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Poaceascoma aquaticum sp. nov. (Lentitheciaceae), a new species from submerged bamboo in freshwater

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Abstract

Poaceascoma aquaticum sp. nov. (Lentitheciaceae) was found on decaying bamboo submerged in a freshwater stream in northern Thailand. The new species is introduced based on its unique morphology and phylogeny. *Poaceascoma aquaticum* is characterized by black, semi-immersed or erumpent, papillate ascomata, bitunicate, cylindrical to cylindric-clavate, short pedicellate asci and filiform, pale brown to brown ascospores. Phylogenetic analyses of combined LSU, SSU and RPB2 sequence data shows that *Poaceascoma aquaticum* forms a robust clade with *P. helicoides* in the family Lentitheciaceae (Pleosporales). *Poaceascoma aquaticum* is described, illustrated and compared with related taxa.

Key words: Aquatic fungi, Lentitheciaceae, Phylogeny, Taxonomy

Introduction

Freshwater ascomycetes are an ecological assemblage that occur on submerged or partially submerged woody substrates in freshwater habitats, including both sexual and asexual species (Shearer 1993, 2001, Hyde *et al.* 2015). These taxa play a key role in the degradation of organic carbon (Wong *et al*. 1998; Gulis *et al*. 2006; Krauss *et al*. 2011). Both sexual and asexual morphs of ascomycetes as well as a few basidiomycetes have been found on various substrates in freshwater in lentic (lakes, ponds) and lotic (rivers, streams, creeks, peat swamps) habitats (Wong *et al.* 1999, Cai *et al.* 2003a, b, Jones & Choeyklin 2008, Hyde *et al*. 2015). The freshwater fungi of the Asian region have been particularly well-studied with collections made from Australia, China, Malaysia, Philippines and Thailand (Nawawi 1985, Kuthubutheen 1987, Hyde 1995, Sivichai *et al.* 2000, Ho *et al.* 2001, 2002, Cai *et al.* 2003a, b, Goh and Tsui 2003, Luo *et al*. 2004, Sivichai & Jones 2004, Jones *et al*. 2007, Hu *et al*. 2010, Kurniawati *et al*. 2010); and the data are summarized by Hyde *et al*. (2015). Despite this, collections in unstudied streams will often result in the discovery of new species.

This manuscript is part of a study on the taxonomy and diversity of microfungi on substrates in freshwater, along a north-south latitudinal gradient from China through to New Zealand (Hyde *et al*. 2015). We have previously introduced several new taxa (Luo *et al.* 2015, Su *et al.* 2015a, b, Liu *et al.* 2015a). In this paper, we introduce a new species, *Poaceascoma aquaticum,* in the family Lentitheciaceae (Pleosporales). The new species is defined based on its unique morphological characters as well as support from phylogenetic analyses of combined LSU, SSU and RPB2 sequence data.

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Materials and methods

Isolation and morphology

Decaying bamboo was collected in November 2013 from a freshwater stream in Chiang Rai Province, Thailand and returned to the laboratory in plastic Ziploc bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. The samples were processed and examined following the methods described by Taylor and Hyde (2003). Morphological observations were made under a Nikon SMZ-171 dissecting microscope and captured by using a Cannon EOS 600D camera on a Nikon Eclipse 80i compound microscope.

Single ascospore isolations were made to obtain pure cultures as described in Chomnunti *et al*. (2014). The pure cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Dali University Culture Collection (DLUCC). Herbarium specimens are deposited at the herbarium of Mae Fah Luang University (MFLU) and the herbaria of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Faces of fungi numbers are as detailed in Jayasiri *et al*. (2015).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh fungal mycelium grown on PDA at 25°C. The EZ gene™ Fungal gDNA kit (GD2416) was used to extract DNA according to the manufacturer's instructions. The primer pair LROR and LR7 was used to amplify partial large subunits of the nuclear ribosomal RNA gene (LSU) (Vilgalys & Hester 1990). The PCR thermal cycle program for LSU, SSU amplification were as follows: initially 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50 °C for 40 seconds, elongation at 72 °C for 90 seconds, and final extension at 72°C for 10 minutes. The PCR thermal cycle program for the partial RNA polymerase second largest subunit (*RPB2*) was followed as initially 95 °C for 5 mins, followed by 40 cycle of denaturation at 95 °C for 1 mins, annealing at 52 °C for 2 mins, elongation at 72 °C for 90 seconds, and finalextension at 72°C for 10 mins. PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amershamproduct code: 27–9602–01). The PCR products were observed on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were carried at Shanghai Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, P.R. China).

Phylogenetic analysis

The sequences generated from this study were analyzed with sequence data from related taxa and others from families in the suborder Massarineae (Pleosporales) as obtained from GenBank using the dataset in Phookamsak *et al*. (2015). The consensus sequences were then initially aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and further improved using Bioedit v.5.0.6 (Hall 2001) and ClustalX v. 1.83 (Thompson *et al*. 1997) to allow maximum alignment and maximum sequence similarity.

A maximum likelihood analysis was performed using RAxMLGUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 100 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMA substitution model.

Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

Bayesian analyses were performed by using PAUP v.4.0b10 (Swofford 2002) and MrBayes v3.0b4 (Ronquist & Huelsenbeck 2003). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.0b4 (Liu *et al.* 2012). Six simultaneous Markov Chains were run for 1 m generations and trees were sampled every 100th generation (Resulting 10000 total trees) (Cai *et al.* 2006). The first 2000 trees representing the burn-in phase of the analyses were discarded and the remaining 8000 (post burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai *et al.* 2006, Liu *et al.* 2012).

Trees were viewed in Treeview (Page 1996). Sequences derived in this study are deposited in GenBank (Table 1).

TABLE 1. Isolates used in this study and their GenBank accession numbers. The ex-type strains are in bold; the newly generated sequences are indicated in red.

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
Deniquelata barringtoniae **T/Ts**	**MFLUCC 11-0422**	**JX254655**	**JX254656**	–
Didymosphaeria rubi-ulmifolii **T**	**CBS 100299**	**JX496124**	**AY642523**	–
Falciformispora lignatilis **Ts**	BCC 21117	GU371826	GU371834	–
Falciformispora lignatilis **Ts**	BCC 21118	GU371827	GU371835	–
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**	–
Keissleriella poagena **T**	**CBS 136767**	**KJ869170**	–	–
Keissleriella trichophoricola **T**	**CBS 136770**	**KJ869171**	–	–
Lentithecium aquaticum **I T**	**CBS 123099**	**GU301823**	**GU296156**	–
Lentithecium arundinaceum	CBS 619.86	GU301824	GU296157	–
Lentithecium fluviatile **Ts**	CBS 122367	GU301825	GU296158	–
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**	–
Neottiosporina paspali	CBS 331.37	EU754172	EU754073	GU371779
Ophiosphaerella sasicola	MAFF 239644	AB524599	AB524458	–
Palmiascoma gregariascomum **T/Ts**	**MFLUCC 11–0175**	**KP744495**	**KP753958**	–
Paraconiothyrium minitans	CBS 122788	EU754173	EU754074	GU371776
Paraphaeosphaeria michotii **T/Ts**	**MFLUCC 13-0349**	**KJ939282**	**KJ939285**	–
Phaeodothis winteri	CBS 182.58	GU301857	GU296183	–
Poaceascoma aquaticum **T/Ts**	**MFLUCC 14-0048**	**KT324690**	**KT324691**	**KT373846**
Prosthemium canba	JCM 16966	AB553760	AB553646	–
Setoseptoria phragmitis **T/Ts**	**CBS 114802**	**KF251752**	–	–
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**	–

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.

Results

Phylogeny

Combined analyses of LSU, SSU and RPB2 sequence data were used to determine the taxonomic placement of our strain. The dataset comprised 45 taxa with *Melanomma pulvis-pyrius* (CBS 124080) as the outgroup taxon and the manually adjusted dataset comprised 2998 characters including gaps. 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.

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, Phookamsak *et al.* 2015). *Poaceascoma aquaticum* clusters with *P. helicoides* with high support values (99% MP, 99% ML and 1.00 PP), basal to *Stagonospora macropycnidia* (CBS 114202), *Setoseptoria phragmitis* which clustered with *Lentithecium arundinaceum* (95% MP, 97% ML and 1.00 PP) and may be the asexual morph of *Lentithecium*.

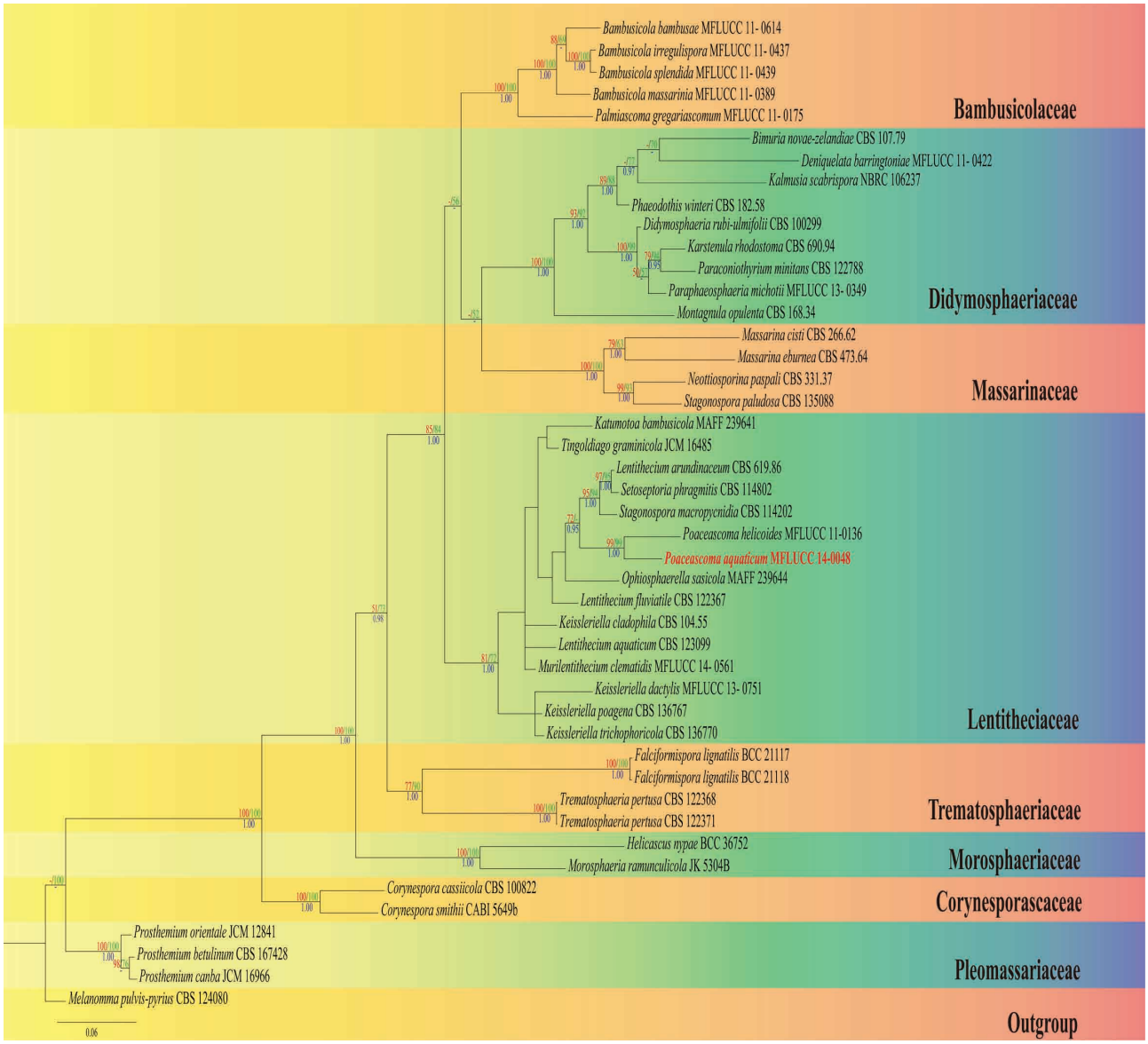

FIGURE 1. Phylogram generated from maximum likelihood analysis (RAxML) based on combined LSU, SSU and RPB2 sequenced data of the families in the suborder Massarineae. Bootstrap support values for maximum likelihood (ML, red) and maximum parsimony (MP, green) equal to or greater than 70% are given above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

Taxonomy

Poaceascoma aquaticum Z.L. Luo & K.D. Hyde, *sp. nov.* **FIGURE 2.**

Index Fungorum: IF551351

Facesoffungi number: FoF00910

Etymology: With reference to the habitat of this taxon.

Holotype: MFLU 15−0075

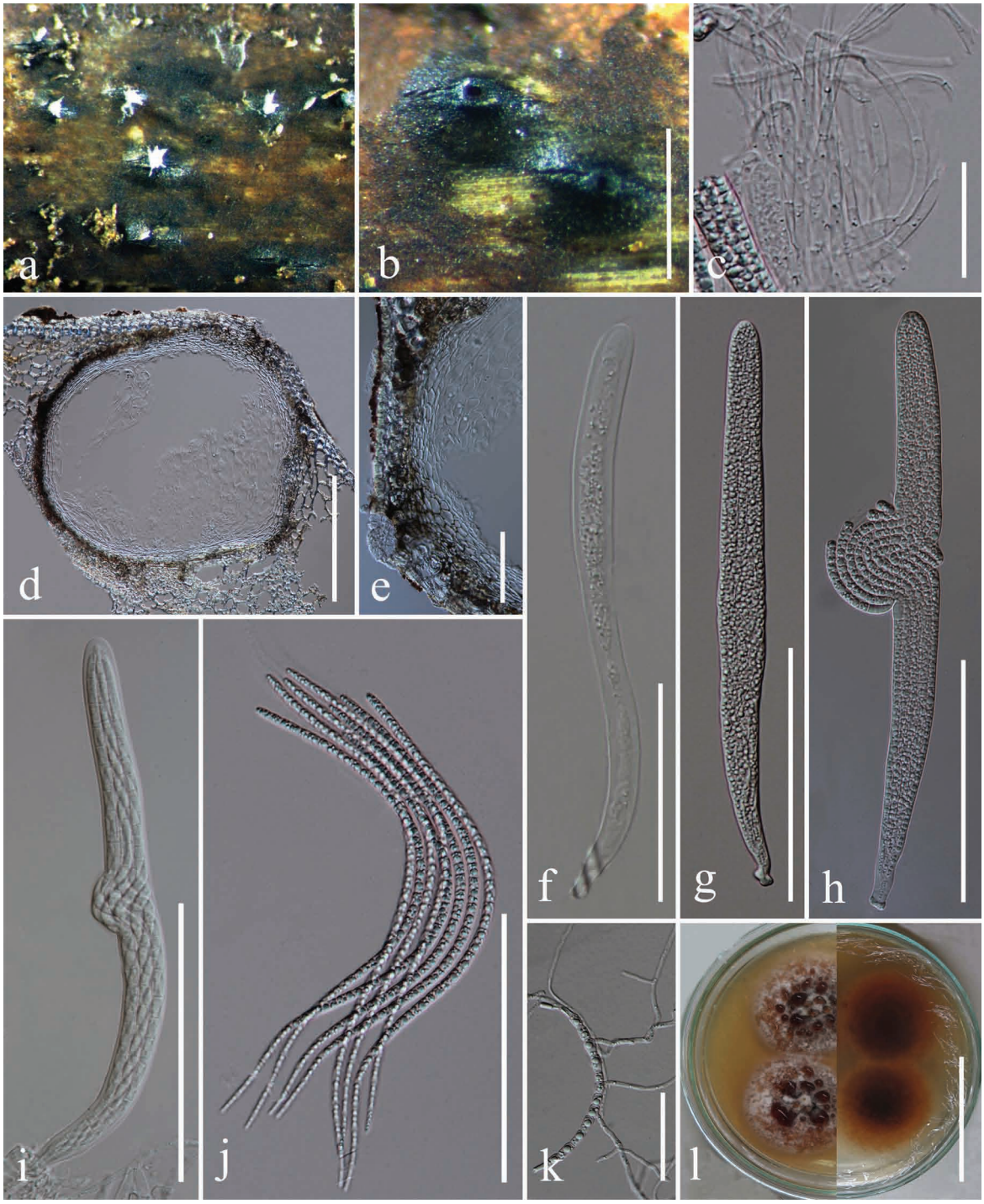

FIGURE 2. *Poaceascoma aquaticum* (MFLU 15−0075, **holotype**). **a**, **b**. Ascomata on submerged wood. **c**. Pseudoparaphyses. **d.** Sections through ascomata. **e.** Section of peridium. **f**–**i.** Asci. **j.** Ascospores. **k.** Germinating ascospore. **l.** Colony on MEA. Scale bars: b = 500 µm, c = 20 µm, d, f–j = 100 µm, e = 50 µm, k = 20 µm, l = 30 mm.

Saprobic on decaying bamboo submerged in freshwater. **Sexual morph**: *Ascomata* 280−380 µm high, 310−500 µm diam, scattered, solitary, semi-immersed or erumpent through host tissue, uniloculate, globose to subglobose, glabrous, black, papilla, visible as raised, dark spots on host surface, ostiole central, with periphyses. *Peridium* 23−40 µm wide, of equal thickness, composed of several layers of pseudoparenchymatous cells, outer layer comprising several layers of pale black cells, arranged in a *textura angularis,* inner layer comprising several layers of hyaline, flattened cells, arranged in a *textura prismatica*. *Hamathecium* composed of numerous, 2−4.5 µm wide, filamentous, broad, cellular pseudoparaphyses, with distinct septa, embedded in a mucilaginous matrix. *Asci* 184.5–210.5 ×12–16 µm ($\bar{x}$ = 197.5 × 14 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, pedicellate, apically rounded with an ocular chamber. *Ascospores* 214–231 × 4.2–5.2 µm ($\bar{x}$ = 223 × 4.7 µm, n = 20), fasciculate, arranged spirally in ascus, filiform, widest at the rounded apex and tapering towards the rounded base, pale brown to brown, 21–36-septate, not constricted at the septa, smooth-walled. **Asexual morph**: Undetermined.

Material examined:—THAILAND. Chiang Rai Province: (N 20°3’39”, E 99°52’29”), saprobic on decaying bamboo submerged in a stream, November 2013, Z.L. Luo ZL-26 (MFLU 15–0075!, **holotype**); ex-type culture, MFLUCC 14-0048, DLUCC; *ibid.* (HKAS 86450, **isotype**).

Notes:—The scolecosporous *Poaceascoma* species was collected from decaying culms of bamboo submerged in a freshwater stream in northern Thailand. There are many genera forming filiform ascospores in Pleosporales, such as *Leptospora*, *Ophiobolus* and *Ophiosphaerella* (Shoemaker 1976, Boonmee *et al.* 2011, Zhang *et al.* 2012, Wijayawardene *et al*. 2014, Hyde *et al*. 2013, Ariyawansa *et al*. 2014, 2015). Phookamsak *et al*. (2015) introduced a new genus *Poaceascoma* in Lentitheciaceae, Pleosporales to accommodate the species associated with Poaceae which formed setose ascoma and filiform ascospores. *Poaceascoma aquaticum* resembles *P. helicoides* in having uni-loculate, immersed ascomata, a peridium of *textura angularis* to *textura prismatica* and filiform, multi-septate ascospores arranged spirally in the ascus. However, *P. aquaticum* differs from *P. helicoides* in having a thinner, pale black to hyaline peridium without setae, wider and longer asci ($\bar{x}$ = 197.5 × 14 µm, versus 173.5 × 9.4 µm) and ascospores ($\bar{x}$ = 223 × 4.7 µm, versus 178.1 × 2.4 µm). The phylogenetic data also confirmed them as distinct taxa (Figure 1).

Discussion

Zhang *et al*. (2012) introduced the family Lentitheciaceae in the order Pleosporales to accommodate massarina-like species, typified by *Lentithecium* with *L. fluviatile* as the type species. Species of the Lentitheciaceae occur on herbaceous plants such as *Phragmites* (*Lentithecium fluviatile* (Aptroot & Van Ryck.) K.D. Hyde *et al*., *L. arundinaceum* (Sowerby) K.D. Hyde *et al*., *Tingoldiago graminicola* K. Hirayama & Kaz. Tanaka) and on submerged wood *(Lentithecium aquaticum* Yin. Zhang *et al*.) in freshwater environments (Zhang *et al*. 2012). *Katumotoa bambusicola* Kaz . Tanaka & Y. Harada and *Ophiosphaerella sasicola* (Nagas. & Y. Otani) Shoemaker & C.E. Babc. are bambusicolous species known from Japan (Nagasawa & Otani 1977; Tanaka & Harada 2005). Hyde *et al.* (2013) included *Katumotoa*, *Keissleriella*, *Lentithecium, Setoseptoria* and *Tingoldiago* in *Lentitheciaceae* based on multigene phylogenetic analyses. Phookamsak *et al.* (2015) introduced a new genus *Poaceascoma* to accommodate the ophiosphaeriella-like taxa in Lentitheciaceae and mentioned that the scolecosporous fungi were polyphyletic in the class Dothideomycetes. Presently, *Katumotoa*, *Keissleriella*, *Lentithecium, Setoseptoria*, *Tingoldiago* and *Poaceascoma* are accepted in the family (Hyde *et al.* 2013, Liu *et al* 2015b, Phookamsak *et al.* 2015). We included all of these genera of Lentitheciaceae in the phylogenetic tree (Figure. 1) to determine the placement of our taxon. Several clades in Lentitheciaceae were not well-resolved, which may be due to limited sequence data in GenBank, however the genus *Poaceascoma* is strongly supported.

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