead stem of *Heracleum sphondylium*, 6 Jun. 2014, E. Camporesi (**holotype** MFLU 14-0725, ex-holotype living culture MFLUCC 14-0518).

Notes: The genus *Heracleicola* was introduced by Ariyawansa *et al.* (2015) to accommodate a single species *Heracleicola premilcurensis*, which is located in the genus *Ascochyta* based on combined LSU and ITS analysis (Supplementary Fig. S1) in the present study. *Heracleicola* is therefore synonymised under *Ascochyta*, and a new combination in *Ascochyta* proposed.

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

*Boeremia exigua* var. *opuli* Q. Chen, Crous & L. Cai, var. nov. MycoBank MB818957. Fig. 5.

*Etymology*: Named after the host species from which the holotype was collected, *Viburmum opulus*.

Conidiomata pycnidial, solitary or aggregated, globose to subglobose, brown, covered with hyphae, produced on the agar surface or (semi-)immersed,  $245-360 \times 200-305 \mu$ m. Ostiole single, slightly papillate. Pycnidial wall pseudoparenchymatous, composed of isodiametric cells, 4-5 layers,  $20-37.5 \mu$ m thick. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform,  $4-9(-10) \times 4-7.5 \mu$ m. Conidia oblong to cylindrical, obovoid, incidentally slightly curved, or reniform, smooth- and thin-walled, hyaline, aseptate,  $5.5-9.5 \times 2.5-4 \mu$ m, with 2 or several minute guttules. Conidia matrix cream. *Culture characteristics*: Colonies on OA, 70–76 mm diam after 7 d, margin regular, covered by white floccose aerial mycelia, white with a pale green concentric ring, pale olivaceous near the centre; reverse reddish brown, grey near the centre. Colonies on MEA 70–75 mm diam after 7 d, margin regular, aerial mycelia white, velvety, olivaceous; reverse concolourous. Colonies on PDA, 65–80 mm diam after 7 d, margin regular, aerial mycelia white, felty, in some sectors covered by a low mat of floccose white to grey aerial mycelia, olivaceous near the centre; reverse olivaceous, with a buff margin. Application of NaOH results in a pale green discolouration of the agar.

Specimens examined: **USA**, from seedlings of *Viburmum opulus*, 2014, W.J. Duan (**holotype** HMAS 247147, dried culture, ex-holotype living culture CGMCC 3.18354 = LC 8117); *ibid.* LC 8118.

Notes: Boeremia exigua var. opuli is phylogenetically closely related to B. exigua var. exigua, B. exigua var. forsythiae, B. exigua var. glivescens and B. exigua var. viburni (Fig. 1). Although similar in conidial dimensions, pycnidia of B. exigua var. opuli (245-360 × 200-305 µm) are much larger than those of the other four varieties (75-200 µm; van der Aa et al. 2000). Boeremia exigua var. opuli also differs from those four varieties in seven positions in the rpb2 locus. Varieties of B. exigua are morphologically very similar and phylogenetically closely related to each other. Boeremia exigua var. exigua and var. forsythia have a wide host range, while other varieties appear host specific to a certain group of plants, such as var. coffeae to Coffea arabica (Rubiaceae), var. forsythia to Forsythia hybrids (Oleaceae), var. heteromorpha to Nerium oleander and Vinca spp. (Apocynaceae), var. linicola to Linum usitatissimum (Linaceae), var. populi to Populus and Salix (Salicaceae), and var. viburni to Viburnum spp. and occasionally Lonicera sp. (Caprifoliaceae) (Boerema et al. 2004). Besides, B. exigua var. pseudolilacis has been found only on Syringa vulgaris (Oleaceae; Aveskamp et al. 2010) and var. opuli only on Viburnum opulus. A host-range determination of B. exigua

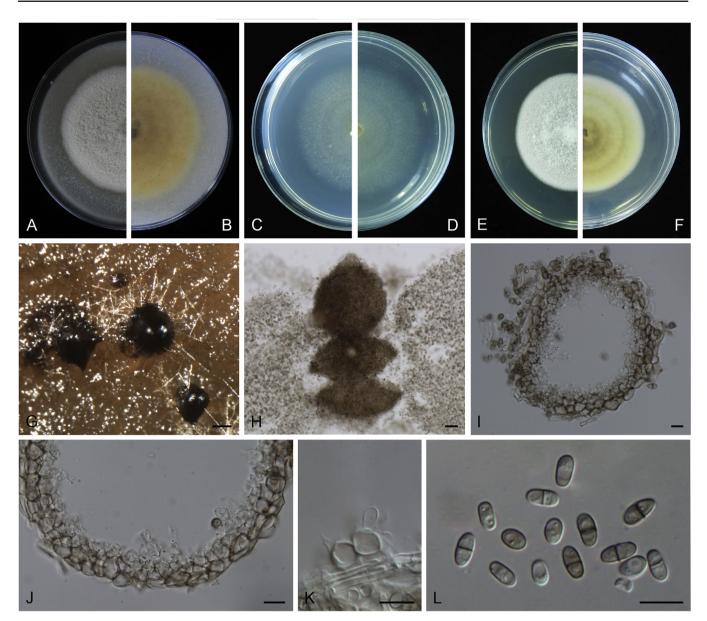


Fig. 6. Calophoma rosae (CGMCC 3.18347). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K. Conidiogenous cells. L. Conidia. Scale bars: G = 100 µm; H = 40 µm; I–L = 10 µm.

var. *rhapontica* indicates that this variety also has a very narrow host range (Berner *et al.* 2015). Thus, the plant generic interrelatedness is presumed to be the basis for susceptibility to *Boeremia exigua* varieties (Berner *et al.* 2015).

Calophoma Q. Chen & L. Cai, Stud. Mycol. 82: 191. 2015.

*Calophoma rosae* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818976. Fig. 6A.

*Etymology*: Named after the host genus *Rosa*, from which the holotype was isolated.

*Leaf spots* amphigenous, circular to irregular, up to 15 mm diam, occurring on or close to the tip of the leaf, brown, surrounded by a dark purple border (Fig. 7). *Conidiomata* pycnidial, mostly aggregated but sometimes solitary, globose to subglobose, brown, glabrous or covered with some hyphal outgrowths, semiimmersed in or superficial on the agar, ostiolate, (110–)  $130-210 \times (110-)130-180 \mu m$ . *Ostiole* single, sometimes with short necks, slightly papillate. *Pycnidial wall*  pseudoparenchymatous, composed of isodiametric cells, 3-4 layers, 11-20 µm thick, pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform,  $6.5-7 \times 7-8.5$  µm. *Conidia* ellipsoidal to oblong, smooth- and thin-walled, 0-1-septate, hyaline, later becoming pale brown with ageing,  $6-10 \times 3-4.5$  µm, eguttulate or sometimes with several guttules. *Conidial matrix* initially buff, gradually becoming dark brown.

*Culture characteristics*: Colonies on OA, 35–40 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense, white; reverse buff. Colonies on MEA 33–35 mm diam after 7 d, margin regular, aerial mycelia sparse, flattened, white; reverse concolourous. Colonies on PDA, 30–36 mm diam after 7 d, margin regular, aerial mycelia covering the whole colony, floccose, dense, white; reverse yellowish green, with concentric rings. NaOH test negative.

Specimens examined: China, Qinghai, Xunhua, from leaves of *Rosa* sp., 2 Sep. 2013, Q. Chen (holotype HMAS 247148, dried culture, ex-holotype living culture CGMCC 3.18347 = LC 5169); *ibid.* LC 8119.





Fig. 7. Symptoms on diseased leaves. A. Calophoma rosae on Rosa sp. B. Didymella infuscatispora on Chrysanthemum indicum. C. Didymella ocimicola on Ocimum sp. D. Didymella sinensis on Cerasus pseudocerasus. E. Epicoccum dendrobii on Dendrobium fimbriatum. F. Epicoccum duchesneae on Duchesnea indica. G. Stagonosporopsis bomiensis on Boraginaceae. H. Epicoccum viticis on Vitex negundo. I. Epicoccum layuense on Perilla sp. J. Heterophoma verbascicola on Verbascum thapsus. K. Stagonosporopsis papillata on Rumex nepalensis.

Notes: Calophoma rosae is phylogenetically closely related to C. clematidis-rectae and C. vodakii (Fig. 1). Morphologically C. rosae differs from C. clematidis-rectae in having larger conidiogenous cells ( $6.5-7 \times 7-8.5 \mu m vs. 3-5 \times 2.5-4.5 \mu m$ ), larger conidia (6–10 × 3–4.5  $\mu$ m vs. 3–8 × 2–3.5  $\mu$ m) (Aveskamp *et al.* 2010), and from *C. vodakii* in having shorter and wider conidia (6.5–7 × 7–8.5  $\mu$ m vs. 14–22 × 4–4.5  $\mu$ m; Saccardo & Trotter 1913, Müller 1953).

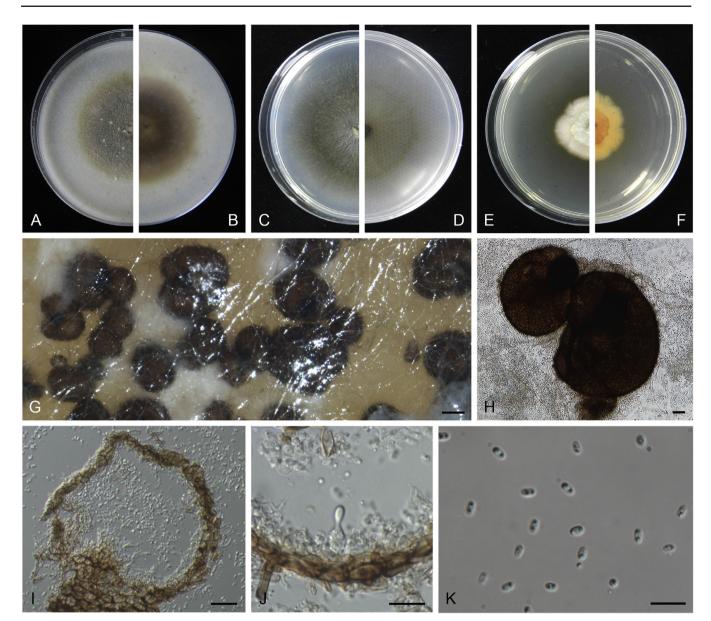


Fig. 8. Didymella aeria (CGMCC 3.18353). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K. Conidia. Scale bars: G = 200 µm; H = 30 µm; I = 20 µm; J–K = 10 µm.

*Calophoma rosae* is the first and only record thus far from the *Rosaceae*, while most species in this genus are associated with species of *Ranunculaceae*.

*Didymella* Sacc. ex Sacc., Syll. Fung. 1: 545. 1882, emend. Q. Chen & L. Cai, Stud. Mycol. 82: 173. 2015.

*Didymella aeria* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818968. Fig. 8.

*Etymology*: Name linked to the fact that this species was collected from air.

Conidiomata pycnidial, solitary or aggregated, globose to subglobose, later becoming irregular, brown, glabrous, superficial or semi-immersed,  $155-375(-460) \times 130-340(-460) \mu m$ . Ostiole single, with a short neck, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3 layers,  $8.5-25 \mu m$  thick, brownpigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform,  $5-7 \times 4.5-6 \mu m$ . Conidia ellipsoidal, smooth- and thin-walled, hyaline, aseptate,  $3-5 \times 2-3 \mu m$ , with 2 large dull green polar guttules. *Conidial matrix* salmon.

*Culture characteristics*: Colonies on OA, 55–60 mm diam after 7 d, margin regular, white aerial mycelia sparse, brownish olivaceous; reverse white to reddish brown. Colonies on MEA 44–48 mm diam after 7 d, margin regular, white to olivaceous, with sparse white aerial mycelia spreading over the colony; reverse concolourous. Colonies on PDA, 15–20 mm diam after 7 d, margin irregular, fluffy to felty, white; reverse amber to saffron. NaOH spot test: a brown discolouration on MEA.

Specimens examined: China, Guizhou, Zunyi, Shuanghe Cave National Geopark, from air, 8 May 2015, Z.F. Zhang (holotype HMAS 247149, dried culture, ex-holotype living culture CGMCC 3.18353 = LC 7441); *ibid.* LC 8120.

*Notes*: The most closely related species to *Didymella aeria* are *D. sinensis* and *D. pomorum* (Fig. 1), but with respectively 33 bp and 55 bp differences in four sequenced loci. *Didymella aeria* produces hyaline conidia measuring  $3-5 \times 2-3 \mu m$ , while *D. pomorum* produces longer, brown conidia ( $4-8 \times 1.5-3 \mu m$ ; Boerema 1993). The asexual morph of *D. sinensis* was



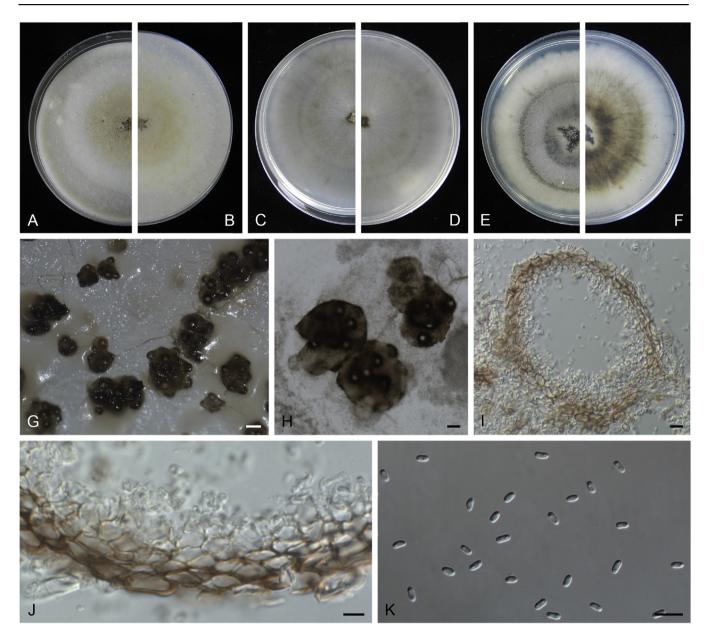


Fig. 9. Didymella aquatica (CGMCC 3.18349). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia sporulating on OA. H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K. Conidia. Scale bars: G = 100 µm; H = 50 µm; I, K = 10 µm; J = 5 µm.

unfortunately not observed. *Didymella aeria* was trapped from air in a Karst cave in China.

*Didymella aquatica* Q. Chen, Crous & L. Cai, **sp. nov.** Myco-Bank MB818973. Fig. 9.

*Etymology*: Name derived from the substrate where the holotype was collected, water.

*Conidiomata* pycnidial, solitary, sometimes aggregated, globose to subglobose, brown, glabrous or covered with some hyphal outgrowths, superficial, ostiolate,  $105-355 \times 95-315 \mu m$ . *Ostioles* 2–13, sometimes elongated as a short neck, up to 50.5  $\mu m$  long, papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–5 layers, 14–35  $\mu m$  thick, outer wall 2–3-layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform, 4–5 × 3.5–5  $\mu m$ . *Conidia* ellipsoidal to oblong, smooth- and thinwalled, hyaline, aseptate, 4–5.5 × 2–3  $\mu m$ , with 2 distinct polar guttules. *Conidial matrix* cream.

*Culture characteristics*: Colonies on OA, 15–40 mm diam after 7 d, margin regular, covered by velvety aerial mycelia, flat, white to amber; reverse concolourous. Colonies on MEA 46–53 mm diam after 7 d, margin regular, white to pale green, with sparse aerial mycelia near the centre; reverse concolourous. Colonies on PDA, 54–56 mm diam after 7 d, margin regular, floccose to felty, white to grey, iron grey near the centre; reverse white, hazel to brown. NaOH test negative.

Specimens examined: China, Guizhou, Kuankuoshui National Geopark, water, 23 Jul. 2014, Z.F. Zhang (holotype HMAS 247150, dried culture, ex-holotype living culture CGMCC 3.18349 = LC 5556); *ibid.* LC 5555.

*Notes*: *Didymella aquatica* formed a distinct lineage sister to *D. macrophylla*, with 6 bp differences in both *rpb2* and *tub2* loci. Morphologically, *D. aquatica* is clearly differentiated from *D. macrophylla* in producing smaller conidiogenous cells  $(4-5 \times 3.5-5 \ \mu m \ vs. \ 6-8 \times 4.5-8 \ \mu m)$ , longer and narrower conidia  $(4-5.5 \times 2-3 \ \mu m \ vs. \ 1.5-2.5 \times 3.5-5.5 \ \mu m)$ , and in the number of conidiomatal ostioles  $(2-13 \ vs. \ 1)$ . This is the first *Didymella* species known from water.

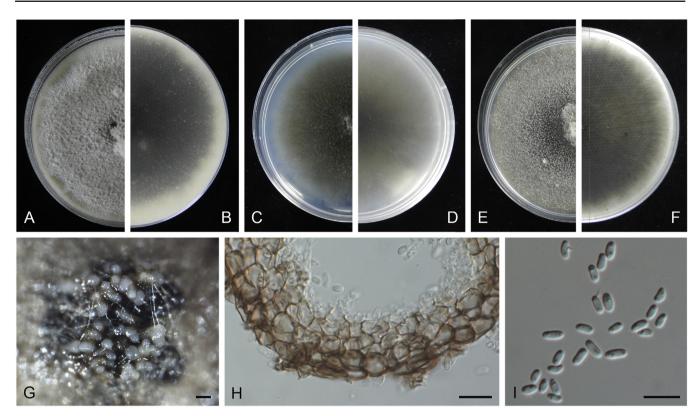


Fig. 10. Didymella chloroguttulata (CGMCC 3.18351). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Section of pycnidial wall. I. Conidia. Scale bars: G = 200 µm; H–I = 10 µm.

*Didymella chloroguttulata* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818970. Fig. 10.

*Etymology*: Latin, *chloro-* = green, referring to the two green guttules of the conidia.

Conidiomata pycnidial, confluent, globose to subglobose, brown, glabrous, superficial,  $145-260(-410) \times 130-230(-365) \mu m$ . Ostiole single, sometimes with a short neck, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3-4 layers,  $14.5-22 \mu m$  thick, pigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform,  $5.5-8 \times 4-6.5 \mu m$ . Conidia oblong to cylindrical, incidentally slightly curved, smooth- and thin-walled, hyaline, aseptate,  $4-6 \times 2-3 \mu m$ , with 2-3 dull green polar guttules. Conidial exudates not recorded.

*Culture characteristics*: Colonies on OA, 54–57 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense, grey; reverse black. Colonies on MEA 44–47 mm diam after 7 d, margin regular, white aerial mycelia sparse, fluffy, greenish brown; reverse concolourous. Colonies on PDA, 57–62 mm diam after 7 d, margin regular, floccose, grey to leaden-black; reverse leaden-black. NaOH spot test: a pale reddish brown discolouration on MEA.

Specimens examined: China, Guizhou, Zunyi, Shuanghe Cave National Geopark, air, 8 May 2015, Z.F. Zhang (holotype HMAS 247151, dried culture, exholotype living culture CGMCC 3.18351 = LC 7435); *ibid.* LC 8122.

Notes: Didymella chloroguttulata is characterised by having two to three dull green polar guttules in its oblong to cylindrical conidia and sometimes having conidiomata with a short neck. In the phylogenetic tree, it formed a distinct clade sister to *D. dactylidis* and *D. rhei* (Fig. 1). *Didymella chloroguttulata* is well distinguished from these two species in the NaOH reactions (pale reddish brown discolouration on *D. chloroguttulata*, slight greenish discolouration on *D. dactylidis*, and no effect on *D. rhei*) (de Gruyter *et al.* 2002, Aveskamp *et al.* 2010).

*Didymella ellipsoidea* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818971. Fig. 11.

Etymology: Name refers to its ellipsoidal conidia.

Conidiomata pycnidial, solitary, globose to subglobose, brown, glabrous or covered with some hyphal outgrowths, superficial, ostiolate,  $335-400(-460) \times 290-340(-440) \mu m$ . Ostioles 1–8, often developing to elongated short necks, up to 80  $\mu m$  long, papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, 23.5–50  $\mu m$  thick, pigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, 5.5–7.5 × 4.5–6.5  $\mu m$ . Conidia ellipsoidal, smoothand thin-walled, hyaline, aseptate, 3–4.5 × 2–3  $\mu m$ , with 2 pale green guttules. Conidial matrix cream.

*Culture characteristics*: Colonies on OA, 56–62 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense, white to pale brown; reverse white to greenish brown. Colonies on MEA 61–64 mm diam after 7 d, margin regular, white aerial mycelia sparse, fluffy, grey to olivaceous; reverse concolourous. Colonies on PDA, 52–54 mm diam after 7 d, margin regular, floccose, grey to leaden-black, with a white concentric ring near the centre; reverse concolourous. NaOH test negative.

Specimen examined: China, Guizhou, Zunyi, Shuanghe Cave National Geopark, air, 8 May 2015, Z.F. Zhang (holotype HMAS 247152, dried culture, ex-holotype living culture CGMCC 3.18350 = LC 7434); *ibid.* LC 8123.



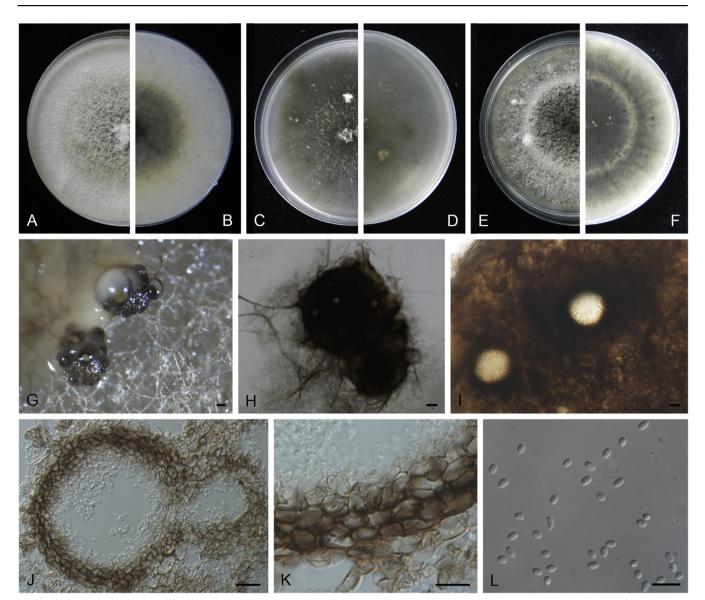


Fig. 11. *Didymella ellipsoidea* (CGMCC 3.18350). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia sporulating on OA. H. Pycnidia. I. Ostioles on pycnidium. J. Section of pycnidium. K. Section of pycnidial wall. L. Conidia. Scale bars: G = 100 μm; H = 50 μm; I, K–L = 10 μm; J = 20 μm.

*Notes*: This species is represented by two isolates trapped from air in a Karst cave which cluster in a distinct lineage clearly differentiated from other species in *Didymella* (Fig. 1). Morphologically, *Didymella ellipsoidea* is distinguishable from its closest neighbours, *D. viburnicola*, in producing wider conidia (3–4.5 × 2–3 µm vs. 3.5–5.5 × 1.5–2 µm; de Gruyter & Noordeloos 1992), from *D. macrostoma* in having shorter conidia (3–4.5 × 2–3 µm vs. 4–11 × 2–4 µm; de Gruyter *et al.* 2002), and from *D. pteridis* in producing larger conidiogenous cells (5.5–7.5 × 4.5–6.5 µm vs. 4–5 × 3.5–4.5 µm).

*Didymella ilicicola* Q. Chen, Crous & L. Cai, **sp. nov.** Myco-Bank MB818969. Fig. 12.

*Etymology*: Name derived from *llex*, the plant from which the holotype was collected.

*Conidiomata* pycnidial, solitary or aggregated, (sub-)globose to flask-shaped, or obpyriform, brown, later becoming irregular when matured, covered with hyphal outgrowths, mostly

(80-)erumpent, sometimes semi-immersed, ostiolate, 150-200 × (70-)150-180 µm. Ostioles 2-3, elongated as short papillate necks. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 2-5 layers, 15-20 µm thick, outer wall 2-3-layers pigmented. Conidiogenous cells phialidic, hyaline. smooth, ampulliform to doliiform. 4.5-8 × 3.5-5 µm. Conidia ellipsoidal to oblong, smooth- and thin-walled, hyaline, aseptate, 3-4 × 1.5-2.5 µm, with two minute guttules. Conidial matrix cream to buff.

*Culture characteristics*: Colonies on OA, 43–50 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, white to pale buff, with a dull green concentric ring near the centre; reverse reddish brown to buff, with a brown concentric ring. Colonies on MEA 56–65 mm diam after 7 d, margin irregular, white, aerial mycelia sparse; reverse concolourous. Colonies on PDA, 62–65 mm diam after 7 d, margin regular, felty to floccose, dense, white to pale yellow; reverse white to buff with some pale reddish brown tings in concentric rings. NaOH test negative.

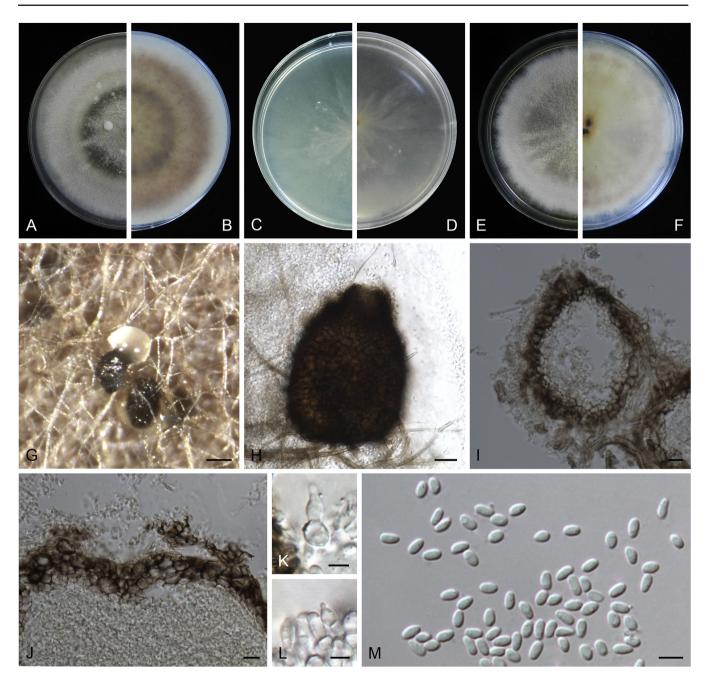


Fig. 12. Didymella ilicicola (CGMCC 3.18355). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K–L. Conidiogenous cells. M. Conidia. Scale bars: G = 100 μm; H = 20 μm; I–J = 10 μm; K–M = 5 μm.

Specimens examined: Italy, from seedlings of *llex chinensis*, 2013, W.J. Duan (holotype HMAS 247153, dried culture, ex-holotype living culture CGMCC 3.18355 = LC 8126); *ibid.* LC 8127.

Notes: Didymella ilicicola clustered in a clade together with *D. subherbarum* and *D. pedeiae* (Fig. 1), but with 1 bp and 9 bp differences in ITS and *tub2* respectively from *D. subherbarum* (lack of *rpb2* sequence), and 26 bp and 12 bp differences in *rpb2* and *tub2* respectively from *D. pedeiae*. Morphologically, *D. ilicicola* differs from *D. subherbarum* in producing shorter conidia ( $3-4 \times 1.5-2.5 \mu m vs. 4-6.5 \times 1.5-2 \mu m$ ; de Gruyter *et al.* 1993), and from *D. pedeiae* in producing larger conidiogenous cells ( $4.5-8 \times 3.5-5 \mu m vs. 3.5-4.5 \times 3-4 \mu m$ ; Aveskamp *et al.* 2010).

*Didymella infuscatispora* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818974. Fig. 13. *Etymology*: Latin, *infuscat-* = brownish, referring to the colour of its conidia.

Leaf spots amphigenous, irregular, 3–11 mm diam, extending along leaf margin to the whole leaf, dark grey to dark brown (Fig. 7B). Conidiomata pycnidial, solitary, globose to subglobose, brown, later becoming irregular when matured, covered with some hyphal outgrowths, superficial, ostiolate, (50-)95–265 ×  $(20-)75-165 \ \mu m$ . Ostiole single, sometimes elongated as short necks, slightly papillate. Pycnidial wall pseudoparenchymatous, composed of isodiametric cells, 2–3 layers, 13.5–23  $\mu m$  thick. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, 6–8.5 × 5.5–8  $\mu m$ . Conidia globose to broadly ellipsoidal, oblong, smooth- and thin-walled, hyaline, later becoming pale brown, mostly aseptate, occasionally 1-septate, 5–8.5 × 3.5–5.5  $\mu m$ , with several indistinct minute guttules. Conidial matrix dark brown.

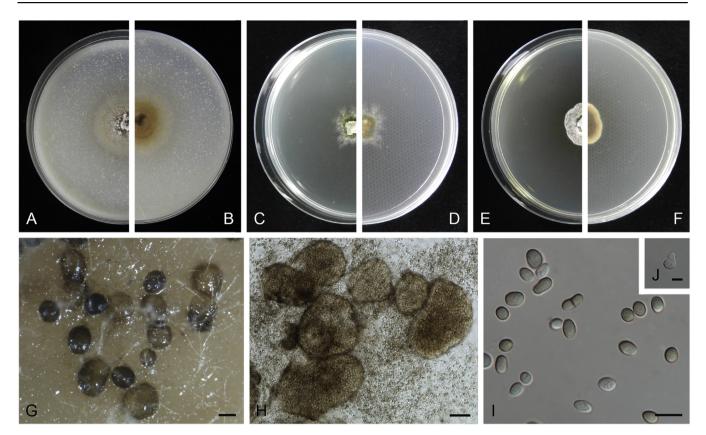


Fig. 13. Didymella infuscatispora (CGMCC 3.18356). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. J. Conidiogenous cell. Scale bars: G = 200 µm; H = 50 µm; I = 10 µm; J = 2.5 µm.

*Culture characteristics*: Colonies on OA, 15–20 mm diam after 7 d, margin regular, covered by felty aerial mycelia, white to buff, pale brown near the centre; reverse white to amber, hazel near the centre. Colonies on MEA 10–15 mm diam after 7 d, margin irregular, aerial mycelia sparse, white to pale green; reverse white to pale green, yellowish brown near the centre. Colonies on PDA, 14–16 mm diam after 7 d, margin regular, aerial mycelia felty, flat, white to pale brown; reverse buff to brown. NaOH test negative.

Specimens examined: China, Tibet, Lulang, on leaves of *Chrysanthemum indicum*, 15 Jun. 2015, Q. Chen (holotype HMAS 247154, dried culture, ex-holotype living culture CGMCC 3.18356 = LC 8128); *ibid*. LC 8129.

*Note*: This species clustered in a distinct lineage separated from other species in this genus, and is characterised by pale brown and broadly ellipsoidal conidia and a dark brown conidial matrix when mature.

*Didymella macrophylla* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB819189. Fig. 14.

*Etymology*: Named after the host species *Hydrangea macro-phylla*, from which the holotype was collected.

Conidiomata pycnidial, mostly solitary, sometime aggregated, globose to subglobose, pale brown, glabrous, semi-immersed or immersed in agar, ostiolate,  $(80-)120-200 \times (60-)100-150 \mu m$ . Ostiole single, slightly papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 1-2 layers,  $7-15 \mu m$  thick, pigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform,  $6-8 \times 4.5-8 \mu m$ . Conidia obovoid, ellipsoidal to oblong, smooth-

and thin-walled, hyaline, aseptate,  $3.5-5.5 \times 1.5-2.5 \mu m$ , with two polar guttules. *Conidial matrix* buff.

*Culture characteristics*: Colonies on OA, 44–46 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, white to grey, yellowish grey near the centre; reverse grey to greyish yellow. Colonies on MEA 54–60 mm diam after 7 d, margin regular, covered by white aerial mycelia, fluffy; reverse concolourous. Colonies on PDA, 59–68 mm diam after 7 d, margin regular, aerial mycelia floccose, dense, white to yellowish grey; reverse dark brown with pale olivaceous margin. NaOH test negative.

Specimens examined: **Italy**, *Hydrangea macrophylla*, 2013, W.J. Duan (**holotype** HMAS 247155, dried culture, ex-holotype living culture CGMCC 3.18357 = LC8131); *ibid*. LC 8132.

Note: Didymella macrophylla is phylogenetically most closely related to *D. aquatica* (Fig. 1), which is discussed under the notes of the latter species.

*Didymella ocimicola* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB819127. Fig. 15.

*Etymology*: Name derived from *Ocimum*, the plant host from which the holotype was collected.

*Leaf spots* amphigenous, irregular, 8–15 mm diam, next to or close to the leaf margin, pale brown (Fig. 7C). *Conidiomata* pycnidial, solitary, sometimes aggregated, globose to flask-shaped, brownish olivaceous, covered by some hyphal outgrowths, superficial or semi-immersed, ostiolate,  $100-235 \times 95-180 \mu m$ . *Ostiole* single, with an elongated neck, slightly papillate or non-

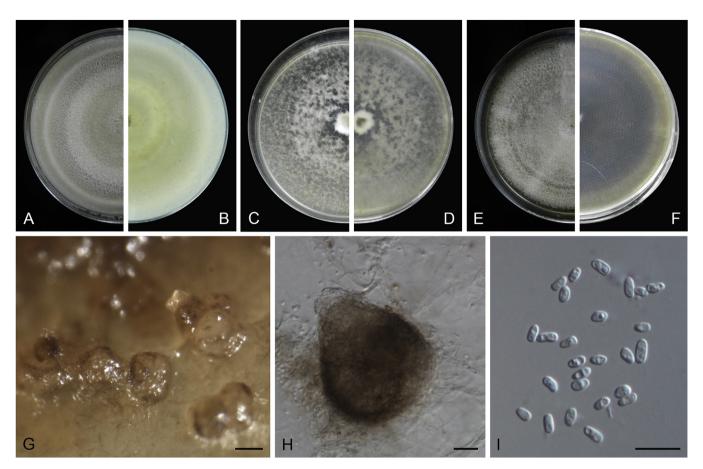


Fig. 14. Didymella macrophylla (CGMCC 3.18357). A-B. Colony on OA (front and reverse). C-D. Colony on MEA (front and reverse). E-F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Conidia. Scale bars: G = 200 μm; H-I = 10 μm.

papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 2–5 layers, 12–35.5  $\mu$ m thick, brownpigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform, 5–5.5 × 3.5–5  $\mu$ m. *Conidia* globose to broadly ellipsoidal, smooth- and thin-walled, hyaline, aseptate, 4–6.5 × 3–4.5  $\mu$ m, with one to several distinct guttules. Conidial exudates not recorded.

*Culture characteristics*: Colonies on OA, 10–15 mm diam after 7 d, margin regular, aerial mycelia floccose, flat, white to buff; reverse concolourous. Colonies on MEA 9–12 mm diam after 7 d, margin irregular, aerial mycelia floccose, white, dull green; reverse white to dull green. Colonies on PDA, 10–15 mm diam after 7 d, margin regular, aerial mycelia floccose, white; reverse olivaceous with white to pale brown patches. NaOH test negative.

Specimens examined: China, Tibet, Lulang, on leaves of *Ocimum* sp., 15 Jun. 2015, Q. Chen (holotype HMAS 247156, dried culture, ex-holotype living culture CGMCC 3.18358 = LC 8137); *ibid.* LC 8138.

Notes: Didymella ocimicola grouped closely with *D. chenopodii* and *D. senecionicola* (Fig. 1), but differs from *D. chenopodii* in smaller conidiogenous cells  $(5-5.5 \times 3.5-5 \,\mu\text{m vs.} 4-8 \times 4-6 \,\mu\text{m})$  and wider conidia  $(4-6.5 \times 3-4.5 \,\mu\text{m vs.} 5-5.5 \times 2-2.2 \,\mu\text{m})$  and from *D. senecionicola* in wider conidia  $(4-6.5 \times 3-4.5 \,\mu\text{m vs.} 4-6.5 \times 1.5-2.5 \,\mu\text{m})$  (de Gruyter *et al.* 1993). *Didymella ocimicola* has 44 bp and 30 bp differences in three loci (lack of *rpb2* sequence) from *D. chenopodii* and *D. senecionicola* respectively.

*Didymella pteridis* L.W. Hou, Crous & L. Cai, **sp. nov.** Myco-Bank MB820002. Fig. 16.

*Etymology*: Named after the host genus *Pteris*, from which the holotype was collected.

Conidiomata pycnidial, mainly solitary, sometimes aggregated, (sub-)globose or flask-shaped, glabrous or with some mycelial superficial semi-immersed, ostiolate, outgrowths, or 170-350(-430) × 150-330 µm. Ostiole single, papillate, sometimes elongated as a short neck, with dark colour near the ostioles. Pycnidial wall pseduoparenchymatous, composed of oblong to isodiametric cells, 3-6 layers, 9-28 µm thick, with outer 1-2-layers pigmented. Conidiogenous cell phialidic, hyaline, smooth, ampulliform to doliiform, 4-5 × 3.5-4.5 µm. Conidia ovoid to broadly oval, smooth- and thin-walled, hyaline, aseptate,  $(3-)4-6 \times 2.5-3.5 \mu m$ , with two polar guttules. Conidial matrix pale salmon.

*Culture characteristics*: Colonies on OA, 58–60 mm diam after 7 d, margin regular, aerial mycelia flat, cinnamon to hazel, mycelia sparse in some furrowed zone, pycnidia abundant near the margin; reverse buff to pale olivaceous. Colonies on MEA 20–25 mm diam after 7 d, margin regular, aerial mycelia floccose, white, grey near the centre, pale salmon conidial matrix appeared near the centre; reverse yellow in outer ring, changing towards the centre from saffron, hazel, greyish brown to brown. Colonies on PDA, 65–68 mm diam after 7 d, margin regular, densely covered by floccose aerial mycelia, greenish brown, with

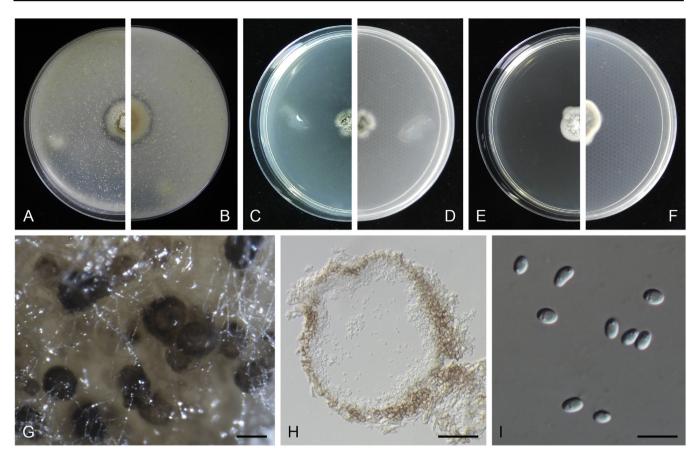


Fig. 15. Didymella ocimicola (CGMCC 3.18358). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Section of pycnidium. I. Conidia. Scale bars: G = 100 μm; H = 50 μm; I = 10 μm.

some white mycelial pellets scattering over the colony; reverse dark brown, buff near the margin. NaOH spot test: a pale reddish brown discolouration on MEA.

Specimen examined: **The Netherlands**, Wageningen, Alphen aan de Rijn, from a leaf of *Pteris* sp., deposited in CBS Apr. 1996 (**holotype** CBS H-23013, dried culture, ex-holotype living culture CBS 379.96).

Notes: CBS 379.96 was originally identified as "Didymella adianticola", which is currently a synonym of Paraboeremia adianticola. CBS 379.96 is well distinguished from Pa. adianticola both in morphology and phylogeny. Didymella pteridis produces pycnidia with a single ostiole and shorter conidiogenous cells  $(4-5 \times 3.5-4.5 \ \mu\text{m})$ , different from Pa. adianticola (pycnidia with 1–3 ostioles, conidiogenous cells  $5.5-7 \times 3-6.5 \ \mu\text{m}$ ; Chen et al. 2015a). Thus, we introduce CBS 379.96 as a new species, D. pteridis. Didymella pteridis is closely related to D. viburnicola in the multi-locus phylogenetic analyses (Fig. 1), but D. pteridis is differentiated from the latter by wider conidia ( $3-6 \times 2.5-3.5 \ \mu\text{m}$  vs.  $3.6-5.6 \times 1.6-2.2 \ \mu\text{m}$ ) and the colour of its conidial matrix (pale salmon vs. whitish) (de Gruyter & Noordeloos 1992).

*Didymella segeticola* (Q. Chen) Q. Chen, Crous & L. Cai, comb. nov. MycoBank MB819327.

Basionym: Phoma segeticola Q. Chen, Phytotaxa 197: 274. 2015.

Description: Chen et al. (2015b).

Specimens examined: China, Hubei, Shennongjia Forest Region, on diseased leaves of *Cirsium segetum*, 1 Aug. 2011, K. Zhang (holotype HMAS 245746, exholotype living culture CGMCC 3.17489); *ibid.* CGMCC 3.17498 = LC 1635; *ibid.* LC 1633; *ibid.* LC 1634.

*Notes*: This species was introduced as *Phoma segeticola*, before the comprehensive revision of *Didymellaceae* (Chen *et al.* 2015b). Under current circumstance of *Didymellaceae*, it belongs to *Didymella*. *Didymella segeticola* is closely related to *D. bellidis*, and has 12 bp differences in four loci from the latter. Morphologically, *D. segeticola* could be distinguished from the latter in producing wider conidia (4.5–7 × 2.5–4 µm vs. 4–6.5 × 2–2.5 µm; Chen *et al.* 2015b).

*Didymella sinensis* Q. Chen, Crous & L. Cai, **sp. nov.** Myco-Bank MB818967. Fig. 17.

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

Leaf spots amphigenous, angular to irregular, 3–5 mm diam, scatter over the leaf, dark brown to black (Fig. 7D). Ascomata aggregated, globose to irregular, brown, small, up to 170 µm diam, papillate. *Pseudothecial wall* 18–29.5 µm thick, outer wall consisting of 2–5 layers of cells of *textura angularis*. *Pseudoparaphyses* hyaline, 1.5–2 µm diam, septate. *Asci* bitunicate, clavate to short cylindrical,  $32-52 \times 8.5-16$  µm. *Ascospores* biseriate, ellipsoidal, straight to slightly curved,  $12-18 \times 4.5-7.5$  µm, hyaline, smooth, apex obtuse, base broadly obtuse to subobtuse, medianly 1-septate, upper cell often wider than lower cell, slightly constricted at the septum.

*Culture characteristics*: Colonies on OA, 49–52 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, grey to black; reverse black. Colonies on MEA 57–60 mm diam after 7 d, margin regular, greyish brown; reverse concolourous. Colonies on PDA, 56–60 mm diam after 7 d, margin regular, pale

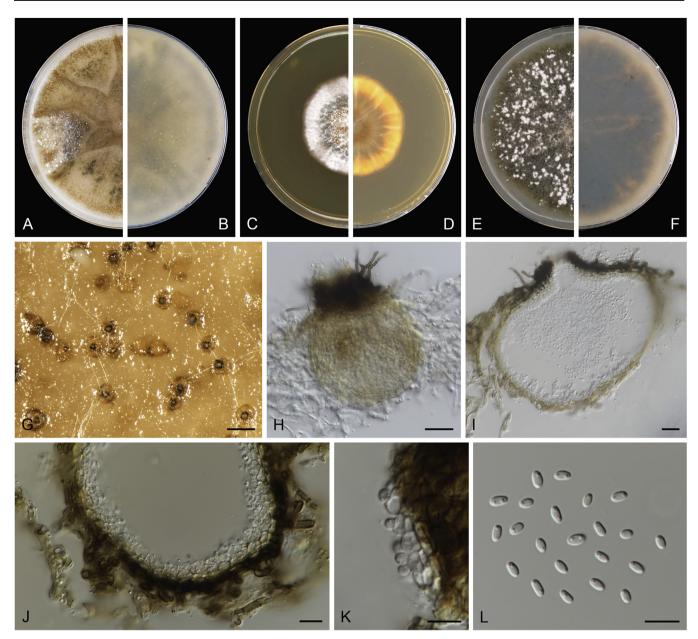


Fig. 16. Didymella pteridis (CBS 379.96). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K. Conidiogenous cells. L. Conidia. Scale bars: G = 300 µm; H = 40 µm; I = 20 µm; J–L = 10 µm.

grey, with brownish olivaceous margin; reverse dark brown. NaOH spot test: a hazel discolouration on MEA.

Specimens examined: China, Guizhou, Huangguoshu waterfall, on leaves of *Cerasus pseudocerasus*, 21 Jul. 2014, Q. Chen (holotype HMAS 247157, dried culture, ex-holotype living culture CGMCC 3.18348 = LC 5210); Guizhou, Kuankuoshui National Geopark, *Urticaceae*, 20 Jul. 2014, Q. Chen, LC 5246; Guizhou, Xingyi, on leaves of *Dendrobium officinale*, 4 Jul. 2015, Q. Chen, LC 8142; *ibid.* LC 8143.

Notes: Didymella sinensis has only been observed as a sexual morph, which is not common among species of Didymellaceae. Four isolates from diseased leaves of three host plants in different families were collected, i.e. Cerasus pseudocerasus (Rosaceae), Dendrobium officinale (Orchidaceae) and Urticaceae, indicating an opportunistic pathogen with very broad host range.

*Didymella suiyangensis* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818972. Fig. 18. *Etymology*: Epithet derived from the location of origin, Suiyang County in Guizhou, China.

Conidiomata pycnidial, solitary, sometimes aggregated, globose to irregular, brown, covered by some hyphal outgrowths, superficial or semi-immersed, ostiolate,  $(65-)90-240 \times 55-180 \mu m$ . Ostiole single, slightly papillate or non-papillate. *Pycnidial wall* pseudo-parenchymatous, composed of oblong to isodiametric cells, 2–4 layers,  $15-36.5 \mu m$  thick, outer wall 2-layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform,  $4-4.5 \times 3-4 \mu m$ . *Conidia* ellipsoidal to oblong, smooth- and thinwalled, hyaline, aseptate,  $3.5-7 \times 2-3 \mu m$ , with indistinct guttules. *Conidial matrix* cream.

*Culture characteristics*: Colonies on OA, 52–55 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, sparsely, white to buff; reverse concolourous. Colonies on MEA 59–64 mm diam after 7 d, margin regular, floccose, pale grey to greenish olivaceous; reverse white to yellowish green. Colonies



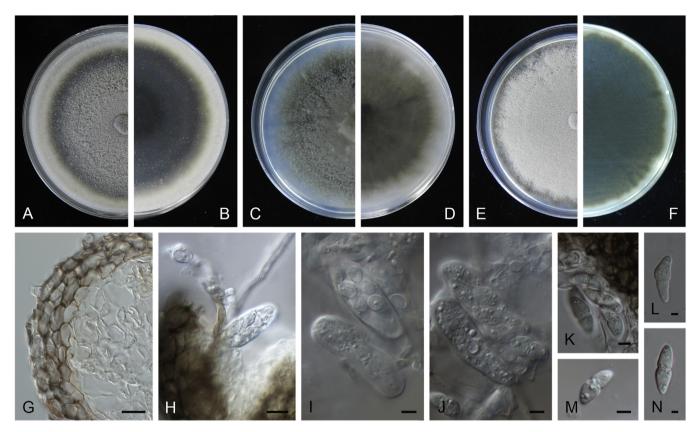


Fig. 17. Didymella sinensis (CGMCC 3.18348). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Section of pseudothecial wall. H. Asci forming in ascomata. I–J. Asci. K–N. Ascospores. Scale bar: G–H = 10 µm; I–K, M = 5 µm; L, N = 2.5 µm.

on PDA, 57–61 mm diam after 7 d, margin regular, floccose, white to pale greyish brown; reverse white to hazel. NaOH spot test: a reddish brown discolouration on MEA.

Specimens examined: China, Guizhou, Zunyi, Shuanghe Cave National Geopark, air, 8 May 2015, Z.F. Zhang (holotype HMAS 247158, dried culture, exholotype living culture CGMCC 3.18352 = LC 7439); *ibid.* LC 8144.

Notes: Didymella suiyangensis formed a distinct clade sister to *D. bellidis* and *D. segeticola* (Fig. 1), with respectively 18 bp and 19 bp differences in four loci from the latter two species. However, *D. suiyangensis* is differentiated from *D. bellidis* and *D. segeticola* in producing narrower conidiogenous cells  $(4-4.5 \times 3-4 \ \mu m \ vs. 3-6 \times 4-8 \ \mu m \ and 5-6.5 \times 4-5.5 \ \mu m)$ , and the number of ostioles (1 vs. 1–5 and 1–2, respectively). Moreover, the NaOH reactions on MEA showed a reddish brown discolouration on *D. suiyangensis*, but green to red on *D. bellidis* and negative on *D. segeticola* (de Gruyter *et al.* 1993, Chen *et al.* 2015b).

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

*Epicoccum camelliae* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818958.

*Etymology*: Name refers to the host genus from which the holotype was collected, *Camellia*.

Cultures sterile. *Epicoccum camelliae* differs from its closest phylogenetic neighbour *E. viticis* by unique fixed alleles in three loci

based on alignments of the separate loci deposited in TreeBASE (S20724): LSU positions: 66(T), 398(T); *tub2* positions: 30(T), 258(C); *rpb2* positions: 47(C), 95(C), 197(A), 419(C), 554(A).

Specimens examined: China, Jiangxi, Ganzhou, leaves of *Camellia sinensis*, 7 Sep. 2013, Y. Zhang (holotype HMAS 247159, dried culture, culture ex-holotype CGMCC 3.18343 = LC 4858); *ibid*. LC4862.

*Notes: Epicoccum camelliae* is closely related to *E. viticis* with a high support value in the phylogenetic tree (Fig. 1), and has 10 bp differences in four loci from the latter. Two isolates of this species are both from *Camellia sinensis*, one as endophyte in healthy leaves and the other as pathogenic fungus from diseased leaves. Both isolates proved to be sterile on the defined media used in this study.

*Epicoccum dendrobii* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818964. Fig. 19.

Etymology: Named after the host plant, Dendrobium.

Leaf spots amphigenous, subcircular, up to 10 mm diam, black (Fig. 7E). Conidiomata sporodochial, aggregated, semi-immersed or superficial, clavate, pale brown. Hyphae septate, frequently branched,  $2.5-4.5 \mu m$ . Conidia globose, aseptate and smooth when young, later becoming multicellular-phragmosporous, verrucose, subglobose-pyriform, brown, with a basal cell,  $11-19 \mu m$  diam.

*Culture characteristics*: Colonies on OA, 58–64 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense,

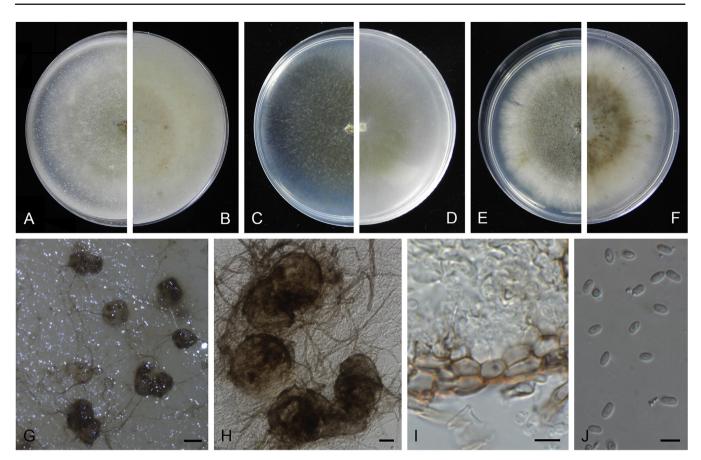


Fig. 18. Didymella suiyangensis (CGMCC 3.18352). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidial wall. J. Conidia. Scale bars: G = 300 µm; H = 30 µm; H = 30 µm; I–J = 5 µm.

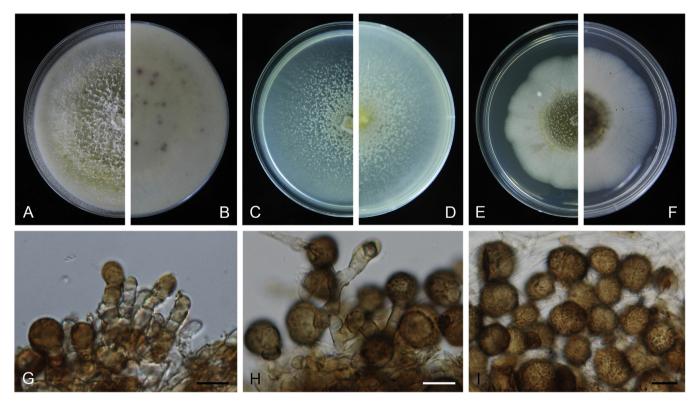


Fig. 19. Epicoccum dendrobii (CGMCC 3.18359). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Sporodochia. H–I. Conidia. Scale bars: G–I = 10 μm.

white to greyish yellow; reverse white to pale grey, with some purple dots scattered over the colony. Colonies on MEA 65–68 mm diam after 7 d, margin regular, grey, with sparse white aerial mycelia; reverse white to yellow. Colonies on PDA, 34–38 mm diam after 7 d, margin regular, aerial mycelia felty to floccose, flat, white to buff, olivaceous near the centre; reverse pale salmon, hazel to brown near the centre. NaOH test negative.

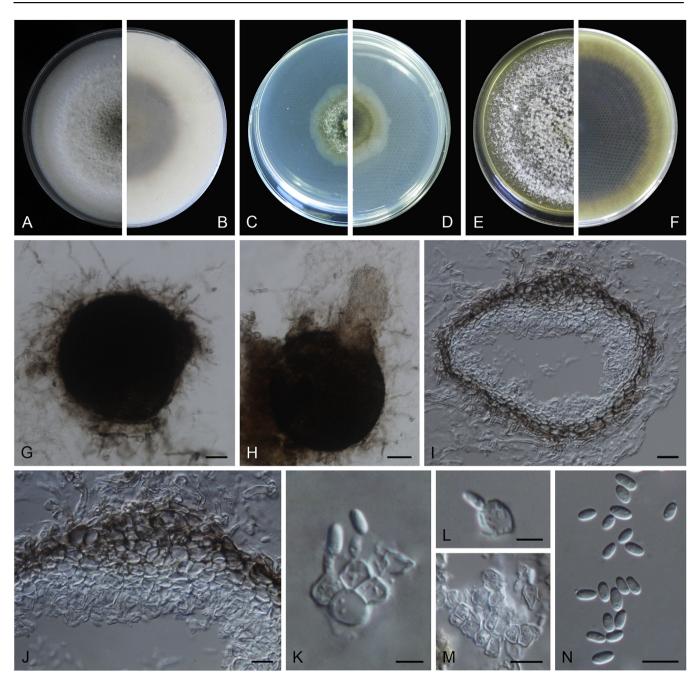


Fig. 20. Epicoccum duchesneae (CGMCC 3.18345). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G–H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K–M. Conidiogenous cells. N. Conidia. Scale bars: G–I = 40 µm; J, M–N = 10 µm; K–L = 5 µm.

Specimens examined: China, Guizhou, Xingyi, on leaves of *Dendrobium fimbriatum*, 4 Jul. 2015, Q. Chen (holotype HMAS 247160, dried culture, exholotype living culture CGMCC 3.18359 = LC 8145); *ibid.* LC 8146.

Notes: Epicoccum dendrobii formed a distinct clade basal to *E. nigrum*, *E. poae* and *E. layuense* (Fig. 1). These species all produce typical epicoccoid conidia (multicellular-phragmosporous, verrucose), with phoma-like conidia only observed in *E. nigrum. Epicoccum dendrobii* differs in the length of its epicoccoid conidia (11–19 µm) from *E. nigrum* (15–35 µm; Punithalingam *et al.* 1972) and *E. poae* (10–23 µm), and in its NaOH reaction (negative) from *E. layuense* (a pale reddish brown discolouration on MEA, with a yellowish brown margin).

*Epicoccum duchesneae* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818966. Fig. 20. *Etymology*: Name derived from *Duchesnea*, the plant genus from which the holotype was collected.

Leaf spots amphigenous, circular to irregular, 2–5 mm diam, yellowish brown, surrounded by a purple border (Fig. 7F). Conidiomata pycnidial, solitary, globose to subglobose, covered with hyphal outgrowths, immersed in agar, ostiolate, (150-) 170–270 ×  $(100-)150-230 \ \mu$ m. Ostiole single, sometimes with an elongated, pale brown neck, slightly papillate. Pycnidial wall pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, 13–30  $\mu$ m thick, outer wall of 2–3 pigmented layers. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, 4.5–9.5 × 3.5–7  $\mu$ m. Conidia ellipsoidal to oblong, smooth- and thin-walled, hyaline, aseptate, 2.5–3.5 × 1.5–2  $\mu$ m, egutullate or sometimes with 1(–3) small guttules. Conidial matrix whitish to salmon.

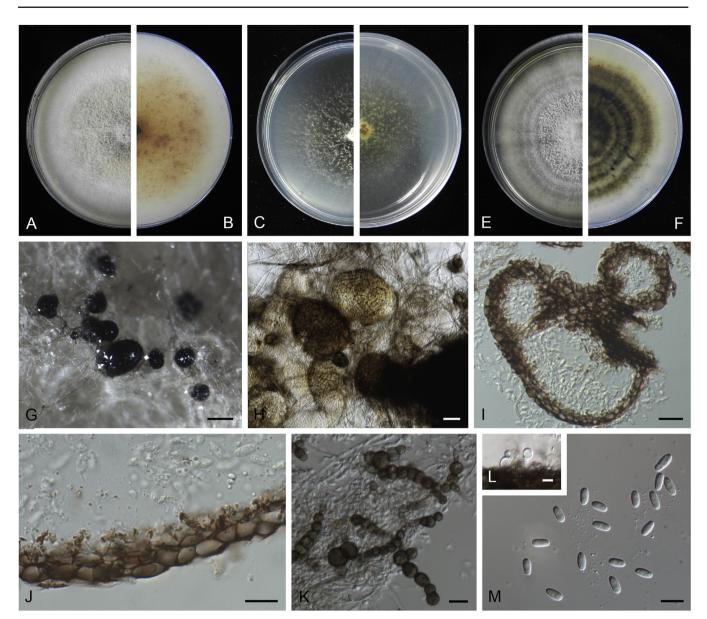


Fig. 21. *Epicoccum hordei* (CGMCC 3.18360). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K. Chlamydospores. L. Conidiogenous cells. M. Conidia. Scale bars: G = 300 μm; H = 30 μm; I, K = 20 μm; J, M = 10 μm; L = 5 μm.

*Culture characteristics*: Colonies on OA, 62–65 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, white to grey, greyish brown near the centre; reverse white to dark brown. Colonies on MEA 24–27 mm diam after 7 d, margin regular, covered by white, sparse floccose aerial mycelia, grey to pale olivaceous; reverse concolourous. Colonies on PDA, 55–60 mm diam after 7 d, margin regular, aerial mycelia covering the whole colony, floccose, white to grey; reverse greenish olivaceous to dark brown. Application of NaOH results in a pale olivaceous discolouration of the agar.

Specimens examined: China, Jiangxi, Ganzhou, on leaves of *Duchesnea indica*, 12 May 2013, Q. Chen (holotype HMAS 247161, dried culture, ex-holotype living culture CGMCC 3.18345 = LC 5139); *ibid.* LC 8147.

Notes: Epicoccum duchesneae formed a distinct lineage close to *E. huancayense* (Fig. 1). *Epicoccum duchesneae* differs in producing smaller conidia from *E. huancayense*,  $2.5-3.5 \times 1.5-2 \ \mu m \ vs. \ (4-)5-8(-12) \times 2.5-4.5 \ \mu m \ (de Gruyter$ *et al.*1998).

*Epicoccum hordei* Q. Chen, Crous & L. Cai, **sp. nov.** Myco-Bank MB818961. Fig. 21.

*Etymology*: Named after the host genus *Hordeum*, from which the holotype was isolated.

Conidiomata pycnidial, solitary or aggregated, globose to subglobose, glabrous, semi-immersed or on the surface of agar, ostiolate,  $(85-)115-190(-260) \times (70-)95-180 \mu m$ . Ostiole single, non-papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3 layers, 11–18.5 µm thick, pigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, 7–8.5 × 5.5–7.5 µm. Conidia obovoid, ellipsoidal to oblong, cylindrical, smooth- and thin-walled, hyaline, aseptate,  $6.5-9 \times 3-4 \mu m$ , with several minute guttules. Conidial matrix pale brown. Chlamydospores unicellular, produced on the agar, yellowish brown to dark brown, intercalary, in chains, globose to subglobose,  $6-21.5 \mu m$  diam, thick-walled.

*Culture characteristics*: Colonies on OA, 58-62 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, white to



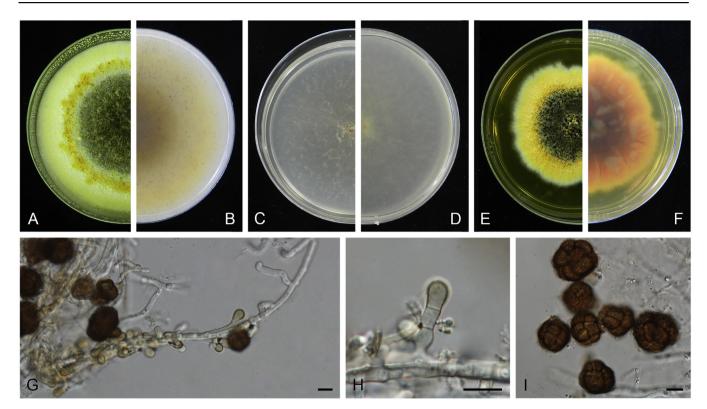


Fig. 22. Epicoccum italicum (CGMCC 3.18361). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G–H. Sporodochia. I. Conidia. Scale bars: G–I = 10 µm.

grey, pale olivaceous near the centre; reverse white to amber. Colonies on MEA 43–46 mm diam after 7 d, margin regular, aerial mycelia white, fluffy to floccose, grey to greenish yellow; reverse concolourous. Colonies on PDA, 54–56 mm diam after 7 d, margin regular, aerial mycelia floccose, white to grey, with pale olivaceous concentric rings; reverse pale greenish brown to olivaceous, with concentric rings. Application of NaOH results in a pale brown discolouration of the agar.

Specimens examined: Australia, on seeds of Hordeum vulgare, 2014, W.J. Duan (holotype HMAS 247162, dried culture, ex-holotype living culture CGMCC 3.18360 = LC 8148); *ibid.* LC 8149.

*Notes*: Isolates of this species clustered in a lineage closely related to *E. pimprinum* (49 bp differences in four sequenced loci) (Fig. 1). Morphologically, *E. hordei* differs in the colour of its conidial matrix (pale brown) from *E. pimprinum* (salmon) and the absence of elongated necks of pycnidia (with pronounced necks in *E. pimprinum*) (Boerema 1993).

*Epicoccum italicum* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818965. Fig. 22.

*Etymology*: Named after the country where the holotype was collected, Italy.

Conidiomata sporodochial, aggregated, semi-immersed or superficial, clavate, yellowish brown. *Hyphae* septate, branched,  $3.5-5 \mu m$ . *Conidia* multicellular-phragmosporous, verrucose, subglobose-pyriform, brown, with a basal cell,  $12.5-28 \mu m$  diam.

*Culture characteristics*: Colonies on OA, 48–50 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense, white to yellow, dark iron-grey near the centre, with a pale yellow halo near the margin, and a yellow concentre ring; reverse buff to yellowish brown. Colonies on MEA 50–55 mm diam after 7 d,

margin regular, grey to pale yellowish green, with sparse white aerial mycelia; reverse concolourous. Colonies on PDA, 35–39 mm diam after 7 d, margin irregular, aerial mycelia floccose, yellow with a white margin, black near the centre; reverse salmon to saffron, with a yellow margin. Application of NaOH results in a yellow discolouration of the agar.

Specimens examined: Italy, on seedlings of Acca sellowiana, 2013, W.J. Duan (holotype HMAS 247163, dried culture, ex-holotype living culture CGMCC 3.18361 = LC 8150); *ibid.* LC 8151.

*Notes*: Phylogenetically, *Epicoccum italicum* formed a distinct lineage closely related to *E. dendrobii*. Morphologically, the two species could be distinguished in the length of epicoccoid conidia ( $12.5-28 \mu m$  in *E. italicum* vs.  $11-19 \mu m$  in *E. dendrobii*), and the results of NaOH test (a yellow discolouration in *E. italicum* vs. negative in *E. dendrobii*).

*Epicoccum latusicollum* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818960. Fig. 23.

*Etymology*: Name refers to the wide neck of pycnidia, *latus* = wide, *collum* = neck.

Conidiomata pycnidial, mostly solitary, sometime aggregated, globose to subglobose or pyriform, glabrous, produced on the agar surface, ostiolate,  $110-155 \times 90-130 \mu m$ . Ostioles 1-2, sometimes elongated as a short, slightly papillate neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3-4 cell layers of which outer 2-3 are brown pigmented,  $15-20 \mu m$  thick. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform,  $5-8 \times 4-5.5 \mu m$ . Conidia ellipsoidal to oblong, smooth- and thin-walled, hyaline, aseptate,  $4-6.5 \times 2-3 \mu m$ , guttulate. Conidial matrix buff.

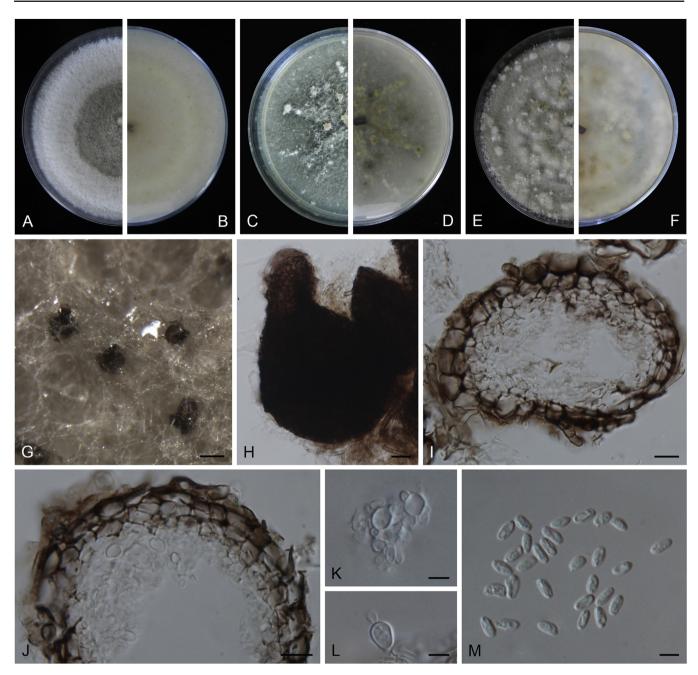


Fig. 23. Epicoccum latusicollum (CGMCC 3.18346). A-B. Colony on OA (front and reverse). C-D. Colony on MEA (front and reverse). E-F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K-L. Conidiogenous cells. M. Conidia. Scale bars: G = 100 μm; H = 20 μm; I-J = 10 μm; K-M = 5 μm.

*Culture characteristics*: Colonies on OA, 70–72 mm diam after 7 d, margin regular, flattened, whole colony covered by floccose aerial mycelia, white, grey to smoke grey near the centre; reverse white to buff. Colonies on MEA 75–80 mm diam after 7 d, margin regular, aerial mycelia floccose, greyish dull green, forming several mycelial pellets, white or pale salmon; reverse grey, with some yellow sections. Colonies on PDA, 80–85 mm diam after 7 d, margin regular, floccose aerial mycelia covering the whole colony, dense, white to grey, forming several white mycelial pellets; reverse white to hazel. NaOH spot test: a green discolouration on MEA, later changing to three colour layers, via dark green, pale red to purple, from the centre to the outer ring.

Specimens examined: China, Jiangxi, Ganzhou, on leaves of Vitex negundo, 25 Apr. 2013, Q. Chen, LC 5124; Jiangxi, Ganzhou, endophyte of Camellia sinensis, 7 Sep. 2013, Y. Zhang, LC 4859; Shandong, Jining, on leaves of Sorghum bicolor, 3 Aug. 2013, N. Zhou (holotype HMAS 247164, dried culture, ex-holotype living culture CGMCC 3.18346 = LC 5158). Japan, *Podocarpus macrophyllus*, 2013, W.J. Duan, LC 8152; *ibid*. LC 8153; on stem of *Acer palmatum*, LC 8154.

*Notes*: Isolates of *Epicoccum latusicollum* clustered in a sister clade to *E. camelliae*, *E. sorghinum* and *E. viticis* (Fig. 1). Although the conidial dimensions are similar in these species, *E. latusicollum* differs in 1 bp in ITS, 14 bp in *rpb2* and 5 bp in *tub2* from *E. camelliae*; 16 bp in *rpb2* and 7 bp in *tub2* from *E. sorghinum*; and 1 bp in ITS, 1 bp in LSU, 16 bp in *rpb2* and 4 bp in *tub2* from *E. viticis*.

This is the first report of an *Epicoccum* species from *Acer* palmatum (Aceraceae), *Podocarpus* macrophyllus (*Podocarpaceae*) and *Vitex* negundo (*Verbenaceae*).

*Epicoccum layuense* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818963. Fig. 24.



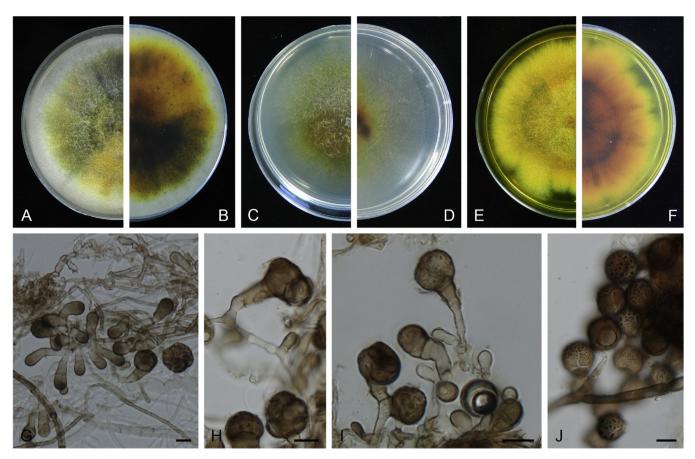


Fig. 24. Epicoccum layuense (CGMCC 3.18362). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Sporodochia. H–I. Conidia producing on sporodochia. J. Conidia. Scale bars: G–I = 10 µm.

*Etymology*: Epithet derived from the location of origin, Layue Village in Tibet, China.

*Leaf spots* distinct, angular to irregular, up to 12 mm diam, dark brown. *Conidiomata* sporodochial, aggregated, superficial, clavate, brown (Fig. 7I). *Hyphae* septate, branched,  $2-5.5 \mu m$ . *Conidia* multicellular-phragmosporous, verrucose, subglobose-pyriform, with a basal cell, dark brown,  $13-19.5 \mu m$  diam.

*Culture characteristics*: Colonies on OA, 27–37 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, yellow to greenish yellow, olivaceous; reverse yellow to saffron, with dark brown sections. Colonies on MEA 30–43 mm diam after 7 d, margin irregular, aerial mycelia white, floccose, greenish yellow to pale brown; reverse concolourous. Colonies on PDA, 47–55 mm diam after 7 d, margin irregular, aerial mycelia floccose, bright yellow; reverse yellow to pale brown, with a brown concentric ring near the centre. NaOH spot test: a pale reddish brown discolouration on MEA, with a yellowish brown margin.

Specimens examined: China, Tibet, Lulang, on leaves of *Perilla* sp., 15 Jun. 2015, Q. Chen (holotype HMAS 247165, dried culture, ex-holotype living culture CGMCC 3.18362 = LC 8155); *ibid.* LC 8156.

*Notes*: This species is phylogenetically closely related to *E. nigrum* and *E. poae*, but *E. layuense* has differences at 19 positions from *E. nigrum* and 14 positions from *E. poae* in the multi-locus sequences of their ex-type strains. Morphologically, *E. layuense* produces smaller epicoccoid conidia than *E. nigrum* (13–19.5 µm vs. 15–35 µm; Punithalingam *et al.* 1972), and they

also differ in their NaOH reactions (a pale reddish brown discolouration on *E. layuense*, but pale brown on *E. poae*).

*Epicoccum poae* Q. Chen, Crous & L. Cai, *sp. nov.* MycoBank MB818962. Fig. 25.

*Etymology*: Name derived from *Poa*, the plant genus from which the holotype was collected.

*Conidiomata* sporodochial, aggregated, superficial, clavate, brown. *Hyphae* septate, branched, 2–3 µm. *Conidia* multicellular-phragmosporous, verrucose, subglobose-pyriform, with a basal cell, dark brown, 10–23 µm diam.

*Culture characteristics*: Colonies on OA, 49–51 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, yellow, reddish brown to brown near the centre, with a white margin; reverse yellow to saffron, with some brown sections. Colonies on MEA 25–27 mm diam after 7 d, margin irregular, aerial mycelia white to greenish yellow, fluffy to floccose, grey to greenish yellow; reverse white to greenish yellow. Colonies on PDA, 20–22 mm diam after 7 d, margin irregular, aerial mycelia flattened, brownish yellow, with a white margin; reverse yellow to saffron, brown towards the centre. NaOH spot test: a pale brown discolouration on MEA.

Specimens examined: **USA**, on seeds of *Poa annua*, Oct. 2014, X.M. Wang, strain isolated by Q. Chen (**holotype** HMAS 247166, dried culture, ex-holotype living culture CGMCC 3.18363 = LC 8160); *ibid.* LC 8161, LC 8162.

Notes: Epicoccum poae is phylogenetically closely related to E. nigrum (Fig. 1), but differs in producing smaller epicoccoid

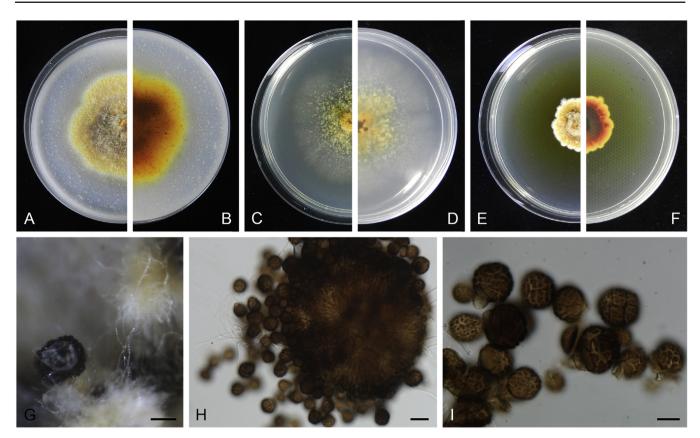


Fig. 25. Epicoccum poae (CGMCC 3.18363). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Conidia producing on OA. H–I. Conidia. Scale bars: G = 100 µm; H = 20 µm; I = 10 µm.

conidia (10–23 µm vs. 15–35 µm; Punithalingam *et al.* 1972). Furthermore, *E. poae* hasn't been observed to have phoma-like conidia, while *E. nigrum* readily produces short-cylindrical conidia,  $3-7(-10) \times 1.5-3(-3.5)$  µm (Punithalingam *et al.* 1972).

*Epicoccum viticis* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818959. Fig. 26.

*Etymology*: Name derived from *Vitex*, the plant genus from which the holotype was collected.

Leaf spots amphigenous, circular to irregular, 2-8 mm diam, close to the leaf margin, reddish brown, single lesions may coalesce to form larger lesions and become dark brown (Fig. 7H). Conidiomata pycnidial, aggregated or sometimes solitary, (sub-)globose, glabrous, produced on the agar surface, 120-200 × 100-175 µm. Ostioles 1-2, papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 2-3 cell layers, outer 1-2 layers brown pigmented, 8-16 µm thick. Conidiogenous cells phialidic, hyaline. smooth, ampulliform to doliiform, 5.5-9 × 3-6 µm. Conidia ellipsoidal to obovoid, smooth- and thinwalled, hyaline, aseptate,  $3.5-6 \times 2-3 \mu m$ , with two minute polar guttules. Conidial matrix buff to cinnamon.

*Culture characteristics*: Colonies on OA, 48–67 mm diam after 7 d, margin regular, aerial mycelia floccose, white to grey, with a greyish olivaceous concentric ring; reverse white to pale olivaceous, with a broad greyish olivaceous concentric ring. Colonies on MEA 75–80 mm diam after 7 d, margin regular, aerial mycelia fluffy to floccose, grey to pale yellowish green; reverse concolourous. Colonies on PDA, 70–75 mm diam after 7 d, margin regular,

floccose aerial mycelia covering the whole colony, grey; reverse white to buff, with some dull green dots. NaOH test negative.

Specimens examined: Australia, Darwin, Northern Territory University, Greenhouse, from *Andropogon gayanus*, 2002, A. Hollingsworth, BRIP 29294 = LC 5257. China, Jiangxi, Ganzhou, on leaves of *Vitex negundo*, 25 Apr. 2013, Q. Chen (holotype HMAS 247167, dried culture, ex-holotype living culture CGMCC 3.18344 = LC 5126).

*Note: Epicoccum viticis* is phylogenetically closely related to *E. camelliae* (Fig. 1), with 10 bp differences in four sequenced loci.

Heterophoma Q. Chen & L. Cai, Stud. Mycol. 82: 165. 2015.

*Heterophoma verbascicola* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB819128. Fig. 27.

*Etymology*: Named after the host genus from which the holotype was collected, *Verbascum*.

Leaf spots amphigenous, angular to irregular, 2-7 mm diam, scattered over the leaf, brown, with a pale yellow diffuse halo (Fig. 7J). Conidiomata pycnidial, aggregated or solitary, globose to subglobose or obpyriform, brown, covered with some hyphal outgrowths, semi-immersed or superficial, ostiolate, 120-300 × (100-)150-300 µm. Ostioles 2-3, elongated as short necks, slightly papillate or non-papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 2-3 layers, 7-20 µm thick, outer wall 1-2-layers pigmented. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, 5.5-6 × 3.5-5 µm. Conidia ellipsoidal to oblong, smooth- and thin-walled, hyaline, aseptate, incidentally



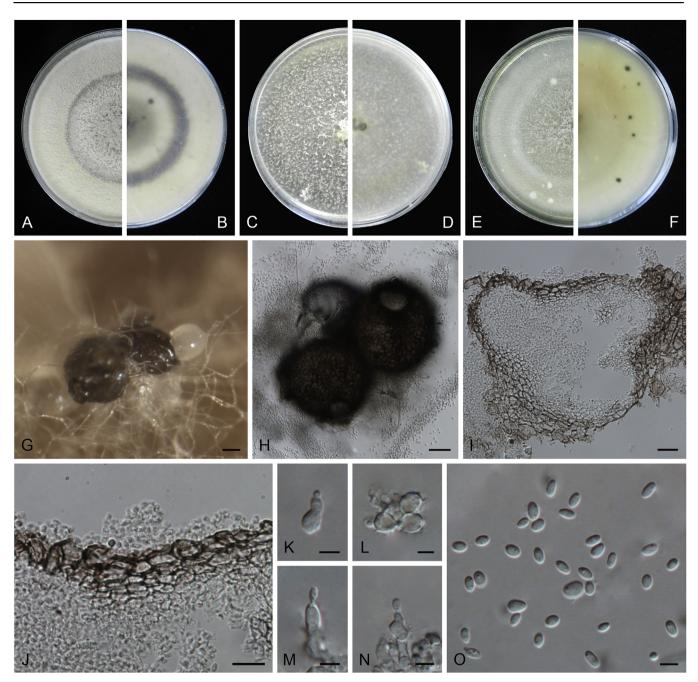


Fig. 26. Epicoccum viticis (CGMCC 3.18344). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidium. J. Section of pycnidial wall. K–N. Conidiogenous cells. O. Conidia. Scale bars: G–H = 40 μm; I = 20 μm; J = 10 μm; K–O = 5 μm.

produce 1-septate large conidia,  $3.5-6(-8) \times 1.5-3.5 \mu m$ , with 1–2 guttules. *Conidial matrix* cream to buff.

*Culture characteristics*: Colonies on OA, 40–45 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, white, with grey margin, greyish olivaceous near the centre; reverse white to pale olivaceous with a broad white concentric ring. Colonies on MEA 50–52 mm diam after 7 d, margin regular, aerial mycelia fluffy to floccose, white; reverse concolourous. Colonies on PDA, 50–53 mm diam after 7 d, margin irregular, crenate, dense, felty, white to mouse-grey; reverse white to hazel, with brown concentric rings. NaOH test negative.

Specimens examined: China, Tibet, Lulang, on leaves of Verbascum thapsus, 15 Jun. 2015, Q. Chen (holotype HMAS 247168, dried culture, ex-holotype living culture CGMCC 3.18364 = LC 8163); *ibid.* LC 8164.

*Notes: Heterophoma verbascicola* is phylogenetically closely related to *H. novae-verbascicola*, but is distinguishable from the latter species in its slightly narrower conidiogenous cells  $(5.5-6 \times 3.5-5 \,\mu\text{m} \text{ vs. } 2-6 \times 4-6 \,\mu\text{m}; \text{de Gruyter et al. } 1993)$  and larger conidia  $(3.5-8 \times 1.5-3.5 \,\mu\text{m} \text{ vs. } 3.5-5.5 \times 1.5-2.5 \,\mu\text{m})$ . Moreover, the NaOH test showed a yellowish green discolouration that became reddish in *H. novae-verbascicola*, but remained negative in *H. verbascicola*.

Neoascochyta Q. Chen & L. Cai, Stud. Mycol. 82: 198. 2015.

*Neoascochyta argentina* L.W. Hou, Crous & L. Cai, **sp. nov.** MycoBank MB820003. Fig. 28.

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

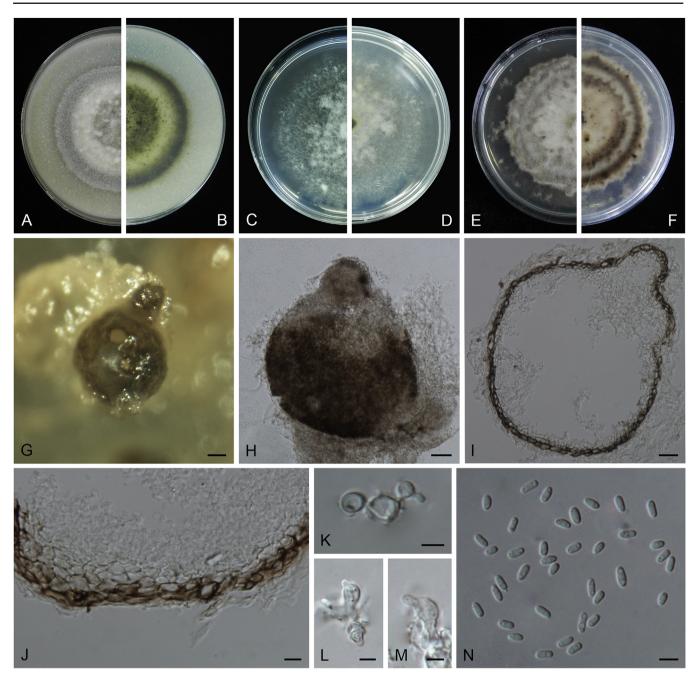


Fig. 27. Heterophoma verbascicola (CGMCC 3.18364). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidium forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K–M. Conidiogenous cells. N. Conidia. Scale bars: G–H = 40 μm; I = 20 μm; J–N = 5 μm.

Conidiomata pycnidial, solitary or aggregated, (sub-)globose of flask-shaped, glabrous, semi-immersed or immersed, ostiolate,  $210-390 \times 140-270 \,\mu$ m. Ostioles 1–3, sometimes elongated as a long neck (up to 350  $\mu$ m), papillate. *Pycnidial wall* pseduopar-enchymatous, composed of oblong to isodiametric cells, 4–6 layers, with outer 3–4-layers pigmented, 14.5–52  $\mu$ m thick. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform, 7.5–14.5 × 6–13.5  $\mu$ m. *Conidia* cylindrical, smooth- and thinwalled, hyaline, 0–1-septate, (10.5–)11.5–14.5(–16) × 3–5  $\mu$ m, guttulate. *Conidial matrix* whitish cream.

*Culture characteristics*: Colonies on OA, 50–55 mm diam after 7 d, margin regular, densely covered by floccose aerial mycelia, greyish olivaceous, with some white zones near the margin; reverse greyish black. Colonies on MEA 55–60 mm diam after 7 d, margin regular, densely covered by woolly aerial mycelia, fawn, white near margin; reverse brown. Colonies on PDA,

60–65 mm diam after 7 d, margin regular, covered by pale grey aerial mycelia, floccose, dark olivaceous near the margin; reverse greyish brown. NaOH spot test: a pale reddish brown discolouration on MEA.

Specimen examined: Argentina, Tandil, from a leaf of *Triticum aestivum*, Oct. 2002 (holotype CBS H-23014, dried culture, ex-holotype living culture CBS 112524).

Notes: CBS 112524 was initially received as "Ascochyta hordei". However, this isolate clustered in the Neoascochyta clade, and produces much smaller conidia (10.5–16 × 3–5 µm) than As. hordei (15–22 × 3.5–4.5 µm; Punithalingam 1979). Therefore, Neoa. argentina is introduced as a new species, based on isolate CBS 112524. Neoascochyta argentina is well distinguished from its most closely related species Neoa. triticicola by its smaller conidia (10.5–16 × 3–5 µm vs. 16.5–27 × 5–8.5 µm).

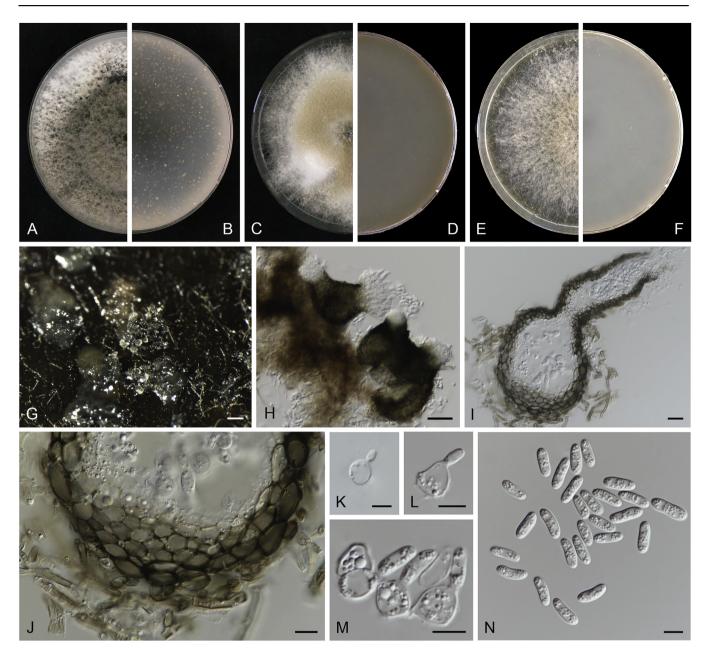


Fig. 28. Neoascochyta argentina (CBS 112524). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K–M. Conidiogenous cells. N. Conidia. Scale bars: G = 100 μm; H = 50 μm; I = 20 μm; J–N = 10 μm.

*Neoascochyta triticicola* L.W. Hou, Crous & L. Cai, **sp. nov.** MycoBank MB820004. Fig. 29.

*Etymology*: Name refers to the host genus *Triticum*, from which the holotype was collected.

Conidiomata pycnidial, mostly confluent, flask-shaped, glabrous or sometimes with hyphal outgrows, superficial or semi-immersed on the agar,  $(170-)230-420(-620) \times 160-430 \mu m$ ; conidiomata becoming black, irregular with age, and ostiolate. Ostioles 1-3(-5), developing to conspicuously elongated necks (up to 400 µm tall), papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4-6 layers, with outer 2-3-layers pigmented,  $25-40 \mu m$  thick. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform,  $8.5-13 \times (4.5-)7.5-12(-13) \mu m$ . *Conidia* bacilliform to fusiform, smooth- and thin-walled, hyaline, mainly uniseptate, occasionally aseptate,  $(16.5-)20-27 \times 5-8.5 \mu m$ , guttulate. *Conidial matrix* whitish cream to pale salmon.

*Culture characteristics*: Colonies on OA, 55–65 mm diam after 7 d, margin regular, aerial mycelia floccose, greyish black, with some greyish mycelia tufts; reverse concolourous. Colonies on MEA 40–55 mm diam after 7 d, margin irregular, slightly lobate, covered by floccose mycelia, white, greyish olivaceous to greyish pink near the centre; reverse dark brown, saffron near the margin. Colonies on PDA, 55–65 mm diam after 7 d, margin irregular, slightly lobate, covered by floccose, greenish black mycelia, with erected tufts of white mycelia; reverse greyish olivaceous. NaOH spot test: a pale reddish brown discolouration on MEA.

Specimen examined: South Africa, Heilbron, from *Triticum aestivum*, deposited in CBS Sep. 1974, W.J. Jooste (holotype CBS H-9008, ex-holotype living culture CBS 544.74).

*Notes*: Isolate CBS 544.74 was originally identified as "*Ascochyta hordei*" but clustered in the *Neoascochyta* clade. Morphologically, it differs in producing larger conidia  $(16.5-27 \times 5-8.5 \ \mu\text{m})$  from *Ascochyta hordei*  $(15-22 \times 3.5-4.5 \ \mu\text{m})$ ; Punithalingam 1979).



Fig. 29. Neoascochyta triticicola (CBS 544.74). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I–J. Section of pycnidia. K. Section of pycnidial wall. L–N. Conidiogenous cells. O–P. Conidia. Scale bars: G = 500 μm; H = 200 μm; I–J = 50 μm; K–L, O = 10 μm; M–N, P = 5 μm.

Therefore, we introduce CBS 544.74 as a new species, *Neoa. triticicola*. In *Neoascochyta*, it should be compared with *Neoa. argentina*, which is discussed under the notes of the latter species.

*Neoascochyta soli* Q. Chen, Crous & L. Cai, **sp. nov.** Myco-Bank MB818975. Fig. 30.

*Etymology*: Name derived from the substrate where the holotype was collected, soil.

Conidiomata pycnidial, aggregated or solitary, globose to subglobose, dark brown, glabrous, superficial, ostiolate, (135–) 390–630 × (110–)340–565 µm. Ostiole single, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, 18–42 µm thick, outer wall of 1–2-pigmented layers. *Conidiogenous cells*  phialidic, hyaline, smooth, ampulliform to doliiform,  $6-10.5 \times 5.5-9 \mu m$ . *Conidia* ellipsoidal to oblong, smooth- and thin-walled, hyaline, aseptate,  $7-10 \times 3-4 \mu m$ , with 2 to several polar guttules. Conidial exudates not recorded.

*Culture characteristics*: Colonies on OA, 62–64 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense, white, greyish olivaceous near the centre; reverse white to iron grey. Colonies on MEA 45–47 mm diam after 7 d, margin irregular, grey, white near the centre; reverse white to olivaceous, forming concentric rings. Colonies on PDA, 50–53 mm diam after 7 d, margin regular, aerial mycelia fluffy, white to olivaceous; reverse concolourous. NaOH test negative.

Specimens examined: China, Guizhou, Kuankuoshui National Geopark, soil, 23 Jul. 2014, Z.F. Zhang (holotype HMAS 247169, dried culture, ex-holotype living culture CGMCC 3.18365 = LC 8165); *ibid.* LC 8166.



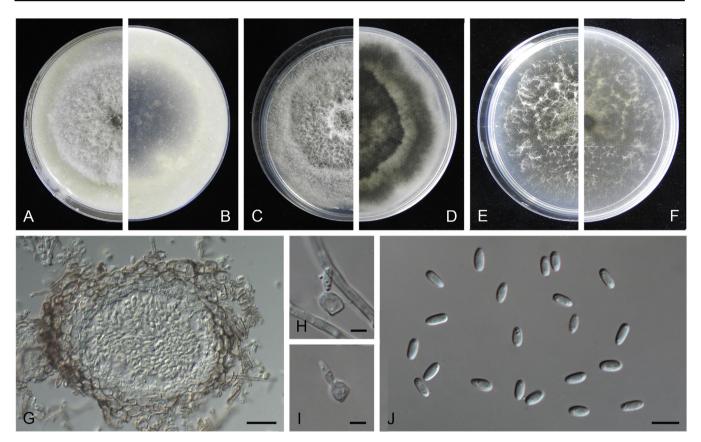


Fig. 30. Neoascochyta soli (CGMCC 3.18365). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Section of pycnidium. H–I. Conidiogenous cells. J. Conidia. Scale bars: G = 20 µm; H–I = 5 µm; J = 10 µm.

*Notes: Neoascochyta soli* clustered with *Neoa. paspali* in a distinct clade in this genus, but can be differentiated from the latter in producing larger conidiogenous cells  $(6-10.5 \times 5.5-9 \ \mu\text{m} \ \text{vs.} \ 4-6 \times 4-6 \ \mu\text{m})$ . In addition, the test of metabolite E production was negative for *Neoa. soli*, while a green to bluish discolouration, becoming red, appeared in *Neoa. paspali* (de Gruyter *et al.* 1998).

Neodidymelliopsis Q. Chen & L. Cai, Stud. Mycol. 82: 207. 2015.

*Neodidymelliopsis achlydis* L.W. Hou, Crous & L. Cai, **sp. nov.** MycoBank MB820005. Fig. 31.

*Etymology*: Named after the host genus *Achlys*, from which the holotype was collected.

Conidiomata pycnidial, solitary or aggregated, (sub-)globose, glabrous, semi-immersed or superficial, ostiolate, (150–)  $300-550(-630) \times (120-)250-500(-630) \mu m$ . Ostioles 1–5, slightly papillate. Pycnidial wall pseudoparenchymatous, composed of oblong to isodiametric cells, 4–9 layers, with outer 2–4-layers pigmented,  $30-80 \mu m$  thick. Conidiogenous cells phialidic, hyaline, smooth, ampulliform to doliiform, (4–)  $6.5-10 \times (3.5-)4.5-6.5 \mu m$ . Conidia oblong to cylindrical, incidentally slightly curved, smooth- and thin-walled, hyaline, aseptate,  $7.5-10(-18) \times 2-3.5(-5) \mu m$ , with two polar guttules. Conidial matrix whitish cream.

*Culture characteristics*: Colonies on OA, 45–50 mm diam after 7 d, margin regular, aerial mycelia floccose, white to pale brown; reverse pale salmon, with some pale olivaceous tinges near the

centre. Colonies on MEA 40–45 mm diam after 7 d, margin regular, aerial mycelia floccose and compact, white to pale grey; reverse saffron to pale yellowish brown, yellow near margin. Colonies on PDA, 50–52 mm diam after 7 d, margin regular, densely covered by floccose, grey aerial mycelia, white near the margin; reverse pale brown to brown. NaOH spot test: a dull green discolouration with a reddish brown margin on MEA.

Specimen examined: Canada, British Columbia, from a leaf of Achlys triphylla, Jun. 1976, J. Gremmen (holotype CBS H-23015, dried culture, ex-holotype living culture CBS 256.77).

Notes: Isolate CBS 256.77 was received as "Ascochyta achlvdis", which was from the same host (Achlvs triphvlla) and the same location (Canada) as reported for Ascochyta achlydis (Dearness 1916). However, it produces narrower and aseptate conidia compared to the uniseptate conidia of As. achlydis  $(7.5-18 \times 2-5 \ \mu m \ vs. \ 14-20 \times 5-6.5 \ \mu m; \ Dearness \ 1916),$ and is obviously a different species. Phylogenetically, CBS 256.77 clustered in the Neodidymelliopsis clade, closely related to Neod. polemonii and Neod. xanthina (Fig. 1), and has differences at six positions from Neod. polemonii and 12 positions from Neod. xanthina in multi-locus sequences of their ex-type strains. We therefore introduce a new species, Neod. achlydis based on CBS 256.77. Morphologically, Neod. achlydis produces pycnidia with 1-5 ostioles, while Neod. xanthina only has pycnidia with a single ostiole (Boerema et al. 2004). Neodidymelliopsis achlydis differs from Neod. polemonii in its whitish cream conidial matrix from whitish/smoke grey of Neod. polemonii (Boerema et al. 2004). Neodidymelliopsis achlydis is also well distinguished from Neod. polemonii and Neod. xanthina in the NaOH reactions (dull green with pale reddish brown margin in Neod. achlydis, pale sienna to rust colour in Neod.

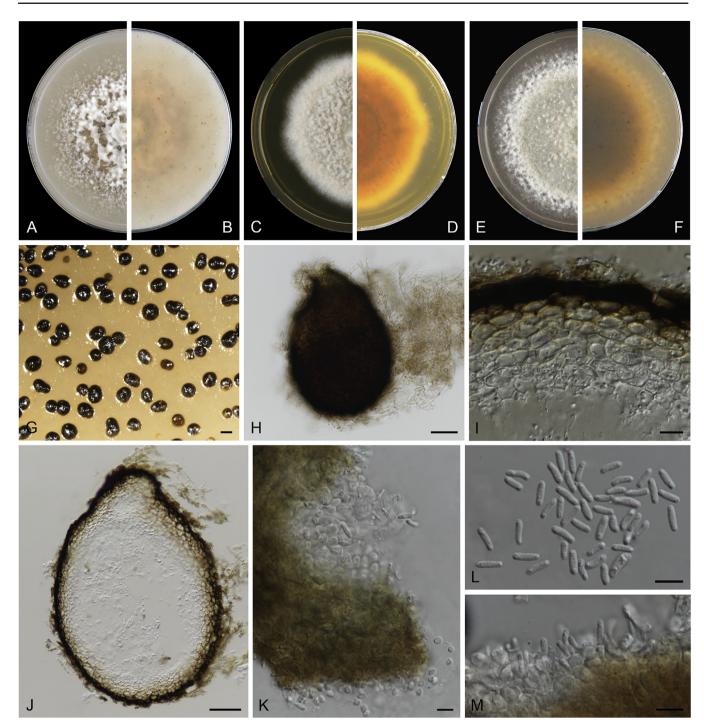


Fig. 31. Neodidymelliopsis achlydis (CBS 256.77). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidium. I. Section of pycnidial wall. J. Section of pycnidium. K, M. Conidiogenous cells. L. Conidia. Scale bars: G = 500 μm; H, J = 50 μm; I, K–M = 10 μm.

polemonii, reddish brown discolouration in Neod. xanthina; Boerema et al. 2004).

*Neodidymelliopsis longicolla* L.W. Hou, Crous & L. Cai, **sp. nov.** MycoBank MB820006. Fig. 32.

Etymology: Name refers to the elongated, long ostiolar necks.

Conidiomata pycnidial, solitary or aggregated, globose to flaskshaped, glabrous or with some hyphal outgrows, superficial or semi-immersed, ostiolate,  $200-490 \times 150-360 \mu m$ . Ostioles 1–3, developing into elongated necks, up to 250 µm tall, papillate. Pycnidial wall pseudoparenchymatous, composed of isodiametric cells, 4–7 layers, outer 3–6-layers pigmented, 20–45 µm thick. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform, 4.5–6.5 × 4.5–6 µm. *Conidia* oblong to cylindrical, smooth- and thin-walled, initially aseptate and hyaline, later becoming 1-septated and pale brown, somewhat constricted at the septum,  $12-15(-16.5) \times 4-7$  µm, guttulate. *Conidial matrix* brown.

*Culture characteristics*: Colonies on OA, 45–52 mm diam after 7 d, margin regular, aerial mycelia white and woolly, greenish olivaceous; reverse darker brown. Colonies on MEA 55–57 mm diam after 7 d, margin regular, covered by floccose, white aerial mycelia, black pycnidia visible; reverse brown, saffron near the margin. Colonies on PDA, 55–60 mm diam after 7 d, margin

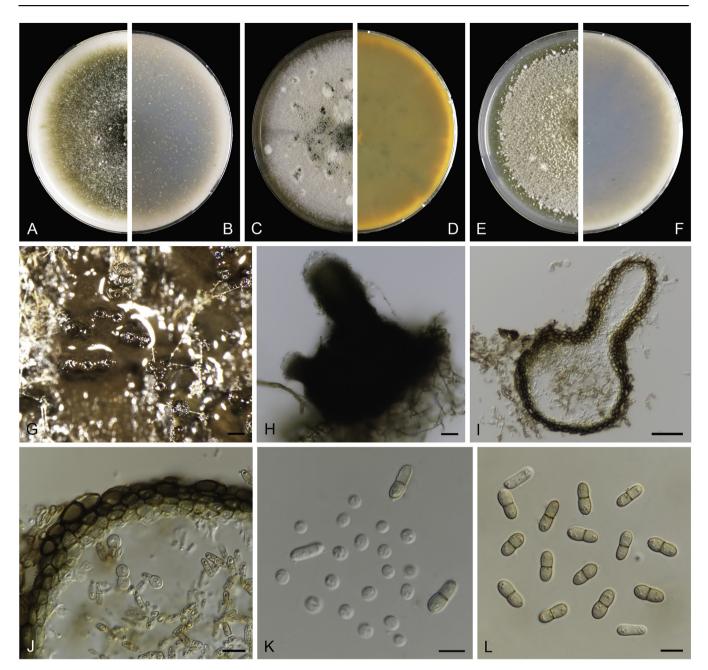


Fig. 32. Neodidymelliopsis longicolla (CBS 382.96). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K. Conidiogenous cells and conidia. L. Conidia. Scale bars: G = 250 µm; H–I = 50 µm; J = 5 µm; K–L = 10 µm.

regular, densely covered by floccose aerial mycelia, grey, greenish olivaceous near the margin; reverse dark brown, pale brown near the margin. Application of NaOH results in a pale reddish brown discolouration on MEA.

Specimen examined: Israel, En Avdat, Negev desert, from soil in desert, Feb. 1996, A. van Iperen (holotype CBS H-23016, dried culture, ex-holotype living culture CBS 382.96).

Notes: CBS 382.96 was deposited as "Ascochyta scotinospora", but differs from As. scotinospora by its larger pycnidia (200–490 × 150–360 µm vs. 140 µm diam) and forming elongated long necks (Punithalingam 1979). Phylogenetically, it clustered in the Neodidymelliopsis clade, basal to Neod. achlydis, Neod. polemonii and Neod. xanthina (Fig. 1). Hence, CBS 382.96 was described as a new species, Neod. longicolla. Neodidymelliopsis longicolla differs from Neod. achlydis in its septate conidia (mainly 1-septated vs. aseptate) and colour of its conidial matrix (brown vs. whitish cream); from *Neod. polemonii* in producing wider conidia (12–16.5 × 4–7  $\mu$ m vs. 4.5–7.5 × 1.5–4  $\mu$ m; Chen *et al.* 2015a); from *Neod. xanthina* in the number of pycnidial ostioles (1–3 vs. 1; Boerema *et al.* 2004).

*Phoma* Sacc. emend. Q. Chen & L. Cai, Stud. Mycol. 82: 194. 2015.

*Phoma herbarum* Westend., Bull. Acad. R. Sci. Belg., Cl. Sci. 19(3): 118. 1852, emend. Q. Chen & L. Cai, Stud. Mycol. 82: 195. 2015.

Synonyms: Phoma neerlandica Q. Chen & L. Cai, Stud. Mycol. 82: 197. 2015.

Atradidymella muscivora M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.

Phoma muscivora M.L. Davey & Currah, Amer. J. Bot. 96: 1283. 2009.

Phoma cruris-hominis Punith., Nova Hedwigia 31: 135. 1979.

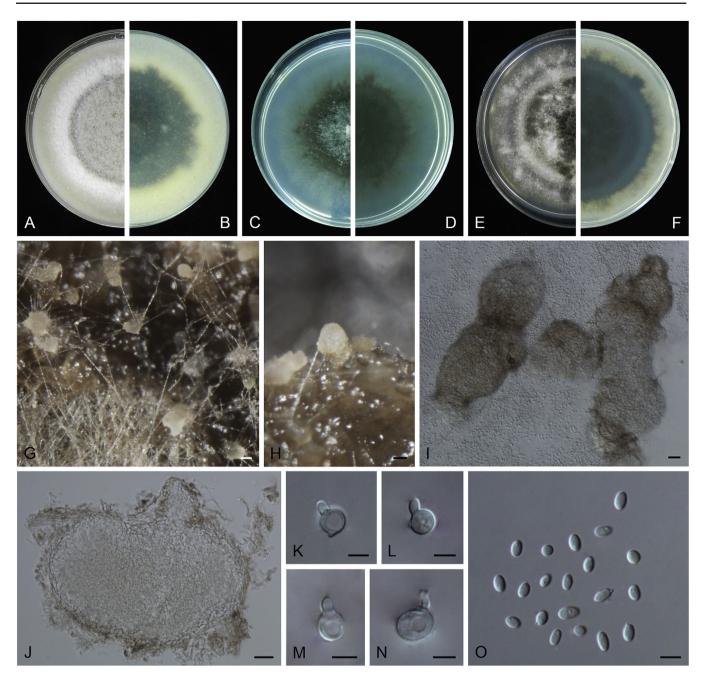


Fig. 33. Stagonosporopsis bomiensis (CGMCC 3.18366). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G–H. Pycnidium forming on OA. I. Pycnidia. J. Section of pycnidium. K–N. Conidigenous cells. O. Conidia. Scale bars: G–H = 40 µm; I–J = 20 µm; K–O = 5 µm.

Specimens examined: Canada, Alberta, Wolf Lake, from gametophytes of *Polytrichum juniperinum*, 2008, M.L. Davey, UAMH 10909 = CBS 127589. Switzerland, Kt. Graubünden, from *Achillea millefolium*, deposited in CBS Mar. 1951, E. Müller, CBS 304.51. The Netherlands, Emmeloord, from the stem of *Rosa multiflora* cv. Cathayensis, deposited in CBS Dec. 1975, G.H. Boerema, CBS 615.75 = PD 73/665 = IMI 199779; Emmeloord, from a leaf of *Delphinium* sp., deposited in CBS Feb. 1996, culture ex-holotype of "*Phoma neerlandica*" CBS 134.96 = PD 84/676; Naaldwijk, from a stem base of *Nerium* sp., deposited in CBS Sep. 1991, J. de Gruyter, CBS 502.91 = PD 82/276. UK, from a leg of woman, Apr. 1977, Y.M. Clayton, CBS 377.92 = IMI 213845; near Dumfries, from die-back of *Picea excelsa*, deposited in CBS Oct. 1937, T.R. Peace, CBS 274.37.

Notes: Phoma neerlandica was regarded distinct from *P. herbarum* based on its slightly longer and occasionally uniseptate conidia (Chen *et al.* 2015a). Similar to many other species in *Didymellaceae*, such an overlapping morphology often caused confusion with regards to species boundaries.

Unfortunately, a sequencing error occurred in the *tub2* sequence of CBS 134.96, which was not detected in the subsequent control and processing steps. *Phoma neerlandica* therefore became a name introduced with ambiguous data, and is therefore reduced to synonymy.

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

Stagonosporopsis bomiensis Q. Chen, Crous & L. Cai, sp. nov. MycoBank MB818955. Fig. 33.

*Etymology*: Epithet derived from its location of origin, Bomi in Tibet, China.

Leaf spots amphigenous, circular to irregular, 2–5 mm diam, scattered over the leaf, brown, surrounded by a greenish yellow

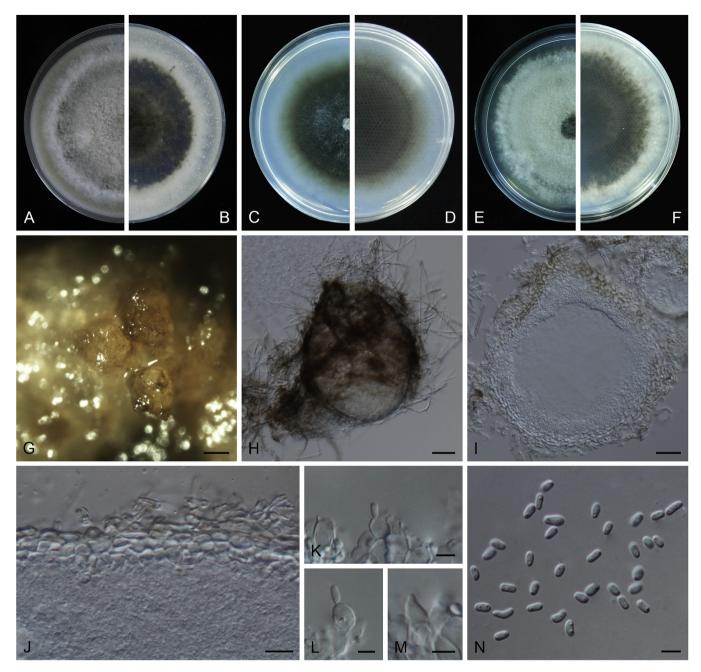


Fig. 34. Stagonosporopsis papillata (CGMCC 3.18367). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidium forming on OA. H. Pycnidium. I. Section of pycnidium. J. Section of pycnidial wall. K–M. Conidiogenous cells. N. Conidia. Scale bars: G = 100 μm; H = 40 μm; I–J = 10 μm; K–N = 5 μm.

border, single lesions may coalesce to form larger lesions till the whole leaf and getting dark brown (Fig. 7G). *Conidiomata* pycnidial, solitary, sometimes aggregated, globose to sub-globose, pale brown, glabrous, superficial, ostiolate,  $100-200 \times 100-180 \ \mu m$ . *Ostiole* single, with an elongated neck, slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3 layers, 20–30  $\mu m$  thick, outer wall 1–2-layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform, 5–8 × 4.5–7  $\mu m$ . *Conidia* ovoid to ellipsoidal, smooth-and thin-walled, hyaline, aseptate, 3.5–6.5 × 2–3.5  $\mu m$ , with 1–2 distinct polar guttules. *Conidial matrix* buff.

*Culture characteristics*: Colonies on OA, 35–43 mm diam after 7 d, margin regular, covered by floccose aerial mycelia, dense,

white to greyish olivaceous; reverse white to olivaceous. Colonies on MEA 45–47 mm diam after 7 d, margin irregular, olivaceous, with sparse white aerial mycelia near the centre; reverse concolourous. Colonies on PDA, 53–55 mm diam after 7 d, margin regular, aerial mycelia floccose, white to olivaceous, forming concentric rings; reverse olivaceous with pale green margin. NaOH test negative.

Specimens examined: China, Tibet, Bomi, leaves of *Boraginaceae*, 14 Jun. 2015, Q. Chen (holotype HMAS 247170, dried culture, ex-holotype living culture CGMCC 3.18366 = LC 8167); *ibid.* LC 8168.

Notes: Stagonosporopsis bomiensis is most closely related to S. papillata, another novel species collected from Tibet. However, S. bomiensis is distinguishable from S. papillata by having slightly shorter and wider conidia (3.5–6.5 × 2–3.5  $\mu m$  vs. 3.5–9 × 1.5–3  $\mu m$ ), and based on its number of ostioles (1 vs. 2–3).

This is the first record of a *Stagonosporopsis* species on a member of the *Boraginaceae*.

*Stagonosporopsis papillata* Q. Chen, Crous & L. Cai, **sp. nov.** MycoBank MB818954. Fig. 34.

Etymology: Name refers to its papillate pycnidia.

*Leaf spots* amphigenous, angular to irregular, 2–8 mm diam, reddish brown, indefinite border (Fig. 7K). *Conidiomata* pycnidial, solitary or aggregated, yellowish brown to brown, globose to subglobose or obpyriform, with hyphal outgrowths, semi-immersed in the agar, ostiolate,  $(130-)200-280 \times (100-)$  150–250 µm. *Ostioles* 2–3, slightly papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3 layers, 10–15(–20) µm thick, outer wall 1–2-layers pigmented. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to doliiform, 5–8.5 × 4–7.5 µm. *Conidia* ellipsoidal to oblong, incidentally slightly curved, smooth- and thin-walled, hyaline, aseptate, 3.5–6.5(–9) × 1.5–3 µm, with two large polar guttules. *Conidial matrix* buff.

*Culture characteristics*: Colonies on OA, 44–50 mm diam after 7 d, margin regular, covered by white, dense aerial mycelia, grey near the centre, with white margin; reverse olivaceous with white margin. Colonies on MEA 50–52 mm diam after 7 d, margin regular, dull green, aerial mycelia sparsely; reverse concolourous. Colonies on PDA, 55–57 mm diam after 7 d, margin regular, aerial mycelia covering the whole colony, white; reverse olivaceous with white margin. NaOH test negative.

Specimens examined: China, Tibet, Bomi, on leaves of *Rumex nepalensis*, 14 Jun. 2015, Q. Chen (holotype HMAS 247171, dried culture, ex-holotype living culture CGMCC 3.18367 = LC 8169); *ibid.* LC 8170; Bomi, on leaves of *Boraginaceae*, 14 Jun. 2015, Q. Chen, LC 8171.

*Notes: Stagonosporopsis papillata* is phylogenetically allied to *S. bomiensis* and *S. dorenboschii* (Fig. 1). Morphological differences between *S. papillata* and *S. bomiensis* are discussed under the latter species. *Stagonosporopsis papillata* could be differentiated from *S. dorenboschii* by producing slightly larger conidiogenous cells ( $5-8.5 \times 4-7.5 \mu m vs. 4-6 \times 3-6 \mu m$ ) and conidia ( $3.5-9 \times 1.5-3 \mu m vs. 3-5.5 \times 1.5-2.5 \mu m$ ; de Gruyter & Noordeloos 1992).

## DISCUSSION

The *Didymellaceae* has recently undergone extensive revision based on its phylogenetic relationships (Aveskamp *et al.* 2009a, b, 2010, de Gruyter *et al.* 2009, Chen *et al.* 2015a). In this study, 32 new taxa and two new combinations are proposed in nine genera, mostly based on specimens collected from Asia.

The majority of members in *Didymellaceae* are plant associated fungi. So far, only a few species were reported from other substrates, such as *Phoma herbarum*, *Didymella glomerata*, *D. pomorum* from inorganic materials including asbestos, cement, paint, etc. (Aveskamp *et al.* 2008), *D. eucalyptica* from water, *D. gardeniae* from air, and *Leptosphaerulina australis* from soil (Aveskamp et al. 2010). In the present study, several new species, namely Allophoma oligotrophica, Didymella aeria, D. aquatica, D. chloroguttulata, D. ellipsoidea, D. suiyangensis were collected from substrates such as air, soil, water and limestone from caves in South-west China, a typical environment with relatively low temperature, low nutrition, high humidity, and absolute darkness (Zhang et al. 2017). All these species are oligotrophic fungi except D. aquatic. It is interesting that many of these new species present pale green to dull green polar guttules which are not often observed in other species, while few other recognizable morphological differences could be observed.

The 360 isolates belonging to 194 taxa investigated in this study represent a large collection of Didymellaceae, which occurred on 163 different host genera within 70 families. Our results indicated that Asteraceae, Fabaceae, Poaceae, Ranunculaceae, Rosaceae and Solanaceae were the six most common host families associated with Didymellaceae (Fig. 2). Based on currently available data, several genera exhibited a certain level of host-specificity, i.e. Ascochyta species show relatively high host specificity to Fabaceae, Neoascochyta to Poaceae and Neomicrosphaeropsis to Tamaricaceae. Heterophoma species appear somewhat specific to Scrophulariaceae, as well as Phomatodes to Brassicaceae. Other genera appear to have a rather broad range of host families. Among the five apparently host-specific genera listed above, Neoascochyta is located in the earliest divergent clade in Didymellaceae, followed by Phomatodes, Ascochyta, Neomicrosphaeropsis and Heterophoma. Surprisingly, this evolutionary direction is consistent with that of their respective host families, i.e. Poaceae as earliest, followed by Brassicaceae, Fabaceae, Tamaricaceae and Scrophulariaceae (Bremer et al. 2009). Our data suggest, therefore, a general trend of coevolution in the host-specific groups in Didymellaceae.

Nine new species belonging to Epicoccum and 10 in Didymella are proposed in this paper, which reflect the high diversity of species in these two genera. The most remarkable feature of Epicoccum species is the formation of the darkly pigmented multiseptate conidia (dictyochlamydospores) from sporodochia. Of the nine new taxa, four were only observed as typical Epicoccum conidia, while the pycnidial morphs proved to be absent. These four species could also produce yellowish pigments that diffuse into culture media. In addition, six of the new Epicoccum species showed positive reactions in the NaOH test, that detects the production of metabolite E. Epicoccum camelliae is likely an opportunistic pathogen that could asymptomatically colonise plants as a potential destructive invader, as we obtained two strains, one from a healthy leaf, and another from a diseased leaf. Among the 10 new species described in the sexual genus Didymella, D. sinensis was recorded as sexual morph in all the single ascospore isolates obtained from three different hosts, while the asexual morph was not observed, revealing the homothallic nature of this species.

In spite of the good performance on the resolution of genera and species in *Didymellaceae* using the combined four loci, LSU, ITS, *rpb2* and *tub2*, there are still several taxa or species complexes that await further assessment, such as the *Boeremia exigua* varieties (Abeln *et al.* 2002, Aveskamp *et al.* 2009b) and the *Epicoccum nigrum* complex (Fávaro *et al.* 2011). Additional loci and more isolates are required for a future study to clarify their phylogenetic relationships as well as species boundaries.

Following the 17 genera accepted in *Didymellaceae* by Chen et al. (2015a), *Briansuttonomyces* (Crous and Groenewald 2016) and *Neomicrosphaeropsis* (Thambugala et al. 2017) were subsequently embedded in this family based on the multi-locus

phylogenies, which were confirmed in this paper. However, the introduction of several other genera are in need of reassessment. The monotypic genus Heracleicola erected by Ariyawansa et al. (2015) is herewith reduced to synonymy with Ascochyta, according to our combined LSU and ITS sequences analysis (Supplementary Fig. S1). Another genus, Neodidymella, was established in the same paper without any sequence data provided, although a tree was presented which suggested a very close relationship to Boeremia (Ariyawansa et al. 2015). Wijayawardene et al. (2016) proposed another monotypic genus Didymellocamarosporium, typified with Didymelloc. tamaricis, for which only SSU and LSU sequences could be obtained from public sequence repositories. The tree presented in Wijayawardene et al. (2016) provided little information as most of the Didymellaceae members were ignored in their analysis. We conducted a phylogenetic analysis using LSU sequences (Supplementary Fig. S2) and Didymelloc. tamaricis was embedded within the genus Neomicrosphaeropsis that was established later in the same year (Thambugala et al. 2017). Morphologically, Didymellocamarosporium tamaricis produces large brown, muriformly septate conidia  $(13-21.5 \times 7-9.5 \mu m)$ , which is obviously different from Neomicrosphaeropsis species and other genera in Didymellaceae.

The genera Endocoryneum and Pseudohendersonia were established by Petrak (1922) and Crous & Palm (1999) respectively, with their familial placements undetermined. Recently two new species, Endocoryneum festucae and Pseudohendersonia galiorum were introduced and they were placed in Didymellaceae by Wijayawardene et al. (2016), in which however, only LSU sequences were provided. Our analysis on the basis of LSU sequences revealed that E. festucae belonged to Stagonosporopsis, and formed an unexpected very long terminal branch (Supplementary Fig. S2). The sequence of E. festucae (access number: KU848203) needs to be verified. The phylogenetic relationships of Pseudohendersonia galiorum can also not be clarified solely based on the LSU sequence provided in Wijayawardene et al. (2016). From the morphological aspect, E. festucae and P. galiorum both produce large brown conidia (30-37 × 9-12 µm, and  $9-15 \times 3.5-5.5 \mu m$ ), with 3-4 transverse septa (Wijayawardene et al. 2016), obviously atypical for Didymellaceae. Due to the lack of sequences and the morphological divergence. their taxonomic placement remains unresolved, and these genera can thus not be accepted in *Didymellaceae*.

Our study, together with that of Aveskamp *et al.* (2010) and Chen *et al.* (2015a), have set the foundation for the systematics and taxonomy of *Didymellaceae*. Most of the genera included in this family have been well circumscribed, and useful molecular loci have been identified for species delimitation. The present study adds further indication that a large number of unknown *Didymellaceae* species exist in nature, especially from previously ignored ecosystems.

## ACKNOWLEDGEMENTS

This study was financially supported by the Project for Fundamental Research on Science and Technology, MOST (2014FY120100) and NSFC 31600023. Jiang-Rui Jiang, Zhi-Feng Zhang, Nan Zhou and Fang Liu are thanked for help with sample collection, as well as providing cultures. Roger G. Shivas and Bevan S. Weir are acknowledged for providing fungal cultures. Da-Chuan Bao and Bing Liu are thanked for helping in identifying the various host plants. Xiao-Ling Zhang is thanked for helping with the statistical analysis. Ling-Wei Hou acknowledges CAS GJHZ1310 for supporting her visit to the Westerdijk Fungal Biodiversity Institute.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.simyco.2017.06.002.

## REFERENCES

- Abeln ECA, Stax AM, de Gruyter J, et al. (2002). Genetic differentiation of Phoma exigua varieties by means of AFLP fingerprints. Mycological Research 106: 419–427.
- Ariyawansa HA, Hyde KD, Jayasiri SC, et al. (2015). Fungal diversity notes 111–252–taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75: 27–274.
- Aveskamp MM, de Gruyter J, Crous PW (2008). Biology and recent developments in the systematics of *Phoma*, a complex genus of major quarantine significance. *Fungal Diversity* **31**: 1–18.
- Aveskamp MM, de Gruyter J, Woudenberg JHC, et al. (2010). Highlights of the Didymellaceae: A polyphasic approach to characterise Phoma and related pleosporalean genera. Studies in Mycology 65: 1–60.
- Aveskamp MM, Verkley GJM, de Gruyter J, et al. (2009a). DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. *Mycologia* **101**: 363–382.
- Aveskamp MM, Woudenberg JHC, de Gruyter J, et al. (2009b). Development of taxon-specific sequence characterized amplified region (SCAR) markers based on actin sequences and DNA amplification fingerprinting (DAF): a case study in the *Phoma exigua* species complex. *Molecular Plant Pathology* **10**: 403–414.
- Berner D, Cavin C, Woudenberg JHC, et al. (2015). Assessment of Boeremia exigua var. rhapontica, as a biological control agent of Russian knapweed (Rhaponticum repens). Biological Control 81: 65–75.
- Boerema GH (1993). Contributions towards a monograph of *Phoma* (Coelomycetes) – II. Section *Peyronellaea*. *Persoonia* 15: 197–221.
- Boerema GH, de Gruyer J, Noordeloos ME, et al. (2004). Phoma identification manual. Differentiation of specific and infra-specific taxa in culture. CABI Publishing, Wallingford, UK.
- Bremer B, Bremer K, Chase M, et al. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society **161**: 105–121.
- Cai L, Hyde KD, Taylor PWJ, et al. (2009). A polyphasic approach for studying *Colletotrichum. Fungal Diversity* **39**: 183–204.
- Chen Q, Jiang JR, Zhang GZ, et al. (2015a). Resolving the Phoma enigma. Studies in Mycology 82: 137–217.
- Chen Q, Zhang K, Zhang GZ, et al. (2015b). A polyphasic approach to characterise two novel species of *Phoma* (*Didymellaceae*) from China. *Phytotaxa* **197**: 267–281.
- Choi YW, Hyde KD, Ho WH (1999). Single spore isolation of fungi. *Fungal Diversity* **3**: 29–38.
- Crous PW, Gams W, Stalpers JA, *et al.* (2004). MycoBank: an online initiative to launch mycology into the 21<sup>st</sup> century. *Studies in Mycology* **50**: 19–22.
- Crous PW, Groenewald JZ (2016). They seldom occur alone. *Fungal Biology* **120**: 1392–1415.
- Crous PW, Palm ME (1999). Systematics of selected foliicolous fungi associated with leaf spots of *Proteaceae*. *Mycological Research* **103**: 1299–1304.
- Crous PW, Verkley GJM, Groenewald JZ, et al. (2009). Fungal biodiversity. In: CBS Laboratory Manual Series, 1. Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands.
- Cubero OF, Crespo A, Fatehi J, et al. (1999). DNA extraction and PCR amplification method suitable for fresh, herbarium stored, lichenized, and other fungi. *Plant Systematics and Evolution* **216**: 243–249.
- De Gruyter J, Aveskamp MM, Woudenberg JHC, et al. (2009). Molecular phylogeny of *Phoma* and allied anamorph genera: Towards a reclassification of the *Phoma* complex. *Mycological Research* **113**: 508–519.
- De Gruyter J, Boerema GH, van der Aa HA (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) VI 2. Section *Phyllostictoides*: Outline of its taxa. *Persoonia* **18**: 1–53.
- De Gruyter J, Noordeloos ME (1992). Contributions towards a monograph of Phoma (Coelomycetes) – I. 1. Section Phoma: taxa with very small conidia in vitro. Personia 15: 71–92.
- De Gruyter J, Noordeloos ME, Boerema GH (1993). Contributions towards a monograph of *Phoma* (Coelomycetes) I. 2. Section *Phoma*: additional

taxa with very small conidia and taxa with conidia up to 7  $\mu m$  long. Persoonia 15: 369-400.

- De Gruyter J, Noordeloos ME, Boerema GH (1998). Contributions towards a monograph of *Phoma* (Coelomycetes) I. 3. Section *Phoma*: taxa with conidia longer than 7 μm. *Persoonia* **16**: 471–490.
- De Hoog GS, Gerrits van den Ende AHG (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41**: 183–189.

Dearness J (1916). New or noteworthy species of fungi. Mycologia 8: 98-107.

- Fávaro LCdL, de Melo FL, Aguilar-Vildoso CI, et al. (2011). Polyphasic analysis of intraspecific diversity in *Epicoccum nigrum* warrants reclassification into separate species. *PLoS One* **6**: e14828.
- Gomes RR, Glienke C, Videira SIR, et al. (2013). Diaporthe: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia **31**: 1–41.
- Jiang JR, Chen Q, Cai L (2017). Polyphasic characterisation of three novel species of Paraboeremia. Mycological Progress 16: 285–295.
- Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology* and Evolution 30: 772–780.
- Liu YJ, Whelen S, Hall BD (1999). Phylogenetic relationships among ascomycetes evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* **16**: 1799–1808.
- Lombard L, Houbraken J, Decock C, et al. (2016). Generic hyper-diversity in Stachybotriaceae. Persoonia 36: 156-246.
- Müller E (1953). Kulturversuche mit Ascomyceten I. Sydowia 7: 325-334.
- Nylander JAA (2004). *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Petrak F (1922). Mykologische Notizen. IV. Annales Mycologici **20**: 300–345.

- Punithalingam E (1979). Graminicolous Ascochyta species. Mycological Papers 142: 1–214.
- Punithalingam E, Tulloch M, Leach CM (1972). Phoma epicoccina sp. nov. on Dactylis glomerata. Transactions of the British Mycological Society 59: 341–345.
- Rayner RW (1970). A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey, UK.
- Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.
- Ronquist F, Teslenko M, van der Mark P, et al. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology **61**: 539–542.
- Saccardo PA (1902). Sylloge Fungorum omnium hucusque cognitorum: Supplementum Universale, Pars V: 16: 1–1291. Padova, Italy.

- Saccardo PA, Trotter A (1913). Sylloge Fungorum omnium hucusque cognitorum: Supplementum Universale, Pars IX: 22: 1–1612. Padova, Italy.
- Smith H, Wingfield MJ, Coutinho TA, et al. (1996). Sphaeropsis sapinea and Botryosphaeria dothidea endophytic in Pinus spp. and Eucalyptus spp. in South Africa. South African Journal of Botany 62: 86–88.
- Stamatakis A, Alachiotis N (2010). Time and memory efficient likelihood-based tree searched on phylogenomic alignments with missing data. *Bioinformatics* 26: i132–i139.
- Sung G-H, Sung J-M, Hywel-Jones NL, et al. (2007). A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution 44: 1204–1223.
- Tamura K, Stecher G, Peterson D, et al. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolutionary* 30: 2725–2729.
- Thambugala KM, Daranagama DA, Phillips AJL, et al. (2017). Microfungi on Tamarix. Fungal Diversity 82: 239–306.
- Van der Aa HA, Boerema GH, de Gruyer J (2000). Contributions towards a monograph of *Phoma* (Coelomycetes) – VI-1. Section *Phyllostictoides*: characteristics and nomenclature of its type species *Phoma exigua*. *Persoonia* 17: 435–456.
- Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
- Wainwright M, Al-Talhi A (1999). Selective isolation and oligotrophic growth of Candida on nutrient-free silica gel medium. Journal of Medical Microbiology 48, 1130–1130.
- White TJ, Bruns T, Lee S, et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, California, USA: 315–322.
- Wijayawardene NN, Hyde KD, Wanasinghe DN, et al. (2016). Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Diversity 77: 1–316.
- Woudenberg JHC, Aveskamp MM, de Gruyter J, et al. (2009). Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. Persoonia 22: 56–62.
- Zhang K, Su YY, Cai L (2013). An optimized protocol of single spore isolation for fungi. *Cryptogamie, Mycologie* 34: 349–356.
- Zhang ZF, Liu F, Zhou X, et al. (2017). Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. *Persoonia* **39**: 1–31.

