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Leaf litter saprobic Didymellaceae (Dothideomycetes): *Leptosphaerulina longiflori* sp., nov. and *Didymella sinensis*, a new record from *Roystonea regia*

Tennakoon DS[1,2,3,4], Thambugala KM[5], De Silva NI[2,3,4,6], Kuo CH[1*] and Hyde KD [2,3,4,6]

[1]*Department of Plant Medicine, National Chiayi University, 300 Syuefu Road, Chiayi City 60004, Taiwan*
[2]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand*
[4]*Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan China*
[5]*Industrial Science and Management (International Program), Faculty of Science and Technology, Thammasat University (Rangsit Center), Klong Luang, Pathumthani 12121, Thailand*
[6]*Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*

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Abstract

Taxonomic studies of leaf litter inhabiting fungi resulted in two saprobic members of Dothideomycetes being collected from Fanlu Township area, Dahu forest, Chiayi in Taiwan (Elevation 630 m). Morphology coupled with combined gene analysis of a LSU, ITS and RPB2 DNA sequence data, showed that they belong to the family Didymellaceae. A new species, *Leptosphaerulina longiflori* from dead leaves of *Lilium longiflorum* and a new host record of *Didymella sinensis* from dead leaves of *Roystonea regia* are herein described. *Leptosphaerulina longiflori* is distinguished from other *Leptosphaerulina* species based on distinct size differences in ascomata, asci, ascospores and DNA sequence data. Both species are compared with other similar species and comprehensive descriptions and micrographs are provided.

Key words – 1 new species – *Lilium* – pleosporales – phylogeny – taxonomy

Introduction

Forest leaf litter is a hidden world of activities as it provides substrates for a variety of living things, from the smallest bacteria and fungi, to the largest macro-invertebrates. In particular, it acts as a protective layer against microhabitat fluctuations, erosion, soil compaction and creates a microclimate that is favourable for fungal fruiting-body production (Eaton et al. 2004, Koide et al. 2005, Sayer 2005, Shirouzu et al. 2009, Promputtha et al. 2017). We have been carrying out studies of fungal species inhabiting leaf litter and have described numerous new species of Dothideomycetes (Ariyawansa et al. 2015, Hyde et al. 2017, 2018, Wanasinghe et al 2017, Tennakoon et al. 2018a, Pem et al. 2018, 2019, Phookamsak et al. 2019).

The family Didymellaceae is considered as one of the species-rich families in the order Pleosporales and includes species that inhabit a wide range of ecosystems (Chen et al. 2017, Hyde

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Corresponding Author: Chang-Hsin Kuo – e-mail – chkuo@mail.ncyu.edu.tw

et al. 2017, Tibpromma et al. 2017, Thambugala et al. 2018). De Gruyter et al. (2009) introduced Didymellaceae to accommodate the type species *Didymella exigua*, together with some *Phoma* or *Phoma*-like genera which constituted a strongly supported clade in the phylogenetic tree. Didymellaceae species are characterized by immersed, rarely superficial, separate or gregarious, globose to flattened, ostiolate ascomata, with a few to several layers of pseudoparenchymatous cells. Asci are 8-spored, bitunicate, cylindrical to clavate or saccate, and ascospores are mostly hyaline or brownish and 1-septate to multiseptate (Aveskamp et al. 2010, Zhang et al. 2012, Hyde et al. 2013, Chen et al. 2015, Jayasiri et al. 2017).

The members of Didymellaceae play a vital role as saprobes, endophytes and pathogens of wide range of host species (Zhang et al. 2012, Hyde et al. 2013, 2016, Chen et al. 2017). Zhang et al. (2009) included Didymellaceae in the order Pleosporales within the suborder Pleosporineae. Aveskamp et al. (2010) revised the taxonomy of Didymellaceae species based on multi-gene analyses and included eleven genera. Subsequently, many researchers added more genera (Zhang et al. 2012, Hyde et al. 2013, Ariyawansa et al. 2015, Valenzuela-Lopez et al. 2018). Chen et al. (2015) introduced nine new genera and accepted 17 genera. Currently, the family comprises 27 genera including *Allophoma* Q. Chen & L. Cai, *Ascochyta* Lib., *Boeremia* Aveskamp et al., *Calophoma* Q. Chen & L. Cai, *Chaetasbolisia* Speg., *Didymella* Speg., *Didymellocamarosporium* Wijayaw. & K.D. Hyde, *Didysimulans* Tibpromma et al., *Endocoryneum* Petr., *Epicoccum* Link, *Heterophoma* Q. Chen & L. Cai, *Leptosphaerulina* McAlpine, *Macroventuria* Aa, *Mixtura* O.E. Erikss. & J.Z. Yue, *Monascostroma* Hohn., *Neoascochyta* Q. Chen & L. Cai, *Neodidymelliopsis* Q. Chen & L. Cai, *Neomicrosphaeropsis* Thambug., Camporesi & K.D. Hyde, *Nothophoma* Q. Chen & L. Cai, *Paraboeremia* Q. Chen & L. Cai, *Peyronellaea* Gold. ex Togliani, *Phaeomycocentrospora* Crous et al., *Phoma* Sacc., *Phomatodes* Q. Chen & L. Cai, *Platychora* Petr., *Pseudohendersonia* Crous & M.E. Palm, *Stagonosporopsis* Died., *Xenodidymella* Q. Chen & L. Cai (Thambugala et al. 2017, Wijayawardene et al. 2018).

In this study, two dothideomycetous species were collected from Dahu forest, Chiayi in Taiwan and morphological characters and DNA sequence data were analyzed to establish their taxonomic affinities.

Materials and methods

Sample collection, morphological studies and isolation

Decaying leaf litter samples of *Lilium longiflorum* Thunb. and *Roystonea regia* (Kunth) O.F. Cook were collected from Dahu forest area in Chiayi, Taiwan and brought to the laboratory in Zip lock plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature (25°C) for two days. The samples were examined following the methods described by Tennakoon et al. (2018b). Morphological observations were made using an AXIOSKOP 2 PLUS compound microscope and images were taken with an AXIOSKOP 2 PLUS compound microscope equipped with a Canon AXIOCAM 506 COLOR digital camera. Permanent slides were prepared by mounting fungal material in lactoglycerol and sealed by applying nail-polish around the margins of cover slips. All measurements were made with ZEN2 (blue edition) and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single ascospore isolations were carried out following the method described in Chomnunti et al. (2014). Germinated spores were individually transferred to potato dextrose agar (PDA) plates and grown at 25°C in the daylight. Isolates including accession numbers of gene sequences are listed in Table 1. Cultures are deposited in the culture collection of Mae Fah Luang University, Chiang Rai, Thailand and Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan. Specimens are deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Faces of Fungi and Index Fungorum numbers are provided as outlined in Jayasiri et al. (2015) and Index Fungorum (2019).

DNA extraction and PCR amplification

Fungal isolates were grown on PDA for 30 days at 25°C in the dark. The genomic DNA was extracted using a DNA extraction kit (E.Z.N.A Fungal DNA Mini Kit, D3390-02, Omega Bio-Tek) following the manufacturer's protocol. The DNA product was kept at 4°C for DNA amplification and maintained at -20°C for long term storage. DNA was amplified by Polymerase Chain Reaction (PCR) for three genes, the large subunit (28S, LSU), small subunit (18S, SSU), internal transcribed spacers (ITS1-5.8S-ITS2) and RNA polymerase II second largest subunit (RPB2). The LSU gene was amplified by using the primers LROR and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994); nuclear ITS was amplified by using the primers ITS5 and ITS4 (White et al. 1990) and the RPB2 gene was amplified by using primers fRPB2-5F and fRPB2-7cR (Liu et al. 1999, Sung et al. 2007).The amplification reactions were performed in 25µl of total reaction that contained 9.5 µl of sterilized water, 12.5 µl of 2×Power Taq PCR MasterMix (Tri-I Biotech, Taipei, Taiwan), 1 µl of each forward and reverse primers and 1 µl of DNA template. The polymerase chain reaction (PCR) thermal cycle program for ITS and LSU were as detailed by Cai et al. (2005) and RPB2 genes amplification was as suggested by Chen et al. (2015). The PCR products were analyzed by 1.5% agarose gels containing the Safeview DNA stain (GeneMark, Taipei, Taiwan) to confirm the expected molecular weight of a single amplification product. PCR products were purified and Sanger sequenced with primers mentioned above by Tri-I Biotech, Taipei, Taiwan. Nucleotide sequences were deposited in GenBank (Table 1).

Table 1 GenBank and culture collection accession numbers of species included in the phylogenetic study. The newly generated sequence is shown in bold.

Species	Strain/Voucher no.	GenBank accession no.		
		LSU	ITS	RPB2
Didymella aquatica	CGMCC 3.18349	KY742209	KY742055	-
D. arachidicola	CBS 333.75	GU237996	GU237833	KT389598
D. aurea	CBS 269.93	GU237999	GU237818	KT389599
D. bellidis	CBS 714.85	GU238046	GU237904	KP330417
D. boeremae	CBS 109942	GU238048	FJ426982	KT389600
D. calidophila	CBS 448.83	GU238052	FJ427059	-
D. chenopodii	CBS 128.93	GU238055	GU237775	KT389602
D. chloroguttulata	CGMCC 3.18351	KY742211	KY742057	KY742142
D. curtisii	CBS 251.92	GU238013	FJ427038	-
D. dimorpha	CBS 346.82	GU238068	GU237835	-
D. eriobotryae	MFLUCC 16–0489	MG967667	MG967669	-
D. exigua	CBS 183.55	EU754155	GU237794	GU357800
D. gardeniae	CBS 626.68	GQ387595	FJ427003	KT389606
D. heteroderae	CBS 109.92	GU238002	FJ426983	KT389601
D. ilicicola	CGMCC 3.18355	KY742219	KY742065	KY742150
D. infuscatispora	CGMCC 3.18356	KY742221	KY742067	KY742152
D. longicolla	CBS 124514	GU238095	GU237767	-
D. macrophylla	CGMCC 3.18357	KY742224	KY742070	KY742154
D. magnoliae	MFLUCC18–1560	MK348033	MK347814	MK434852
D. mascrostoma	CBS 529.66	GU238098	GU237885	-
D. molleriana	CBS 109179	GU238066	GU237744	-
D. molleriana	CBS 229.79	GU238067	GU237802	KP330418
D. musae	CBS 463.69	GU238011	FJ427026	-
D. negriana	CBS 358.71	GU238116	GU237838	KT389610
D. ocimicola	CGMCC 3.18358	KY742232	KY742078	-

Table 1 Continued.

Species	Strain/Voucher no.	GenBank accession no.		
		LSU	ITS	RPB2
D. pedeiae	CBS 124517	GU238127	GU237770	KT389612
D. pinodes	CBS 525.77	GU238023	GU237883	KT389614
D. poaceicola	MFLUCC 13–0212	KX954395	KX965726	KX898364
D. pomorum	CBS 285.76	GU238025	FJ427053	KT389615
D. protuberans	CBS 381.96	GU238029	GU237853	KT389620
D. pteridis	CBS 379.96	KT389722	KT389504	KT389624
D. rumicicola	CBS 683.79	KT389721	KT389503	KT389622
D. sancta	CBS 281.83	GU238030	FJ427063	KT389623
D. segeticola	CGMCC 3.17489	KP330455	KP330443	KP330414
D. senecionicola	CBS 160.78	GU238143	GU237787	-
D. sinensis	CGMCC 3.18348	KY742239	KY742085	KY742165
D. sinensis	**MFLUCC 17–1778**	**MK503810**	**MK503799**	**MK503804**
D. subglomerata	CBS 110.92	GU238032	FJ427080	KT389626
D. subherbarum	CBS 250.92	GU238145	GU237809	-
D. suiyangensis	CGMCC 3.18352	KY742243	KY742089	-
D. viburnicola	CBS 523.73	GU238155	GU237879	KP330430
D. americana	CBS 568.97	GU237991	FJ426974	-
D. brunneospora	CBS 115.58	KT389723	KT389505	KT389625
Epicoccum nigrum	CBS 173.73	GU237975	FJ426996	KT389632
E. plurivorum	CBS 558.81	GU238132	GU237888	KT389634
Leptosphaerulina americana	CBS 213.55	GU237981	GU237799	KT389641
L. arachidicola	CBS 275.59	GU237983	GU237820	-
L. australis	CBS 317.83	EU754166	GU237829	GU371790
L. australis	CBS 311.51	FJ795508	-	GU456357
L. longiflori	**MFLUCC 18–1641**	**MK503811**	**MK503800**	**MK503805**
L. longiflori	**FU310115**	**MK503812**	**MK503801**	**MK503806**
L. saccharicola	ICMP:19875	KF670716	KF670717	KF670714
L. trifolii	CBS 235.58	GU237982	GU237806	-
Neomicrosphaeropsis italica	MFLUCC 15–0485	KU729854	KU900318	KU674820
N. italica	MFLUCC 15–0484	KU729853	KU900319	KU695539
N. novorossica	MFLUCC 14–0578	KX198710	KX198709	-
N. rossica	MFLUCC 14–0586	KU729855	KU752192	-
Nothophoma anigozanthi	CBS 381.91	GU238039	GU237852	KT389655
N. arachidis-hypogaeae	CBS 125.93	MH874048	MH862388	KT389656
N. gossypiicola	CBS 377.67	GU238079	GU237845	KT389658
N. infossa	CBS123395	FJ899743	FJ427025	KT389659
N. macrospora	UTHSC:DI16-276	LN880537	LN880536	LT593073
N. multilocularis	AUMC-H-0002.17	KY996744	-	-
N. quercina	CBS 633.92	EU754127	GU237900	KT389657
N. quercina	MFLUCC 16–1392	KY053897	KY053896	KY053898
N. raii	A189	MG590069	MF664467	
N. variabilis	UTHSC DI16-285	-	LT592939	LT593078

Phylogenetic analysis

Taxa with the highest similarities to our strains were determined with standard nucleotide BLASTn searches in GenBank (http://www.ncbi.nlm.nih.gov/). The other sequences used in the analyses were obtained from the recent publications (Jayasiri et al. 2017, Thambugala et al. 2018, Wanasinghe et al. 2018). The combined dataset consists of 68 taxa including our newly generated taxa. *Epicoccum nigrum* (CBS 173.73) and *E. plurivorum* (CBS 558.81) were selected as out-group taxa. The multiple alignments were made by MAFFT v. 7.036 (Katoh & Standley 2013), and adjusted manually for improvement where necessary using BioEdit v. 7.2 (Hall 1999) and ClustalX v. 1.83 (Thompson et al. 1997). Modeltest v. 2.0 (Nylander 2004) following Akaike Information Criterion was used to determine the best-fit model of evolution for each data set for Bayesian and Maximum Likelihood analyses.

Maximum likelihood trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. Maximum parsimony analysis (MP) was performed in PAUP v. 4.0b10 (Swofford 2002), with the heuristic search option and 1,000 random replicates. Maxtrees was set to 1000 and branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria as detailed in Jeewon et al. (2002, 2003), Cai et al. (2005) and Wang et al. (2007). The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were meaningfully different.

A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Posterior Probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation and 10,000 trees were obtained. The first 2,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 8,000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) (Cai et al. 2006). Bootstrap support values for maximum likelihood (ML), maximum parsimony (MP) higher than 60 % and Bayesian posterior probabilities (BYPP) greater than 0.90 are given above each branch respectively. Phylograms were visualized with FigTree v1.4.0 (Rambaut 2012) and annotated in Microsoft Power Point (2010). The final alignment and trees were deposited in TreeBASE, submission ID: 23978.

Results

The LSU, ITS and RPB2 combined analyses comprised 2432 characters, of which 2060 characters are constant, 270 characters are parsimony-informative, while 102 variable characters are parsimony-uninformative in the maximum parsimony (MP) analysis (In the most parsimonious tree, TL = 1412, CI = 0.361, RI = 0.656, RC = 0.237, HI = 0.639). The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -10444.131665. The matrix had 463 distinct alignment patterns, with 17.1 % of undetermined characters or gaps. Estimated base frequencies; A = 0.249037, C = 0.225464, G = 0.279496, T = 0.246003; substitution rates AC = 1.263042, AG = 5.753864, AT = 1.584288, CG = 0.936575, CT = 14.099119, GT = 1.000; proportion of invariable sites I = 0.756805; gamma distribution shape parameter $\alpha = 0.534605$. The Bayesian analysis resulted 10000 trees after 1000000 generations. All analyses (ML, MP and BYPP) gave similar results of the generic placements in agreement with previous studies based on multi-gene analyses (Chen et al. 2015, Jayasiri et al. 2017, Wanasinghe et al. 2018).

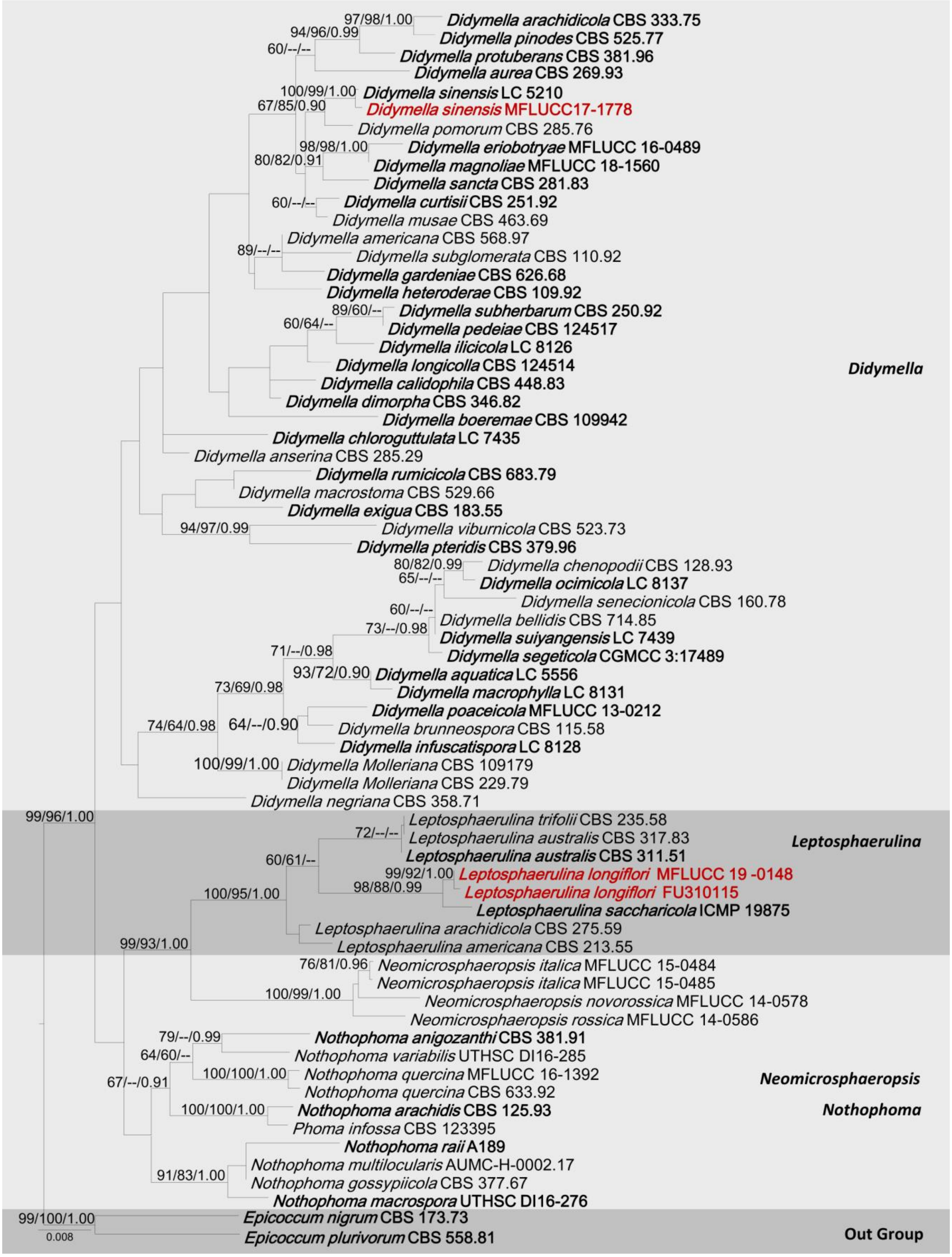

Fig. 1 – RAxML tree based on a combined dataset of LSU, ITS and RPB2 partial sequences. Bootstrap support values for ML, MP higher than 60 % and BYPP greater than 0.90 are given above each branch respectively. The new isolates are in red. Ex-type strains are in bold. The tree is rooted *Epicoccum nigrum* (CBS 173.73) and *E. plurivorum* (CBS 558.81).

Taxonomy

Didymella sinensis Qian Chen, Crous & L. Cai, Stud. Mycol. 87: 138 (2017) Fig. 2

Index Fungorum Number: IF 818967; Facesoffungi number: FoF05828

Saprobic on dead leaves of *Roystonea regia* (Kunth) O.F. Cook. Sexual morph: See Chen et al. (2017). Asexual morph: Unknown.

Culture characteristics – Colonies on PDA reaching 10 mm diameter after 2 weeks at 25–30°C, colonies medium dense, circular, convex, surface slightly rough with edge entire, effuse, velvety to hairy, colony from above: light brown to gray at the margin, gray centre; reverse, brown at the margin, gray at the centre; mycelium light brown to grayish with tufting; not producing pigments in PDA.

Material examined – Taiwan, Chiayi, Fanlu Township area, Dahu forest, dead leaves of *Roystonea regia* (Arecaceae), 20 July 2017, D.S. Tennakoon, DS001 (MFLU 17–0759), living culture (MFLUCC17–1778).

Notes – In this study, a new isolate of *Didymella sinensis* was collected from dead leaves of *Roystonea regia* (Arecaceae) in Taiwan. The new collection shares a close phylogenetic relationship with *Didymella sinensis* (LC–5210) in our combined phylogeny using LSU, ITS and RPB2 sequence data with strong bootstrap support (100% ML, 99% MP and 1.00 BYPP) (Fig. 1). The new isolate MFLUCC 17–1778 is morphologically similar to *Didymella sinensis* (LC-5210) in having immersed, globose, ostiolate ascomata with dense pseudoparaphyses, cylindrical to clavate, 8-spored asci and hyaline, 1-sepate, asymmetrical ascospores (Chen et al. 2017). *Didymella sinensis* has been previously reported from *Cerasus pseudocerasus* (Rosaceae), *Dendrobium officinale* (Orchidaceae) and Urticaceae sp. (Chen et al. 2017), but it has not been reported from *Roystonea regia* (Arecaceae). Thus, we provide the new host record of *Didymella sinensis* for the family Arecaceae in Taiwan.

Leptosphaerulina longiflori Tennakoon, C.H. Kuo & K.D. Hyde, sp. nov. Fig. 3

Index Fungorum Number: IF 556240; Facesoffungi number: FoF05820

Etymology – Name reflects the host *Lilium longiflorum*, from which the holotype was collected.

Holotype – MFLU 18–2527

Saprobic on dead leaves of *Lilium longiflorum* Thunb. Sexual morph: *Ascomata* 35–45 μm high, 40–50 μm diam., pseudothecial, solitary, scattered or sometimes clustered, immersed to erumpent, visible as slightly raised, visible as brown spots on host surface, uniloculate, brown to dark brown, globose to subglobose, pseudoparenchymatous. *Ostiole* central, with a minute papilla. *Peridium* 5–8 μm wide, comprising several layers of dark brown to lightly pigmented, cells of *textura angularis*, with outer layers composed of thick-walled, brown, somewhat flattened cells, becoming lighter towards the inner layers of hyaline cells. *Hamathecium* lacking pseudoparaphyses. *Asci* (23.5–) 25–30(−31) × (19–) 20–24 (−24.5) μm ($\bar{x}$ = 28.5 × 22.8 μm, n=25), 8-spored, bitunicate, fissitunicate, broadly obovoid, short pedicellate, apically rounded, with well-developed ocular chamber. *Ascospores* (9.5–) 10–13 (−13.2) × 3–4 μm ($\bar{x}$ = 11.4 × 3.5 μm, n=30), overlapping or irregularly triseriate, ellipsoid to obovoid, hyaline, muriform, with 3–4 transverse septa, and 1–2 longitudinal septa, usually widest in the second cell, smooth-walled, with small guttules, surrounded by a distinctive structured mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Colonies on PDA reaching 20 mm diameter after 2 weeks at 25–30°C, colonies medium dense, circular, convex, surface slightly rough with edge entire, effuse, velvety to hairy, margin well-defined, colony from above: light brown to gray at the margin, dark brown to black at the centre; reverse, light brown at the margin, dark brown to black at the centre; mycelium light brown to dark brown with tufting; not producing pigments in PDA.

Material examined – Taiwan, Chiayi, Fanlu Township area, Dahu forest, dead leaves of *Lilium longiflorum* (Liliaceae), 20 June 2018, D.S. Tennakoon, XP052 (MFLU18–2527 holotype;

MFLU 19-0796 isotype), ex-type living culture (MFLUCC 19–0148, FU310115)

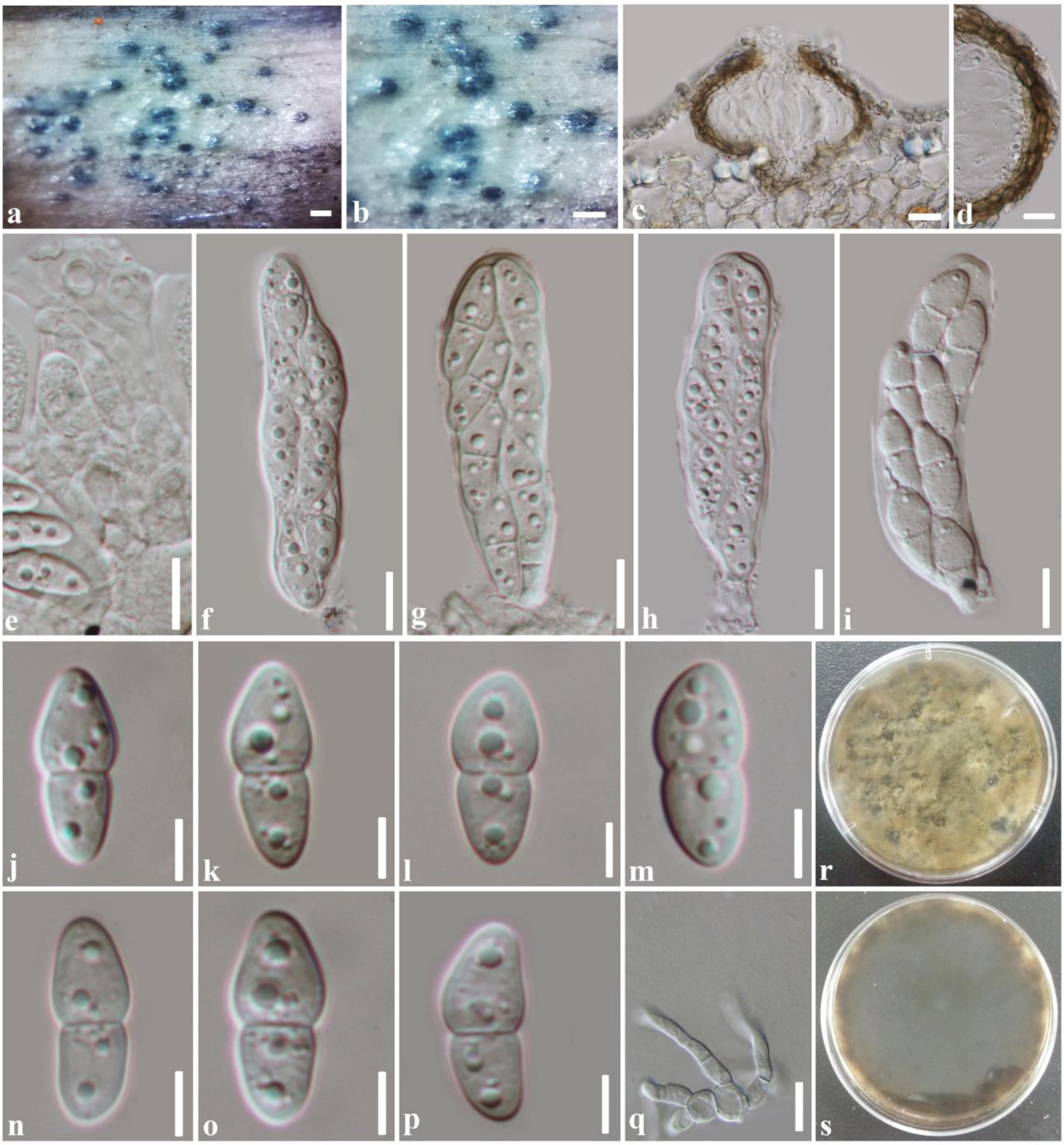

Fig. 2 – *Didymella sinensis* (new record, MFLU 17–0759). a Appearance of ascomata on host. b Close-up of ascomata. c Section of ascoma. d Section of peridium. e Pseudoparaphyses. f–i Asci. j–p Ascospores. q Germinated ascospore. r Colony from above. s Colony from below. Scale bars: a, b = 100 μm, c = 20 μm, d–i = 10 μm, j–q = 5 μm.

Notes – The morphological characteristics of *Leptosphaerulina longiflori* fit into the generic concept of *Leptosphaerulina* in having immersed to erumpent, ostiolate ascomata, 8-spored, bitunicate asci and hyaline, muriform ascospores (Abler 2003, Zhang et al. 2012, Phookamsak et al. 2013). A multi-gene phylogeny generated herein indicates that *Leptosphaerulina longiflori* forms a strongly supported lineage (98% ML, 88% MP, 0.99 BYPP) close to *L. saccharicola* (MFLUCC 11–0169) (Fig. 1). However, *Leptosphaerulina longiflori* is distinct from *L. saccharicola* in having smaller ascomata (35–45 × 40–50 μm), asci (28.5 × 22.8 μm) and ascospores (11.4×3.5 μm), as compared to *L. saccharicola* which has larger ascomata (70–110 × 100–140 µm), asci (67.9×39.4 µm) and ascospores (29.6×11 µm) (Phookamsak et al. 2013). *Leptosphaerulina saccharicola* also differs from *L. longiflori* in terms of host association, as the former has been reported from the living leaves of *Saccharum officinarum* (Phookamsak et al. 2013). This is the first report of *Leptosphaerulina* species from *Lilium longiflori* and even from the family Liliaceae. The main morphological differences of *Leptosphaerulina* species are presented in Table 2.

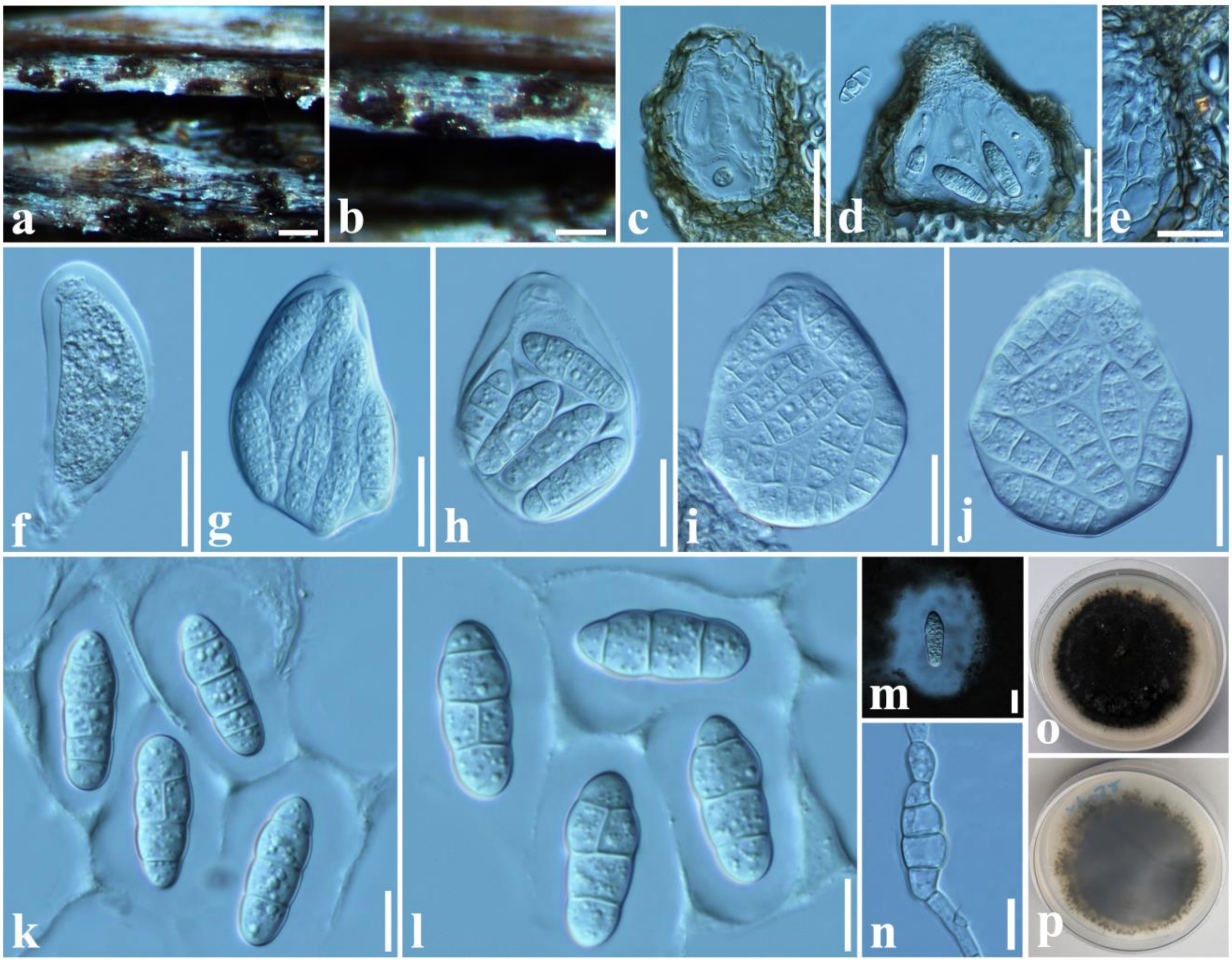

Fig. 3 – *Leptosphaerulina longiflori* (holotype, MFLU18–2527). a Appearance of ascomata on host. b Close-up of ascomata. c, d Vertical sections through ascoma. e Section of peridium. f–j Asci. k, l Ascospores. m Ascospore stained in Indian ink showing a mucilaginous sheath. n Germinated ascospore. o Colony from above. p Colony from below. Scale bars: a, b = 50 µm, c–d = 20 µm, e = 5 µm, f–j = 10 µm, k–n = 5 µm.

Discussion

The genus *Leptosphaerulina* was introduced by McAlpine (1902) to accommodate *L. australis* as the type species, which was recorded on *Prunus armeniaca* L. leaves. *Leptosphaerulina* species are characterized by small, immersed ascomata, obpyriform asci with a large ocular chamber and an apical ring, as well as muriformly septate ascospores which may be hyaline or pigmented (Zhang et al. 2012, Hyde et al. 2013, Phookamsak et al. 2013). Based on morphological characters, *Leptosphaerulina* has been placed in different families, including Pseudosphaeriaceae (Höhnel 1907, Luttrell 1955, Graham & Luttrell 1961, Barr 1982) and Pleosporaceae (Eriksson & Hawksworth 1998, Kirk et al. 2001, Eriksson 2005). However, current phylogenies have confirmed the placement of *Leptosphaerulina* in Didymellaceae (Aveskamp et al. 2010, Zhang et al. 2012, Hyde et al. 2013, Phookamsak et al. 2013, Chen et al. 2017). The asexual morph of *Leptosphaerulina* has been reported as *Pithomyces* species (Hyde et al. 2011, Phookamsak et al. 2013, Wijayawardene et al. 2017a, b). There are 61 *Leptosphaerulina* epithets in Index Fungorum (2019), but few species have molecular data.

Table 2 Synopsis of recorded *Leptosphaerulina* species.

Species	**Size (µm)**			**Septation**		**Host**	**Reference**
	Ascospores	**Asci**	**Ascomata (diam)**	**Transverse septa**	**Longitudinal septa**		
L. americana	34-49 × 13-18	101-106 × 45-48	126-140	5-6	2-5	*Trifolium* sp., *Phleum* sp.	Graham & Luttrell (1961)
L. arachidicola	23-40 × 11-17	53-87 × 28-42	64-140	3.5	0-2	*Arachis* spp.	Graham & Luttrell (1961)
L. australis	30-32 × 11	75-80 × 28-50	150	5	2	*Agrostis* sp. *Brassica* sp.	McAlpine (1902)
L. calamagrostidis	19-23.1 × 6.3-7.3	63-81.9 × 14.7-16.8	126-175	3-5	1-3	*Calamagrostis*sp.	Pisareva (1964)
L. chartarum	23-27 × 7-12	100-150 × 60-100	**-**	3	1	*Galenia procumbens*	Roux (1986)
L. longiflori	**10-13 × 3-4**	**25-30 × 20-24**	**40-50**	**3-4**	**1-2**	***Lilium longiflorum***	**This study**
L. olivaceogrisea	14-20 × 5-9	45-65 × 15-20(-29)	60-170	3-6	1-2	*Carex firma*, *Dryas*sp.	Nograsek (1990)
L. oryzae	28-30 × 10-11	60-78 × 38-51	120-170	4-5	2-3	*Oryza sativa*	Phookamsak et al. (2013)
L. saccharicola	27-32 × 10-11.5	60-80 × 35-45	100-140	4	0-2	*Saccharum* sp.	Phookamsak et al. (2013)
L. trifolii	25-49 × 11-21	62-95 × 42-59	124-207	3-4	0-2	*Arachis* sp. *Arundinaria* sp.	Graham & Luttrell (1961

Leptosphaerulina species seem to be cosmopolitan in distribution since they have been recorded from both temperate and tropical countries (i.e. Canada, China, Colombia, Georgia, India, Indonesia, Japan, Kenya, Netherlands, Peru, Taiwan, Thailand, USA) (Phookamsak et al. 2013, Chen et al. 2017, Farr & Rossman 2019). Host specificity aspects of *Leptosphaerulina* species have not yet been investigated as species have been recorded from various plant families in both monocotyledons and dicotyledons (i.e. Brassicaceae, Combretaceae, Cupressaceae, Euphorbiaceae, Fabeceae, Myrtaceae, Nyctaginaceae, Poaceae (Farr & Rossman 2019). The morphological characters of *Leptosphaerulina* are similar to *Pleospora*

(Pleosporaceae), but differ in having smaller ascomata (Table 2) and hyaline ascospores that only become pigmented after discharge, whereas the ascospores of *Pleospora* become brown within the asci (Zhang et al. 2012, Ariyawansa et al. 2015).

This study incorporates both morphological and phylogenetic approach based on DNA sequence data (LSU, SSU and RPB2) and provides insights into the taxonomic novelties of *Leptosphaerulina longiflori,* collected from *Lilium longiflorum* (Liliaceae) in Taiwan. This is the first report of *Leptosphaerulina* species recorded from the family Liliaceae. Chen et al. (2015) emended *Didymella* to accommodate *Peyronellaea* and several other phoma-like species that are phylogenetically related to *D. exigua*, the type species of *Didymella*. *Didymella* species are characterized by immersed or erumpent, globose or flattened and ostiolate ascomata with dense pseudoparaphyses, cylindrical or clavate, 8-spored asci and hyaline, 1-septate (symmetrical or asymmetrical) ascospores. Many *Didymella* species have been reported worldwide on a wide range of hosts and substrates (Aveskamp et al. 2010, Chen et al. 2015, 2017, Thambugala et al. 2017, 2018, Farr & Rossman 2019).

Combined phylogenetic analyses herein, with a larger taxon sampling, provide a better resolution of interspecific relationships of *Didymella* within Didymellaceae. It is also noted that phylogeny recovered herein is also agreed with previously established ones in that *Didymella* within the Pleosporales (Chen et al. 2015, Thambugala et al. 2018). Our new record of *Didymella sinensis* (MFLUCC17–1778), grouped in a well-supported clade (80% ML, 82% MP and 0.91 BYPP) with other *Didymella* species (Fig. 1). In particularly, it shows a close affinity with *Didymella sinensis* (LC-5210), with high support (98% ML, 99% MP and 0.99 BYPP). Morphological characters of our collection are similar to LC-5210 in having immersed, globose, ostiolate ascomata with dense pseudoparaphyses, cylindrical to clavate, 8-spored asci and hyaline, 1-septate, asymmetrical ascospores (Chen et al. 2017). Therefore, we consider our collection as a new record of *Didymella sinensis* from dead leaves of *Roystonea regia* (Arecaceae) from Taiwan.

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