



Orbiliaceae from Thailand

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Abstract

The family Orbiliaceae is characterized by small, yellowish, sessile to sub-stipitate apothecia, inoperculate asci and asymmetrical globose to fusoid ascospores. Morphological and phylogenetic studies were carried out on new collections of Orbiliaceae from Thailand and revealed *Hyalorbilia erythro stigma*, *Hyalorbilia inflatula*, *Orbilia stipitata* sp. nov., *Orbilia leucostigma* and *Orbilia caudata*. Our new species is confirmed to be divergent from other Orbiliaceae species based on morphological examination and molecular phylogenetic analyses of ITS and LSU sequence data. Descriptions and figures are provided for the taxa which are also compared with allied taxa.

Key words – apothecia – discomycetes – inoperculate – phylogeny – taxonomy

Introduction

The family Orbiliaceae was established by Nannfeldt (1932). Previously, this family has been treated as a member of Leotiomycetes (Korf 1973, Spooner 1987) and Eriksson et al. (2003) transferred this family into a new class Orbiliomycetes. The recent studies on this class include Yu et al. (2011), Guo et al. (2014), Qiao et al. (2015), Quijada et al. (2012, 2014, 2015, 2016), Quijada & Baral (2017), Baral et al. (2017). A recent account of the family is provided by Ekanayaka et al. (2017). This family is wide spread, mostly saprobic on various plant substrates. Some species have the ability to trap invertebrates by means of adhesive devices or constricting rings (Li et al. 2006).

The present study provides morphological descriptions of five Orbiliaceae species from Thailand, viz. *Hyalorbilia erythro stigma*, *Hyalorbilia inflatula*, *Orbilia stipitata* sp. nov., *Orbilia leucostigma* and *Orbilia caudata*. Phylogenetic analyses inferred from LSU and ITS sequence data support the molecular lineages for taxa of *Orbilia*, corresponding to morphological features.

Materials & Methods

Sample collection specimen examination and deposition

Five specimens of *Orbilia* were collected from southern Thailand in 2014 to 2015. Macroscopic and microscopic characters of the specimens were recorded. A Motic SMZ-168 stereo microscope was used to observe structures of the apothecia. Thin hand sections of apothecia were made with a razor blade and mounted in water. A Nikon ECLIPSE 80i compound microscope was

used to observe the microscopic characters. Photomicrography was carried out with a Canon 450D digital camera fitted to the microscope. Measurements of paraphyses, asci and ascospores were made from materials mounted in water and the mean values were used in the descriptions. Measurements were made with the Taro soft (R) Image Frame Work v. 0.9.7 program and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems Inc.). The type specimens are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and in the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Facesoffungi and Index Fungorum numbers were registered as described in Jayasiri et al. (2015) and Index Fungorum (2017).

DNA extraction, PCR and sequencing

Genomic DNA was extracted directly from the apothecia using a Plant DNA Rapid Extraction Kit (Bio Teke corporation, Beijing, China). Polymerase chain reactions (PCR) for this study were carried out for the internal transcribed spacer (ITS), using ITS4 and ITS5 (White et al. 1990) and LROR and LR5 (Vilgalys & Hester 1990) for the nuclear ribosomal large subunit (LSU) primers. The PCR mixtures (25 μ L) contained ddH₂O (11 μ L), PCR Master Mix (QinKe Co., China) (11 μ L; 2 \times), DNA template (1 μ L), each primer (1 μ L; 10 μ M). PCR amplification conditions for both gene regions were consisted an initial denaturation step of 5 min at 94 °C, 35 cycles consisted of denaturation at 94 °C for 1 minute, annealing at 53 °C for 50 seconds and elongation at 72 °C for 3 minute and final extension step of 7 minutes at 72 °C. The PCR products were viewed on 1 % agarose electrophoresis gels, stained with ethidium bromide. PCR products were sent to a commercial sequencing provider, Qinke in Kunming, China.

Sequence alignment and phylogenetic analysis

Newly generated sequences in this study were subjected to a standard BLAST search of GenBank for rough identification. For the phylogenetic analysis, sequences belonging to ITS and LSU gene regions from representative Orbiliaceae species and the out-group taxon *Tuber albidum* Fr., were downloaded from GenBank (Table 1). The newly generated sequences are deposited in GenBank (Table 1). The consensus sequences for each gene were aligned using MAFFT v. 6.864b (<http://mafft.cbrc.jp/alignment/server/index.html>). The alignment was improved manually where necessary using Bioedit (Hall 2004). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Maximum likelihood (ML) phylogenetic analyses were performed in the CIPRES web portal (Miller et al. 2010) using RAxML-HPC2 Workflow on XSEDE (8.2.9) tool. The bootstrap analysis for each ML tree was performed with 1000 thorough bootstrap replicates with the same parameter settings using combined alignment of ITS and LSU gene regions. The resultant trees were viewed with FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). Maximum parsimony analysis (MP) was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) for the combined ITS and LSU gene regions. Ambiguously aligned regions were excluded from the analysis, gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Branches of zero length were collapsed and all equally most parsimonious trees were saved. Descriptive tree statistics such as tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], and homoplasy index [HI], were calculated.

Posterior probabilities in Bayesian inference (BI) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). The GTR+ G substitution model selected by MrModel Test. Four simultaneous Markov chains were run for 25,000,000 generations and trees were sampled every 100th generation. The MCMC heated chain was set with a “temperature” value of 0.15. The distribution of loglikelihood scores were 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 2009). All sampled topologies beneath the asymptote (20 %) were

discarded as part of a burn-in procedure, while the remaining trees (20,000,000) were used for calculating posterior probabilities in the majority rule consensus tree.

The resultant trees were viewed with FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bayesian posterior probabilities (BPP) equal or greater than 0.9 are given as the first set of numbers above the nodes, Maximum likelihood bootstrap values (MLBP) equal or greater than 70% are given as the second set of numbers above the nodes and maximum-parsimony bootstrap values (MPBP) equal or greater than 70% are given as the third set of numbers near the nodes (Fig. 1).

Table 1 Taxa used in the two gene phylogenetic analyses and ITS and LSU GenBank accession numbers (Newly generated sequences are in bold).

Species name	Strain number	GenBank number
<i>Amphosoma atroolivacea</i>	G.M. 2010-09-03	KT380069
<i>Amphosoma resinicola</i>	H.B. 6992a	KT222389
<i>Arthrobotrys superba</i>	CBS 109 52	KT215210
<i>Arthrobotrys flagrans</i>	CBS 583 91	AY261132/AF106520
<i>Arthrobotrys oligospora</i>	ATCC 96709	EF445989
<i>Arthrobotrys scaphoides</i>	CBS 226 52	NR_145361
<i>Brachyphoris brevistipitata</i>	CBS 113946	KT215221
<i>Brachyphoris lignatilis</i>	YMFT 1.00596	KT215222
<i>Dactylella cylindrospora</i>	CBS 325 70	NR_145394
<i>Dactylella intermedia</i>	CBS109506	DQ494359
<i>Dactylella zhongdianensis</i>	H.B. 9641	KT222436
<i>Dactylellina leptospora</i>	CBS 100579	KT215199
<i>Dactylellina mammillata</i>	CBS229.54	AY902802/AY902794
<i>Dactylellina parvicollis</i>	CBS 317 94	KT215206
<i>Dactylellina pauca</i>	CBS 642 80	KT215205
<i>Drechslerella bembicodes</i>	HB 7165b	KT215295
<i>Drechslerella brochopaga</i>	ATCC 96710	EF445987
<i>Drechslerella dactyloides</i>	CNU091026	GU130301/GU130300
<i>Drechslerella doedycoides</i>	ATCC 96778	EF445992
<i>Gamsylella arcuata</i>	CBS 174 89	KT215197
<i>Gamsylella gephyropaga</i>	ATCC 96677	EF445990
<i>Gamsylella lobata</i>	CBS 329.94	AF106524
<i>Gamsylella phymatopaga</i>	CBS 568 95	KT215203
<i>Helicoon sessile</i>	A327	KY659207
<i>Hyalorbilia cf. inflatula</i>	MFLU 15-0167/ HD007	MG599270/MG599273
<i>Hyalorbilia latispora</i>	H.B. 9902c	KT222386
<i>Hyalorbilia polypori</i>	HB 7557	KT215223
<i>Hyalorbilia ulicicola</i>	HMAS 139526	DQ656691/DQ656646
<i>Lecophagus longisporus</i>	CBS 845 91	KT215220
<i>Lilapila oculispora</i>	H.B. 9631a	KT222383
<i>Lilapila oculispora</i>	G.M. 2011-09-19	KT222384
<i>Orbilia aethiopica</i>	HB 9246a	KT222424
<i>Orbilia alba</i>	H.B. 9645a	KT222438
<i>Orbilia albidorosea</i>	HB 6615a	KT215254

Table 1 Continued.

Species name	Strain number	GenBank number
<i>Orbilia albovinosa</i>	HB 7231f	KT215255
<i>Orbilia anigozanthi</i>	HB 8831	KT222374
<i>Orbilia aprilis</i>	HB 6801	KT215268
<i>Orbilia aristata</i>	HB 6713	KT596782
<i>Orbilia asomatica</i>	TFCMic 21258	KT222399
<i>Orbilia aurantiorubra</i>	HB 6815a	KF741595
<i>Orbilia auricolor</i>	HB 6664	KT215294
<i>Orbilia australiensis</i>	HB 7208b	KT215262
<i>Orbilia beltraniae</i>	TFC Mic. 24363	KT222405
<i>Orbilia beltraniae</i>	TFC Mic. 23890	KT222406
<i>Orbilia bomiensis</i>	–	DQ656686/DQ656629
<i>Orbilia brachychiti</i>	HB 7578a	KT215257
<i>Orbilia canadensis</i>	HB 6826	KT215277
<i>Orbilia cardui</i>	H.B. 9891	KT222402
<i>Orbilia carminorosea</i>	HB 8777a	KT222423
<i>Orbilia carpoboloides</i>	HB 6639c	KT215248
<i>Orbilia caulicola</i>	G.M. 2013-07-30	KT380067/KT380063
<i>Orbilia caudata</i>	MFLU 16-0580/ HD018	MG599271/MG599274
<i>Orbilia clavuliformis</i>	HB 6714	KT215271
<i>Orbilia comma</i>	HB 6639b	KT215258
<i>Orbilia corculispora</i>	HB 6279	KT215273
<i>Orbilia cotoneastri</i>	CBS 116281	KT215288
<i>Orbilia crenatomarginata</i>	HB 9265	KM248772
<i>Orbilia cryptogena</i>	HB 7397a	KT215272
<i>Orbilia cucumispora</i>	HB 6762	KT215231
<i>Orbilia denticulata</i>	HB 6725	KT215256
<i>Orbilia digitalina</i>	HB 7566a	KT596781
<i>Orbilia dryadum</i>	HB 6876a	KT215281
<i>Orbilia ebuli</i>	HB 9550	KT222425
<i>Orbilia epipora</i>	HB 9039	KT222431
<i>Orbilia eucalypti</i>	HB 6662	KT215285
<i>Orbilia euonymi</i>	H.B. 9152	KT222444
<i>Orbilia farnesiana</i>	HB 8997h	KT222421
<i>Orbilia flavida</i>	HB 6716	KT215228
<i>Orbilia flavidorosella</i>	G.M. 2012-9-18	KT222391
<i>Orbilia fusiformis</i>	YMF1.01848	EF026115/EF026114
<i>Orbilia gambelii</i>	HB 6466	KT215249
<i>Orbilia hesperidea</i>	HB 6694	KT215263
<i>Orbilia inaequalis</i>	HB 6963a	KT215283
<i>Orbilia lentiformis</i>	HB 6569c	KT215225
<i>Orbilia leucostigma</i>	HB 6810	KT215282
<i>Orbilia liliputiana</i>	HB 6905	KT215284
<i>Orbilia luteorubella</i>	YMF1.01843	DQ480728/DQ480727

Table 1 Continued.

Species name	Strain number	GenBank number
<i>Orbilia mammillata</i>	H.B. 7165c	KT215290
<i>Orbilia microserpens</i>	HB 6519a	KT215251
<i>Orbilia microsoma</i>	HB 6738a	KT215278
<i>Orbilia multimaeandrina</i>	HB 6737a	KT215243
<i>Orbilia multimicrosoma</i>	HB 9582a	KT380094
<i>Orbilia multiurosperma</i>	HB 6493a	KT215245
<i>Orbilia myriofusiclava</i>	HB 7237a	KT215274
<i>Orbilia myriosphaera</i>	HB 6679a	KT215233
<i>Orbilia nemaspora</i>	sp.4201	DQ656700/DQ656630
<i>Orbilia octoserpentina</i>	HB 6609b	KT215237
<i>Orbilia oudemansii</i>	HB 6972a	KT215292
<i>Orbilia ovoidea</i>	HB 6489a	KT215275
<i>Orbilia paracylindrospora</i>	HB 9484	KT222430
<i>Orbilia patellarioides</i>	HB 6490a	KT215224
<i>Orbilia phragmotricha</i>	HB 7535a	KT215259
<i>Orbilia pilifera</i>	HB 8362a	KT222364
<i>Orbilia pleioaustraliensis</i>	HB 6277e	KT215261
<i>Orbilia plesteuonymi</i>	HB 6494a	KT215234
<i>Orbilia pluristomachia</i>	HB 6617a	KT215232
<i>Orbilia polyspora</i>	HB 7243b	KT215276
<i>Orbilia quaestiformis</i>	HB 7121	KT215246
<i>Orbilia quercus</i>	HMAS 88783	DQ656669/AY804213
<i>Orbilia rectispora</i>	HB 7142	KT215289
<i>Orbilia rosea</i>	HB 6756a	KM199779
<i>Orbilia rubrovacuolata</i>	HB 6598a	KT215291
<i>Orbilia sarraziniana</i>	HB 7235	KM199780
<i>Orbilia serpentina</i>	HB 6609d	KT215238
<i>Orbilia spermoides</i>	HB 7517	KT380068
<i>Orbilia sphaerospora</i>	HB 9129	KT222429
<i>Orbilia stipitata</i>	MFLU 15-0229/ HD011	MG599272/MG599275
<i>Orbilia subaristata</i>	H.B. 6685a	KT215270
<i>Orbilia subocellata</i>	H.B. 6474	KT215227
<i>Orbilia subvinosa</i>	HB 6748f	KT215265
<i>Orbilia subvitalbae</i>	HB 6504a	KT215250
<i>Orbilia vinosa</i>	AFTOL-ID 905	DQ470952/DQ491511
<i>Orbilia xanthoguttulata</i>	Oxa79	KF768637
<i>Orbilia xinjiangensis</i>	HB 9646	KT222435
<i>Tridentaria implicans</i>	CBS 320 56	KT215279
<i>Tridentaria subuliphora</i>	CBS 700 93	KT215280
<i>Tuber albidum</i>	CBS 272 72	KT215193
<i>Vermispora fusarina</i>	CBS 382 84	HQ110699/DQ494377

Results

Phylogenetic analyses

A phylogenetic tree for *Orbilbia* species was generated using the combined ITS and LSU dataset and comprised 1632 characters including alignment gaps for 117 ingroup and one outgroup taxon. Of the 1632 characters, 563 were constant, 229 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 840 parsimony-informative characters resulted in most parsimonious tree (TL = 10400; CI = 0.223, RI = 0.501, RC = 0.111, HI = 0.777). The final likelihood value of best scoring tree was -43441.239297. The matrix had 1217 distinct alignment patterns, with 29.52% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.262, C = 0.227, G = 0.261, T = 0.251; substitution rates AC = 1.347511, AG = 1.974455, AT = 1.253581, CG = 0.889264, CT = 4.161195, GT = 1.000000; gamma distribution shape parameter α = 0.384574. The trees from MP, ML and BI analyses showed the same topologies (Fig. 1).

Taxonomy

Orbilbia stipitata Ekanayaka & K.D. Hyde, sp. nov.

Fig. 2

Index Fungorum number: IF554114; Facesoffungi number: FoF 03954

Etymology – The specific epithet *stipitata* refers to the ascospore shape.

Holotype – MFLU 15-0229

Description – Saprobiic on wood. Sexual morph: *Apothecia* 0.8–1.2 mm diam., scattered, yellow to cream, disc flat, superficial, margin smooth, discoid with a wide attachment subsessile to shortly stipitate. *Ectal excipulum* 18–23 μm wide (\bar{x} = 21 μm , n=10) at upper flanks, composed of thin-walled *textura globulosa*. *Medullary excipulum* 30–45 μm wide (\bar{x} = 36 μm , n=10) with few cell layers comprising with hyaline, gelatinized cells of *textura angularis*, sharply delimited from ectal excipulum. *Hymenium* hyaline. *Paraphyses* medium to strongly clavate, septate, unbranched, swollen at the apices, terminal cell 1–1.5 μm width (\bar{x} = 1.3 μm , n=20). *Asci* 28–35 \times 4–5 μm (\bar{x} = 31.8 \times 4.6 μm , n=30), 8-spored, with thin apex; cylindric-clavate, with a short- to long-stalked bifurcate base. *Ascospores* 8–11 \times 0.8–1.3 μm (\bar{x} = 9.8 \times 1.1 μm , n=40), 2-3-seriate, cylindric-subcylindric, immature spores are straight and mature spores are strongly curved, apex obtuse, 1-2 guttules present at both poles. Asexual morph: Unknown.

Material examined – THAILAND, Kun Korn waterfall, Chiang Rai Province, on unidentified wood, 20 January 2015, A.H. Ekanayaka, HD0011 (MFLU 15-0229, **holotype**).

Notes: — *Orbilbia stipitata* is morphologically similar to *O. vermiformis* and *O. arcospora* (Yu et al. 2007, Su et al. 2011). However, it differs by having larger (*O. vermiformis*, 39.3–62 \times 3.8–5 μm ; *O. arcospora*, 42–53 \times 3.4–4.4 μm) asci (Yu et al. 2007, Su et al. 2011). *Orbilbia stipitata* is phylogenetically close to *O. nemaspora* 4201 (Baral et al. 2017), but they differ in 15 base pairs in the ITS and LSU regions.

Hyalorbilia cf. inflatula (P. Karst.) Baral & G. Marson

Fig. 3

Facesoffungi number: FoF03955

Description – Saprobiic on wood. Sexual morph: *Apothecia* 0.4–2 mm diam., gregarious, brownish yellow, discoid to deeply cupulate when mature, superficial, margin smooth, sessile with wide attachment. *Ectal excipulum* 10–15 μm wide at lower flanks, composed of large, thin-walled, yellowish cells of *textura angularis-prismatica*. *Medullary excipulum* 38–45 μm wide at lower flanks, composed of large, thin-walled, hyaline, gelatinized cells of *textura angularis-prismatica*, not sharply delimited from ectal excipulum. *Hymenium* hyaline, asci and paraphyses glued together and made a thick layer. *Paraphyses* uninflated, slightly septate at the base, unbranched, terminal cell 2–3 μm wide (\bar{x} = 1.8 μm , n=20). *Asci* 25–30 \times 3–4 μm (\bar{x} = 25.9 \times 3.6 μm , n=30), 8-spored, biseriate,

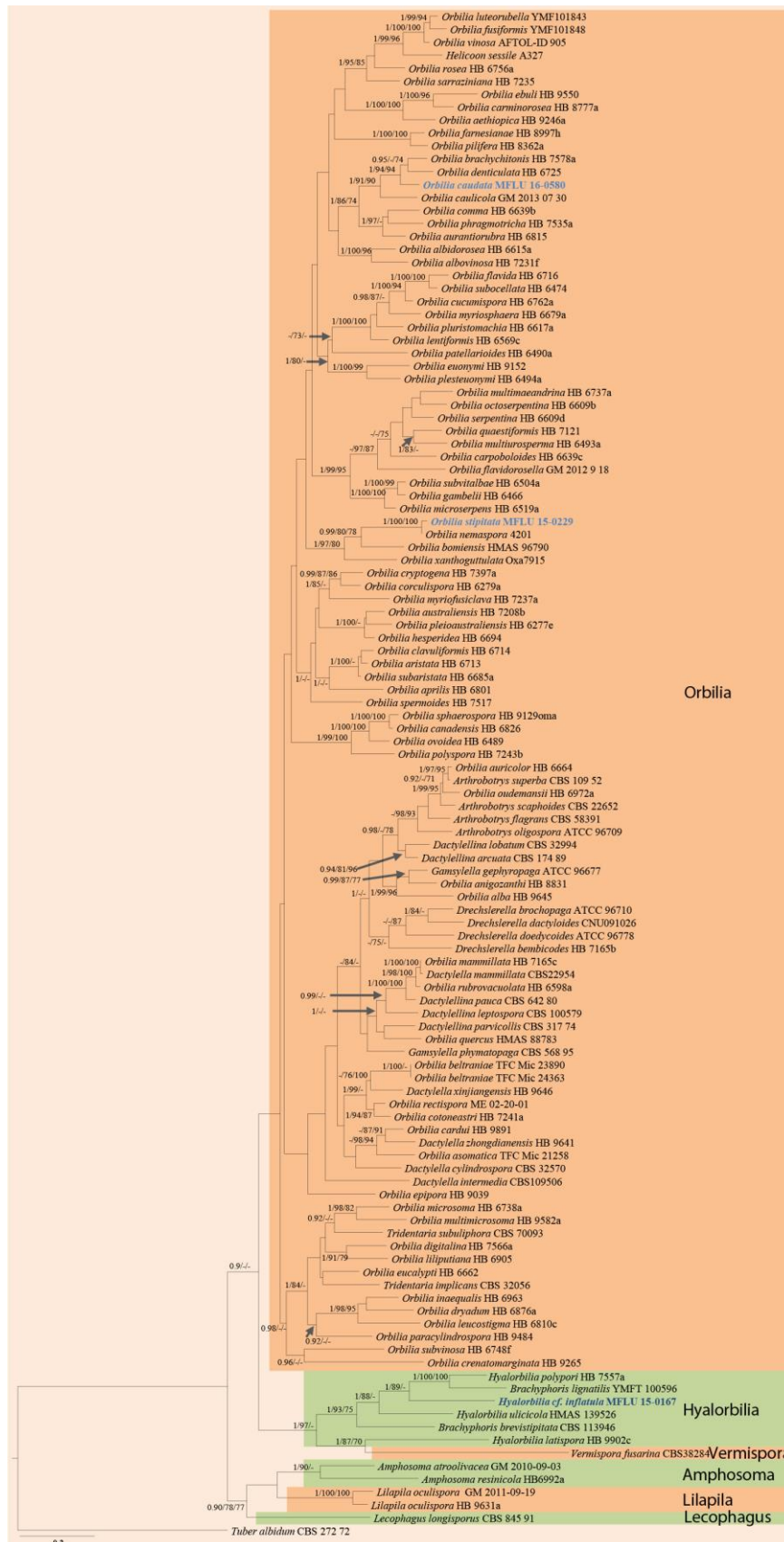


Figure 1 – Phylogram generated from maximum likelihood analysis of sequences of Orbiliaceae based on ITS and LSU sequence data. BPP \geq 0.90 are given as the first set of numbers, MLBP \geq 70% are given as the second set of numbers and MPBP \geq 70% are given as the third set of numbers, near the nodes. Strain/culture numbers are given after the taxon names. The newly generated sequences are in blue bold. The tree was rooted with *Tuber albidum* (CBS 272-72).

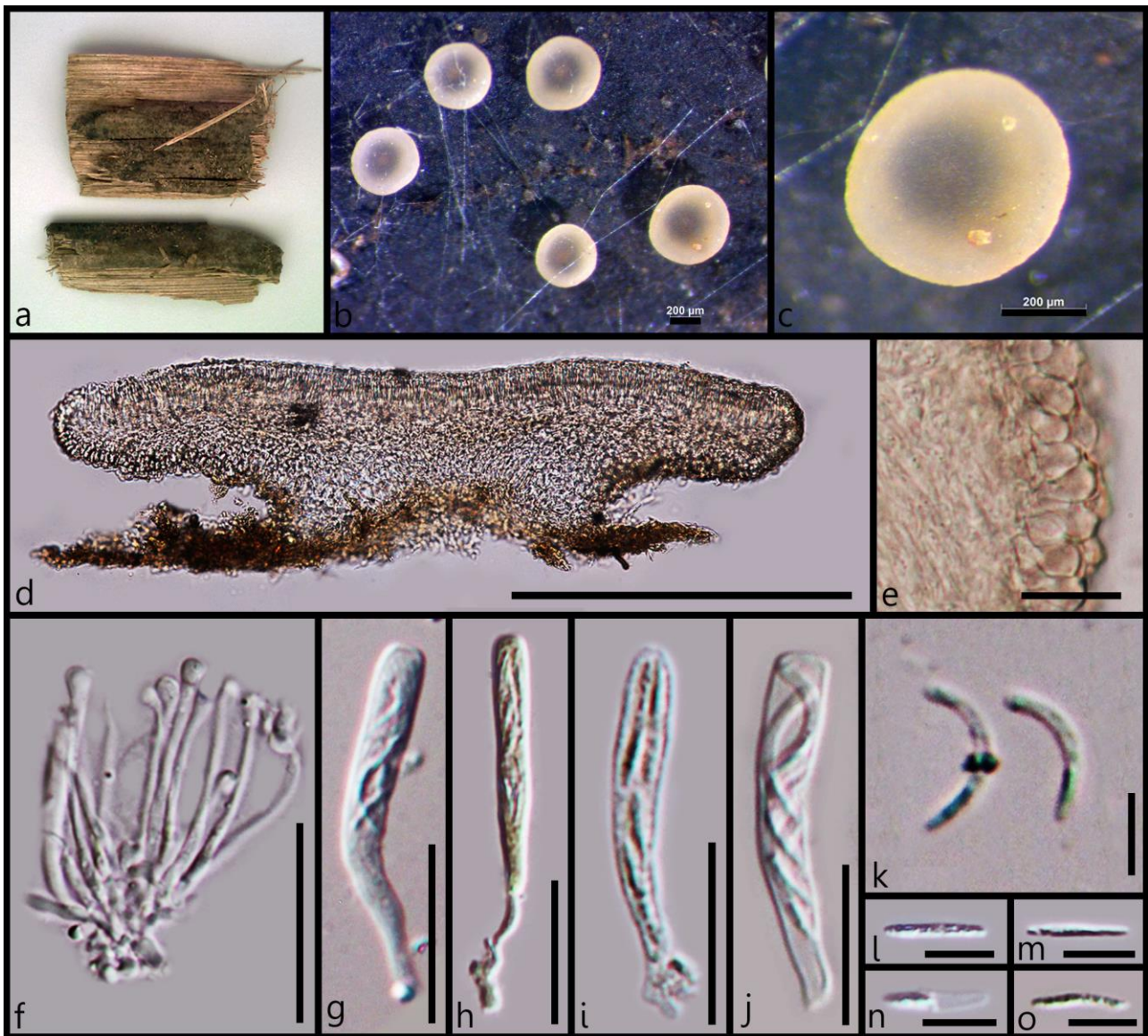


Figure 2 – Morphology of *Orbilia stipitata* (Holotype MFLU 15-0229) a Substrate. b,c Fresh apothecia. d Median section of an apothecia. e Excipular cells at upper flanks. f Paraphyses. g, h, i, j Asci, k-o: Ascospores. Scale bars: b, c = 200 μ m, d = 300 μ m, e = 15 μ m, f = 10 μ m, g-j = 15 μ m, k-o = 5 μ m.

cylindric-clavate, 2-3-seriate, short pedicellate with croziers. *Ascospores* 4.5–5.5 \times 0.5–1.3 μ m (\bar{x} = 5.2 \times 1.1 μ m, n=40), cylindrical, strongly to slightly curved, both ends rounded to obtuse, eguttulate or 1-2 guttules present. Asexual morph: Undetermined.

Material examined – THAILAND, Mae Fah Luang University, Chiang Rai Province, on unidentified wood, 15 January 2015, A.H. Ekanayaka, HD007 (MFLU 15-0167).

Notes – Sequence data from type specimen of *H. inflatula* is not available. However, the description for *H. inflatula* in Quijada et al. (2015) is identical with our isolate. Therefore, we place our collection under *H. inflatula*. For details about differences of *H. inflatula* with morphological related species see Quijada et al. (2015).

Orbilia caudata Starbäck Bih. Kongl. Svenska Vetensk.-Akad. Handl., Afd. 3, 25(1): 8, 1899.

Fig. 4

Facesoffungi number: FoF03956

Description – Saprobic on wood. Sexual morph: *Apothecia* 0.2–0.7 mm diam., superficial, scattered to subgregarious, yellow to orange, margin smooth, disc flat, superficial, sessile with wide

attachment. *Ectal excipulum* 12–16 μm wide at lower flanks, composed of large, thin-walled, yellowish cells of *textura angularis-globulosa*. *Medullary excipulum* 28–35 μm wide at lower flanks, composed of large, thin-walled, hyaline, gelatinized cells of *textura angularis-intricata*, not sharply delimited from ectal excipulum. *Hymenium* hyaline. *Paraphyses* 1.5 – 2.0 μm wide (\bar{x} = 1.8 μm , n=20) at the middle, medium clavate, numerous, filamentous, septate, branched at the base, slightly swollen at the apices, terminal cell 2 – 3 μm wide (\bar{x} = 2.7 μm , n=20). *Asci* 30–40 \times 4.0–5.5 μm (\bar{x} = 34.7 \times 5.1 μm , n=30) 8-spored, 1-2-seriate, cylindrical, apices truncate with a long-stalked bifurcate base. *Ascospores* 7–9 \times 1.4–2 μm (\bar{x} = 8.1 \times 1.6 μm , n=40), asymmetrical, fusoid to ellipsoid, apex obtuse, tapered, sometimes form an inflated tail, straight to slightly-medium curved, base obtuse, hyaline, aseptate, smooth-walled, guttulate. Asexual morph: Undetermined.

Material examined – THAILAND, Mae Fah Luang University, Chiang Rai Province, on unidentified wood, 18 March 2015, A.H. Ekanayaka, HD018 (MFLU 16-0580).

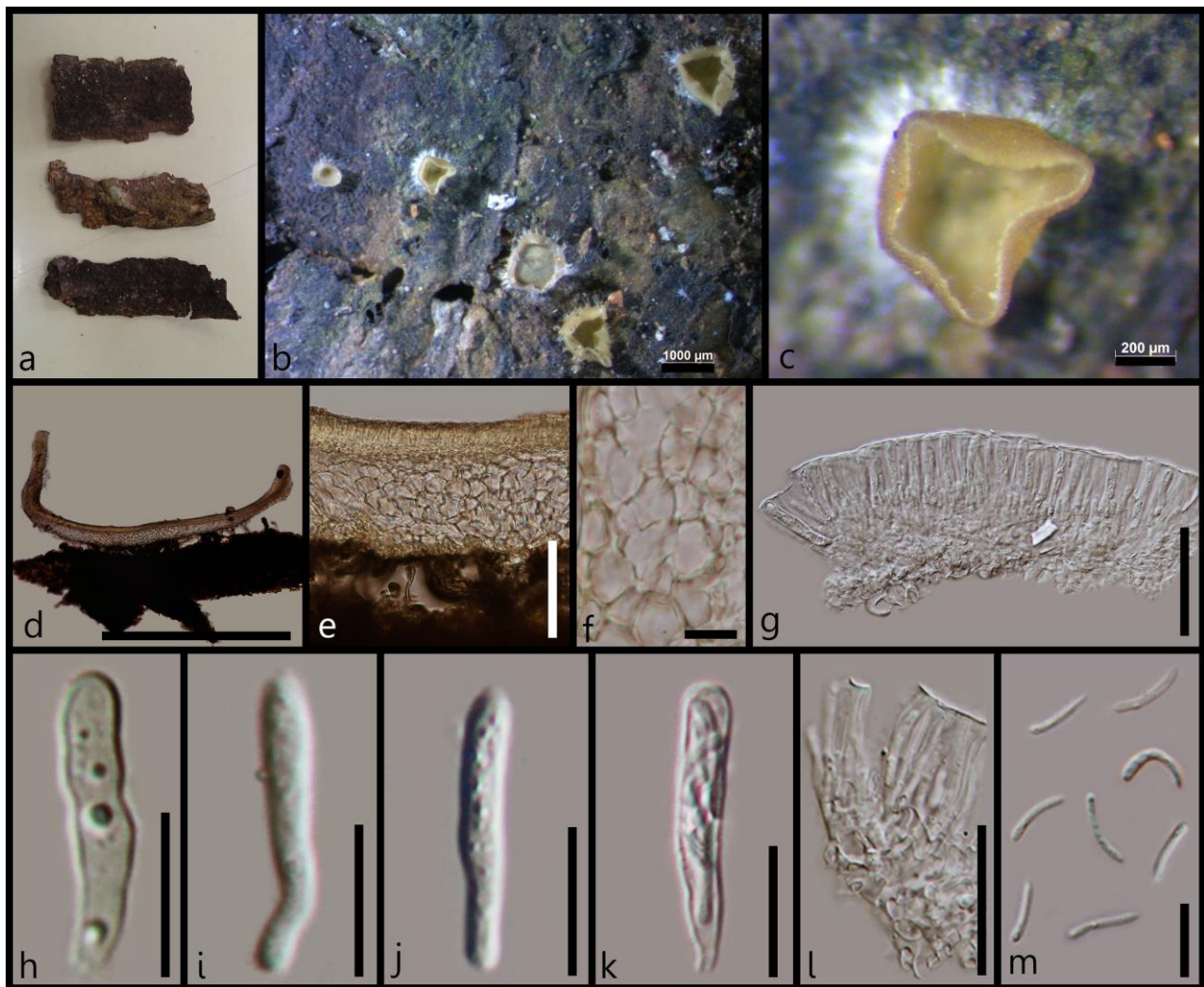


Figure 3 – Morphology of *Hyalorbilia inflatula* (MFLU 15-0167) a Substrate. b, c Rehydrated apothecia. d Median section of an apothecia. e, f Excipular cells at lower flanks. g Hymenium layer. h, i, j, k Asci. l Paraphyses. m Ascospores. Scale bars: b= 1000 μm , c = 200 μm , d = 1000 μm , e=75 μm , f= 10 μm , g = 40 μm , h-k = 15 μm , l= 25 μm , m= 05 μm .

Notes – *Orbilbia pisciformis* is similar to *O. caudata* mainly in ascospore shape, but differs in having straight ascospores with subacute apices (Quijada et al. 2012). *Orbilbia caudata* is morphologically and phylogenetically close to *O. comma*. However, *O. comma* differs from *O. caudata* by having shorter ascospores (5.8–7.6 \times 1.4–1.7 μm) and longer asci (47–65 \times 4–4.8 μm) (Zhang et al. 2009, Quijada et al. 2012).



Figure 4 – Morphology of *Orbilia caudata* (MFLU 16-0580) a Substrate. b,c Fresh apothecia. d Median section of an apothecia. e Excipular cells at upper flanks. f Paraphyses. g-j Asci. k-o Ascospores. Scale bars: b= 500 µm, c, d = 200 µm, e=100 µm, f= 20 µm, g-j = 15 µm, k-o= 5 µm.

Hyalorbilia erythrostroma (W. Phillips) Baral & G. Marson 2001

Fig. 5

Facesoffungi number: FoF03957

Description – Fungicolous on *Annulohypoxyton* sp. Sexual morph: *Apothecia* 220–250 × 380–430 µm (height × width) superficial, scattered to subgregarious, bright yellow, short stipitate, disc slightly concave, superficial, margin smooth. *Ectal excipulum* 9–14 µm wide at margins and upper flanks, composed of large, thin-walled, hyaline cells of *textura angularis-globulosa*. *Medullary excipulum* 18–25 µm wide at lower flanks, composed of large, thin-walled, hyaline, gelatinized cells of *textura angularis-intricata*, not sharply delimited from ectal excipulum. *Hymenium* hyaline, asci and paraphyses glued together and made a thick gelatinous layer. *Paraphyses* slightly to medium clavate, septate, terminal cell 1.8 – 2.5 µm width (\bar{x} = 2.2 µm, n=20), unbranched. *Asci* 30–33 × 3–3.5 µm (\bar{x} = 31.4 × 3.3 µm, n=30) 8-spored, cylindrical clavate, short pedicellate, arising from croziers, truncate apex uniseriate. *Ascospore* 1.6–2.2 × 1.7–2.3 µm (\bar{x} = 2.0 × 2.1 µm, n=40), globose, subglobose to ellipsoid, hyaline, aseptate, 1-2 guttules present, smooth-walled. Asexual morph: Undetermined.

Material examined – THAILAND, Mae Fah Luang University, Chiang Rai Province, on *Annulohypoxyton* sp., 20 April 2015, A.H. Ekanayaka, HD028 (MFLU 16-0589).

Notes – *Hyalorbilia brevistipitata* is morphologically similar to *H. erythrostroma*. However, *H. brevistipitata* differs from *H. erythrostroma* by having flat apothecia, and shorter stipe, smaller asci (18-25 × 3-3.5 μm) and smaller ascospores (1.5-1.8 × 1.5-1.8 μm) (Liu et al. 2005). We were not able to obtain molecular data for this collection.



Figure 5 – Morphology of *Hyalorbilia erythrostroma* (MFLU 16-0589) a Substrate. b,c. Fresh apothecia. d Median section of an apothecia. e Excipular cells at upper flanks. f Paraphyses. g, h, i, j Asci. k-o Ascospores. (Structures are mounted in Congo red reagent). Scale bars: b,d= 200 μm, c = 100 μm, e=40 μm, f-j= 10 μm, k-l = 5 μm.

Orbilbia leucostigma (Fr.) Fr., Summa veg. Scand., Sectio Post. (Stockholm): 357 (1849) Fig. 6
Facesoffungi number: FoF03958

Description – Saprobic on wood. Sexual morph: *Apothecia* 350–400 μm, superficial, sessile, scattered, yellow, sessile, disc flat or slightly concave, margin smooth. *Receptacle* turbinate yellow when fresh, yellowish orange when dry. *Excipulum* 15–20 μm wide (\bar{x} = 18 μm, n=10) *Ectal excipulum* composed of large, thin-walled cells of *textura angularis*. *Medullary excipulum* 25–30 μm wide (\bar{x} = 28 μm, n=10) hyaline cells of *textura angularis* to *intricata*. *Hymenium* hyaline. *Paraphyses* 1 – 2 μm wide at the middle (\bar{x} = 1.4 μm, n=20), numerous, filamentous, septate, hyaline, branched at the base, swollen at the apices, strongly to very strongly capitate, terminal cell 2.5 – 4 μm width (\bar{x} = 3.5 μm, n=20). *Asci* 35–45 × 5.5–6.5 μm (\bar{x} = 41.2 × 6.1 μm, n=30) 8-spored, cylindrical clavate, 8-spored, 1-2-seriate, with a short to long-stalked bifurcate base.

Ascospores 3–3.5 × 1.2–1.6 μm (\bar{x} = 3.2 × 1.4 μm, n=40), reniform to cashew-shaped, smooth walled. Asexual morph: Undetermined.

Material examined – THAILAND, Mae Fah Luang University, Chiang Rai Province, on unidentified wood, 20 April 2015, A.H. Ekanayaka, HD035 (MFLU 16-1861).

Notes – We were not able to obtain molecular data for this collection. However, by considering the morphological similarity of our collection with *O. leucostigma* (Fries 1849), we named our collection as *Orbilia leucostigma*. *Orbilia leucostigma* is morphologically similar to *O. xanthostigma*, but *O. xanthostigma* differs by having larger ascospores (6–8.5 × 1–1.4 μm) with warts (Guo et al. 2014, Quijada et al. 2016).

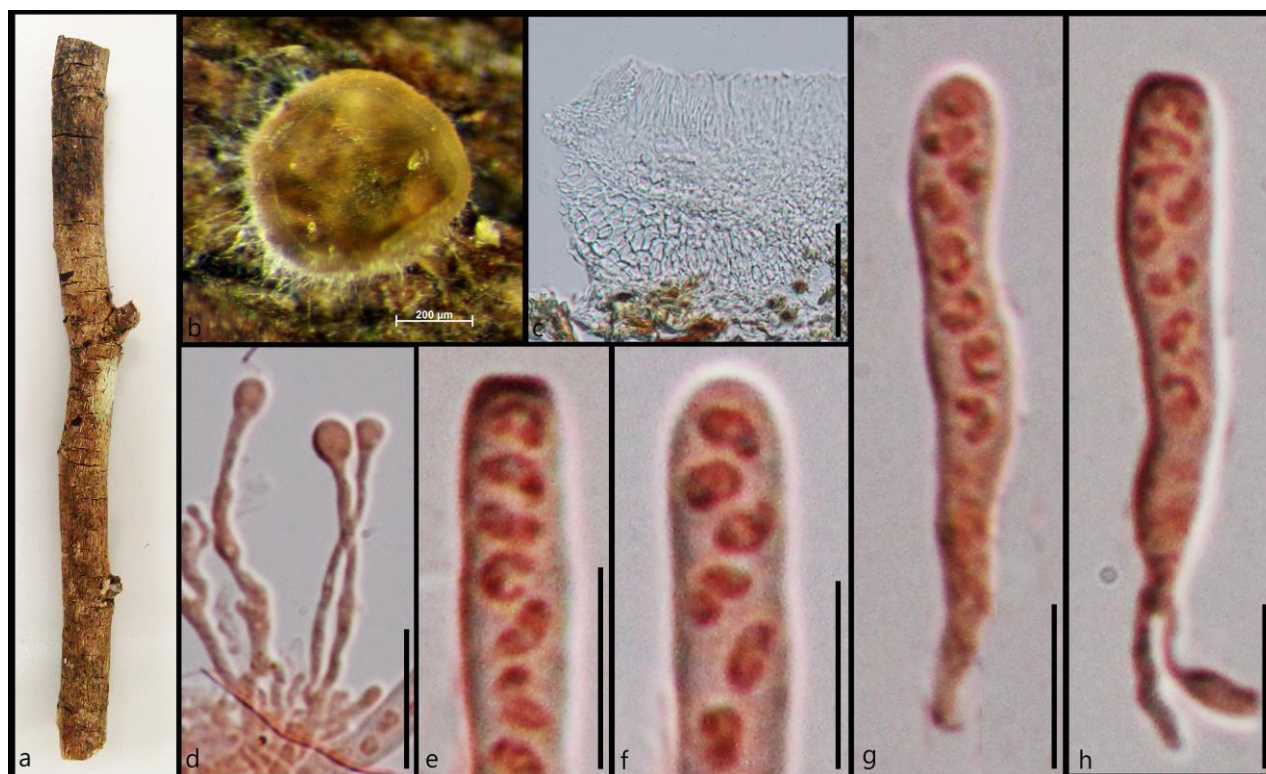


Figure 6 – Morphology of *Orbilia leucostigma* (MFLU 16-1861) a Substrate. b Fresh apothecia. c Excipular cells at upper flanks. d Paraphyses. e, f Asci. k-o Close up of ascus apex with ascospores. (d-h are mounted in congo red reagent) Scale bars: b= 200 μm, c = 45 μm, d = 35 μm, e and f=5 μm, g and h= 10.

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