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Three new *Hermatomyces* species (*Lophiotremataceae*) on *Pandanus odorifer* from Southern Thailand

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Abstract

Investigations on microfungi on *Pandanus odorifer* (*Pandanaceae*) from southern Thailand resulted in the discovery of three new species of *Hermatomyces* (*Lophiotremataceae*). Phylogenetic analyses of combined LSU, ITS, SSU, RPB2 and TEF1 sequence data showed that our new taxa cluster with *Hermatomyces* species and were well separated from *Aquasubmersa* and *Lophiotrema* species in *Lophiotremataceae.* We introduce the new species, *Hermatomyces krabiensis*, *H. pandanicola* and *H. saikhuensis*, with illustrated accounts. Evidence for demarcation of the three new species is provided using morphology and phylogenetic analyses. This is the first report of *Hermatomyces* on *Pandanus* species.

Keywords: asexual-morph, multi-genes phylogenetics, *Pandanaceae*, taxonomy

Introduction

We are studying the microfungi associated with *Pandanaceae* (Whitton *et al*. 2012, Tibpromma *et al*. 2016) and in this paper report three new taxa of *Hermatomyces* collected from southern Thailand. The genus *Hermatomyces* was introduced by Spegazzini (1911) with *H. tucumanensis* as the type species and presently there are six species noted in Index Fungorum (2016). The genus is characterized by lenticular to cylindrical, muriform conidia, often with subhyaline to pale brown peripheral cells, and dark brown central cells. Conidia are cylindrical and comprise 1–4 columns with 2–11 cells and are irregularly pigmented (Castañeda & Heredia 2000, Leão–Ferreira *et al*. 2013, Doilom *et al*. 2016).

Based on molecular data Doilom *et al*. (2016) accommodated *Hermatomyces* in *Lophiotremataceae* and introduced two new species from teak. The family *Lophiotremataceae* was introduced by Hirayama & Tanaka (2011) with *Lophiotrema* Sacc. 1878 as the type genus. In earlier studies, *Lophiotrema* was placed in *Lophiostomataceae*, but Hirayama & Tanaka (2011) showed the *Lophiotrema* clade was distantly placed from *Lophiostomataceae* and introduced the new family based on evidence from analyses of LSU and SSU sequence data. The family *Lophiotremataceae* now comprises *Aquasubmersa*, *Hermatomyces* and *Lophiotrema* (Doilom *et al*. 2016, Hyde *et al*. 2016).

The objectives of the present study were to introduce three new species of *Hermatomyces*, collected from Krabi, Phang Nga and Prachuap Khiri Khan Provinces of Thailand, during 2015, and evaluate the taxonomic and phylogenetic position of these new taxa using analysis of combined ITS, LSU, RPB2, SSU and TEF1 sequence data.

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Materials and methods

Sample collection and specimen examination

Fresh specimens of *Pandanus odorifer* (Forssk.) Kuntze (*Pandanaceae*) (Fig. 1), in the form of dead and fallen leaves, were collected from southern Thailand (Krabi, Phang Nga and Prachuap Khiri Khan Provinces) during 2015. The leaves were brought to the laboratory in zip-lock bags and examined following the methods outlined by Tibpromma *et al*. (2016). All photomicrographs of microscopic fungal structures were measured using Tarosoft® Image Framework program v.0.9.0.7.

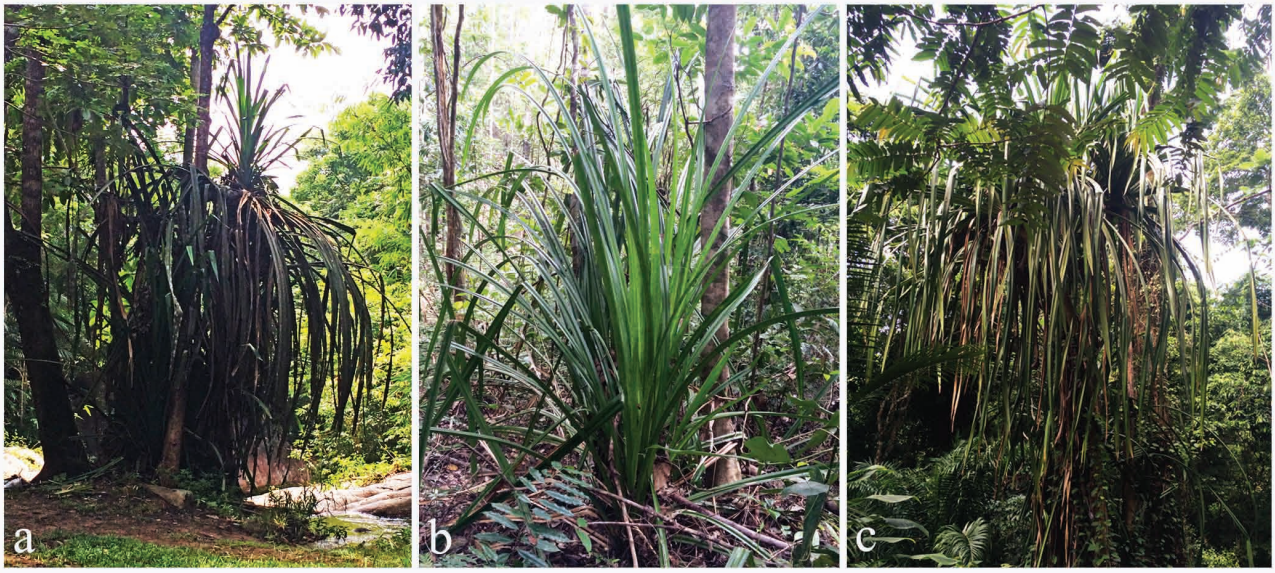

FIGURE 1. ***Pandanus odorifer*** (*Pandanaceae*). **a** Sai Khu waterfall, Prachuap Khiri Khan Province. **b** Muang District, Krabi Province. **c** Thap Put District, Phang Nga Province.

Culturing the fungi

Malt extract agar (MEA; 20 g/L of malt extract, 16 g/L of agar, 20 g/L of dextrose, 6 g/L of peptone) was used as the medium for culturing the isolated fungi. Single spore isolation was performed as described by Chomnunti *et al*. (2014). Germinating ascospores were aseptically transferred to MEA media plates after 24 hours. The cultures were incubated at room temperature 22–25 ºC for 4–6 weeks and colonies were then observed. Herbarium specimens were dried using silica gel and deposited in Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and Kunming Institute of Botany Academia Sinica (HKAS). Ex-type cultures were deposited at the Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi numbers (FoF) and Index Fungorum (IF) numbers were registered as described by Jayasiri *et al*. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and DNA sequencing

Isolates were grown on MEA at room temperature for 4 weeks, and the fungal mycelium was scraped off and transferred to 1.5 mL Micro-centrifuge tubes and kept at -20 ºC. The fungal genomic DNA extraction followed the protocol of Biospin Fungal Genomic DNA extraction Kit–BSC14S1 (BioFlux, P.R. China). Polymerase chain reaction (PCR) was used to amplify partial gene regions (ITS, LSU, RPB2, SSU and TEF1) using primers and conditions as shown in Table 1. The total volume of PCR mixtures for amplifications were 25 µL containing 8.5 µL ddH_2O, 12.5 µL2× Easy Taq PCR Super Mix (mixture of Easy Taq TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China), 2 µL of DNA template, 1 µL of each forward and reverse primers (10 pM). The quality of PCR products were checked on 1% agarose gel electrophoresis stained with 4S green nucleic acid (Life Science Products & Services, Cat. No: A616694). Purification and sequencing of PCR products were carried out by Sangon Biotech Co., Shanghai, China.

Phylogenetic analyses

SSU, LSU RPB2, TEF1 and ITS sequence data generated in this study were subjected to BLAST searches in the nucleotide database of GenBank (www http://blast.ncbi.nlm.nih.gov/) to examine their most likely taxa resemblance.

Sequence data were retrieved from GenBank based on recent publications (Doilom *et al*. 2016, Hyde *et al*. 2016). *Dothidotthia aspera* (Ellis & Everh.) M.E. Barr 1989 (CPC 12933) was used as the outgroup taxon (Table 2). Raw forward and reverse sequences were assembled using Geneious. Pro.v4.8.5 and sequence alignments were done with MAFFT v.6.864b (Katoh & Standley 2016) and alignments were manually improved if necessary. The sequence datasets were combined using BioEdit v.7.2.5 (Hall 2004). The phylogenetic analyses were performed by using Randomized Accelerated Maximum Likelihood (RAxML) and performed maximum-parsimony (MP). Bootstrap support for the branches was generated with 1,000 replicates. A maximum likelihood (ML) analysis was performed using RAxMLGUI v. 1.3 (Silvestro & Michalak 2010) with 1,000 rapid bootstrap replicates using the GTR+GAMMA model of nucleotide substitution. The maximum-parsimony (MP) analysis was performed using PAUP v. 4.0b10 (Swofford 2003), bootstrap analysis with 1,000 replicates. All multiple, equally parsimonious trees were saved and descriptive tree statistics for parsimony consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated. The robustness of the best parsimonious tree was estimated by a bootstrap (BT) value with 1,000 replicates, each with 100 replicates of random stepwise addition of taxa. The trees were figured in Treeview v.1.6.6 and edited using Microsoft Office PowerPoint 2007 and Adobe illustrator CS3 (Adobe Systems, USA).

TABLE 1. Details of genes/loci with PCR primers and protocols

Genes/loci	PCR primers (forward/reverse)	PCR conditions	References
ITS	ITS5/ITS4	[a1]94 °C: 30 s, 52 °C: 50 s, 72 °C: 1 min (35 cycles)[b]	White *et al*. (1990)
LSU	LROR/LR5	[a1]94 °C: 30 s, 55 °C: 50 s, 72 °C: 1 min (35 cycles)[b]	Vilgalys & Hester (1990)
RPB2	fRPB2-5f/fRPB2–7cR	[a2]95 °C: 1 min, 52 °C: 2 min, 72 °C: 1.30 s (35 cycles)[b]	Liu *et al*. (1999)
SSU	NS1/NS4	[a1]94 °C: 30 s, 55 °C: 50 s, 72 °C: 1 min (35 cycles)[b]	White *et al.* (1990)
TEF1	EF1–983F/EF1–2218R	[a1]94 °C: 30 s, 52 °C: 50 s, 72 °C: 1 min (35 cycles)[b]	Rehner (2001)

[a1] Initiation step of 94 °C: 3 min

[a2] Initiation step of 95 °C: 5 min

[b] final elongation step of 72 °C: 10 min and final hold at 4 °C

Results

Phylogenetic analyses

Phylogenetic analyses were performed using a combined alignment of SSU, LSU RPB2, TEF1 and ITS sequence data, comprising 52 taxa, including the outgroup taxon (Table 2). The dataset comprised 4,170 characters including coded alignment gaps; 2,873 are constant, and 975 are parsimony informative in the MP analysis. The phylogenetic analyses of the combined data matrix showed considerably high bootstrap support and well-resolved clades. Bootstrap support (BS) values of MP (equal to or above 70% based on 1,000 replicates) are shown above branches (TL=3319, CI=0.564, RI=0.703, RC=0.396, HI=0.436) and bootstrap support (BS) values of ML (equal to or above 70 % based on 1,000 replicates) are shown (Fig. 2) from the best score generated from the MP analysis. The phylogenetic analyses showed our taxa group with *Hermatomyces* species in a well separated clade with *Aquasubmersa*, *Hermatomyces* and *Lophiotrema* in *Lophiotremataceae*. Our new taxa are well separated in *Hermatomyces* as *H. krabiensis* (99% in MP, 94% in ML), *H. pandanicola* (98% in MP, 96% in ML) and *H. saikhuensis* (99% in MP, 71% in ML).

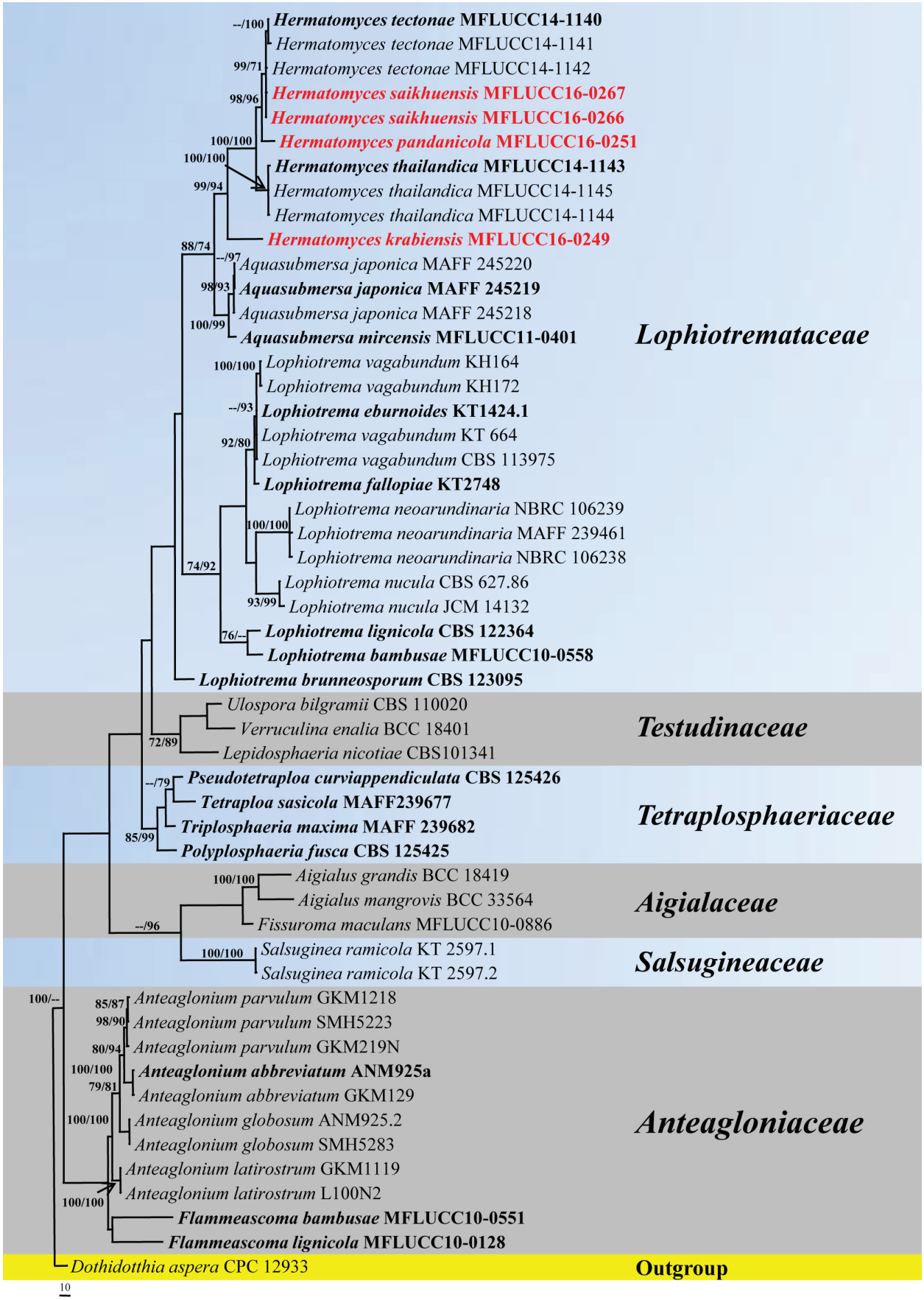

FIGURE 2. The best MP tree based on analysis of combined SSU, LSU RPB2, TEF1 and ITS sequence data. Bootstrap support values for maximum-parsimony (left) and maximum likelihood (right) greater than 70 % are given at the nodes. The tree is rooted with *Dothidotthia aspera* (CPC 12933). Ex-type strains are in bold. The newly generated sequences are in red.

TABLE 2. Strains and GenBank accession numbers used in the phylogenetic analyses (New taxa are indicated with an asterisk).

Species	Culture collection	GenBank accession numbers				
		LSU	ITS	SSU	TEF1	RPB2
Aigialus grandis	BCC 18419	GU479779	–	GU479743	GU479843	GU479818
Aigialus mangrovis	BCC 33564	GU479777	–	GU479742	GU479841	GU479816
Anteaglonium abbreviatum	ANM925a	GQ221877	–	–	GQ221924	–
Anteaglonium abbreviatum	GKM129	GQ221881	–	–	GQ221915	–
Anteaglonium globosum	ANM925.2	GQ221879	–	–		–
Anteaglonium globosum	SMH5283	GQ221911	–	–	GQ221919	–
Anteaglonium latirostrum	L100N2	–	–	–	GQ221938	–
Anteaglonium latirostrum	GKM 1119	–	–	–	GQ221937	–
Anteaglonium parvulum	GKM 1218	GQ221880	–	–	GQ221922	–
Anteaglonium parvulum	GKM 219N	–	–	–	GQ221916	–
Anteaglonium parvulum	SMH5223	GQ221909	–	–	GQ221918	–
Aquasubmersa japonica	MAFF 245218	LC061586	LC061591	LC061581	–	–
Aquasubmersa japonica	MAFF 245219	LC061587	LC061592	LC061582	–	–
Aquasubmersa japonica	MAFF 245220	LC061588	LC061593	LC061583	–	–
Aquasubmersa mircensis	MFLUCC11-0401	JX276955	JX276954	JX276956	–	–
Dothidotthia aspera	CPC 12933	EU673276	–	EU673228	–	–
Fissuroma maculans	MFLUCC10-0886	JN846724	JN846710	JN846734	–	–
Flammeascoma bambusae	MFLUCC10-0551	KP744485	KP744440	KP753952	–	–
Flammeascoma lignicola	MFLUCC10-0128	KT324583	KT324582	KT324584	KT324585	KT324586
*Hermatomyces krabiensis**	MFLUCC16-0249	KX525742	KX525750	KX525746	KX525758	KX525754
*Hermatomyces pandanicola**	MFLUCC16-0251	KX525743	KX525751	KX525747	KX525759	KX525755
*Hermatomyces saikhuensis**	MFLUCC16-0266	KX525740	KX525748	KX525744	KX525756	KX525752
*Hermatomyces saikhuensis**	MFLUCC16-0267	KX525741	KX525749	KX525745	KX525757	KX525753
Hermatomyces tectonae	MFLUCC14-1140	KU764695	KU144917	KU712465	KU872757	KU712486
Hermatomyces tectonae	MFLUCC14-1141	KU764696	KU144918	KU712465	KU872758	KU712487
Hermatomyces tectonae	MFLUCC14-1142	KU764697	KU144919	KU712465	KU872759	KU712488
Hermatomyces thailandica	MFLUCC14-1143	KU764698	KU144920	KU712465	KU872760	KU712489
Hermatomyces thailandica	MFLUCC14-1144	KU764699	KU144921	KU712465	KU872761	KU712490
Hermatomyces thailandica	MFLUCC14-1145	KU764700	KU144922	KU712465	KU872762	KU712491
Lepidosphaeria nicotiae	CBS101341	DQ678067	–	–	DQ677910	DQ677963
Lophiotrema bambusae	MFLUCC10-0558	KX672154	KX672149	KX672159	KX672162	KX672161
Lophiotrema brunneosporum	CBS 123095	FJ795444	–	GU296165	GU349071	–
Lophiotrema eburnoides	KT1424.1	LC001707	LC001709	LC001706	–	–
Lophiotrema fallopiae	KT2748	LC149915	LC149913	LC149911	–	–
Lophiotrema lignicola	CBS 122364	FJ795445	–	GU296166	GU349072	FJ795462
Lophiotrema neoarundinaria	NBRC 106238	AB524597	AB524787	AB524456	AB524818	AB539097
Lophiotrema neoarundinaria	NBRC 106239	AB524598	–	AB524457	–	–

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TABLE 2. (Continued)

Species	Culture collection	GenBank accession numbers				
		LSU	ITS	SSU	TEF1	RPB2
Lophiotrema neoarundinaria	MAFF 239461	AB524596	AB524786	AB524455	AB524817	AB539096
Lophiotrema nucula	CBS 627.86	FJ795446	–	GU296167	–	FJ795463
Lophiotrema nucula	JCM 14132	AB619021	–	AB618703	–	–
Lophiotrema vagabundum	JCM 14138	AB619024	–	AB618706	–	–
Lophiotrema vagabundum	JCM 17674	AB619022	–	AB618704	–	–
Lophiotrema vagabundum	JCM 17675	AB619023	–	AB618705	–	–
Lophiotrema vagabundum	CBS 113975	AB619025	–	AB618707	–	–
Polyplosphaeria fusca	CBS 125425	AB524607	AB524791	AB524466	AB524822	–
Pseudotetraploa curviappendiculata	CBS 125426	AB524610	AB524794	AB524469	AB524825	–
Salsuginea ramicola	NBRC 106257	GU479801	–	GU479768	GU479862	GU479834
Salsuginea ramicola	NBRC 106256	GU479800	–	GU479767	GU479861	GU479833
Tetraplosphaeria sasicola	MAFF 239677	AB524631	AB524807	AB524490	AB524838	–
Triplosphaeria maxima	MAFF 239682	AB524637	AB524812	AB524496	AB524843	–
Ulospora bilgramii	CBS 110020	DQ384108	–	DQ384071	–	–
Verruculina enalia	BCC 18401	GU479802	–	GU479770	GU479863	GU479835

ABBREVIATIONS: **ANM**: A.N. Miller; **BCC**: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Patumwan, Bangkok, Thailand; **CBS**: Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands; **CPC**: Collection of Pedro Crous housed at CBS; **GKM**: G.K. Mugambi; **JCM**: Japan Collection of Microorganisms; **KT**: K. Tanaka; **MAFF**: Ministry of Agriculture, Forestry and Fisheries; **MFLUCC**: Mae Fah Luang University culture collection; **NBRC**: NITE Biological Research Center, National Institute of Technology and Evaluation, Kisarazu-shi, Chilba Prefecture, Japan; **SMH**: S.M. Huhndorf.

Taxonomy

Hermatomyces krabiensis Tibpromma, D.J. Bhat & K.D. Hyde, ***sp. nov.*** (Fig. 3)

Index Fungorum number: IF552307, *Facesoffungi number*: FoF02479

Etymology:—refers to the province (Krabi) in Thailand where the holotype was collected.

Holotype:—MFLU16-1885

Saprobic on dead leaves of *Pandanus odorifer*. **Sexual morph:** Undetermined. **Asexual morph:** *Colonies* on natural substrate dry, blackish brown, velvety, circular, dull, consisting of a sterile mycelial outer zone and a round, glistening, abundantly sporulating centre, with conidia readily liberated when disturbed. *Mycelium* 1.0–3.7 µm wide, superficial, composed of a network of branched, septate, brown, thick-walled hyphae. *Conidiophores* 3.1–8.4 µm long, 2.1–3.3 µm wide, micronematous, straight or flexuous, hyaline, septate, smooth, unbranched, arising from prostrate hyphae at the centre of circular colony. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal, cylindrical, hyaline to subhyaline. *Conidia* dimorphic, thick-walled, smooth: *lenticular conidia* 24.3–32.5 µm high, 12.1–21.3 µm diam wide ($\overline{x}$ = 28.5 × 17.2 µm, n = 30), multiseptate, with central cells dark brown to black, with peripheral cells subhyaline to pale brown, slightly constricted at the septa, smooth, in side view composed of one column of 4–6 cells, hyaline to light brown at the lower and upper cells, often carrying remnant of conidiogenous cell at base; *cylindrical conidia*: 20.4–26.4 µm high, × 8.6–19.7 µm wide in broadest part of lower cells, ($\overline{x}$ = 23.2 × 14.2 µm, n = 20), with 1–2 columns of 2–3 cells arising from a common basal cell, each column, with rectangular to globose cells, constricted at septa, pale to subhyaline, granulate, smooth, terminating with a dark brown, turbinate upper cell.

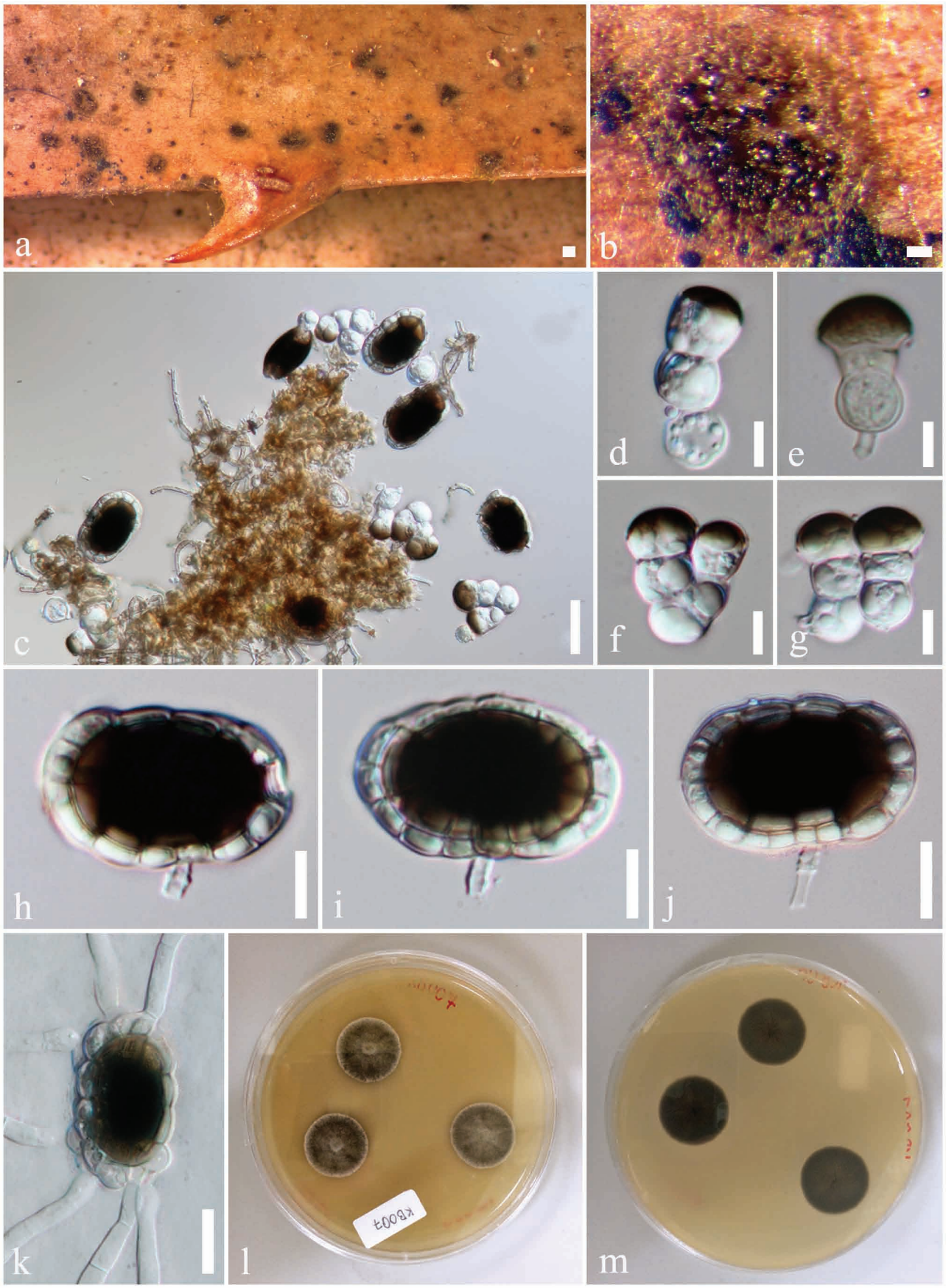

FIGURE 3. ***Hermatomyces krabiensis*** (MFLU16-1885, holotype). **a, b** Colonies on substrate. **c** Attached conidia. **d–g** Cylindrical conidia. **h–j** Lenticular conidia. **k** Germinated lenticular conidium. **l, m** Colony on MEA after 7 days. Scale bars: a = 200 µm, b = 50 µm, c = 20 µm, d–k = 10 µm.

Culture characteristics:—Colonies on MEA at room temperature (22–25 ˚C) reaching 4 cm in 1 week, circular with entire, white-grey mycelium with white in the margin, smooth at surface and raised, not sporulating in culture even after 4 months.

Material examined:—THAILAND, Krabi Province, on dead leaves of *Pandanus odorifer* (*Pandanaceae*), 14 December 2015, S. Tibpromma & K.D. Hyde KB007 (MFLU16-1885, **holotype**; HKAS94528, **isotype**); ex-type living culture, MFLUCC16-0249.

Notes:**—***Hermatomyces krabiensis* shares a similar morphology with *H. amphisporus*. Both taxa produce lenticular and cylindrical conidia. *Hermatomyces krabiensis* however, is distinct, with cylindrical conidia bearing 1–2 columns of 2–3 cells arising from a common basal cell. In *H. amphisporus,* the conidia are composed of 6–11 cells arranged in 4 rows (Castañeda & Heredia 2000).

Hermatomyces pandanicola Tibpromma, D.J. Bhat & K.D. Hyde, ***sp. nov.*** (Fig. 4)

Index Fungorum number: IF552308, *Facesoffungi number*: FoF02480

Etymology:—named for its occurrence on the host plant *Pandanus*

Holotype:—MFLU16-1896

Saprobic on dead leaves of *Pandanus odorifer*. **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* on natural substrate dry, blackish brown, velvety, circular, doughnut-shaped, dull, consisting of a sterile mycelial outer zone and a round, glistening, abundantly sporulating centre, conidia readily liberated when disturbed. *Mycelium* 1.2–2.8 µm wide, superficial, composed of a network of branched, septate, brown, thick-walled hyphae. *Conidiophores* 1.2–9.8 µm long, 1.3–3.7 µm wide, micronematous, straight or flexuous, hyaline, smooth, unbranched, arising from prostrate hyphae at the centre of circular colony. *Conidiogenous* cells holoblastic, monoblastic, integrated, terminal, cylindrical, hyaline. *Conidia dimorphic*, thick-walled, smooth: *lenticular conidia* 12–15.7 µm high, 20–30.1 µm diam wide ($\overline{x}$ = 19.2 × 24.8 µm, n = 30), with central cells dark brown to black, with peripheral cells subhyaline to pale brown, not constricted at septa, smooth, in lateral view obovoid, guttulate, hyaline to light brown at lower and upper ends, dark brown at central cells; *cylindrical conidia* 13.2–20.6 µm high, 8.9–11.9 µm wide in broadest part of lower cells ($\overline{x}$ = 16.6 × 10.1 µm, n = 20), granulate, with 2 rows of 2 cells in each column arising from a common basal cell, lower cells usually hyaline, cylindrical, swollen, constricted at septa, turbinate and dark brown at upper cell.

Culture characteristics:—Colonies on MEA at room temperature (22–25 ˚C) reaching 1.5 cm in 1 week, irregular with wavy margin, wrinkled on media, white-grey, not sporulating in culture after 4 months.

Material examined:—THAILAND, Phang Nga Province, Thap Put District, on dead leaves of *Pandanus odorifer* (*Pandanaceae*), 17 December 2015, S. Tibpromma & K.D. Hyde, KB018 (MFLU16-1896, **holotype,** HKAS94530, **isotype**); ex-type living culture, MFLUCC16-0251.

Notes:—*Hermatomyces pandanicola* is compared with *H. indicus,* as both produce lenticular and cylindrical or turbinate conidia. The lenticular conidia in both species are similar in shape, size and colour. However, the cylindrical conidia of *H. pandanicola* have 2 rows of 2 cells in each column, arising from a common basal cell (13.2–20.6 × 8.9–11.9 µm), with lower cells usually hyaline, cylindrical, swollen, constricted at septa, turbinate and dark brown at upper surface. In *H. indicus* the conidia are composed of 6–7 cells in 2 rows (22.4–35.4 × 11.4–21.6 µm) (Prasher & Prasher 2014). The morphology of *H. pandanicola* is supplemented with molecular data in this study.

Hermatomyces saikhuensis Tibpromma, D.J. Bhat & K.D. Hyde***, sp. nov.*** (Fig. 5)

Index Fungorum number: IF552309, *Facesoffungi number*: FoF02481

Etymology:—refers to the Sai Khu waterfall where the fungus was collected.

Holotype:—MFLU16-1930

Saprobic on dead leaves of *Pandanus odorifer*. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on natural substrate dry, blackish brown, velvety, circular, doughnut-shaped, dull, consisting of a sterile mycelial outer zone and a round, glistening, abundantly sporulating centre, conidia readily liberated when agitated. *Mycelium* 1.1–2.5 µm wide, superficial, composed of a network of branched, septate, brown, thick-walled, smooth hyphae. *Conidiophores* 3–5.2 µm long, 1.7–2.8 µm wide, micronematous, straight or flexuous, pale brown, smooth, unbranched, arising from prostrate hyphae at the centre of circular colony. *Conidiogenous* cells holoblastic, monoblastic, integrated, terminal, cylindrical, hyaline to subhyaline. *Conidia* monomorphic, thick-walled, smooth, lenticular on top view, fattened and

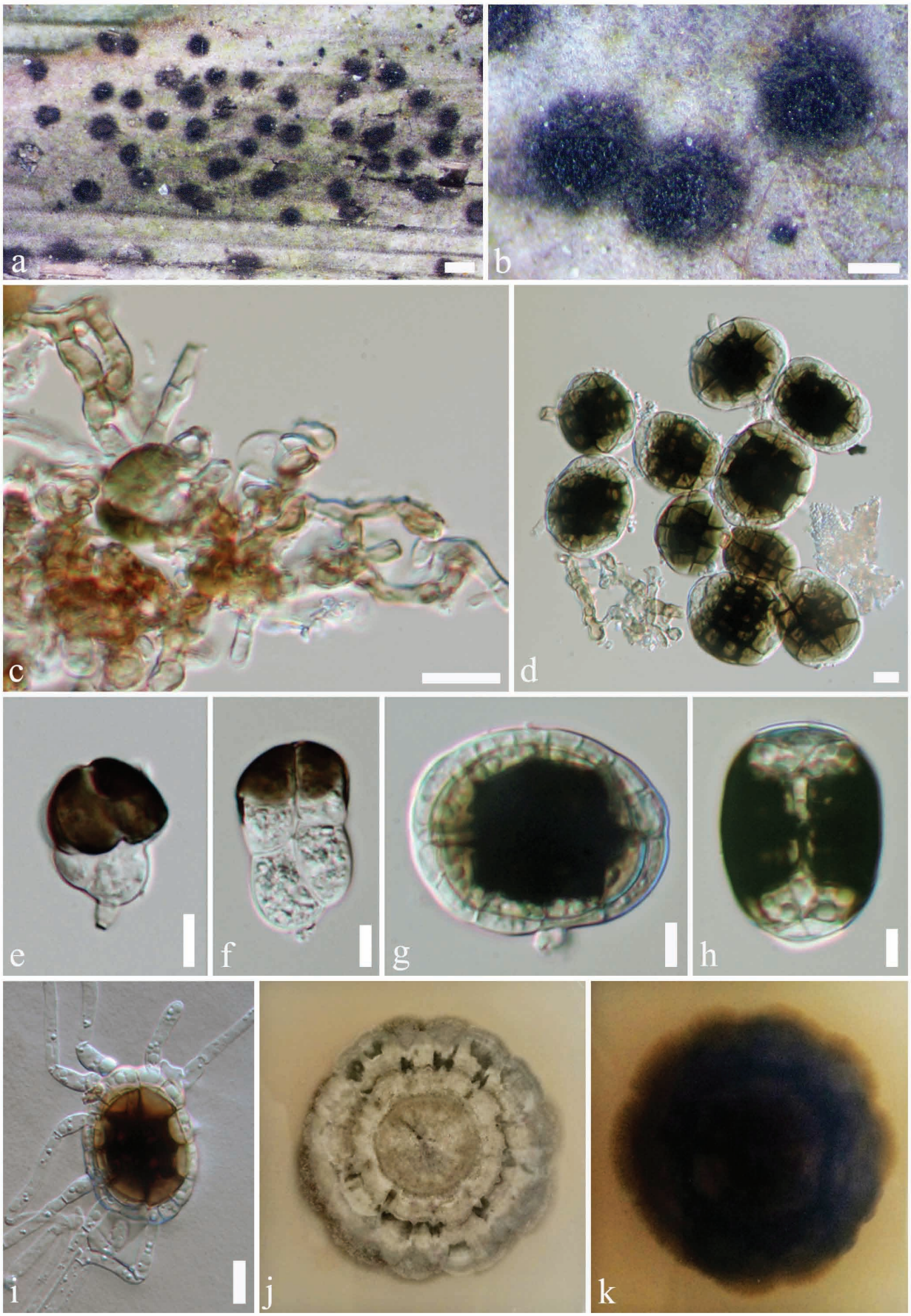

FIGURE 4 ***Hermatomyces pandanicola*** (MFLU16-1896, holotype). **a, b** Colonies on substrate. **c** Mycelium. **d** Conidia and conidiophores **e, f** Cylindrical conidia. **g, h** Lenticular conidia **i** Germinated conidium. **j, k** Colony on MEA after 7 days. Scale bars: a = 500 µm, b = 200 µm, c = 10 µm, d = 20 µm, e-h = 20 µm, i = 10 µm.

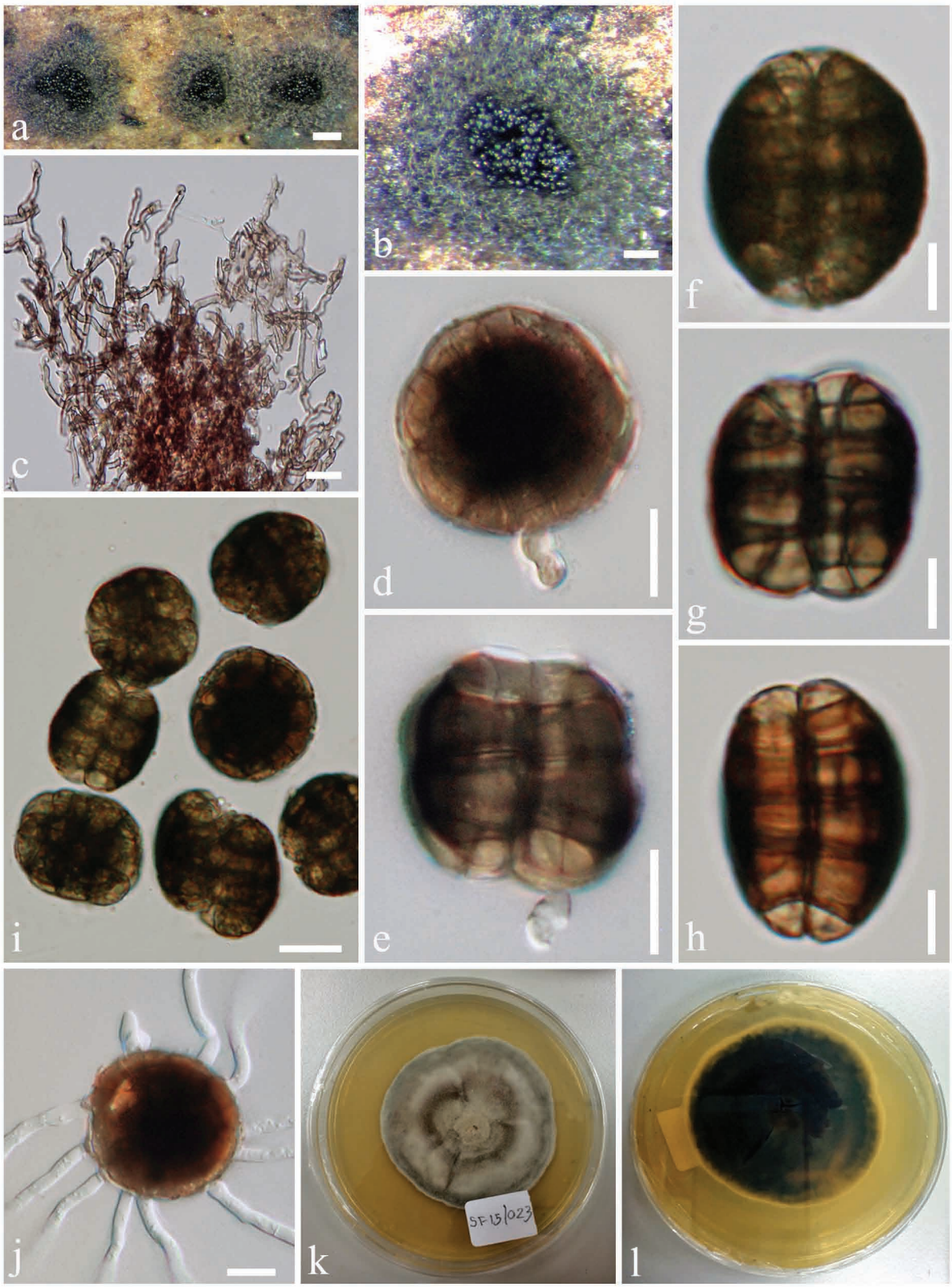

FIGURE 5. ***Hermatomyces saikhuensis*** (MFLU16-1930, holotype). **a, b** Colonies on substrate. **c** Mycelium. **d, e** Conidia and conidiogenous cells. **f–i** Lenticular conidia. **j** Germinated conidium. **k, l** Colony on MEA after 7 days. Scale bars: a = 200 µm, b = 100 µm, c = 10 µm, d–h = 5 µm, i = 10 µm, j = 5 µm.

disk-shaped in lateral view, 14.2–21.4 µm high, 11.2–19.3 µm wide ($\bar{x}$ = 17.5 × 17 µm, n = 30), with central cells dark brown to black, with peripheral cells subhyaline to pale brown. Two halves of the disk-shaped conidia symmetrically

adpressed, forming a deep constriction at lower and upper end in lateral view, each half with 6–7 cells, hyaline to light brown at lower and upper cells, dark brown in middle cells.

Culture characteristics:—Colonies on MEA at room temperature (22–25 ˚C) reaching 2.5 cm in 1 week, circular with wavy margin, white-grey, smooth surface and raised, not sporulating in culture after 4 months.

Material examined:—THAILAND, Prachuap Khiri Khan Province, Sai Khu waterfall, on dead leaves of *Pandanus odorifer* (*Pandanaceae*), 30 July 2015, S. Tibpromma & K.D. Hyde, SF15-015 (MFLU16-1930, **holotype,** HKAS94531, **isotype**); ex-type living culture, MFLUCC16-0266.

Notes:—Similar to *Hermatomyces saikhuensis*, both *H. sphaericum* and *H. tucumanensis* produce only one type of conidia (Castañeda & Heredia 2000). However, *Hermatomyces saikhuensis* can be differentiated by its shape, colour and size of conidia. In *H. sphaericum* the conidia are lenticular to elliptical, with pale peripheral cells (Castañeda & Heredia 2000). *Hermatomyces tucumanensis* has subglobose to broadly oval conidia with pale peripheral cells (Castañeda & Heredia 2000). In *H. saikhuensis* the lenticular conidia with subhyaline to pale brown peripheral cells are paler than the previously described species. In size, the conidia of *H. saikhuensis* (14.2–21.4 μm) are smaller than both *H. sphaericum* (26–31 × 25–50 μm) and *H. tucumanensis* (29–42 × 19–25 μm).

Discussion

According to the morphological characteristics, the three taxa collected on *Pandanus odorifer* in southern Thailand belong in the genus *Hermatomyces* (Spegazzini 1911). *Hermatomyces krabiensis, H. pandanicola* and *H. saikhuensis* are introduced as new species based on both morphological characteristics and phylogenetic analyses. *Hermatomyces krabiensis*, *H. pandanicola*, and *H. saikhuensis,* compared with similar species of *Hermatomyces,*have several unique morphological characteristics that separate them from other *Hermatomyces* species. The new species are well-separated from other genera in the family *Lophiotremataceae* in the phylogenetic analyses and group within *Hermatomyces* with high bootstrap support (*H. krabiensis* 99% in MP, 94% in ML*, H. pandanicola* 98% in MP, 96% in ML and *H. saikhuensis* 99% in MP, 71% in ML) and also well separated from the two species described from teak (*H. tectonae* and *H. thailandica*, Doilom *et al*. 2016). The phylogenetic tree obtained in this study shows similar results as in previous studies by Doilom *et al*. (2016) and Hyde *et al*. (2016). Moreover, this is the first report of *Hermatomyces* on *Pandanus* species and details of host and distribution of all *Hermatomyces* species are listed in Table 3.

TABLE 3. Known *Hermatomyces* species with host, location and relevent references

Name	Host	Country	References
Hermatomyces amphisporus	*Cyathea* sp.	Mexico	Castañeda & Heredia (2000)
Hermatomyces dimorphus	Unknown	India	Rao & De Hoog (1986)
Hermatomyces indicus	*Phoenix rupicola*	India	Prasher & Prasher (2014)
Hermatomyces krabiensis	*Pandanus odorifer*	Thailand	This study
Hermatomyces pandanicola	*Pandanus odorifer*	Thailand	This study
Hermatomyces saikhuensis	*Pandanus odorifer*	Thailand	This study
Hermatomyces tectonae	*Tectona grandis*	Thailand	Doilom *et al*. (2016)
Hermatomyces thailandica	*Tectona grandis*	Thailand	Doilom *et al*. (2016)
Hermatomyces tucumanensis	*Smilax campestris*	Argentina	Leão-Ferreira *et al*. (2013)
Hermatomyces uniseriatus	Unknown	Brazil	Leão-Ferreira *et al*. (2013)

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