Cryptogamie, Mycologie, 2015, 36 (2): 225-236

Poaceascoma helicoides gen et sp. nov., a new genus with scolecospores in Lentitheciaceae

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Abstract – An ophiosphaerella-like species was collected from dead stems of a grass (Poaceae) in Northern Thailand. Combined analysis of LSU, SSU and *RPB2* gene data, showed that the species clusters with *Lentithecium arundinaceum*, *Setoseptoria phragmitis* and *Stagonospora macropycnidia* in the family Lentitheciaceae and is close to *Katumotoa bambusicola* and *Ophiosphaerella sasicola*. Therefore, a monotypic genus, *Poaceascoma* is introduced to accommodate the scolecosporous species *Poaceascoma helicoides*. The species has similar morphological characters to the genera *Acanthophiobolus*, *Leptospora* and *Ophiosphaerella* and these genera are compared.

Lentitheciaceae / Leptospora / Ophiosphaerella / phylogeny

INTRODUCTION

Lentitheciaceae was introduced by Zhang *et al.* (2012) to accommodate massarina-like species in the suborder Massarineae. In the recent monograph of Dothideomycetes (Hyde *et al.*, 2013), the family Lentitheciaceae comprised the genera *Lentithecium, Katumotoa, Keissleriella* and *Tingoldiago* and all species had fusiform to cylindrical, 1-3-septate ascospores and mostly occurred on grasses. A single species with filiform ascospores, *Ophiosphaerella sasicola* (Nagas. & Y. Otani) Shoemaker & C.E. Babc., seemed oddly placed, while a *Stagonospora macropycnidia* Cunnell, an asexual species, also clustered in the family. The asexual morph genus *Setoseptoria* was introduced for stagonospora-like or dendrophoma-like taxa (Quaedvlieg *et al.*, 2013), while Wanasinghe *et al.* (2014) introduced a new genus, *Murilentithecium* Wanasinghe *et al.* to accommodate a single species with

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doi/10.7872/crym/v36.iss2.2015.225

muriform ascospores and reported its asexual morph as coelomycetous, with hyaline to brown, muriform conidia. Liu *et al.* (2015) have also added *Keissleriella sparticola* Singtripop & K.D. Hyde in Lentitheciaceae. While introducing a new species of *Keissleriella* (Lentitheciaceae) from a dead stem of *Dactylis* sp. collected in Italy, Singtripop *et al.* (2015) showed five species clustering in *Keissleriella* and five strains of two species clustering in *Lentithecium. Lentithecium arundinaceum*, which may require new genus status, and *Ophiosphaerella sasicola* also clustered in the family, along with *Katumotoa*, *Tingoldiago* and *Stagonospora macropycnidia. Katumotoa*, *Lentithecium*, *Setoseptoria* and *Tingoldiago* were accepted in Lentitheciaceae by Wijayawardene *et al.* (2014)

Taxa with bitunicate asci and filiform ascospores had traditionally been placed in *Leptospora, Ophiosphaerella* and *Ophiobolus* and there are more than 400 epithets for these genera in Index Fungorum (2015). The genera *Ophiosphaerella* and *Ophiobolus* have generally thought to belong in Phaeosphaeriaceae (Phookamsak *et al.*, 2014b), while the placement of *Leptospora* is unresolved. Molecular data from a putatively named strain of *L. rubella* has placed it in Phaeosphaeriaceae (Câmara *et al.*, 2003; Crous *et al.*, 2006). *Ophiobolus* has long filiform ascospores with two distinctive central swellings and often splits into two part spores on release from the ascus; the neck is interesting as it is a crest, almost similar to that found in *Lophiostoma* species (Hyde *et al.*, 2013; Phookamsak *et al.*, 2014b). *Ophiosphaerella* species on the other hand, have long filiform ascospores without swellings (Phookamsak *et al.*, 2014b). Such genera with bitunicate asci and filiform ascospores have different ascoma morphology and are probably polyphyletic and are likely to have evolved across the range of families of Dothideomycetes.

Molecular data for taxa of *Ophiosphaerella* and *Ophiobolus* are few and also contradictory, with different species clustering in different clades in Phaeosphaeriaceae, but also in other families (Phookamsak *et al.*, 2014b). Two strains of *Ophiosphaerella agrostidis* Dern. *et al.* clustered in a well-supported clade in Phaeosphaeriaceae and probably represents *Ophiosphaerella sensu stricto.* The putative strains of *Ophiosphaerella herpotricha* (Fr.) J. Walker, however, clustered in a distant clade. Strains of *Ophiobolus cirsii* (P. Karst.) Sacc. and *O. erythrosporus* (Riess) G. Winter were also distantly clustered in the tree (Ariyawansa *et al.*, 2014; Phookamsak *et al.*, 2014b). Phookamsak *et al.* (2014b) and Ariyawansa *et al.* (2014) showed that *Ophiosphaerella* and *Ophiobolus* species are polyphyletic in Phaeosphaeriaceae and likely to comprise several genera. *Ophiosphaerella sasicola* on the other hand, which is also typical of *Ophiosphaerella* and *Ophiobolus* species, clustered in Lentitheciaceae, confirming the polyphyletic nature of this ascomycete form.

We collected an ophiosphaerella-like species from *Digitaria sanguinalis* (L.) Scop. in Thailand and isolated cultures from single ascospores. Phylogenetic analyses showed the species to belong in the family Lentitheciaceae, placed near *Katumotoa bambusicola* and *Ophiosphaerella sasicola.* There are clearly one or more lineages of Lentitheciaceae with filiform ascospores. In this paper we introduce a new genus of scolecosporous in Lentitheciaceae to accommodate this new taxon.

MATERIALS AND METHODS

Isolation and identification. The fungus was collected from dead stems of *Digitaria sanguinalis* in Phayao Province, Thailand and returned to laboratory in an

envelope. Examination, observations and description were made following the methods described in Phookamsak *et al.* (2014a, b). A pure culture was obtained from a single spore isolate following the protocols in Chomnunti *et al.* (2014). The pure culture is deposited in Mae Fah Luang University Culture Collection (MFLUCC) in Thailand, and duplicated in the International Collection of Microorganisms from Plants (ICMP), Landcare Research, New Zealand. A herbarium specimen was dried by using silica gel and deposited in Mae Fah Luang University (MFLU), Chiang Rai, Thailand.

Micro-morphological characters were captured by using a Cannon 550D digital camera under a Nikon ECLIPSE 80i compound microscope with DIC microscopy and a Sony DSC-T110 digital camera was used to captured macro-morphological characters under an Olympus SZH10 stereomicroscope. Squash mount preparations were made to determine the micro-morphology, such as asci, ascospores and pseudoparaphyses, while free hand sections were made for obtaining the ascoma and peridium structures. Melzer's reagent was used to stain the ascus apical rings, whereas Indian ink was used to stain mucilaginous sheaths surrounding the ascospores. A photographic plate was edited and combined using program Adobe Photoshop version CS5 (Adobe Systems Inc., The United States) and morphological characters measured in Tarosoft (R) Image Frame Work version 0.9.7. Permanent slides were prepared by adding lactoglycerol and sealed with clear nail polish (Phookamsak *et al.*, 2014a, b).

DNA extraction, PCR amplification and sequencing. The genomic DNA was obtained from fresh mycelium using a DNA extraction kit (A Biospin Fungus Genomic DNA Extraction Kit, BioFlux®, China) following the protocols in manufacturer's instructions (Hangzhou, P.R. China) (Phookamsak *et al.*, 2013, 2014a, b). DNA amplification was obtained by polymerase chain reaction (PCR) using the respective gene primers (ITS, LSU, SSU, *RPB2*, and *TEF1*) and DNA amplification procedures described in Phookamsak *et al.* (2013, 2014a, b). The PCR products were checked for quality using 1% agarose gel electrophoresis stained with ethidium bromide and sent to sequence at Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China) (Phookamsak *et al.*, 2013, 2014a, b).

Phylogenetic analyses. The newly generated sequences were analyzed with additional sequences obtained from GenBank (Table 1). LSU, SSU and *RPB2* single gene datasets were aligned with MAFFT: multiple sequence alignment software version 7.215 (Katoh & Standley, 2015: http://mafft.cbrc.jp/alignment/server/) and was optimized manually where necessary in MEGA6 version 6.0 (Tamura *et al.*, 2013). The alignment was converted to NEXUS file for maximum parsimony analysis using ClustalX2 v. 1.83 (Thompson *et al.*, 1997) and PHYLIP file for maximum likelihood analysis (RAxML) using ALTER (alignment transformation environment: http://sing.ei.uvigo.es/ALTER/; 2015). The phylogenetic trees were made using maximum likelihood (RAxML), maximum parsimony (MP) and Bayesian analyses.

Maximum likelihood analysis (RAxML) was carried out using RaxmlGUI v.1.0 (Silvestro & Michalak, 2011). The available substitution models comprised a generalized time reversible (GTR) for nucleotides with a discrete gamma distribution (Silvestro & Michalak, 2012). A discrete GAMMA (Yang, 1994) was complemented for each substitution model with four rate classes (Stamatakis *et al.*, 2008). Rapid bootstrap analysis (Stamatakis *et al.*, 2008) and search for a best-scoring ML tree were applied (Silvestro & Michalak, 2012). The best scoring tree was selected with a final ML optimization likelihood value of -23148.159572.

Table 1. Isolates used in this study and their GenBank accession numbers. The ex-type and ex-epitype strains are in bold; the newly generated sequences are indicated in pale blue/gray

Taxon	*Culture/voucher*	*GenBank Accession Number*		
		LSU	*SSU*	*RPB2*
***Bambusicola bambusae*^T**	**MFLUCC 11-0614**	**JX442035**	**JX442039**	**KP761718**
***Bambusicola irregulispora*^T**	**MFLUCC 11-0437**	**JX442036**	**JX442040**	**KP761719**
***Bambusicola massarinia*^T/Ts**	**MFLUCC 11-0389**	**JX442037**	**JX442041**	**KP761716**
***Bambusicola splendida*^T**	**MFLUCC 11-0439**	**JX442038**	**JX442042**	**KP761717**
***Bimuria novae-zelandiae*^T/Ts**	**CBS 107.79**	**AY016356**	**AY016338**	**DQ470917**
Corynespora cassiicola	CBS 100822	GU301808	GU296144	GU371742
Corynespora smithii	CABI 5649b	GU323201	–	GU371783
Falciformispora lignatilis^Ts	BCC 21118	GU371827	GU371835	–
Halomassarina thalassiae	JK 5262D	GU301816	–	–
Helicascus nypae	BCC 36751	GU479788	GU479754	GU479826
Helicascus nypae	BCC 36752	GU479789	GU479755	GU479827
***Kalmusia scabrispora*^T**	**NBRC 106237**	**AB524594**	**AB524453**	**AB539094**
Karstenula rhodostoma	CBS 690.94	GU301821	GU296154	GU371788
***Katumotoa bambusicola*^T/Ts**	**MAFF 239641**	**AB524595**	**AB524454**	**AB539095**
***Keissleriella cladophila*^T**	**CBS 104.55**	**GU301822**	**GU296155**	–
***Keissleriella dactylis*^T**	**MFUCC 13-0751**	**KP197668**	**KP197666**	**KP998464**
Keissleriella genistae	CBS 113798	GU205222	GU205242	–
Keissleriella rara	CBS 118429	GU479791	GU479757	–
***Lentithecium aquaticum* I^T**	**CBS 123099**	**GU301823**	**GU296156**	–
Lentithecium arundinaceum	CBS 619.86	GU301824	GU296157	–
Lentithecium arundinaceum	CBS 123131	GU456320	GU456298	–
Lentithecium fluviatile^Ts	CBS 122367	GU301825	GU296158	–
Lentithecium fluviatile^Ts	CBS 123090	FJ795450	FJ795492	FJ795467
Lentithecium lineare	IFRD 2008	FJ795435	FJ795478	–
Massarina cisti	CBS 266.62	FJ795447	FJ795490	FJ795464
Massarina eburnea	CBS 473.64	GU301840	GU296170	GU371732
***Melanomma pulvis-pyrius*^T/Ts**	**CBS 124080**	**GU456323**	**GU456302**	**GU456350**
Montagnula opulenta	CBS 168.34	DQ678086	AF164370	DQ677984
Morosphaeria ramunculicola	JK 5304B	GU479794	GU479760	GU479831
***Murilentithecium clematidis*^T/Ts**	MFLUCC 14-0561	KM408758	KM408760	KM454446
Murilentithecium clematidis	MFLUCC 14-0562	KM408759	KM408761	KM454447
Neottiosporina paspali	CBS 331.37	EU754172	EU754073	GU371779
Ophiosphaerella sasicola	MAFF 239644	AB524599	AB524458	AB539098
***Palmiascoma gregariascomum*^T/Ts**	**MFLUCC 11-0175**	**KP744495**	**KP753958**	**KP998466**
Paraconiothyrium minitans	CBS 122788	EU754173	EU754074	GU371776
***Paraphaeosphaeria michotii*^T/Ts**	**MFLUCC 13-0349**	**KJ939282**	**KJ939285**	**KP998465**
Phaeodothis winteri	CBS 182.58	GU301857	GU296183	DQ677970
***Poaceascoma helicoides*^T/Ts**	MFLUCC 11-0136	KP998462	KP998463	KP998460
***Setoseptoria phragmitis*^T/Ts**	**CBS 114802**	**KF251752**	–	**KF252254**
Stagonospora macropycnidia	CBS 114202	GU301873	GU296198	–
***Stagonospora paludosa*^T/Ts**	**CBS135088**	**KF251760**	–	**KF252262**
***Trematosphaeria pertusa*^T/Ts**	**CBS 122368**	**FJ201990**	**FJ201991**	**FJ795476**
Trematosphaeria pertusa^Ts	CBS 122371	FJ201992	FJ201993	GU371801
***Tingoldiago graminicola*^T/Ts**	**JCM 16485**	**AB521743**	**AB521726**	–
Tingoldiago graminicola^Ts	JCM 16486	AB521745	AB521728	–

Abbreviations: **BCC**: BIOTEC Culture Collection, Bangkok, Thailand; **CABI**: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **JCM**: The Japan Collection of Microorganisms, Japan; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC**: NITE Biological Resource Centre, Japan; Culture and specimen abbreviations: **JK**: J. Kohlmeyer; **T**: ex-type/ex-epitype isolates; **Ts**: type species.

Maximum parsimony (MP) was performed using PAUP v. 4.0b10 (Swofford, 2002). The heuristic search option used 100 replicates of random additional sequences and tree-bisection reconnection (TBR) of branch-swapping algorithm. The starting tree (S) was obtained via stepwise addition with the number of trees held at each step during stepwise addition, treated as one. All characters have equal weight and gaps were treated as missing data. Maxtrees was setup at 1000 with branches collapsed when the minimum branch length was zero. The calculation of consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were included in the analysis for generating trees under different optimality criteria. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa, 1989) were performed to determine significant parsimonious trees. The robustness of the most parsimonious tree was estimated based on 1000 bootstrap replications (Ariyawansa *et al.*, 2013; Phookamsak *et al.*, 2014b).

Bayesian analysis was analyzed via the CIPRES Science Gateway version 3.3 (http://www.phylo.org/). Bayesian command was generated from Fabox-an online fasta sequence toolbox (http://users-birc.au.dk/biopv/php/fabox/; Miller *et al.*, 2010) as a MrBayes input file from fasta (fasta2mrbayes) in data conversion block. Posterior probabilities (PP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes 3.2.3 on XSEDE tool. The following parameters were used: Setting Nst to 6 with rates GAMMA, two parallel runs with four chains, carried out for 4000000 generations with sample frequency in every 1000 generations, the sump burnin and sumt burnin phases were treated as 400 and using a relative burnin of 25% for diagnostics. Other parameters were left as default (Huelsenbeck & Ronquist, 2001; Boonmee *et al.*, 2014). The Convergence diagnostic was determined by Estimated Sample Size (ESS) and Potential Scale Reduction Factor (PSRF) which average PSRF for parameter values (excluding NA and > 10.0) = 1.000.

Phylograms were visualized in Treeview (Page, 1996) and reorganized in Microsoft power point (2007) and Adobe Photoshop version CS5 (Adobe Systems Inc., The United States). The new sequences generated in this study have been submitted to GenBank. The resulting alignment and tree is deposited in TreeBASE, submission ID: 17319 (http://www.treebase.org/).

RESULTS AND DISCUSSION

Phylogenetic analyses

The combined LSU, SSU and *RPB2* gene dataset comprises 45 taxa from Bambusicolaceae, Corynesporascaceae, Didymosphaeriaceae, Lentithecіaceae, Massarinaceae, Morosphaeriaceae, and Trematosphaeriaceae in the suborder Massarineae (Pleosporales, Dothideomycetes). *Melanomma pulvis-pyrius* (Pers.) Fuckel (CBS 124080) is selected as the outgroup taxon. Phylogenetic analyses obtained from maximum likelihood (RAxML), maximum parsimony (MP) and Bayesian analyses showed similar topologies and were not significantly different. The best scoring RAxML tree was selected to represent the relationships among taxa and is shown in Figure 1. Bootstrap support values for maximum likelihood (ML, blue) and maximum parsimony (MP, green), equal to or greater than 70%, are given above the nodes. The values for the Bayesian posterior probabilities from MCMC analyses (BYPP, red), equal or higher than 95% are given below the nodes.

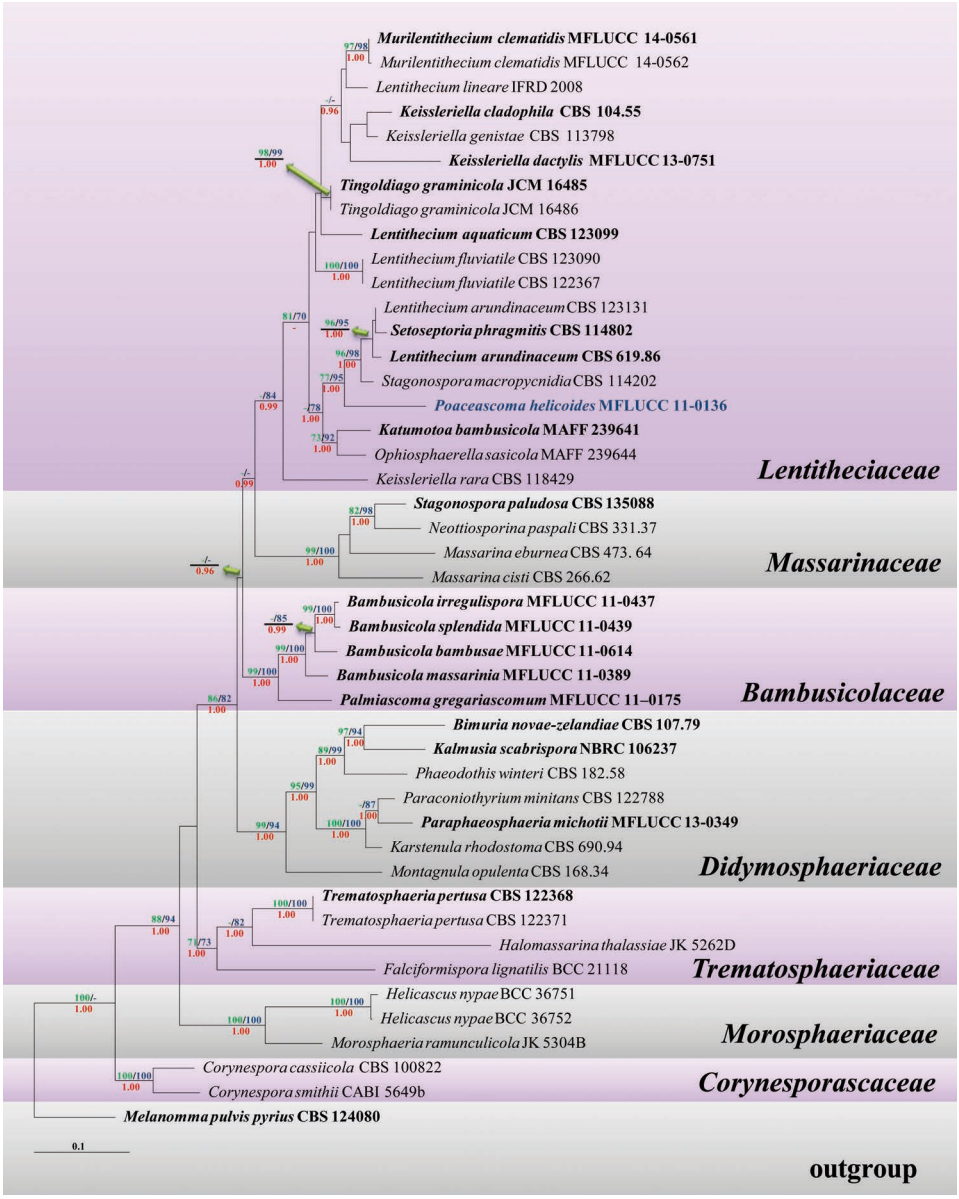

Fig. 1. Phylogram generated from maximum likelihood analysis (RAxML) based on combined LSU, SSU and *RPB2* sequenced data of the families in suborder Massarineae. Bootstrap support values for maximum likelihood (ML, blue) and maximum parsimony (MP, green) equal to or greater than 70% are given above the nodes. The values of the Bayesian posterior probabilities from MCMC analyses (BYPP, red) equal or higher than 95% are given below the nodes. The tree is rooted to *Melanomma pulvis-pyrius* (CBS 124080). Ex-type and ex-epitype strains are in bold. Newly generated sequences are indicated in blue-gray.

The phylogenic analyses obtained from maximum likelihood (RAxML), maximum parsimony (MP) and Bayesian analyses gave similar results for related families in the suborder Massarineae (Pleosporales) as in previous studies (Hyde *et al.*, 2013; Singtripop *et al.*, 2015; Wanasinghe *et al.*, 2014; Wijayawardene *et al.*, 2014). *Poaceascoma helicoides* formed a monophyletic clade (77% MP, 95% ML and 1.00 PP) basal to *Stagonospora macropycnidia* (CBS 114202), *Setoseptoria phragmitis* Quaedvlieg *et al.* (CBS 114802) and *Lentithecium arundinaceum* and is close to *Katumotoa bambusicola* (MAFF 239641) and *Ophiosphaerella sasicola* (MAFF 239644). *Setoseptoria phragmitis* clusters with *Lentithecium arundinaceum* (96% MP, 95% ML and 1.00 PP) and may be the asexual morph of *Lentithecium*. *Stagonospora macropycnidia* has similar morphology to species of *Setoseptoria*. *Stagonospora macropycnidia* may therefore need to be synonymized under *Setoseptoria* as molecular data places the type species of *Stagonospora, St. paludosa* (Sacc. & Speg.) Sacc. in Massarinaceae (Quaedvlieg *et al.*, 2013). The generic type of *Lentithecium, L. fluviatile* (Aptroot & Van Ryck.) K.D. Hyde *et al.* clusters in a single clade, separated from the type strain of *L. aquaticum* Ying Zhang *et al.* in Lentitheciaceae. The type species of *Murilentithecium, M. clematidis* (MFLUCC 14-0561 and MFLUCC 14-0562) forms a clade with *Lentithecium lineare. Lentithecium* species are therefore not monophyletic in this study. The natural classification of species in *Lentithecium* need further study to clarify their natural placement. The generic type of *Tingoldiago, T. graminicola*, forms strongly monophyletic clade (99% ML, 98% MP and 1.00 PP) in Lentitheciaceae, while *Keissleriella cladophila, K. dactylis* and *K. genistae* form a clade close to *Murilentithecium clematidis* and *Lentithecium lineare*. Several clades in Lentitheciaceae were not well-resolved, which may be due to limited sequence data in GenBank. Bambusicolaceae and Massarinaceae do not form well-resolved clades in ML and MP analyses, but these two families form a strongly supported clade in the Bayesian analysis. The family Trematosphaeriaceae forms a well-resolved clade in the MP analysis but forms a weakly-supported clade in ML and Bayesian analyses. The other families form well-resolved clades in the suborder Massarineae, Pleosporales.

Taxonomy

Poaceascoma Phookamsak & K.D. Hyde, **gen. nov.**

Index Fungorum number: IF551141
Facesoffungi number: FoF 00622
Etymology: The generic epithet "*Poaceascoma*" refers to the taxon forming ascomata with turf-like surrounded ascomata and scolecosporous ascospores on dead stems and roots of *Digitaria sanguinalis* (L.) Scop. (Poaceae).

Saprobic on Poaceae. Sexual morph: *Ascomata* solitary to gregarious, semi-immersed to erumpent, uni-loculate, globose to subglobose, ostiole central, with short to long papilla. *Papilla* erumpent, exposed parts covered with raised brown tufts of hyphae. *Peridium* thick walled, of equal thickness, composed of several layers of dark brown to black, pseudoparenchymatous cells, arranged in a *textura angularis* to *textura prismatica*. *Hamathecium* composed of dense, cellular pseudoparaphyses, with distinct septa, not constricted at the septa, anastomosing at the apex, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, elongate-cylindrical, short pedicelate, apically rounded with ocular chamber. *Ascospores* fasciculate, spirally arranged within the ascus, filiform, hyaline, multi-

septate, not constricted at the septa, smooth-walled, ascospores are often longer than asci. Asexual morph: Undetermined.

Notes: *Poaceascoma* is introduced to accommodate the Dothideomycete species associated with Poaceae which form setose ascoma with filiform ascospores. The genus is designated as a monotypic genus typified by *Poaceascoma helicoides* Phookamsak & K.D. Hyde. Various generic segregates form filiform ascospores, but most genera are classified in Sordariomycetes (Shoemaker 1976). In Dothideomycetes, there are also many genera forming filiform ascospores, such as *Acanthophiobolus*, *Leptospora*, *Ophiobolus*, and *Ophiosphaerella* (Shoemaker, 1976; Boonmee *et al.*, 2011, 2014; Zhang *et al.*, 2012). *Ophiobolus* and *Ophiosphaerella* are accommodated in Phaeosphaeriaceae (Ariyawansa *et al.*, 2014; Phookamsak *et al.*, 2014b,) based on their phylogeny, whereas *Acanthophiobolus* is placed in Tubeufiaceae due to its morphological characters (Kirk *et al.*, 2008; Lumbsch & Huhndorf, 2010; Boonmee *et al.*, 2011, 2014; Hyde *et al.*, 2013).

Leptospora is a poorly known genus which stains the host red, with aseptate, filiform ascospores loosely twisted in the ascus and glabrous ascomata with reddish papilla (Rabenhorst, 1857; W.J. Li, personal observations). The generic type of *Leptospora*, *L. porphyrogona* (Tode) Rabenh. is currently listed as a heterotypic synonym of *L. rubella* (Pers.) Rabenh. *Poaceascoma* differs from *Leptospora* in having turfs of hyphae surrounding the ascomata and ascospores arranged in tight spirals in the asci and does not stain the host red. W.J. Li (personal observation) examined *Leptospora rubella* (Pers.) Rabenh, of Fries specimens [Fries, Scleromyceti sueciae exs. No. 240, Sweden, on wood, K(M) 181455] and found that the ascospores of *L. rubella* are brown to yellowish brown, and fasciculate (not spirally arranged) in the asci. *Leptospora rubella* therefore, differs from *Poaceascoma* and molecular analysis of *L. rubella* shows it is related to the family Phaeosphaeriaceae (Câmara *et al.*, 2003; Crous *et al.*, 2006).

Poaceascoma is similar to *Acanthophiobolus* due to its setose ascomata with ascospores spirally arranged in the asci (Boonmee *et al.*, 2011, 2014). However, *Acanthophiobolus* has rather smaller ascomata than *Poaceascoma* and ascoma are apapillate, and superficial on the rotten cloth, while *Poaceascoma* has semi-immersed to erumpent ascomata with short to long beaks on grasses. *Ophiosphaerella sasicola* has a similar morphology to *Poaceascoma helicoides* (Shoemaker & Babcock, 1989), thus some *Ophiosphaerella* species such as *Ophiosphaerella sasicola* may need to synonymize under the new genus. In this paper we therefore introduce a new genus *Poaceascoma* to accommodate ophiosphaerella-like species in Lentitheciaceae. Descriptions and illustrations are provided.

Type species: *Poaceascoma helicoides* Phookamsak & K.D. Hyde

Poaceascoma helicoides Phookamsak & K.D. Hyde, **sp. nov.** **Fig. 2**

Index Fungorum number: IF551142

Facesoffungi number: FoF 00623

Etymology: The specific epithet "*helicoides*" refers to the ascospores arranged in a spiral in the ascus.

HOLOTYPUS: MFLU11-0172

Saprobic on grass culms and roots. Sexual morph: *Ascomata* 270-360 µm high, 320-450 µm diam., solitary to gregarious, semi-immersed to erumpent, visible as raised, dark spots on host surface, uni-loculate, globose to subglobose, ostiole papillate. *Papilla* 160-340 µm high, 110-180 µm diam, conical to cylindrical,

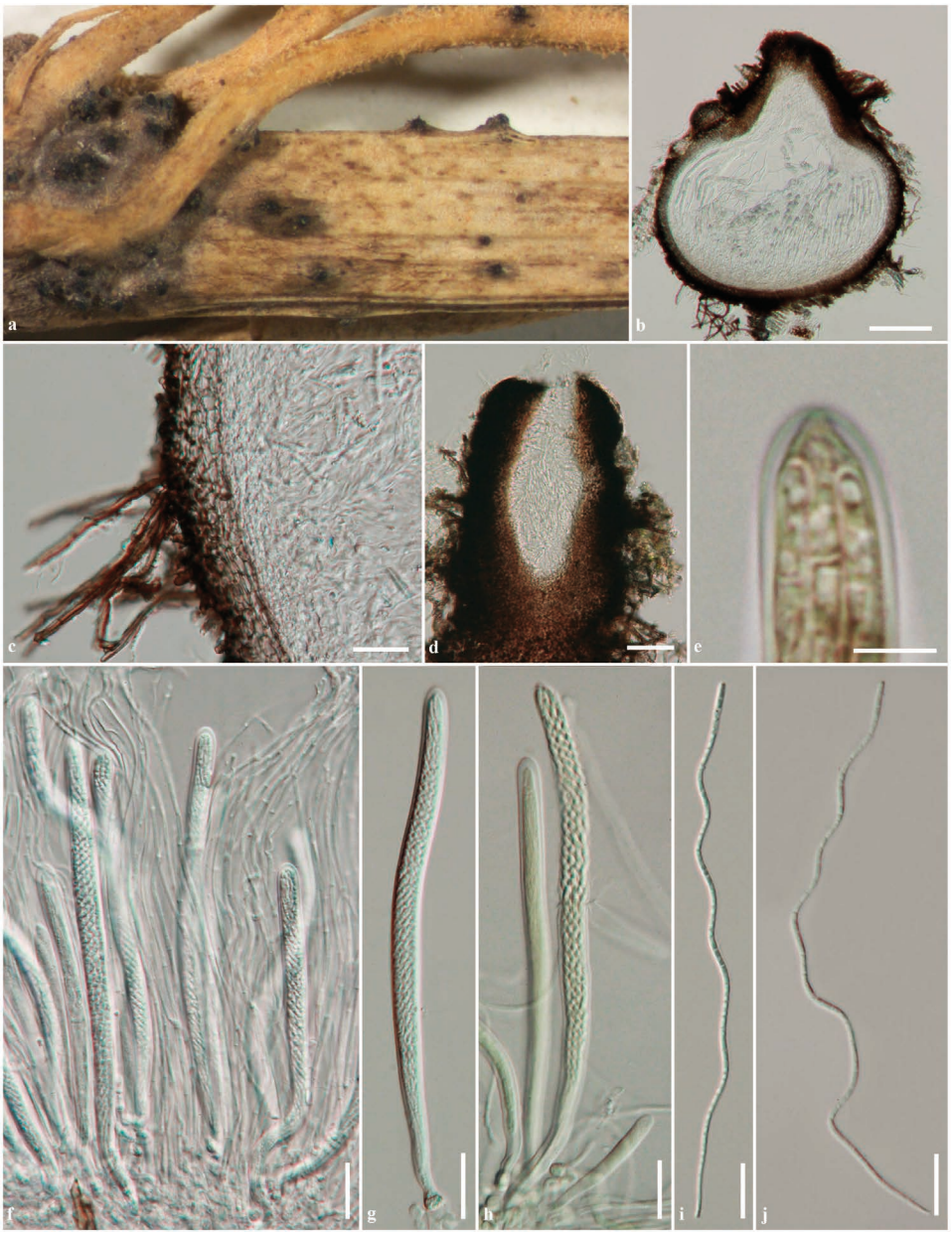

Fig. 2. *Poaceascoma helicoides* (MFLU11-0172, holotype) **a.** Ascomata visible as black spots on host surface. **b.** Section through an ascoma. **c.** Section through peridium. **d.** Section through neck. **e.** Ocular chamber stained in Melzer's reagent. **f.** Asci with pseudoparaphyses. **g.** Ascus. **h.** Asci stained with Melzer's reagent. **i-j.** Ascospores. Scale bars: b = 100 µm, c, d f, g, h, i, j = 20 µm, e = 5 µm.

erumpent, exposed parts covered with raised brown tufts of hyphae. *Peridium* 20-50 μm wide, with thick walls of equal thickness, composed of several layers of dark brown to black, pseudoparenchymatous cells arranged in a *textura angularis* to *textura prismatica*. *Hamathecium* composed of dense, 1-2.5 μm diam., narrow, cellular pseudoparaphyses, with distinct septa, not constricted at the septa, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* (150-)160-185(-190) × 8.5-10(-11) μm ($\bar{x}$ = 173.5 × 9.4 μm, n = 30), 8-spored, bitunicate, fissitunicate, elongate-cylindrical, short pedicellate, with rounded to obtuse pedicel, apically rounded, with indistinct ocular chamber. *Ascospores* (148-)150-185(-215) × 2-2.5 μm ($\bar{x}$ = 178.1 × 2.4 μm, n = 30), spirally arranged in the ascus, filiform, wider in the upper part, narrow towards the ends, hyaline, multi-septate, with 29-33 septa, continuous, smooth-walled. Asexual morph: Undetermined.

Culture characters: Colonies on PDA 33-34 mm diam after 4 weeks at 25-30°C, colonies dense, circular, slightly raised to umbonate, dull with entire edge, fluffy to floccose, smooth, not producing pigments. Colonies from above site are white to cream at the margin, pale grey in the centre; reverse white to yellowish brown at the margin, dark brown in the centre, slightly radiating.

Material examined: THAILAND: Phayao Prov., Mae Jai District, on dead stem of *Digitaria sanguinalis* (L.) Scop., 19 August 2010, R. Phookamsak RP0052 (MFLU 11-0172, **holotype**), isotype will be deposited in BBH, extype living culture = MFLUCC 11-0136 = ICMP.

Acknowledgements. We would like to thank The Royal Golden Jubilee Ph. D. Program (PHD/0090/2551) under Thailand Research Fund for financial support. The Humidtropics, a CGIAR Research Program that aims to develop new opportunities for improved livelihoods in a sustainable environment and Mae Fah Luang University (grant for study Dothideomycetes No. 56101020032) are gratefully thanked for partially funding and laboratory support in this work. K.D. Hyde appreciates to The Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. International Fungal Research & Development Centre, Research Institute of Resource Insects, Chinese Academy of Forestry and Jun-Bo Yang, Plant Germplasm and Genomics Center in Germplasm Bank of Wild Species, Kunming Institute of Botany would be appreciated for the molecular laboratory support. Shaun Pennycook, Chatsachee Chatpapamon, Saranyaphat Boonmee and Supalak Yacharoen are gratefully thanked for informative name information, cultures preparation and general assistance.

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