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Kwanghwana miscanthi Karun., C.H.Kuo &
K.D.Hyde, gen. et sp. nov. (Phaeosphaeriaceae,
Pleosporales) on *Miscanthus floridulus* (Labill.)
Warb. ex K.Schum. & Lauterb. (Poaceae)

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on *Miscanthus floridulus* (Labill.) Warb.
ex K.Schum. & Lauterb. (Poaceae)**

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ABSTRACT

We are studying microfungi growing on Poaceae hosts in different geographical regions and in this study we introduce *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, gen. et sp. nov., based on phylogenetic analyses of DNA sequences of four loci used in combination (three ribosomal (LSU, SSU and ITS) and one protein coding region (TEF1- α)) and on morphological characters. This new genus, including a single species isolated from *Miscanthus floridulus* (Labill.) Warb. ex K.Schum. & Lauterb., is molecularly distinct from closely related genera, *Neostagonospora* C.L.Yang, X.L.Xu & K.D.Hyde, *Setophoma* Gruyter, Aveskamp & Verkley and *Edenia* M.C.González, A.L.Anaya, Glenn, Saucedo & Hanlin. *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov., also differs from *Neostagonospora* and *Setophoma* by having subglobose ascomata, irregularly thickened peridium, cellular, septate and branched pseudoparaphyses, and fusiform, broadly fusoid, 3-septate ascospores.

KEY WORDS

Dothideomycetes,
graminicolous fungi,
Taiwan,
new species.

RÉSUMÉ

Kwanghwana miscanthi Karun., C.H.Kuo & K.D.Hyde, gen. et sp. nov. (Phaeosphaeriaceae, Pleosporales) sur *Miscanthus floridulus* (Labill.) Warb. ex K.Schum. & Lauterb. (Poaceae).

Nous étudions les microchampignons qui poussent sur les hôtes de Poaceae dans différentes régions géographiques et dans cette étude nous introduisons *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, gen. et sp. nov., basé sur des analyses phylogénétiques des séquences d'ADN de quatre loci utilisés en combinaison (trois ribosomes (LSU, SSU et ITS) et une région codant pour une protéine (TEF1- α)) et sur des caractères morphologiques. Ce nouveau genre, qui comprend une seule espèce isolée de *Miscanthus floridulus* (Labill.) Warb. ex K.Schum. & Lauterb., se distingue moléculairement de genres étroitement apparentés, à savoir *Neostagonospora* C.L.Yang, X.L.Xu & K.D.Hyde, *Setophoma* Gruyter, Aveskamp & Verkley et *Edenia* M.C.González, A.L.Anaya, Glenn, Saucedo & Hanlin. *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov., se distingue également de *Neostagonospora* et *Setophoma* par ses ascomates sous-globulaires, son périidium épaissi de façon irrégulière, ses pseudoparaphyses cellulaires, septées et ramifiées, et ses ascospores fusiformes, largement fusoides, à trois états.

MOTS CLÉS

Dothideomycetes,
champignons
graminicoles,
Taiwan,
espèce nouvelle.

INTRODUCTION

Studies on the taxonomy and phylogeny of fungi on Poaceae have revealed many new taxa (Dai *et al.* 2016; Thambugala *et al.* 2017; Karunarathna *et al.* 2017b, 2019). A checklist on grass fungi in Thailand (Goonasekara *et al.* 2018) revealed 98 species belonging to Ascomycota among 15 orders, 37 families and 68 genera. Furthermore, Dai *et al.* (2016) reported 43 Ascomycota Caval.-Sm. species on bamboo. Recent studies have shown an abundance of the family Phaeosphaeriaceae on grasses (Karunarathna *et al.* 2017a; Thambugala *et al.* 2017; Yang *et al.* 2019).

Pleosporales is the largest order in Dothideomycetes and comprises 55 families based on multi-gene phylogenetic analyses (Wijayawardene *et al.* 2018). Phaeosphaeriaceae is the largest family of the order Pleosporales and is a heterogeneous assemblage of genera in the order. Species in Phaeosphaeriaceae can be endophytic, epiphytic or saprobic, especially on monocotyledons (e.g. Cannaceae, Cyperaceae, Juncaceae, Poaceae) (Hyde *et al.* 2013; Phookamsak *et al.* 2014b). Species of this family have also been reported as pathogens causing leaf spots on various hosts (Quaedvlieg *et al.* 2013; Phookamsak *et al.* 2014a; Li *et al.* 2016). In a recent consensus study of fungi on grasses, Thambugala *et al.* (2017) introduced a new genus and 15 new species in the family Phaeosphaeriaceae among the 50 taxa studied. Currently taxonomy and phylogeny of fungi

in Taiwan is broadly being studied (Ariyawansa *et al.* 2018; Karunarathna *et al.* 2019; Tennakoon *et al.* 2019).

The current study was initiated to identify new taxa from grasses in Taiwan. In this paper, a new grass inhabiting genus, *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov., is introduced. All taxonomic arrangements proposed herein are supported with detailed phylogenetic and morphological analyses.

MATERIALS AND METHODS

PLANT SAMPLE COLLECTION, MORPHOLOGICAL STUDIES AND ISOLATION

Decaying *Miscanthus floridulus* (Labill.) Warb. ex K.Schum. & Lauterb. specimens were collected from Kwang Hwa, in Chiayi Province, Taiwan. Specimens were processed and examined for the presence of fungi. Hand-cut sections of fungal fruiting structures were mounted in water for microscopic studies and photomicrography. Specimens were examined with an AXI-OSKOP 2 PLUS compound microscope and photographed with a Canon AXIOM 506 COLOR digital camera fitted to the microscope. Measurements of morphological characters were made with the ZEN2 (blue edition) program and images used for figures processed with Adobe Photoshop CS3 Extended version 10.0 (Adobe Systems, United States).

Single spore isolation was carried out following the spore suspension method described in Chomnunti *et al.* (2014). Germinated spores were individually transferred to potato dextrose agar (PDA) plates and grown at room temperature (20–25°C). Colony colour and the other characters were observed. The specimen was deposited in the Mae Fah Luang University Herbarium (MFLU). Living cultures were deposited in the Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan and National Chiayi University Culture Collection.

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING FOR FUNGAL ISOLATES

Genomic DNA was extracted from fresh fungal mycelium grown on PDA media at 20°C for four weeks using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, P. R. China) following the instructions of the manufacturer.

The DNA amplification was performed by polymerase chain reaction (PCR) for the partial sequences of five genes, the 28S large subunit rDNA (LSU) was amplified by using the primer pair LR0R and LR5 (Vilgalys & Hester 1990), internal transcribed spacers (ITS1, 5.8S, ITS2) was amplified by using the primers ITS5 and ITS4 (White *et al.* 1990), 18S small subunit rDNA (SSU) was amplified by using the primer pair NS1 and NS4 (White *et al.* 1990) and translation elongation factor 1- α (TEF1- α) was amplified with primers EF1-983F and EF1-2218R (Rehner 2001). The amplification reaction was performed for the 25 μ L of total PCR reaction mixture containing 9.5 μ L of sterilized deionized water, 12.5 μ L of 2 $\times$ Power Taq PCR MasterMix (Bioteke Co., China), 1 μ L from each of forward and reverse primers and 1 μ L of DNA template. The primers and the PCR amplification conditions for LSU, SSU, ITS and TEF1- α were as follow. 3 min at 95°C, then 30 cycles of 95°C for 30 s, 55°C for 50 s, 72°C for 30 s, and final extension for 10 min at 72°C. The quality of PCR amplification was confirmed on 1% agarose gels electrophoresis stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (Tri-I Biotech, Taipei, Taiwan). The nucleotide sequence data acquired were deposited in GenBank (Table 1).

PHYLOGENETIC ANALYSES

Phylogenetic analyses were conducted based on combined genes of ITS, LSU, SSU and TEF1- α sequence data. The topologies of the trees obtained from each gene were compared prior to combine gene analysis to check for any incongruence in overall topology. The reference nucleotide sequences (Table 1) of representative genera and recently published data in Phaeosphaeriaceae were retrieved from GenBank (Phookamsak *et al.* 2017; Yang *et al.* 2019). The single gene sequences were initially aligned by MAFFT V.7.036 (<http://mafft.cbrc.jp/alignment/server/>) (Katoh *et al.* 2017), improved manually where necessary and multiple alignments were combined using Bioedit v.7.2 (Hall 1999). Phylogenetic reconstructions of the combined gene trees were performed using maximum likelihood (ML) and Bayesian inference (BI) criteria.

Maximum likelihood analysis was generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis *et al.* 2008;

Stamatakis 2014) in the CIPRES Science Gateway platform (Miller *et al.* 2010) using GTR + I + G model of evolution.

Evolutionary models for phylogenetic analyses and Bayesian information criterion were obtained using the jModeltest 2.1.10 v20160303 (Darriba *et al.* 2012) on jModelTest2 on XSEDE in the CIPRES Science Gateway platform (Miller *et al.* 2010).

Bayesian inference analysis was conducted with MrBayes on XSEDE, MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001) in the CIPRES Science Gateway platform (Miller *et al.* 2010) to evaluate Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: six simultaneous Markov chains were run for 2 000 000 generations and trees were sampled every 100th generation. The distribution of log-likelihood scores were examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using Tracer v. 1.6 program (Rambaut *et al.* 2014). First 10% of generated trees representing the burn-in phase were discarded and the remaining trees were used to calculate posterior probabilities of the majority rule consensus tree.

RESULTS

PHYLOGENY

The combined LSU, SSU, ITS and TEF1- α gene dataset comprised 83 taxa, including new taxon and other taxa from Phaeosphaeriaceae (Fig. 1). Phylogenetic trees obtained from ML and BI analyses yielded trees with similar overall topologies at the generic level and are in agreement with previous studies (Phookamsak *et al.* 2017; Yang *et al.* 2019).

The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -21740.110940. The matrix had 1017 distinct alignment patterns, with 26.21% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined LSU, SSU, ITS and TEF1- α were: estimated base frequencies A = 0.243869, C = 0.234065, G = 0.265185, T = 0.256880; substitution rates AC = 1.307686, AG = 3.037850, AT = 2.369585, CG = 0.756328, CT = 6.216514, GT = 1.000000; proportion of invariable sites I = 0.657196; gamma distribution shape parameter α = 0.517010. The Bayesian analysis resulted in 20 001 trees after 2 000 000 generations. The first 2000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 18 001 trees were used for calculating posterior probabilities in the majority rule consensus tree. The average standard deviation of split frequencies was 0.005413.

TAXONOMY

In this section, the new genus (*Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov.) is described and the new species (*Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov.) are illustrated and described from holotype (holo-, MFLU[MFLU18-1950]), and detailed illustrations are provided for the new species.

TABLE 1. — GenBank accession numbers and culture accession numbers of isolates included in this study. The newly generated sequence is shown in black **bold**. Notes: (†) indicates Ex-type strains. Abbreviations: **CBS**, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CCTU**, Culture Collection of Tabriz University, Tabriz, Iran; **CPC**, P. W. Crous; **JK**, J. Kohlmeyer; **KUMCC**, Kunming Institute of Botany Culture Collection, Chinese Academy of Sciences, Kunming, China; **MFLU**, Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **UTHSC**, Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States.

Species	Strain/Voucher no.	GenBank accession no.				References
		LSU	SSU	ITS	TEF1- α	
<i>Allophaeosphaeria muriformia</i>	MFLUCC 13-0277	KX910089	KX950400	KX926415	–	Liu <i>et al.</i> 2015
<i>Banksiophoma australiensis</i> (†)	CBS 142163	KY979794	–	KY979739	–	Crous <i>et al.</i> 2017
<i>Bhatellae rosae</i> (†)	MFLUCC 17-0664	MG828989	MG829101	MG828873	–	Wanasinghe <i>et al.</i> 2018
<i>Chaetosphaeronema achilleae</i> (†)	MFLUCC 16-0476	KX765266	–	KX765265	–	Hyde <i>et al.</i> 2016
<i>Chaetosphaeronema hispidulum</i> (†)	CBS 216.75	KF251652	EU754045	KF251148	–	de Gruyter <i>et al.</i> 2009; Quaedvlieg <i>et al.</i> 2013
<i>Dematiopleospora cirsii</i> (†)	MFLUCC 13-0615	KX274250	–	KX274243	KX284708	Hyde <i>et al.</i> 2016
<i>Dematiopleospora fusiformis</i> ^v	MFLU 15-2133	KY239030	KY239028	KY239029	–	Huang <i>et al.</i> 2017
<i>Dematiopleospora mariae</i> (†)	MFLUCC 13-0612	KJ749653	KJ749652	KJ749654	KJ749655	Wanasinghe <i>et al.</i> 2014b
<i>Dlhawksworthia alliariae</i> (†)	MFLUCC 13-0070	KX494877	KX494878	KX494876	–	Hyde <i>et al.</i> 2016
<i>Dlhawksworthia clematidicola</i> (†)	MFLUCC 14-0910	MG829011	MG829120	MG828901	MG829202	Wanasinghe <i>et al.</i> 2018
<i>Dlhawksworthia lonicera</i> (†)	MFLUCC 14-0955	MG829012	MG829121	MG828902	MG829203	Wanasinghe <i>et al.</i> 2018
<i>Edenia gomezpompae</i>	AM04	KM246015	–	KM246160	–	Gonzalez <i>et al.</i> 2007
<i>Edenia gomezpompae</i> (†)	CBS 124106	FJ839654	–	FJ839619	–	Crous <i>et al.</i> 2009
<i>Edenia</i> sp.	UTHSC: DI16-264	LN907407	–	LT796858	LT797098	Valenzuela-Lopez <i>et al.</i> 2017
<i>Edenia</i> sp.	UTHSC: DI16-260	LN907403	–	LT796855	LT797095	Valenzuela-Lopez <i>et al.</i> 2017
<i>Equiseticola fusispora</i> (†)	MFLUCC 14-0522	KU987669	KU987670	KU987668	MG520895	Abd-Elisalam <i>et al.</i> 2016; Phookamsak <i>et al.</i> 2017
<i>Galiicola pseudophaeosphaeria</i> (†)	MFLU 14-0524	KT326693	–	KT326692	MG520896	Phookamsak <i>et al.</i> 2017
<i>Kwanghwana miscanthi</i>(†)FU31017		MK503823	MK503829	MK503817	MT009126	In this study
<i>Leptospora rubella</i>	CPC 11006	DQ195792	DQ195803	DQ195780	–	Crous <i>et al.</i> 2006
<i>Leptospora thailandica</i> (†)	MFLUCC 16-0385	KX655549	KX655554	KX655559	KX655564	Hyde <i>et al.</i> 2016
<i>Loratospora aestuarii</i>	JK 5535B	GU301838	GU296168	–	–	Schoch <i>et al.</i> 2009
<i>Melnikia anthoxanthii</i> (†)	MFLUCC 14-1010	KU848204	KU848205	–	–	Wijayawardene <i>et al.</i> 2016
“ <i>Muriphaeosphaeria ambrosiae</i> (†)”	MFLU 15-1971	KX765264	–	KX765267	–	Hyde <i>et al.</i> 2016
<i>Muriphaeosphaeria galatellae</i>	MFLUCC 15-0769	KT438330	KT438332	–	–	Phukhamsakda <i>et al.</i> 2015
<i>Neosetophoma clematidis</i> (†)	MFLUCC 13-0734	KP684153	KP684154	KP744450	–	Liu <i>et al.</i> 2015
<i>Neosetophoma rosae</i>	MFLUCC 17-0844	MG829035	MG829141	MG828926	MG829219	Wanasinghe <i>et al.</i> 2018
<i>Neostagonospora caricis</i> (†)	CBS 135092	KF251667	–	KF251163	–	Quaedvlieg <i>et al.</i> 2013
<i>Neostagonospora phragmitis</i> (†)	MFLUCC 16-0493	KX910090	KX950401	KX926416	MG520902	Phookamsak <i>et al.</i> 2017; Thambugala <i>et al.</i> 2017
<i>Neostagonosporaella sichuanensis</i> (†)	MFLUCC 18-1228	MH368073	MH368079	MH368088	MK313851	Yang <i>et al.</i> 2019
<i>Neostagonosporaella sichuanensis</i>	MFLUCC 18-1231	MH368074	MH368080	MH368089	–	Yang <i>et al.</i> 2019
<i>Neostagonosporaella sichuanensis</i>	MFLU 18-1223	MH394690	MH394687	MK296469	MK313854	Yang <i>et al.</i> 2019
<i>Neosulcatispora agaves</i> (†)	CPC 26407	KT950867	–	KT950853	–	Crous <i>et al.</i> 2015
<i>Nodulosphaeria guttulatum</i> (†)	MFLUCC 15-0069	KY496726	KY501115	KY496746	KY514394	Tibpromma <i>et al.</i> 2017
<i>Nodulosphaeria multiseptata</i> (†)	MFLUCC 15-0078	KY496728	KY501116	KY496748	KY514396	Tibpromma <i>et al.</i> 2017
<i>Nodulosphaeria scabiosae</i> (†)	MFLUCC 14-1111	KU708846	KU708842	KU708850	KU708854	Mapook <i>et al.</i> 2016
<i>Ophiobolopsis italica</i> (†)	MFLUCC 17-1791	MG520959	MG520977	MG520939	MG520903	Phookamsak <i>et al.</i> 2017
<i>Ophiobolus artemisiae</i> (†)	MFLU 15-1966	MG520960	MG520978	MG520940	MG520904	Phookamsak <i>et al.</i> 2017

TABLE 1. — Continuation.

Species	Strain/Voucher no.	GenBank accession no.				References
		LSU	SSU	ITS	TEF1- α	
<i>Ophiobolus disseminans</i> ^(T)	MFLUCC 17-1787	MG520961	MG520980	MG520941	MG520906	Phookamsak <i>et al.</i> 2017
<i>Ophiobolus italicus</i> ^(T)	MFLUCC 14-0526	KY496727	–	KY496747	KY514395	Tibpromma <i>et al.</i> 2017
<i>Ophiobolus rossicus</i> ^(T)	MFLU 17-1639	MG520964	MG520983	MG520944	MG520909	Phookamsak <i>et al.</i> 2017
<i>Ophiobolus rudis</i>	CBS 650.86	GU301812	AF164356	KY090650	GU349012	Liew <i>et al.</i> 2000; Schoch <i>et al.</i> 2009; Ahmed <i>et al.</i> 2017
<i>Ophiobolus senecionis</i> ^(T)	MFLUCC 13-0575	KT728366	–	KT728365	–	Tibpromma <i>et al.</i> 2015
<i>Ophiosimulans tanacetii</i> ^(T)	MFLUCC 14-0525	KU738891	KU738892	KU738890	MG520910	Tibpromma <i>et al.</i> 2016; Phookamsak <i>et al.</i> 2017
<i>Ophiosphaerella agrostidis</i>	MFLUCC 11-0152	KM434281	KM434290	KM434271	KM434299	Phookamsak <i>et al.</i> 2014a
<i>Ophiosphaerella aquatica</i> ^(T)	MFLUCC 14-0033	KX767089	KX767090	KX767088	MG520911	Ariyawansa <i>et al.</i> 2015; Phookamsak <i>et al.</i> 2017
<i>Paraophiobolus arundinis</i> ^(T)	MFLUCC 17-1789	MG520965	MG520984	MG520945	MG520912	Phookamsak <i>et al.</i> 2017
<i>Paraophiobolus plantaginis</i> ^(T)	MFLUCC 17-0245	KY815010	KY815012	KY797641	MG520913	Hyde <i>et al.</i> 2017; Phookamsak <i>et al.</i> 2017
<i>Paraphoma chrysanthemicola</i> ^(T)	CBS 522.66	GQ387582	GQ387521	KF251166	–	de Gruyter <i>et al.</i> 2010; Quaedvlieg <i>et al.</i> 2013
<i>Paraphoma radicina</i> ^(T)	CBS 111.79	KF251676	EU754092	KF251172	–	de Gruyter <i>et al.</i> 2009; Quaedvlieg <i>et al.</i> 2013
<i>Parastagonospora minima</i> ^(T)	MFLUCC 13-0376	KU058723	MG520986	KU058713	MG520916	Li <i>et al.</i> 2015; Phookamsak <i>et al.</i> 2017
<i>Parastagonospora uniseptata</i> ^(T)	MFLUCC 13-0387	KU058725	MG520987	KU058715	MG520917	Li <i>et al.</i> 2015; Phookamsak <i>et al.</i> 2017
<i>Parastagonospora fallopiae</i>	CCTU 1151.1	MH460546	–	MH460544	–	Bakhshi <i>et al.</i> 2019
<i>Phaeopoacea festucae</i> ^(T)	MFLUCC 17-0056	KY824767	KY824769	KY824766	–	Thambugala <i>et al.</i> 2017
<i>Phaeopoacea phragmiticola</i>	CBS 459.84	KF251691	KY090700	KF251188	–	Quaedvlieg <i>et al.</i> 2013; Ahmed <i>et al.</i> 2017
<i>Phaeosphaeria oryzae</i> ^(T)	CBS 110110	KF251689	GQ387530	KF251186	–	de Gruyter <i>et al.</i> 2010; Quaedvlieg <i>et al.</i> 2013
<i>Phaeosphaeria thysanolaenica</i> ^(T)	MFLUCC 10-0563	KM434276	KM434286	KM434266	KM434295	Phookamsak <i>et al.</i> 2014a
<i>Phaeosphaeriopsis glaucopunctata</i> ^(T)	MFLUCC 13-0265	KJ522477	KJ522481	KJ522473	MG520918	Thambugala <i>et al.</i> 2014; Phookamsak <i>et al.</i> 2017
<i>Phaeosphaeriopsis triseptata</i> ^(T)	MFLUCC 13-0271	KJ522479	KJ522484	KJ522475	MG520919	Thambugala <i>et al.</i> 2014; Phookamsak <i>et al.</i> 2017
<i>Poaceicola arundinis</i>	MFLU 16-0158	MG829057	MG829162	MG828947	MG829229	Wanasinghe <i>et al.</i> 2018
<i>Poaceicola forlicesenica</i> ^(T)	MFLUCC 15-0470	KX910095	KX950406	KX926422	MG520922	Phookamsak <i>et al.</i> 2017; Thambugala <i>et al.</i> 2017
<i>Pseudoophiobolus achilleae</i> ^(T)	MFLU 17-0925	MG520966	–	MG520946	–	Phookamsak <i>et al.</i> 2017
<i>Pseudoophiobolus galii</i> ^(T)	MFLUCC 17-2257	MG520967	MG520989	MG520947	MG520926	Phookamsak <i>et al.</i> 2017
<i>Pseudoophiobolus urticicola</i> ^(T)	KUMCC 17-0168	MG520975	MG520996	MG520955	MG520933	Phookamsak <i>et al.</i> 2017
<i>Pseudophaeosphaeria rubi</i> ^(T)	MFLUCC 14-0259	KX765299	KX765300	KX765298	–	Hyde <i>et al.</i> 2016
<i>Setomelanomma holmii</i>	CBS 110217	GQ387633	GQ387572	KT389542	GU349028	Schoch <i>et al.</i> 2009; de Gruyter <i>et al.</i> 2010; Chen <i>et al.</i> 2015
<i>Setophoma chromolaena</i> ^(T)	CBS 135105	KF251747	–	KF251244	–	Quaedvlieg <i>et al.</i> 2013

TABLE 1. — Continuation.

Species	Strain/Voucher no.	GenBank accession no.				References
		LSU	SSU	ITS	TEF1- α	
<i>Setophoma sacchari</i> ^(T)	CBS 333.39	GQ387586	GQ387525	KF251245	–	de Gruyter <i>et al.</i> 2010
<i>Setophoma sacchari</i>	MFLUCC 12-0241	KJ476147	KJ476149	KJ476145	KJ461318	Phookamsak <i>et al.</i> 2014b
<i>Setophoma sacchari</i>	MFLUCC 11-0154	KJ476146	KJ476148	KJ476144	KJ461319	Phookamsak <i>et al.</i> 2014b
<i>Setophoma vernoniae</i> ^(T)	CPC 23123	KJ869198	–	KJ869141	–	Crous <i>et al.</i> 2014
<i>Sulcisporea pleurospora</i>	CBS 460.84	–	–	AF439498	–	Câmara <i>et al.</i> 2002
<i>Sulcisporea supratumida</i> ^(T)	MFLUCC 14-0995	KP271444	KP271445	KP271443	–	Senanayake <i>et al.</i> 2018
<i>Tintelnotia destructans</i> ^(T)	CBS 127737	KY090664	KY090698	KY090652	–	Ahmed <i>et al.</i> 2017
<i>Tintelnotia opuntiae</i> ^(T)	CBS 376.91	GU238123	GU238226	KY090651	–	Aveskamp <i>et al.</i> 2010; Ahmed <i>et al.</i> 2017
<i>Vrystaatia aloecicola</i> ^(T)	CBS 135107	KF251781	–	KF251278	–	Quaedvlieg <i>et al.</i> 2013
<i>Wojnowiciella dactylidis</i> ^(T)	MFLUCC 13-0735	KP684149	KP684150	KP744470	–	Liu <i>et al.</i> 2015
<i>Wojnowiciella spartii</i> ^(T)	MFLUCC 13-0402	KU058729	MG520998	KU058719	MG520937	Li <i>et al.</i> 2015; Phookamsak <i>et al.</i> 2017
<i>Xenoseptoria neosaccardoi</i>	CBS 120.43	KF251783	–	KF251280	–	Quaedvlieg <i>et al.</i> 2013
<i>Xenoseptoria neosaccardoi</i> ^(T)	CBS 128665	KF251784	–	KF251281	–	Quaedvlieg <i>et al.</i> 2013
<i>Yunnanensis phragmitis</i>	MFLUCC 17-0315	MF684863	MF684867	MF684862	MF683624	Karunarathna <i>et al.</i> 2017a
<i>Yunnanensis phragmitis</i> ^(T)	MFLUCC 17-1361	MF684865	MF684864	MF684869	–	Karunarathna <i>et al.</i> 2017a

Family PHAEOSPHAERIACEAE M.E.Barr

Kwanghwana Karun., C.H.Kuo & K.D.Hyde, gen. nov.

INDEX FUNGORUM NUMBER. — IF557100.

FACESOFFUNGI NUMBER. — FoF 07775.

ETYMOLOGY. — Refers to the collection site Kwang Hwa.

TYPE SPECIES. — *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov.

DESCRIPTION

Associated with *Miscanthus floridulus* (Poaceae) leaf sheath.

Sexual state

Ascomata. Immersed or semi-immersed, visible as minute black dots on host surface, uniloculate, subglobose, brown to dark brown, solitary; centrally ostiolate, circular, papillate.

Peridium. With 7–9 layers at the apex and 3–4 layers at the base, thinner towards the base, the outer layers composed of brown, thick-walled cells of *textura angularis*, the inner layers composed of hyaline to pale brown, thin-walled cells of *textura prismatica*.

Hamathecium. Composed of numerous, hyaline, frequently septate, broadly cellular wide pseudoparaphyses, often constricted at the septa, branching at the mid-point, embedded in mucilage.

Asci. 8-spored, bitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded with well-developed narrow ocular chamber, smooth-walled, arising from the base of the ascoma.

Ascospores. Fusiform, broadly fusoid with rounded ends, hyaline, overlapping or irregularly biseriate, 3-septate, the cells above and below are broader, upper cell is comparatively broader, smooth-walled with large guttules. *Ascospores* germinate within 12 hours on PDA, germinating from end cells.

Asexual state

Unknown.

NOTES

The new genus *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov., is morphologically and phylogenetically well supported (100% ML/1.00 BYPP).

Genus *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde

Kwanghwana miscanthi Karun., C.H.Kuo & K.D.Hyde, sp. nov.
(Fig. 2)

INDEX FUNGORUM NUMBER. — IF557101.

FACESOFFUNGI NUMBER. — FoF 07776.

ETYMOLOGY. — Refers to the generic name for the host.

HOLOTYPE. — MFLU 18-1950.

CULTURE CHARACTERS. — Colonies on PDA slow growing at 20–25°C, circular, white at the growing edge, light to dark pink in the centre; reverse white at the edge, the centre dense, convex or dome-shaped to umbonate, with entire edge, glabrous with tiny granular on surface, no pigment produced.

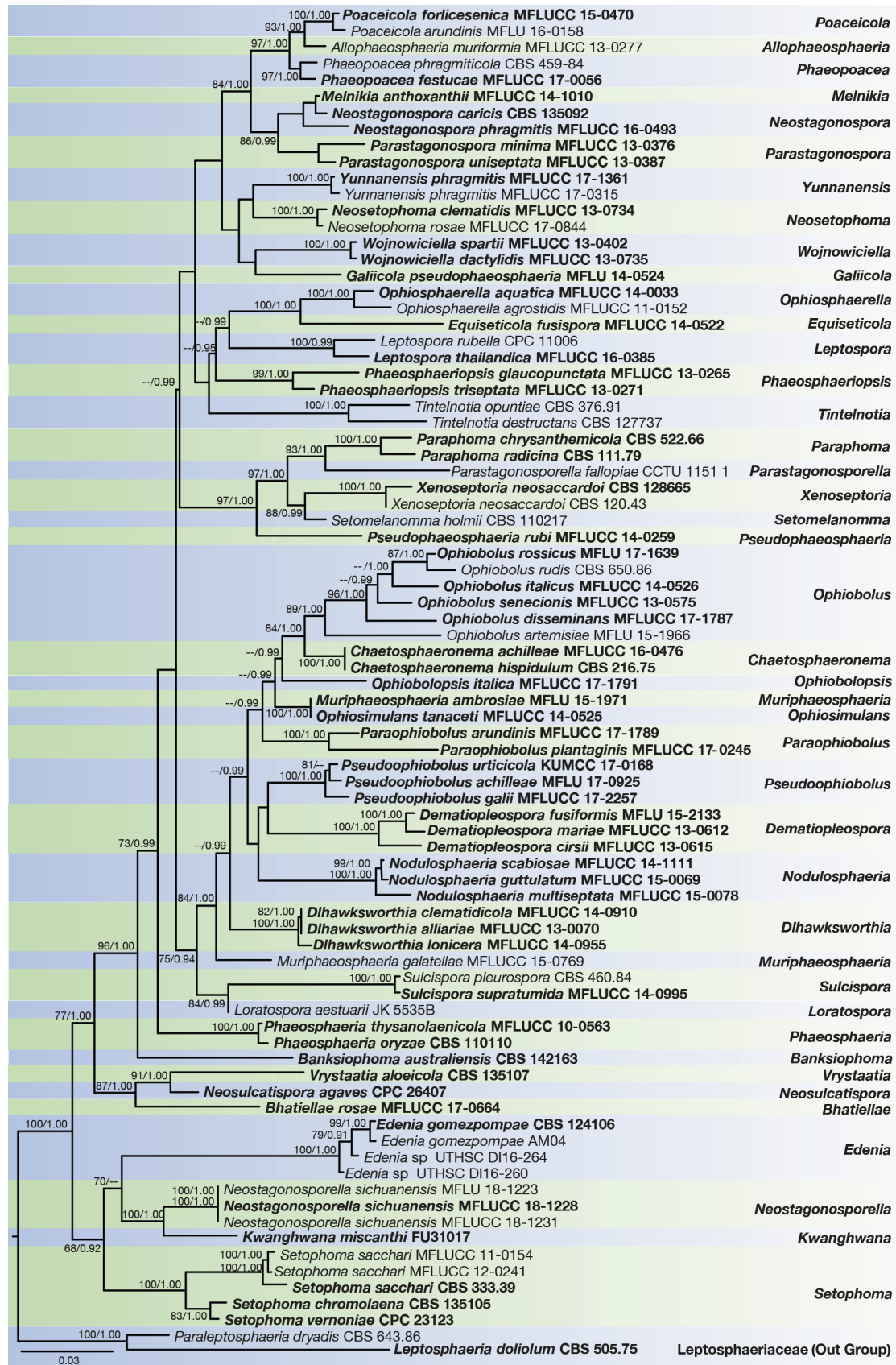


FIG. 1. — Phylogram generated from RAxML analysis based on a combined dataset of LSU, SSU, ITS and TEF1- α partial sequences. Bootstrap support values for maximum likelihood higher than 60% and Bayesian posterior probabilities (BYPP) greater than 0.90 are displayed as above the nodes respectively. The ex-type strains are in bold. New strain is indicated in red. The tree is rooted to *Leptosphaeria dolium* (Pers.) Ces. & De Not. (CBS 505.75) and *Paraleptosphaeria dryadis* (Johanson) Gruyter, Aveskamp & Verkley (CBS 643.86).

TABLE 2. — Morphological comparison of *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov., *Setophoma sacchari* (Bitanc.) Gruyter, Aveskamp & Verkley and *Neostagonospora sichuanensis* C.L.Yang, X.L.Xu & K.D.Hyde.

Characters	<i>Kwanghwana miscanthi</i> Karun., C.H.Kuo & K.D.Hyde, sp. nov.	<i>Setophoma sacchari</i> (Bitanc.) Gruyter, Aveskamp & Verkley	<i>Neostagonospora sichuanensis</i> C.L.Yang, X.L.Xu & K.D.Hyde
Ascomata (size)	130–150 µm high × 160–170 µm diam.	120–180 µm high × 140–190 µm diam	Width (0.5–) 1.4–2.37 (–4.5) × 0.81 1.28 mm ($\bar{x}$ = 1.89 × 1.05 mm, n = 50), 230–340 µm ($\bar{x}$ = 290 µm, n = 20) high
Ascomata (appearance)	Immersed, uniloculate, subglobose, solitary, centrally ostiolate, circular, papillate	Immersed or semi-immersed, uniloculate, globose to subglobose, solitary or gregarious, centrally ostiolate, papillate	Immersed, multi-loculate, ellipsoidal, to globose to subglobose or irregular in shape, solitary to gregarious, generating rhombus to sub- rhombus pale yellow stripes on the ascostromatal fringe, centrally ostiolate, papillate
Peridium	20–30 µm wide, with 7–9 layers at the apex and 3–4 layers at the base, thinner towards the base, <i>textura</i> <i>angularis</i> to <i>textura prismatica</i>	8.5–15 µm wide, with 3–5 layers, <i>textura angularis</i> to <i>textura prismatica</i>	18–35 µm wide, nearly equal in thickness, <i>textura angularis</i> to <i>textura prismatica</i> , with inner hyaline layer
Hamathecium	Numerous, hyaline, frequently septate, broadly cellular 2–3 µm wide pseudoparaphyses, often constricted at the septa, branching at the mid- point, embedded in mucilage	Numerous, hyaline, frequently septate, broadly cellular 1.5–3 µm wide pseudoparaphyses, often constricted at the septa, branching at the apex, embedded in mucilage	Hyaline, septate, filamentous, narrow, 1–2 µm ($\bar{x}$ = 1.59 µm, n = 50) wide, anastomosing pseudoparaphyses, septate, branched, embedded in a hyaline gelatinous matrix
Asci	60–70 × 10–13 µm ($\bar{x}$ = 67.5 × 11 µm), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded with well-developed narrowly ocular chamber, smooth-walled, arising from the base of ascoma	60–75 (–85) × 12–15 (–17) µm ($\bar{x}$ = 67.3 × 14.1 µm, n = 25), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded with well-developed narrowly ocular chamber (1–2.5 µm wide), smooth- walled, arising from the base of ascoma	90–125 × 12.5–14 µm ($\bar{x}$ = 108.1 × 13.3 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short-pedicellate, apically rounded, with an ocular chamber
Ascospores	16–18.5 × 5–6 µm ($\bar{x}$ = 17 × 5 µm), fusiform, broadly fusoid with rounded ends, hyaline, overlapping or irregularly biseriate, 3-septate, the cells above and below are broader, upper cell is comparatively broader, smooth-walled with large guttules	20–23 (–25) × 5–6 µm ($\bar{x}$ = 22.5 × 5.6 µm, n = 30), fusiform, narrowly fusoid with rounded ends, hyaline, overlapping or irregularly biseriate, 3-septate, the cells above and below are broader, upper cell is comparatively broader, smooth-walled with large guttules	30–35 × 6–7 µm ($\bar{x}$ = 31.9 × 6.6 µm, n = 50), fusiform or lengthened long fusiform, narrower towards the ends, sometimes narrower at lower end, hyaline, partially overlapping, 5–8 transversely septate, mostly 7-septate, slightly constricted at the septa, nearly equidistant between septa, smooth-walled, guttulate, surrounded by a mucilaginous sheath, 5–9 µm thick ($\bar{x}$ = 6.9 µm, n = 30)
Hosts	<i>Miscanthus floridulus</i> (Labill.) Warb. ex K.Schum. & Lauterb. (Poaceae)	<i>Saccharum officinarum</i> L. (Poaceae)	<i>Phyllostachys heteroclada</i> Oliv. (Poaceae)
References	This study	Phookamsak <i>et al.</i> (2014b)	Yang <i>et al.</i> (2019)

MATERIAL EXAMINED. — **Taiwan**, Chiayi Province, Kwang Hwa, alt. 1330 m, 23°30.084'N, 120°41.73.5'E on *Miscanthus floridulus* (Poaceae) leaf sheath, 17.III.2018, A. Karunarathna, AKTW 05 (holo-, MFU[MFU18-1952]), ex-type living culture, FU31017, NCYUCC 19-0372.

DESCRIPTION

Associated with *Miscanthus floridulus* (Poaceae) leaf sheath.

Sexual state

Ascomata. 130–150 µm × 160–170 µm. ($\bar{x}$ = 138 × 168 µm, n = 5), immersed or semi-immersed, visible as minute black

dots on host surface, uniloculate, subglobose, brown to dark brown, solitary; centrally ostiolate, circular, papillate.

Peridium. 20–30 µm wide, with 7–9 layers at the apex and 3–4 layers at the base, thinner towards the base, the outer layers composed of brown, thick-walled cells of *textura angularis*, the inner layers composed of hyaline to pale brown, thin-walled cells of *textura prismatica*.

Hamathecium. Composed of numerous, hyaline, frequently septate, broadly cellular 2–3 µm wide pseudoparaphyses, often constricted at the septa, branching at the mid-point, embedded in mucilage.

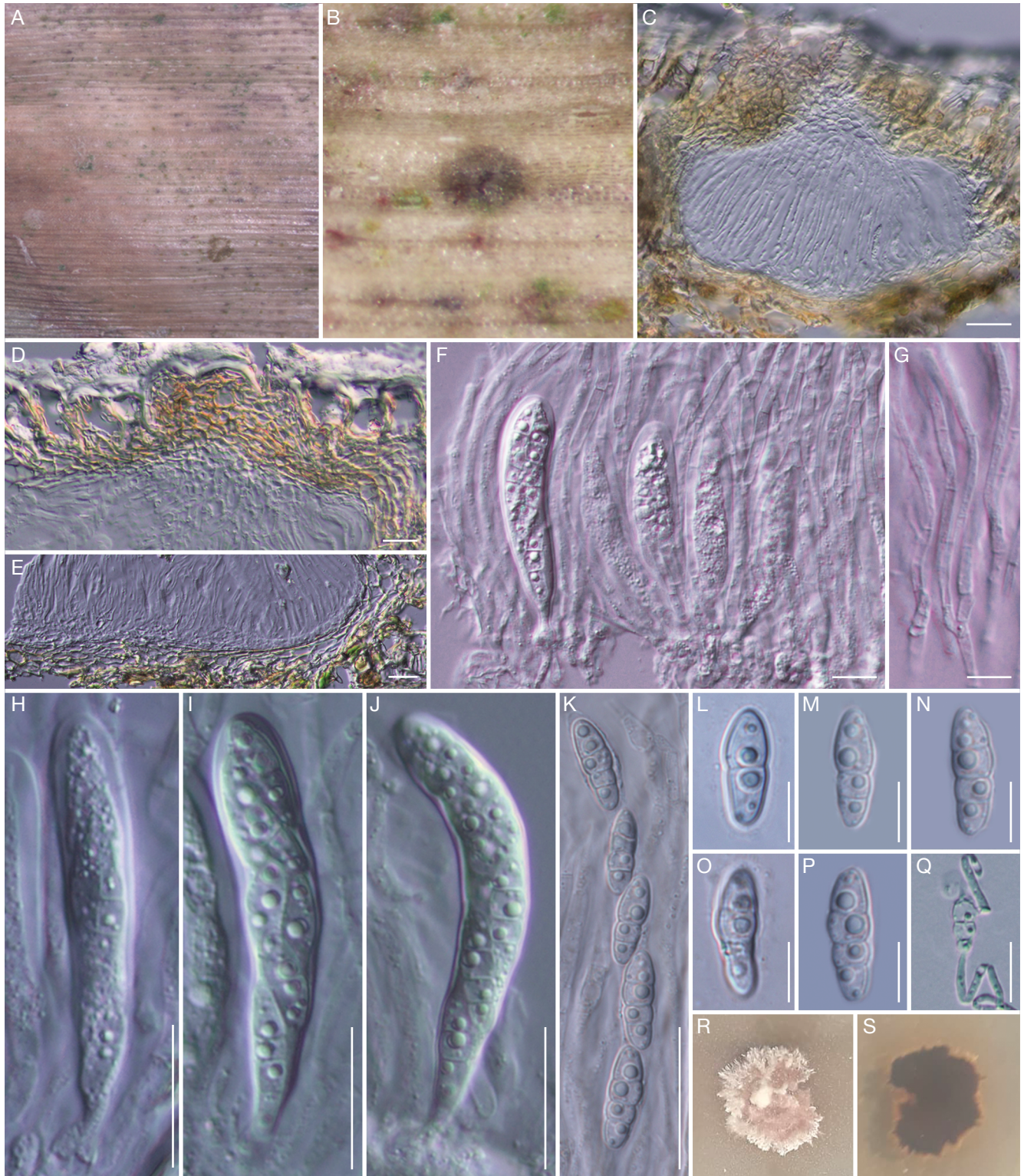


FIG. 2.— *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov. (MFU 18-1950, holotype): **A, B**, appearance of ascomata on the host *Miscanthus floridulus* (Labill.) Warb. ex K.Schum. & Lauterb. (Poaceae) leaf sheath; **C**, section through ascoma; **D**, section through peridium at the apex; **E**, section through peridium at the base; **F**, hamathecium; **G**, Pseudoparaphyses; **H–K**, asci; **L–P**, ascospores; **Q**, germinating ascospore; **R, S**, culture characteristics on PDA (r = from above, s = from below). Scale bars: C, 20 μ m; D–F, 25 μ m; G–K, 20 μ m; L–Q, 25 μ m.

Asci. 60–70 $\times$ 10–13 μ m ($\bar{x}$ = 67.5 $\times$ 11 μ m, n = 30), 8-spored, bitunicate, cylindrical to cylindric-clavate, short pedicellate, apically rounded with well-developed narrowly ocular chamber, smooth-walled, arising from the base of ascoma.

Ascospores. 16–18.5 $\times$ 5–6 μ m ($\bar{x}$ = 17 $\times$ 5 μ m, n = 40), fusi-form, broadly fusoid with rounded ends, hyaline, overlapping or irregularly biseriolate, 3-septate, the cells above and below the middle septum are broader, upper cell is comparatively

broader, smooth-walled with large guttules; germinating within 12 hours on PDA, germinating from end cells.

Asexual state

Unknown.

DISCUSSION

Symbiotic foliar fungal species presence in *Miscanthus sinensis* and *M. floridulus* from Taiwan, based on ITS region was studied by Chiang *et al.*, (2001) and revealed four fungal species which includes, two *Cladosporium* Link species, one *Fusarium* Link and one basidiomycete. Most recent study by Tennakoon *et al.*, (2019) introduced *Phaeosphaeria ampeli* Tennakoon, C.H.Kuo & K.D.Hyde belongs to Phaeosphaeriaceae on *Ficus ampelas* Burm.f from Taiwan with proper taxonomy and phylogenetic study.

The new genus *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov., forms a phylogenetically distinct (100% RAxML/1.00 BYPP) clade in Phaeosphaeriaceae and sister to *Neostagonospora* C.L.Yang, X.L.Xu & K.D.Hyde. The new genus is morphologically also well-supported with its sister clade *Neostagonospora* and adjacent clade *Setophoma* Gruyter, Aveskamp & Verkley. *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov., is phylogenetically more closely related to *Neostagonospora* with a high bootstrap support (100% ML/1.00 BYPP). However, *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov., is distinct from *Neostagonospora sichuanensis* C.L.Yang, X.L.Xu & K.D.Hyde, the type of *Neostagonospora* by its subglobose ascomata, unevenly thickened peridium, numerous, cellular, septate and branched hamathecium and broadly fusoid, 3-septate ascospores (Table 2). *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov., morphologically and phylogenetically differs from *Setophoma*. *Setophoma* was introduced as an asexual genus (de Gruyter *et al.* 2009). Later, Phookamsak *et al.* (2014b) introduced the sexual morph for this genus as *Setophoma sacchari* (Bitanc.) Gruyter, Aveskamp & Verkley. *Kwanghwana miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov., shows similarities in hamathecium characters in having septate, cellular and branched pseudoparaphyses. A morphological comparison of *K. miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov., *N. sichuanensis* and *S. sacchari* is given in Table 2. *Edenia* M.C.González, A.L.Anaya, Glenn, Saucedo & Hanlin was described and introduced to accommodate *Edenia gomez-pompae* M.C.González, A.L.Anaya, Glenn, Saucedo & Hanlin, a sterile endophytic fungus. Therefore, a morphological comparison cannot be performed for this genus (González. *et al.* 2007). Based on both morphology and molecular sequence data, the new genus *Kwanghwana* Karun., C.H.Kuo & K.D.Hyde, gen. nov., is herein introduced and validated with *K. miscanthi* Karun., C.H.Kuo & K.D.Hyde, sp. nov.

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