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Phylogenetic investigations on freshwater fungi in Tubeufiaceae (Tubeufiales) reveals the new genus *Dictyospora* and new species *Chlamydotubeufia aquatica* and *Helicosporium flavum*

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Abstract

Seven new collections of sexual and asexual morphs of Tubeufiaceae from woody substrates in freshwater, were obtained from different regions of Thailand. ITS, LSU and TEF1α sequence data obtained from single spore isolates of these collections were analyzed with other species of Tubeufiaceae. The phylogenetic analyses with combined ITS, LSU and TEF1α data indicated that the collections represented three new species, *Chlamydotubeufia aquatica*, *Dictyospora thailandica* and *Helicosporium flavum*. A new genus, *Dictyospora* is introduced to accommodate *Dictyospora thailandica*. Morphological descriptions and illustrations are provided for the new taxa and compared with similar taxa. A single ascospore isolates of *Dictyospora thailandica* and *Helicosporium flavum* produced asexual conidia in MEA.

Keywords – *Chlamydotubeufia* – *Dictyospora* – *Helicosporium* – lignicolous fungi – phylogeny – taxonomy

Introduction

Freshwater fungi are an important group of organisms involved in nutrient cycling of woody and leaf litter (Hyde et al. 2016). Tubeufiales is a large order comprising more than 20 genera of freshwater ascomycetes and presently incorporates the monotypic family Tubeufiaceae (Doilom et al. 2017). The majority of tubeufiaceous taxa is widely distributed and occurs on woody substrates in terrestrial and freshwater habitats (Hyde and Goh 1998, Ho et al. 2001, Cai et al. 2002) and in peat swamps (Pinnoi et al. 2006, Pinruan et al. 2007). Tubeufiaceae species generally have superficial, globose to subglobose, light to dark pigmented ascomata, often with aerial mycelium and/or setae, cylindrical, clavate asci, multi-septate hyaline ascospores and hyphomycetous, often helicosporous asexual morphs (Kodsueb et al. 2006, Boonmee et al. 2011, 2014, Lu et al 2017a, 2017b, Luo et al 2017). A modern classification of the order based on

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molecular data was provided by Boonmee et al. (2014) and subsequent papers (Doilom et al. 2017, Luo et al. 2017, Lu et al 2017a, b).

We are studying freshwater fungi in water bodies along a north south longitudinal gradient in the Asian region. The aim of the present study is to determine the phylogenetic placement of several collections of tubeufiaceous taxa from Thailand. A new monotypic genus with four collections, a new species of *Chlamydotubeufia* and a new species of *Helicosporium* are introduced, based on molecular and morphological investigations, with detailed descriptions and illustrations.

Materials and methods

Sample collection, morphological studies and isolation

Decaying wood specimens were collected from various regions in Thailand. Morphological examinations were carried out following the methods in Boonmee et al. (2011, 2014). Single spore isolations were performed following the techniques in Chomnunti et al. (2014). Germinated ascospores were transferred to malt extract agar (MEA) plates and incubated at 28 ^{0}C in natural light. Culture morphologies were examined, photographed and measured after 2–4 weeks. The specimens and living cultures were deposited in the Herbarium of Mae Fah Luang University (Herb. MFLU) and Culture Collection of Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand and Thailand Bioresource Research Center (TBRC), Pathum Thani, Thailand. Facesoffungi and Index Fungorum numbers were submitted (Jayasiri et al. 2015, Index Fungorum 2017). New species were justified based on recommendations outlined by Jeewon & Hyde (2016).

DNA extraction and PCR amplification

Genomic DNA was extracted from fungal mycelium grown on MEA at 28 ^{0}C for 60 days using Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturer's protocol (Hangzhou, P.R. China). Three genes were amplified with primers, namely the internal transcribed spacer region of ribosomal DNA (ITS: ITS5/ITS4) (White et al. 1990), large subunit nuclear ribosomal DNA (LSU: LROR/LR5) (Vilgalys & Hester 1990), and the translation elongation factor 1-alpha gene (TEF1α: EF1-983F/EF1-2218R) (Rehner & Buckley 2005). The PCR products were purified and sequenced with the same primers. Amplifications was performed in 25 μl of PCR mixtures containing 9.5 μl ddH2O, 12.5 μl 2 × PCR Master Mix, 1 μl of DNA template, 1 μl of each primer (10 μM) (Lu et al. 2017a). The quality of PCR products were checked on 1 % agarose gel electrophoresis strained with Ethidium bromide. The PCR products were sent for sequencing at Sangon Biotech, Shanghai, China. The nucleotide sequence data acquired were deposited in GenBank (Table 1).

Phylogenetic analysis

Sequence data for related taxa (Table 1) were downloaded from GenBank following Luo et al. (2017). Multiple sequence alignments were produced with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html, Katoh & Standley 2013) and further improved manually where necessary using BioEdit v. 7.0.5.2 (Hall 1999). Ambiguous regions were excluded from the analyses and gaps were treated as missing data. Phylogenetic analyses of individual gene and combined aligned data were generated under maximum likelihood (ML), maximum parsimony (MP) and Bayesian criteria. Parsimony analysis was carried with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002) with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm (Jeewon et al. 2002, 2003). All characters were unordered and of equal weight and gaps were treated as missing data. Max trees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1000 replicates, each with 10 replicates of random.

Table 1 Taxa used in this study and their GenBank accession numbers for ITS, LSU and TEF1α DNA sequence data.

Taxa	Culture accession No.[b]	GenBank Accession No.			References
		ITS	LSU	TEF1α	
Acanthohelicospora aurea	GZCC16-0059	KY321322	KY321325	KY792599	Lu et al. (2017b)
Acanthohelicospora guianense	UAMH 1699	AY916479	AY856891	–	Tsui et al. (2006)
Acanthohelicospora pinicola	MFLUCC 10-0116	KF301526	KF301534	KF301555	Boonmee et al. (2014)
Acanthostigma chiangmaiensis	MFLUCC 10-0125	JN865209	JN865197	KF301560	Boonmee et al. (2014)
Acanthostigma perpusillum	UAMH 7237	AY916492	AY856892	–	Tsui & Berbee (2006)
Acanthostigmina multiseptatum	ANM 665	–[a]	GQ850492	–	Promputtha & Miller (2010)
Acanthostigmina multiseptatum	ANM 475	–	GQ850492	–	Promputtha & Miller (2010)
Aquaphila albicans	BCC 3543	DQ341096	DQ341101	–	Tsui et al. (2007)
Aquaphila albicans	MFLUCC 16-0010	KX454165	KX454166	KY117034	Hyde et al. (2016); Lu et al. (2017a)
Boerlagiomyces macrospora	MFLUCC 12-0388	KU144927	KU764712	KU872750	Doilom et al. (2017)
Botryosphaeria dothidea	CBS 115476	KF766151	DQ678051	DQ767637	Schoch et al. (2006); Slippers et al. (2013)
Chlamydotubeufia aquatica	**MFLUCC 16-1131**	**KY873625**	**KY873620**	**KY873284**	In this study
Chlamydotubeufia chlamydosporum	CBS 160.69	AY916466	AY856875	–	Tsui et al. (2006)
Chlamydotubeufia helicospora	MFLUCC 16-0213	KX454169	KX454170	KY117035	Hyde et al. (2016); Lu et al. (2017a)
Chlamydotubeufia huaikangplaensis	MFLUCC 10-0926	JN865210	JN865198	–	Boonmee et al. (2011)
Chlamydotubeufia huaikangplaensis	MFLUCC 16-0227	KY678765	KY678757	KY792596	(Hyde pers. comm.)
Chlamydotubeufia huaikangplaensis	MFLUCC 16-0023	KY678766	KY678758	KY792597	(Hyde pers. comm.)
Chlamydotubeufia khunkornensis	MFLUCC 10-0117	JN865201	JN865189	–	Boonmee et al. (2011)
Chlamydotubeufia khunkornensis	MFLUCC 10-0118	JN865202	JN865190	KF301564	Boonmee et al. (2011)
Chlamydotubeufia krabiensis	MFLUCC 16-1134	KY678767	KY678759	KY792598	(Hyde pers. comm.)
Dictyospora thailandica	**MFLUCC 16-0001**	**KY873627**	**KY873622**	**KY873286**	In this study
Dictyospora thailandica	**MFLUCC 16-0215**	**KY873628**	**KY873623**	**KY873287**	In this study
Dictyospora thailandica	**MFLUCC 11-0512**	**KF301528**	**KF301536**	**–**	In this study
Dictyospora thailandica	**MFLUCC 11-0509**	**KF301527**	**KF301535**	**–**	In thid study
Helicangiospora lignicola	MFLUCC 11-0378	KF301523	KF301531	KF301552	Boonmee et al. (2014)
Helicoma chiangraiense	MFLUCC10-0115	JN865200	JN865188	–	Boonmee et al. (2011)
Helicoma khunkornensis	MFLUCC 10-0119	JN865203	JN865191	KF301559	Boonmee et al. (2011)
Helicoma muelleri	CBS 964.69	AY916453	AY856877	–	Tsui et al. (2006)
Helicomyces indicum	CBS 374.93	AY916477	AY856885	–	Tsui & Berbee (2006)
Helicomyces paludosa	CBS 120503	DQ341095	DQ341103	–	Tsui et al. (2007)
Helicomyces roseus	CBS 283.51	AY916464	AY856881	–	Tsui & Berbee (2006)
Helicomyces roseus	KUMCC 15-0281	KY320526	KY320543	KY320559	Luo et al. (2017)

Helicomyces talbotii	MUCL 33010	AY916465	AY856874	–	Tsui & Barbee (2006)
Helicosporium vegetum	NBRC 9014	AY916489	AY856903	–	Tsui & Berbee (2006)
Helicosporium vegetum	CBS 254.75	–	DQ470982	DQ471105	Spatafora et al. (2006)
Helicosporium flavum	**MFLUCC 16-1230**	**KY873626**	**KY873621**	**KY873285**	In this study
Helicosporium guianense	CBS 269.52	AY916479	AY856891	–	Tsui & Berbee (2006)
Helicosporium luteosporum	MFLUCC 16-0226	KY321324	KY321327	KY792601	Lu et al. (2017b)
Helicosporium luteosporum	MFLUCC 16-1233	–	KY873624	–	In this study
Helicosporium patagonica	BBB MVB 573	JN127358	JN127359	–	Sanchez et al. (2011)
Helicosporium vaccinii	CBS 216.90	AY916486	AY856879	–	Sanchez et al. (2011)
Helicosporium vegetum	NBRC 30345	–	AY856896	–	Tsui et al. (2007)
Helicosporium vegetum	CBS 941.72	AY916488	AY856883	–	Tsui et al. (2007)
Helicosporium vegetum	BCC 3332	AY916490	AY856907	–	Tsui et al. (2007)
Helicosporium vegetum	BCC 8125	AY916491	AY856909	–	Tsui et al. (2007)
Manoharachariella tectonae	MFLUCC12-0170	KF301529	KF301537	KU872762	Boonmee et al. (2014)
Muripulchra aquatica	KUMCC 15-0276	KY320534	KY320551	KY320564	Luo et al. (2017)
Muripulchra aquatica	MFLUCC 15-0249	KY320532	KY320549	–	Luo et al. (2017)
Neoacanthostigma fusiforme	MFLUCC 11-0510	KF301529	KF301537	–	Boonmee et al. (2014)
Neoacanthostigma septoconstrictum	ANM 536.1	GQ856143	GQ850491	–	Promputtha & Miller (2010)
Neohelicomyces aquaticus	KUMCC15-0463	KY320529	KY320546	KY320562	Luo et al. (2017)
Neohelicomyces aquaticus	MFLUCC16-0993	KY320528	KY320545	KY320561	Luo et al. (2017)
Neohelicomyces submersus	MFLUCC 16-1106	KY320530	KY320547	–	Luo et al. (2017)
Tamhinispora indica	NFCCI 2924	KC469282	KC469283	–	Rajeshkumar & Sharma (2013)
Thaxteriellopsis lignicola	MFLUCC 10-0122	JN865206	JN865194	KF301563	Boonmee et al. (2011)
Thaxteriellopsis lignicola	MFLUCC 10-0124	JN865208	JN865196	KF301561	Boonmee et al. (2011)
Tubeufia chiangmaiensis	MFLUCC 11-0514	KF301530	KF301538	KF301557	Boonmee et al. (2014)
Tubeufia filiformis	MFLUCC 16-1135	KY092416	KY092411	KY117032	Lu et al. (2017a)
Tubeufia javanica	MFLUCC 12-0545	KJ880034	KJ880036	KJ880037	Boonmee et al. (2014)
Tubeufia latispora	MFLUCC 16-0027	KY092417	KY092412	KY117033	Lu et al. (2017a)

Notes: new sequences are in bold.

[a] No data in GenBank.

[b]*ANM*, A.N. Miller; *BCC*, BIOTEC Culture Collection, Thailand; *CBS*, Centraalbureau voor Schimmel cultures, Utrecht, The Netherlands; *GZCC*, Guizhou Culture Collection, Guizhou Academy of Agricultural Sciences, Guiyang, China; *KUMCC*, Culture collection of Kunming Institute of Botany, Kunming, China; *MFLUCC*, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; *MUCL*, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; *NBRC*, the NITE Biological Resource Center; *NFCCI*, the National Fungal Culture Collection of India. *UAMH*, UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada.

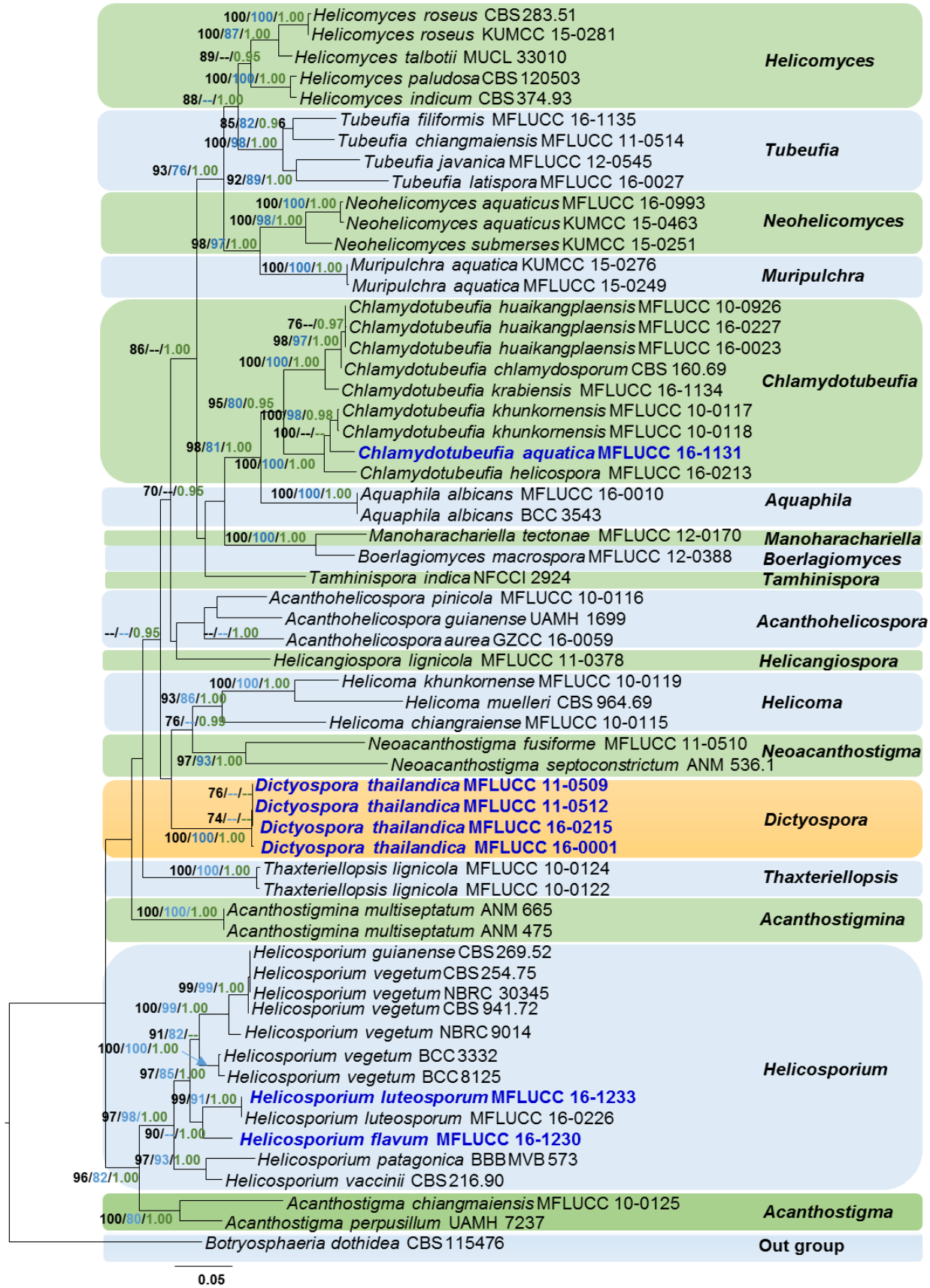

Fig.1 – RAxML phylogenetic tree based on combined LSU, ITS and TEF1α sequence data from taxa of the family Tubeufiaceae. Bootstrap support values for ML (black), MP (blue) equal to or greater than 75 % and BYPP (green) equal to or greater than 0.95 are given above each branch respectively. The tree is rooted to *Botryosphaeria dothidea* (CBS 115476). The new taxa in this study are highlighted in blue bold and all ex-type strains are in bold.

stepwise addition of taxa (Hillis & Bull 1993, Jeewon et al 2013). The evolutionary models for Bayesian analysis and maximum-likelihood were selected independently for each locus using Mr Modeltest v. 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC) implemented in PAUP v. 4.0b10. GTR+I+G model is resulted in each locus for Bayesian analysis and maximum-likelihood by AIC in Mr Modeltest as the best-fit model. Bayesian analysis was conducted with Mr Bayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC).Six simultaneous Markov chains were run for 5000000 generations and trees were sampled every 1000 generation. The distribution of log-likelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.5 (Rambaut & Drummond 2007). First 20 % of generated trees were discarded and the remaining 80 % of trees were used to calculate posterior probabilities of the majority rule consensus tree. Maximum likelihood trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform with default parameters and bootstrapping with 1000 replicates (Miller et al. 2010). Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft power point (2007) and Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA). The finalized alignment and tree were deposited in TreeBASE, submission ID: 20603 (http://www.treebase.org/).

Results and Discussion

Phylogenetic analysis

The combined LSU, ITS and TEF1α gene dataset comprised 60 sequences with relevant taxa in Tubeufiaceae including our new strains. After exclusion of ambiguous regions, the combined dataset included 2312 characters including gaps. RAxML analysis yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -17327.386055. The matrix had 920 distinct alignment patterns, with 30.61 % of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.235035, C = 0.255864, G = 0.270076, T = 0.239024; substitution rates AC = 0.862840, AG = 3.454395, AT = 2.545071, CG = 0.753876, CT = 8.271214, GT = 1.000; proportion of invariable sites I = 0.576976; gamma distribution shape parameter α = 0.636858. The maximum parsimonious dataset consists of 593 parsimony-informative and 162 parsimony-uninformative characters. The parsimony analysis of the data matrix resulted in five thousand equally parsimonious trees with a length of 3233 steps (CI = 0. 363, RI = 0. 616, RC = 0. 223, HI = 0. 637) in the first tree. Tree topology of the maximum parsimony, Bayesian analysis was almost compatible with the ML tree and the best scoring RAxML tree, is shown (Fig. 1).

The *Chlamydotubeufia* Clade comprises five species and is a well-supported clade (98% ML, 81% MP, 1.00 BYPP) within Tubeufiaceae. It is a well-defined genus comprising both asexual and sexual morphs. The new taxon *C. aquatica* forms a well-separated (100% ML) lineage sister to *C. khunkornensis* Boonmee & K.D. Hyde.

The new genus *Dictyospora* is monotypic and typified by the new species *Dictyospora thailandica* (MFLUCC 16-0001). It forms a separate clade apart from all the other genera of Tubeufiaceae and basal to a clade comprising genera *Helicoma* and *Neoacanthostigma*.

The *Helicosporium* Clade comprises seven species including a new species *Helicosporium flavum* (MFLUCC 16-1230). *Helicosporium flavum* clustered with *H. luteosporum* (MFLUCC 16-1233) with good support (90% MP, 1.00 BYPP), but is clearly a distinct taxon.

Taxonomy

Chlamydotubeufia aquatica Brahamanage, Y.Z. Lu, Boonmee & K.D. Hyde, *sp. nov.* Fig. 2

Index Fungorum Number – IF 553177; Facesoffungi Number − FoF 03261

Etymology − Name referring the aquatic habitat, of which the species was collected

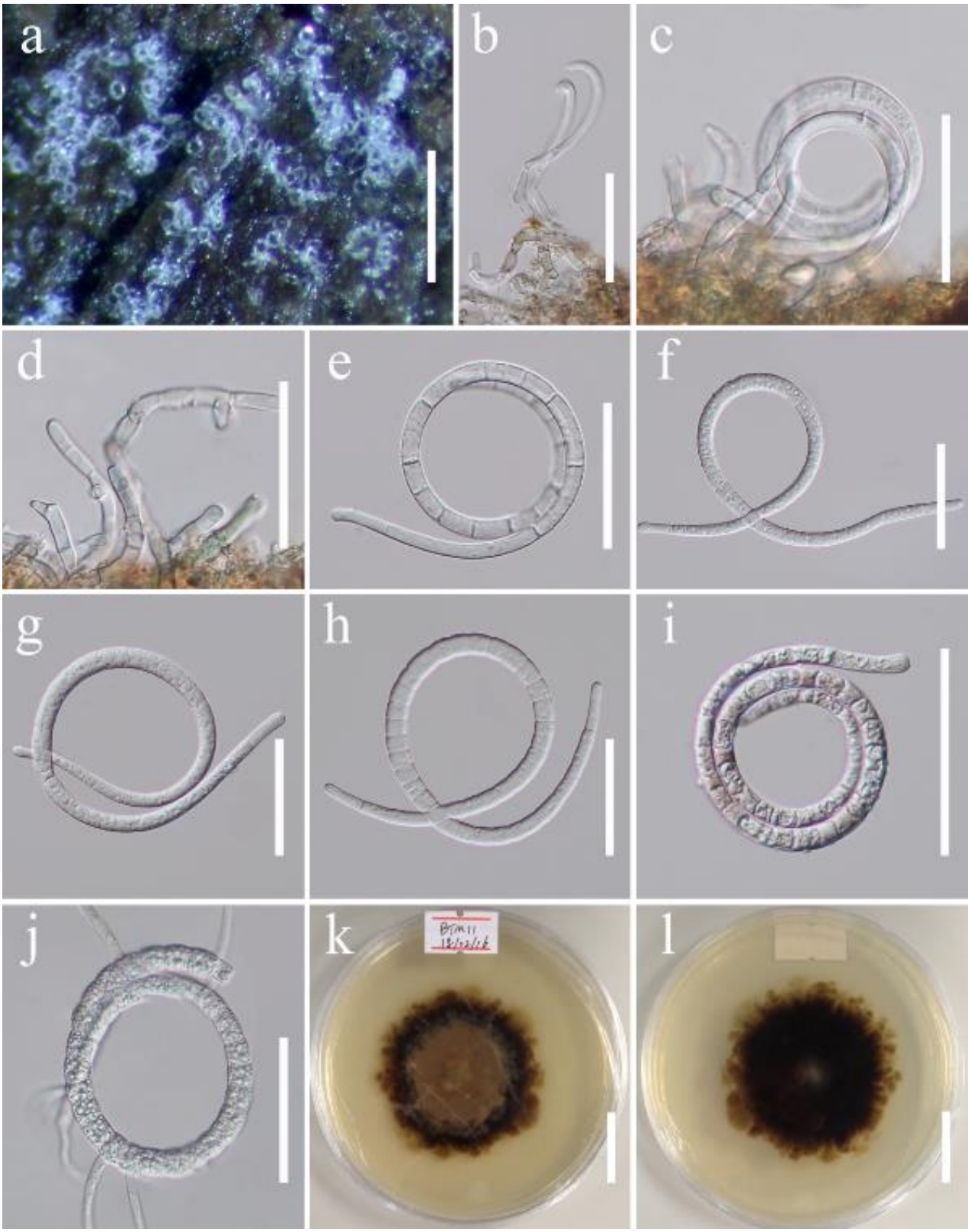

Fig. 2 – ***Chlamydotubeufia aquatica*** (MFLU 17-0501, **holotype**). a Conidia arise directly from hyphal cells on natural substrate. b, c Conidia attached to conidiophores. d Conidiophores and conidiogenous cells. e–i Conidia. j Germinating conidium. k, l Culture colonies on PDA at 30 days. Scale bars – a = 500 µm, b–j = 50 µm, k–l = 20 mm.

Holotype − MFLU 17-0501

Saprobic on submerged decaying wood. Mycelium partly immersed, dark brown, branched, septate, with masses of crowded conidia. **Sexual morph**: Undetermined. **Asexual morph**:

hyphomycetous, helicosporous. *Conidiophores* 28–69 × 4–4.5 μm ($\bar{x}$ = 48 × 4.5 μm, n = 20), mononematous, erect, septate, short, hyaline to pale brown, smooth-walled. *Conidiogenous cells* 11–14 × 4–6 μm ($\bar{x}$ = 14 × 5 μm, n = 10), monoblastic, holoblastic, integrated, terminal, cylindrical, each with single conidium. *Conidia* 62–89 μm diam. and conidial filament 4.5–9 μm wide ($\bar{x}$ = 74 × 6.5 μm, n = 20), with 144–392 μm long, coiled 2½–3 times when tightly coiled, becoming loosely coiled in the water, rounded at apical end, up to 60-septate, slightly constricted at septa, hyaline, smooth-walled.

Culture characteristics − Conidia germinating on water agar (WA) within 24 h and germ tubes produced from conidia. Colonies growing on MEA, reaching 5 mm in 7 days at 28 °C, slightly convex, undulating to raised, dentate, with lobate edges, brown. Mycelium superficial and partially immersed, branched, septate, smooth, hyaline, pale brown to brown.

Material examined − THAILAND, Krabi, Plai Praya, Khao To, Ban Bang Thao Mae, on submerged decaying wood, 17 December 2015, S. Boonmee, BTM11 (MFLU 17-0501, **holotype**), isotype in BBH, ex-type culture, MFLUCC 16–1131, TBRC.

Notes − *Chlamydotubeufia aquatica* is morphologically similar to *C. helicospora* Boonmee, Y.Z. Lu & K.D. Hyde. However, *C. aquatica* differs from *C. helicospora* in having longer conidiophores (28–69 × 4–4.5 μm vs 15–25 × 4–6 μm) and shorter conidial filaments (144–392 μm vs 405–546 μm). Phylogenetic analysis placed *C. aquatica* in a node of species *C. khunkornensis* Boonmee & K.D. Hyde (100% ML), while *C. helicospora* is placed in a basal lineage of the genus (Fig. 1). *Chlamydotubeufia aquatica* is therefore assigned as a new species in this genus based on morphology and phylogeny evidences.

Dictyospora Brahamanage, Y.Z. Lu, Boonmee & K.D. Hyde, *gen. nov.*

Index Fungorum Number – IF 553178; Facesoffungi Number − FoF 03262

Etymology – Name referring to the feature of dictyosporous conidia.

Saprobic on submerged decaying wood. **Sexual morph**: *Ascomata* superficial, seated on a subiculum, solitary, scattered, globose to subglobose, black, surrounded by brown to black setae. *Peridium* composed of several-layers of cells of *textura angularis*, with outer layer cells darkened and inner layer cells pale brown to hyaline. *Hamathecium* comprising numerous filiform, septate, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate, cylindrical, short-pedicellate, apically rounded. *Ascospores* fasciculate, broadly fusiform, elongate-cylindrical to subfusiform, slightly curved, tapering towards rounded ends, long multi-septate, hyaline, smooth-walled. **Asexual morph**: hyphomycetous, dictyochlamydosporous. *Conidia* broadly oval to ellipsoid, dictyoseptate, pale brown when immature, becoming dark to black when mature.

Type species − ***Dictyospora thailandica*** Brahamanage, Y.Z. Lu, Boonmee & K.D. Hyde

Notes – *Dictyospora* appears to shares similar morphological characters with species of Tubeufiaceae. *Dictyospora* consists of a monotypic species *Dictyospora thailandica* and formed a well-separated clade from all other genera within the Tubeufiaceae. Morphologically, *D. thailandica* is characterized by superficial, globose to subglobose, setiferous ascomata, cylindrical asci and septate ascospores, while the asexual morph has similar characters with species in *Chlamydotubeufia*. However, a new genus *Dictyospora* is introduced based on multigene phylogenetic evidence (Fig. 1).

Dictyospora thailandica Brahamanage, Y.Z. Lu, Boonmee & K.D. Hyde, *sp. nov.* Figs. 3–5

Index Fungorum Number – IF 553179; Facesoffungi Number − FoF 03263

Etymology − Name referring to the host locality country Thailand.

Holotype − MFLU 17-0500

Saprobic on submerged decaying wood. **Sexual morph**: *Ascomata* 173–212 μm high × 165–206 μm diam. ($\bar{x}$ = 195 × 188 μm, n = 5), superficial, seated on a subiculum, solitary, scattered, globose to subglobose, dark brown to black, surrounded by brown to black setae, with 53–95 (–103) × 3–5 μm septate, tapering towards an acute end at apex.

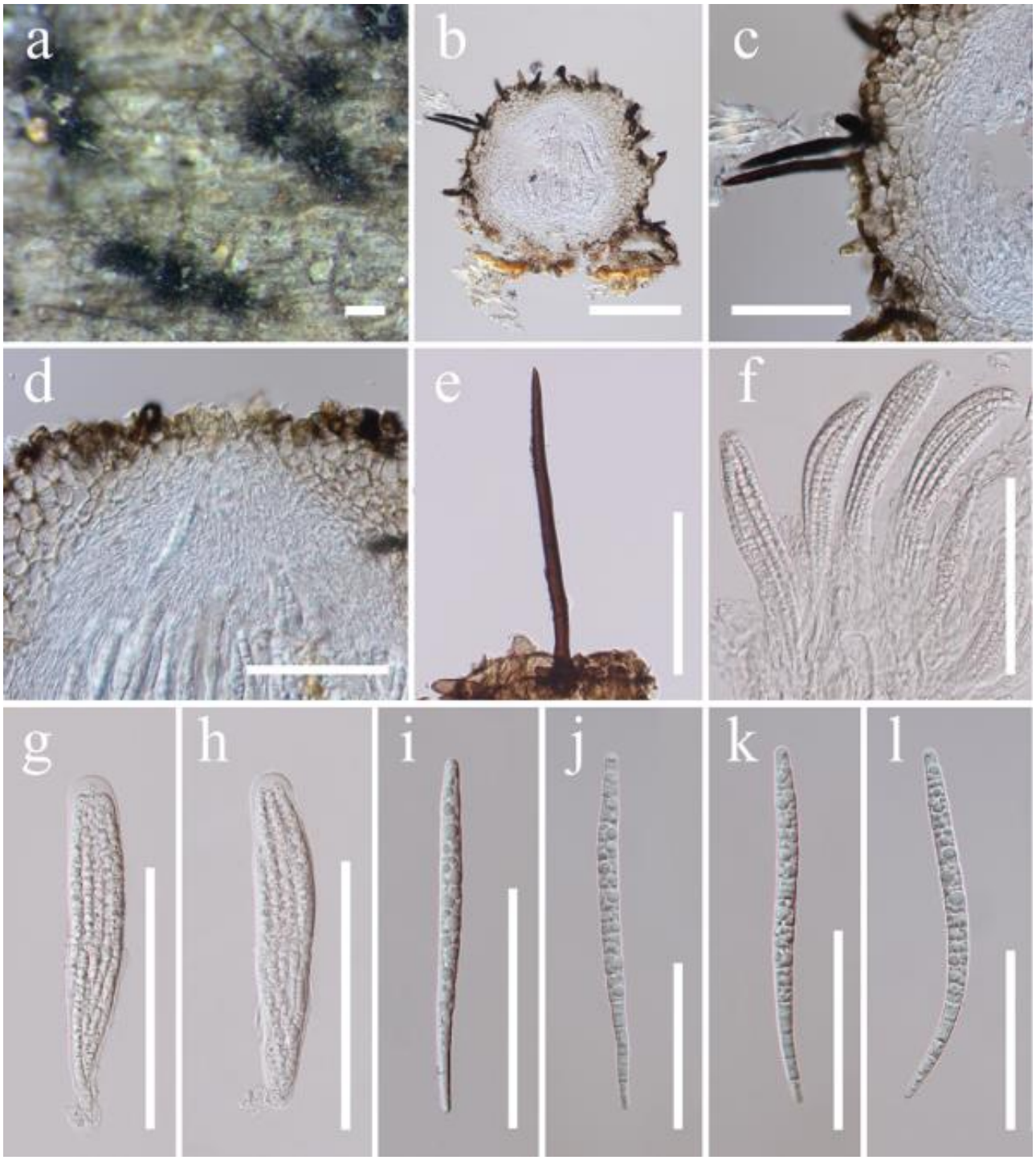

Fig. 3 – ***Dictyospora thailandica*** (MFLU 17-0500, **holotype**). a Appearance of ascomata on wood substrate. b Vertical section through ascoma. c Peridium. d Close up of ostiole. e Setae.f–h Asci. i–l Ascospores. Scale bars – a = 200 µm, b, f–h = 100 µm, c–e, i–l = 50 µm.

Peridium 21–38 µm wide, composed of several layers of hyaline to brown-cells of *textura angularis*, with outer layer darkened cells and inner layer pale brown to hyaline cells. *Hamathecium* comprising numerous filiform, septate, hyaline pseudoparaphyses. *Asci* 129–182 × 18–24.5 µm ($\bar{x}$ = 152 × 21 µm, n = 20), 8-spored, bitunicate, cylindrical, apically rounded, short-pedicellate or sessile. *Ascospores* 76–107 × 4.5–7 µm ($\bar{x}$ = 95 × 5.5 µm, n = 50), fasciculate, broadly fusiform, cylindrical to long.

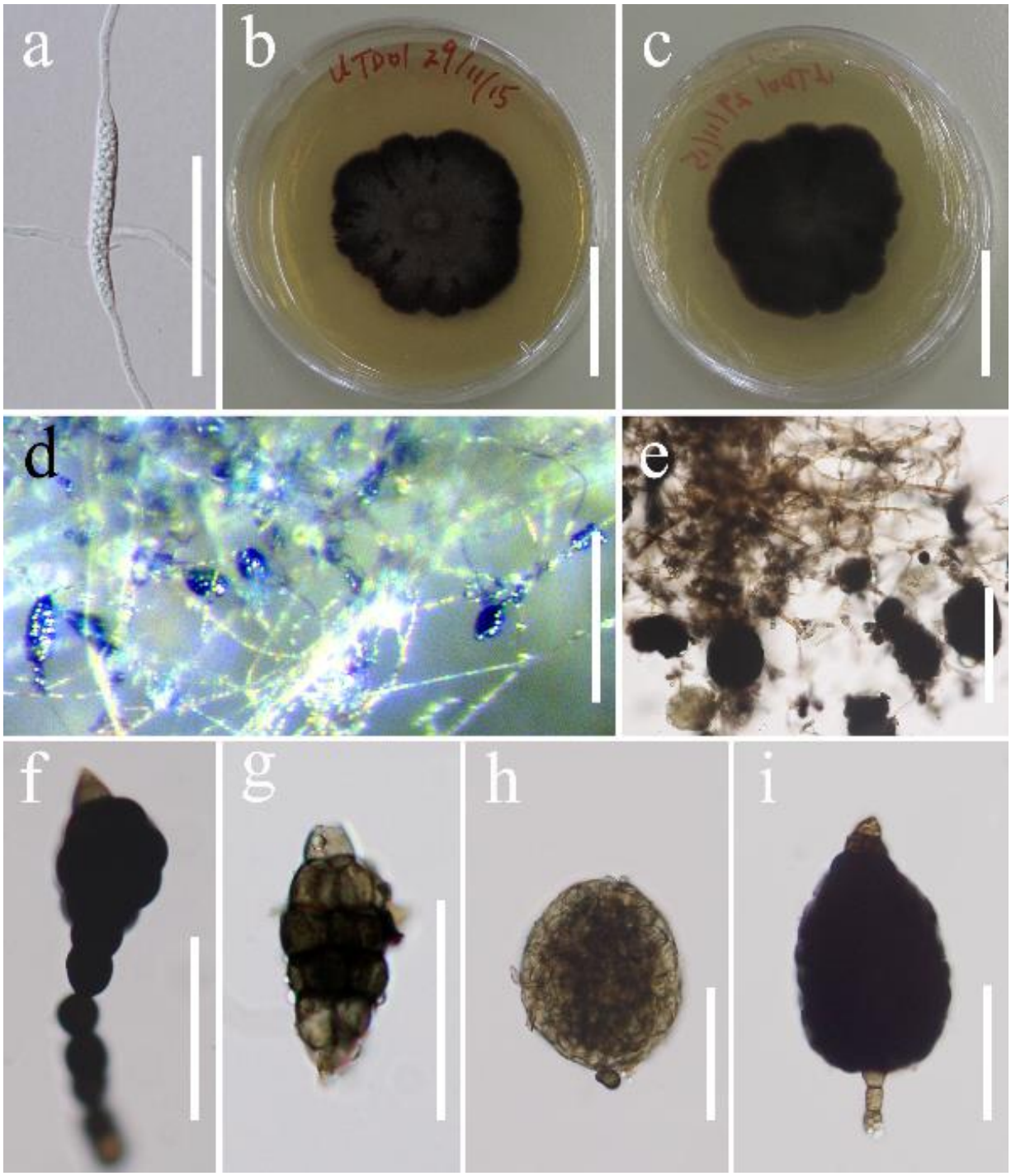

Fig. 4 – ***Dictyospora thailandica*** on MEA after 8 weeks (MFLUCC 16-0001, **holotype**). a Germinating ascospore. b, c Culture on PDA. d Conidia on colony. e Conidiophores with conidia. f–i Mature and immature conidia. Scale bars – a, f–i = 50 µm, b–c = 20 mm, d = 200 µm, e = 100 µm.

sub fusiform, elongate, slightly curved, tapering towards rounded ends, phragmoseptate, hyaline, guttulate, smooth-walled. Asexual morph: hyphomycetous, dictyosporous. Conidia 51–100 × 39–62 µm (= 72.5 × 51 µm, n = 20), blastic, broadly oval to ellipsoid, dictyoseptate, pale brown when immature, becoming dark to black when mature.

Culture characteristics − Ascospores germinating on WA within 12 h. Colonies growing on malt extract agar (MEA), reaching 18 mm in 2 weeks at 28°C, effuse, dentate and lobate edges, darkly brown. Mycelium superficial and partly immersed, branched, septate, pale brown to brown, smooth-walled, due to the development of muriform chlamydospores.

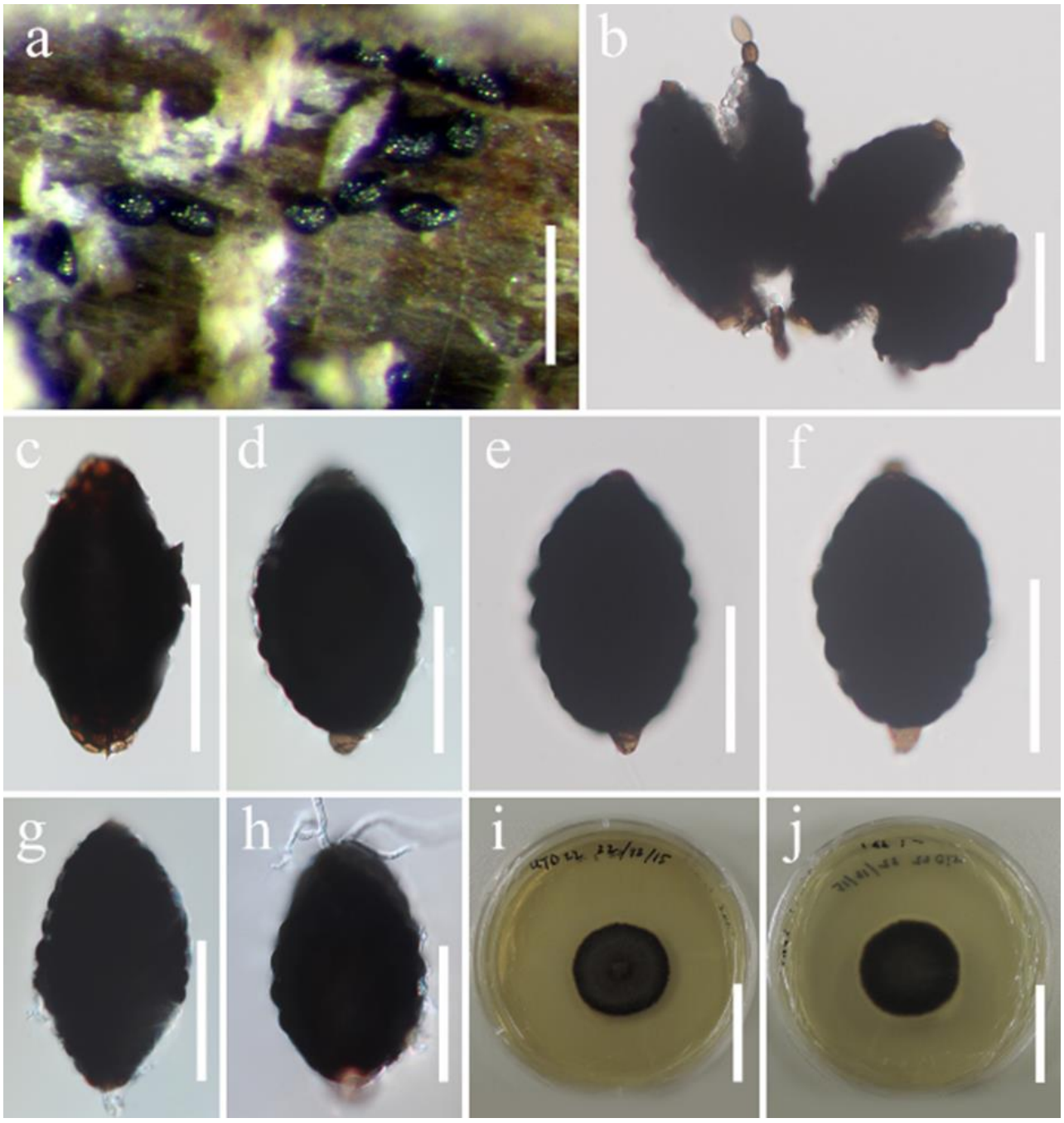

Fig. 5 – ***Dictyospora thailandica*** on natural substrate (MFLU 17-0500, **paratype**). a Conidia arise directly from mycelium on natural substrate. b–g Mature and immature conidia. h Germinating conidium. i, j Culture on PDA. Scale bars – a = 200 µm, b–h = 50 mm, i–j = 20.

Material examined − THAILAND, Uttaradit, Laplae, Mae Phun, Ban Ton Klua, on submerged decaying wood, 24 October 2015, S. Boonmee, UTD01 (MFLU 17-0500, **holotype**), isotype in BBH, ex-type living culture, MFLUCC 16−0001 and UTD 22 (MFLU 17-0703, **paratype**), ex-type living culture, MFLUCC 16−0215, TBRC.

Note − *Dictyospora thailandica* is similar with *Chlamydotubeufia huaikangplaensis* Boonmee & K.D. Hyde in having continued sexual and asexual states. However, the sexual characteristics of *D. thailandica* are distinctly differed in the features of ascomata, asci and ascospores. In addition, multigene phylogenies placed it in a clearly defined monophyletic clade (100% ML, 100% MP, 1.00 BYPP), distant from *Chlamydotubeufia* clade. A new species *D. thailandica* is therefore introduced with morphological details and phylogenetic placement (Figs. 1, 3, 4 and 5).

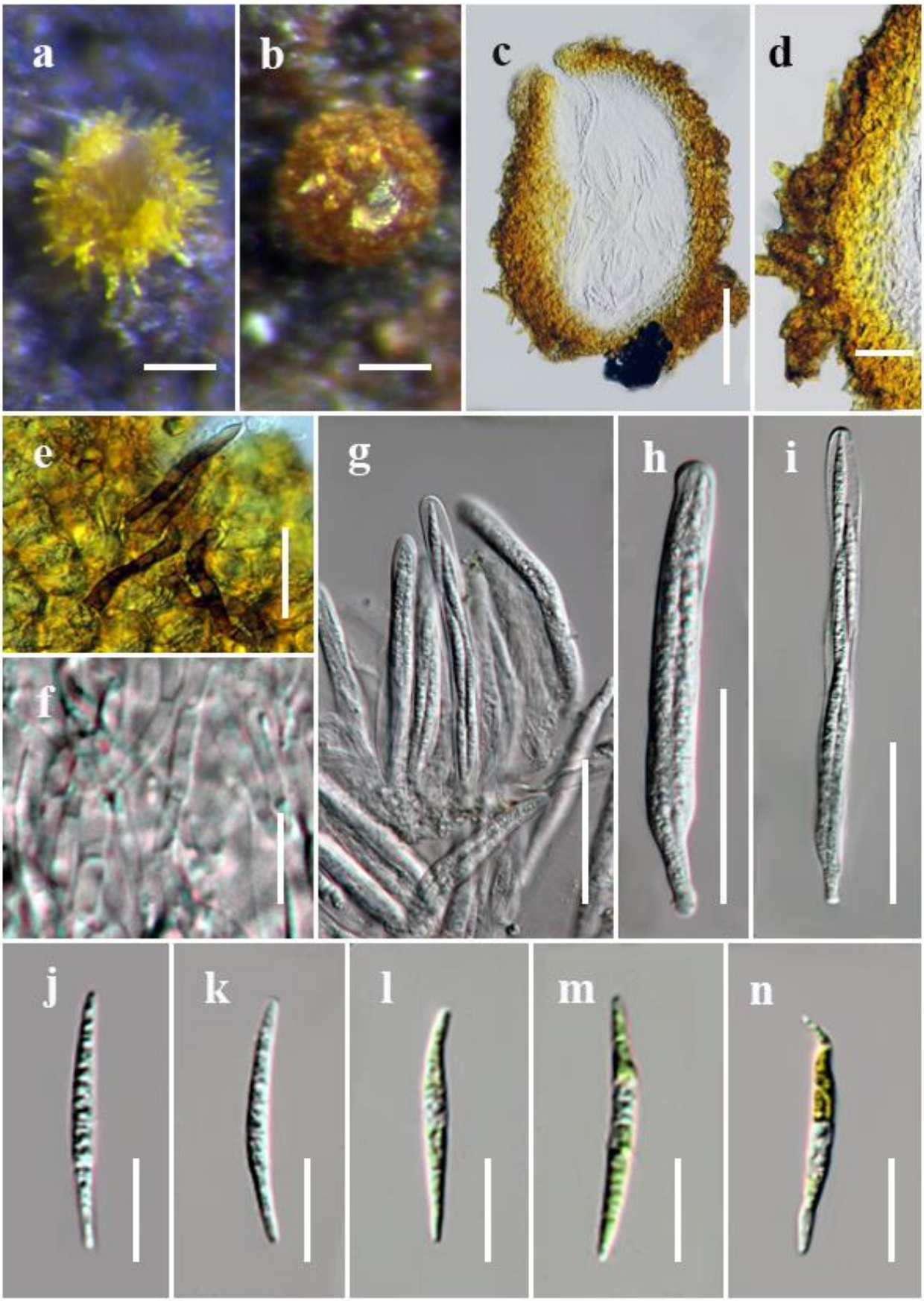

Fig. 6 – ***Helicosporium flavum*** (MFLUCC16-1230, **holotype**). a–b Ascoma on wood substrate. b Close up of ostiole in top view. c Cross section of Ascoma. d Peridium with short setae. e setae. f Hamathecium pseudoparaphyses. g–i Asci. j–n Ascospores. Scale bars – c = 100 µm, d = 5 µm, e–g = 50 µm, h–i =50, i–m = 20 µm.

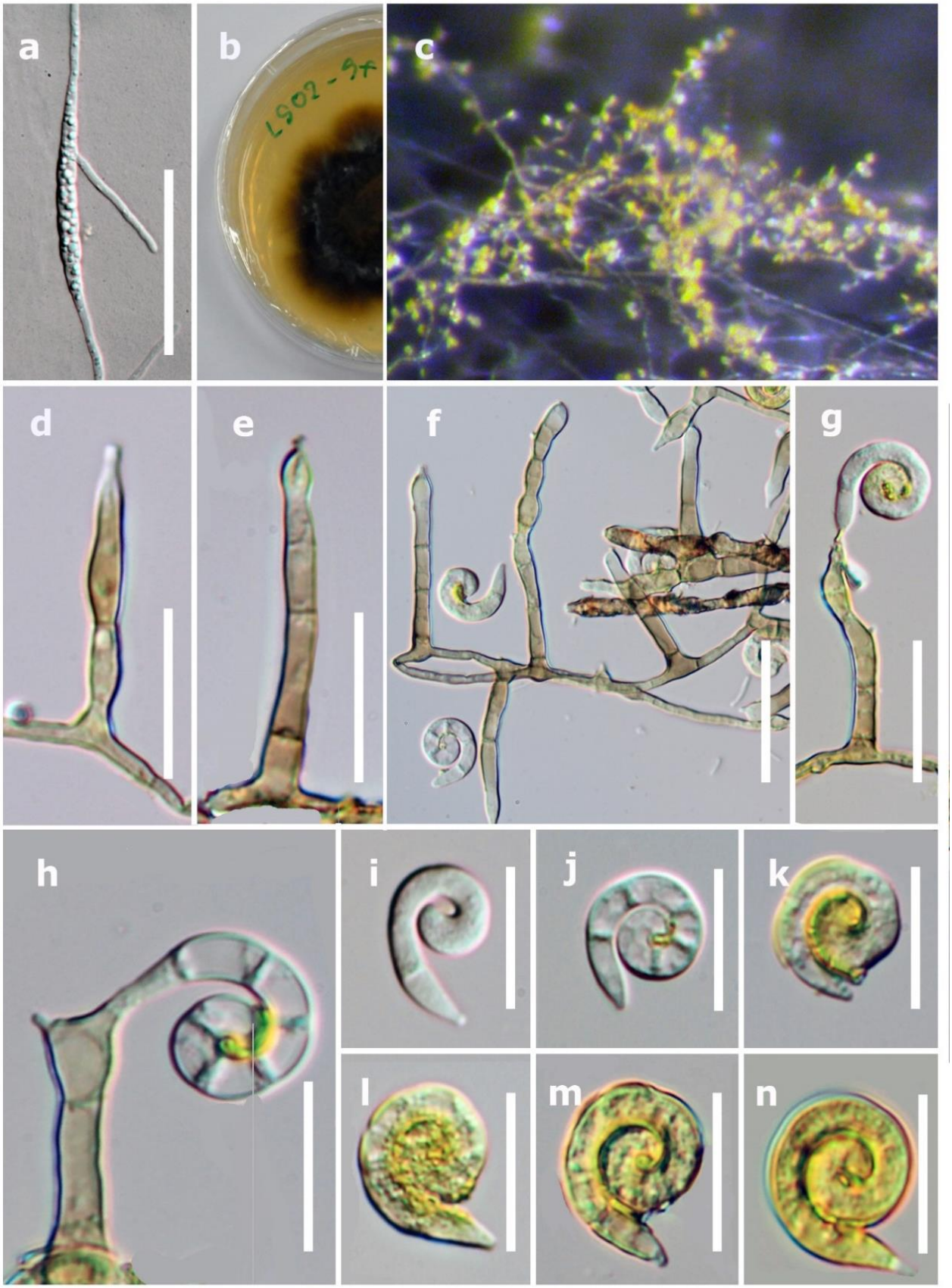

Fig. 7 – ***Helicosporium flavum*** on MEA culture (MFLU 17-0704, **holotype**). a Germinating ascospore. b Culture on MEA from surface. c Colony on MEA. d–f Conidiophores. g, h Conidiophores with the attached conidia. i–n Conidia. Scale bars – a, i–n = 10 µm, c = 200 µm, d, h = 50 µm, f–g = 100 µm.

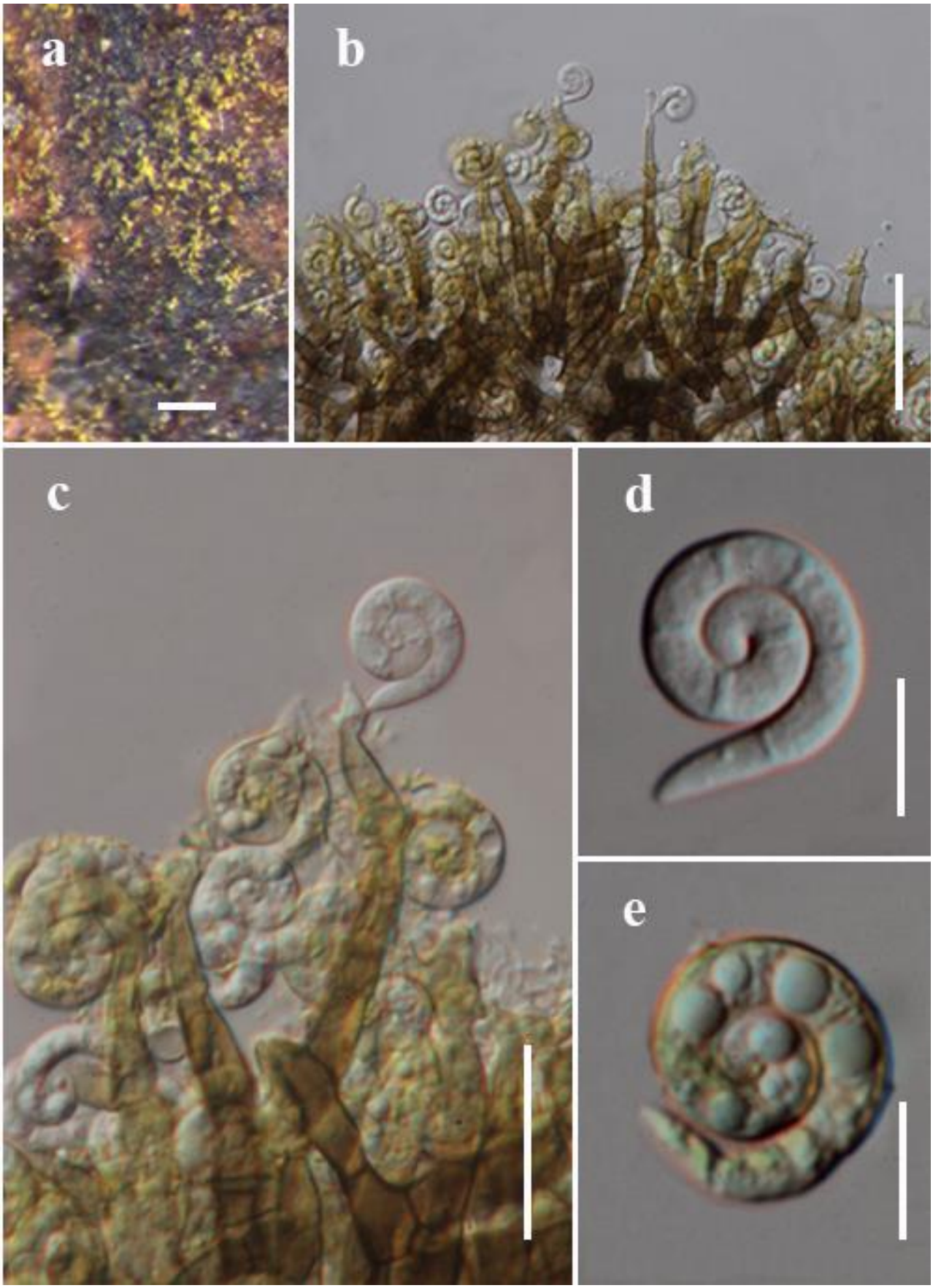

Fig. 8 – ***Helicosporium flavum*** on substrate (MFLU 17-0704, **holotype**)**.** a Colony on wood substrate. b, c Conidiophores with apical conidia on natural substrate. d, e Conidia. Scale bars – a = 50 µm, b = 100 µm, c = 50 µm, d–e = 10 µm.

Helicosporium flavum Brahamanage, Y.Z. Lu, Boonmee & K.D. Hyde, *sp. nov.* Figs 6,7

Index Fungorum Number – IF 553180; Facesoffungi Number − FoF 03264

Etymology − Name reflects the bright yellow to brown yellow ascomata

Holotype − MFLU 17-0704

Saprobic on submerged decaying wood. **Sexual morph**: *Ascomata* 320–350 µm high, 220–260 µm diam., superficial, seated on a subiculum, solitary, scattered, globose to subglobose, bright yellow to brown yellow, with central narrow ostiole, comprising short projections of setae-like. *Peridium* 50–70 µm wide, composed of several layers of hyaline to bright yellow-cells of *textura angularis*, outer layer yellow cells and inner layer pale yellow to hyaline cells. *Hamathecium* comprising numerous, 2–2.5 µm wide, filiform, septate, branched, hyaline pseudoparaphyses. *Asci* 70–130 × 12–16 µm ($\bar{x}$ = 105 × 14 µm, n = 20) 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short-pedicellate, apically rounded. *Ascospores* 40–60 ×8–12 µm ($\bar{x}$ = 50 × 10 µm, n = 20), overlapping 2–3 seriate, elongate-fusiform, tapering towards narrow, widest at the central part, subacute ends, straight to slightly curved, multi-septate, hyaline when young, becoming yellowish when mature, smooth walled. **Asexual morph**: hyphomycetous, helicosporous. *Conidiophores* 36–48×6.5–7.5 µm ($\bar{x}$ = 52 × 7 µm, n = 20), macronematous, erect, straight or slightly flexuous, septate, unbranched, tapering toward narrow subacute apex, pale brown to brown, smooth-walled. *Conidiogenous cells* holoblastic, monoblastic to polyblastic, terminal, integrated, ampulliform, with tooth-like protuberance, pale brown to brown. *Conidia* 18–30 µm diameter, with conidial filament 6.0–7.0 µm wide ($\bar{x}$ = 26×50 µm, n = 20), tightly coiled (1–1½) times hyaline to yellow, tapering towards the flattened end with a basal scar, septate, slightly constricted at septum, smooth-walled.

Culture characteristics − Ascospores germinating on WA within 24 h and germ tubes produced from both ends. Colonies growing slowly on MEA, reaching 4.5 mm in 2 weeks at 28 C, effuse, velvety to hairy, edge fimbriate, olive to olive brown, dark brown in MEA media. Mycelium superficial and partially immersed, branched, septate, pale brown to olivaceous brown, smooth-walled.

Material examined − THAILAND, Cahanthabori, Laem sing, on submerged decaying wood, 7 July 2015, S. Boonmee, (MFLU 17-0704, **holotype**) isotype in BBH, ex-type living culture, MFLUCC 16-1230 = TBRC.

Notes – *Helicosporium flavum* shares similar with *H. vegetum* Nees (= *Tubeufia cerea* (Berk. & M.A. Curtis) Höhn = *H. cereum*) in having sexual and asexual states (Boonmee et al. 2014). *Helicosporium flavum* is characterized by bright yellow ascomata with hyphal appendages on the surface, long cylindrical asci, yellow ascospores and having asexual conidia as helicoma-like with yellow-pigmented (Figs. 6-8). These characters are different from all species in *Helicosporium*. Phylogenetically, *H. flavum* clustered with *H. luteosporum* with good support (90% ML, 1.00 BYPP), but their morphological characters are clearly distinguishable (Lu et al. 2017b). *Helicosporium flavum* is therefore introduced as a new species in this study.

Acknowledgments

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