Phytotaxa 227 (1): 055–065
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.227.1.6

Muriphaeosphaeria galatellae gen. et sp. nov. in *Phaeosphaeriaceae* (*Pleosporales*)

CHAYANARD PHUKHAMSAKDA[1,2], HIRAN A. ARIYAWANSA[1,2], RUNGTIWA PHOOKAMSAK[1,2], PUTARAK CHOMNUNTI[1,2], TIMUR S. BULGAKOV[6], JUN-BO YANG[5], DARBHE J. BHAT[7], ALI H. BAHKALI[8] & KEVIN D. HYDE[1,2,3,8]

[1]*Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*
[4]*World Agroforestry Centre, East and Central Asia, Kunming 650201, Yunnan, China*
[5]*Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, China*
[6]*Academy of Biology and Biotechnology (Department of Biological Sciences), Southern Federal University, Rostov-on-Don 344090, Russia*
[7]*Formerly Department of Botany, Goa University, Goa, India; No. 128/1–J, Azad Housing Society, Curca, Goa Velha, India*
[8]*Department of Botany and Microbiology, King Saudi University, Riyadh, Saudi Arabia*
[1,2,3,8] *Author for correspondence: kdhyde3@gmail.com*

Abstract

Muriphaeosphaeria galatellae was collected from *Galatella villosa* in Russia and is introduced as a novel monotypic genus and species in the family *Phaeosphaeriaceae* (*Pleosporales*). *Muriphaeosphaeria* is distinct from other genera of the family *Phaeosphaeriaceae* by its unique suite of characters such as, superficial ascomata with an ascomatal wall comprising thin-walled brown cells, cellular pseudoparaphyses, muriform ascospores; and conidiomata with a thick hyaline inner wall layer producing cylindrical to subclavate, 1−3-transversely septate, brown conidia. The asexual morph of *M. galatellae* developed in cultures when grown on sterilized pine needles and bamboo pieces. A phylogenetic analysis based on combined LSU, SSU and ITS sequence data showed that *M. galatellae* forms a distinct lineage in *Phaeosphaeriaceae*. The new genus and species are introduced and compared with other taxa in the family.

Keywords: Dothideomycetes, *Galatella villosa*, Holomorph, New genus, Russia

Introduction

Dothideomycetes is the largest class of Ascomycota, characterized by bitunicate and mostly fissitunicate asci (Berbee 1996, Kirk *et al*. 2008, Hyde *et al.* 2013). *Pleosporales* is considered as the largest order in the class, comprising a quarter of all dothideomycetous species (Kirk *et al.* 2008, Zhang *et al.* 2012). Species of *Pleosporales* can be epiphytes, endophytes, parasites on plants, hyperparasites on fungi, and saprobes on dead plant litter (Barr 1979, Taylor *et al.* 2000, Schoch *et al.* 2009, Wijayawardene *et al*. 2014, Ariyawansa *et al*. 2014a, 2015). The family *Phaeosphaeriaceae,* introduced by Barr (1979), is one of the largest families in the order *Pleosporales*. The family was initially characterized by immersed to superficial, globose to subglobose ascomata with short papilla, bitunicate asci, and hyaline, yellowish or brown, uni or multi-septate, muriform ascospores (Shoemaker 1984, Shoemaker & Babcock 1989, 1992, Zhang *et al.* 2012). Phookamsak *et al.* (2014) revised the family *Phaeosphaeriaceae* and accepted 30 genera based on both morphology and phylogeny. Although species of *Phaeosphaeriaceae* are mostly found on monocotyledonous hosts as pathogens or saprobes (Câmara *et al*. 2002, Hyde *et al*. 2013, Quaedvlieg *et al.* 2013, Thambugala *et al*. 2014) some also occur on dicotyledons (Wanasinghe *et al.* 2014, Liu *et al.* 2015). Currently 17 asexual genera have been reported in the family *Phaeosphaeriaceae* by Phookamsak *et al.* (2014).

We have been studying the families of *Pleosporales* in order to provide a natural classification of this large taxon (Zhang *et al.* 2012, Phookamsak *et al.* 2013, 2014a, Wijayawardene *et al.* 2014, Ariyawansa *et al.* 2014a, b, c, 2015). In this study, we introduce a new genus *Muriphaeosphaeria*, with *M. galatellae* as the type species, the specimen of

Accepted by Sajeewa Maharachchikumbura: 1 Sept. 2015; published: 18 Sept. 2015

which was collected from the Rostov region of Russia. The new genus is compared with other genera in the family *Phaeosphaeriaceae* and its uniqueness is confirmed based on both morphology and molecular data.

Material and Methods

Sample collection, morphological study and isolation

The specimen was collected from the Rostov region of Russia, in a natural sanctuary 'Persianovskaya steppe' on dead and dying stems of *Galatella villosa* (L.) Rchb.f. The fungus was examined under a Motic SMZ 168 Series stereo-microscope. Vertical free-hand sections were made by a razor blade and placed on a droplet of sterilized water on a glass slide (Gupta *et al.* 2013). Microscopic characters were observed and photo-micrographed using a Nikon ECLIPSE80i compound microscope fitted to Cannon 600D digital camera. The measurements were determined using Taro soft ® Image Framework program version.0.9.7. Photo-plates were made using Photoshop version CS5.1.

Pure cultures were obtained from single ascospores on 2% potato dextrose agar (PDA; 39 g/L in distilled water, Difco potato dextrose) as described in Chomnunti *et al.* (2014). Growth rate of colonies were measured after 7 days and up to 4 weeks at 16 °C. The asexual morph was obtained by placing agar squares with mycelia on water agar with sterile bamboo pieces (Phookamsak *et al.* 2014a) and sterile pine needles (Crous *et al*. 2006, Liu 2011) and incubating at 16 °C in the dark for 8 weeks. The type specimen of the new genus is deposited in Mae Fah Luang University (MFLU) herbarium, Chiang Rai, Thailand. Ex-type living cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in CBS Fungal Biodiversity Centre, the Netherlands under material transfer agreement no. MTA0038.

DNA extraction, amplification and sequencing

Fungal isolates were grown on PDA medium at 25 ± 2 °C for 4 weeks. Genomic DNA was extracted from the growing mycelium, following the manufacturer's protocol from Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P. R. China). In this study, several gene regions were amplified using known primer pairs. LROR and LR5 were used to amplify region of nuclear large subunit rDNA (LSU) (Vilgalys & Hester 1990). NS1 and NS4 were used to amplify region of nuclear small subunit rDNA (SSU), and internal transcribed spacer region (ITS) was amplified by using ITS5 and ITS4 primer pairs (White *et al.* 1990).

The amplification reactions were performed in 25 µl of total reaction which contained 9.5 µl of sterilized water, 12.5 µl of 2× Easy Taq PCR Super Mix (mixture of *Easy Taq* TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China), 1 µl of each forward and reverse primers, and 1 µl of DNA template. PCR amplification conditions were set as follows; an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, primer annealing at 55 °C for 50 sec, primer extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR products were checked on 1% Agarose gel electrophoresis stained with Ethidium bromide. PCR products were purified and sent for sequencing at Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China).

Phylogeny analysis

Taxa used in the analyses were obtained through recent publications (Phookamsak *et al.* 2014) and individual LSU, SSU and ITS genes of *Muriphaeosphaeria galatellae* sexual morph and asexual morph were checked using the BLAST search engine tool of NCBI to reveal the closest matches in GenBank. DNA alignments were performed by using Bioedit version 7.0.9 (Hall 1999), MEGA version 5 and version 6 (Tamura *et al*. 2011, 2013), and MAFFT version 7.220 (Katoh *et al*. 2013) online sequence alignment (mafft.cbrc.jp/alignment/server) and further aligned manually. Combined LSU, SSU and ITS gene sequence data were used in the analysis to increase the phylogenetic accuracy and missing data was treated as gaps. Maximum parsimony analysis (MP) was performed by PAUP1.0b10 software (Swofford 2002), with heuristic search option 1,000 random replicates. Maxtrees were setup to 5000 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. Maximum likelihood analyses (ML) included 1000 bootstrap replicates run using RAxML (O'meara *et al*. 2006, Stamatakis 2006) implemented in raxmlGUI version v.1.3.1 (Silvestro & Michalak 2011). The search strategy was set to rapid bootstrapping. Analysis carried out by general time reversible (GTR) model nucleotide substitution and applied with gamma-distributed heterogeneity with 4 discrete implementation rate (Guindon *et al.* 2010). Bootstrap values (BT) were indicated for clade stability for MP and ML analysis values, equal or greater than 50% were given above node.

To perform Bayesian analysis, the model of evolution was performed by using MrModeltest 2.2 (Nylander 2004). The Nucleotide substitution models selected for individual and combined datasets were GTR+I+G. Posterior probabilities (PP) (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Four simultaneous Markov chains were run for 3,500,000 generations and trees were sampled every 100th generation; a total 35,000 trees were obtained. The first 7,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01) (Zhaxybayeva & Gogarten 2002, Ariyawansa *et al*. 2015).

Phylogenetic trees and data files were viewed in MEGA v. 6 (Tamura *et al.* 2013), TreeView v. 1.6.6 (Page 1996) and FigTree v. 1.4 (Rambaut and Drummond 2008). Facesoffungi numbers are as detailed in Jayasiri *et al*. (2015).

Results and Discussion

Phylogenetic analysis

Partial nucleotides of LSU, SSU and ITS dataset comprising 58 strains in *Phaeosphaeriaceae* were used to determine the generic placement of *Muriphaeosphaeria galatellae*. *Didymella exigua* (CBS 183.55) was used as the outgroup taxon (FIG. 1). The individual LSU, SSU and ITS single gene tree initial performed and observed in similar topology, these were not significantly different (data not shown). Therefore genes were combined The maximum parsimony dataset consists of 2252 characters with 1745 characters as constant information, 179 characters as variable characters are parsimony-uninformative, and 328 characters were count as parsimony-informative character. The most parsimonious tree showed TL= 1941, CI=0.412, RI=0.59, RC=0.243, HI=0.588 values. The best scoring tree was selected with a final likelihood value of In: -11885.672296 and the result is presented in FIG. 1.

The strain *Muriphaeosphaeria galatellae* clustered in the family *Phaeosphaeriaceae,* phylogeny analyses of two isolates formed strongly supported values (95% MP/100% ML/ 0.97 PP) to confirmed same species. *M. galatellae* formed a sister clade to *Entodesmium rude* (CBS 650.86), but separated from other genera in the family with high bootstrap support (70% ML/ 1.00 PP). Phylogenetic trees obtained from Maximum Likelihood and Bayesian 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, 2014a, Wijayawardene *et al.* 2014, Ariyawansa *et al.* 2014a, b, c, 2015), while the Maximum parsimony analysis (MP) tree has some differences with some internal clades, i.e. *Nodulosphaeria modesta*, clustered with *Dermatiopleospora mariae* and *Ophiobolus erythrosporus*, but in ML and PP analysis *Nodulosphaeria modesta* forms a separate branch but still in the same clade. This is not unexpected as divergence in evolutionary rates and the presence of missing data affects all these differently. Nevertheless, we describe the new taxon based on agreement in support for all three computational methods. The new sequence data is deposited in GenBank (TABLE 1).

TABLE 1. Fungal strain used in the phylogenetic analysis.

Taxon	Strain number	GenBank accession numbers		
		LSU	SSU	ITS
Amarenomyces ammophilae	CBS 114595	GU301859	GU296185	KF766146
Ampelomyces quisqualis	CBS 129.79	EU754128	EU754029	HQ108038
Allophaeosphaeria dactylidis	MFLUCC 13-0618	KP744473	KP753946	KP744432
Allophaeosphaeria muriformia	MFLUCC 13-0349	KP765681	KP765682	KP765680
Chaetosphaeronema coonsii	CBS 141.84	GQ387575	GQ387514	–
Chaetosphaeronema coonsii	CBS 559.78	EU754196	EU754097	–
Chaetosphaeronema hispidulum	CBS 216.75	KF251652	EU754045	KF251148
Dermatiopleospora mariae	MFLUCC 13-0612	KJ749653	KJ749652	KJ749654
Didymella exigua	CBS 183.55	EU754155	EU754056	GU237794
Entodesmium rude	CBS 650.86	GU301812	AF164356	–
Loratospora aestuarii	JK 5535B	GU301838	GU296168	–

...continued on the next page

TABLE 1. (Continued)

Taxon	Strain number	GenBank accession numbers		
		LSU	SSU	ITS
Muriophaeosphaeria galatellae	MFLUCC 14-0614/ CBS 140021	**KT438329**	**KT438331**	**KT438333**
Muriophaeosphaeria galatellae	MFLUCC 15-0769	**KT438330**	**KT438332**	**–**
Neosetophoma samarorum	CBS 138.96	KF251664	GQ387517	KF251160
Neosetophoma clematidis	MFLUCC 13-0734	KP684153	KP684154	KP744450
Neosetophoma italica	MFLUCC 13-0388	KP711361	KP711366	KP711356
Neostagonospora caricis	CBS 135092	KF251667	–	KF251163
Neostagonospora elegiae	CBS 135101	KF251668	–	KF251164
Nodulosphaeria modesta	MFLUCC 13-0728	KP744493	KP753957	–
Ophiobolus cirsii	MFLUCC 13-0218	KM014662	KM014663	KM014664
Ophiobolus erythrosporus	MFLUCC 12-2225	KM014665	KM014666	–
Ophiosphaeriella agrostidis	MFLUCC 11-0152	KM434281	KM434290	KM434271
Ophiosphaeriella agrostidis	MFLUCC 12-0007	KM434282	KM434291	KM434272
Paraphoma radicina	CBS 111.79	KF251676	EU754092	KF251172
Parastagonospora caricis	S615	KF251680	–	KF251176
Parastagonospora nodorum	CBS 110109	KF251681	EU754076	KF251177
Parastagonospora poae	CBS 135089	KF251682	–	KF251178
Phaeosphaeria papayae	CBS 135416	KF251690	–	KF251187
Phaeosphaeria alpina	CBS 456.84	KF251684	–	KF251181
Phaeosphaeria caricicola	CBS 603.86	GQ387590	GQ387529	KF251182
Phaeosphaeria chiangraina	MFLUCC 13-0231	KM434280	KM434289	KM434270
Phaeosphaeria juncicola	CBS 110108	KF251686	–	KF251183
Phaeosphaeria juncophila	CBS 575.86	GU456328	GU456307	AF439488
Phaeosphaeria musae	CBS 120026	GU301862	GU296186	DQ885894
Phaeosphaeria oryzae	CBS 110110	KF251689	GQ387530	KF251186
Phaeosphaeria oryzae	MFLUCC 11-0170	KM434279	–	KM434269
Phaeosphaeria phragmiticola	CBS 459.84	KF251691	–	KF251188
Phaeosphaeria pontiformis	CBS 117487	KF251692	–	KF251189
Phaeosphaeria thysanolaenicola	MFLUCC 10-0563	KM434276	KM434286	KM434266
Phaeosphaeria typharum	CBS 296.54	KF251695	–	KF251192
Phaeosphaeria vagans	CBS 604.86	KF251696	–	KF251193
Phaeosphaeriopsis dracaenicola	MFLUCC 11-0157	KM434283	KM434292	KM434273
Phaeosphaeriopsis glaucopunctata	MFLUCC 13-0265	KJ522477	KJ522481	KJ522473
Phaeosphaeriopsis triseptata	MFLUCC 13-0271	KJ522479	KJ522484	KJ522475
Phoma haematocycla	CBS 175.93	GU238080	GU238219	–
Sclerostagonospora cycadis	CBS 123538	FJ372410	–	FJ372393
Sclerostagonospora phragmiticola	CBS 338.86	KF251733	–	KF251230
Scolicosporium minkeviciusii	MFLUCC 12-0089	KF366382	KF366383	–
Setomelanomma holmii	CBS 110217	GU301871	GU296196	–
Setophoma chromolaena	CBS 135105T/CPC 18553	KF251747	–	KF251244
Setophoma sacchari	CBS 333.39	KF251748	GQ387525	KF251245
Setophoma terrestris	CBS 335.29	KF251749	GQ387526	KF251246
Sulcispora pleurospora	MFLUCC 14-0995	KP271444	KP271445	KP271443
Vrystaatia aloeicola	CBS 135107	KF251781	–	KF251278
Wojnowicia viburni	MFLUCC 12-0733/ICMP 19778	KC594287	KC594288	KC594286
Wojnowicia dactylidicola	MFLUCC 13-0738	KP684147	KP684148	KP744469
Wojnowicia dactylidis	MFLUCC 13-0735	KP684149	KP684150	KP744470
Wojnowicia lonicerae	MFLUCC 12-0737	KP684151	KP684152	KP744471
Xenoseptoria neosaccardoi	CBS 128665	KF251784	–	KF251281

Abbreviations: CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; ICMP: International Collection of Microorganisms from Plants, Lincoin, New Zealand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; JK: J. Kohlmeyer; S: Working collection of William Quaedvlieg

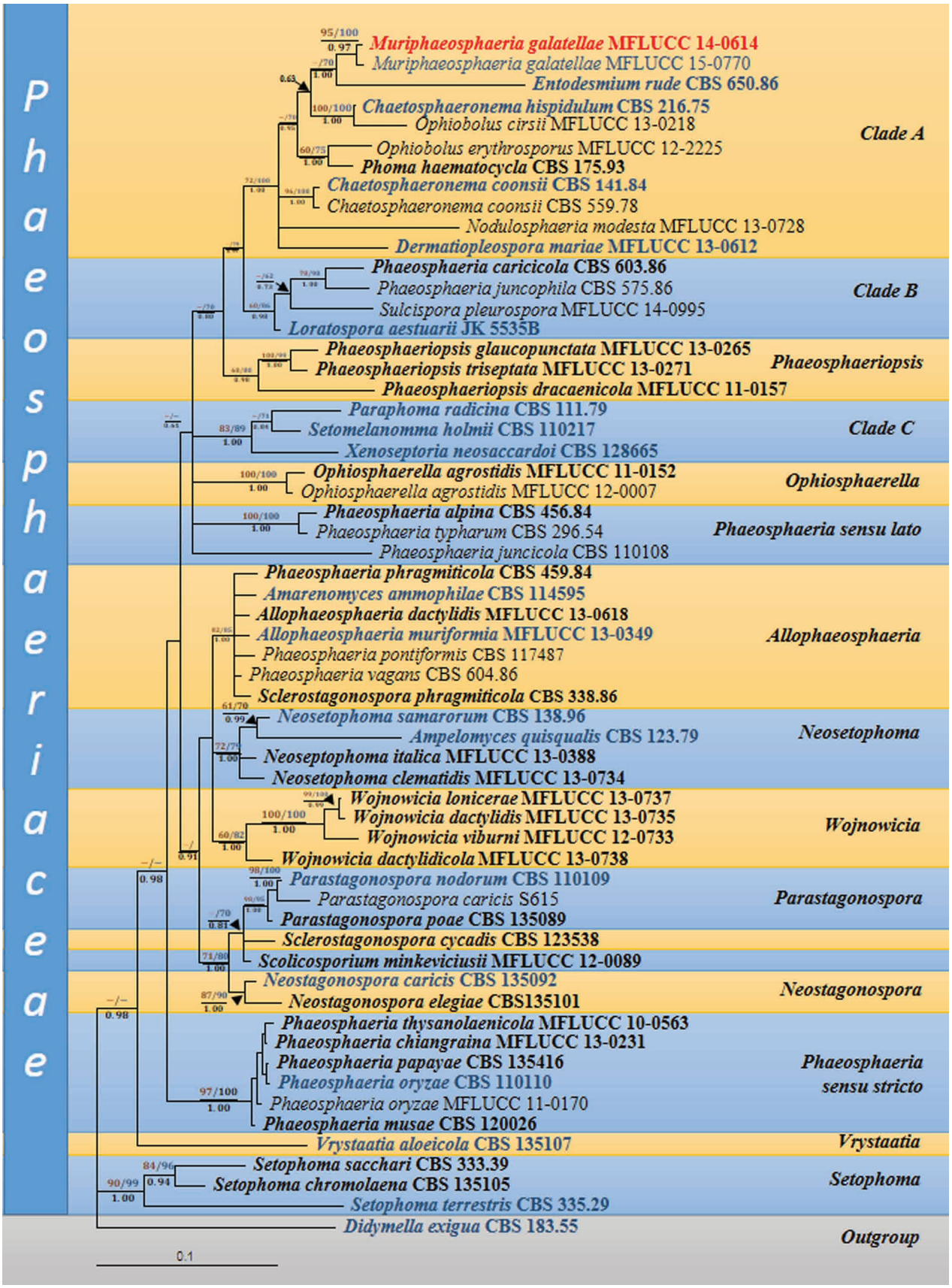

FIGURE 1. Bayesian majority (50%) consensus rule tree based on combined partial LSU, SSU and ITS gene datasets. Bootstrap values from maximum parsimony (MP, left above), and maximum likelihood (ML, right above) of more than 60% are given above the nodes. Bayesian posterior probabilities (PP, below) of more than 0.95 are given below the nodes. The tree is rooted to *Didymella exigua* (CBS183.55). *Muriphaeosphaeria galatellae* (strain MFLUCC 14−0614) is indicated in *red bold*. The type species of each genus is indicated in *blue bold*, ex-type strains are *bold*.

Taxonomy

The monotypic genus *Muriphaeosphaeria,* typified by *M. galatellae,* is introduced in the family *Phaeosphaeriaceae*. It has a distinct morphology compared to other genera in *Phaeosphaeriaceae* and is also supported by distinctive DNA data.

Muriphaeosphaeria C. Phukhamsakda, T. Bulgakov & K.D. Hyde, *gen. nov.*
Index Fungorum Number IF551291; Facesoffungi number FoF 00868

Etymology:—The generic epithet is in reference to the muriform ascospores.

Saprobic on dead and dying stems. ***Sexual morph*:** *Ascomata* coriaceous, superficial, solitary, scattered, globose to cupulate, black to dark brown, smooth-walled, ostiolate. *Ostiole* central, lacking periphyses. *Peridium* multi-layered, dark to light brown, comprising cells of *textura angularis*, with inner hyaline layer. *Hamathecium* comprising long, filamentous, narrow, septate pseudoparaphyses surrounding asci. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short-pedicellate, apically rounded with an ocular chamber clearly visible when immature. *Ascospores* bi-seriate, partially overlapping, obovoid to sub-fusiform, narrow towards ends, slightly curved, transversely septate, with a longitudinal septuma in the central cells, slightly constricted at the central septum, light brown to yellowish, granulate, smooth-walled. ***Asexual morph:*** Coelomycetous. *Conidiomata* superficial, dark brown to black, globose, uniloculate, solitary, scattered, ostiolate. *Pycnidial* wall thick-walled, multi-layered, with inner layer comprising several cell-layers, comprising brown-walled cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic with percurrent annelidic proliferations, integrated, oblong, hyaline, brown when mature, smooth-walled, and formed from the inner layer of pycnidial wall. *Conidia* oblong to cylindrical, narrowly rounded at both ends, transversely septate, pale brown when mature.

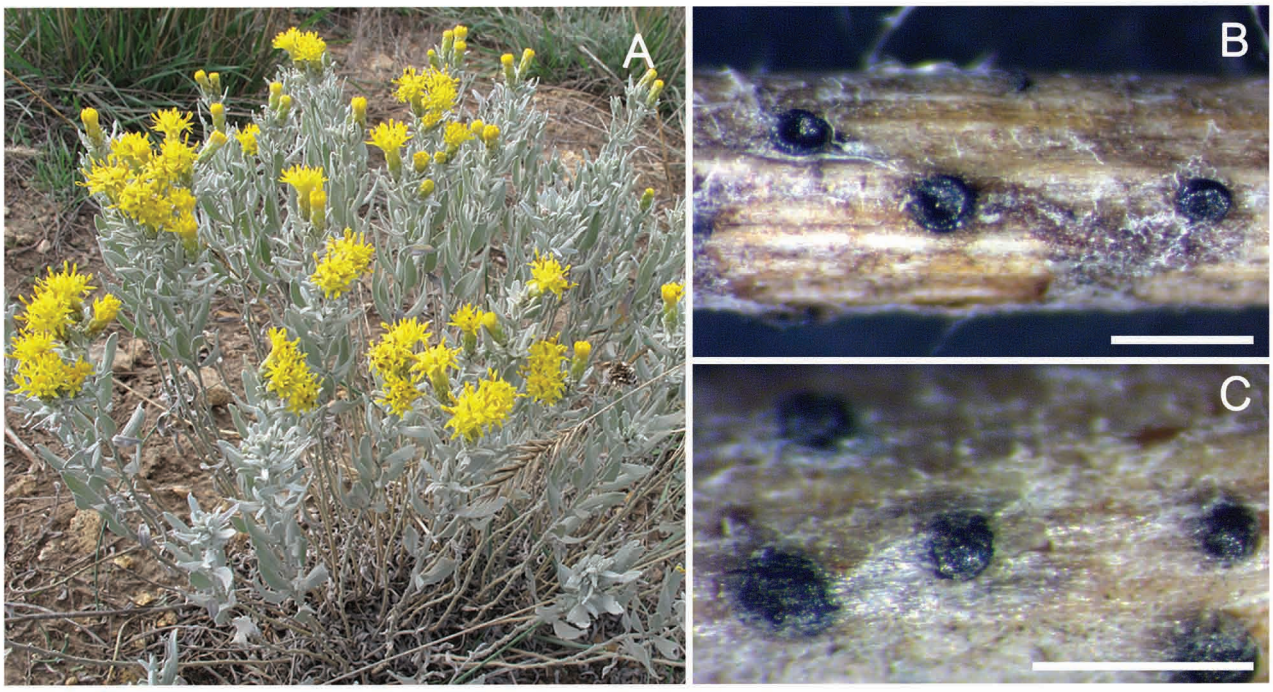

FIGURE 2. ***Muriphaeosphaeria galatellae*** (MFLU 15–0087, **holotype**) **A**. *Galatella villosa* (L.) Rchb.f. summer. **B.** Superficial ascomata of sexual state (MFLUCC 14−0614). **C.** Conidiomata on host (MFLUCC 15−0769). Scale bars: B, C = 500 *μm*.

Muriphaeosphaeria galatellae Phukhamsakda, Bulgakov & K.D. Hyde, *sp. nov.* FIG. 3
Index Fungorum Number IF551292; Facesoffungi number FoF 00643

Etymology:—Named after the host genus from which it was isolated, *Galatella*.

Holotype: MFLU 15−0087

Saprobic on dead and dying stems of *Galatella* villosa (L.) Rchb.f. ***Sexual morph*:** *Ascomata* 114−180 *μm* high × 167–263 *μm* wide, oriaceous, superficial, flat at the base, globose to cupulate, black to dark brown, solitary, scattered,

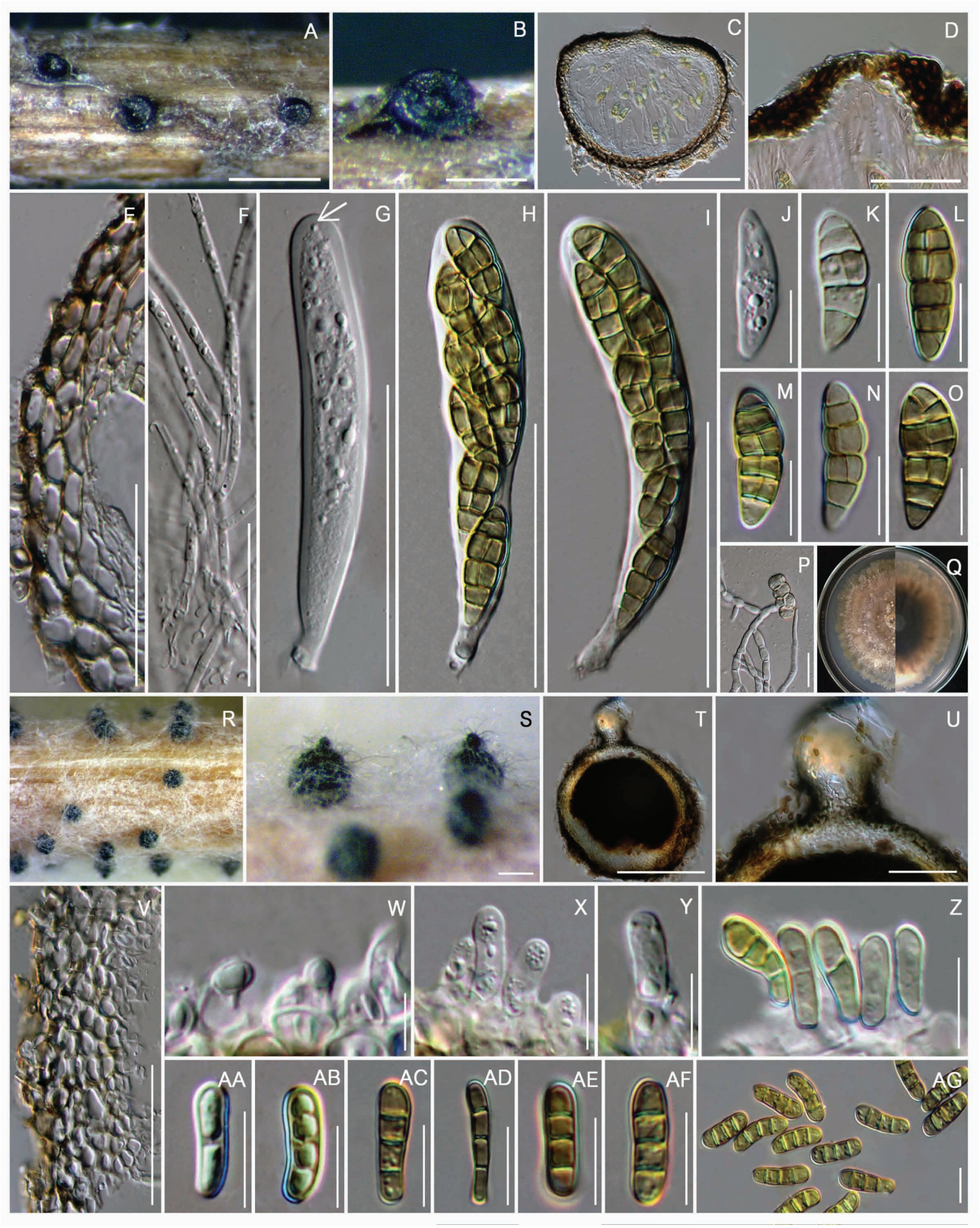

FIGURE 3. ***Muriphaeosphaeria galatellae*** (MFLU 15-0087, **holotype**) **A.** Superficial ascomata of sexual state on host. **B.** Close up of ascoma. **C.** Vertical section of ascoma. **D.** Section of papilla. **E.** Section through peridium. **F.** Pseudoparaphyses **G.** Immature ascus with ocular chamber. **H–I.** Mature asci. **J–O.** Ascospores. **P.** Germinating ascospore. **Q.** Culture characters on PDA, left from above. **R.** Conidiomata forming on bamboo pieces on WA after 8 weeks. **S.** Close up of conidiomata. **T.** Vertical section through conidiomata. **U.** Ostiole. **V.** Section through peridium. **W–Z.** Conidiogenous cells and developing conidia. **AA–AG.** Conidia. Scale bars: A = 500 *μm,* B, S, T = 200 *μm*, C = 100 *μm*, E, G–I, U–V = 50 *μm*, D, F = 20 *μm*, J–O, X–Z, AA–AG = 10 *μm*, W = 5 *μm*.

without a papilla, smooth-walled. *Ostiole* 16−22 *μm* high × 22−32 *μm* wide, slightly raised, centrally located, lacking periphyses. *Peridium* 12–26 *μm* wide, composed of 3–5 wall layers, with outer part comprising dark brown to light brown cells of *textura angularis*, inner layer comprising thin-walled, hyaline cells. *Hamathecium* of dense, 2–3 *μm* ($\bar{x}$ = 2.7 *μm*, n = 20) wide, filamentous, septate, branched, pseudoparaphyses surrounding asci. *Asci* 53–86 × 9–17 *μm* ($\bar{x}$ = 77.5 × 13.6 *μm*, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short-pedicellate, apically rounded with an ocular chamber, clearly visible when immature. *Ascospores* 13–27 × 4–11 *μm* ($\bar{x}$ = 22.1 × 7.8 *μm*, n=50), bi-seriate, partially overlapping, obovoid to sub-fusiform, or clavate, narrow towards ends, slightly curved, 4–5-tranversely septate and with 1 longitudinal septa in the central 2–3 cells, constricted at three central septa, slightly constricted at other septa, with second cell from apex enlarged in the centre, light brown to yellowish, granulate, smooth-walled, without a mucilaginous sheath. ***Asexual morph****:* Produced on sterilized bamboo pieces and pine needles on water agar. *Conidiomata* 233−293 μm high × 202–332 *μm* wide diam., dark brown to black, globose, superficial on substrate, covered by dense vegetative hyphae, uniloculate, solitary to scattered, ostiolate. *Pycnidial* wall 41−54 *μm* wide (up to 85 *μm* wide at apex), thick-walled, two-layered, with outer region comprising 2-layers of brown-walled cells of *textura angularis*, with inner region comprising up to 7 layers of thick, hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 1−3 × 2−3 *μm*, holoblastic with percurrent proliferations, annellidic, integrated, oblong, hyaline, and formed from the inner layer of pycnidium wall. *Conidia* 10–17 × 2–6 *μm* ($\bar{x}$ = 14.2 × 4.6 *μm*, n = 50), cylindrical to subclavate, rounded at both ends, sometimes slightly curved, with 1−3 transverse septa, initially hyaline, brown when mature, smooth-walled.

Culture characters:—Ascospore germinating on PDA within 24 hours, with germ tubes developed from the end cells of ascospores. Colonies on PDA reaching 60 mm diam after 4 weeks. Culture incubated at 16 °C, white at first, becoming pale green after 2 weeks, olive-green, convex with papillate surface, with circular, friable margin.

Material examined:—RUSSIA, Rostov region (rus. Rostovskaya Oblast), Oktyabrsky District (rus. Oktyabr'sky raion), natural sanctuary 'Persianovskaya steppe', dead and drying stems of *Galatella villosa* (L.) Rchb.f. (*Asteraceae*) (FIG. 2A), 26 April 2014, T.S. Bulgakov (MFLU, **holotype** 15−0087; HKAS 88183, **isotype**); ex-type living culture, MFLUCC 14–0614 = MFLUCC 15−0769 = CBS 140021.

Notes:—The genus *Muriphaeosphaeria* is a monotypic and characterized by superficial, globose to cupulate, ostiolate ascomata, thick-walled peridium, filamentous, septate, pseudoparaphyses, bitunicate, fissitunicate, short and narrowly pedicellate asci and muriform ascospores. The asexual morph of the genus (MFLUCC 15−0769, FIG. 2C) found in same pieces of host and also formed in culture (MFLUCC 14−0614, FIG. 2B, FIG. 3) is coelomycetous with pycnidial, globose, superficial, uniloculate, ostiolate, thick-walled conidiomata, with an inner wall-layer comprising up to 7 hyaline cell-layers, annelledic conidiogenous cells, and subclavate, 1−3-transverse septate, brown conidia. The connectively of sexual and as asexual morphs is proven by phylogenetic analysis (FIG. 1). *Muriphaeosphaeria galatellae* forms a sister clade with a putative strain of *Entodesmium rude* (CBS 650.86, FIG. 1), but the morphology is obviously distinct. *E. rude* has long necked, periphysate ostiole, thick-walled peridium, fasciculate, scolecosporous, filiform, 18−20-septate ascospores (Phookamsak *et al.* 2014b). *Muriphaeosphaeria galatellae* shares similarities with *Dematiopleospora mariae* (Wanasinghe *et al*. 2014) in having superficial ascomata and muriform ascospores, but differs in having thick-walled ascomata, without loose hyphae surrounding the ostiole, filamentous, cellular pseudoparaphyses, short and simple pedicellate asci and ascospores with rounded ends, while *D. mariae* contains hyphae around the ostiole and furcated pedicellate asci. Both taxa cluster in the clade A, with in the family in molecular analysis (FIG. 1). Thus, it is evident that more taxa are needed to be studied to resolve the relationship within the clade.

Acknowledgements

Chayanard Phukhamsakda would like to thank Royal Golden Jubilee Ph. D. Program under Thailand Research Fund, for the award of a scholarship no. PHD/0020/2557 to study towards a PhD. We thank the National Research Council of Thailand (grant for Dothideomycetes No: 58201020010) for supporting studies on Dothideomycetes. Kevin D. Hyde thanks the Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. We would like to thank Plant Germplasm and Genomics Center in Germplasm Bank of Wild Species, Kunming Institute of Botany for help in molecular work. Chayanard Phukhamsakda is grateful to Saowaluck Tibpromma, Jian Kui Liu, and Sinang Hongsanun for they valuable suggestions. The authors extend their sincere appreciations to the Deanship of Scientific Research at King Saud University for its funding this Prolific Research group (PRG-1436-09).

References

Ariyawansa, H.A., David, L.H., Hyde, K.D., Jones, E.B.G., Maharachchikumbura, S.S.N., Manamgoda, D.S., Thambugala, K.M., Udayanga D., Camporesi, E., Daranagama, A., Jayawardena, R., Liu, J.K., McKenzie, E.H.C., Phookamsak, R., Senanayake, I.C., Shivas, R.G., Tian, Q. & Xu, J.-C. (2014a) Epitypification and neotypification: guidelines with appropriate and inappropriate examples. *Fungal Diversity* 69: 57–91.
http://dx.doi.org/10.1007/s13225-014-0315-4

Ariyawansa, H.A., Tanaka, K., Thambugala, K.M., Phookamsak, R., Tian, Q., Camporesi, E., Hongsanan, S., Monkai, J., Wanasinghe, D.N., Mapook, A., Chukeatirote, E., Kang, J.C., Xu, J.-C., Mckenzie, E.H.C., Jones, E.B.G. & Hyde, K.D. (2014b) A molecular phylogenetic reappraisal of the *Didymosphaeriaceae* (= *Montagnulaceae*). *Fungal Diversity* 68: 69–104.
http://dx.doi.org/10.1007/s13225-014-0305-6

Ariyawansa, H.A., Thambugala, K.M, Manamgoda, D.S., Jayawardena, R., Camporesi, E., Boonmee, S., Wanasinghe, D.N., Phookamsak, R., Hongsanan, S., Singtripop, C., Chukeatirote, E., Kang, J.C., Jones, E.B.G. & Hyde, K.D. (2015) Towards a natural classification and backbone tree for *Pleosporaceae*. *Fungal Diversity* 71: 85–139.
http://dx.doi.org/10.1007/s13225-015-0323-z

Ariyawansa, H.A., Thambugala, K.M., Kang, J.C., Alias, S.A., Chukeatirote, E. & Hyde, K.D. (2014c) Towards a natural classification of Dothideomycetes 2: The genera *Cucurbidothis, Heterosphaeriopsis, Hyalosphaera, Navicella* and *Pleiostomellina* (Dothideomycetes *incertae sedis*). *Phytotaxa* 176 (1): 7–17.
http://dx.doi.org/10.11646/phytotaxa.176.1.4

Barr, M.E. (1979) A classification of Loculoascomycetes. *Mycologia* 71: 935–957.
http://dx.doi.org/10.2307/3759283

Berbee, M.L. (1996) Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18s rRNA gene sequence data. *Molecular Biology and Evolution* 13: 462–670.
http://dx.doi.org/10.1093/oxfordjournals.molbev.a025607

Câmara, M.P., Palm, M.E., Van-Berkum, P. & O'neill, N.R. (2002) Molecular phylogeny of *Leptosphaeria* and *Phaeosphaeria*. *Mycologia* 94: 630–640.
http://dx.doi.org/10.2307/3761714

Chomnunti, P., Hongsanan, S., Hudson, B.A., Tian, Q., Peroh, D., Dhami, M.K., Alias, A.S., Xu, J., Liu, X., Stadler, M. & Hyde, K.D. (2014) The Sooty Moulds. *Fungal Diversity* 66: 1–36.
http://dx.doi.org/10.1007/s13225-014-0278-5

Crous, P.W., Bernard, S., Michael, J.W., John, R., Walter, F.O.M., Alan, J.L.P., Artur, A., Treena, B., Paul, B. & Johannes, Z.G. (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in mycology* 55: 235–253.
http://dx.doi.org/10.3114/sim.55.1.235.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic biology* 59 (3):307–321.
http://dx.doi.org/10.1093/sysbio/syq010

Gupta, V.K. & Tuohy, M.G. (2013) Laboratory Protocols in Fungal Biology. *Springer* New York, London, England, pp. 114–115.
http://dx.doi.org/10.1007/978-1-4614-2356-0

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

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
http://dx.doi.org/10.1093/bioinformatics/17.8.754

Hyde, K.D., Jones, E.B.G., Liu, J.K, Ariyawansa, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I.C., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirrehudson, B., Alias, S.A., Aptroot, A., Bahkali, A., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., Hoog, S.D., Kang, J.C., Knudsen, K., Li, W.J., Li, X.H., Liu, Z.Y., Mapook, A., Mckenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A, Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yacharoen, S., Yan, J.Y. & Zhang, M. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313.
http://dx.doi.org/10.1007/s13225-013-0263-4

Hyde, K.D., Mckenzie, E.H.C. & Koko, T.W. (2011) Towards incorporating anamorphic fungi in a natural classification–checklist and notes for 2010. *Mycosphere* 2: 1–88.

Katoh, K., Standley, K. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30 (4): 772–780. Available from: http://mbe.oxfordjournals.org/ http://dx.doi.org/10.1093/molbev/mst010

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's Dictionary of the Fungi, 10th edn*. CABI, Wallingford, UK, pp. 221, 518, 547.

Liu, D. (2011) *Molecular detection of human fungal pathogens.* CRC Press, London, England, p. 111

Liu, J.K., Hyde, K.D., Jones, E.B.G., Ariyawansa, H.A., Bhat, D.J., Boonmee, S., Maharachchikumbura, S.S.N., Mckenzie, E.H.C., Phookamsak, R., Phukhamsakda, C., Shenoy, B.D., Abdel-Wahab, M.A., Buyck, B., Chen, J., Chethana, K.W.T., Singtripop, C., Dai, D.Q., Dai, Y.C., Daranagama, D.A., Dissanayake, A.J., Doilom, M., D'souza, M.J., Fan, X.L., Goonasekara, I.D., Hirayama, K., Hongsanan, S., Jayasiri, S.C., Jayawardena, R.S., Karunarathna, S.C., Li, W.J., Mapook, A., Norphanphoun, C., Pang, K.L., Perera, R.H., Peršoh, D., Pinruan, U., Senanayake, I.C., Somrithipol, S., Suetrong, S., Tanaka, K., Thambugala, K.M., Tian, Q., Tibpromma, S., Udayanga, D., Wijayawardene, N.N., Wanasinghe, D., Wisitrassameewong, K., Zeng, X.Y., Abdel-Aziz, F.A., Adamčík, S., Bahkali, A.H., Boonyuen, N., Bulgakov, T., Callac, P., Chomnunti, P., Greiner, K., Hashimoto, A., Hofstetter, V., Kang, J.C., Lewis, D., Li, X.H., Liu, X.X., Liu, Z.Y., Matsumura, M., Mortimer, P.E., Rambold, G., Randrianjohany, E., Sato, G., Sri-Indrasutdhi, V., Tian, C.M., Verbeken, A., Brackel, W.V., Wang, Y., Wen, T.C., Xu, J.C., Yan, J.Y., Zhao, R.L. & Camporesi, E. (2015) Fungal Diversity Notes 1–110: Taxonomic and phylogenetic contributions to fungal species. *Fungal Diversity* 72: 1–197. http://dx.doi.org/10.1007/s13225-015-0324-y

Nylander, J.A.A. (2004) *MrModeltest 2.0.* Program distributed by the author. Evolutionary Biology Centre, Uppsala University

O'meara, B.C., Ané, C., Sanderson, M.J. & Wainwright, P.C. (2006) Testing for different rates of continuous trait evolution using likelihood. *Evolution* 60: 922–933. http://dx.doi.org/10.1111/j.0014-3820.2006.tb01171.x

Phookamsak, R., Liu, J.K., Chukeatirote, E., Mckenzie, E.H.C. & Hyde K.D. (2013) Phylogeny and morphology of *Leptosphaerulina saccharicola sp. nov.* and *Pleosphaerulina oryzae* and relationships with *Pithomyces*. *Cryptogamie Mycologie* 34: 303–319. http://dx.doi.org/10.7872/crym.v34.iss4.2013.303

Phookamsak, R., Liu, J.K., Manamgoda, D.S., Chukeatirote, E., Mckenzie, E.H.C. & Hyde, K.D. (2014a) The sexual state of *Setophoma*. *Phytotaxa* 176: 260–269. http://dx.doi.org/10.11646/phytotaxa.176.1.25

Phookamsak, R., Liu, J.K., Mckenzie, E.H.C., Manamgoda, D.S., Ariyawansa, H., Thambugala, K.M., Dai, D.Q., Camporesi, E., Chukeatirote, E., Wijayawardene, N.N., Bahkali, A.H., Mortimer, P.E., Xu, J.C. & Hyde, K.D. (2014b) Revision of *Phaeosphaeriaceae. Fungal Diversity* 68: 159–238. http://dx.doi.org/10.1007/s13225-014-0308-3

Quaedvlieg, W., Verkley, G.J.M., Shin, H.D., Barreto, R.W., Alfenas, A.C., Swart, W.J., Groenewald, J.Z. & Crous, P.W. (2013) Sizing up *Septoria*. *Studies in Mycology* 75: 307–390. http://dx.doi.org/10.3114/sim0017

Rambaut, A. & Drummond, A. (2008) FigTree: Tree figure drawing tool, version 1.2. 2. *Institute of Evolutionary Biology*, University of Edinburgh

Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311. http://dx.doi.org/10.1007/BF02338839

Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W.A., Burgess, T.I., De-Gruyter, J., De-Hoog, G.S., Dixon, L.J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, S., Hirayama, K., Hosoya, T., Huhndorf, S.M., Hyde, K.D., Jones, E.B.G., Kohlmeyer, J., Kruys, Å., Li, Y.M., Lücking, R., Lumbsch, H.T., Marvanová, L., Mbatchou, J.S., Mcvay, A.H., Miller, A.N., Mugambi, G.K., Muggia, L., Nelsen, M.P., Nelson, P., Owensby, C.A., Phillips, A.J.L., Phongpaichit, S., Pointing, S.B., Pujade-Renaud, V., Raja, H.A., Rivas, P.E., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L., Shearer, C.A., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-Kohlmeyer, B., Wingfield, M.J., Wood, A.R., Woudenberg, J.H.C., Yonezawa, H., Zhang, Y. & Spatafora, J.W. (2009) A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1–15. http://dx.doi.org/10.3114/sim.2009.64.01

Shoemaker, R.A. & Babcock, C.E. (1989) *Phaeosphaeria*. *Canadian Journal of Botany* 67: 1500–1599. http://dx.doi.org/10.1139/b89-199

Shoemaker, R.A. & Babcock, C.E. (1992) Applanodictyosporous *Pleosporales*: *Clathrospora, Comoclathris, GraphyIlium, Macrospora*, and *Platysporoides*. *Canadian Journal of Botany* 70: 1617–1658. http://dx.doi.org/10.1139/b92-204

Shoemaker, R.A. (1984) Canadian and some extralimital *Nodulosphaeria* and *Entodesmium* species. *Canadian Journal of Botany* 62: 2730–2753.

http://dx.doi.org/10.1139/b84-367

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

Stamatakis, A. (2006) Raxml-vi-hpc: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. http://dx.doi.org/10.1093/bioinformatics/btl446

Subashini, C.J., Ariyawansa, H.A., Liu J.K., Jones, E.B.G. & Hyde, K.D. (2015) The Faces of Fungi database: Fungal names linked with morphology, molecular and human attributes, Database. *Fungal Diversity (Inpress)*

Swofford, D.L. (2002) *PAUP: Phylogenetic analysis using parsimony and other methods version 4.0 b10.* Sinauer Associates. Sunderland.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* 28: 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution* 30: 2725–2729. http://dx.doi.org/10.1093/molbev/mst197

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S. & Fisher, M.C. (2000) Phylogenetic Species Recognition and Species Concepts in Fungi. *Fungal Genetics and Biology* 31: 21–32. http://dx.doi.org/10.1006/fgbi.2000.1228

Thambugala, K.M., Camporesi, E., Ariyawansa, H.A., Phookamsak, R., Liu, Z.Y. & Hyde, K.D. (2014) Phylogeny and morphology of *Phaeosphaeriopsis triseptata sp. nov.,* and *Phaeosphaeriopsis glaucopunctata*. *Phytotaxa* 176: 238–250. http://dx.doi.org/10.11646/phytotaxa.176.1.23

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.

Wanasinghe, D.N., Jones, E.G., Camporesi, E., Boonmee, S., Karunarathna, S.C., Thines, M., Mortimer, P.E., Xu, J.-C. & Hyde, K.D. (2014) *Dematiopleospora mariae gen. sp. nov*., from *Ononis Spinosa* in Italy. *Cryptogamie, Mycologie* 35: 105–117. http://dx.doi.org/10.7872/crym.v35.iss2.2014.105

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

Wijayawardene, D.N.N., Camporesi, E., Song, Y., Dai, D.Q., Bhat, D.J., Mckenzie, E.H.C., Chukeatirote, E., Mel'nik, V.A., Wang, Y. & Hyde, K.D. (2013) Multi-gene analyses reveal taxonomic placement of *Scolicosporium minkeviciusii* in *Phaeosphaeriaceae (Pleosporales)*. *Cryptogamie, Mycologie* 34: 357–366. http://dx.doi.org/10.7872/crym.v34.iss2.2013.357

Wijayawardene, D.N.N., Crous, P.W., Kirk, P.M., Hawksworth, D.L., Boonmee, S., Braun, U., Chomnunti, P., Dai, D.Q., D'souza, M.J., Diederich, P., Dissanayake, A., Doilom, M., Hongsanan, S., Jones, E.B.G., Groenewald, J.Z., Jayawardena, R., Lawrey, J.D., Liu, J.K., Lücking, R., Madrid, H., Manamgoda, D.S., Muggia, L., Nelsen, M.P., Phookamsak, R., Suetrong, S., Tanaka, K., Thambugala, K.M., Wikee, S., Zhang, Y., Aptroot, A., Ariyawansa, H.A., Bahkali, A.H., Bhat, J.D., Gueidan, C., De-Hoog, G.S., Knudsen, K., Mckenzie, E.H.C., Miller, A.N., Mortimer, P.E., Wanasinghe, D.N., Phillips, A.J.L., Raja, H.A., Slippers, B., Shivas, R.S., Taylor, J.E., Wang, Y., Woudenberg, J.H.C., Piątek, M., Cai, L., Jaklitsch, W.M. & Hyde, K.D. (2014) Naming and outline of Dothideomycetes 2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69: 1–55. http://dx.doi.org/10.1007/s13225-014-0309-2

Zhang, Y., Crous, P.W, Schoch, C.L. & Hyde, K.D. (2012) *Pleosporales*. *Fungal Diversity* 52: 1–225. http://dx.doi.org/10.1007/s13225-011-0117-x

Zhang, Y., Schoch, C.L., Fournier, J., Crous, P.W., De-Gruyter, J., Woudenberg, J.H.C., Hirayama, K., Tanaka, K., Pointing, S.B. & Hyde, K.D. (2009) Multilocus phylogeny of the *Pleosporales:* a taxonomic, ecological and evolutionary reevaluation. *Studies in Mycology* 64: 85–102. http://dx.doi.org/10.3114/sim.2009.64.04

Zhaxybayeva, O. & Gogarten, J.P. (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3 (1): 4. http://dx.doi.org/10.1186/1471-2164-3-4