Mycological Progress (2021) 20:701–720
https://doi.org/10.1007/s11557-021-01692-x

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ORIGINAL ARTICLE

Phylogenetic assessment and taxonomic revision of *Halobyssothecium* and *Lentithecium* (Lentitheciaceae, Pleosporales)

Mark Seasat Calabon[1,2] · E.B. Gareth Jones[3] · Kevin D. Hyde[1,4] · Saranyaphat Boonmee[1,2] · Sanja Tibell[5] · Leif Tibell[5] · Ka-Lai Pang[6] · Rungtiwa Phookamsak[7,8,9,10]

Received: 3 January 2021 / Revised: 2 March 2021 / Accepted: 4 March 2021

Abstract

Our studies on lignicolous aquatic fungi in Thailand, Sweden, and the UK resulted in the collection of three new *Halobyssothecium* species (*H. bambusicola*, *H. phragmitis*, *H. versicolor*) assigned to Lentitheciaceae (Pleosporales, Dothideomycetes). Multi-loci phylogenetic analyses of the combined large subunit, small subunit, internal transcribed spacers of ribosomal DNA, and the translation elongation factor 1-alpha sequence data enabled a revision of the taxa assigned to *Lentithecium* and the transfer of *L. cangshanense*, *L. carbonneanum*, *L. kunmingense*, *L. unicellulare*, and *L. voraginesporum* to *Halobyssothecium*. Collection of an asexual morph of *L. lineare* and phylogenetic analysis confirmed its taxonomic placement in *Keisslerella*. Detailed descriptions and illustrations of *H. bambusicola*, *H. phragmitis*, and *H. versicolor* are provided.

Keywords 3 new taxa · Dothideomycetes · Freshwater fungi · Marine fungi · Multi-locus phylogeny

Introduction

Pleosporales, typified by *Pleospora herbarum* (Pers.) Rabenh. (Pleosporaceae), was formally established by Luttrell and Barr (in Barr 1987) and characterized by perithecioid ascomata, usually with a papillate apex, ostiolate, cellular pseudoparaphyses, and bitunicate asci. Phylogenetic studies of Pleosporales have been provided by Schoch et al. (2009), Zhang et al. (2009a, 2012), Hyde et al. (2013), Liu et al. (2017), and Hongsanan et al. (2020). Lumbsch and Huhndorf (2010) included 28 families and 175 genera in Pleosporales, with 12 genera listed under Pleosporales, genera *incertae sedis*. Hyde et al. (2013) accepted 88 families in Pleosporales. Wijayawardene et al. (2020) and Hongsanan

Section Editor: Roland Kirschner

✉ E.B. Gareth Jones
torperadgj@gmail.com

[1] Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

[2] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

[3] Department of Botany and Microbiology, College of Science, King Saud University, P.O Box 2455, Riyadh 11451, Kingdom of Saudi Arabia

[4] Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Guangzhou 510225Guangdong Province, People's Republic of China

[5] Systematic Biology, Department of Organismal Biology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

[6] Institute of Marine Biology and Centre of Excellence for the Oceans, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan

[7] CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming, People's Republic of China

[8] East and Central Asia Regional Office, World Agroforestry Centre (ICRAF), Kunming 650201, Yunnan, People's Republic of China

[9] Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Honghe County, Yunnan, People's Republic of China

[10] Research Center of Microbial Diversity and Sustainable Utilization, Faculty of Sciences, Chiang Mai University, Chiang Mai 50200, Thailand

et al. (2020) included 91 families in Pleosporales. Ecologically, the order includes saprotrophs, parasites, pathogens, epiphytes, and endophytes (Hongsanan et al. 2020).

Zhang et al. (2009b) established Lentitheciaceae with *Lentithecium fluviatile* (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Ying Zhang as the genus and species type, and included *L. arundinaceum* (Sowerby) K.D. Hyde, J. Fourn. & Ying Zhang, *L. aquaticum* Ying Zhang, J. Fourn. & K.D. Hyde, *Stagonospora macropycnidia* Cunnell, *Wettsteinina lacustris* (Fuckel) Shoemaker & C.E. Babc., *Keissleriella cladophila* (Niessl) Corbaz, and *Katumotoa bambusicola* Kaz. Tanaka & Y. Harada. Suetrong et al. (2009) also referred *Massarina phragmiticola* Poon & K.D. Hyde to the new family. Lentitheciaceous taxa are saprobic on herbaceous and woody plants having narrow peridia, fusiform to broadly cylindrical pseudoparaphyses, hyaline ascospores with 1–3-transverse septa and containing refractive globules, surrounded by a mucilaginous sheath or extended appendage-like sheaths and asexual morphs producing stagonospora-like or dendrophoma-like asexual morphs (Zhang et al. 2012; Hyde et al. 2013; Wanasinghe et al. 2014). Fourteen genera from different habitats are included in Lentitheciaceae based on molecular data: *Darksidea* (Knapp et al. 2015), *Halobyssothecium* (Dayarathne et al. 2018), *Katumotoa* (Tanaka and Harada 2005), *Keissleriella* (Höhnel 1919), *Lentithecium* (Zhang et al. 2009b), *Murilentithecium* (Wanasinghe et al. 2014), *Neoophiosphaerella* (Tanaka et al. 2015), *Phragmocamarosporium* (Wijayawardene et al. 2015), *Pleurophoma* (de Gruyter et al. 2009; Crous et al. 2015), *Poaceascoma* (Phookamsak et al. 2015), *Pseudomurilentithecium* (Hyde et al. 2020b), *Setoseptoria* (Quaedvlieg et al. 2013), *Tingoldiago* (Hirayama et al. 2010), and *Towyspora* (Li et al. 2016).

Lentithecium was proposed to accommodate *Massarina arundinacea* (Sowerby) Leuchtm., *M. fluviatilis* Aptroot & Van Ryck., and *Keissleriella linearis* E. Müll. ex Dennis (Zhang et al. 2009b). The genus currently contains ten species that were described from aquatic habitats, seven from freshwater, and three from marine environments. *Lentithecium* species have been described from submerged wood (Tanaka et al. 2005, 2015; Hyde et al. 2016; Su et al. 2016; Crous et al. 2018) and submerged parts of plant host species (*Juncus*, *Phragmites*, *Fraxinus*, *Alnus*, and *Platanus*) (Kohlmeyer et al. 1996; Van Ryckegem and Aptroot 2001; Suetrong et al. 2009; Zhang et al. 2009b). *Lentithecium* is characterized by its immersed to semi-immersed, globose to subglobose ascomata, a thin peridium, cellular pseudoparaphyses, short pedicellate asci and fusoid or filiform, subglobose, hyaline, brown, uni- to multi-septate ascospores, usually surrounded by a sheath (Zhang et al. 2009b; Hyde et al. 2013, 2016).

Halobyssothecium was introduced by Dayarathne et al. (2018) to accommodate several taxa variously described under *Pleospora obiones* P. Crouan & H. Crouan by Crouan and Crouan (1867) and *Leptosphaeria discors* Sacc. & Ellis by Saccardo (1882). This "taxon" had been assigned to various genera: *Metasphaeria* (Saccardo 1883), *Heptameria* (Cooke 1889), and *Passeriniella* (Apinis and Chesters 1964; Hyde and Mouzouras 1988; Khashnobish and Shearer 1996). Various studies have shown that *Pleospora obiones*/*Leptosphaeria discors* are synonyms, but clearly do not belong in any of these genera (Khashnobish and Shearer 1996). Jones (1962), Cavaliere (1968), and Webber (1970) reported *Leptosphaeria discors* collections with larger ascospores than those by Crouan and Crouan (1867) indicating that there might be a second morphologically similar species. *Halobyssothecium obiones* (P. Crouan & H. Crouan) Dayarathne, E.B.G. Jones & K.D. Hyde has a worldwide distribution in temperate regions and occurs as a saprobe of *Agropyron junceiforme*, *Halimione portulacoides*, *Spartina* species, on intertidal wood, bamboo, and exposed test panels of *Betula pubescens* and *Fagus sylvatica* (Kohlmeyer and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced *Halobyssothecium estuariae* B. Devadatha, Calabon, K.D. Hyde & E.B.G. Jones collected on a dead culm of *Phragmites communis* from Slebech Estuary, Pembrokeshire, UK, which resembles the generic type *H. obiones* in possessing subglobose or ellipsoidal, carbonaceous ascomata, conical papilla and ascospores with brown central cells and hyaline end cells (Dayarathne et al. 2018). However, *H. estuariae* is distinct from *H. obiones* in having a longer and narrower papilla (65–85 × 55–85 vs. 25–35 × 130–145 μm) and smaller ascospores (20–44 × 4–9 vs. 28–47 × 10–18 μm). *Halobyssothecium obiones* and *H. estuariae* differ by 5.1% (22/431 bp) in ITS and 3.12% (28/895 bp) in *TEF1-α* sequence data.

Keissleriella, typified by *K. aesculi* (Höhn.) Höhn., is characterized by an ostiolar neck covered by short dark setae (Tanaka et al. 2015; Hongsanan et al. 2020). *Keissleriella* is the most speciose genus in Lentitheciaceae with 46 epithets listed in Species Fungorum (http://www.speciesfungorum.org/Names/Names.asp; accessed on December 2020) and 38 morphological species, 25 of which have molecular data. Sequence data for the type species of *Keissleriella* is unavailable, but phylogenetic studies confirmed its placement within Lentitheciaceae (Tanaka et al. 2015; Tibpromma et al. 2017; Hongsanan et al. 2020).

In the present study, a phylogenetic tree of taxa in Lentitheciaceae was constructed based on sequence data of four loci (LSU, SSU, ITS, *TEF1-α*) to reevaluate the taxonomic status of *Halobyssothecium* and *Lentithecium*. The latest treatments and updated accounts of Lentitheciaceae in Dayarathne et al. (2018), Hongsanan et al. (2020), and Wijayawardene et al. (2020) are followed in this paper. The insights from the multi-loci analyses and morphological observations reveal three new species of *Halobyssothecium* and

confirm the taxonomic placement of *Lentithecium lineare* (E. Müll. ex Dennis) K.D. Hyde, J. Fourn. & Ying Zhang in *Keissleriella*, and *L. cangshanense* Z.L. Luo, X.J. Su & K.D. Hyde, *L. carbonneanum* J. Fourn., Raja & Oberlies, *L. kunmingense* Dong, H. Zhang & K.D. Hyde, *L. unicellulare* Abdel-Aziz and *L. voraginesporum* Abdel-Wahab, Bahkali & E.B.G. Jones in *Halobyssothecium*. The transfers are made, and descriptions, photographic plates, and multi-loci phylogenetic analyses are provided.

Materials and methods

Sample collection, morphological observation, and fungal isolation

Samples of submerged decayed wood were collected from a freshwater stream in Chiang Mai, Thailand. Dead and decaying *Halimione portulacoides* was collected from Hayling Island bridge, Hampshire, UK. Drift culms and stems of *Phragmites* sp. were obtained from Sudersand and Kappelshamnsviken in Gotland, Sweden. The samples were observed using a stereomicroscope for the presence of fruiting bodies. Micromorphological features were photographed using a Motic SMZ 168 Series dissection microscope for fungal structures on the woody substrate while microscopic characters were documented using a Nikon Eclipse 80i microscope. Single spore isolation was used to obtain pure cultures and colonial characteristics described. Herbarium-type specimens are deposited in Mae Fah Luang University (MFLU). Ex-type and ex-paratype living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC). The new species and combinations were registered in Faces of Fungi (http://www.facesoffungi.org/; Jayasiri et al. 2015) and Index Fungorum database (http://www.indexfungorum.org/names/IndexFungorumRegisterName.asp).

DNA extraction, PCR amplification, and sequencing

Fungal mycelia from pure cultures grown in malt extract agar (MEA) for 30 days were scraped using a sterilized scalpel and kept in a sterilized 1.5 mL microcentrifuge tube. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's protocol. Polymerase chain reaction (PCR) was used to amplify four markers: the large subunit (LSU), small subunit (SSU), internal transcribed spacers (ITS) of rDNA, and the translation elongation factor 1-alpha gene (*TEF1-α*). The LSU was amplified using the primers LR0R and LR5 (Vilgalys and Hester 1990). The SSU was amplified using the primers NS1 and NS4 (White et al. 1990). For ITS, primers ITS5 and ITS4 were used (White et al., 1990). *TEF1-α* was amplified using primers EF1-983F and EF1-2218R (Rehner 2001). Polymerase chain reaction was performed in a volume of 25 μl, which contained 12.5 μl of 2× Power Taq PCR Master Mix (Bioteke Co., China), 1 μl of each primer (10 pM), 1 μl genomic DNA, and 9.5 μl double-distilled water (ddH_2O). The PCR thermal cycle program for LSU, SSU, ITS, and *TEF1-α* amplification were as follows: initial denaturing step of 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 45 seconds, annealing at 56 °C for 50 seconds, elongation at 72 °C for 1 min, and final extension at 72 °C for 10 min. Agarose gel electrophoresis was done to confirm the presence of amplicons at the expected molecular weight. PCR products were purified and sequenced with the primers mentioned above at a commercial sequencing provider (Beijing Qingke Biotechnology Co., Ltd). A BLASTn search of the newly generated sequences was carried out to exclude contamination and to search for related taxa in GenBank database (www.ncbi.nlm.nih.gov/blast/).

Phylogenetic analyses

The taxa table was assembled based on the closest matches from the BLASTn search results and from recently published data in Dayarathne et al. (2018) and Devadatha et al. (2020). Sequences generated from the four markers were analyzed along with other sequences retrieved from GenBank (Table 1). Four datasets, one for each marker, were aligned with MAFFT v. 7 using the web server (http://mafft.cbrc.jp/alignment/server; Katoh et al. 2019) with the following settings: L-INS-i tree-based iterative refinement methods, 20PAM/k = 2 scoring matrix for nucleotide sequences and 1.53 gap opening penalty. Alignment was further refined manually, where necessary, using BioEdit v.7.0.9.0 (Hall 1999). Aligned sequences were automatically trimmed using TrimAl v. 1.3 on the web server (http://phylemon.bioinfo.cipf.es/utilities.html). The online tool "ALTER" (Glez-Peña et al. 2010) was used to convert the alignment file to phylip and nexus formats. Phylogenetic analyses of both individual and combined gene data were performed using maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI).

Maximum parsimony (MP) analysis was performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm in PAUP* 4.0b4 (Swofford 2002). All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BS) analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa (Hillis and Bull 1993). Descriptive tree statistics for parsimony (tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RC], and homoplasy index [HI]) were calculated for trees generated under different optimality criteria.

Table 1 Taxa used in this study for the analysis of combined LSU, SSU, ITS rDNA, and *TEF1-α* sequence data and their GenBank accession numbers. The newly generated sequences are indicated with asterisk (*) and the ex-type strains are indicated in bold

Species	Strain/voucher number	GenBank accession number			
		LSU	SSU	ITS	*TEF1-α*
Bambusicola bambusae	**MFLUCC 11–0614**	**JX442035**	**JX442039**	**NR_121546**	**KP761722**
Bambusicola irregulispora	**MFLUCC 11–0437**	**JX442036**	**JX442040**	**NR_121547**	**KP761723**
Bambusicola massarinia	**MFLUCC 11–0389**	**JX442037**	**JX442041**	**JX442033**	**KP761725**
Bambusicola splendida	**MFLUCC 11–0439**	**JX442038**	**JX442042**	**NR121549**	**KP761726**
Bimuria novae-zelandiae	CBS 107.79	**AY016356**	**AY016338**	**NR_159620**	DQ471087
Byssothecium circinans	CBS 675.92	GU205217	GU205235	–	GU349061
Corynespora cassiicola	CBS 100822	GU301808	GU296144	–	GU349052
Corynespora smithii	CABI5649b	GU323201	–	–	GU349018
Darksidea alpha	**CBS 135650**	**KP184019**	**KP184049**	**NR_137619**	**KP184166**
Darksidea beta	**CBS 135637**	**KP184023**	**KP184074**	**NR_137957**	**KP184189**
Darksidea delta	**CBS 135638**	**KP184024**	**KP184069**	**NR_137075**	**KP184184**
Darksidea epsilon	**CBS 135658**	**KP184029**	**KP184070**	**NR_137959**	**KP184186**
Darksidea gamma	**CBS 135634**	**KP184031**	**KP184073**	**NR_137587**	**KP184188**
Darksidea zeta	**CBS 135640**	**KP184013**	**KP184071**	**NR_137958**	**KP184191**
Falciformispora lignatilis	BCC 21117	GU371826	GU371834	KF432942	GU371819
Falciformispora lignatilis	BCC 21118	GU371827	GU371835	KF432943	GU371820
Halobyssothecium bambusicola*	**MFLUCC 20–0226**	**MT068489**	**MT068494**	**MN833419**	**MT477868**
Halobyssothecium cangshanense	**DLUCC 0143**	**KU991149**	**KU991150**	–	–
Halobyssothecium carbonneanum	**CBS 144076**	**MH069699**	–	**MH062991**	–
Halobyssothecium estuariae	**MFLUCC 19–0386**	**MN598871**	**MN598868**	**MN598890**	**MN597050**
Halobyssothecium estuariae	**MFLUCC 19–0387**	**MN598872**	**MN598869**	**MN598891**	**MN597051**
Halobyssothecium kunmingense	**KUMCC 19–0101**	**MN913732**	**MT864313**	**MT627715**	**MT954408**
Halobyssothecium obiones	20AV2566	–	–	KX263862	–
Halobyssothecium obiones	27AV2385	–	–	KX263864	–
Halobyssothecium obiones	**MFLUCC 15–0381**	**MH376744**	**MH376745**	**MH377060**	**MH376746**
Halobyssothecium phragmitis*	**MFLUCC 20–0223**	**MT068486**	**MT068491**	**MT232435**	**MT477865**
*Halobyssothecium phragmitis**	MFLUCC 20–0225	MT068488	MT068493	MT232437	MT477867
Halobyssothecium unicellulare	MD129	KX505375	KX505373	–	–
Halobyssothecium unicellulare	**MD6004**	**KX505376**	**KX505374**	–	–
Halobyssothecium versicolor*	**MFLUCC 20–0222**	**MT068485**	**MW346047**	**MT232434**	**MT477864**
Halobyssothecium voraginesporum	**CBS H-22560**	**NG_066171**	**NG_063065**	–	–
Helicascus nypae	**BCC 36752**	**GU479789**	**GU479755**	–	**GU479855**
Kalmusia scabrispora	KT2202	AB524594	AB524453	LC014576	AB539107

Table 1 (continued)

Species	Strain/voucher number	GenBank accession number			
		LSU	SSU	ITS	*TEF1-α*
Karstenula rhodostoma	CBS 690.94	GU301821	GU296154	–	GU349067
Katumotoa bambusicola	**KT 1517a**	**AB524595**	**AB524454**	**LC014560**	**AB539108**
Keissleriella bambusicola	**KUMCC 18–0122**	**MK995880**	**MK995878**	**MK995881**	**MN213156**
Keissleriella breviasca	KT 581	AB807587	AB797297	AB811454	AB808566
Keissleriella breviasca	**KT 649**	**AB807588**	**AB797298**	AB811455	**AB808567**
Keissleriella camporesiana	**MFLUCC 15–0029**	**MN401741**	**MN401743**	**MN401745**	**MN397907**
Keissleriella camporesii	**MFLUCC 15–0117**	**MN252886**	**MN252907**	**MN252879**	–
Keissleriella caraganae	**KUMCC 18–0164**	**MK359439**	**MK359444**	**MK359434**	**MK359073**
Keissleriella cirsii	**MFLUCC 16–0454**	**KY497780**	**KY497782**	**KY497783**	**KY497786**
Keissleriella cladophila	**CBS 104.55**	**GU301822**	**GU296155**	**MH857391**	**GU349043**
Keissleriella culmifida	KT2308	AB807591	AB797301	LC014561	AB808570
Keissleriella culmifida	KT2642	AB807592	AB797302	LC014562	AB808571
Keissleriella dactylidicola	**MFLUCC 13–0866**	**KT315506**	**KT315505**	–	**KT315507**
Keissleriella dactylidis	**MFLUCC 13–0751**	**KP197668**	**KP197666**	**KP197667**	**KP197669**
Keissleriella genistae	CBS 113798	GU205222	GU205242	–	–
Keissleriella gloeospora	KT829	AB807589	AB797299	LC014563	AB808568
Keissleriella linearis	IFRD2008	FJ795435	FJ795478	–	–
Keissleriella linearis	MFLUCC 19–0410	MN598873	MN598870	MN598892	MN607978
*Keissleriella linearis**	MFLUCC 20–0224	MT068487	MT068492	MT232436	MT477866
Keissleriella phragmiticola	CPC 33249	MT223903	–	MT223808	MT223715
Keissleriella phragmiticola	**MFLUCC 17–0779**	**MG829014**	–	**MG828904**	–
Keissleriella poagena	CBS 136767	KJ869170	–	KJ869112	–
Keissleriella quadriseptata	**KT2292**	**AB807593**	**AB797303**	**AB811456**	**AB808572**
Keissleriella rara	CBS 118429	GU479791	GU479757	–	–
Keissleriella rosacearum	**MFLUCC 15–0045**	**MG829015**	**MG829123**	–	–
Keissleriella rosae	**MFLUCC 15–0180**	**MG829016**	**MG922549**	–	–
Keissleriella rosarum	**MFLUCC 15–0089**	**MG829017**	**MG829124**	**MG828905**	–
Keissleriella sp.	KT895	AB807590	AB797300	–	AB808569
Keissleriella sparticola	**MFLUCC 14–0196**	**KP639571**	–	–	–
Keissleriella tamaricicola	**MFLUCC 14–0168**	**KU900300**	–	**KU900328**	–
Keissleriella taminensis	KT571	AB807595	AB797305	LC014564	AB808574
Keissleriella taminensis	KT594	AB807596	AB797306	–	–
Keissleriella taminensis	KT678	AB807597	AB797307	LC014565	AB808575
Keissleriella trichophoricola	**CBS 136770**	**KJ869171**	–	**KJ869113**	–

Table 1 (continued)

Species	Strain/voucher number	GenBank accession number			
		LSU	SSU	ITS	*TEF1-α*
Keissleriella yonaguniensis	**HHUF 30138**	**NG_059402**	**NG_064856**	**NR_155212**	**AB808573**
Latorua caligans	**CBS 576.65**	**MH870362**	–	**MH858723**	–
Latorua grootfonteinensis	**CBS 369.72**	**NG_058181**	–	–	–
Lentithecium aquaticum	**CBS 123099**	**GU301823**	**GU296156**	**NR_160229**	**GU349068**
Lentithecium clioninum	**KT1149A**	**AB807540**	**AB797250**	**LC014566**	**AB808515**
Lentithecium clioninum	KT1220	AB807541	AB797251	LC014567	AB808516
Lentithecium fluviatile	CBS 122367	FJ795451	FJ795493	–	GU349074
Lentithecium fluviatile	CBS 123090	FJ795450	FJ795492	–	–
Lentithecium pseudoclioninum	KT1111	AB807544	AB797254	AB809632	AB808520
Longipedicellata aptrootii	**MFLUCC 10–0297**	**KU238894**	**KU238895**	**KU238893**	**KU238892**
Longipedicellata aptrootii	MFLUCC 18–0988	MN913744	–	MT627733	–
Macrodiplodiopsis desmazieri	**CBS 140062**	**NG_058182**	–	**NR_132924**	–
Massarina cisti	**CBS 266.62**	**FJ795447**	**FJ795490**	**LC014568**	**AB808514**
Massarina eburnea	CBS 139697	AB521735	AB521718	LC014569	AB808517
Massarina eburnea	CBS 473.64	GU301840	GU296170	AF383959	GU349040
Montagnula opulenta	AFTOL-ID 1734	DQ678086	AF164370	AF383966	–
Morosphaeria ramunculicola	**JK5304B**	**GU479794**	**GU479760**	–	–
Multiseptospora thailandica	**MFLUCC 11–0183**	**NG_059554**	**KP753955**	**NR_148080**	**KU705657**
Murilentithecium clematidis	MFLUCC 14–0561	KM408758	KM408760	KM408756	KM454444
Murilentithecium clematidis	**MFLUCC 14–0562**	**KM408759**	**KM408761**	**KM408757**	**KM454445**
Murilentithecium lonicerae	**MFLUCC 18–0675**	**MK214373**	**MK214376**	**MK214370**	**MK214379**
Murilentithecium rosae	**MFLUCC 15–0044**	**MG829030**	**MG829137**	**MG828920**	–
Neoophiosphaerella sasicola	**KT1706**	**AB524599**	**AB524458**	**LC014577**	**AB539111**
Palmiascoma gregariascomum	**MFLUCC 11–0175**	**KP744495**	**KP753958**	**KP744452**	–
Parabambusicola thysanolaenae	**KUMCC 18–0147**	**NG_066435**	**NG_067681**	**NR_164044**	**MK098209**
Parabambusicola thysanolaenae	KUMCC 18–0148	MK098198	MK098202	MK098193	MK098211
Paraconiothyrium brasiliense	**CBS 100299**	**JX496124**	**AY642523**	**JX496011**	–
Paraphaeosphaeria michotii	**MFLUCC 13–0349**	**KJ939282**	**KJ939285**	**KJ939279**	–
Paraphaeosphaeria minitans	CBS 122788	EU754173	EU754074	–	GU349083
Phaeodothis winteri	CBS 182.58	GU301857	GU296183	–	DQ677917
Phragmocamarosporium hederae	**MFLUCC 13–0552**	**KP842915**	**KP842918**	–	–
Phragmocamarosporium platani	**MFLUCC 14–1191**	**KP842916**	**KP842919**	–	–
Phragmocamarosporium rosae	**MFLUCC 17–0797**	**MG829051**	**MG829156**	–	**MG829225**
Pleohelicoon fagi	**MFLUCC 15–0182**	**NG_066320**	**NG_065791**	**NR_163353**	–

Table 1 (continued)

Species	Strain/voucher number	GenBank accession number			
		LSU	SSU	ITS	*TEF1-α*
Pleomonodictys descalsii	**CBS 142298**	**KY853522**	–	**KY853461**	–
Pleurophoma ossicola	**CBS139905**	**KR476769**	–	**KR476736**	–
Pleurophoma ossicola	CPC24985	KR476770	–	KR476737	–
Pleurophoma pleurospora	**CBS130329**	**JF740327**	–	–	–
Poaceascoma aquaticum	**MFLUCC 14–0048**	**KT324690**	**KT324691**	–	–
Poaceascoma halophila	**MFLUCC 15–0949**	**MF615399**	**MF615400**	–	–
Poaceascoma helicoides	**MFLUCC 11–0136**	**KP998462**	**KP998463**	**KP998459**	**KP998461**
Poaceascoma taiwanense	**MFLUCC 18–0083**	**MG831567**	**MG831568**	**MG831569**	
Pseudomurilentithecium camporesii	**MFLUCC 14–1118**	**MN638846**	**MN638850**	**MN638861**	**MN648730**
Pseudoxylomyces elegans	KT 2887	AB807598	AB797308	–	AB808576
Setoseptoria arundelensis	**MFLUCC 17–0759**	**MG829073**	**MG829173**	**MG828962**	–
Setoseptoria arundinacea	CBS 123131	GU456320	GU456298	–	GU456281
Setoseptoria arundinacea	CBS 619.86	GU301824	GU296157	–	–
Setoseptoria englandensis	**MFLUCC 17–0778**	**MG829074**	**MG829174**	**MG828963**	–
Setoseptoria lulworthcovensis	**MFLU 18–0110**	**MG829075**	**MG829175**	–	–
Setoseptoria magniarundinacea	KT1174	AB807576	AB797286	LC014596	AB808552
Setoseptoria phragmitis	**CBS 114802**	**KF251752**	–	**KF251249**	**KF253199**
Setoseptoria phragmitis	CBS 114966	KF251753	–	KF251250	KF253200
Setoseptoria scirpi	**MFLUCC 14–0811**	**KY770982**	**KY770980**	**MF939637**	**KY770981**
Splanchnonema platani	CBS 221.37	MH867404	–	MH855894	DQ677908
Splanchnonema platani	CBS 222.37	KR909316	KR909318	KR909311	KR909319
Stagonospora macropycnidia	CBS 114202	GU301873	GU296198	–	GU349026
Tingoldiago clavata	MFLUCC 19–0495	MN857180	MN857188	MN857184	–
Tingoldiago clavata	**MFLUCC 19–0496**	**MN857178**	**MN857186**	**MN857182**	–
Tingoldiago clavata	MFLUCC 19–0498	MN857179	MN857187	MN857183	–
Tingoldiago graminicola	KH155	AB521745	AB521728	LC014599	AB808562
Tingoldiago graminicola	**KH68**	**AB521743**	**AB521726**	**LC014598**	**AB808561**
Tingoldiago graminicola	KT891	AB521744	AB521727	LC014600	AB808563
Tingoldiago hydei	**MFLUCC 19–0499**	**MN857177**	–	**MN857181**	–
Towyspora aestuari	**MFLUCC 15–1274**	**KU248852**	**KU248853**	**NR_148095**	–
Trematosphaeria pertusa	**CBS 122368**	**FJ201990**	**FJ201991**	**KF015668**	**KF015701**
Trematosphaeria pertusa	CBS 122371	GU301876	GU348999	KF015669	KF015702

Maximum likelihood analysis was performed using RAxML-HPC2 on XSEDE on the CIPRES web portal (Stamatakis 2006, 2014; Stamatakis et al. 2008) (http://www.phylo.org/portal2/; Miller et al. 2010). The GTR+GAMMA model of nucleotide evolution was used. RAxML rapid bootstrapping of 1,000 replicates was performed. The best-fit evolutionary models for individual and combined datasets were estimated under the Akaike Information Criterion (AIC) using jModeltest 2.1.10 on the CIPRES web portal and each resulted to the GTR+I+G model (Nylander 2004; Darriba et al. 2012). Bayesian inference analyses were performed using MrBayes v. 3.2.6 on XSEDE at the CIPRES webportal (Ronquist and Huelsenbeck 2003), using the parameter setting of two parallel runs, four chains, the run for 4,000,000 generations at which point the standard deviation of split frequencies was below 0.01. Trees were sampled every 1,000 generations and all other parameters were left as default. Bayesian analysis resulted in 4,000 trees after the run wherein the first 1,000 trees, 25% of the total, were in the burn-in phase and were discarded. The remaining 3,000 trees were used to calculate the posterior probability (PP). Newly generated sequences have been deposited in GenBank (Table 1).

Genealogical concordance phylogenetic species recognition analysis

New species and their most closely related species were analyzed using the Genealogical concordance phylogenetic species recognition (GCPSR) model. A pairwise homoplasy index (PHI) (Bruen et al. 2006) test was performed in SplitsTree4 (Huson 1998; Huson and Bryant 2006) as described by Quaedvlieg et al. (2014). This was done to determine the recombination level within phylogenetically closely related species using a four-locus concatenated dataset for new species of *Halobyssothecium*. The test detects incompatibility between pairs of sites regarding whether there is genealogical history that can be inferred parsimoniously that does not involve any recurrent or convergent mutations. Pairwise homoplasy index below a 0.05 threshold ($\Phi w < 0.05$) indicates that there is significant recombination present in the dataset. The relationships between closely related species were visualized by constructing a split graph, using both the LogDet transformation and splits decomposition options.

Results

Phylogenetic analyses

The combined LSU, SSU, ITS and *TEF1-α* dataset comprised of 133 taxa from Lentitheciaceae, with *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei (CBS 100822) and *C. smithii* (Berk. & Broome) M.B. Ellis (CABI5649b) as outgroup taxa (Table 1). The analyzed dataset, after trimming, comprised a total 3,578 characters including gaps (LSU = 1,274 bp, SSU = 916 bp, ITS = 473 bp, *TEF1-α* = 915 bp) with 1,632 distinct alignment patterns and 28.64% proportion of gaps and completely undetermined characters, 2,235 constant, 414 parsimony uninformative and 940 parsimony informative characters. The MP analysis resulted a single most parsimonious tree (TL = 5,457, CI = 0.364, RI = 0.674, RC = 0.245, HI = 0.636). The ML analysis for the combined dataset provided the best scoring tree (Fig. 1) with a final ML optimization likelihood value of -32434.024914 (ln). Parameters for the GTR+I+G model of the combined LSU, SSU, ITS and *TEF1-α* dataset are as follows: estimated base frequencies; A = 0.241074, C = 0.248510, G = 0.273533, T = 0.236882; substitution rates AC = 1.038579, AG = 2.219296, AT = 1.397250, CG = 1.151737, CT = 6.450277, GT = 1.000000; gamma distribution shape parameter α = 0.228421. The Bayesian analysis indicated the average standard deviation of split frequencies at the end of total MCMC generations is 0.007035. Phylogenetic analyses of the combined data matrix resulted in well-resolved clades (Fig. 1). The tree topologies resulted from maximum likelihood (ML), maximum parsimony (MP), and Bayesian posterior probabilities (BYPP) analyses were congruent.

In the phylogenetic analysis (Fig. 1), *Halobyssothecium* formed a well-supported monophyletic clade, separate from *Lentithecium* (99% ML, 95% MP, 1.00 BYPP). Three novel *Halobyssothecium* species, *H. bambusicola*, *H. phragmitis* and *H. versicolor* grouped with the other *Halobyssothecium* species in Lentitheciaceae. Moreover, five species of *Lentithecium* (*L. cangshanense*, *L. carbonneanum*, *L. kunmingense*, *L. unicellulare*, *L. voraginesporum*) clustered with *Halobyssothecium*. Therefore, these five *Lentithecium* species are transferred to *Halobyssothecium* in this study. *Halobyssothecium bambusicola* MFLUCC 20–0226 and *H. kunmingense* KUMCC 19–0101 were strongly supported as sister species (100% ML, 100% MP, 1.00 BYPP) and clustered with *H. phragmitis* (MFLUCC 20–0223, MFLUCC 20–0225) with high support (93% ML, 80% MP, 1.00 BYPP). *Halobyssothecium versicolor* MFLUCC 20–0222 forms a distinct lineage and basal to other *Halobyssothecium* species. *Lentithecium clioninum* (Kaz. Tanaka, Sat. Hatak. & Y. Harada) Kaz. Tanaka & K. Hiray. and *L. pseudoclioninum* Kaz. Tanaka & K. Hiray. clustered together with *L. fluviatile*, the type species of *Lentithecium* (99% ML, 96% MP, 1.00 BYPP). Furthermore, *L. lineare* MFLUCC 20–0224 clustered with the other two strains of *L. lineare* (IFRD2008, MFLUCC 19–0410) (100% ML, 100% MP, 1.00 BYPP).

The relationships between the three new species of *Halobyssothecium* were visualized by constructing a split graph and PHI-test revealed significant genetic recombination levels between two strains of *H. phragmitis* suggesting that

they are conspecific. The presence of recombination among fungal isolates is the hallmark that these belong to the same biological species. No significant recombination events were observed between *H. bambusicola*, *H. kunmingense*, and *H. phragmitis* indicating that these are different species (Fig. 2). PHI-test returns the probability of observing the data under the null hypothesis of no recombination.

Taxonomy

Halobyssothecium Dayar., E.B.G. Jones & K.D. Hyde

Saprobic on salt marsh halophytes and submerged decaying wood in aquatic habitats. **Sexual morph**: *Ascomata* immersed, semi-immersed or erumpent, scattered to clustered, globose to subglobose or ellipsoidal, carbonaceous, dark brown to black, gregarious, ostiolate. *Peridium* comprising of only pseudoparenchyma or two layers: outer layer of brown, inner layer of elongated, hyaline cells. *Pseudoparaphyses* cellular, septate, branched. *Asci* 8-spored, bitunicate, fissitunicate, cylindric-clavate to subcylindrical, short pedicellate, thick-walled, with or without an ocular chamber. *Ascospores* overlapping uni- to bi-seriate, clavate, ellipsoid, subcylindrical ovoid or fusoid with rounded ends, versicolored, initially hyaline when young to pale brown, golden brown or brown when mature, end cells hyaline, central cells brown, 1–3-septate, constricted at the septa, guttulate, slightly curved, lacking gelatinous sheath or appendages, slimy material without well-defined sheath. **Asexual morph**: *Coelomycetous*. *Conidiomata* pycnidial, immersed, erumpent at maturity, solitary or aggregated, unilocular, globose to subglobose, ellipsoidal, dark brown to black, ostiolate. *Ostiole* single, circular to subcylindrical, papillate, dark brown to black, centrally located. *Conidiomatal wall* composed of thick-walled, dark brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, determinate, smooth-walled, hyaline, aseptate, globose to subglobose, ellipsoidal, cylindrical to subcylindrical. *Conidia* spherical to globose, subglobose, ovate to obovate, ellipsoidal, clavate to subclavate, lageniform, hyaline, aseptate, straight to slightly curved, guttulate, smooth, and thick-walled. *Chlamydospores* apical, rarely intercalary, single or in chains, branching, filamentous, filiform to narrowly fusiform straight or curved, catenate, rarely solitary, branched, septate, with thickened septa, brown to dark brown at the septa, smooth-walled.

Type species: *Halobyssothecium obiones* (P. Crouan & H. Crouan) Dayar., E.B.G. Jones & K.D. Hyde, Mycological Progress 17 (10): 1165 (2018)

Notes: Two species were included in *Halobyssothecium*, *H. obiones* and *H. estuariae* (Dayarathne et al. 2018; Devadatha et al. 2020), collected from various host substrates in temperate regions. In the present study, three collections of morphologically distinct isolates were encountered, two were asexual morphs (*H. bambusicola* and *H. phragmitis*) and one sexual morph (*H. versicolor*), which advances the current understanding of how complex the genus is. The complexity was noted by Devadatha et al. (2020) based on previous collections by various authors. For instance, two morphologically similar taxa of *H. obiones* were collected but differed in ascospore measurements (24–38 × 8–14 μm vs. 38–56 × 16–22 μm) (Jones 1962; Cavaliere 1968; Webber 1970), but no sequence data was available at that time to distinguish them. *Halobyssothecium versicolor* agrees with the generic description of the genus and its placement in the phylogenetic tree redefines what comprises *Halobyssothecium*. Currently, the *Lentithecium* clade includes *L. fluviatile*, *L. clioninum* and *L. pseudoclioninum*, while *L. cangshanense*, *L. carbonneanum*, *L. kunmingense, L. unicellulare*, and *L. voraginesporum* grouped within the *Halobyssothecium* clade and are transferred herein.

Halobyssothecium bambusicola M.S. Calabon, Boonmee, E.B.G. Jones & K.D. Hyde, sp. nov. (Fig. 3)

Index Fungorum number: IF558089; *Facesoffungi number*: FoF 09430

Etymology: the specific epithet "*bambusicola*" refers to the host, of which the fungus was collected

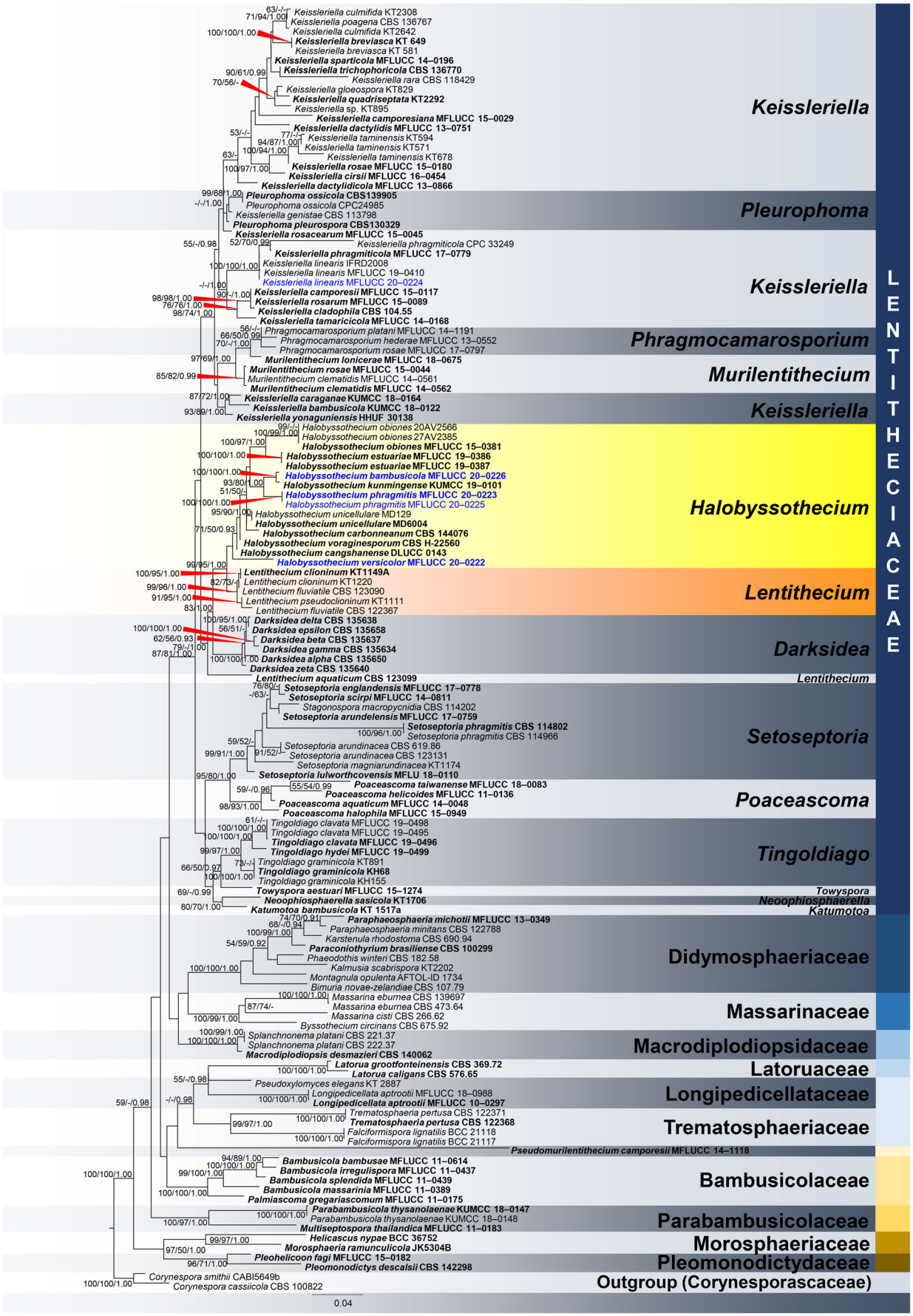
Holotype: MFLU 20–0549

Saprobic on decaying bamboo culms submerged in freshwater habitat. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 350–470 μm high, 230–260 μm wide ($\bar{x}$ = 415.4 × 238.6, *n* = 10), pycnidial, immersed, erumpent at maturity, solitary or aggregated, globose, unilocular, dark brown to black, ostiolate. *Ostiole* 150–160 × 170–180 μm, single, circular to subcylindrical, centrally located. *Conidiomatal wall* 14–28 μm, composed of thick-walled, dark brown cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 6–45 × 2–5 μm ($\bar{x}$ = 19.7 × 3.3, *n* = 30), enteroblastic, phialidic, hyaline, aseptate, cylindrical to subcylindrical. *Conidia* 6–12 × 5–10 μm ($\bar{x}$ = 8.7 × 6.8, *n* = 50), spherical to globose, obovate, ellipsoidal, subclavate, hyaline, aseptate, guttulate, smooth, and thick-walled.

Culture characteristics: On MEA, colony circular with filamentous margin, reaching 25–30 mm diam. in 25 days at 25 °C, brown to grayish brown from above, yellowish brown to dark brown from below, surface rough, dry, raised, with dense mycelia, edge filiform.

Material examined: THAILAND, Chiang Mai Province, on submerged bamboo culm in a stream, 11 February 2019, M.S. Calabon (MFLU 20–0549, holotype), ex-type living culture MFLUCC 20–0226

Notes: Several species of freshwater fungi growing on submerged bamboo have been recorded, e.g. *Acrodictys liputii* L.


Keissleriella culmifida KT2308
Keissleriella poagena CBS 136767
Keissleriella culmifida KT2642
Keissleriella breviasca KT 649
Keissleriella breviasca KT 581
Keissleriella sparticola MFLUCC 14–0196
Keissleriella trichophoricola CBS 136770
Keissleriella rara CBS 118429
Keissleriella gloeospora KT829
Keissleriella quadriseptata KT2292
Keissleriella sp. KT895
Keissleriella camporesiana MFLUCC 15–0029
Keissleriella dactylidis MFLUCC 13–0751
Keissleriella taminensis KT594
Keissleriella taminensis KT571
Keissleriella taminensis KT678
Keissleriella rosae MFLUCC 15–0180
Keissleriella cirsii MFLUCC 16–0454
Keissleriella dactylidicola MFLUCC 13–0866
Pleurophoma ossicola CBS139905
Pleurophoma ossicola CPC24985
Keissleriella genistae CBS 113798
Pleurophoma pleurospora CBS130329
Keissleriella rosacearum MFLUCC 15–0045
Keissleriella phragmiticola CPC 33249
Keissleriella phragmiticola MFLUCC 17–0779
Keissleriella linearis IFRD2008
Keissleriella linearis MFLUCC 19–0410
Keissleriella linearis MFLUCC 20–0224
Keissleriella camporesii MFLUCC 15–0117
Keissleriella rosarum MFLUCC 15–0089
Keissleriella cladophila CBS 104.55
Keissleriella tamaricicola MFLUCC 14–0168
Phragmocamarosporium platani MFLUCC 14–1191
Phragmocamarosporium hederae MFLUCC 13–0552
Phragmocamarosporium rosae MFLUCC 17–0797
Murilentithecium lonicerae MFLUCC 18–0675
Murilentithecium rosae MFLUCC 15–0044
Murilentithecium clematidis MFLUCC 14–0561
Murilentithecium clematidis MFLUCC 14–0562
Keissleriella caraganae KUMCC 18–0164
Keissleriella bambusicola KUMCC 18–0122
Keissleriella yonaguniensis HHUF 30138
Halobyssothecium obiones 20AV2566
Halobyssothecium obiones 27AV2385
Halobyssothecium obiones MFLUCC 15–0381
Halobyssothecium estuariae MFLUCC 19–0386
Halobyssothecium estuariae MFLUCC 19–0387
Halobyssothecium bambusicola MFLUCC 20–0226
Halobyssothecium kunmingense KUMCC 19–0101
Halobyssothecium phragmitis MFLUCC 20–0223
Halobyssothecium phragmitis MFLUCC 20–0225
Halobyssothecium unicellulare MD129
Halobyssothecium unicellulare MD6004
Halobyssothecium carbonneanum CBS 144076
Halobyssothecium voraginesporum CBS H-22560
Halobyssothecium cangshanense DLUCC 0143
Halobyssothecium versicolor MFLUCC 20–0222
Lentithecium clioninum KT1149A
Lentithecium clioninum KT1220
Lentithecium fluviatile CBS 123090
Lentithecium pseudoclioninum KT1111
Lentithecium fluviatile CBS 122367
Darksidea delta CBS 135638
Darksidea epsilon CBS 135658
Darksidea beta CBS 135637
Darksidea gamma CBS 135634
Darksidea alpha CBS 135650
Darksidea zeta CBS 135640
Lentithecium aquaticum CBS 123099
Setoseptoria englandensis MFLUCC 17–0778
Setoseptoria scirpi MFLUCC 14–0811
Stagonospora macropycnidia CBS 114202
Setoseptoria arundelensis MFLUCC 17–0759
Setoseptoria phragmitis CBS 114802
Setoseptoria phragmitis CBS 114966
Setoseptoria arundinacea CBS 619.86
Setoseptoria arundinacea CBS 123131
Setoseptoria magniarundinacea KT1174
Setoseptoria lulworthcovensis MFLU 18–0110
Poaceascoma taiwanense MFLUCC 18–0083
Poaceascoma helicoides MFLUCC 11–0136
Poaceascoma aquaticum MFLUCC 14–0048
Poaceascoma halophila MFLUCC 15–0949
Tingoldiago clavata MFLUCC 19–0498
Tingoldiago clavata MFLUCC 19–0495
Tingoldiago clavata MFLUCC 19–0496
Tingoldiago hydei MFLUCC 19–0499
Tingoldiago graminicola KT891
Tingoldiago graminicola KH68
Tingoldiago graminicola KH155
Towyspora aestuari MFLUCC 15–1274
Neoophiosphaerella sasicola KT1706
Katumotoa bambusicola KT 1517a
Paraphaeosphaeria michotii MFLUCC 13–0349
Paraphaeosphaeria minitans CBS 122788
Karstenula rhodostoma CBS 690.94
Paraconiothyrium brasiliense CBS 100299
Phaeodothis winteri CBS 182.58
Kalmusia scabrispora KT2202
Montagnula opulenta AFTOL-ID 1734
Bimuria novae-zelandiae CBS 107.79
Massarina eburnea CBS 139697
Massarina eburnea CBS 473.64
Massarina cisti CBS 266.62
Byssothecium circinans CBS 675.92
Splanchnonema platani CBS 221.37
Splanchnonema platani CBS 222.37
Macrodiplodiopsis desmazieri CBS 140062
Latorua grootfonteinensis CBS 369.72
Latorua caligans CBS 576.65
Pseudoxylomyces elegans KT 2887
Longipedicellata aptrootii MFLUCC 18–0988
Longipedicellata aptrootii MFLUCC 10–0297
Trematosphaeria pertusa CBS 122371
Trematosphaeria pertusa CBS 122368
Falciformispora lignatilis BCC 21118
Falciformispora lignatilis BCC 21117
Pseudomurilentithecium camporesii MFLUCC 14–1118
Bambusicola bambusae MFLUCC 11–0614
Bambusicola irregulispora MFLUCC 11–0437
Bambusicola splendida MFLUCC 11–0439
Bambusicola massarinia MFLUCC 11–0389
Palmiascoma gregariascomum MFLUCC 11–0175
Parabambusicola thysanolaenae KUMCC 18–0147
Parabambusicola thysanolaenae KUMCC 18–0148
Multiseptospora thailandica MFLUCC 11–0183
Helicascus nypae BCC 36752
Morosphaeria ramunculicola JK5304B
Pleohelicoon fagi MFLUCC 15–0182
Pleomonodictys descalsii CBS 142298
Corynespora smithii CABI5649b
Corynespora cassiicola CBS 100822
0.04
Keissleriella
Pleurophoma
Keissleriella
Phragmocamarosporium
Murilentithecium
Keissleriella
Halobyssothecium
Lentithecium
Darksidea
Lentithecium
Setoseptoria
Poaceascoma
Tingoldiago
Towyspora
Neoophiosphaerella
Katumotoa
Didymosphaeriaceae
Massarinaceae
Macrodiplodiopsidaceae
Latoruaceae
Longipedicellataceae
Trematosphaeriaceae
Bambusicolaceae
Parabambusicolaceae
Morosphaeriaceae
Pleomonodictydaceae
Outgroup (Corynesporascaceae)
LENTITHECIACEAE

◀ **Fig. 1** Phylogenetic tree generated from maximum likelihood (ML) analysis based on LSU, SSU, ITS and *TEF1-α* sequence data for the species from Lentitheciaceae and closely related families in Pleosporales. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) higher than 50% and Bayesian posterior probabilities (BYPP) greater than 0.90 are indicated above the nodes in this order. The new isolates are represented in blue. The ex-type strains are indicated in bold. The tree is rooted to *Corynespora cassiicola* (CBS 100822) and *C. smithii* (CABI5649b) (Corynesporascaceae). Bar = 0.04 estimated number of nucleotide substitutions per site per branch

Cai, K.Q. Zhang, McKenzie, W.H. Ho & K.D. Hyde, *Annulatascus liputii* L. Cai & K.D. Hyde, *Ascoyunnania aquatica* L. Cai & K.D. Hyde, *Cataractispora receptaculorum* W.H. Ho, K.D. Hyde & Hodgkiss, *Dictyosporella thailandensis* W. Dong, H. Zhang & K.D. Hyde, *Fluminicola saprophytica* W. Dong, H. Zhang & K.D. Hyde, *Dictyochaeta curvispora* L. Cai, McKenzie & K.D. Hyde, *D. plovercovensis* Goh & K.D. Hyde, *Diluviicola aquatica* W. Dong, H. Zhang & K.D. Hyde, *Linocarpon bambusicola* L. Cai & K.D. Hyde, *Ophioceras guttulatum* C.K.M. Tsui, H.Y.M. Leung, K.D. Hyde & Hodgkiss, *Microthecium sepedonioides* (Preuss) Y. Marín, Stchigel, Guarro & Cano, *Payosphaeria minuta* H.Y.M. Leung, *Pseudoproboscispora thailandensis* W. Dong, H. Zhang & K.D. Hyde, and *Saccardoella minuta* L. Cai & K.D. Hyde (Cai et al. 2002a, b, 2003, 2004, 2005, 2006; Ho et al. 2004; Zhang et al. 2017).

Halobyssothecium bambusicola closely resembles *H. kunmingense*, and *H. unicellulare*. *Halobyssothecium kunmingense* has wider conidiomata (210–250 × 320–350 μm vs. 350–470 × 230–260 μm), a thicker peridium (60–80 μm vs 14–28 μm), smaller conidiogenous cells (5–19 × 2–5 μm vs. 6–45 × 2–5 μm) and larger globose to obovoidal conidia (8–14 × 5–8 μm vs 6–12 × 5–10) with large guttules, compared to *H. bambusicola* (Dong et al. 2020). The guidelines on species delimitation for new species by Jeewon and Hyde (2016) was followed and pairwise comparison of ribosomal ITS sequences showed 15 nucleotide base pair differences among the 800 nucleotides analyzed between *H. bambusicola* and *H. kunmingense*. Moreover, a SplitsTree analysis supports the introduction of *H. bambusicola* (Fig. 2).

Halobyssothecium bambusicola has larger conidiomata (350–470 μm high × 230–260 μm wide vs. 115–235 μm high × 140–235 μm wide) and larger globose to obovoidal conidia (6–12 × 5–8 μm vs. 6–9 × 4–5 μm) with large guttules as compared to *H. unicellulare* (Hyde et al. 2016). *Halobyssothecium bambusicola* differs from *H. phragmitis* in conidial shape (globose to obovate conidia vs. ovoid to fusoid-ellipsoidal). *Halobyssothecium bambusicola* and *H. phragmitis* differ by 6.84% (36/526 bp) and 2.63% (25/952 bp) in ITS and *TEF1-α*, respectively. The multigene phylogenetic analysis placed *H. bambusicola* within *Halobyssothecium* in a well-supported subclade with *H. kunmingense* (100% ML, 100% MP, 1.00 BYPP) and the coelomycetous marine *H. phragmitis* (93% ML, 80% MP, 1.00 BYPP).

Halobyssothecium phragmitis **M.S. Calabon, E.B.G. Jones, S. Tibell & K.D. Hyde, sp. nov. (Fig. 4)**

Index Fungorum number: IF558090; *Facesoffungi number*: FoF 09431

Etymology: In reference to the host genus *Phragmites*, from which the species was isolated.

Holotype: MFLU 20–0550

Saprobic on dead *Phragmites* culm and stem. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 205–340 μm high, 215–280 μm wide, solitary, scattered, immersed to slightly immersed, pycnidial, subglobose to ellipsoidal, unilocular, black, with indistinct ostioles. *Ostioles* 82–96 μm, central, circular, papillate, dark brown to black. *Conidiomatal wall* 13.3–31 μm, thick-walled, 7–9 layers, comprising of dark brown cells, of *textura angularis*, inner layer comprising hyaline gelatinous layer, thickening at the upper and basal zone. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 8–18 × 1–5 μm ($\bar{x}$ = 11.6 × 3.2 μm, *n* = 20), enteroblastic, phialidic, cylindrical to lageniform, determinate, hyaline, formed from inner layers of conidiomata. *Conidia* 9–19 × 2–6 μm ($\bar{x}$ = 13.7–4.1 μm, *n* = 50), cylindrical, fusoid-ellipsoidal, straight or slightly curved, hyaline, aseptate to 1–2-septate, unicellular, mostly with one large central guttule per cell, smooth-walled.

Culture characteristics: Conidia germinated on MEA within 24 h. Colonies on MEA, reaching 10–12 mm diam. in 14 days at 25 °C. Mycelium superficial, white, flattened, hairy, dense, circular, flattened, margin entire; reverse pale brown.

Material examined: SWEDEN, Gotland, Kappelshamnsviken, on dead *Phragmites* culm (Poaceae), 7 March 2019, E.B.G. Jones, GJ653 (MFLU 20–0550,

Fig. 2 Results of the pairwise homoplasy index (PHI) test of three novel *Halobyssothecium* species using both LogDet transformation and splits decomposition. PHI test results (Φw) < 0.05 indicating significant recombination within the dataset

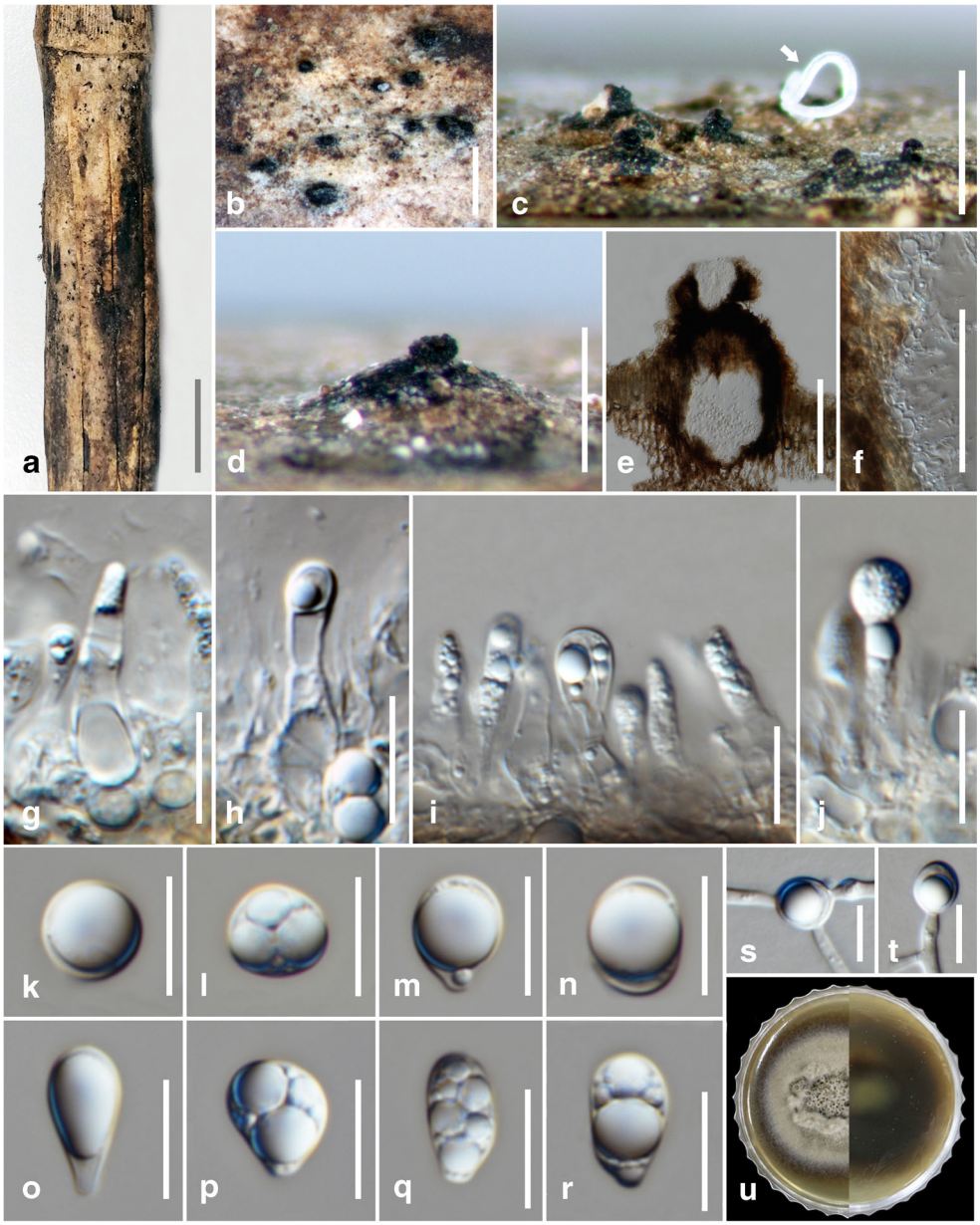


Fig. 3 *Halobyssothecium bambusicola* (MFLU 20–0549, holotype). **a** Host. **b–d** Appearance of conidiomata on host surface releasing conidia in a cirrus (arrow). **e** Vertical section of conidioma. **f** Conidiomatal wall. **g–j** Developing conidia attach to conidiogenous cell. **k–r** Conidia. **s–t** Germinated conidia. **u** Colony on MEA (obverse, reverse). Scale bars: **a** = 200 mm; **b** = 1 mm; **c–e** = 500 μm; **f** = 50 μm; **g–t** = 10 μm

holotype), ex-type living cultures MFLUCC 20–0223; ibid, Sudersand, on dead *Phragmites* (Poaceae) stem, 7 March 2019, E.B.G. Jones, GJ659 (MFLU 20–0552, paratype), ex-paratype living culture MFLUCC 20–0225.

Notes: *Halobyssothecium phragmitis* resembles *Stagonospora macropycnidia* but the former species has smaller conidiomata (205–340 μm high × 215–280 μm wide vs. 410–1020 μm high × 120–380 μm wide), and smaller conidia (9–19 × 2–6 μm vs. 22–42 × 2.5–5 μm) (Cunnell 1961). *Setoseptoria phragmitis* Quaedvl., Verkley & Crous is distinct from *H. phragmitis* with smaller conidiomata (up to 200 μm vs. 205–340 μm) and longer subcylindrical conidia (19–38 × 3.5–4 μm vs. 9–19 × 2–6 μm) (Quaedvlieg et al. 2013). *Phragmocamarosporium platani* Wijayaw., Yong Wang bis & K.D. Hyde differs from *H. phragmitis* with smaller conidiomata (100–320 μm high, 150–300 μm diam. vs. 205–340 μm high, 215–280 μm wide) and larger brown conspicuous phragmospores (12–13 × 5–7.5 μm vs. 9–19 × 2–6 μm) (Wijayawardene et al. 2015). *Pleurophoma ossicola* Crous, Krawczynski & H.-G. Wagner differs from *H. phragmitis* with smaller conidia (3–5 × 1.5–2 μm vs. 9–19 × 2–6 μm) (Crous et al. 2015). *Murilentithecium clematidis* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde is distinct from *H. phragmitis* with larger conidiomata (0.5–1.5 mm diam vs. 205–340 μm) (Wanasinghe et al. 2014). *Keissleriella quadriseptata* Kaz. Tanaka & K. Hiray. differs from *H. phragmitis* with larger cylindrical conidia (25–32 × 6–8.5 μm vs. 9–19 × 2–6 μm) (Tanaka et al. 2015). Based on multi-loci phylogenetic analyses, the above mentioned species are phylogenetically distinct to *H. phragmitis*.

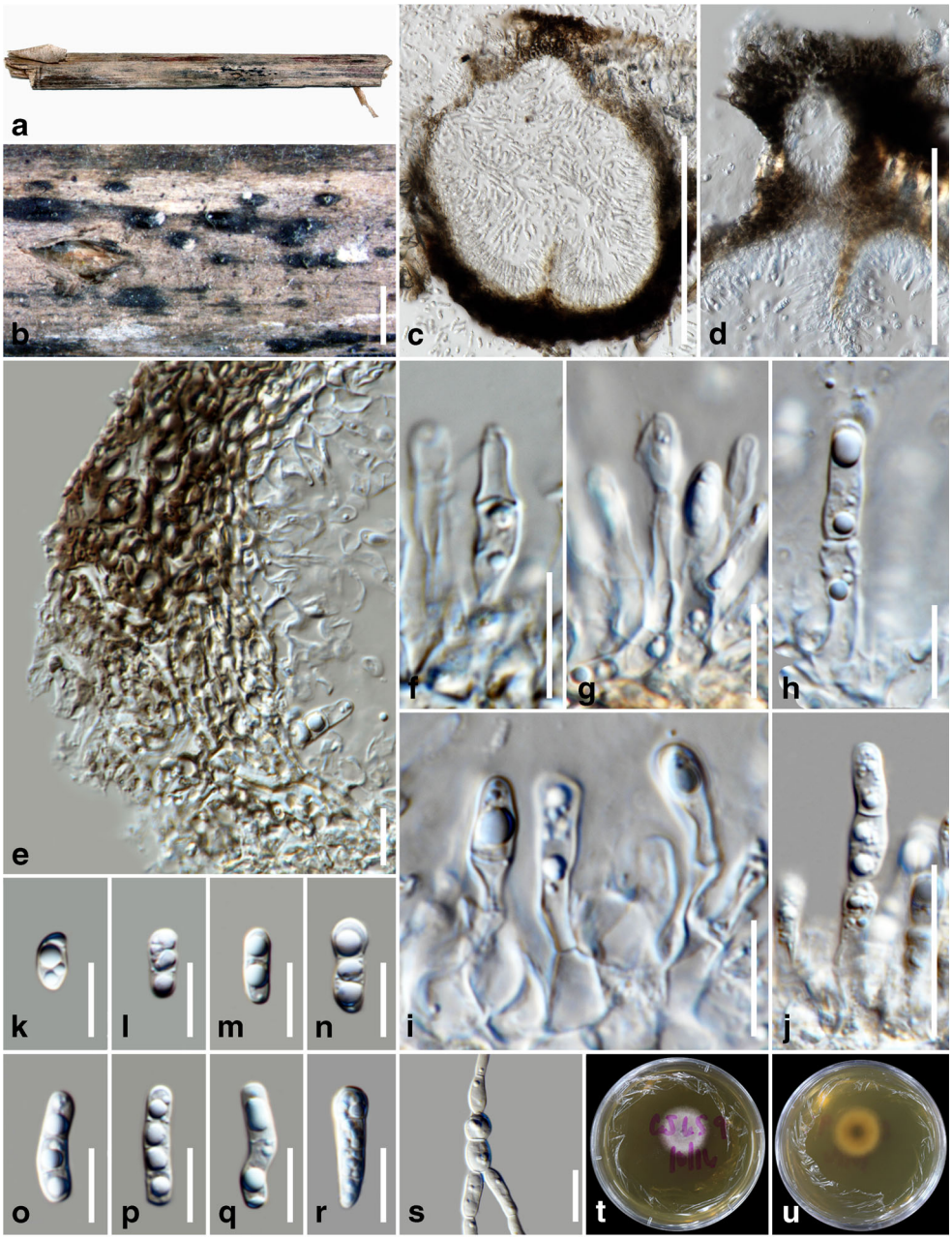


Fig. 4 *Halobyssothecium phragmitis* (MFLU 20–0550, holotype). **a** Host. **b** Appearance of conidiomata on host surface. **c** Vertical section of conidioma. **d** Ostiole. **e** Conidiomatal wall. **f–j** Developing conidia attach to conidiogenous cells. **k–r** Conidia. **s** Germinated conidium. **t–u** Colony on MEA: from **t** obverse, **u** reverse. Scale bars: **b**, **c** = 200 μm, **d** = 100 μm, **e–s** = 10 μm

Halobyssothecium phragmitis is phylogenetically close to *H. bambusicola* and *H. kunmingense* (93% ML, 80% MP, 1.00 BYPP). It differs from the latter with ovoidal to fusoid-ellipsoidal conidia. *Halobyssothecium kunmingense* has 14 base pair differences (800 bp, 1.75%) with *H. bambusicola* in ITS region.

Halobyssothecium versicolor M.S. Calabon, E.B.G. Jones & K.D. Hyde, sp. nov. (Fig. 5)

Index Fungorum number: IF558091; *Facesoffungi number*: FoF 09432

Etymology: Referring to the versicolored ascospore

Holotype: MFLU 19–0676

Saprobic on *Halimione portulacoides* in intertidal habitat. **Sexual morph**: *Ascomata* 265–510 μm high, 365–530 μm wide ($\overline{x}$ = 408 × 459, *n* = 10), superficial to semi-immersed, clustered, sometimes solitary, scattered, subglobose or ellipsoidal, dark brown to black, carbonaceous, conspicuous at the surface, uni- to bi-loculate, ostiolate, with periphyses. *Ostiolar neck* 105–190 μm long, 95–175 μm wide ($\overline{x}$ = 150 × 135, *n* = 10) central, papillate, rounded, short, crest-like, dark brown, composed of several layers of pseudoparenchymatous cells. *Peridium* 37–94 μm thick, comprising two layers: outer layer of brown pseudoparenchyma; inner layer of elongated, hyaline cells. *Pseudoparaphyses* 2–3 μm wide, septate, hyaline,

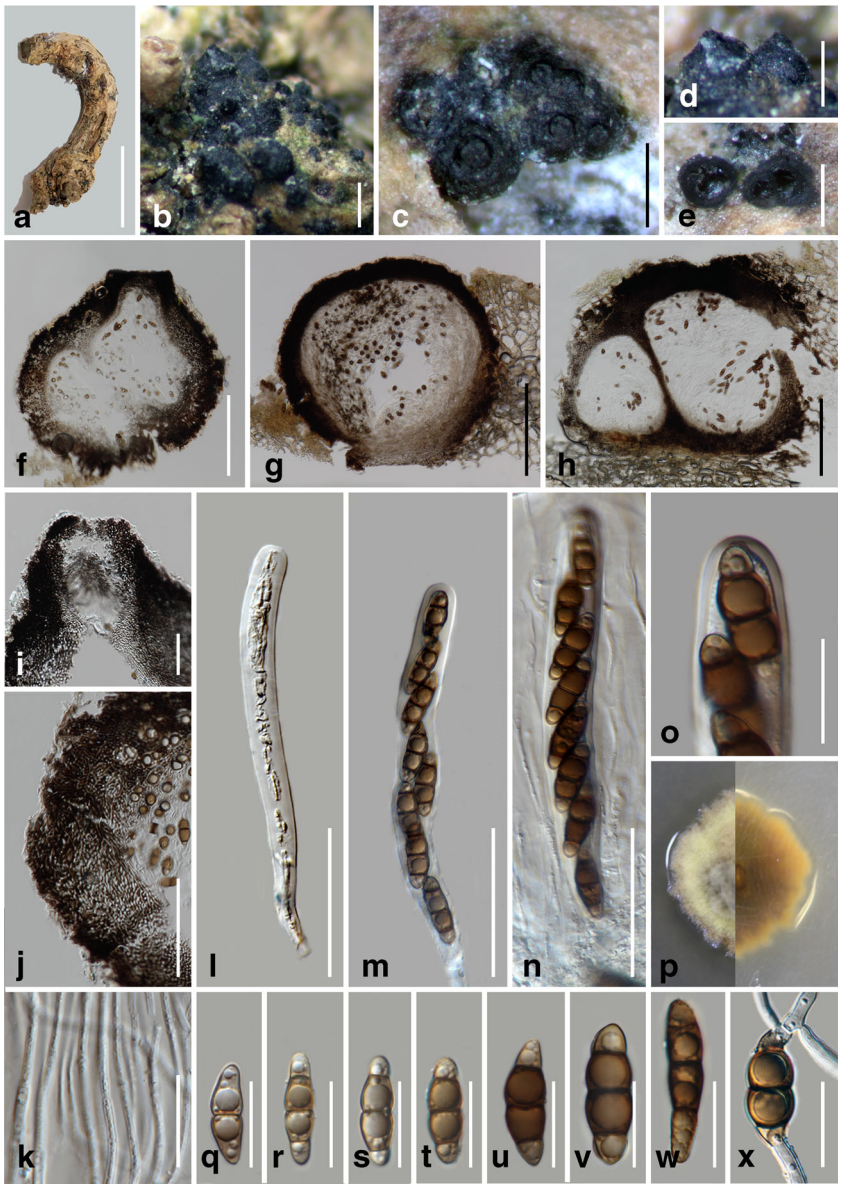


Fig. 5 *Halobyssothecium versicolor* (MFLU 19–0676, holotype). **a** Host. **b–e** Appearance of ascomata on the host. **f–h** Sections of ascomata. **i** Ostiole. **j** Section of peridium. **k** Pseudoparaphyses. **l–n** Asci. **o** Apex of ascus. **p** Colony on MEA. **q–w** Ascospores. **x** Germinated conidium. Scale bar: **a** = 20 mm, **b–e** = 500 μm, **f–h** = 200 μm, **i–n** = 50 μm, **o**, **q–x** = 20 μm

filiform, branched and anastomosing above the asci. *Asci* 137–173 × 17–12 μm ($\overline{x}$ = 153.4 × 14.7 μm, *n* = 20), 8-spored, clavate to subcylindrical, short pedicellate with an ocular chamber. *Ascospores* 18–41 × 6–12 μm ($\overline{x}$ = 27.4 × 8.6, *n* = 20), overlapping, uniseriate to biseriately arranged, versicolored, central cells are pale brown to dark brown, end cells hyaline, 1-septate at an early stage, 3-septate when mature, and constricted at the septa, slightly curved, lacking gelatinous sheaths or appendages. **Asexual morph**: Undetermined.

Culture characteristics: Ascospores germinated on MEA within 24 h. Colonies on MEA, reaching 10–15 mm diam. in 15 days at 25 °C. Mycelium superficial, initially pale yellow, becoming yellowish brown with age, hairy, effuse with wavy edge, dense, circular, raised, undulate, reverse dark yellowish brown.

Material examined: UK, Hampshire, Hayling Island bridge, on dead *Halimione portulacoides* (Amaranthaceae), 28 February 2019, E.B.G Jones, GJ597 (MFLU 19–0676, holotype), ex-type living cultures MFLUCC 20–0222.

Notes: *Halobyssothecium versicolor* resembles *H. obiones* and *H. estuariae* in having versicolored ascospores with brown central cells and hyaline end cells. *Halobyssothecium versicolor* differs from *H. obiones* with larger ascomata (265–510 μm high, 365–530 μm diam. vs. 360–400 μm high, 340–380 μm diam.) and smaller ascospores (18–41 × 6–12 μm vs. 28–47 × 10–18 μm) (Dayarathne et al. 2018). The asexual morph was not observed in the culture but *Halobyssothecium* species have xylomyces-like chlamydospores (Devadatha et al. 2020) and phoma-like conidia (Kohlmeyer and Kohlmeyer 1979; Calado et al. 2015).

Phylogenetic analysis shows that *Halobyssothecium versicolor* clustered within Lentitheciaceae and basal to other *Halobyssothecium* species. *Halobyssothecium versicolor* is phylogenetically close to *H. bambusicola*, *H. kunmingense*, and *H. phragmitis*. A comparison of ITS and *TEF1-α* sequence data of *H. versicolor* differs by 40 (8.97%, 446 bp) and 56 (6.26%, 895 bp) base pairs with *H. obiones*, type species of the genus.

Keissleriella linearis **E. Müll. ex Dennis, Kew Bulletin 19 (1): 120 (1964)**

Facesoffungi number: FoF 09433

Saprobic on decaying culm of *Phragmites* sp. **Sexual morph**: [for descriptions and illustrations, see Dennis (1964)]. **Asexual morph**: *Conidiomata* 42.6–53 μm high, 15.5–22.2 μm wide ($\bar{x}$ = 51 × 18.8 μm, *n* = 10), black, solitary, scattered, immersed, pycnidial, subglobose to ellipsoidal, unilocular. *Conidiomatal wall* 2.2–7.4 μm, thick-walled, 7–9 layers, comprising of dark brown cells, of *textura angularis* to *textura globosa*, inner layer comprising hyaline gelatinous layer, thickening at the upper zone. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4.1–7.7 × 1.6–3.8 μm, ($\bar{x}$ = 5.8 × 3.1 μm, *n* = 20), enteroblastic, phialidic, cylindrical to lageniform, determinate, hyaline. *Conidia* 4.0–6.5 × 1.0–2.5 μm ($\bar{x}$ = 5.4 × 1.8 μm, *n* = 50), ovoid, obovoidal, cylindrical, fusoid-ellipsoidal, straight, or slightly curved, unicellular, hyaline, guttulate, smooth-walled. *Beta conidia* not observed. For illustrations of morphological characters, refer to Tibell et al. (2020).

Culture characteristics: Ascospores germinated on MEA within 24 h. Colonies on MEA, reaching 22–24 × 26–28 mm diam. in 25 days at 25 °C. Mycelium superficial, white to grayish white with age, hairy, effuse with wavy edge, dense, circular, raised, undulate to filiform with age; reverse dark yellowish brown.

Material examined: SWEDEN, Gotland, Kappelshamnsviken, on dead *Phragmites* culm (Poaceae), 7 March 2019, E.B.G. Jones, GJ654 (MFLU 20–0551), living culture MFLUCC 20–0224.

Notes: *Keisslerialla linearis* (MFLUCC 20–0224) groups with two strains of *K. linearis* (IFRD2008, MFLUCC 19–0410) with strong bootstrap support (100% ML, 100% MP, 1.00 BYPP; Fig. 1). The new strain is an asexual morph of *K. linearis* observed from *Phragmites* sp. in Sweden (Tibell et al. 2020). In the phylogenetic analysis, *K. linearis* clustered with other *Keissleriella* species. A comparison of the LSU and SSU sequence data of *K. linearis* (IFRD2008) and the new strain (MFLUCC 20–0224) revealed no nucleotide differences.

New combinations

Halobyssothecium cangshanense **(Z.L. Luo, X.J. Su & K.D. Hyde) M.S. Calabon, K.D. Hyde & E.B.G. Jones, comb. nov.**

Index Fungorum number: IF558092; *Facesoffungi number*: FoF 09434

Basionym: Lentithecium cangshanense Z.L. Luo, X.J. Su & K.D. Hyde, Phytotaxa 267 (1): 65 (2016)

Sexual morph: Descriptions and illustrations refer to Su et al. (2016). *Asexual morph*: Undetermined

Distribution: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream.

Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.

Halobyssothecium carbonneanum **(J. Fourn., Raja & Oberlies) M.S. Calabon, K.D. Hyde & E.B.G. Jones, comb. nov.**

Index Fungorum number: IF558093; *Facesoffungi number*: FoF 09435

Basionym: Lentithecium carbonneanum J. Fourn., Raja & Oberlies, Persoonia 40: 295 (2018)

Sexual morph: Descriptions and illustrations refer to Crous et al. (2018). *Asexual morph*: Undetermined

Distribution: FRANCE, Haute-Garonne, Carbonne, SW of route du Lançon, artificial lake in a gravel pit, on submerged decorticated branch of *Populus*.

Notes: Holotype ILLS 81639. ITS, LSU and *RPB2* sequence data are available.

Halobyssothecium kunmingense **(W. Dong, H. Zhang & K.D. Hyde) M.S. Calabon, Boonmee, K.D. Hyde & E.B.G. Jones, comb. nov.**

Index Fungorum number: IF556948; *Facesoffungi number*: FoF 09436

Basionym: *Lentithecium kunmingense* W. Dong, H. Zhang & K.D. Hyde

Sexual morph: Undetermined. *Asexual morph*: Descriptions and illustrations refer to Dong et al. (2020)

Distribution: CHINA, Yunnan Province, Kunming University of Science and Technology, on submerged wood in a stream (Dong et al. 2020).

Notes: HKAS 102150. LSU, SSU, ITS, *TEF1-α* sequence data are available.

Halobyssothecium unicellulare **(Abdel-Aziz) M.S. Calabon, K.D. Hyde & E.B.G. Jones, comb. nov.**

Index Fungorum number: IF558094; *Facesoffungi number*: FoF 09437

Basionym: Lentithecium unicellulare Abdel-Aziz, Fungal Diversity 80: 53 (2016)

Sexual morph: Undetermined. *Asexual morph*: Descriptions and illustrations refer to Hyde et al. (2016)

Distribution: EGYPT, Sohag City, on decayed wood submerged in the River Nile (Hyde et al. 2016).

Notes: Holotype CBS H-22674. LSU and SSU sequence data are available.

***Halobyssothecium voraginesporum* (Abdel-Wahab, Bahkali & E.B.G. Jones) M.S. Calabon, K.D. Hyde & E.B.G. Jones, comb. nov.**

Index Fungorum number: IF558095; *Facesoffungi number*: FoF 09438

Basionym: Lentithecium voraginesporum Abdel-Wahab, Bahkali & E.B.G. Jones, Fungal Diversity 80: 53 (2016)

Sexual morph: Descriptions and illustrations refer to Hyde et al. (2016). *Asexual morph*: Undetermined

Distribution: SAUDI ARABIA, Arabian Gulf, Tarut mangroves, on submerged, decayed *Phragmites australis* (Poaceae), stem inside the mangrove stand (Hyde et al. 2016)

Notes: Holotype CBS H-22560. LSU and SSU sequence data are available.

Notes for *Lentitheciaceae*

***Lentithecium aquaticum* Ying Zhang, J. Fourn. & K.D. Hyde, Fungal Diversity 38: 234 (2009)**

Phylogenetic analysis shows that *Lentithecium aquaticum* does not cluster within the *Lentithecium* clade but forms a weakly supported subclade basal to *Darksidea* species. Further collections are required to establish the taxonomic position of *L. aquaticum*.

***Pseudomurilentithecium camporesii* Mapook & K.D. Hyde, Fungal Diversity 100: 69 (2020)**

In the phylogenetic analysis (Fig. 1), *Psedomurilentithecium camporesii* does not cluster within Lentitheciaceae but forms a weakly supported clade basal to Latoruaceae, Longipedicellataceae, and Trematosphaeriaceae. Broader taxon sampling, including other families in Pleosporales, is necessary to confirm its placement.

***Keissleriella caudata* (E. Müll.) Corbaz, Phytopathologische Zeitschrift 28 (4): 411 (1957)**

Preliminary phylogenetic analysis shows that *Keissleriella caudata* does not group with other *Keissleriella* species, but clusters instead with *Corynespora* species. Only ITS sequence data of *K. caudata* is available in GenBank with an accession number MH857034. BLAST analysis did not show any *Keissleriella* species in the first 100 closely related sequence data. A fresh collection of specimens and additional DNA sequence data are required to confirm its placement within Pleosporales.

Discussion

Since *Lentithecium* was established for *L. fluviatile* (≡ *Massarina fluviatilis*), ten additional species have been introduced from lotic and lentic freshwater (Zhang et al. 2009b; Tanaka et al. 2015; Hyde et al. 2016; Su et al. 2016; Crous et al. 2018), as well as marine (Suetrong et al. 2009; Zhang et al. 2009b; Hyde et al. 2016) habitats and from different hosts. *Lentithecium arundinaceum* (≡ *Massarina arundinacea*), whose phylogenetic position was unclear for a long time and has been assigned to various genera (i.e., *Ampullina*, *Heptameria*, *Leptosphaeria*, *Lophiostoma*, *Massarina*, *Metasphaeria*, *Peripherostoma*, *Phaeosphaeria*, *Pleospora*, *Rhopographus*, *Sphaeria*, *Sphaeropsis*), was transferred by Tanaka et al. (2015) to *Setoseptoria*. *Setoseptoria arundinacea* clustered with other *Setoseptoria* species in the phylogenetic analysis (Fig. 1).

Multi-locus phylogenetic analysis shows that the three *Lentithecium* species, *L. aquaticum*, *L. lineare* and *L. rarum* (Kohlm., Volkm.-Kohlm. & O.E. Erikss.) Suetrong, Sakay., E.B.G. Jones, Kohlm. & Volkm.-Kohlm. do not group with other *Lentithecium* species, which was also reported by Tanaka et al. (2015), Devadatha et al. (2020), Dong et al. (2020), and Wijayawardene et al. (2020). *Lentithecium aquaticum*, a species introduced by Zhang et al. (2009b) based on LSU, SSU and *RPB2* sequence data, forms a weakly supported clade basal to *Darksidea* and *Lentithecium*, which confirms the observations of Tanaka et al. (2015) (Fig. 1). Dayarathne et al. (2018) and Devadatha et al. (2020) showed that *Lentithecium aquaticum* clustered within *Setoseptoria* and the asexual morph *Stagonospora macropycnidia*, while Crous et al. (2018) confirmed that it does not group in *Lentithecium*.

Keissleriella rara was transferred to *Lentithecium* by Suetrong et al. (2009) together with *K. cladophila* and *Massarina phragmiticola*. The present phylogenetic analysis shows that *Lentithecium rarum* clustered in *Keissleriella* as sister taxon to *K. trichophoricola* Crous & Quaedvl. (Fig. 1). The same placement was observed also by Singtripop et al. (2015). *Keissleriella linearis* was transferred by Zhang et al. (2009b) to *Lentithecium* based on LSU and SSU sequence data. *Keissleriella linearis*, in common with other *Keissleriella* species, has short brown setae around the apex of the ascomatal ostiole, but Zhang et al. (2009b) opined that the presence of setae has little phylogenetic significance. In their phylogenetic analysis, other species and strains of *Keissleriella* were not included. Singtripop et al. (2015) reexamined the type specimen of *L. lineare* and transferred it to *Keissleriella* based on

morphology and LSU sequence data, and this is in agreement with recent studies by Tanaka et al. (2015), Hyde et al. (2016) and the present study. However, Dayarathne et al. (2018) and Devadatha et al. (2020) placed *L. lineare* in the *Lentithecium* clade. The recent discovery of the asexual morph of *L. lineare* by Tibell et al. (2020) and the phylogenetic analysis based on the four-locus sequence dataset in the present study supports its taxonomic placement in *Keissleriella*.

The continuous discovery of novel fungal species has significantly contributed to the revision of fungal taxa (Arzanlou et al. 2007; Boonmee et al. 2011; Tanaka et al. 2015; Hashimoto et al. 2017; Hyde et al. 2018, 2020a,b,c). Phylogenetic analysis of the newly discovered *Halobyssothecium* species, including all the members of *Lentitheciaceae*, with molecular data supports the transfer of *Lentithecium cangshanense*, *L. carbonneanum*, *L. kunmingense*, *L. unicellulare*, and *L. voraginesporum* to *Halobyssothecium*. In the present placement, members of *Halobyssothecium* have brown and versicolored ascospores without sheath and hyaline conidia, while *Lentithecium* species possess hyaline ascospores with mucilaginous sheaths.

Key to *Halobyssothecium* species

- 1 Asexual morph.....2
- 1* Sexual morph.....5
- 2 Conidia, globose to obovate.....3
- 2* Conidia, ellipsoidal to cylindrical.....*H. phragmitis*
- 3 Conidiomata > 350 μm long.....*H. bambusicola*
- 3* Conidiomata < 350 μm long.....4
- 4 Conidiomata 210–250 × 320–350 μm...*H. kunmingense*
- 4* Conidiomata 115–235 × 140–235 μm...*H. unicellulare*
- 5 Ascospores, brown.....6
- 5* Ascospores, versicolored.....8
- 6 Asci > 100 μm long.....*H. carbonneanum*
- 6* Asci < 100 μm long.....7
- 7 Asci 38–50 × 8–10 μm.....*H. voraginesporum*
- 7* Asci 65–78 × 11–13 μm.....*H. cangshanense*
- 8 Asci > 200 μm high.....9
- 8* Asci < 200 μm high.....*H. versicolor*
- 9 Asci 180–214 × 12–16 μm.....*H. obiones*
- 9* Asci 120–235 × 10–25 μm.....*H. estuariae*

Acknowledgments MS Calabon is grateful to Mushroom Research Foundation and Department of Science and Technology – Science Education Institute (Philippines). The authors are grateful to Dr. Eleni Gentekaki, Dr. Bandarupalli Devadatha, Dr. Mingkwan Doilom, Chun-Fang Liao, and Er-Fu Yang for their comments and suggestions to improve the manuscript, and for helping with the molecular analysis of the samples. W. Dong, H. Zhang, and K.D. Hyde are thanked for the release of data on *Lentithecium kunmingense* W. Dong, H. Zhang & K.D. Hyde prior to publication of their paper.

Author contribution Conceptualization: Mark S. Calabon, E.B. Gareth Jones, and Kevin D. Hyde. Methodology: Mark S. Calabon, E.B. Gareth Jones, and Kevin D. Hyde. Formal analysis and investigation: Mark S. Calabon, E.B. Gareth Jones, Kevin D. Hyde, Ka-Lai Pang, and Sanja Tibell. Resources: E.B. Gareth Jones, Kevin D. Hyde, and Rungtiwa Phookamsak. Writing—original draft preparation: Mark S. Calabon. Writing—review and editing: E.B. Gareth Jones, Kevin D. Hyde, Saranyaphat Boonmee, Sanja Tibell, Leif Tibell, Ka-Lai Pang, and Rungtiwa Phookamsak. Supervision: E.B. Gareth Jones and Kevin D. Hyde. Funding acquisition: E.B. Gareth Jones, Kevin D. Hyde, Saranyaphat Boonmee, and Rungtiwa Phookamsak. All authors have read and agreed to the published version of the manuscript.

Funding Mark Calabon is grateful to the 5th batch of Postdoctoral Orientation Training Personnel in Yunnan Province (grant no.: Y934283261) and the 64th batch of China Postdoctoral Science Foundation (grant no.: Y913082271). E. B Gareth Jones is supported under the Distinguished Scientist Fellowship Program (DSFP), King Saud University, Kingdom of Saudi Arabia. The Swedish Species Initiative ("ArtDatabanken") is thanked for support within the project "Marine fungi in Sweden" (SLU.dha.2017.4.3-73). Kevin D. Hyde thanks the Thailand Research Fund grant entitled "Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion" (Grant No. RDG6130001). Rungtiwa Phookamsak is supported by CAS President's International Fellowship Initiative (PIFI) for Young Staff 2019–2021 (grant number 2019FY0003), The Yunnan Provincial Department of Human Resources and Social Security (Grant No. Y836181261), and The National Science Foundation of China (NSFC) project code 31850410489.

Data availability All data generated or analyzed in this study are included in this article. All alignments and trees from this study are available from TreeBASE (accession number 27520) and all sequences generated here are available from GenBank with accession numbers: MT232434–MT232437, MN833419 (ITS); MT068485–MT068489 (LSU); MT068491–MT068494, MW346047 (SSU); MT477864–MT477868 (*TEF1-α*).

Declarations

Conflict of interest The authors declare no competing interests.

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