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Scolecocyhalosporium thailandense sp. nov. (Parabambusicolaceae, Pleosporales) collected on *Imperata* sp. (Poaceae) in northern Thailand

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Abstract

In the present study, a new species, *Scolecocyhalosporium thailandense*, is introduced based on morphological and molecular approaches. The species was found as a saprobe occurring on *Imperata* sp. (Poaceae) in terrestrial habitats in Chiang Rai Province, Thailand. This species is characterized by solitary, semi-immersed to erumpent, subglobose to ampulliform, papillate ascomata, dark brown pseudoparenchymatous peridium, fissitunicate, cylindrical to subcylindrical asci embedded in a hyaline, filamentous to cellular pseudoparaphysate hamathecium, and filiform, yellowish, septate ascospores. Phylogenetic analyses based on a concatenated ITS, LSU, SSU, and *TEF1-α* sequence matrix demonstrated that *S. thailandense* formed a well-resolved clade with *S. submersum* (the type species of this genus) and *Scolecocyhalosporium* sp. within the Parabambusicolaceae. Therefore, *S. thailandense* is introduced herein as the second species of the genus *Scolecocyhalosporium*. Morphological characteristics, illustrations, and updated phylogenetic analyses are provided, and notes on species distinctiveness with closely related taxa are discussed.

Keywords: A novel species, Dothideomycetes, Fungi on grasses, Taxonomy, Thai mycota

Introduction

Grass-inhabiting fungi have been relatively well-studied worldwide since the 19th century (Parbery 1967, Sivanesan 1987, Clay 1988, Wong & Hyde 2001, Bhilabutra *et al.* 2010, Purahong & Hyde 2011, Dai *et al.* 2017, Dayarathne *et al.* 2017, Thambugala *et al.* 2017, Goonasekara *et al.* 2018, Karunarathna *et al.* 2022). However, comprehensive taxonomic studies of grass-inhabiting fungi based on morphological and molecular approaches, especially ascomycetous fungi, have been well-investigated over the last decade. Although, these studies were restricted to some fungal genera or specific hosts (Manamgoda *et al.* 2011, Phookamsak *et al.* 2015b, Tanaka *et al.* 2015, Dai *et al.* 2017, Dayarathne *et al.*

2017, Marin-Felix *et al.* 2017, Thambugala *et al.* 2017, Crouch *et al.* 2022). *Imperata* (Poaceae) is considered one of the top ten most problematic weeds in the world as it causes over 62–90% yield losses of different crops (MacDonald 2004, Estrada & Flory 2015, Javaid *et al.* 2015, Kato-Noguchi 2022). However, taxonomic studies of fungi associated with this plant are poorly documented. According to Karunaratna *et al.* (2022), less than 20 fungal species have been reported from *Imperata* species worldwide.

Taxa in the family Parabambusicolaceae are relatively well-studied based on morphological characteristics and multigene phylogenetic analyses, and the family is classified in the suborder Massarineae, order Pleosporales, class Dothideomycetes (Hongsanan *et al.* 2020). Presently 12 genera are accommodated in this family, which include *Aquastroma* Kaz. Tanaka & K. Hiray. (Tanaka *et al.* 2015), *Lonicericola* Phookamsak, Jayasiri & K.D. Hyde (Phookamsak *et al.* 2019), *Multilocularia* Phookamsak, Ariyaw. & K.D. Hyde (Li *et al.* 2016), *Multiseptospora* Phookamsak & K.D. Hyde (Liu *et al.* 2015), *Neoaquastroma* Wanas., E.B.G. Jones & K.D. Hyde (Wanasinghe *et al.* 2017), *Neomultiseptospora* N. Xie, Phookamsak & Hongsanan (Xie *et al.* 2022), *Parabambusicola* Kaz. Tanaka & K. Hiray. (Tanaka *et al.* 2015), *Paramonodictys* N.G. Liu, K.D. Hyde & J.K. Liu (Hyde *et al.* 2020), *Paramultiseptospora* Phookamsak & H.B. Jiang (Phookamsak *et al.* 2022), *Paratrimmatostroma* Jayasiri *et al.* (Phookamsak *et al.* 2019), *Pseudomonodictys* Doilom *et al.* (Ariyawansa *et al.* 2015a), and *Scolecohyalosporium* N. Xie, Phookamsak & Hongsanan (Xie *et al.* 2022).

Species of Parabambusicolaceae are saprobic in nature and occur on a wide range of hosts in both aquatic and terrestrial environments, and are mainly distributed in China (Guizhou and Yunnan), Japan, and Thailand (Ariyawansa *et al.* 2015a, Liu *et al.* 2015, Tanaka *et al.* 2015, Li *et al.* 2016, Phukhamsakda *et al.* 2018, Phookamsak *et al.* 2019, 2022, Samarakoon *et al.* 2019, Dong *et al.* 2020, Hyde *et al.* 2020, Yasanthika *et al.* 2020, Bao *et al.* 2022, Wijayawardene *et al.* 2022, Xie *et al.* 2022, Yang *et al.* 2022). Tanaka *et al.* (2015) erected the family to accommodate two novel genera, *Aquastroma* and *Parabambusicola*, and also included two unidentified species of *Monodictys* and *Multiseptospora* in this family. Subsequently, several monotypic genera viz. *Lonicericola*, *Multilocularia*, *Neoaquastroma*, *Neomultiseptospora*, *Paramonodictys*, *Paramultiseptospora*, *Paratrimmatostroma*, *Pseudomonodictys*, and *Scolecohyalosporium* were introduced in this family (Li *et al.* 2016, Wanasinghe *et al.* 2017, Phookamsak *et al.* 2019, 2022, Hyde *et al.* 2020, Xie *et al.* 2022). However, the species numbers of these genera have increased in recent years (Phukhamsakda *et al.* 2018, Samarakoon *et al.* 2019, Yasanthika *et al.* 2020, Bao *et al.* 2022, Wijayawardene *et al.* 2022, Yang *et al.* 2022). There are currently 24 species accommodated in Parabambusicolaceae (Ariyawansa *et al.* 2015a, Liu *et al.* 2015, Tanaka *et al.* 2015, Li *et al.* 2016, Wanasinghe *et al.* 2017, Phukhamsakda *et al.* 2018, Phookamsak *et al.* 2019, 2022, Samarakoon *et al.* 2019, Dong *et al.* 2020, Hyde *et al.* 2020, Yasanthika *et al.* 2020, Bao *et al.* 2022, Wijayawardene *et al.* 2022, Xie *et al.* 2022, Yang *et al.* 2022).

Scolecohyalosporium was introduced as a monotypic genus by Xie *et al.* (2022) and is typified by *S. submersum* Phookamsak, Hongsanan & N. Xie. *Scolecohyalosporium submersum* was documented as a saprobe that occurred on grass submerged in a freshwater stream in Xishuangbanna, Yunnan, China. Only the sexual morph is known for the genus. It is characterized by solitary, scattered or clustered, erumpent to superficial, uni-loculate, conical to ovoid, glabrous, rough-walled ascogonia, with central, carbonaceous ostiole perched on truncate apex; composed of small, dark brown to black, pseudoparenchymatous celled peridium; fissitunicate, long cylindrical, short pedicellate asci, embedded in a dense, filamentous, branched, septate, cellular pseudoparaphysate hamathecium, and spiral, hyaline, filiform, multi-septate, smooth-walled ascospores (Xie *et al.* 2022).

Morphologically, *Scolecohyalosporium* can be distinguished from other sexual morph genera in Parabambusicolaceae in having scolecosporous ascospores, whereas other sexual genera of Parabambusicolaceae have phragmosporous ascospores (Liu *et al.* 2015, Tanaka *et al.* 2015, Li *et al.* 2016, Wanasinghe *et al.* 2017, Phookamsak *et al.* 2019, 2022, Xie *et al.* 2022). *Scolecohyalosporium* was introduced to accommodate a single species with a different morphology from other known genera in Parabambusicolaceae. The genus was well-established based on multigene phylogenetic evidence (Xie *et al.* 2022). This study aims to introduce a novel species associated with *Imperata* (Poaceae) in Thailand based on a morpho-molecular approach.

Materials and methods

Sample collection, isolation and morphological examination

Grass samples used in our study were collected from Chiang Rai, Thailand, in June 2010 and brought to the laboratory for observation and isolation. The fungus was found on a dead culm of *Imperata* sp. (Poaceae). Ascogonia visualized

on the host surface were observed under an Olympus SZH10 stereomicroscope and photographed by a Sony DSC-T110 digital camera. Micro-morphological characteristics (e.g., ascomata, peridial structure, pseudoparaphyses, asci, and ascospores) were examined under a Nikon ECLIPSE 80i compound microscope with DIC microscopy and captured by a Canon 550D digital camera fitted to a Nikon ECLIPSE 80i compound microscope. The ascospores were stained with India Ink to check the mucilaginous sheath or appendages. Measurements of ascomata, peridia, pseudoparaphyses, asci, and ascospores were made with Tarosoft (R) Image Frame Work program. The photographic plate of morphological characteristics was edited and combined using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA). The specimen was dried with silica gel in a paper bag for preservation as a fugarium and deposited at the Mae Fah Luang University (MFLU) herbarium, Chiang Rai, Thailand.

Pure fungal colonies were obtained by single spore isolation following the spore suspension technique (Senanayake *et al.* 2020). Two sets of five ascospores germinated on water agar medium (WA; 15 g/l sterile distilled water) and, after overnight incubation, were each transferred to new malt extract agar plates (MEA; 33.6 g/l sterile distilled water, Difco malt extract, USA) and potato dextrose agar plates (PDA; 39 g/l sterile distilled water, Difco potato dextrose, USA). The plates were then incubated at room temperature (25–35°C) for 1–4 weeks. The characteristics and growth of fungal colonies and sporulation *in vitro* were observed at two and four-week intervals. The pure cultures were preserved in PDA for short-term storage, and in sterilized distilled water and 10% glycerol for long-term storage. The cultures were then deposited at Mae Fah Luang University Culture Collection (MFLUCC). Index Fungorum number and Faces of Fungi number were registered for the new taxon following the guidelines described in the Index Fungorum (Index Fungorum 2023) and the Faces of Fungi (Jayasiri *et al.* 2015) databases. The justification of new species was followed as in Jeewon & Hyde (2016).

DNA extraction, PCR amplification and sequencing

Fungal genomic DNA was extracted from fresh mycelium using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, P.R. China), following the protocol guidelines provided. Amplicons were generated by polymerase chain reaction (PCR) with the target genes. Primers ITS5 and ITS4 (White *et al.* 1990) were used to amplify the internal transcribed spacers region of ribosomal DNA (ITS; ITS1-5.8S-ITS2), LR0R and LR5 (Vilgalys & Hester 1990) were used to amplify the partial 28S large subunit nuclear ribosomal DNA (LSU), NS1 and NS4 (White *et al.* 1990) were used to amplify the partial 18S small subunit rDNA (SSU), and EF1-983F and EF1-2218R (Rehner 2001) were used to amplify the translation elongation factor 1-alpha (*TEF1-α*). The total volume of PCR components was 25 µL, containing 2 µL of DNA template (50 ng/µL), 1 µL of each forward and reward primer, 12.5 µL of PCR MasterMix (mixture of *EasyTaq*™ DNA Polymerase, dNTPs, and optimized buffer; Beijing TransGen Biotech Co., Ltd., Chaoyang District, Beijing, China) and 8.5 µL of the sterilized double-distilled water (ddH₂O). The PCR thermal cycling of these genes was set as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 50 sec, elongation at 72 °C for 1 min, final extension at 72 °C for 10 min and hold on at 4 °C. Amplicons were sent to TsingKe Biological Technology, Kunming City, Yunnan Province, China for purification and sequencing.

Phylogenetic analyses

Consensus sequences were assembled by BioEdit 7.1.3.0 (Hall 1999) and subjected to the nucleotide BLAST search in the GenBank database (www.ncbi.nlm.nih.gov/blast/) for preliminary identification. Reference sequences were retrieved from the nucleotide BLAST search results and relevant publications (Phookamsak *et al.* 2022, Xie *et al.* 2022; TABLE 1). Individual gene datasets of ITS, LSU, SSU, and *TEF1-α* gene regions were aligned using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>; Katoh *et al.* 2019). Ambiguous sites were trimmed and manually edited where necessary with BioEdit 7.1.3.0 (Hall 1999). Aligned individual gene datasets were first analyzed using maximum likelihood (ML) to check the compatibility of tree topologies. A concatenated ITS, LSU, SSU, and *TEF1-α* sequence dataset was further analyzed by maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses to determine phylogenetic relationships of the novel species with other related taxa in Parabambusicolaceae.

Maximum likelihood (ML) analysis was implemented using the Randomized Axelerated Maximum Likelihood program on raxmlGUI v. 7.4.2 graphical interface (Silvestro & Michalak 2012) using the GTR + GAMMAI model of nucleotide substitution with 1000 rapid bootstrap replicates (Miller *et al.* 2010). Maximum parsimony (MP) analysis was executed by PAUP v.4.0b10 (Swofford 2002) using the heuristic search option. Starting tree(s) were obtained via stepwise additions with 100 replicates of randomized sequence addition. The tree-bisection reconnection (TBR) was used to determine the branch-swapping algorithm. All characters were of type unordered and had equal weight. Gaps were treated as missing data, and multistate taxa were interpreted as uncertainty. MaxTree(s) setting was 1,000, and

branches collapsed if the maximum branch length was zero. The stability of the tree was evaluated by 1,000 bootstrap replications. All parsimonious trees were saved. Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC], and Homoplasy Index [HI] were calculated by Kishino–Hasegawa tests (KHT) to determine the generated trees under different optimality criteria.

Bayesian inference (BI) was analyzed by MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). MrModeltest v. 2.3 (Nylander 2004) was used to determine the best-fit model of nucleotide substitution and incorporated into the analysis, of which GTR+I+G was the best-fit model for a combined dataset under the Akaike Information Criterion (AIC). Thus, GTR+I+G model was selected using the following parameters: Prset statefreqpr = dirichlet (1,1,1,1); Lset nst = 6 rates = invgamma for BI analysis. Bayesian posterior probabilities (PP) were computed by Markov chain Monte Carlo sampling (BMCMC) (Huelsenbeck & Ronquist 2001, Zhaxybayeva & Gogarten 2002, Ronquist *et al.* 2012). Two parallel runs with six simultaneous Markov chains were run for 2,000,000 generations, and trees were sampled every 100th generation (resulting in 20,001 trees). The first 4,000 trees were set as burn-in and were discarded. The remaining trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. Phylograms were visualized by FigTree v1.4.0 program (Rambaut 2012) and edited in Microsoft Office PowerPoint 2016 (Microsoft Inc., Redmond, WA, USA). The photographic tree was made using Adobe Photoshop CS6 software (Adobe Systems, USA). The nucleotide sequences of the new taxon were registered for the GenBank accession numbers. The final alignment and tree were submitted in TreeBASE (<https://www.treebase.org>; accessed on 14 February 2023) under submission ID: 30132 (<http://purl.org/phylo/treebase/phylovs/study/TB2:S30132?x-access-code=b9542e81d558e99e065163c86da6cad0&format=html>).

Results

Phylogenetic analyses

Phylogenetic relationships of *Scolecophyalosporium thailandense* with related species in Parabambusicolaceae and other related families in suborder Massarineae were clarified based on maximum likelihood, maximum parsimony and Bayesian inference analyses of a concatenated ITS, LSU, SSU, and *TEF1- α* sequence dataset. The data matrix comprises 69 taxa, including 68 ingroup taxa in families Bambusicolaceae, Dictyosporiaceae, Didymosphaeriaceae, Lentitheciaceae, Macrodiplodiopsidaceae, Parabambusicolaceae, Sulcatisporaceae, and Trematosphaeriaceae. While *Melanomma pulvis-pyrius* CBS 124080 (Melanommataceae) was selected as the outgroup taxon. A concatenated sequence matrix consists of 3,828 total characters, including gaps (ITS = 1–600 bp, LSU = 601–1,486 bp, SSU = 1,487–2,890 bp, and *TEF1- α* = 2,891–3,828 bp). RAxML analysis has 1,389 distinct alignment patterns, with a 27.35% proportion of gaps and completely undetermined characters. The final ML optimization likelihood was -25879.239798, and the gamma distribution shape parameter alpha was 0.195009. The Tree-Length was 2.831996 with substitution rates AC = 1.166385, AG = 2.525122, AT = 1.396055, CG = 0.983314, CT = 5.725257, GT = 1.000000 and the estimated base frequencies were as follows: A = 0.236098, C = 0.253834, G = 0.272190, T = 0.237878. The best-scoring RAxML tree was selected as a backbone tree to represent the phylogenetic relationships of the new taxon with other related species in this study (FIGURE 1).

Maximum parsimony (MP) analysis retained 29 maximum parsimonious trees, of which the first parsimonious tree was selected as the best tree for the Kishino–Hasegawa test (TL = 4289, CI = 0.402, RI = 0.702, RC = 0.283, HI = 0.598). Of 3,828 total characters, 2,664 were constant, 385 variable characters were parsimony-uninformative, and 779 characters were parsimony-informative. Bayesian posterior probabilities (PP) from MCMC were evaluated with the final average standard deviation of split frequencies = 0.005954.

Phylogenetic analyses based on maximum likelihood, maximum parsimony, and Bayesian inference criteria resulted in similar overall tree topologies. The new species, *Scolecophyalosporium thailandense*, clustered with *S. submersum* (KUN-HKAS 122242, KUMCC 21-0412, and KUMCC 21-0413) and *Scolecophyalosporium* sp. (KUMCC 21-0507, and KUMCC 21-0508) with significant support (85% ML, 92% MP and 1.00 PP; FIGURE 1). Species of *Scolecophyalosporium* formed a well-separated subclade within Parabambusicolaceae. However, the phylogenetic relationships between *Scolecophyalosporium* and other related genera (*viz.* *Neomultiseptospora*, *Multiseptospora*, and *Paramultiseptospora*, respectively) were not well-resolved in the present study. Furthermore, most genera in Parabambusicolaceae formed well-resolved subclades, except for monotypic genera *Aquastroma* and *Pseudomonodictys*. *Aquastroma* formed a single lineage basal to the genus *Paramonodictys* with low support. Whereas, *Pseudomonodictys* formed a separate branch basal to *Lonicericola* and *Paratrimmatostroma* with low support. More taxon sampling of these genera is acquired for resolving better phylogenetic resolutions in further studies.

TABLE 1. Taxa information used in the present phylogenetic analyses and their corresponding GenBank accession numbers. The ex-type strains are indicated by superscript “T” after the taxon name. The new taxon name is indicated in black bold, and unavailable information is represented by “n/a”.

Taxon name	Strain/specimen no.	Host	Origin	GenBank accession numbers			
				ITS	LSU	SSU	<i>TEFI-α</i>
<i>Aquastroma magniostiolata</i> ^T	MAFF 243824/ HHUF 30122	Woody plant	Japan	NR_153583	NG_056936	NG_061000	AB808486
<i>Bambusicola loculata</i> ^T	MFLUCC 13-0856	Bamboo	Thailand	NR_153609	NG_069267	NG_065061	KP761724
<i>Bambusicola massarinia</i> ^T	MFLUCC 11-0389	Bamboo	Thailand	NR_121548	NG_058658	NG_061198	KP761725
<i>Camarographium koreanum</i> ^T	CBS 117159	<i>Cornus kousa</i>	South Korea	JQ044432	JQ044451	n/a	n/a
<i>Dendryphiella fasciculata</i> ^T	MFLUCC 17-1074	Decaying wood	Thailand	NR_154044	NG_059177	n/a	n/a
<i>Dictyocheirospora aquatica</i> ^T	KUMCC 15-0305	Decaying submerged wood	Yunnan, China	NR154030	KY320513	n/a	n/a
<i>Dictyosporium elegans</i>	NBRC 32502	n/a	n/a	DQ018087	DQ018100	DQ018079	n/a
<i>Didymocrea sadasivani</i> ^T	CBS 438.65	Soil under <i>Oryza sativa</i>	India	MH858658	DQ384103	DQ384074	n/a
<i>Didymosphaeria rubi- ulmifolii</i> ^T	MFLUCC 14-0023	<i>Rubus ulmifolius</i>	Italy	n/a	KJ436586	NG_063557	n/a
<i>Falciformispora lignatilis</i>	BCC 21117	n/a	n/a	KF432942	GU371826	GU371834	GU371819
<i>Katumotoa bambusicola</i> ^T	MAFF 239641/ HHUF 28661	<i>Sasa kurilensis</i>	Japan	NR_154103	NG_059386	NG_060989	AB539108
<i>Lonicericola fuyuanensis</i> ^T	MFLU 19-2850	<i>Caprifolioideae</i> sp.	Yunnan, China	NR_172419	NG_073809	NG_070329	MN938324
<i>Lonicericola hyaloseptispora</i> ^T	KUMCC 18-0149	<i>Lonicera maackii</i>	Yunnan, China	NR_164294	NG_066434	NG_067680	n/a
<i>Lonicericola hyaloseptispora</i> ^T	KUMCC 18-0150	<i>Lonicera maackii</i>	Yunnan, China	MK098194	MK098200	MK098206	MK098210
<i>Lonicericola qujingensis</i> ^T	GMBCC1178	<i>Magnolia grandiflora</i>	Yunnan, China	NR_182717	OM855602	NG_148904	OM857556
<i>Macrodiplodiopsis desmazieri</i> ^T	CBS 140062	<i>Platanus</i> sp.	Switzerland	NR_132924	NG_058182	n/a	n/a
<i>Magnicamarosporium iriomotense</i> ^T	MAFF 243827/ HHUF 30125	<i>Diplospora dubia</i>	Japan	NR_153445	NG_059389	NG_060999	AB808485
<i>Melanomma pulvis-pyrius</i> ^T	CBS 124080/ IFRD2001	<i>Salix caprea</i>	France	MH863349	MH874873	GU456302	GU456265
<i>Monodictys</i> sp.	JO 10	<i>Phragmites</i> sp.	Japan	n/a	AB807552	AB797262	AB808528
<i>Multilocularia bambusae</i> ^T	MFLUCC 11-0180	Bamboo	Thailand	NR_148099	NG_059654	NG_061229	KU705656
<i>Multiseptospora thailandica</i> ^T	MFLUCC 11-0183	<i>Thysanolaena maxima</i>	Thailand	NR_148080	NG_059554	KP753955	KU705657
<i>Multiseptospora thailandica</i>	MFLUCC 11-0204	<i>Thysanolaena maxima</i>	Thailand	KU693447	KU693440	KU693444	KU705659
<i>Multiseptospora thailandica</i>	MFLUCC 12-0006	<i>Thysanolaena maxima</i>	Thailand	KU693448	KU693441	KU693445	KU705660
<i>Multiseptospora thysanolaenae</i> ^T	MFLUCC 11-0202	<i>Thysanolaena maxima</i>	Thailand	n/a	NG_059655	NG_063600	KU705658
<i>Neoaquastroma bauhiniae</i> ^T	MFLUCC 16-0398	<i>Phanera variegata</i>	Thailand	NR_165217	NG_067814	NG_070696	MH028247
<i>Neoaquastroma bauhiniae</i>	MFLUCC 17-2205	<i>Bauhinia purpurea</i>	Thailand	MH025953	MH023320	MH023316	MH028248

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TABLE 1. (Continued)

Taxon name	Strain/specimen no.	Host	Origin	GenBank accession numbers			
				ITS	LSU	SSU	<i>TEFI-a</i>
<i>Neoaquastroma guttulatum</i> ^T	MFLUCC 14-0917	Herbaceous plant	Thailand	KX949739	KX949740	KX949741	KX949742
<i>Neoaquastroma krabiense</i> ^T	MFLUCC 16-0419	<i>Barringtonia acutangula</i>	Thailand	NR_165218	NG_067815	NG_067670	MH028249
<i>Neobambusicola strelitziae</i> ^T	CBS 138869	<i>Strelitzia nicolai</i>	South Africa	NR_137945	NG_058125	n/a	MG976037
<i>Neomultiseptospora yunnanensis</i> ^T	KUMCC 21-0411	Bamboo	Yunnan, China	OL898884	OL898925	OL898890	OL964282
<i>Neomultiseptospora yunnanensis</i> ^T	KUN-HKAS 122240	Bamboo	Yunnan, China	OL898885	OL898886	OL898891	OL964283
<i>Palmiascoma gregariascomum</i> ^T	MFLUCC 11-0175	Palm	Thailand	NR_154316	NG_059557	KP753958	n/a
<i>Parabambusicola aquatica</i> ^T	MFLUCC 18-1140	Submerged wood	Thailand	NR_171877	NG_073791	n/a	n/a
<i>Parabambusicola bambusina</i>	MAFF 239462/ H 4321	<i>Sasa kurilensis</i>	Japan	n/a	AB807536	AB797246	AB808511
<i>Parabambusicola bambusina</i>	MAFF 243823/ KH 139	<i>Sasa</i> sp.	Japan	n/a	AB807537	AB797247	AB808512
<i>Parabambusicola bambusina</i>	MAFF 243822/ KT 2637	<i>Sasa kurilensis</i>	Japan	n/a	AB807538	AB797248	AB808513
<i>Parabambusicola hongheensis</i> ^T	KUMCC 21-0410	Bamboo	Yunnan, China	OL898880	OL898921	OL898886	n/a
<i>Parabambusicola thysanolaenae</i> ^T	KUMCC 18-0147	<i>Thysanolaena maxima</i>	Yunnan, China	NR_164044	NG_066435	NG_067681	MK098209
<i>Parabambusicola thysanolaenae</i>	KUMCC 18-0148	<i>Thysanolaena maxima</i>	Yunnan, China	MK098193	MK098198	MK098202	MK098211
<i>Paraconiothyrium estuarinum</i> ^T	CBS 109850	An estuarine sediment polluted with industrial discharges	Brazil	NR_166007	MH874432	AY642522	n/a
<i>Paraphaeosphaeria michotii</i> ^T	MFLUCC 13-0349	Poaceae sp.	Italy	NR_155640	NG_059522	KJ939285	n/a
<i>Paramonodictys hongheensis</i> ^T	KUMCC 21-0343	<i>Mangifera indica</i>	Yunnan, China	ON350762	NG_081549	NG_081438	OL505582
<i>Paramonodictys hongheensis</i>	KUMCC 21-0346	<i>Mangifera indica</i>	Yunnan, China	OL436235	OL436224	OL436225	OL505583
<i>Paramonodictys solitarius</i> ^T	GZCC 20-0007	Decaying wood	Guizhou, China	MN901152	MN897835	MN901118	MT023012
<i>Paramonodictys solitarius</i>	MFLUCC 17-2353	Submerged wood	Thailand	MT627707	MN913703	MT864299	MT954397
<i>Paramonodictys solitarius</i>	MAFF 243826/ KH 331	Herbaceous plant	Japan	n/a	AB807553	AB797263	AB808529
<i>Paramonodictys yunnanensis</i> ^T	KUMCC 21-0337	<i>Mangifera indica</i>	Yunnan, China	NR_175767	NG_081538	NG_081426	OL505585
<i>Paramonodictys yunnanensis</i>	KUMCC 21-0347	<i>Mangifera indica</i>	Yunnan, China	OL436233	OL436228	OL436234	OL505586
<i>Paramultiseptospora bambusae</i> ^T	KUN-HKAS 122241A	Bamboo	Yunnan, China	ON077075	ON077064	ON077070	ON075058

.....continued on the next page

TABLE 1. (Continued)

Taxon name	Strain/specimen no.	Host	Origin	GenBank accession numbers			
				ITS	LSU	SSU	<i>TEFI-a</i>
<i>Paramultiseptospora bambusae</i>	KUN-HKAS 122241B	Bamboo	Yunnan, China	ON077076	ON077065	ON077071	ON075059
<i>Paratrimmatostroma kunmingensis</i> ^T	KUN-HKAS 102224A	Fern	Yunnan, China	MK098192	MK098196	MK098204	MK098208
<i>Paratrimmatostroma kunmingensis</i> ^T	KUN-HKAS 102224B	Fern	Yunnan, China	MK098195	MK098201	MK098207	n/a
<i>Poaceascoma helicoides</i> ^T	MFLUCC 11-0136	<i>Digitaria sanguinalis</i>	Thailand	NR_154317	NG_059565	NG_061205	KP998461
<i>Pseudochaetosphaeronema larense</i> ^T	CBS 640.73	Mycetoma of <i>Homo sapiens</i>	Venezuela	NR_132038	NG_057978	NG_061147	KF015684
<i>Pseudocoleophoma calamagrostidis</i> ^T	CBS 139700/ HHUF 30450	<i>Calamagrostis matsumurae</i>	Japan	NR_154375	NG_059804	NG_061264	LC014614
<i>Pseudomonodictys tectonae</i> ^T	MFLUCC 12-0552	<i>Tectona grandis</i>	Thailand	n/a	NG_059590	NG_061213	KT285571
<i>Scolecohyalosporium</i> sp.	KUMCC 21-0507	Dead wood	Yunnan, China	OQ158949	OQ170871	OQ168222	n/a
<i>Scolecohyalosporium</i> sp.	KUMCC 21-0508	Dead wood	Yunnan, China	OQ158950	OQ170872	OQ168223	n/a
<i>Scolecohyalosporium submersum</i> ^T	KUMCC 21-0412	Submerged grass	Yunnan, China	OL898883	OL898924	OL898889	OL964281
<i>Scolecohyalosporium submersum</i> ^T	KUMCC 21-0413	Submerged grass	Yunnan, China	OL898881	OL898922	OL898887	OL964279
<i>Scolecohyalosporium submersum</i> ^T	KUN-HKAS 122242	Submerged grass	Yunnan, China	OL898882	OL898923	OL898888	OL964280
<i>Scolecohyalosporium thailandense</i>^T	MFLUCC 11-0128	<i>Imperata</i> sp.	Thailand	OQ437403	OQ437404	OQ437402	OQ435570
<i>Setoseptoria phragmitis</i> ^T	CBS 114802	Phragmites australis	Hong Kong, China	KF251249	KF251752	n/a	KF253199
<i>Spegazzinia tessarthra</i>	SH 287/ HHUF 27691	Balsa wood	Japan	n/a	AB807584	AB797294	AB808560
<i>Sulcatispora acerina</i> ^T	KT 2982/ HHUF 30449	<i>Acer palmatum</i>	Japan	LC014597	LC014610	LC014605	LC014615
<i>Sulcatispora berchemiae</i> ^T	KT 1607/ HHUF 29097	<i>Berchemia racemosa</i>	Japan	NR_153444	NG_059390	NG_064843	AB808509
<i>Tingoldiagio graminicola</i> ^T	KH 68/ HHUF 30009	<i>Phragmites australis</i>	Japan	n/a	AB521743	AB521726	AB808561
<i>Trematosphaeria grisea</i> ^T	CBS 332.50	Mycetoma of <i>Homo sapiens</i>	Chile	NR_132039	NG_057979	NG_062930	KF015698
<i>Trematosphaeria pertusa</i> ^T	CBS 122368	<i>Fraxinus excelsior</i>	France	NR_132040	NG_057809	n/a	n/a

Abbreviation BCC: Belgian Coordinated Collections of Microorganisms; **CBS**: the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **GZCC**: Guizhou Culture Collection, Guizhou, China; **H**: Y. Harada; **HHUF**: Herbarium of Hiroasaki University, Japan; **IFRD**: International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China; **JO**: J. Onodera; **KH**: K. Hirayama; **KT**: Kazuaki Tanaka, Japan; **KUMCC**: Kunming Institute of Botany Culture Collection, Yunnan, China; **KUN-HKAS**: Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Yunnan, China; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC**: NITE Biological Resource Centre, Japan; **SH**: S. Hatakeyama.

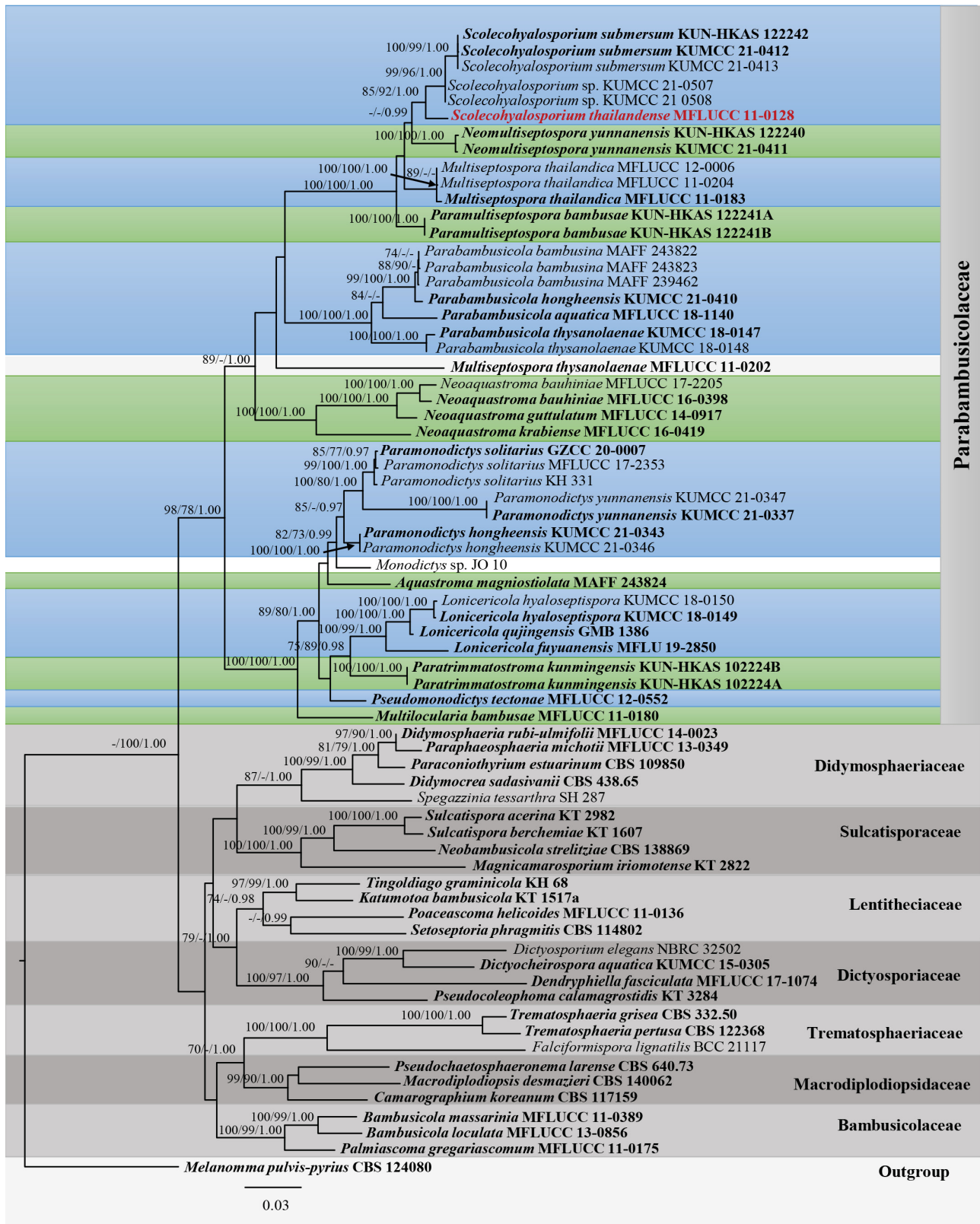


FIGURE 1. Phylogram generated by RAxML analysis based on a concatenated ITS, LSU, SSU, and *TEF1- α* sequence matrix representing the phylogenetic relationships of a novel species in Parabambusicolaceae and related families in suborder Massarineae. The tree is rooted with *Melanomma pulvis-pyrius* (CBS 124080). Bootstrap support values for ML and MP equal to or greater than 70%, and the Bayesian posterior probabilities equal to or higher than 0.95 PP are indicated at the nodes as ML/MP/PP. Support values lower than 70% and 0.95 were indicated by a hyphen (-). Ex-type strains are in black bold, and the newly generated strain is indicated in red bold.

Taxonomy

Scolecocyhalosporium thailandense Phookamsak & Hongsanan, *sp. nov.* FIGURE 2

Index Fungorum number: IF 900245; *Facesoffungi number:* FoF 14015

Etymology: The specific epithet “*thailandense*” refers to the locality in Thailand, where the holotype was collected.

Holotype: MFLU 11-0164

Saprobic on dead culm of *Imperata* sp. (Poaceae). **Sexual morph** *Ascomata* 180–250 µm high (including papilla), 145–370 µm diam., scattered solitary, semi-immersed to erumpent through the host tissue, visible as a black, shiny knob on host surface, subglobose to ampulliform, or irregular in shape, uni-loculate, glabrous, with a central, rounded to truncate ostiole, perched on a subconical to mammiform apical papilla, filled with hyaline periphyses. *Peridium* 10–30 µm wide, of equal thickness, composed of several layers of flattened, dark brown pseudoparenchymatous cells, paler towards the inner layers, arranged in *textura angularis* to *textura prismatica*. *Hamathecium* composed of numerous, 2–4 µm wide, filamentous, septate, broad, cellular pseudoparaphyses, constricted at the septa, tapering towards the apex, anastomosed above the asci, embedded in a hyaline gelatinous matrix. *Asci* 138–160(–170) × 7–10 µm (\bar{x} = 153.7 × 9.1 µm, n = 25), 8-spored, bitunicate, cylindrical to subcylindric-clavate, subsessile to short pedicellate, apically rounded, with subconical ocular chamber clearly visible in the immature state. *Ascospores* (135–)140–160(–166) × 2–3 µm (\bar{x} = 150.7 × 2.8 µm, n = 15), overlapping, parallelly to spirally arranged, filiform, tapering towards the lower cell, pale yellowish to yellowish, curved, smooth-walled, with 15–25 non-constricted septa, lacking a mucilaginous sheath. **Asexual morph** Undetermined.

Culture characteristics:—Colonies on PDA reaching 32–35 mm diam. after 2 weeks at room temperature (25–35 °C). Colony dense, circular, slightly raised, to low convex, surface smooth, with edge entire, floccose; from above, dark brown to greenish-black; from below, black; not producing pigmentation on agar medium.

Material examined:—Thailand, Chiang Rai Province, Mae Fah Luang District, Doi Tung, on a dead culm of *Imperata* sp. (Poaceae), 29 June 2010, R. Phookamsak, RP0044 (MFLU 11-0164, **holotype!**), ex-type living culture = MFLUCC 11-0128.

Notes:—Based on the nucleotide BLAST search of ITS sequence, *Scolecocyhalosporium thailandense* (strain MFLUCC 11-0128) showed the closest similarity with *Multiseptospora* sp. strain SFC20220920-G141 with 98.23% similarity (Identities = 556/566 bp, with one gap), and *Scolecocyhalosporium* sp. GCR-2022a isolate KUMCC 21-0508 (97.96% similarity, Identities = 528/539 bp, with one gap) and isolate KUMCC 21-0507 (97.95% similarity, Identities = 525/536 bp, with one gap). *Scolecocyhalosporium thailandense* (strain MFLUCC 11-0128) is also similar to the generic type, *S. submersum* strain HKAS 122242 (strain from type material), with 97.15% similarity (Identities = 478/492 bp, with one gap). The nucleotide BLAST search of LSU sequence indicated that *S. thailandense* (strain MFLUCC 11-0128) is similar to *S. submersum* strains HKAS 122242, KUMCC 21-0412, and KUMCC 21-0413 with 98.78% similarity (Identities = 809/819 bp, with one gap), and to *Scolecocyhalosporium* sp. GCR-2022a (isolates KUMCC 21-0508 and KUMCC 21-0507) with 98.66% similarity (Identities = 808/819 bp, with one gap). The nucleotide BLAST search of *TEF1-α* sequence indicated that *S. thailandense* (strain MFLUCC 11-0128) has the closest similarity with *Neomultiseptospora yunnanensis* (strains KUN-HKAS 122240 and KUMCC 21-0411) with 97.04% similarity (Identities = 885/912 bp, with no gap), to *Paramultiseptospora bambusae* (voucher KUN-HKAS 122241B and KUN-HKAS 122241A) with 96.80% similarity (Identities = 907/937 bp, with no gap), and to *Multiseptospora thailandica* strain MFLUCC 12-0006 with 96.69% similarity (Identities = 818/846 bp, with no gap). *Scolecocyhalosporium thailandense* is also similar to the generic type, *S. submersum* (strains KUN-HKAS 122242 and KUMCC 21-0413), with 96.63% similarity (Identities = 888/919 bp, with no gap).

Based on a nucleotide pairwise comparison, *Scolecocyhalosporium thailandense* (strain MFLUCC 11-0128) differs from *S. submersum* (KUMCC 21-0412, ex-type strain) in 17/465 bp of ITS (3.65%), 10/850 bp of LSU (1.18%), and 33/928 bp of *TEF1-α* (3.56%). The species is also different from *Scolecocyhalosporium* sp. GCR-2022a (isolates KUMCC 21-0508 and KUMCC 21-0507) in 10/465 bp of ITS (2.15%) and 11/818 bp of LSU (1.34%), while *TEF1-α* sequence data of *Scolecocyhalosporium* sp. GCR-2022a (isolates KUMCC 21-0508 and KUMCC 21-0507) is unavailable.

Morphologically, *Scolecocyhalosporium thailandense* can be easily distinguished from *S. submersum* (HKAS 122242, generic type) in having smaller sized ascomata (180–250 × 145–370 µm vs. 370–480 × 380–600 µm), asci (138–160(–170) × 7–10 µm vs. (200–)250–300(–370) × 7–9(–11) µm), and ascospores ((135–)140–160(–166) × 2–3

μm vs. $(230\text{--}260\text{--}285\text{--}315) \times 1.5\text{--}2.2 \mu\text{m}$) (Xie *et al.* 2022). *Scolecocyhalosporium thailandense* also differs from *S. submersum* in having pale yellowish to yellowish and 15–25-septate ascospores, whereas *S. submersum* has hyaline and multi-septate ascospores (up to 20 septa) (Xie *et al.* 2022). Multigene phylogenetic evidence also supported their distinctiveness (FIGURE 1). Therefore, *S. thailandense* is introduced herein as the new species following the justification guidelines of Jeewon & Hyde (2016).

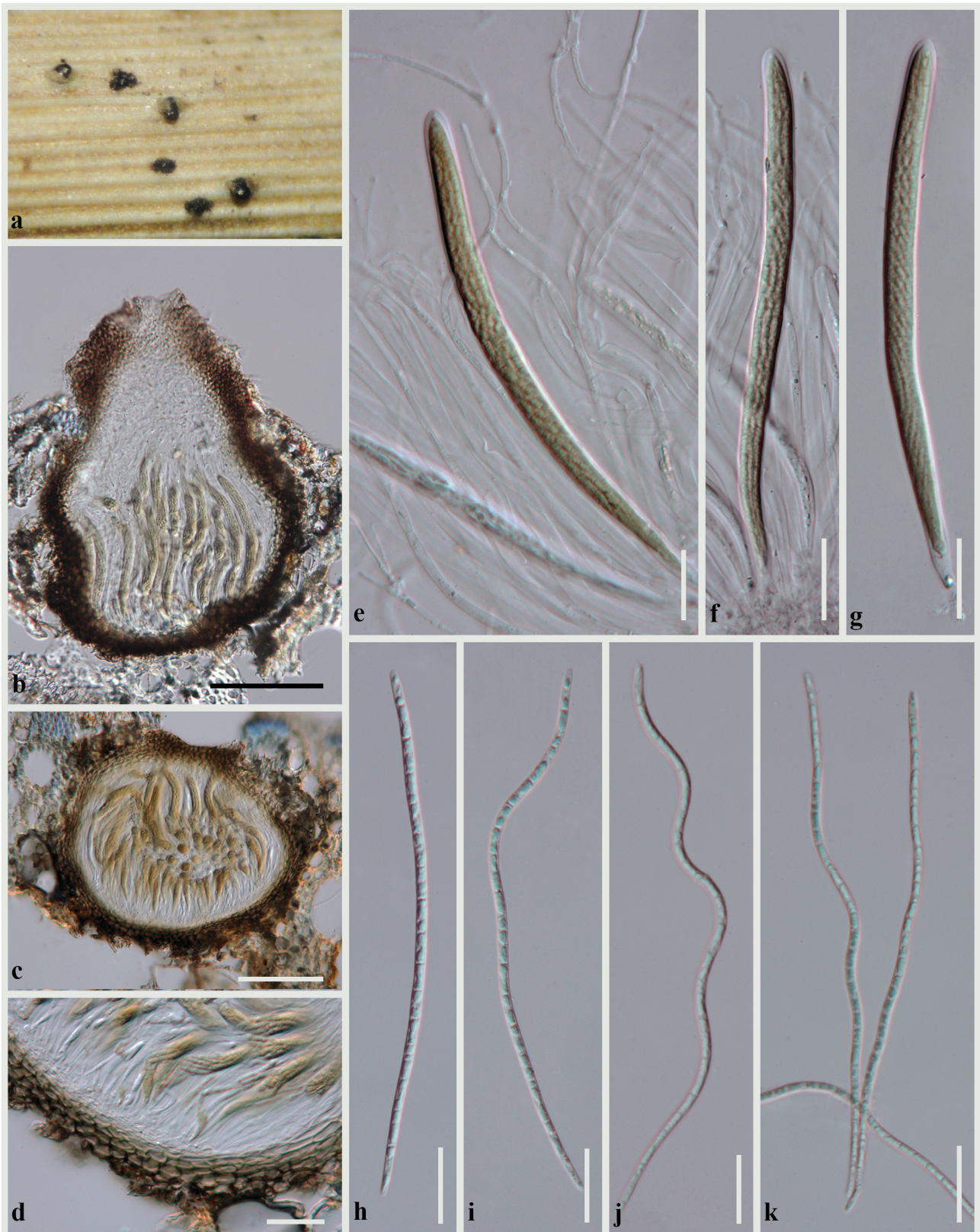


FIGURE 2. *Scolecocyhalosporium thailandense* (MFLU 11-0164, holotype). **a.** Appearance of ascomata on the host surface. **b, c.** Vertical section through ascomata. **d.** Section through peridium. **e, f.** Asci embedded in pseudoparaphyses. **g.** Ascus. **h–k.** Ascospores. Scale bars: **b, c** = 100 μm , **d–k** = 20 μm .

Discussion

Taxonomic studies of Dothideomycetes on *Imperata* sp. in Thailand are not well studied and publicized. Goonasekara *et al.* (2018) provided a checklist of microfungi associated with grasses in Thailand and reported only three Dothideomycete species on *Imperata* sp., including *Curvularia variabilis* Y. Marín, Senwana & Crous (Marin-Felix *et al.* 2017), an ophiostroma-like species (Phookamsak 2016) and *Stagonospora imperaticola* Phukhams., Thambug. & K.D. Hyde (Thambugala *et al.* 2017). Hence, it is highly possible that there are many undescribed species awaiting discovery on this grass.

Scolecospore genera such as *Neophiosphaerella* Kaz. Tanaka & K. Hiray, *Poaceascoma* Phookamsak & K.D. Hyde in Lentitheciaceae (Phookamsak *et al.* 2015a, Tanaka *et al.* 2015), *Alternariaster* E.G. Simmons in Leptosphaeriaceae (Ariyawansa *et al.* 2015b) and *Leptospora* Rabenh., *Nodulosphaeria* Rabenh., *Ophiobolus* Riess, *Ophiobolopsis* Phookamsak *et al.*, *Ophiosimulans* Tibpromma, Camporesi & K.D. Hyde, *Ophiosphaerella* Speg., *Paraophiobolus* Phookamsak, Wanas. & K.D. Hyde, *Pseudoophiobolus* Phookamsak, Wanas. & K.D. Hyde in Phaeosphaeriaceae showed to be polyphyletic in the Pleosporales based on a combined ITS, LSU, SSU, *TEF1- α* and *RPB2* phylogenetic analyses (Ariyawansa *et al.* 2015a, Hyde *et al.* 2016, Mapook *et al.* 2016, Tibpromma *et al.* 2016, Phookamsak *et al.* 2017). However, these genera can be distinguished from each other based on morphological features and multigene phylogenetic evidence. In the present study, the new species, *Scolecophyalosporium thailandense*, is introduced as the second species of the genus *Scolecophyalosporium*. This new finding supports the phylogenetic placement of *Scolecophyalosporium* in Parabambusicolaceae as the first scolecospore genus in this family. Both *S. submersum* and *S. thailandense* were collected from grasses (Poaceae), while *Scolecophyalosporium* sp. GCR-2022a was reported on dead wood. However, *S. submersum* was found as a saprobe submerged in a freshwater stream in Xishuangbanna, Yunnan, China (*ca.* 660 msl), whereas *S. thailandense* as a saprobe in a terrestrial environment in Chiang Rai, Thailand (*ca.* 1,300 msl). This shows that the members of the genus can live in a wide range of hosts, habitats, and elevations.

Multigene phylogenetic analyses demonstrated that *Scolecophyalosporium* formed a well-resolved subclade within the Parabambusicolaceae and is closely related to *Multiseptospora*, *Neomultiseptospora*, and *Paramultiseptospora*, concurring with Phookamsak *et al.* (2022) and Xie *et al.* (2022). However, *Scolecophyalosporium* is unique in having scolecospore ascospores, whereas these three genera shared similar morphologies in having phragmosporous ascospores (Phookamsak *et al.* 2022, Xie *et al.* 2022). *Multiseptospora*, *Neomultiseptospora*, and *Paramultiseptospora* were introduced as monotypic genera whose morphologies do not significantly support their distinctiveness, but their generic statuses have relied on molecular phylogeny. Phylogenetic relationships of these genera are not well-resolved, which may be affected by insufficient species number. Therefore, more species of these genera need to be studied to resolve their generic statuses.

In the present study, two unidentified species of *Monodictys* previously identified by Tanaka *et al.* (2015) were found clustered within the *Paramonodictys* with significant support (FIGURE 1). *Monodictys* sp. strain KH 331 was identified as *Paramonodictys solitarius* by Hyde *et al.* (2020) based on multigene phylogenetic analyses, which provided evidence that this strain formed a high support subclade with the ex-type strain of *P. solitarius* (GZCC 20-0007). While *Monodictys* sp. strain JO 10 formed a separate branch basal to the genus *Paramonodictys* with significant support (82% ML, 73% MP, and 0.99 PP) in the present study. *Monodictys* sp. strain JO 10 possibly belongs to the genus *Paramonodictys* based on phylogenetic affinity. However, the morphological characteristics of this taxon have not yet been studied. Furthermore, the phylogenetic placement of the type species of *Monodictys* (*M. putredinis* (Wallr.) S. Hughes) has not yet been clarified. Therefore, *Monodictys* sp. strain JO 10 is tentatively instated in *Monodictys*, pending further study of its novelty.

Multiseptospora thysanolaenae (strain MFLUCC 11-0202) formed a distinct lineage and separated from *M. thailandica* (the generic type) in the present study, concurring with previous studies (Hyde *et al.* 2020, Yasanthika *et al.* 2020, Phookamsak *et al.* 2022, Xie *et al.* 2022). Xie *et al.* (2022) suggested that *M. thysanolaenae* may represent a distinct genus from *M. thailandica* based on phylogenetic evidence. However, the sequence data available in GenBank is not well-verified. Thus, the congeneric status of these two species needs to be clarified.

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