Mycosphere 6 (6): 716–728 (2015)
www.mycosphere.org
Copyright © 2015

Article

ISSN 2077 7019
Mycosphere
Online Edition

Doi 10.5943/mycosphere/6/6/7

Two novel species of *Vagicola* (*Phaeosphaeriaceae*) from Italy

Jayasiri SC[1], Wanasinghe DN[1,2], Ariyawansa HA[3], Jones EBG[4], Kang JC[5], Promputtha I[6], Bahkali AH[4], Bhat J[7,8], Camporesi E[9] and Hyde KD[1, 2, 4]

[1]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*World Agro forestry Centre East and Central Asia Office, 132 Lanhei Road, Kunming 650201, China*
[3]*Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang, 550006, Guizhou, China*
[4]*Botany and Microbiology Department, College of Science, King Saud University, Riyadh, 1145, Saudi Arabia*
[5]*Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang 550025, Guizhou Province, China*
[6]*Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand*
[7]*No. 128/1-J, Azad Housing Society, Curca, P.O. Goa Velha, 403108, India*
[8]*Department of Botany, Goa University, Goa, 403 206, India*
[9]*A.M.B. GruppoMicologicoForlivese "Antonio Cicognani", Via Roma 18, Forlì, Italy; A.M.B. CircoloMicologico "Giovanni Carini", C.P. 314, Brescia, Italy; Società per gliStudiNaturalisticidella Romagna, C.P. 144, Bagnacavallo (RA), Italy*

Jayasiri SC, Wanasinghe DN, Ariyawansa HA, Jones EBG, Kang JC, Promputtha I, Bahkali AH, Bhat J, Camporesi E, Hyde KD 2015 – Two novel species of *Vagicola* (*Phaeosphaeriaceae*) from Italy. Mycosphere 6(6), 716–728, Doi 10.5943/mycosphere/6/6/7

Abstract

Phaeosphaeriaceae is a large and important family in the order Pleosporales, comprising economically important plant pathogens. Species may also be endophytes or saprobes on plant hosts. Two new species referable to *Vagicola*, *Phaeosphaeriaceae* are introduced in this paper based on analyses of LSU and ITS sequence data and their unique morphology. Most *Phaeosphaeriaceae* species grow on monocotyledons; *Vagicola dactylidis* and *V. chlamydospora* are also saprobic on grasses (*Poaceae*). *Vagicola chlamydospora* formed asexual structures in a culture. The new species are described and illustrated and compared with other taxa.

Key words – LSU – ITS – monocotyledons – multigene analyses – Poaceae

Introduction

Phaeosphaeriaceae is a large family in the order Pleosporales (Hyde et al. 2013, Phookamsak et al. 2014). Members of this group grow mainly on monocotyledons, but some species have also been reported on dicotyledons (Shoemaker and Babcock 1989, Schoch et al. 2006, Zhang et al. 2009, 2012, De Gruyter et al. 2010, Hyde et al. 2013, Wijayawardene et al. 2014). The family was introduced by Barr (1979) and recent studies have shown it to be a natural group comprising 25 genera (Ariyawansa et al. 2015). Ariyawansa et al. (2015) and Phukhamsakda et al. (2015) have provided the latest backbone trees for the family. The asexual morphs are coelomycetous (Zhang et al. 2009, Phookamsak et al. 2014, Wijayawardene et al. 2014, Li et al. 2015). The family *Phaeosphaeriaceae* has a cosmopolitan distribution, and species are generally

Submitted 24 October 2015, Accepted 19 November 2015, Published online 24 November 2015
Corresponding Author: Itthayakorn Promputtha – e-mail – ppam118@gmail.com

necrotrophic, plant pathogens or saprobes on a wide range of plants Shoemaker and Babcock 1989, Carson 2005, Stukenbrock et al. 2006, Cannon and Kirk 2007.

The genus *Phaeosphaeria* was introduced by Miyake (1909). Miyake (1909) treated 114 species of *Phaeosphaeria* and accommodated them in six subgenera, viz. *Ovispora*, *Fusispora*, *Phaeosphaeria*, *Spathispora*, *Vagispora* and *Sicispora* based on the differences in ascospore shape, number of septa and the gelatinous sheaths on spores (Eriksson 1967, Shoemaker & Babcock 1989). The morphological characters of taxa in this genus are often ambiguous and can be confused with other taxa in the *Leptosphaeriaceae* and *Montagnulaceae*, and with genera in the family itself (Hyde et al. 2013, Phookamsak et al. 2014). Multigene phylogenetic analyses were carried out to confirm the placement of this group by Zhang et al. (2009), Phookamsak et al. (2014) and Ariyawansa et al. (2015). In this paper, we introduce two new species in *Vagicola* from Italy, which were found on dead culms of *Dactylis* sp. (*Poaceae*). Combined analyses of LSU and ITS sequence data using maximum-likelihood (ML) and maximum-parsimony (MP) clearly showed these species grouped in *Phaeosphaeriaceae* with strong statistical support. In this paper, the two new species are described and illustrated and compared with similar taxa.

Material and Methods

Collections, morphology and isolation

Specimens were collected in Italy by Erio Campesori. Study of gross morphology and photomicrography were carried out under a stereomicroscope. Sections of ascoma were made free-hand. Several specimens were used to observe the asci and ascospore characters and slides were preserved in lactoglycerol. Micro-morphological characters were observed under a compound microscope (Nikon Eclipse Ni), and measurements made using Tarosoft (R) Image Frame Work v. 0.9.7. Single spore isolation was carried out following the method of Chomnunti et al. (2014). Type specimens of the new species are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand, and ex-type cultures in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany (KIB). Facesoffungi numbers and Index Fungorum numbers are as outlined in Jayasiri et al. (2015) and Index Fungorum (2015).

Establishing the asexual morphs

Circular (0.5 cm) agar blocks from growing colony margins were cut and placed on fresh Malt Extract Agar (MEA) plates as described in Phooksamak et al. (2015). Asexual structures produced on Malt Extract Agar were observed after eight weeks of incubation, under light, at 20° C.

DNA isolation, amplification and sequencing

Fungal isolates were grown on 2% MEA for 20 days at 16°C. Genomic DNA was extracted from the growing mycelium using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China); following the instructions of the manufacturer (Hangzhou, P.R. China). DNA sequence data was obtained from the internal transcribe spacer (ITS), large subunits of the nuclear ribosomal RNA genes (LSU). Primer sets used for these genes were as follows: ITS: ITS5/ITS4; LSU: LR0R/LR5 (Liu et al. 1999; Sung et al. 2007). The amplification was performed following the instructions, and were set up for initial denaturation of 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 45 s at 52°C and 90 s at 72°C, and a final extension period of 10 min at 72°C. PCR-products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were done by Majorbio Co., China. DNA sequence data were obtained from the large subunit rDNA (LSU) and internal transcribed spacers will amplify by primer pairs ITS5 and ITS4 (White et al. 1990). Primer sequences and database are available in GenBank. For *Vagicola dactylidis* single spore isolation was not successful. Therefore fungal DNA was isolated directly from the ascomata.

Phylogenetic analysis

Sequences data were downloaded from GenBank to supplement the dataset (Table 1) (Phookamsak et al. 2014, Ariyawansa et al. 2015). The represented sequences including those newly obtained were aligned using with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html) and improved manually where necessary using Bioedit (Hall 1999). *Didymella exigua* was selected as outgroup taxon. The model of evolution was carried out using MrModeltest 2.2 (Nylander 2004). Maximum likelihood analysis was performed by using raxmlGUIv.0.9b2 (Silvestro and Michalak 2011). The search strategy was set to rapid bootstrapping and the analysis carried out using the GTRGAMMAI model of nucleotide substitution. The number of replicates was inferred using the stopping criterion (Pattengale et al. 2009). Maximum Likelihood bootstrap values equal or greater than 70% are given as the first set of numbers above the nodes (Fig. 1). PAUPv4.0b10 was used to conduct the parsimony analysis to obtain the phylogenetic trees. Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were setup to 500 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. Kishino-Hasegawa tests (KHT) (Kishino and Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum-parsimony bootstrap values equal or greater than 70% are given as the second set of numbers above the nodes (Fig. 1).

Results and Discussion

Molecular phylogeny

The combined LSU and ITS dataset comprising 57 strains of species of *Phaeosphaeriaceae* were used to determine the generic placement of our two strains as *Vagicola dactylidis* and *V. chlamydospora*. The phylogenetic trees obtained from Maximum Likelihood and Parsimony analysis yielded trees with similar overall topology at subclass and family relationships, in agreement with previous work based on Maximum Likelihood analysis (Zhang et al. 2012; Phookamsak et al. 2013, 2014, Ariyawansa et al. 2014a, b, c, 2015, Wijayawardene et al. 2013, Phukhamsakda et al. 2015). Individual LSU and ITS single gene trees were initially made and had a similar topology (data not shown). Therefore the genes were combined. The maximum parsimony dataset consists of 1360 characters with 987 characters as constant information, 113 characters as variable characters are parsimony-uninformative, and 260 characters were count as parsimony-informative character. The most parsimonious tree showed TL = 1873, CI = 0.343, RI = 0.613, RC = 0.210, HI = 0.657 values. The best scoring tree is presented in Figure 1. The strains of *Vagicola dactylidis* and *V. chlamydospora* clustered in the family *Phaeosphaeriaceae. Vagicola dactylidis* and *V. chlamydospora* formed a sister clade with *V. vagans* (CBS 604.86) with 52% ML and 50% MP support, but separate from other genera in the family. The new sequence data are deposited in GenBank (Table 1).

Taxonomy

Vagicola K.W.T. Chethana and K.D. Hyde, in Ariyawansa et al. Fungal Diversity (2015)

= *Phaeosphaeria* subgen. *Vagispora* Shoemaker & Babcock, Can. J. Bot. 67: 1500–1599 (1989)

Type species: *Vagicola vagans* (Niessl) O. Eriksson, Chethana & K.D. Hyde, *comb. nov.*

Basionym: *Pleospora vagans* Niessl, Verh. Naturf. Ver. Briinn 14: 174. 1876

≡ *Phaeosphaeria vagans* (Niessl) O.E. Erikss., Ark. Bot. 6: 430 (1967)

Vagicola chlamydospora Jayasiri, Camporesi & K.D. Hyde, **sp. nov.** Fig. 2

Index Fungorum Number: IF551683

Facesoffungi Number: FoF 01323

Fig. 1 – RAxML Maximum Likelihood phylogenetic tree based on a combined LSU and ITS sequence dataset. Bootstrap support values for Maximum Likelihood (ML) greater than 70% and Maximum-Parsimony bootstrap values above 70% are given above and below the nodes respectively. The tree is rooted to *Didymella exigua* (CBS 18355).

Table 1 Taxa used in the phylogenetic analysis and GenBank accession numbers (LSU and ITS) and species. New sequences are in bold.

Taxon	Voucher/culture numbers	GenBank accession numbers	
		LSU	ITS
Ampelomyces quisqualis	CBS 129.79	EU754128	HQ108038
Allophaeosphaeria cytisi	MFLUCC 15-0649	KT306950	KT306947
Allophaeosphaeria clematidis	MFLUCC 15-0652	KT306953	KT306949
Allophaeosphaeria dactylidis	MFLUCC 13-0618	KP744473	KP744432
Allophaeosphaeria subcylindrospora	MFLUCC 13-0380	KT314183	KT314184
Allophaeosphaeria muriformia	MFLUCC 13-0349	KP765681	KP765680
Chaetosphaeronema hispidulum	CBS 216.75	KF251652	KF251148
Coniothyrium dolichi	CBS 124140	GQ387611	JF740183
Coniothyrium glycines	CBS 124141	KF251714	KF251211
Dermatiopleospora mariae	MFLUCC 13-0612	KJ749653	KJ749654
Dermatiopleospora luzulae	MFLUCC 14-0932	KT306951	–
Didymella exigua	CBS 183.55	EU754155	GU237794
Diederichomyces cladoniicola	CBS 128026	JQ238628	KP170642
Diederichomyces caloplacae	CBS 129338	JQ238643	KP170639
Diederichomyces ficuzzae	CBS 128019	JQ238616	KP170647
Entodesmium rude	CBS 650.86	GU301812	–
Entodesmium artemisiae	MFLUCC 14-1156	KT315509	KT315508
Galliicola pseudophaeosphaeria	MFLUCC 14-0524	KT326693	KT326692
Leptospora rubella	CPC 11006	DQ195792	DQ195780
Loratospora aestuarii	JK 5535B	GU301838	–
Loratospora luzulae	MFLUCC 14 0826	KT328495	KT328497
Muriophaeosphaeria galatellae	MFLUCC 14-0614	KT438329	KT438333
Neosetophoma samarorum	CBS 139.96	GQ387579	KF251161
Neosetophoma samarorum	CBS 138.96	KF251664	KF251160
Neostagonospora caricis	CBS 135092	KF251667	KF251163
Neostagonospora elegiae	CBS 135101	KF251668	KF251164
Neosphaerellopsis thailandica	CPC 21659	KP170721	KP170652
Nodulosphaeria modesta	MFLUCC 11-0461	KM434285	KM434275
Nodulosphaeria senecionis	MFLUCC 15-1297	KT290257	KT290257
Ophiobolus cirsii	MFLUCC 13-0218	KM014662	KM014664
Ophiobolus erythrosporus	MFLUCC 12-2225	KM014665	KM491547
Ophiosphaerella aquatica	MFLUCC 14-0033		
Ophiosphaerella agrostidis	MFLUCC 11-0152	KM434281	KM434271
Ophiosphaerella agrostidis	MFLUCC 12-0007	KM434282	KM434272
Paraphoma dioscoreae	CBS 135100	KF251671	KF251167
Paraphoma chrysanthemicola	CBS 172.70	KF251669	KF251165
Paraphoma radicina	CBS 111.79	KF251676	KF251172
Parastagonospora caricis	S6150/CBS135671	KF251680	KF251176
Parastagonospora nodorum	CBS 110109	KF251681	KF251177
Parastagonospora poae	CBS 135089	KF251682	KF251178
Phaeosphaeria papayae	CBS 135416	KF251690	KF251187
Phaeosphaeria alpina	CBS 456.84	KF251684	KF251181
Phaeosphaeria avenaria	DAOM 226215	AY544684	–

Taxon	Voucher/culture numbers	GenBank accession numbers	
		LSU	ITS
Phaeosphaeria eustoma	CBS 573.86	DQ678063	–
Phaeosphaeria chiangraina	MFLUCC 13-0231	KM434280	KM434270
Phaeosphaeria musae	MFLUCC 11-0133	KM434277	–
Phaeosphaeria musae	CBS 120026	GU301862	DQ885894
Phaeosphaeria nigrans	CBS 307.79	KF251687	–
Phaeosphaeria oryzae	CBS 110110	KF251689	KF251186
Phaeosphaeria oryzae	MFLUCC 11-0170	KM434279	KM434269
Phaeosphaeria thysanolaenicola	MFLUCC 10-0563	KM434276	KM434266
Phaeosphaeria typharum	CBS 296.54	KF251695	KF251192
Phaeosphaeria vagans	CBS 604.86	KF251696	KF251193
Phaeosphaeriopsis musae	CBS 120026	GU301862	DQ885894
Phaeosphaeriopsis dracaenicola	MFLUCC 11-0157	KM434283	KM434273
Phaeosphaeriopsis glaucopunctata	MFLUCC 13-0265	KJ522477	KJ522473
Phaeosphaeriopsis triseptata	MFLUCC 13-0271	KJ522479	KJ522475
Populocrescentia forlicesenensis	MFLUCC 15-0651	KT306952	KT306948
Sclerostagonospora cycadis	CBS 123538	FJ372410	FJ372393
Scolicosporium minkeviciusii	MFLUCC 12-0089	KF366382	–
Septoriella phragmitis	CBS 140065	KR873279	KR873251
Septoriella oudemansii	CBS 138012	KJ869224	KR873250
Septoriella poae	CBS 136766	KJ869169	KJ869111
Septoriella leuchtmannii	CBS 459.84	KF251691	KF251188
Setophoma achromolaena	CBS 135105T/CPC 18553	KF251747	KF251244
Setophoma sacchari	CBS 333.39	KF251748	KF251245
Setophoma sacchari	MFLUCC11-0154	KJ476146	KJ476144
Setophoma terrestris	CBS 335.29	KF251749	KF251246
Sulcispora pleospora	MFLUCC 14-0995		
Stagonospora neglecta	CBS 343.86	EU754218	AJ496630
Stagonospora foliicola	CBS 110111	KF251759	KF251256
Vagicola chlamydospora	**MFLUCC 15-0177**	**KU163654**	**KU163658**
Vagicola dactylidis	**MFLU 15-2720**	**KU163656**	**KU163657**
Vagicola vagans	CBS 604.86	KF251696	KF251193
Vrystaatia aloeicola	CBS 135107	KF251781	KF251278
Wojnowiciella viburni	MFLUCC 12-0733/ICMP 19778	KC594287	KC594286
Wojnowicia dactylisicola	MFLUCC 13-0738	KP684147	KP744469
Wojnowicia dactylisii	MFLUCC 13-0735	KP684149	KP744470
Wojnowicia lonicerae	MFLUCC 13-0737	KP684151	KP744471
Wojnowiciella eucalypti	CBS 139904	KR476774	KR476741
Xenophoma puncteliae	JDL-2012a/CBS 128022	JQ238619	JQ238617
Xenoseptoria neosaccardoi	CBS 128665	KF251784	KF251281

CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC Working collection of Pedro Crous housed at CBS; DAOM Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; ICMP International Collection of Microorganisms from Plants, New Zealand; JK: J. Kohlmeyer; MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

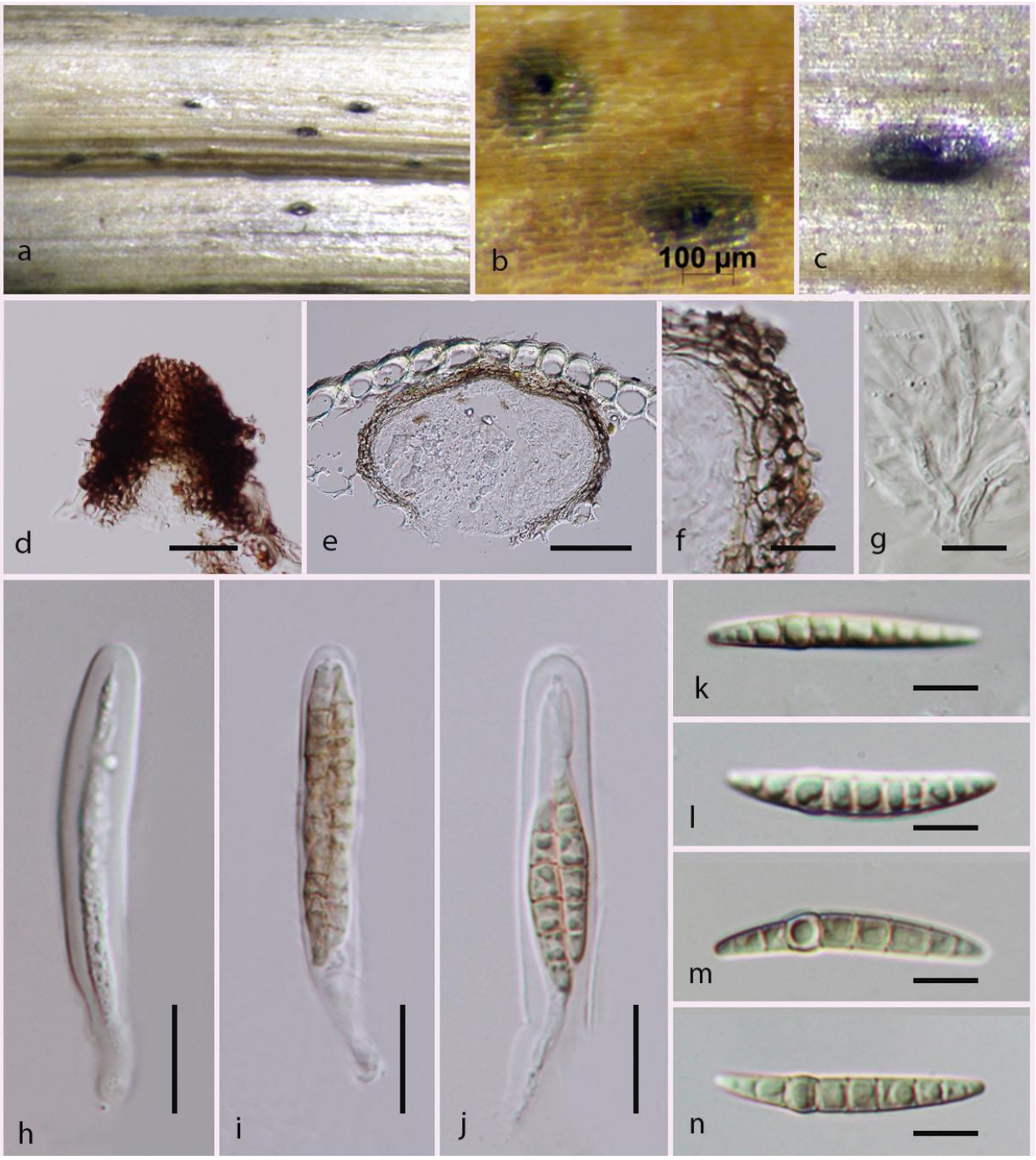

Fig. 2 – *Vagicola chlamydospora* (MFLU 15-1399, **holotype)** a-c Ascomata developing on surface of host. d Papilla. e Section through the ascoma. f Peridium. g Pseudoparaphyses. h-j Asci. k-n Ascospores. Scale bars: d = 30 µm, e = 50 µm, f, h-j = 10 µm, g, k-n = 5 µm.

Holotype – MFLU 15-1399

Etymology – With reference to chlamydospores-like asexual morph formed in culture

Saprobic on *Dactylis* sp. **Sexual morph**: *Ascomata* 121–156 µm high, 177–208 µm diam., scattered or sometimes clustered, immersed, visible as raised, black dots on the host surface, uni- to bi-loculate, subglobose, brown to dark brown, ostiole central, with a minute papilla. *Peridium* 21–27 µm wide, thin-walled, of equal thickness, composed of 2–5 layers of brown to dark brown, pseudoparenchymatous cells, arranged in *textura angularis* to inner layer composed of brown cells of *textura prismatica*. *Hamathecium* composed of numerous, 1.7–2.3 µm wide, filiform, broad, cellular pseudoparaphyses, with distinct septa, slightly constricted at the septa, embedded in a

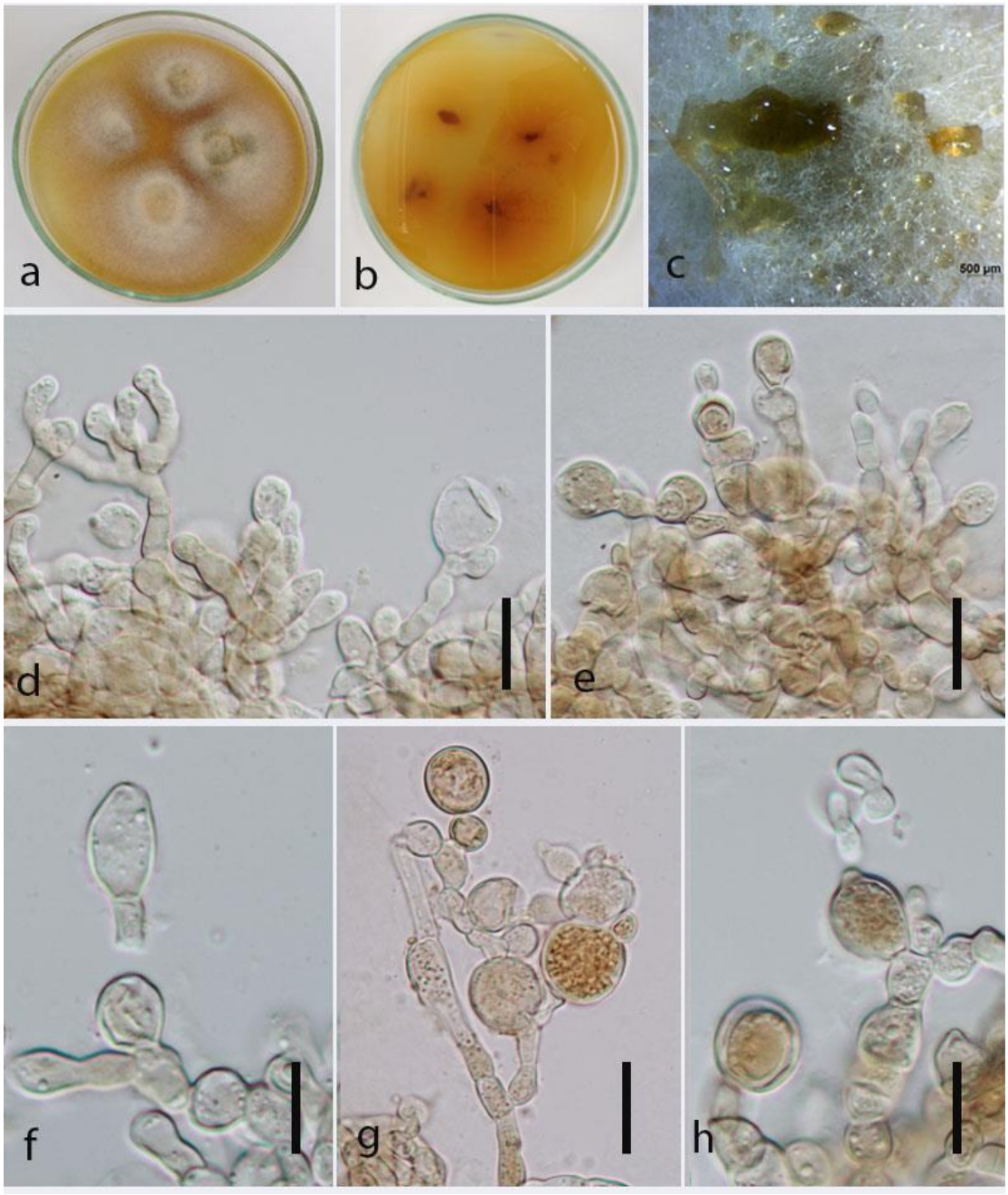

Fig. 3 – *Vagicola chlamydospora* asexual morph in culture (MFLUCC 15-0177, **ex-type culture**). a Fungal mycelium in MEA from above. b Fungal mycelium in MEA from below. c Close up view of fruiting body in culture. d-h Asexual spores formation structures. Scale bars: d-h = 10 µm.

gelatinous matrix. *Asci* 54–57 × 9–10.6 µm (x = 57 × 9.6 µm, n = 20), 8-spored, bitunicate, fissitunicate, broadly cylindrical or cylindric-clavate, sessile to subsessile, apically rounded with an ocular chamber. *Ascospores* 21–32 × 3.7–5.7 µm (x = 30 × 4.6 µm, n = 30), overlapping 1–3-seriate, phragmosporous, narrowly fusiform, with obtuse ends, with guttules, hyaline to yellowish-brown, 9-septate, slightly curved, constricted at the septa, smooth-walled, lacking a sheath or appendages. **Asexual morph:** *Colonies* on MEA effuse, white to pale yellow. *Mycelium* 2–3 µm wide, prostrate, composed of septate, branched, smooth, hyaline, hyphae. *Conidiophores* micro- to macronematous, erect, flexuous, smooth, composed of beaded, variedly-sized cells. *Conidiogenous cells* 5–9 × 3–6 µm holoblastic, globose to subglobose, smooth, subhyaline. *Conidia* 9–12 × 10–13 µm (x = 10 × 11 µm, n = 20), globose, solitary, thick-walled, chlamydospore-like, with dense cytoplasm (Fig 3).

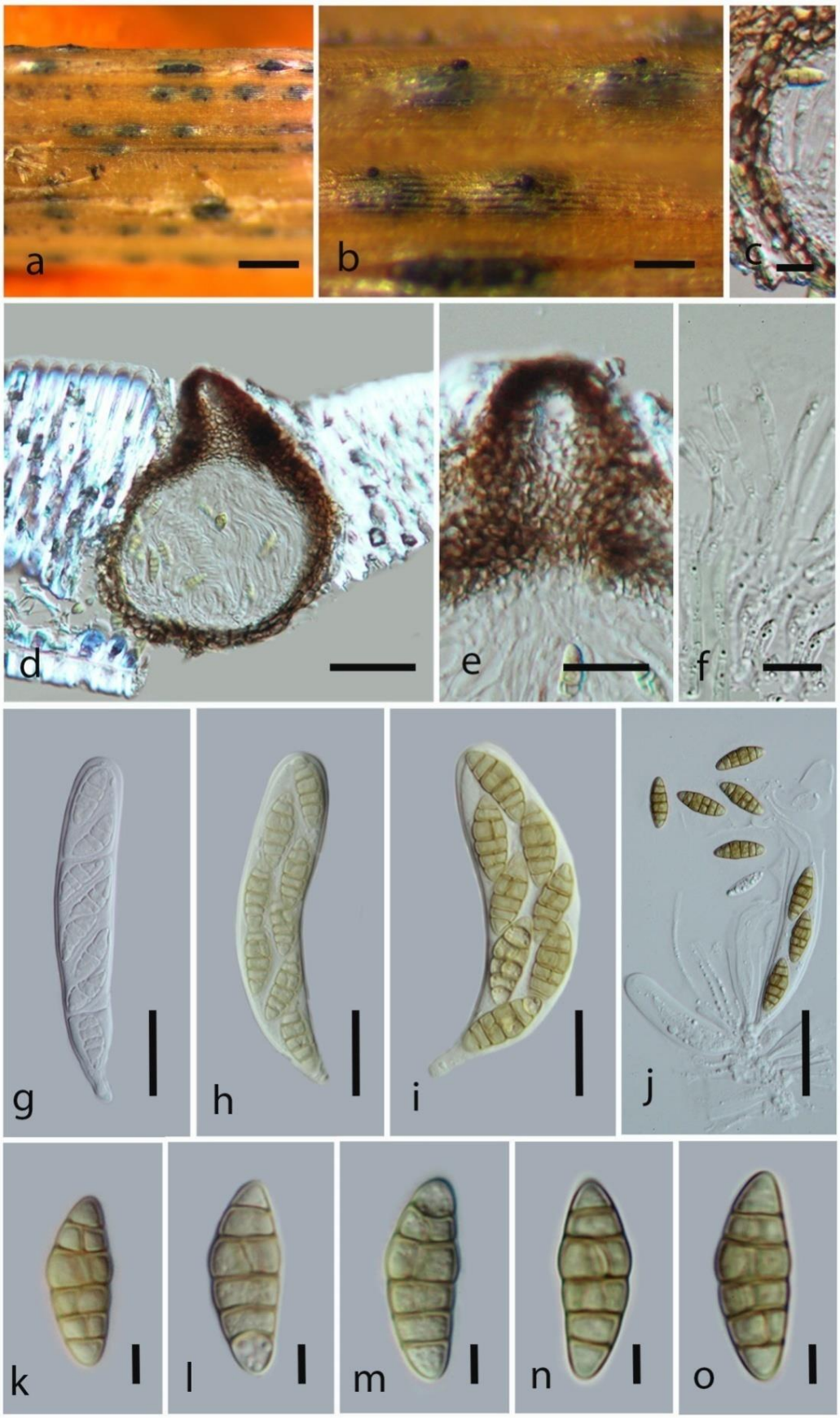

Fig. 4 – *Vagicola dactylidis* **(holotype).** a, b Ascomata developing on surface of host. c Peridium. d Section through ascoma e Papilla. f Pseudoparaphyses. g-j Asci. k-o Ascospores. Scale bars: a = 500 µm, b = 200 µm, c = 10 µm, d = 50 µm, e = 20 µm, g-j = 20 µm, g, f, k-o = 5 µm.

Material examined – ITALY, Province of Forlì-Cesena, near Poderone - Corniolo, dead stem of *Dactylis* sp. (*Poaceae*), 21 October 2014, Erio Camporesi IT 2188 (MFLU 15-1399, **holotype**), (isotype in KUN), ex-type culture, MFLUCC 15-0177, Genbank accession numbers: LSU- KU163654, ITS-KU163658, SSU-KU163655.

Culture characters – Ascospores germinating on MEA within 36 h. Colonies growing on MEA, reaching 2 cm diam. in 1 week at 16°C. Mycelium superficial, felty, gummy, edge undulate, from above white, reverse yellow colour.

Notes – *Vagicola chlamydospora* resembles to *Vagicola vagans* in having similar ascomata, as black coloured dots of ostiole visible with immersed ascomata when viewed on the host surface, *textura angularis* to *textura prismatica* brown colour cells peridium. and broad cellular pseudoparaphyses but differs in processing longitudinal septa and narrow ascospores. The spores in *Vagicola chlamydospora* are similar to *Nodulosphaeria*, but differ in the morphology of ascomata and phylogenetic analyses. In this study we observed chlamydospore-like asexual morph in culture (Fig. 3).

Vagicola dactylidis Wanasinghe, Jayasiri, Camporesi & K.D. Hyde, **sp. nov.** Fig. 4

Index Fungorum Number: IF551684

Facesoffungi Number: FoF 01324

Holotype – MFLU 15-2720

Etymology – With reference to the host occurrence

Saprobic on dead stem of *Dactylis* sp. **Sexual morph:** *Ascomata* 120–180 µm high, 110–160 µm diam. ($\overline{x}$ = 153.9 × 141.3 µm, n = 10), solitary, scattered, superficial, globose to subglobose, dark brown to black, coriaceous, ostiolate. *Ostiole* 50–60 µm high, 20–30 µm diam. ($\overline{x}$ = 55.5 × 27.5 µm, n = 5), papillate, black, smooth, filled with dark brown cells. *Peridium* 9–12 µm wide at the base, 12–16 µm wide in sides, comprising 3–4 layers, comprising blackish to dark brown, thick-walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2–2.5 µm (n = 30) wide, filamentous, branched, septate, pseudoparaphyses. *Asci* 70–120 × 15–21 µm ($\overline{x}$ = 84.4 × 18.7 µm, n = 40), 8-spored, bitunicate, fissitunicate, broadly-clavate, with a short, orbicular pedicel, rounded at apex, with minute ocular chamber. *Ascospores* 19–23 × 6–9 µm ($\overline{x}$ = 20.7 × 7.4 µm, n = 50), obliquely bi-seriate, initially hyaline, becoming yellowish brown at maturity, broadly fusiform, 5-trans-septate, with a longitudinal septa in between second and third trans-septate, constricted at the central septa, weakly constricted at the other septa, with conical and narrowly rounded ends, lacking a mucilaginous sheath. **Asexual morph:** Undetermined.

Material examined – ITALY, Province of Arezzo, Bagno di Cetica, dead stem of *Dactylis* sp. (Poaceae), 8 October 2012, Erio Camporesi. IT 799 (MFLU 15-2720, **holotype**), (isotype in KUN), GenBank accession numbers: LSU – KU163656, ITS – KU163657.

Notes – *Vagicola dactylidis* is more similar to the type species, *V. vagans*, than *V. chlamydospora*, in having ascospores with vertical septa. *Dactylis* is the host plant of both *Vagicola chlamydospora* and *dactylidis*. Two other species associated with *Dactylis* in Phaeosphaeriaceae are *Phaeosphaeria huronensis* (Shoemaker & Babcock 1989) and *Ophiosphaerella herpotricha* (Phookamsak et al. 2014), however, they share no morphological characters.

Acknowledgements

This work was supported by the International Research Group Program (IRG-14-27), Deanship of Scientific Research, King Saud University, Saudi Arabia. K.D. Hyde thanks the Chinese Academy of Sciences, [project number 2013T2S003], for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. MFLU [grant number 56101020032] is thanked for supporting studies on Dothideomycetes. We are grateful to the Mushroom Research Foundation, Chiang Rai, Thailand. Subashini C. Jayasiri is grateful to Mr. and (Late) Mrs. Jayasiri and S.P.R.D. Lasantha for their valuable suggestions.

References

Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B, Chethana KWT, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Lücking R, Ghobad-Nejhad M, Niskanen T, Thambugala KM, Voigt K, Zhao RL, Li GJ, Doilom M, Boonmee S, Yang ZL, Cai Q, Cui YY, Bahkali AH, Chen J, Cui BK, Chen JJ, Dayarathne MC, Dissanayake AJ, Ekanayaka AH, Hashimoto A, Hongsanan S, Jones EBG, Larsson E, Li WJ, Li QR, Liu JK, Luo ZL, Maharachchikumbura SSN, Mapook A, McKenzie EHC, Norphanphoun C, Konta S, Pang KL, Perera RH, Phookamsak R, Phukhamsakda C, Pinruan U, Randrianjohany E, Singtripop C, Tanaka K, Tian CM, Tibpromma S, Abdel-Wahab MA, Wanasinghe DN, Wijayawardene NN, Zhang JF, Zhang H, Abdel-Aziz FA, Wedin M, Westberg M, Ammirati JF, Bulgakov TS, Lima DX, Callaghan TM, Callac P, Chang CH, Coca LF, Dal-Forno M, Dollhofer V, Fliegerová K, Greiner K, Griffith GW, Ho HM, Hofstetter V, Jeewon R, Kang JC, Wen TC, Kirk PM, Kytövuori I, Lawrey JD, Xing J, Li H, Liu ZY, Liu XZ, Liimatainen K, Lumbsch HT, Matsumura M, Moncada B, Nuankaew S, Parnmen S, de Azevedo Santiago ALCM, Sommai S, Song Y, de Souza CAF, de Souza-Motta CM, Su HY, Suetrong S, Wang Y, Wei SF, Wen TC, Yuan HS, Zhou LW, Réblová M, Fournier J, Camporesi E, Luangsa-ard JJ, Tasanathai K, Khonsanit A, Thanakitpipattana D, Somrithipol S, Diederich P, Millanes AM, Common RS, Stadler M, Yan JY, Li XH, Lee HW, Nguyen TTT, Lee HB, Battistin E, Marsico O, Vizzini A, Vila, J, Ercole E, Eberhardt U, Simonini G, Wen HA, Chen XH. 2015 – Fungal Diversity Notes 111–252—taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75(1) (DOI 10.1007/s13225-015-0346-5)

Ariyawansa H, Phookamsak R, Tibpromma S, Kang JC, Hyde KD. 2014a – A molecular and morphological reassessment of *Diademaceae*. The Scientific World Journal 2014 1–11 (DOI 10.1155/2014/675348)

Ariyawansa HA, Camporesi E, Thambugala KM, Mapook A, Kang JC, Alias SA, Chukeatirote E, Thines M, McKenzie EHC, Hyde KD 2014b – Confusion surrounding *Didymosphaeria* – phylogenetic and morphological evidence suggest *Didymosphaeriaceae* is not a distinct family. Phytotaxa 176, 102–119 (DOI 10.11646/phytotaxa.181.1.1)

Ariyawansa HA, Kang JC, Alias SA, Chukeatirote E, Hyde KD 2014c – *Pyrenophora*. Mycosphere 5, 351–362 (DOI 10.5943/mycosphere/5/2/9)

Barr ME. 1979 – A classification of Loculoascomycetes. Mycologia 71, 935–957 (DOI 10.2307/3759283)

Cannon PF, Kirk PM. 2007 – Fungal Families of the World. CAB. International, Wallingford, Oxfordshire, U.K.

Carson ML. 2005 – Yield loss potential of *Phaeosphaeria* leaf spot of maize caused by *Phaeosphaeria maydis* in the United States. Plant Disease 89, 986–988 (DOI 10.1094/PD-89-0986)

Chomnunti P, Hongsanan S, Hudson BA, Tian Q, Persoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, Hyde, KD. 2014 – The Sooty Moulds. Fungal Diversity 66, 1–36 (DOI 10.1007/s13225-014-0278-5)

De Gruyter J, Woudenberg JH, Aveskamp MM, Verkley GJ, Groenewald JZ, Crous PW. 2010 – Systematic reappraisal of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*. Mycologia 102, 1066–1081 (DOI 10.3852/09-240)

Eriksson O. 1967 – On graminicolous Pyrenomycetes from Fennoscandia 2. Phragmosporous and scolecosporous species. Arkiv för Botanik, serie 2, 6, 381–440.

Hyde KD, Jones EBG, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jayawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake I, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang, Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bahkali AH, Bezerra JL, Bhat DJ, Camporesi E, Chukeatirote E, Gueidan C,

Hawksworth DL, Hirayama K, Hoog SD, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yan JY, Yacharoen S, Zhang M. 2013 – Families of Dothideomycetes. Fungal Diversity 63, 1–313 (DOI 10.1007/s13225-013-0263-4)

Index Fungorum (2015) http://www.indexfungorum.org/Names/Names. asp. Accessed on November 2015

Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J, Buyck B, Cai L, Dai YC, Abd-Elsalam KA, Ertz D, Hidayat I, Jeewon R, Jones EBG, Bahkali AH, Karunarathna SC, Liu JK, Luangsa-ard JJ, Lumbsch HT, Maharachchikumbura SSN, McKenzie EHC, Moncalvo, JM, Ghobad-Nejhad M, Nilsson H, Pang KA, Pereira OL, Phillips AJL, Raspé O, Rollins AW, Romero AI, Etayo J, Selçuk F, Stephenson SL, Suetrong S, Taylor JE, Tsui CKM, Vizzini A, Abdel-Wahab MA, Wen TC, Boonmee S, Dai DQ, Daranagama DA, Dissanayake AJ, Ekanayaka AH, Fryar SC, Hongsanan S, Jayawardena RS, Li WJ, Perera RH, Phookamsak R, de Silva NI, Thambugala KM, Tian Q, Wijayawardene NN, Zhao RL, Zhao Q, Kang JC, Promputtha I. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74 (DOI 10.1007/s13225-015-0351-8)

Kishino H, Hasegawa M. 1989 – Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA – sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29, 170–179 (DOI 10.1007/BF02100115)

Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Liu YJ, Whelen S, Hall BD. 1999 – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16, 1799–1808.

Miyake I. 1909 – Studies on the parasitic fungi of rice in Japan. Botanical Magazine Tokyo. 23: 85–97.

Nylander JAA. 2004 – MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University

Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. 2009 – How many bootstrap replicates are necessary?. LNCS 5541, 184–200

Phookamsak R, Liu JK, Chukeatirote E, McKenzie EHC, Hyde KD. 2013 – Phylogeny and morphology of *Leptosphaerulina saccharicola sp. nov.* and *Pleosphaerulina oryzae* and relationships with *Pithomyces*. Cryptogamie Mycologie 34, 303–319 (10.7872/crym.v34.iss4.2013.303)

Phookamsak R, Liu JK, McKenzie EHC, Manamgoda DS, Ariyawansa HA, Thambugala, KM, Dai DQ, Camporesi E, Chukeatirote E, Wijayawardene NN, Bahkali AH, Mortimer PE, Xu JC, Hyde KD 2014 – Revision of *Phaeosphaeriaceae*. Fungal Diversity 68, 159–238 (DOI 10.1007/s13225-014-0308-3)

Phookamsak R, Manamgoda DS, Li WJ, Dai DQ, Singtripop C, Hyde KD 2015 – *Poaceascoma helicoides gen et sp. nov.*, a new genus with scolecospores in *Lentitheciaceae*. Cryptogamie Mycologie 36(2), 1–12 (DOI 10.7872/crym/v36.iss2.2015.225)

Phukhamsakda C, Ariyawansa HA, Phookamsak R, Chomnunti P, Bulgakov TS, Yang JB, Bhat DJ, Bahkali AH, Hyde KD. 2015 – *Muriphaeosphaeria galatellae gen. et sp. nov.* in *Phaeosphaeriaceae* (Pleosporales). Phytotaxa 227, 55–65 (DOI 10.11646/phytotaxa.227.1.6)

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. 2006 – A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98, 1041–1052

Shoemaker RA, Babcock CE. 1989 – *Phaeosphaeria*. Canadian Journal of Botany 67, 1500–1599 (DOI 10.1139/b89-199)

Silvestro D, Michalak I. 2011 – raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12 (4), 335–337 (DOI 10.1007/s13127-011-0056-0)

Stukenbrock EH, Banke S, McDonald BA. 2006 – Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*. Molecular Ecology 15, 2895–2904 (DOI 10.1111/j.1365-294X.2006.02986.x)

Sung GH, Sung JM, Hywel Jones NL, Spatafora JW. 2007 – A multigene phylogeny of *Clavicipitaceae* (Ascomycota, fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics & Evolution 44, 1204–1223.

White TJ, Bruns TD, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: Guide Methods Application 18, 315–322 (10.1016/B978-0-12-372180-8.50042-1)

Wijayawardene DNN, Camporesi E, Song Y, Dai DQ, Bhat DJ, McKenzie EHC, Chukeatirote E, Mel'nik VA, Wang Y, Hyde KD. 2013 – Multi-gene analyses reveal taxonomic placement of *Scolicosporium minkeviciusii* in *Phaeosphaeriaceae* (Pleosporales). Cryptogamie Mycologie 34, 357–366 (DOI 10.7872/crym.v35.iss2.2014.105)

Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Dai DQ D'souza MJ, Diederich P, Dissanayake A, Doilom M, Hongsanan S, Jones EBG, Groenewald JZ, Jayawardena R, Lawrey JD, Liu JK, Lücking R, Madrid H, Manamgoda DS, Muggia L, Nelsen MP, Phookamsak R, Suetrong S, Tanaka K, Thambugala KM, Wanasinghe DN, Wikee S, Zhang Y (24), Aptroot A, Ariyawansa HA, Bahkali AH, Bhat DJ, Gueidan C, Chomnunti P, De Hoog GS, Knudsen K, Li WJ, McKenzie EHC, Miller AN, Phillips AJL, Piątek M, Raja HA, Shivas RS, Slippers B, Taylor JE, Tian Q, Wang Y, Woudenberg JHC, Cai L, Jaklitsch WM, Hyde KD. 2014 – Naming and outline of Dothideomycetes 2014 – Fungal Diversity 69(1), 1–55 (DOI 10.1007/s13225-014-0309-2)

Zhang Y, Schoch CL, Fournier J, Crous PW, de Gruyter J, Woudenberg JHC, Hirayama K, Tanaka K, Pointing SB, Spatafora JW, Hyde KD. 2009 – Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. Studies in Mycology 64, 85–102 (DOI 10.3114/sim.2009.64.04)

Zhang Y, Crous PW, Schoch CL, Hyde KD. 2012 – Pleosporales. Fungal Diversity 53, 1–221 (DOI 10.1007/s13225-011-0117-x)