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

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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 *Keissleriella*. 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...
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 macrocypnidia 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 cylindric pseudoparaphyses, hyaline ascosporas 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 (Höhn 1919), Lentithecium (Zhang et al. 2009b), Muri lentithecium (Wanasinghe et al. 2014), Neoo phiosphaerella (Tanaka et al. 2015), Phragmocamarosporium (Wijayawardene et al. 2015), Pleurophoma (de Gruyter et al. 2009; Croux et al. 2015), P. a ceascom a (Pho o kamsak et al. 2015), Pseudomuri lentithecium (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) Leuchtn., M. fl uvialitis 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; Croux et al. 2018) and submerged parts of plant host species (Juncus, Phragmites, Fraxinus, Alnus, and Platanus) (Kohlmeier et al. 1996; Van Ryckegem and Aptroot 2001; Su et al. 2009; Zhang et al. 2009b). Lentithecium is characterized by its immersed to semi-immersed, globose to subglobose ascocoma, a thin peridium, cellular pseudoparaphyses, short pedicellate asci and fusoid or filiform, subglobose, hyaline, brown, uni- to multi-septate ascosporas, 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 Ple spora obiones P. Crouan & H. 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 P asserini ella (Apinis and Chesters 1964; Hyde and Mouzouras 1988; Khashnobish and Shearer 1996). Various studies have shown that Ple spora 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 ascosporas than those by Crouan and Crouan (1867) indicating that there might be a second morphologically similar species. Dayarathne, E.B.G. Jones & K.D. Hyde has a worldwide distribution in temperate regions and occurs as a saprobe of Agropyron junceiforme, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019). Devadatha et al. (2020) introduced 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, Halimone portulacoides, Spartina species, on intertidal wood, bamboo, and exposed test panels of Betula pubescens and Fagus sylvatica (Kohlmeier and Kohlmeyer 1979; Jones et al. 2019).
confirm the taxonomic placement of *Lentithecium lineare* (E. Müll. ex Dennis) K.D. Hyde, J. Fourn. & Ying Zhang in *Keissleriella*, and *L. cana-shanense* Z.L. Luo, X.J. Su & K.D. Hyde, *L. carbo-nearum* 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 *Haloybissothecium*. 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 (ddH2O). 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.
| Species                          | Strain/voucher number | LSU access. | SSU access. | ITS access. | TEF1-α access. |
|---------------------------------|-----------------------|-------------|-------------|-------------|----------------|
| Bambusicola bambusae           | MFLUCC 11–0614        | JX42489     | JX42490     | JX44203     | DQ471087       |
| Bambusicola irregulispora       | MFLUCC 11–0437        | JX42236     | JX42238     | JX44202     | DQ471087       |
| Bambusicola massaarinia         | MFLUCC 11–0389        | JX42240     | JX42241     | JX44201     | DQ471087       |
| Bambusicola splendida           | MFLUCC 11–0439        | JX42242     | JX42243     | JX44200     | DQ471087       |
| Bimuria novae-zelandiae         | CBS 107.79            | AY016356    | AY016356    | –           | GU300025       |
| Byssothecium circinans          | CBS 675.92            | GU205217    | GU205217    | GU349061    | –              |
| Corynespora cassiicola          | CBS 1008.22           | GU290484    | GU290484    | –           | GU349062       |
| Corynespora caespitosa          | CBS 1069.96           | GU32286     | GU32286     | –           | GU349062       |
| Darksidea alpha                 | CBS 135650            | KU991149    | KU991149    | –           | GU349062       |
| Darksidea beta                  | CBS 135638            | KU991150    | KU991150    | –           | GU349062       |
| Darksidea delta                 | CBS 135666            | KU991160    | KU991160    | –           | GU349062       |
| Darksidea epsilon               | CBS 135658            | KU991170    | KU991170    | –           | GU349062       |
| Darksidea gamma                 | CBS 135640            | KU991180    | KU991180    | –           | GU349062       |
| Falciformispora lignatilis      | BCC 21117             | GU371826    | GU371826    | –           | GU349062       |
| Halobyssothecium bambusicola*  | MFLUCC 20–0226        | MT068486    | MT068486    | –           | GU349062       |
| Halobyssothecium cangshanense  | DLUCC 0143            | KU991139    | KU991139    | –           | GU349062       |
| Halobyssothecium carbonneanum  | CBS 144076            | MH069699    | MH069699    | –           | GU349062       |
| Halobyssothecium estuariae      | MFLUCC 19–0386        | MN598871    | MN598871    | –           | GU349062       |
| Halobyssothecium kunmingense   | KUMCC 19–0101         | KV991150    | KV991150    | –           | GU349062       |
| Halobyssothecium obiones       | MFLUCC 15–0431        | MT068491    | MT068491    | –           | GU349062       |
| Halobyssothecium phragmitis*    | MFLUCC 20–0226        | MT068486    | MT068486    | –           | GU349062       |
| Halobyssothecium unicellulare  | MD129                 | KX505374    | KX505374    | –           | GU349062       |
| Halobyssothecium versicolor*    | MFLUCC 20–0226        | MT068491    | MT068491    | –           | GU349062       |
| Halobyssothecium voraginesporum| CBS H-22560           | NG063065    | NG063065    | –           | GU349062       |

*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 an asterisk (*) and the ex-type strains are indicated in bold.
| Species                        | Strain/voucher number | LSU accession number | SSU accession number | ITS accession number | TEF1-α accession number |
|-------------------------------|-----------------------|----------------------|----------------------|----------------------|-------------------------|
| *Karstenula rhodostoma*       | CBS 690.94            | GU301821             | GU296154             | –                    | GU349067                |
| *Katumotoa bambusicola*       | KT 1517a              | AB524595             | AB524454             | LC014560             | AB539108                |
| *Keissleriella bambusicola*   | KUMCC 18–0122         | MK995880             | MK995878             | MK959881             | MN213156                |
| *Keissleriella brevisaca*     | KT 581                | AB807587             | AB797297             | AB811454             | AB808566                |
| *Keissleriella brevisaca*     | 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             | MIB57391             | GU349043                |
| *Keissleriella culmiifida*    | KT2308                | AB807591             | AB797301             | LC014561             | AB808570                |
| *Keissleriella culmiifida*    | 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                |
| *Keissleriellainearis*        | IFRD2008              | FJ795435             | FJ795478             | –                    | –                       |
| *Keissleriellainearis*        | MFLUCC 19–0410        | MN598873             | MN598870             | MN598892             | MN607978                |
| *Keissleriellainearis*        | MFLUCC 20–0224        | MT068487             | MT068492             | MT232436             | MT477866                |
| *Keissleriella phragmiticola* | CPC 33249             | MT223903             | –                    | MT223808             | MT23715                 |
| *Keissleriella phragmiticola* | MFLUCC 17–0779        | MG829014             | –                    | MG829004             | –                       |
| *Keissleriella poogena*       | CBS 136767            | KJ869170             | –                    | KJ869112             | –                       |
| *Keissleriella quadriseptata* | KT 2292               | 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             | –                       |
| 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 grootfooteinensis* | 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 fluvatilie* | CBS 122367            | FJ795451                | FJ795493                | –                       | GU349074    |
| *Lentithecium fluvatilie* | CBS 123090            | FJ795450                | FJ795492                | –                       | –            |
| *Lentithecium pseudoclioninum* | KTI111               | AB807544                | AB797254                | AB809632                | AB808520    |
| *Longipedicellata aprootii* | MFLUCC 10–0297        | KU238894                | KU238895                | KU238893                | KU238892    |
| *Macrodiptodiopsis desmazieri* | CBS 140062           | NG_058182               |                         |–                          | –            |
| *Massarina cistí*        | 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 ramuncučíola* | JK530.4B             | GU479794                | GU479760                | –                       | –            |
| *Multiseptospora thailandica* | MFLUCC 11–0183        | NG_059554               | KP753955                | NR_140808               | KU705657    |
| *Murilentithecium clioninum* | MFLUCC 14–0561        | KM408758                | KM408760                | KM408756                | KM454444    |
| *Murilentithecium clioninum* | MFLUCC 14–0562        | KM408759                | KM408761                | KM408757                | KM454445    |
| *Murilentithecium lonicerae* | MFLUCC 18–0675        | MK214373                | MK214376                | MK214370                | MK214379    |
| *Murilentithecium rosae* | MFLUCC 15–0044        | MG829030                | MG829137                | MG829820                | –            |
| *Neoosphosphaerella sasicola* | KT1706               | AB524599                | AB524458                | LC014577                | AB539111    |
| *Palmascoma gregariuscomum* | MFLUCC 11–0175        | KP744495                | KP753958                | KP744452                | –            |
| *Parabambusicola thysonaenae* | KUMCC 18–0147        | NG_066345               | NG_067681               | NR_164044               | KM208920    |
| *Parabambusicola thysonaenae* | KUMCC 18–0148        | MK098198                | MK098202                | MK098193                | MK098211    |
| *Paraconiothyrium brasiliense* | CBS 100299         | JX496124                | AY642523                | JX496111                | –            |
| *Paraphaeosphaeria michottii* | MFLUCC 13–0349        | KJ939282                | KJ939285                | KJ393279                | –            |
| *Pheodoliths winteri*     | CBS 127788            | EU754173                | EU754074                | –                       | GU349083    |
| *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 |
| Pleomoniectys descalsii | CBS 142298 | KY853522 | – | – | – |
| Pleurophoma ossicola | CBS139905 | KR476769 | – | – | – |
| Pleurophoma ossicola | CPC24985 | KR476770 | – | – | – |
| 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 |
| Pseudoxylosemyces 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 halworthensis | MFLUCC 18–0110 | MG829075 | – | – | – |
| Setoseptoria magniarundinacea | KTI174 | 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 | KR909310 | KR909319 |
| Stagonospora macropycnidia | CBS 114202 | GU301824 | GU296157 | – | 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 | KH168 | AB521743 | AB521726 | LC014598 | AB808561 |
| Tingoldiago graminicola | KT891 | AB521744 | AB521727 | LC014600 | AB808563 |
| Tingoldiago hydei | MFLUCC 19–0499 | MN857177 | – | MN857181 | – |
| Towyspora aestuarii | 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 1,000 generations and all other parameters were left as default. The split frequencies was below 0.01. Trees were sampled every 4,000,000 generations at which point the 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 (BYP) analyses were congruent.

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 (Φw < 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 cassicola (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 (BYP) 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. gangshanense*, *L. carbonneanum*, *L. kunmingense*, *L. unicellulare*, *L. voraginesporum*) clustered with *Halobyssothecium*. Therefore, these five *Lentithecium* species were 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. flaviatile*, 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. **Conidiomatal wall** dark brown to black, centrally located. **Conidiomatal wall** dark brown to black, centrally located. **Ascomata** immersed, semi-immersed, erumpent at maturity, solitary or aggregated, unilocular, 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, asceptate, globose to subglobose, ellipsoidal, cylindrical to subcylindrical. **Conidia** spherical to globose, subglobose, ovate to obovate, ellipsoidal, clavate to subclavate, lageniform, hyaline, asceptate, 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; Caivari 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. elioninum* and *L. pseudocloinionum*, while *L. cangshanense*, *L. carbonneanum*, *L. kunmingense*, *L. unicellulare*, and *L. voragineporum* 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 \((x = 415.4 \times 238.6, n = 10)\), pyrnicidal, immersed, erumpent at maturity, solitary or aggregated, globose, unilocular, 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, asceptate, globose to subglobose, ellipsoidal, cylindrical to subcylindrical. **Conidia** spherical to globose, subglobose, ovate to obovate, ellipsoidal, clavate to subclavate, lageniform, hyaline, asceptate, 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:** Several species of freshwater fungi growing on submerged bamboo have been recorded, e.g. *Acrodictys liputii* L.
kunmingense has wider conidiomata (210 μm vs. 80 μm) and a n d Mycol Progress (2021) 20:701–720
et al. 2004; Zhang et al. 2017). μ 80 K.D. Hyde (Cai et al. 2002a, b, 2003, 2004, 2005, 2006; H o Leung, 2 Saccardoella minuta Zhang & K.D. Hyde, and C. smithii (CABI5649b) (Corynesporascaceae). Bar = 0.04 estimated number of nucleotide substitutions per site per branch.

H. kunmingense

Halobyssothecium bambusicola

Halobyssothecium phragmitis

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 (x̅ = 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 (x̅ = 13.7–4.1 μm, n = 50), cylindrical, fusoid-ellipsoidal, straight or slightly curved, hyaline, aseptate to 1–2-septate, unilocular, 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: S W E D E N, Got land, 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.
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) (Quaedvlvieg 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) (Wanasieghe 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. 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. a Colony on MEA (obverse, reverse). Scale bars: a = 200 mm; b = 1 mm; c–e = 500 μm; f = 50 μm; g–t = 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 (x̄ = 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 (x̄ = 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,
filiform, branched and anastomosing above the asci. *Asci* 137–173 × 17–12 μm ($\bar{x} = 153.4 \times 14.7$ μm, $n = 20$), 8-spored, clavate to subcylindrical, short pedicellate with an ocular chamber. *Ascospores* 18–41 × 6–12 μm ($\bar{x} = 27.4 \times 8.6$, $n = 20$), 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 (Kohlmeier and Kohlmeier 1979; Calado et al. 2015).
Phylogenetic analysis shows that *Halobyssothecium versicolor* clustered within Lentitheciaeaceae 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.

**Notes:** *K. versicolor* was recovered from Phragmites sp. Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream. Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.

**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.

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

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

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

**Notes:** *K. carbonneanum* was recovered from decaying culm of *Phragmites* sp. Material examined: FRANCE, Haute-Garonne, Carbonne, SW of route du Lançon, artificial lake in a gravel pit, on submerged decorticated branch of *Populus*.

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

**Notes:** *K. kunmingense* was recovered from decaying culm of *Phragmites* sp. Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream. Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.

**Notes:** *K. kunmingense* was recovered from decaying culm of *Phragmites* sp. Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream. Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.

**Notes:** *K. cangshanense* was recovered from decaying culm of *Phragmites* sp. Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream. Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.

**Notes:** *K. versicolor* was recovered from Phragmites sp. Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream. Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.

**Notes:** *K. kunmingense* was recovered from decaying culm of *Phragmites* sp. Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream. Notes: Holotype HKAS 84021. LSU and SSU sequence data are available.
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.

Haloscyphus voragensporum (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 voragensporum 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 mangrove, stem inside the mangrove stand (Hyde et al. 2016)

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

Notes for Lentithecaceae

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.

Pseudomurillentithecium camporeii Mapook & K.D. Hyde, Fungal Diversity 100: 69 (2020)

In the phylogenetic analysis (Fig. 1), Pseudomurillentithecium camporeii does not cluster within Lentithecaceae but forms a weakly supported clade basal to Latoruaceae, Longipedicellataceae, and Trematosphaeriaceae. Broader 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. fluvialis (≡ Massarina fluvialis), 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, Metaphoma, Peripherostoma, Phaeosphaeria, Pleospora, Rpogophagus, Sphaeria, Sphaeropsis), was transferred by Tanaka et al. (2015) to Setosepertoria. Setosepertoria arundinacea clustered with other Setosepertoria species in the phylogenetic analysis (Fig. 1).

Multi-locus phylogenetic analysis shows that the three Lentithecium species, L. aquaticum, L. lineare and L. rarum (Kohl., Volkm.-Kohl. & O.E. Erikss.) Suetrong, Sakay, E.B.G. Jones, Kohlm. & Volkm.-Kohl. 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 Setosepertoria and the asexual morph Stagonospora macrospynedia, 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 Lentitheciaceae 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 Lentitheciu species possess hyaline ascospores with mucilaginous sheaths.

**Key to Halobyssothecium species**

1. Asexual morph...............................................................2
2. Sexual morph...............................................................5

1* Conidiomata < 350 μm long........................................4
1* Conidiomata > 350 μm long........................................2
2* Conidia, ellipsoidal to cylindrical..................H. phragmitis
3 Conidiomata > 350 μm long............................H. bambusicola
3* Conidiomata < 350 μm long..........................H. carbonneanum
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............................H. voraginesporum
6 Asci > 100 μm high...................................................7
6* Asci < 100 μm high........................H. estuariae
7 Asci 38–50 × 8–10 μm..............................................H. voraginesporum
7* Asci 65–78 × 11–13 μm.................................H. kunmingense
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

**References**

Apinis AE, Chesters CGC (1964) Ascomycetes of some salt marshes and sand dunes. Trans Br Mycol Soc 47:419–435. https://doi.org/10.1016/s0007-1536(64)80014-0
Arzanlou M, Groenewald JZ, Gams W et al (2007) Phylogenetic and morphotaxonomic revision of Ramichloridium and allied genera. Stud Mycol 58:57–93. https://doi.org/10.3114/sim.2007.58.03
Barr ME (1987) Prodomus to Class Leculoascomycetes. Amherst.
University of Massachusetts, Massachusetts

Boonmee S, Zhang Y, Chomnunti P et al (2011) Revision of lignicolous Tubeufiaceae based on morphological reexamination and phylo-
genetic analysis. Fungal Divers 51:63–102. https://doi.org/10.1007/s13225-011-0147-4

Bruen TC, Philippe H, Bryant D (2006) A simple and robust statistical test for detecting the presence of recombination. Genetics 172:2665–2681. https://doi.org/10.1534/genetics.105.048975

Cai L, Li, J., Hyde KD (2006) Variation between freshwater and terres-
trial fungal communities on decaying bamboo culms. Antonie van Leeuwenhoek. Int J Gen Mol Microbiol 89:293–301. https://doi.org/10.1007/s10482-005-9030-1

Cai L, Lyumong P, Zhang K, Hyde KD (2002a) New species of Annullatascus and Saccardoellia from the Philippines. Mycota 24:255–263

Cai L, Zhang K, Hyde KD (2005) Ascosynnmania aquatica gen. et sp.
nov., a freshwater fungus collected from China and its micryclic conidiation. Fungal Divers 18:1–8. https://doi.org/10.2307/1468376

Cai L, Zhang K, McKenzie EHC et al (2002b) Acroclydia lipput sp. nov.
and Digitodesmium bambusicola sp. nov. from bamboo submerged in the Liput River in the Philippines. Mycologia 97:525–532. https://doi.org/10.101177/0029-5035/2002/0075-0052

Cai L, Zhang K, McKenzie EHC, Hyde KD (2003) Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. Fungal Divers 13–12.

Cai L, Zhang K, McKenzie EHC, Hyde KD (2004) Linocarpon bambusicola sp. nov. and Dictyochaeta curvispora sp. nov. from bamboo submerged in freshwater. Nova Hedwigia 78:439–445. https://doi.org/10.1007/s10029-005-0397-9

Calado MDL, Carvalho L, Pang KL, Barata M (2015) Diversity and ecological characterization of sporulating higher filamentous marine fungi associated with Spartina maritima (Curtis) Fernald in two Portuguese salt marshes. Microb Ecol 70:612–633. https://doi.org/10.1007/s00248-015-0600-0

Cavaliere AR (1968) Marine fungi of Iceland: A preliminary account of Calfiellopsis and Saccardoellia from the Philippines. Mycota 84:255–263

Crouan PL, Crouan HM (1867) Florule du Finistère: Contenant les de-
scriptions de 360 espèces nouvelles de sporagames, de nombreuses observations et une synonymie des plantes cellulaires et vasculaires, et la table des principaux organismes. Société d'histoire naturelle de Brest et Morlaix. 784 p. [Brest, 1867. L. Lavoisier]

Crous PW, Wingfield MJ, Guarro J et al (2015) Fungal Planet description 84:255–267. Mycol Progress (2021) 20:701–720

Dayarathne MC, Wanasinghe DN, Jones EBG et al (2018) A novel ma-
rine genus, Halobythoscytium (Lenthiaceae) and epitypification of Halobythoscytium obiones comb. nov. Mycol Prog 17:1161–1171. https://doi.org/10.1007/s11557-018-1432-3

de Gruyter J, Aveskamp MM, Woudenberg JHC et al (2009) Molecular phylogeny of Phoma and allied anamorph genera: Towards a reclas-
sification of the Phoma complex. Mycol Res 113:508–519. https://doi.org/10.1016/j.mycres.2009.01.002

Dennis RWG (1964) The Fungi of the Isle of Rhum. Kew Bull 19:77–127. https://doi.org/10.2307/4108295

Devadatha B, Calabon MS, Abeywickrama PD et al (2020) Molecular data reveals a new holomorphic marine fungus, Halobythoscytium estuariae, and the asexual morph of Keissleriella phragmiticola. Mycologia 11:167–183. https://doi.org/10.1080/21501203.2019.1700025

Dong W, Wang B, Hyde KD et al (2020) Freshwater Dothideomycetes. Fungal Divers 105:319–575. https://doi.org/10.1007/s13225-020-00463-5

Glez-Peña D, Gómez-Blanco D, Reiboaro-Jato M et al (2010) ALTER: Program-oriented conversion of DNA and protein alignments. Nucleic Acids Res 38:W14–W18. https://doi.org/10.1093/nar/gkq321

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41(1999):95–98. https://doi.org/10.14601/Phytopathol_Meditter-14998u1.29

Hashimoto A, Matsumura M, Hirayama K, Tanaka K (2017) Revision of Lophiostomataceae (Pleosporales, Dothideomycetes): Aquasubmersaceae, Cryptocoryneaceae, and Hermatomyctaceae fam. nov. Persoonia Mol Phylogeny Evol Fungi 39:51–73. https://doi.org/10.3767/persoonia.2017.39.03

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42:182–192. https://doi.org/10.1093/sysbio/42.2.182

Hirayama K, Tanaka K, Raja HA et al (2010) A molecular phylogenetic assessment of Massarinae ingoldiana sensu lato. Mycologia 102: 729–746. https://doi.org/10.3852/09-230

Ho WH, Hyde KD, Hodgkiiss IJ (2004) Cataractispora receptaculorum, a new freshwater ascomycete from Hong Kong. Mycologia 96:411–417. https://doi.org/10.1080/15572536.2005.11832986

Höhnel F (1919) Fragmente zur Mykologie. XXIII Mitteilung, Nr. 1154 bis 1188. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt I 128:535–625

Hongsanan S, Hyde KD, Phookamsak R et al (2020) Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. Mycosphere 11:1553–2107. https://doi.org/10.5943/mycosphere/11/1/13

Huson DH (1998) SplitsTree: Analysing and visualizing evolutionary data. Bioinformatics 14:68–73. https://doi.org/10.1093/bioinformatics/14.1.68

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267

Hyde KD, Chetana KWT, Jayawardena RS et al (2020a) The rise of mycology in Asia. Sci Asia 46:1–11. https://doi.org/10.2306/scienciaasia1513-1874.2020.S001

Hyde KD, Dong Y, Phookamsak R et al (2020b) Fungal diversity notes 1151–1276; taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Divers 100:5–277. https://doi.org/10.1007/s13225-020-00439-5

Hyde KD, Hongsanan S, Jeewon R et al (2016) Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers 80:1–270. https://doi.org/10.1007/s13225-016-0373-x

Hyde KD, Jeewon R, Chen YJ et al (2020c) The numbers of fungi: is the descriptive curve flattening? Fungal Divers 103:219–271. https://doi.org/10.1007/s13225-020-00458-2

Hyde KD, Jones EBG, Liu JK et al (2013) Families of Dothideomycetes. Fungal Divers 63:1–313. https://doi.org/10.1007/s13225-013-0263-4

Hyde KD, Mouzouras R (1988) Passeriniella savoryiopsis sp. nov., a new ascomycete from intertidal mangrove wood. Trans Br Mycol Soc 91:179–185. https://doi.org/10.1016/s0007-1536(88)80024-x

Hyde KD, Norphanshoun C, Chen J et al (2018) Thailand’s amazing diversity: up to 96% of fungi in northern Thailand may be novel.

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Zhang Y, Schoch CL, Fournier J et al (2009a) Multi-locus phylogeny of Pleosporales: A taxonomic, ecological and evolutionary re-evaluation. Stud Mycol 64:85–102.S5. https://doi.org/10.3114/sim.2009.64.04
Zhang Y, Wang HK, Fournier J et al (2009b) Towards a phylogenetic clarification of Lophiosoma/Massarina and morphologically similar genera in the Pleosporales. Fungal Divers 38:225–251

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