



# Lignicolous Freshwater Fungi from Plateau Lakes in China (I): Morphological and Phylogenetic Analyses Reveal Eight Species of Lentitheciaceae, Including New Genus, New Species and New Records

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Abstract: During the investigation of lignicolous freshwater fungi in plateau lakes in Yunnan Province,

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China, eight Lentitheciaceae species were collected from five lakes viz. Luguhu, Qiluhu, Xingyunhu, Cibihu, and Xihu lake. Based on morphological characters and phylogenetic analysis of combined ITS, LSU, SSU, and *tef 1-α* sequence data, a new genus *Paralentithecium*, two new species (*Paralentithecium suae*, and *Setoseptoria suae*), three new records (*Halobyssothecium phragmitis*, *H. unicellulare*, and *Lentithecium yunnanensis*) and three known species viz. *Halobyssothecium aquifusiforme*, *Lentithecium pseudoclioninum*, and *Setoseptoria bambusae* are reported.

Keywords: three new taxa; lignicolous freshwater fungi; plateau lake; phylogeny; taxonomy

## 1. Introduction

Lignicolous freshwater fungi are those fungi that grow on submerged woody debris in freshwater habitats, including lentic (e.g., lakes, ponds, swamps, and pools), lotic (e.g., rivers, streams, creeks, brooks), and other habitats (e.g., cooling tower, tree holes) [1–3]. They play an important role in the material and energy cycle of freshwater ecosystems [4–8]. Lignicolous freshwater fungi are a highly diverse group, with the majority belonging to Dothideomycetes and Sordariomycetes (Ascomycota), and a few species in Eurotiomycetes and Orbiliomycetes [3,9–12]. Lignicolous freshwater fungi have been investigated worldwide, but mainly in lotic habitats of tropical, subtropical, and temperate regions [10,13–16], with a few from lentic habitats [17]. Those fungi in lentic habitats are poorly studied. This study collects submerged decaying wood from plateau lakes in Yunnan, China, to investigate the species diversity of lignicolous freshwater fungi in the lakes.

Yunnan Province is in the southwest of China, it is a low-latitude, high-altitude inland province, and is one of the biodiversity hotspots in the Yunnan–Guizhou Plateau [18]. Yunnan has three climatic zones, tropical (southwest, south, and southeast borders), subtropical (west, middle, and east), and temperate regions (high-elevation area in the northwest) [19]. The special geographical location and climatic conditions endow Yunnan with abundant natural resources. There are plateau cold-resistant biomes in the west and tropical biomes in the south and southwest. Plateau lakes are an important part of terrestrial lakes and an



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). important part of regional water cycling. They are distributed at higher altitudes, have a large number, and have a wide basin area. They are sensitive to climate change and have made outstanding contributions to coping with global climate change and shaping regional biodiversity patterns [20,21]. There have been several biological studies conducted on plateau lakes in Yunnan, such as waterbirds [22,23], invasive fish [24], water plants [25–27], and microorganisms [2,17,28–32]. Yunnan has abundant lignicolous freshwater fungi resources, and from 1986 to 2021, a total of 281 lignicolous freshwater fungi taxa have been reported. These species were mainly reported in lotic habitats (rivers, streams), with only 53 (19%) species from plateau lakes [12].

Species of Lentitheciaceae from freshwater habitats are mainly in *Halobysothecium*, *Lentithecium*, *Setoseptoria*, and *Tingoldiago* [14,15,33–37]. The family was introduced by Zhang et al. [35] to accommodate those lentitheciaceous taxa that have 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 are stagonospora-like or dendrophoma-like [14,38–40]. Currently, more than 100 species are reported in Lentitheciaceae. The last treatment of Lentitheciaceae was provided by Wijayawardene et al. [41] with acceptance of 18 genera: *Crassoascoma* [42], *Darksidea* [43], *Groenewaldia* [44], *Halobysothecium* [45], *Katumotoa* [46], *Keissleriella* [47], *Lentithecium* [35], *Murilentithecium* [40], *Neolentithecia* [48], *Neoophiosphaerella* [34], *Phragmocamarosporium* [49], *Pleurophoma* [50,51], *Poaceascoma* [52], *Pseudokeissleriella* [53], *Pseudomurilentithecium* [54], *Setoseptoria* [37], *Tingoldiago* [55], and *Towyspora* [56].

We are investigating the diversity of lignicolous freshwater fungi from plateau lakes in Yunnan Province, and 13 collections of lentitheciaceous-like taxa were obtained. Based on morphological and multigene phylogenetic analysis, a new genus *Paralentithecium* is introduced to accommodate *P. aquaticum*, and a new taxon *P. suae*, *Setoseptoria suae* sp. nov. and new records *Halobyssothecium* and *Lentithecium* are described and illustrated. The sexual morph of *Halobyssothecium phragmitis* is also introduced.

## 2. Materials and Methods

## 2.1. Samples Collection

The fresh samples were submerged in lake water with a diameter of less than 2 cm and a length of more than 20 cm, including tree trunks, branches, twigs, and rotten branches of grasses. The specimens in this study were collected from Dali City (Cibihu, Xihu, and Erhai Lakes), Lijiang City (Luguhu Lake), and Yuxi City (Xingyunhu and Qiluhu Lakes) in Yunnan. The collected samples were placed in plastic ziplock bags and were taken back to the laboratory for processing.

#### 2.2. Sample Processing and Cultivation

The samples were brought to the laboratory in ziplock bags to avoid moisture loss and then trimmed to 15 cm in length with pruning scissors. Each sample with a label number that is attached to the end of the sample with a thumbtack (Figure 1a). In addition, plastic boxes with the size of  $24 \text{ cm} \times 16 \text{ cm} \times 6 \text{ cm}$  were prepared. First, rinse the inside of the plastic box with sterile water, then wipe the entire plastic box with 75% alcohol. After drying, put two layers of sterilized tissues on the bottom of the box, lay three sterilized straws on the tissues to prevent the sample from directly touching the sterilized tissues, and add an appropriate amount of sterile water (the water soaks sterile tissues, but accumulates at the bottom), and then arrange the processed samples horizontally on the straw, ten samples in each plastic box, and label the boxes in obvious places (Figure 1b,c). The samples were placed on a culture rack and incubated at room temperature for one week (Figure 1d).



**Figure 1.** (a) Sample with a label (arrow indicates sample number); (b) Samples in the plastic box; (c) Plastic box with labels (arrows indicate labels documenting detailed sampling sites and sample order); (d) The samples were incubated on the culture rack.

## 2.3. Morphological Studies and Isolation

Macromorphological characters of samples were observed using Optec SZ 760 compound stereomicroscope (Chongqing Optec Instrument Co., Ltd., Chongqing, China). The temporarily prepared microscope slide was placed under a Nikon ECLIPSE Ni-U compound stereomicroscope (Nikon, Tokyo, Japan) for observation and microscopic morphological photography. The morphology of colonies on native substrates was photographed with a Nikon SMZ1000 stereo-zoom microscope. Indian ink was used to reveal the presence of a gelatinous sheath around the ascospores or conidia. The measurements of photomicrographs were obtained using Tarosoft (R) Image Frame Work version 0.9.7. Images were edited with Adobe Photoshop CS5 Extended version 12.0.0.0 software (Adobe Systems, San Jose, CA, USA).

Single spore isolations were performed as follows: the tip of a sterile toothpick dipped in sterile water was used to capture the conidia of the target colony directly from the specimen; the conidia were then streaked on the surface of water agar (WA, Composition: Agar 20 g/L, Chloramphenicol 0.1 g/L) or potato dextrose agar (PDA, CM123, Composition: Potato infusion 5.0 g/L, Dextrose 20 g/L, Agar 20 g/L, Chloramphenicol 0.1 g/L, from Beijing Bridge Technology Co., Ltd., Beijing, China) and incubated at room temperature overnight. The single germinated conidia were transferred to fresh PDA medium and incubated at room temperature. A few of the remaining germinated spores in the media plate were separated along with agar using a needle and transferred onto water-mounted glass slides for photographs to capture the germination position of the germ tubes.

After finalizing the observation and isolation, the specimens were dried under natural light, wrapped in absorbent paper, and placed in a ziplock bag with mothballs. Specimens were deposited in the herbarium of Kunming Institute of Botany, Academia Sinica (KUN-HKAS). The living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC) and Kunming Institute of Botany Culture Collection (KUNCC). MycoBank numbers are registered in the MycoBank database (https://www.mycobank.org/Registration%20home (accessed on 4 August 2023)). Entries will be added to the Greater Mekong Subregion database [57].

## 2.4. DNA Extraction, PCR Amplification and Sequencing

DNA extraction, PCR amplification, sequencing, and phylogenetic analysis were done following the methods of Dissanayake et al. [58]. Mycelia for DNA extraction from each isolate was grown on PDA for 3–4 weeks at room temperature. Total genomic DNA was extracted from 100 to 300 mg axenic mycelium via scraping from the edges of the growing culture using a sterile scalpel and transferred to a 1.5 mL microcentrifuge tube

using sterilized inoculum needles. The mycelium was ground to a fine powder with liquid nitrogen or quartz sand to break the cells for DNA extraction. When the cultures could not be maintained with some of the collected samples, fruiting structures (20–50 mg) were removed from the natural substrate using a sterile scalpel placed on sterile paper and then transferred to a 1.5 mL microcentrifuge tube. DNA was extracted with the TreliefTM Plant Genomic DNA Kit (TSP101) following the manufacturer's guidelines.

Four gene regions, ITS, LSU, SSU, and *tef* 1- $\alpha$  were amplified using ITS5/ITS4 [59], LR0R/LR5 [60], NS1/NS4 [59], and EF1-983F/EF1-2218R [61] primer pairs, respectively. The PCR mixture contained 12.5 µL of 2× Power Taq PCR MasterMix (a premix and ready-to-use solution, including 0.1 Units/µL Taq DNA Polymerase, 500 µm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mm Tris–HCl pH 8.3, 100 Mm KCl, 3 mM MgCl<sub>2</sub>, stabilizer, and enhancer), 1 µL of each primer including forwarding primer and reverse primer (10 µm), 1 µL template DNA extract and 9.5 µL deionized water. The PCR thermal cycling conditions of ITS and SSU were as follows: 94 °C for s min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 50 s, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min; LSU and *tef* 1- $\alpha$  were as follows: 94 °C for 30 s, elongation at 72 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 50 s, elongation at 72 °C for 3 min, followed by 35 cycles of denaturation at 72 °C for 1 min, and a final extension at 72 °C for 10 min; LSU and *tef* 1- $\alpha$  were as follows: 94 °C for 3 min, followed by 35 cycles of denaturation at 72 °C for 10 min. PCR products were then purified using minicolumns, purification resin, and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). The sequences were carried out at Beijing Tsingke Biological Engineering Technology and Services Co., Ltd. (Beijing, China).

### 2.5. Phylogenetic Analyses

ITS, LSU, SSU, and *tef* 1-α sequence data used for phylogenetic analysis are selected based on the preliminary identification results and the related publications [14,15]. The sequences were aligned using MAFFT online service: multiple alignment program MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/index.html (accessed on 30 August 2023)) [62], and sequence trimming was performed with trimAl v1.2 for Windows, and all parameters were set by default (http://trimal.cgenomics.org for specific operation steps (accessed on 30 August 2023)) [63]. The sequence dataset was combined using SquenceMatrix v.1.7.8 [64]. FASTA alignment formats were changed to PHYLIP and NEXUS formats by the website: ALignment Transformation EnviRonment (ALTER) (http://sing.ei.uvigo.es/ALTER/ (accessed on 30 August 2023)) [65]. The alignments and phylogenetic trees were deposited in TreeBASE (http://www.treebase.org/ (accessed on 31 August 2023), accession number: 30729-30733).

The single-gene phylogenetic tree was obtained based on maximum likelihood (ML) only, and the multigene phylogenetic tree was obtained based on maximum likelihood (ML) and Bayesian criterion (BI). ML tree and BI tree were run on the CIPRES Science Gateway portal [66-69]. MrModeltest v. 2.3 [70] was run under the AIC (Akaike Information Criterion) implemented in PAUP v. 4.0b10. to evaluate the best-fit model in both ML and BI analyses. ML analyses for the datasets were performed with RAxML-HPC2 on XSEDE v. 8.2.10 [66] using the determined best-fit substitution model with 1000 bootstrap iterations. The BI analysis was computed with MrBayes v. 3.2.6 [69]. Six simultaneous Markov chains were run with a suitable number of generations, and trees were sampled every 100th generation, ending the run automatically when the standard deviation of split frequencies dropped below 0.01. Alignment gaps were treated as missing characters in the analysis of the combined data set, where they will occur in relatively conserved regions. Trees were inferred using the heuristic search option with 1000 random sequence additions, with maxtrees set at 1000. Phylogenetic trees were visualized using FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 31 August 2023)), editing and typesetting using Adobe Illustrator (AI) (Adobe Systems Inc., San Jose, CA, USA). The new sequences were submitted in GenBank, and the strain information used in this paper is provided in Table 1.

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Species	Strain/Voucher Number	GenBank Accession Number				
		LSU	SSU	ITS	tef 1-α	
Bambusicola bambusae	MFLUCC 11–0614 <sup>T</sup>	JX442035	JX442039	NR_121546	KP761722	
Bambusicola irregulispora	MFLUCC 11–0437 <sup>T</sup>	JX442036	JX442040	NR_121547	KP761723	
Bambusicola massarinia	MFLUCC 11–0389 <sup>T</sup>	JX442037	JX442041	JX442033	KP761725	
Bambusicola splendida	MFLUCC 11–0439 <sup>T</sup>	JX442038	JX442042	NR121549	KP761726	
Crassoascoma potentillae	UESTCC 21.0010	OK161254	OK161233	OK161237	OK181165	
Crassoascoma potentillae	UESTCC 21.0011	OK161255	OK161234	OK161238	OK181166	
Crassoascoma potentillae	UESTCC 21.0012	OK161256	OK161235	OK161239	OK181167	
Crassoascoma potentillae	CGMCC 3.20483 <sup>T</sup>	OK161257	OK161236	OK161240	OK181168	
Darksidea alpha	CBS 135650 T	KP184019	KP184049	NR_137619	KP184166	
Darksidea beta	CBS 135637 <sup>T</sup>	KP184023	KP184074	NR_137957	KP184189	
Darksidea delta	CBS 135638 <sup>T</sup>	KP184024	KP184069	NR_137075	KP184184	
Darksidea epsilon	CBS 135658 <sup>T</sup>	KP184029	KP184070	NR_137959	KP184186	
Darksidea gamma	CBS 135634 <sup>T</sup>	KP184031	KP184073	NR_137587	KP184188	
Darksidea zeta	CBS 135640 <sup>T</sup>	KP184013	KP184071	NR_137958	KP184191	
Halobyssothecium aquifusiforme	GZCC 20–0481 <sup>T</sup>	OP377925	OP378010	OP377825	OP473005	
Halobyssothecium aquifusiforme	MFLUCC 19–0305	OP377929	OP378014	OP377829	OP473008	
Halobyssothecium aquifusionme Halobyssothecium aquifusiforme	KUNCC 22–12665	OR335346	OR335329	OR335289	OR367662	
Halobyssothecium bambusicola	MFLUCC 20–0226 <sup>T</sup>	MT068489	MT068494	MN833419	MT477868	
Halobyssothecium cangshanense	DLUCC 0143 T	KU991149	KU991150	-	-	
Halobyssothecium cangshanense Halobyssothecium caohaiense	GZCC 19–0482 <sup>T</sup>	MW133831	MW134611	OP377841	OP473019	
Halobyssothecium carbonneanum	CBS 144076 <sup>T</sup>	MH069699	10100104011	MH062991	01475017	
Halobyssothecium caroonneunum Halobyssothecium estuariae	MFLUCC 19–0386 <sup>T</sup>	MN598871	_ MN598868	MN598890	_ MN597050	
Halobyssothecium estuariae	MFLUCC 19–0387 <sup>T</sup>	MN598872	MN598869	MN598891	MN597050	
Halobyssothecium kunmingense	KUMCC 19–0101 <sup>T</sup>	MN913732	MT864313	MT627715	MT954408	
Halobyssothecium kunningense Halobyssothecium obiones	20AV2566	WIIN913732	W11004313	KX263862	WI1934406	
Halobyssothecium obiones	20AV 2385	-	-	KX263864	-	
Halobyssothecium obiones	MFLUCC 15–0381 <sup>T</sup>	_ MH376744	_ MH376745	MH377060	_ MH376746	
Halobyssothecium phragmitis	MFLUCC 15–0581 MFLUCC 20–0223 <sup>T</sup>	MT068486	MT068491	MT232435	MT477865	
Halobyssothecium phragmitis	MFLUCC 20–0225 MFLUCC 20–0225	MT068488	MT068493	MT232435 MT232437	MT477865 MT477867	
Halobyssothecium phragmitis	HKAS 127181	OR506189	OR506192	OR506177	OR513794	
Halobyssothecium thailandica	MFLUCC 21–0062 <sup>T</sup>	MZ433248	MZ429435	MZ429434	UK515/94	
Halobyssothecium unicellulare	MD129	KX505375	KX505373	-	-	
Halobyssothecium unicellulare	KUNCC 22–12413	OR335347	OR335330	OR335290	_	
Halobyssothecium unicellulare	MD6004 <sup>T</sup>	KX505376	KX505374	-	_	
Halobyssothecium uncentume Halobyssothecium versicolor	MFLUCC 20–0222 <sup>T</sup>	MT068485	MW346047	MT232434	MT477864	
Halobyssothecium voraginesporum	CBS H-22560 <sup>T</sup>	KX499520	KX499519	WI1252454	111477004	
Kalmusia scabrispora	KT2202	AB524594	AB524453	_ LC014576		
Karstenula rhodostoma	CBS 690.94	GU301821	GU296154	LC014570	GU349067	
Katumotoa bambusicola	KT 1517a <sup>T</sup>	AB524595	AB524454	LC014560	AB539108	
Keissleriella bambusicola	KUMCC 18–0122 <sup>T</sup>	MK995880	MK995878	MK995881	MN213156	
Keissleriella breviasca	KUMCC 18-0122 KT 581	AB807587	AB797297	AB811454	AB808566	
Keissleriella breviasca	KT 649 <sup>T</sup>	AB807588	AB797297	AB811454 AB811455	AB808567	
Keissleriella camporesiana	MFLUCC 15–0029 <sup>T</sup>	MN401741	MN401743	MN401745	MN397907	
Keissleriella camporesii	MFLUCC 15-0029 MFLUCC 15-0117 <sup>T</sup>	MN252886	MN252907	MN252879	1011 (0)// )0/	
Keissleriella caraganae	KUMCC 18–0117	MK359439	MK359444	MK359434		
Keissleriella cirsii	MFLUCC 16–0454 <sup>T</sup>					
Keissleriella cladophila	CBS 104.55 <sup>T</sup>	KY497780 GU301822	KY497782 CU296155	KY497783 MH857391	KY497786	
			GU296155	MH857391	GU349043	
Keissleriella culmifida Keissleriella culmifida	KT2308 KT2642	AB807591 AB807592	AB797301	LC014561	AB808570	
Keissleriella culmifida Keissleriella daetulidicola	MFLUCC 13–0866 <sup>T</sup>		AB797302 KT315505	LC014562	AB808571 KT315507	
Keissleriella dactylidicola	MFLUCC 13–0866 <sup>T</sup> MFLUCC 13–0751 <sup>T</sup>	KT315506		- VD107667	KT315507 KP197669	
Keissleriella dactylidis		KP197668	KP197666	KP197667	NT 17/009	
Keissleriella genistae Keissleriella glagospora	CBS 113798 KT829	GU205222	GU205242	– L C014542	-	
Keissleriella gloeospora Keissleriella linearis	IFRD2008	AB807589 FJ795435	AB797299 FJ795478	LC014563	AB808568	
Keissierieitti litteuris	II-IND2000	17790400	1 17 9047 0	_		

 Table 1. Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers.

Species	Strain/Vauchar Number	GenBank Accession Number			
	Strain/Voucher Number	LSU	SSU	ITS	tef 1-α
Keissleriella linearis	MFLUCC 19-0410	MN598873	MN598870	MN598892	MN60797
Keissleriella linearis	MFLUCC 20-0224	MT068487	MT068492	MT232436	MT477866
Keissleriella phragmiticola	CPC 33249	MT223903	_	MT223808	MT223715
Keissleriella phragmiticola	MFLUCC 17–0779 <sup>T</sup>	MG829014	_	MG828904	_
Keissleriella poagena	CBS 136767	KJ869170	_	KJ869112	_
Keissleriella quadriseptata	KT2292 <sup>T</sup>	AB807593	AB797303	AB811456	AB808572
Keissleriella rara	CBS 118429	GU479791	GU479757	_	
Keissleriella rosacearum	MFLUCC 15–0045 <sup>T</sup>	MG829015	MG829123	_	_
Keissleriella rosae	MFLUCC 15-0180 <sup>T</sup>	MG829016	MG922549	_	_
Keissleriella rosarum	MFLUCC 15–0180	MG829017	MG829124	MG828905	
Keissleriella sparticola	MFLUCC 14–0196 <sup>T</sup>	KP639571	WIG029124	WIG020903	-
			-	- VI 1000228	—
Keissleriella tamaricicola	MFLUCC 14–0168 <sup>T</sup>	KU900300	- A D707205	KU900328	
Keissleriella taminensis	KT571	AB807595	AB797305	LC014564	AB808574
Keissleriella taminensis	KT594	AB807596	AB797306	-	-
Keissleriella taminensis	КТ678	AB807597	AB797307	LC014565	AB808575
Keissleriella trichophoricola	CBS 136770 <sup>T</sup>	KJ869171	_	KJ869113	_
Keissleriella yonaguniensis	HHUF 30138 <sup>T</sup>	AB807594	AB797304	AB811457	AB808573
<i>Keissleriella</i> sp.	KT895	AB807590	AB797300	-	AB808569
Latorua caligans	CBS 576.65 T	MH870362	-	MH858723	-
Latorua grootfonteinensis	CBS 369.72 <sup>T</sup>	MH877741	-	-	-
Lentithecium clioninum	KT1149A <sup>T</sup>	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	KT1113 T	AB807544	AB797254	AB809632	AB808520
Lentithecium pseudoclioninum	GZCC 19-0483	MW133832	MW134612	OM692194	_
Lentithecium pseudoclioninum	KUNCC 22-12414	OR335348	OR335331	OR335291	_
Lentithecium pseudoclioninum	KUNCC 22-12415	OR335349	OR335331	OR335291	_
Lentithecium yunnanensis	KUNCC 22-10776 <sup>T</sup>	ON227127	ON227123	ON227126	ON228074
Lentithecium yunnanensis	KUNCC 22-10777	ON227124	ON227122	ON227125	ON228075
Lentithecium yunnanensis	KUNCC 22-12420	OR335350	OR335333	OR335293	OR367664
Lentithecium yunnanensis	KUNCC 22-12421	OR335351	OR335334	OR335294	OR367665
Lentithecium yunnanensis	KUNCC 22-12422	OR335352	OR335335	OR335295	OR367666
Longipedicellata aptrootii	MFLUCC 10-0297 <sup>T</sup>	KU238894	KU238895	KU238893	KU238892
Longipedicellata aptrootii	MFLUCC 18–0988	MN913744	_	MT627733	_
Macrodiplodiopsis desmazieri	CBS 140062 <sup>T</sup>	KR873272	_	KR873240	_
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	GU34904
Multiseptospora thailandica	MFLUCC 11–0183 <sup>T</sup>	KP744490	KP753955	KP744447	KU705657
Murilentithecium clematidis	MFLUCC 11-0185 MFLUCC 14-0561	KM408758	KM408760	KM408756	KM45444
Murilentithecium clematidis	MFLUCC 14–0561 MFLUCC 14–0562 <sup>T</sup>	KM408759	KM408761	KM408757	KM45444
Murilentithecium lonicerae	MFLUCC 14–0562 <sup>-</sup> MFLUCC 18–0675 <sup>T</sup>				
		MK214373	MK214376	MK214370	MK214379
Murilentithecium rosae	MFLUCC 15–0044 <sup>T</sup>	MG829030	MG829137	MG828920	-
Neolentithecia changchunensis	CCMJ10012 T	MZ518790	MZ518820	MZ519071	_
Neoophiosphaerella sasicola	KT1706 <sup>T</sup>	AB524599	AB524458	LC014577	AB539111
Parabambusicola thysanolaenae	KUMCC 18-0147 <sup>T</sup>	MK098199	MK098205	MK098190	MK098209
Parabambusicola thysanolaenae	KUMCC 18-0148	MK098198	MK098202	MK098193	MK09821
Paraconiothyrium brasiliense	CBS 100299 T	JX496124	AY642523	JX496011	-
Paraphaeosphaeria michotii	MFLUCC 13–0349 <sup>T</sup>	KJ939282	KJ939285	KJ939279	-
Paraphaeosphaeria minitans	CBS 122788	EU754173	EU754074	-	GU349083
Phragmocamarosporium hederae	MFLUCC 13-0552 T	KP842915	KP842918	_	_
Phragmocamarosporium platani	MFLUCC 14–1191 <sup>T</sup>	KP842916	KP842919	_	_
Phragmocamarosporium rosae	MFLUCC 17–0797 <sup>T</sup>	MG829051	MG829156	_	MG82922
Pleomonodictys descalsii	CBS 142298 <sup>T</sup>	KY853522	_	KY853461	_
Pleomonodictys capensis	CBS 968.97 <sup>T</sup>	KY853521	_	KY853460	

## Table 1. Cont.

$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Species	Strain/Voucher Number	GenBank Accession Number			
Pleurophoma ossicolaCPC24985KR476770-KR476737-Pleurophoma pleurosporaCBS130329 TJF740327Poaccascoma lalophilaMFLUCC 14-048 TKT324691Poaccascoma halophilaMFLUCC 15-0949 TMF613399MF615400Poaccascoma halophilaMFLUCC 18-0083 TKP998462KP998453KP998459KP988451Sto55153Sto55285175GCMCC 3.24265 TOQ32683OQ87574AB297284LC014594AB808550Setoseptoria arundinaceaCBS 123131GU45323GU335335GU335336GR335296OU367667Setoseptoria arundinaceaMAFF 238461GR335353GR335336GR335296OU367667Setoseptoria arundinaceaMAFF 243842 TAB807575AB797284LC014595AB808551Setoseptoria anubusaeKUNCC 22-12416GR335335			LSU	SSU	ITS	tef 1-α
Pleurophoma pleurosporaCBS130329 TJF740327Poaceascoma alquaticumMFLUCC 14-0048 TKT324690KT324691Poaceascoma halphilaMFLUCC 15-0949 TMF615390MF615400Poaceascoma halphilaMFLUCC 11-0136 TKP998462KP998463KP998459KP998461Poaceascoma taitoanenseMFLUCC 18-0083 TMG831566NR_160229GU349068Paralentifhecium aquaticumCBS 123099 TOQ32683OQ875040OQ874972OR367672Pseudokeissleriella bambusicolaCGMCC 3.24265 TOQ732683OQ875040OQ874972OR367672Pseudokeissleriella bambusicolaCGMCC 3.20950 TON614134ON614095ON614135ON639623Pseudokeissleriella bambusicolaUESTCC 22.0028ON614137ON614095ON614135ON639622Setoseptoria arundinaceaCBS 123131GU456320GU35028Setoseptoria arundinaceaMAFF 239460AB807574AB797284LC014594AB808550Setoseptoria arundinaceaMAFF 239460AB807574AB797284LC014594AB808550Setoseptoria bambusaeGZCC 17-0044OP377833OR33536OR335296OR35296Setoseptoria bambusaeKUNCC 22-12416OR335351OR335337OR335297OR367668Setoseptoria bambusaeKUNCC 22-12416OR335351OR335298OR35296-Setoseptoria bambusaeKUNCC 22-12418OR33535OR335296CO14596 </td <td>Pleurophoma ossicola</td> <td>CBS139905 <sup>T</sup></td> <td>KR476769</td> <td>_</td> <td>KR476736</td> <td>_</td>	Pleurophoma ossicola	CBS139905 <sup>T</sup>	KR476769	_	KR476736	_
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Pleurophoma ossicola	CPC24985	KR476770	-	KR476737	_
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Pleurophoma pleurospora	CBS130329 <sup>T</sup>	JF740327	_	_	_
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Poaceascoma aquaticum	MFLUCC 14-0048 T	KT324690	KT324691	-	_
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Poaceascoma halophila	MFLUCC 15–0949 <sup>T</sup>	MF615399	MF615400	-	_
Paralentithecium aquaticumCBS 123099 TGU301823GU296156NR_160229GU349068Paralentithecium suaeCGMCC 3.20265 TOQ732683OQ875940OQ874972OR367672Pseudokeissleriella bambusicolaCGMCC 3.20950 TON614133ON614096ON614135ON639623Pseudokeissleriella bambusicolaUESTCC 22.0028ON614137ON614095ON614133ON639622Setoseptoria arundhaceaCBS 123131GU456320GU456298-GU456281Setoseptoria arundinaceaCBS 619.86GU301824GU296157Setoseptoria arundinaceaMAFF 239460AB807574AB797284LC014594AB808550Setoseptoria arundinaceaMAFF 243842 TAB807575AB797285LC014595AB808551Setoseptoria bambusaeGZC 17-0044OP377919OP378004OP377820OP472999Setoseptoria bambusaeKUNCC 22-12416OR335335OR335337OR335296OR367667Setoseptoria bambusaeKUNCC 22-12417OR335353OR335338OR335298OR367669Setoseptoria langlantundinaceaMFLUCC 17-0778 TMG829075MG829175Setoseptoria langlantundinaceaKT1174AB807576AB79286LC014596AB808552Setoseptoria bambusaeCBS 114802 TKF251753-KF251250KF253200Setoseptoria lulworthcorensisMFLUCC 17-0778 TMG829074MG829174MG82967KF251250Setoseptoria naginarundinaceaKT1174AB807576AB79286 <td>Poaceascoma helicoides</td> <td></td> <td>KP998462</td> <td>KP998463</td> <td>KP998459</td> <td>KP998461</td>	Poaceascoma helicoides		KP998462	KP998463	KP998459	KP998461
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Poaceascoma taiwanense	MFLUCC 18-0083 T	MG831567	MG831568	MG831569	_
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Paralentithecium aquaticum	CBS 123099 <sup>T</sup>	GU301823	GU296156	NR_160229	GU349068
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		CGMCC 3.24265 <sup>T</sup>	OQ732683	OQ875040	OQ874972	OR367672
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Setoseptoria arundinaceaCBS 123131GU456320GU456298-GU456281Setoseptoria arundinaceaCBS 619.86GU301824GU296157Setoseptoria arundinaceaMAFF 239460AB807574AB797284LC014595AB808550Setoseptoria arundinaceaMAFF 243842TAB807575AB797285LC014595AB808551Setoseptoria bambusaeGZCC 17-0044OP377919OP378004OP377820OP472999Setoseptoria bambusaeKUNCC 22-12416OR335335OR335336OR335296OR367667Setoseptoria bambusaeKUNCC 22-12417OR335355OR335337OR335297OR367668Setoseptoria bambusaeKUNCC 22-12417OR335355OR335338OR335298OR367669Setoseptoria anglandensisMFLUCC 17-0778MG829074MG829174MG829863-Setoseptoria nagniarundinaceaKT1174AB807576AB797286LC014596AB808552Setoseptoria phragmitisCBS 114802TKF251753-KF251290KF253200Setoseptoria phragmitisCBS 114966KF251753-KF251250KF253200Setoseptoria scirpiMFLUCC 14-0811TKY770981MR857184MQ627908Splanchnonema plataniCBS 222.37KR909316KR909318KR909311KR909319Tingoldiago clavataMFLUCC 19-0496MN857178MN857187MN857183-Tingoldiago clavataMFLUCC 19-0498MN857179MN857187MN857183- <tr<< td=""><td>Pseudokeissleriella bambusicola</td><td><b>UESTCC 22.0028</b></td><td>ON614137</td><td>ON614095</td><td>ON614134</td><td>ON639622</td></tr<<>	Pseudokeissleriella bambusicola	<b>UESTCC 22.0028</b>	ON614137	ON614095	ON614134	ON639622
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Setoseptoria arundinaceaMAFF 239460AB807574AB797284LC014594AB808550Setoseptoria arundinaceaMAFF 243842 TAB807575AB797285LC014595AB808551Setoseptoria bambusaeGZCC 17-0044OP377919OP378004OP377820OP472999Setoseptoria bambusaeKUNCC 22-12416OR335353OR335336OR335296OR367667Setoseptoria bambusaeKUNCC 22-12417OR335355OR335337OR335298OR367669Setoseptoria englandensisMFLUCC 17-0778 TMG829074MG829174MG828963-Setoseptoria nagniarundinaceaKT1174MS829075MG829175Setoseptoria phragmitisCBS 114802 TKP251752Setoseptoria scirpiMFLUCC 14-0811 TKY770982KY770980MF939637KY770981Setoseptoria scirpiMFLUCC 14-0811 TKY770982KY770980MF939637KY770981Setoseptoria scirpiMFLUCC 19-0495MN857180MN857188MN857184-Tingoldiago clavataMFLUCC 19-0495MN857178MN857186MN857183-Tingoldiago graminicolaKH155AB521743AB521728LC014598AB808562Tingoldiago graminicolaKH891AB521744AB521724LC014598AB808563Tingoldiago graminicolaKH155AB521744AB521727LC014600AB808563Tingoldiago graminicolaKH1615AB521744AB521727LC014600AB808563Tingoldiago graminicola <t< td=""><td>Setoseptoria arundinacea</td><td></td><td>GU456320</td><td>GU456298</td><td>_</td><td>GU456281</td></t<>	Setoseptoria arundinacea		GU456320	GU456298	_	GU456281
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Setoseptoria bambusaeKUNCC 22-12416OR335353OR335336OR335296OR367667Setoseptoria bambusaeKUNCC 22-12417OR335354OR335337OR335297OR367668Setoseptoria bambusaeKUNCC 22-12418OR335355OR335338OR335298OR367669Setoseptoria englandensisMFLU CC 17-0778 TMG829074MG829174MG828963-Setoseptoria lulworthcovensisMFLU 18-0110 TMG829075MG829175Setoseptoria phragmitisCBS 114802 TKF251752-KF25129KF253199Setoseptoria phragmitisCBS 114966KF251753-KF25120KF253200Setoseptoria scirpiMFLUCC 14-0811 TKY770982KY770980MF939637KY770981Setoseptoria suaeCGMCC 3.24266 TOQ874972OQ875041OQ874972OR367673Splanchnonema plataniCBS 222.37KR909316KR909318KR909311KR909319Tingoldiago clavataMFLUCC 19-0495 TMN857180MN857186MN857184-Tingoldiago graminicolaKH155AB521743AB521728LC014599AB808562Tingoldiago graminicolaKH68 TAB521744AB521727LC014600AB808561Tingoldiago hydeiMFLUCC 19-0499 TMN857177-MN857181-	Setoseptoria arundinacea	MAFF 243842 $^{\mathrm{T}}$	AB807575	AB797285	LC014595	AB808551
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Setoseptoria bambusaeKUNCC 22–12418OR335355OR335338OR335298OR367669Setoseptoria englandensisMFLUCC 17–0778 TMG829074MG829174MG828963-Setoseptoria lulworthcovensisMFLU 18–0110 TMG829075MG829175Setoseptoria magniarundinaceaKT1174AB807576AB797286LC014596AB808552Setoseptoria phragmitisCBS 114802 TKF251752-KF251249KF253199Setoseptoria phragmitisCBS 114966KF251753-KF251250KF253200Setoseptoria scirpiMFLUCC 14–0811 TKY770982KY770980MF939637KY770981Setoseptoria suaeCGMCC 3.24266 TOQ874972OQ875041OQ874972OR367673Splanchnonema plataniCBS 221.37MH867404-MH855894DQ677908Splanchnonema plataniCBS 222.37KR909316KR909318KR909311KR909319Tingoldiago clavataMFLUCC 19–0495MN857180MN857188MN857184-Tingoldiago clavataMFLUCC 19–0496 TMN857179MN857187MN857183-Tingoldiago graminicolaKH155AB521745AB521728LC014599AB808562Tingoldiago graminicolaKH68 TAB521744AB521727LC014600AB808563Tingoldiago graminicolaKH68 TAB521744AB521727LC014600AB808563Tingoldiago hydeiMFLUCC 19–0499 TMN857177-MN857181-	Setoseptoria bambusae	KUNCC 22-12416	OR335353	OR335336	OR335296	OR367667
Setoseptoria englandensisMFLUCC 17–0778 TMG829074MG829174MG828963–Setoseptoria lulworthcovensisMFLU 18–0110 TMG829075MG829175––Setoseptoria magniarundinaceaKT1174AB807576AB797286LC014596AB808552Setoseptoria phragmitisCBS 114802 TKF251752–KF251249KF253199Setoseptoria phragmitisCBS 114966KF251753–KF251250KF253200Setoseptoria scirpiMFLUCC 14–0811 TKY770982KY770980MF939637KY770981Setoseptoria suaeCGMCC 3.24266 TOQ874972OQ875041OQ874972OR367673Splanchnonema plataniCBS 221.37MH867404–MH855894DQ677908Splanchnonema plataniCBS 222.37KR909316KR909318KR909311KR909319Tingoldiago clavataMFLUCC 19–0495MN857180MN857188MN857184–Tingoldiago clavataMFLUCC 19–0496 TMN857179MN857187MN857183–Tingoldiago graminicolaKH155AB521745AB521728LC014599AB808562Tingoldiago graminicolaKH68 TAB521743AB521726LC014598AB808561Tingoldiago graminicolaKT891AB521744AB521727LC014600AB808563Tingoldiago hydeiMFLUCC 19–0499 TMN857177–MN857181–	Setoseptoria bambusae	KUNCC 22-12417	OR335354	OR335337	OR335297	OR367668
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				AB521727		AB808563
<i>Towyspora aestuari</i> MFLUCC 15–1274 <sup>T</sup> KU248852 KU248853 NR_148095 –				-		-
	Towyspora aestuari	MFLUCC 15–1274 <sup>T</sup>	KU248852	KU248853	NR_148095	_

## Table 1. Cont.

Notes: The ex-type cultures are indicated using "T" after strain numbers; newly generated sequences are indicated in bold. "-" stands for no sequence data in GenBank.

## 3. Results

## 3.1. Phylogenetic Analysis

The combined ITS, LSU, SSU, and *tef* 1- $\alpha$  dataset comprises 147 taxa, including nine genera of Lentitheciaceae, with *Pleomonodictys capensis* (CBS 968.97) and *P. descalsii* (CBS 142298) as outgroup taxa (Figure 2). The dataset comprised 3777 characters (LSU: 1285 bp; SSU: 1021 bp; ITS: 539 bp; *tef* 1- $\alpha$ : 932 bp, including gaps). Maximum likelihood (ML) analysis and Bayesian analysis produced similar topologies that were consistent across the major clades. The likelihood of the final tree is evaluated and optimized under GAMMA. The best RAxML tree with a final likelihood value of -31,318.755060 is presented (Figure 2). The matrix had 1636 distinct alignment patterns, with 27.52% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.239720, C = 0.248808, G = 0.272414, T = 0.239058; substitution rates AC = 1.212047, AG = 2.534776, AT = 1.388124, CG = 1.249521, CT = 7.002685, GT = 1.000000,  $\alpha$  = 0.226056, Tree-Length: 3.286461. Bayesian analyses generated 4412 trees (average standard deviation of split frequencies: 0.009960)

from which 3310 were sampled after 25% of the trees were discarded as burn-in. The alignment contained a total of 1441 unique site patterns. Bootstrap support values with an ML greater than 75%, and Bayesian posterior probabilities (PP) greater than 0.97 are given above the nodes.

The multigene phylogenetic analyses showed that the 13 fresh collections clustered within Lentitheciaceae. Five known species, *Halobyssothecium aquifusiforme* (KUNCC 22-12665), *H. phragmitis* (KUN-HKAS 127181), *H. unicellulare* (KUNCC 22-12413), *Lentithecium pseudoclioninum* (KUNCC 22-12414 and KUNCC 22-12415), *L. yunnanensis* (KUNCC 22-124201, KUNCC 22-12421 and KUNCC 22-12422) and *Setoseptoria bambusae* (KUNCC 22-12416, KUNCC 22-12417 and KUNCC 22-12418) clustered with their ex-type strains, respectively. *Paralentithecium suae* (KUNCC 22-12412) clustered sister to *P. aquaticum* (CBS 123099) in an independent clade within Lentitheciaceae. *Setoseptoria suae* (KUNCC 22-12419) was placed sister to *S. phragmitis* (CBS 114804 and CBS 114966). Single-gene phylogenies are shown as Supplemental Materials (Figures S1–S4) because they resulted in being less informative and resolutive than those based on the four-loci concatenated tree.

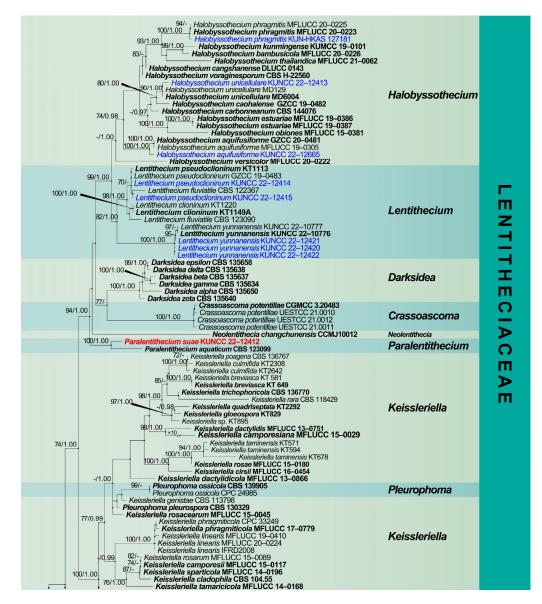
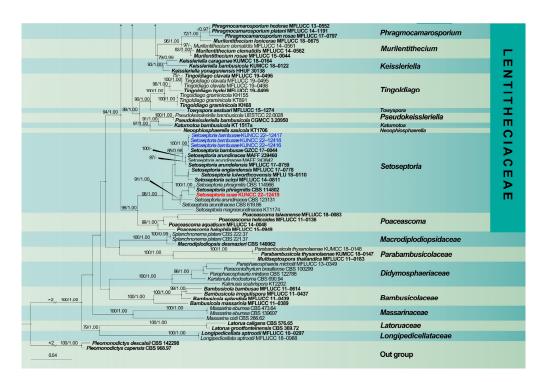


Figure 2. Cont.



**Figure 2.** Maximum likelihood (ML) tree is based on combined LSU, SSU, ITS, and *tef*  $1-\alpha$  sequence data. Bootstrap support values with an ML greater than 70% and Bayesian posterior probabilities (PP) greater than 0.97 are given above the nodes, shown as "ML/PP". The tree is rooted to *Pleomonodictys capensis* (CBS 968.97) and *P. descalsii* (CBS 142298). New species are indicated in red bold, new strains are indicated in blue, and type strains are in black bold.

## 3.2. Taxonomy

*Halobyssothecium aquifusiforme* J. Yang, Jian K. Liu & K.D. Hyde, Fungal Diversity 119: 39 (2023). Figure 3.

Index Fungorum number: IF559450; Facesoffungi number: FoF12783.

Saprobic on submerged decaying wood in a freshwater lake. Asexual morph: Undetermined. Sexual morph: *Ascomata* 354–382 µm high, 328–366 µm wide, immersed, clustered, sometimes solitary, scattered, subglobose or ellipsoidal, dark brown to black, carbonaceous, uniloculate, ostiolate. *Ostiolar* neck central, 86–114 µm long, 138–168 µm wide, papillate, rounded, short, dark brown, composed of several layers of pseudoparenchymatous cells. *Peridium* 22–35 µm thick, composed of several layers of pseudoparenchymatous cells, an outer layer composed of black cells, arranged in a *textura angularis*, inner layer composed of hyaline, flattened cells, arranged in a *textura angularis*. *Pseudoparaphyses* about 2 µm wide, branched, septate, hyaline, filamentous, anastomosing above the asci. *Asci* 97–129 × 13–16 µm ( $\overline{x} = 113 \times 14 \mu$ m, n = 20) µm, 8-spored, clavate to subcylindrical, bitunicate, fissitunicate, apex rounded, short pedicellate, with an ocular chamber. *Ascospores* (20–)24–27 × 7–8 µm ( $\overline{x} = 25 \times 8 \mu$ m, n = 40), overlapping, uniseriate to biseriate, central cells are brown to dark brown, 1-septate when young, 3-septate when mature, constricted at the septa, slightly curved, fusiform, guttulate, conical and narrowly rounded at the ends, one cell on the central septum side is swollen, lacking gelatinous sheaths or appendages.

Culture characteristics: Ascospore germinating on PDA within 12 h. Colonies on PDA reaching 3 cm diameter in 6 weeks at room temperature. Mycelium superficial, initially white, later becoming brown to black, with pale brown dense aerial mycelium on the surface, mastoid, marginal mycelium smooth, sparse, brown to black; from below, light brown at the center, dark brown at the margin.



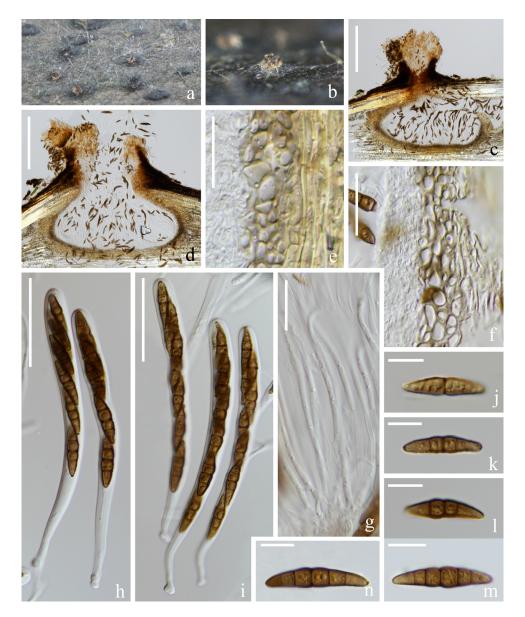
**Figure 3.** *Halobyssothecium aquifusiforme* (KUN-HKAS 1124599). (**a**,**b**) Appearance of ascomata on the host; (**c**,**d**) Sections of ascomata; (**e**) Ostiole; (**f**,**g**) Section of peridium; (**h**) Pseudoparaphyses; (**i**–**n**) Asci; (**o**–**u**) Ascospores; (**v**) Germinated conidium; (**w**,**x**) Colony on MEA, obverse (**w**) and reverse (**x**); Scale bar: (**c**,**d**) = 150  $\mu$ m; (**e**,**i**–**n**) = 30  $\mu$ m; (**f**–**h**) = 20  $\mu$ m; (**o**–**v**) = 10  $\mu$ m.

Material examined: China, Yunnan Province, Dali City, Eryuan County, Cibihu Lake, 26°09′59″ N, 99°55′27″ E (2050 m), on unknown submerged decaying wood, 21 July 2021, S.P. Huang and L.L. Li, L788 (KUN-HKAS 124599), living cultures (KUNCC 22-12665).

Known host and distribution: China, Guizhou Province, Anshun City, Gaodang village, 26.071° N, 105.698° E, Suoluo River, on decaying wood submerged in a freshwater stream HKAS 112638, (holotype), HKAS 112641 (paratype) [15].

Notes: The phylogenetic analysis showed that our new strain, KUNCC 22-12665 clustered sister to *Halobyssothecium aquifusiforme* (GZCC 20-0481 and MFLUCC 19-0305) with 99% ML/1.00 PP supports (Figure 2). Our species is similar to *H. aquifusiforme* in having immersed, subglobose ascomata, and fusiform, guttulate, septate ascospores which are constricted at the septum [15]. We, therefore, identified our new collection as *H. aquifusiforme* and provided detailed descriptions and illustrations for it. *Halobyssothecium aquifusiforme* is an aquatic species that was collected on submerged decaying wood in a freshwater stream in Guizhou, China. Our two new collections were collected from a plateau lake in Yunnan.

Halobyssothecium phragmitis M.S. Calabon, E.B.G. Jones, S. Tibell & K.D. Hyde, Mycological Progress 20: 711 (2021). Figure 4.



**Figure 4.** *Halobyssothecium phragmitis* (KUN-HKAS 124600, new geographic record). (**a**,**b**) Appearance of ascomata on the host; (**c**,**d**) Sections of ascomata; (**e**,**f**) Section of peridium; (**g**) Pseudoparaphyses; (**h**,**i**) Asci; (**j**–**n**) Ascospores. Scale bar: (**c**,**d**) = 200  $\mu$ m; (**e**–**g**) = 20  $\mu$ m; (**h**,**i**) = 40  $\mu$ m; (**j**–**n**) = 10  $\mu$ m.

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

*Saprobic* on submerged decaying wood in a freshwater lake. Sexual morph: *Ascomata* 529–566 µm high, 545–691 µm wide, immersed or semi-immersed, solitary to gregarious,

scattered, subglobose or ellipsoidal, dark brown, subcarbonaceous or coriaceous, uniloculate, with indistinct ostiolate. *Ostiolar neck* 172–265 µm high, 184–320 µm wide, central, papillate, rounded, short, dark brown, composed of several layers of pseudoparenchymatous cells. *Peridium* 26–77 µm thick, composed of several layers of pseudoparenchymatous cells, an outer layer composed of brown cells, arranged in a *textura angularis* and *textura globulosa*, and an inner layer composed of hyaline, flattened cells, arranged in a *textura angularis*. *Pseudoparaphyses* 2–3 µm wide, septate, hyaline, filiform, branched, anastomosing above the asci. *Asci* (102–)111–130(–137) × 10–12 µm ( $\overline{x} = 121 \times 11$ µm, n = 30), 8-spored, clavate to subcylindrical, bitunicate, fissitunicate, short pedicellate with an ocular chamber. *Ascospores* 22–27 × 5–6 µm ( $\overline{x} = 25 \times 6$ µm, n = 40), overlapping, uniseriate to biseriate, fusiform with narrow ends, cells swollen nearly central septum and gradually narrow toward ends, slightly curved, pale brown to dark brown and lightening from central cells to the end cells, 1-septate when young, 5-septate when mature, and constricted at the septa, lacking gelatinous sheaths or appendages. Asexual morph: Coelomycetes [14].

Material examined: China, Yunnan Province, Dali City, Eryuan County, Xihu Lake, 26°00'33″ N, 100°03'35″ E (1970 m), on unknown submerged decaying wood, 8 May 2021, S.P. Huang and L.L Li, L783 (KUN-HKAS 127181).

Known host and distribution: SWEDEN, Gotland, Kappelshamnsviken, on dead *Phragmites culm* (Poaceae), MFLU 20–0550 (holotype); ibid., Sudersand, on dead *Phragmites* (Poaceae) stem, MFLU 20–0552 (paratype) [14].

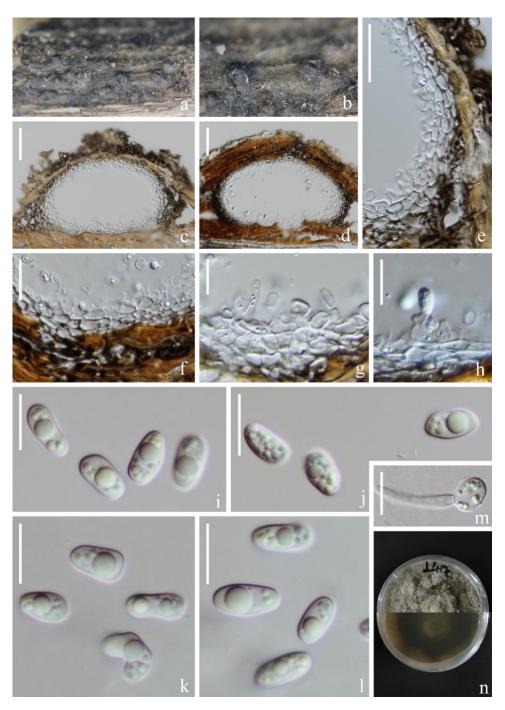
Notes: *Halobyssothecium phragmitis* was introduced by Calabon et al. [14], and only the asexual morph is known. This species was collected on *Phragmites (Poaceae)* culm in Europe. Phylogenetic analysis combined with ITS, LSU, SSU, and *tef 1-a* sequence data showed that our new collection (KUN-HKAS 127181) clustered with two strains of *H. phragmitis* (MFLUCC 20–0223 and MFLUCC 20–0225). The comparison of ITS, LSU, SSU, and *tef 1-a* sequences between our new collection (KUN-HKAS 127181) and the extype of *H. phragmitis* (MFLUCC 20–0226) showed 8 bp, 1 bp, 3 bp, and 3 bp differences, respectively. Morphologically, our new collection is similar to other sexual members of *Halobyssothecium* in having immersed or semi-immersed, subglobose or ellipsoidal, dark brown, subcarbonaceous or coriaceous ascomata, clavate to subcylindrical, bitunicate asci and 3-septate, fusiform ascospores [14,15,45,71]. Based on phylogenetic analysis and morphological evidence, we identified our new collection as *H. phragmitis*, and described its asexual morph. This is the first report of this species in China [14].

Halobyssothecium unicellulare (Abdel-Aziz) M.S. Calabon, K.D. Hyde & E.B.G. Jones, Mycological Progress 20: 715 (2021). Figure 5.

Index Fungorum number: IF558094; Facesoffungi number: FoF 09437

Saprobic on submerged decaying wood in a freshwater lake. Sexual morph: Undetermined. Asexual morph: Coelomycetes. *Conidiomata* 135–178 µm high, 205–242 µm wide, immersed to semi-immersed, most immersed, clustered, sometimes solitary, scattered, subglobose or ellipsoidal, uniloculate, dark brown to black, carbonaceous, short ostiolate, papillate, rounded. *Conidiomatal walls* 14–31 µm thick, composed of several layers of hyaline to black–brown cells of *textura angularis*. *Conidiophores* are reduced to conidiogenous cells. *Conidiogenous cells* 5–12 × 3–5 µm ( $\overline{x} = 8 × 4 µm$ , n = 30), hyaline, thin-walled, holoblastic, smooth, subglobose to pear-shaped, swollen at the base, sometimes one conidiogenous cell producing two conidia. *Conidia* 9–11 × 4–5 µm ( $\overline{x} = 10 × 5 µm$ , n = 60), subglobose, ovate, clavate, ellipsoid, allantoid or irregular, hyaline, aseptate, several small to one big guttulate, smooth-walled.

Culture characteristics: Conidia germinating on PDA within 12 h and germ tubes produced from one end of the conidia. Colonies on PDA, circular, reaching 5 cm in one month at room temperature, flat surface, pale brown to brown in PDA medium. Mycelium superficial, white to brown, hairy, effuse with wavy edge, dense, circular, raised, undulate to filiform with age; reverse light brown in the middle, with a dark brown deposit on the outside.



**Figure 5.** *Halobyssothecium unicellulare* (KUN-HKAS 124589, new geographic record). (**a**,**b**) Appearance of conidiomata on the host. (**c**,**d**) Sections of conidiomata. (**e**,**f**) Conidiomatal wall. (**g**,**h**) Developing conidia attach to conidiogenous cells. (**i**–**l**) Conidia. (**m**) Germinated conidium. (**n**) Colony on PDA, obverse (upper) and reverse (lower). Scale bar: (**c**,**d**) = 40  $\mu$ m, (**e**,**f**) = 20  $\mu$ m, (**g**–**m**) = 10  $\mu$ m.

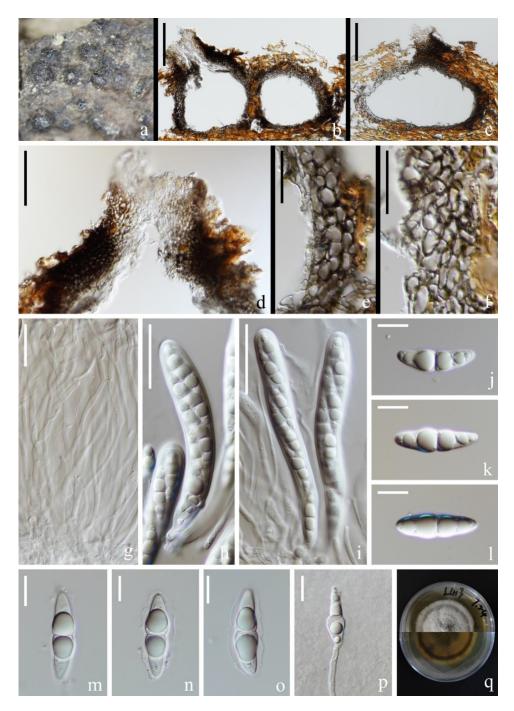
Material examined: China, Yunnan Province, Dali City, Eryuan County, Xihu Lake, 26°00'33″ N, 100°03'35″ E (1970 m), on unknown submerged decaying wood, 8 May 2021, S.P. Huang and L.L Li, L412 (KUN-HKAS 124589), living cultures, KUNCC 22-12413.

Known host and distribution: EGYPT, Sohag City, on decayed wood submerged in the River Nile, CBS H-22674 (holotype) [72].

Notes: The multigene phylogenetic analysis showed that our new collection (KUNCC 22-12413) clustered with the ex-type strain of *Halobyssothecium unicellulare* (MD 6004) with 91% ML/1.00 PP support (Figure 2). Morphologically, our new collection fits well with

the original description of *H. unicellulare* [72]. The nucleotide comparison of LSU and SSU sequence data between our new collection (KUNCC 22-12413) and *H. unicellulare* (MD 6004) revealed 2 bp (including one gap) and 1 bp (including one gap) differences, respectively. We therefore identified it as *H. unicellulare* and it was reported from China for the first time.

*Lentithecium pseudoclioninum* Kaz. Tanaka & K. Hiray, Studies in Mycology 82: 99 (2015). Figure 6.



**Figure 6.** *Lentithecium pseudoclioninum* (KUN-HKAS 124590). (a) Appearance of ascomata on the host; (b,c) Sections of ascomata; (d) Ostiole; (e,f) Section of peridium; (g) Pseudoparaphyses; (h,i) Asci; (j-o) Ascospores; (p) Germinated conidium; (q) Colony on PDA, obverse (upper) and reverse (lower). Scale bar: (b,c) = 100  $\mu$ m; (d) = 50  $\mu$ m; (e–g) = 20  $\mu$ m; (h,i) = 30  $\mu$ m; (j–p) = 10  $\mu$ m.

Index Fungorum number: IF811309; Facesoffungi number: FoF12785.

*Saprobic* on submerged decaying wood in a freshwater lake. Asexual morph: Undetermined. Sexual morph: *Ascomata* 201–310 µm high, 227–274 µm wide, black, semi-immersed, gregarious, erumpent, globose or subglobose, uniloculate, ostiolate. *Ostiolar neck* central, papillate, 92–110 µm long, 100–107 µm wide. *Peridium* 20–32 µm, thick-walled, brown to dark brown cells, composing several layers of pseudoparenchymatous cells of *textura angularis*, outer layers heavily pigmented, inner layers hyaline to pale brown, flattened. *Pseudoparaphyses* 2–3 µm wide, filamentous, branched septate. *Asci* 98–118 × 14–16 µm ( $\bar{x} = 108 \times 15 \mu$ m, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate, slightly curved, pedicellate, apex rounded with a minute ocular chamber. *Ascospores* 28–32 × 8–10 µm ( $\bar{x} = 30 \times 9 \mu$ m, n = 30), overlapping uni- to biseriate, narrowly fusiform, with a nearly median primary septum, constricted at the septum, hyaline, guttulate, usually with 2–4

mucilaginous sheath. Culture characteristics: Ascospore germinating on PDA within 12 h and germ tubes produced from the ends of the spore. Colonies on PDA, circular, reaching 5 cm in one month at room temperature, smooth surface, papillae, brown to dark brown. Mycelium superficial, hairy, smooth, circular, reverse grayish; reverse pale to brown, crack at the middle, flocculent at the edge.

larger guttules, asymmetrical, broadly fusiform, narrowly rounded at the ends, with a

Material examined: China, Yunnan Province, Dali City, Eryuan County, Xihu Lake, 26°00'33" N, 100°03'35" E (1970 m), on unknown submerged decaying wood, 8 May 2021, S.P. Huang and L.L Li, L413 (KUN-HKAS 124590), living cultures (KUNCC 22-12414); ibid., Erhai Lake, 26°00'32" N, 100°03'35" E (1970 m), on unknown submerged decaying wood, 01 April 2021, Z.Q. Zhang, L445 (KUN-HKAS 124593), living cultures (KUNCC 22-12415)

Known host and distribution: JAPAN, Aomori, Hirosaki, Aoki, Mohei pond, on submerged twigs of woody plant, KT 1113 (holotype) and KT 1111 (paratype); China, Guizhou Province, Weining City, Caohai National Nature Reserve, near 26.817° N, 104.217° E, on submerged decaying aquatic plants in Caohai lake, GZAAS 20-0378 [15].

Notes: Our two new collections are morphologically consistent with the holotype of *Lentithecium pseudoclioninum* [34]. In addition, phylogenetic analysis revealed that these two collections clustered with *L. pseudoclioninum* (Figure 2). Based on morphological and phylogenetic evidence, we identified our new collection as *L. pseudoclioninum*. *Lentithecium pseudoclioninum* has been collected on submerged twigs of woody plants in China and Japan [15,34]. Our two specimens were collected from a freshwater plateau lake in Yunnan, China.

*Lentithecium yunnanensis* W.H. Lu, Karun. & Tibpromma, Phytotaxa 554: 108 (2022). Figure 7.

Index Fungorum number: IF559622; Facesoffungi number: FoF 10778.

Saprobic on submerged decaying wood in a freshwater lake. Asexual morph: Undetermined. Sexual morph: *Ascomata* 246–285 µm high, 179–229 µm wide, immersed to semi-immersed, clustered, sometimes solitary, scattered, subglobose or ellipsoidal, dark brown to black, carbonaceous, uni- to bi-loculate, with indistinct ostiolate. *Ostiolar neck* central, papillate, 127–156 µm long, 96–110 µm wide. *Peridium* 11–21 µm thick, composed of several layers of pseudoparenchymatous cells, outer layer composed of back brown to brown cells, arranged in *textura angularis*, inner layer composed of hyaline cells, arranged in *textura angularis*, inner layer composed of hyaline cells, arranged in *textura angularis*. *Pseudoparaphyses* about 2 µm wide, hyaline, filamentous, branched, septate, globose to subglobose swollen at the apex, sometimes swollen at the septum, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 98–117 × 14–15 µm ( $\overline{x} = 108 \times 15$ µm, n = 15), 8-spored, clavate to subcylindrical, bitunicate, apex rounded, short pedicellate with an ocular chamber. *Ascospores* 27–30 × 5–6 µm ( $\overline{x} = 28 \times 6$ µm, n = 30), overlapping, uniseriate to biseriate, hyaline, 1-septate, smooth, constricted at the septa, slightly curved, guttulate, lacking gelatinous sheaths or appendages.

Culture characteristics: Ascospore germinating on PDA within 12 h and germ tubes produced from both ends of the spore. Colonies on PDA, circular, reaching 6 cm in 45 days at room temperature, smooth surface, papillae, brown in PDA medium. Mycelium superficial,



brown to dark brown, hairy, smooth, circular; reverse brown to dark brown, crack at the middle, flocculent at the edge.

**Figure 7.** *Lentithecium yunnanensis* (KUN-HKAS 124597, new habitat records). (**a**–**c**) Appearance of ascomata on the host; (**d**,**e**) Sections of ascomata; (**f**) Section of peridium; (**g**) Pseudoparaphyses; (**h**) Ascomata wall with hypha; (**i**–**k**) Asci; (**l**–**p**) Ascospores; (**q**) Germinated conidium; (**r**) Colony on PDA, obverse (upper) and reverse (lower). Scale bar: (**d**,**e**) = 100  $\mu$ m; (**f**,**g**) = 20  $\mu$ m; (**i**–**k**) = 30  $\mu$ m; (**h**,**l**–**q**) = 10  $\mu$ m.

Material examined: China, Yunnan Province, Dali City, Eryuan County, Xihu Lake, 26°17′37″ N, 99°58′33″ E (2100 m), on unknown submerged decaying wood, 22 July 2021,

L.L. Li, L680 (KUN-HKAS 124598), living cultures (KUNCC 22-12420 = KUNCC 22-12422); ibid., 26°17′24″ N, 99°57′56″ E (2100 m), on unknown submerged decaying wood, 22 July 2021, X.J. Yuan, L679 (KUN-HKAS 124597), living culture (KUNCC 22-12421).

Known host and distribution: China, Yunnan, Kunming, Songhua Dam Reservoir, on dead culms of *Artemisia* sp., HKAS 123192 (holotype) [73].

Notes: *Lentithecium yunnanensis* is a terrestrial species introduced by Lu et al. [73] that occurs on dead culms of *Artemisia* sp. near humid places. We collected two *Lentithecium*-like collections from decaying wood submerged in Xihu Lake, Dali, Yunnan Province. Phylogenetic analysis showed that our two new collections clustered with two strains of *L. yunnanensis* (KUNCC 22-10776 and KUNCC 22-10776). In addition, the morphology of our two collections is similar to the holotype of *L. yunnanensis* in having semi-immersed to immersed, subglobose to globose ascomata with short ostioles, and hyaline, clavate to fusiform, septate ascospores. Therefore, the two new collections were identified as *L. yunnanensis*, which was reported from the freshwater habitat for the first time.

Paralentithecium H.W. Shen, K.D. Hyde & Z.L. Luo gen. nov.

MycoBank number: 849738.

Etymology: referring to the comparable morphological characters to that of Lentithecium.

*Saprobic* on submerged decaying wood in a freshwater lake. Asexual morph: Undetermined. Sexual morph: *Ascomata* immersed to semi-immersed, clustered, sometimes solitary, scattered, subglobose or ellipsoidal, dark brown to black, carbonaceous, uni- to bi-loculate, with indistinct ostiolate. *Peridium* thick, composed of several layers of pseudoparenchymatous cells, an outer layer composed of back brown to brown cells, arranged in *textura angularis*, and an inner layer composed of hyaline cells, arranged in *textura angularis*. *Pseudoparaphyses* thick, hyaline, filamentous, branched, septate, globose to subglobose swollen at the apex, sometimes swollen at the septum, anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, clavate to subcylindrical, bitunicate, apex rounded, short pedicellate with an ocular chamber. *Ascospores* overlapping, uniseriate to biseriate, hyaline, 1-septate, smooth, constricted at the septa, slightly curved, with gelatinous sheaths.

Type species: *Paralentithecium aquaticum* (Yin. Zhang, J. Fourn. & K.D. Hyde) H.W. Shen & Z.L. Luo.

Paralentithecium aquaticum (Yin. Zhang, J. Fourn. & K.D. Hyde) H.W. Shen & Z.L. Luo, comb. nov.

MycoBank number: MB 512791.

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

Known host and distribution: FRANCE, Ariège, Rimont, Peyrau, on submerged wood of *Fraxinus excelsior*; on submerged wood of *Alnus glutinosa*; Le Baup brook, along D 18, on submerged wood of *Platanus* sp. [35].

Notes: *Lentithecium aquaticum* was introduced by Zhang et al. [35] based on phylogenetic analysis and morphological characteristics. The placement of this species was not stable and has been changed by several studies [10,45,71,74]. Previous phylogenetic analyses indicated that *Lentithecium aquaticum* did not cluster with other *Lentithecium* species, and it formed an individual lineage basal to *Darksidea, Halobyssothecium* and *Lentithecium* [10,14,34,41,48,71]. Furthermore, phylogenetic studies of Dayarathne et al. [45] and Devadatha et al. [71] showed that *L. aquaticum* clustered within *Setoseptoria*. Several other studies excluded *L. aquaticum* from *Lentithecium* [10,74]. The latest phylogenetic analysis based on combined ITS, LSU, SSU, and *tef* 1- $\alpha$  genes showed that *L. aquaticum* formed a separate lineage outside of *Lentithecium* [48]. Our phylogenetic analysis shows that *L. aquaticum* clusters with our new collection KUNCC 22-12412 and forms a distinct lineage within Lentitheciaceae with 100 ML/1.00 PP support (Figure 2). Therefore, we propose a new genus, *Paralentithecium* to accommodate *Paralentithecium aquaticum* (*Lentithecium aquaticum*) and a new species *P. suae*.

Paralentithecium suae H.W. Shen, K.D. Hyde & Z.L. Luo sp. nov. Figure 8.



**Figure 8**. *Paralentithecium* suae (KUN-HKAS 124587, holotype). (**a**–**c**) Appearance of ascomata on the host; (**d**) Sections of ascomata; (**e**,**f**) Section of peridium; (**g**,**h**) Pseudoparaphyses; (**i**–**l**) Asci; (**m**–**r**) Ascospores; (**s**) Germinated conidium; (**t**,**u**) Colony on PDA, obverse (**t**) and reverse (**u**). Scale bar: (**d**) = 100  $\mu$ m; (**e**–**l**) = 40  $\mu$ m; (**m**–**s**) = 20  $\mu$ m.

MycoBank number: 849739; Facesoffungi number: FoF 14876.

Etymology: "suae" (Lat.) in memory of the Chinese mycologist Prof. Hong-Yan Su (4 April 1967–3 May 2022).

Holotype: KUN-HKAS 124587.

Saprobic on submerged decaying wood in a freshwater lake. Asexual morph: Undetermined. Sexual morph: Ascomata 212–253  $\mu$ m high, 175–204  $\mu$ m wide, immersed to semi-immersed, clustered, sometimes solitary, scattered, subglobose or ellipsoidal, dark brown to black, carbonaceous, uni- to bi-loculate, with indistinct ostiolate. *Peridium* 17–32  $\mu$ m

thick, composed of several layers of pseudoparenchymatous cells, outer layer composed of bark brown to brown cells, arranged in *textura angularis*, inner layer composed of hyaline cells, arranged in *textura angularis*. *Pseudoparaphyses* 2–3 µm wide, hyaline, filamentous, branched, septate, globose to subglobose swollen at the apex, sometimes swollen at the septum (6–10 µm wide), anastomosing at the apex, embedded in a hyaline gelatinous matrix. *Asci* 104–134 × 24–28 µm ( $\bar{x} = 119 \times 26 \mu$ m, n = 25), 8-spored, clavate to subcylindrical, bitunicate, apex rounded, short pedicellate with an ocular chamber. *Ascospores* 28–34 × 11–14 µm ( $\bar{x} = 31 \times 13 \mu$ m, n = 40), overlapping, uniseriate to biseriate, hyaline, 1-septate, broadly fusiform, smooth, constricted at the septa, slightly curved, guttulate, with gelatinous sheaths.

Culture characteristics: Ascospore germinating on PDA within 12 h and germ tubes produced from both ends of the spore. Colonies on PDA, circular, reaching 4–5 cm in one month at room temperature, smooth surface, papillae, brown to dark brown, olive green in PDA medium. Mycelium superficial, brown to dark brown, hairy, smooth, circular; reverse dark brown, crack at the middle, flocculent at the edge, dark brown with greenish.

Material examined: China, Yunnan Province, Lijiang City, Ninglang County, Luguhu Lake, 27°44′15″ N, 100°45′16″ E (2700 m), on unknown submerged decaying wood, 5 March 2021, Z.Q. Zhang and L. Sha, L184 (KUN-HKAS 124587, holotype), ex-type living cultures (CGMCC 3.24265 = KUNCC 22–12412).

Notes: In our phylogenetic analysis, *Paralentithecium suae* clustered with *P. aquaticum* with 100% ML/1.00 PP support (Figure 2). Comparison of ITS, LSU, SSU, and *tef* 1- $\alpha$  sequences between *Paralentithecium suae* and *P. aquaticum* revealed 11 bp, 4 bp, 4 bp, and 22 bp differences, respectively. *Paralentithecium suae* resembles *P. aquaticum* in having hyaline, 1-septate, broadly fusiform ascospores with gelatinous sheaths [35]. However, *P. suae* is distinct from *P. aquaticum* in having globose to subglobose pseudoparaphyses that are swollen at the apex and sometimes swollen at the septum. In contrast, the pseudoparaphyses of *P. aquaticum* are not swollen. In addition, ascospores of *P. aquaticum* contain four refractive globules, while *P. suae* has ascospores with many small guttules [35]. Therefore, we introduce *P. suae* as a new species.

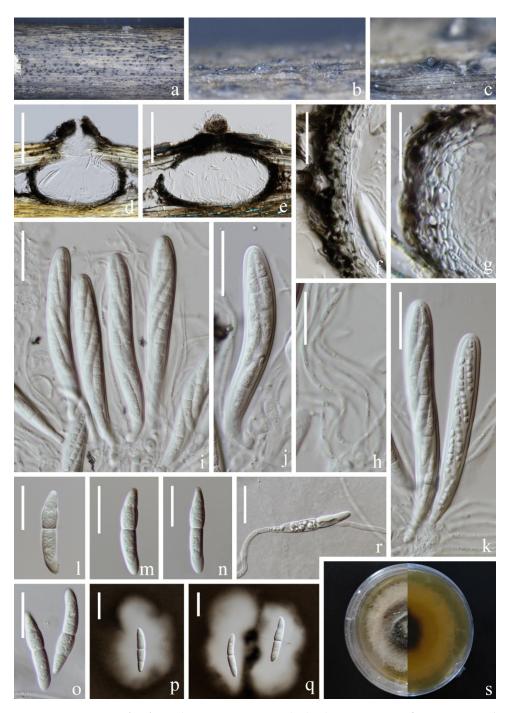
Setoseptoria bambusae J. Yang, Jian K. Liu & K.D. Hyde, Fungal Diversity 119: 44 (2022). Figure 9.

Index Fungorum number: IF559452; Facesoffungi number: FoF12786.

Saprobic on submerged decaying wood in a freshwater lake. Asexual morph: Undetermined. Sexual morph: Ascomata 245–375 µm high, 194–296 µm wide, black, superficial to semi-immersed, gregarious, fully or partly erumpent, globose, uniloculate, ostiolate. Peridium 26–39 µm wide, thick, multi-layered, outer layer most heavily pigmented, comprising blackish to dark brown amorphous layer, middle layer heavily pigmented, inner layer, pale brown to hyaline, cells towards the inside lighter, flattened, thick-walled. Pseudoparaphyses 2–3 µm wide, filamentous, branched septate. Asci 113–128 × 15–19 µm ( $\bar{x} = 120 \times 17$  µm, n = 30), 8-spored, bitunicate, fissitunicate, clavate to cylindric-clavate, pedicellate, apex rounded with a minute ocular chamber. Ascospores 32–40 × 6–8 µm ( $\bar{x} = 36 \times 7$  µm, n = 20), overlapping uni- to biseriate, narrowly fusiform, with a nearly median primary septum, deeply constricted at the septum, hyaline, guttulate, asymmetrical, conical, and narrowly rounded at the ends.

Culture characteristics: Ascospore germinating on PDA within 12 h and germ tubes produced from one end of the spore. Colonies on PDA, circular, reaching 6 cm in 45 days at room temperature, smooth surface, papillae, pale brown in PDA medium. Mycelium superficial, grayish-brown to brown, hairy, smooth, circular; reverse pale brown at the edges, dark brown in the middle, flocculent at the edge.

Material examined: China, Yunnan Province, Yuxi City, Jiangchuan District, Xingyunhu Lake, 24°23'05" N, 102°48'22" E (1720 m), on unknown submerged decaying wood, 10 July 2021, H.W. Shen, L511 (KUN-HKAS 124592), living culture (KUNCC 22–12417); ibid., 24°23'05" N, 102°48'22" E (1720 m), on the submerged stem of *Phragmites* sp. (*Poaceae*), 10 July 2021, S. Luan, L579 (KUN-HKAS 124596), living culture (KUNCC 22–12418); ibid., on



submerged stem of *Phragmites* sp. (*Poaceae*), 10 July 2021, Y.K. Jiang, L474 (KUN-HKAS 124591), living culture (KUNCC 22–12416).

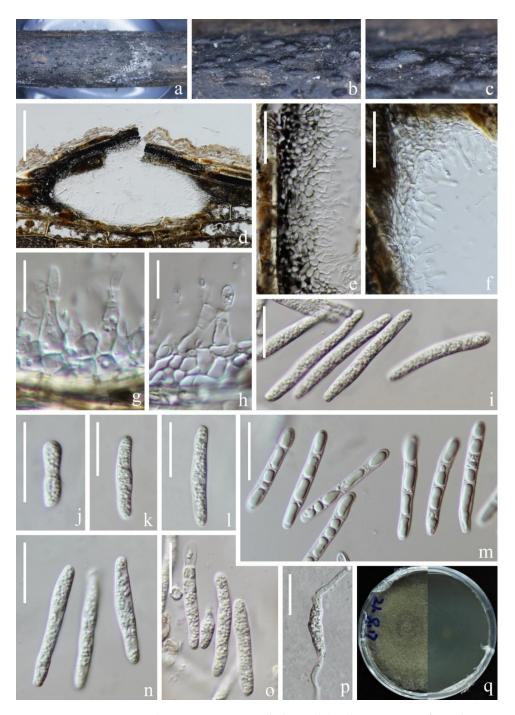
**Figure 9.** *Setoseptoria bambusae* (KUN-HKAS 124592). (**a**–**c**) Appearance of ascomata on the host; (**d**,**e**) Sections of ascomata; (**f**,**g**) Section of peridium; (**h**) Pseudoparaphyses; (**i**–**k**) Asci; (**l**–**o**) Ascospores; (**p**,**q**) Ascospore stained in Indian ink; (**r**) Germinated conidium; (**s**) Colony on PDA, obverse (**left**) and reverse (**right**). Scale bar: (**d**,**e**) = 150  $\mu$ m; (**f**–**k**) = 30  $\mu$ m; (**h**,**i**) = 30  $\mu$ m; (**l**–**r**) = 20  $\mu$ m.

Known host and distribution: China, Guizhou Province, Anshun City, Gaodang Village, 26.071° N, 105.698° E, Suoluo River, on decaying bamboo culms submerged in a freshwater stream, HKAS 112629 (holotype) [15].

Notes: *Setoseptoria bambusae* was introduced by Yang et al. [15] to accommodate two collections, GZCC 17–0044 (ex-type strain) and IFRD500-013 (previously identified as *S*.

*arundinaceae*, without description). In this study, our four new collections clustered with the ex-type strain of *S. bambusae* with 100% ML/1.00 PP statistical support (Figure 2). Furthermore, our collections fit the morphological characteristics of *S. bambusae* except for the size of asci and ascospores, our isolate has shorter asci (113–128 vs. 130–180  $\mu$ m) and longer ascospores (32–40 vs. 28–37  $\mu$ m). Therefore, we identified them as *S. bambusae*. Our four new collections were collected from lentic freshwater habitats. The holotype was collected from lotic habitats.

Setoseptoria suae H.W. Shen, K.D. Hyde & Z.L. Luo sp. nov. Figure 10.



**Figure 10.** *Setoseptoria suae* (KUN-HKAS 124595, holotype). (**a**–**c**) Appearance of conidiomata on the host; (**d**) Sections of conidiomata; (**e**,**f**) Section of peridium; (**g**,**h**) Conidiomata and conidiogenous cells; (**i**–**o**) Conidia; (**p**) Germinated conidium; (**q**) Colony on PDA, obverse (**left**) and reverse (**right**). Scale bar: (**d**) = 100  $\mu$ m; (**e**,**f**) = 30  $\mu$ m; (**g**,**h**) = 10  $\mu$ m; (**i**–**p**) = 10  $\mu$ m.

MycoBank number: 849740; Facesoffungi number: FoF 14877.

Etymology: "suae" (Lat.) in memory of the Chinese mycologist Prof. Hong-Yan Su (4 April 1967–3 May 2022).

Holotype: KUN-HKAS 124595.

Saprobic on submerged decaying wood in a freshwater lake. Sexual morph: Undetermined. Asexual morph: Conidiomata 383–512 µm high, 173–196 µm wide, solitary, scattered, semi-immersed to immersed in the host, pycnidial, subglobose to ellipsoidal, unilocular, black, ostiolate, apapillate. *Ostiole* short, centrally located. *Conidiomatal wall* 33–55 µm wide, thickening at the upper zone, thick-walled, composed of several layers of *textura angularis*, an outer layer comprising brown to dark brown cells, pigmented; inner layer comprising hyaline cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* (4–)7–15(–26) × 4–6 µm ( $\bar{x} = 11 \times 5 µm$ , n = 25), arising from the inner layers of conidiomata, hyaline, enteroblastic, phialidic, determinate, ampuliform, subcylindrical to lageniform. Conidia 33–43 × 4–6 µm ( $\bar{x} = 38 \times 5 µm$ , n = 50), subcylindrical, with obtuse to subobtuse ends, straight or slightly curved, hyaline, (1–)3-septate, euseptate, mostly with one large central guttule per cell when young, with many small guttules in each cell at maturity, slightly constricted at the septum, smooth-walled.

Culture characteristics: Conidia germinated on PDA within 12 h and germ tubes produced from the ends of the spore. Colonies on PDA, circular, reaching 6 cm in one month at room temperature, brown to dark brown. Mycelium superficial, brown to dark brown, hairy, smooth, circular; dark brown from below.

Material examined: China, Yunnan Province, Yuxi City, Tonghai County, Qiluhuhu Lake, 24°08′37″ N, 102°46′24″ E (1800 m), on submerged stem of *Phragmites* sp. (Poaceae), 11 July 2021, H.W. Shen, L570 (KUN-HKAS 124595, holotype), ex-type living cultures (CGMCC 3.24266 = KUNCC 22–12419).

Notes: Phylogenetic analysis showed that *Setoseptoria suae* clustered with *S. phragmitis* with 100% ML/0.99 PP statistical support (Figure 2). The comparison of ITS and LSU sequences between *S. suae* and *S. phragmitis* shows that the similarities are 96.9% (538/555 bp) and 99.9% (826/827 bp), respectively. *Setoseptoria suae* resembles *S. phragmitis* in having immersed, globose conidiomata, hyaline, subcylindrical, smooth, guttulate, (1–)3-septate conidia [37]. However, *Setoseptoria suae* can be distinguished from *S. phragmitis* by its larger conidia (33–43 × 4–6 µm vs. (19–)25–35(–38) × (3.5–)4 µm). In addition, the conidia of *S. phragmitis* mostly have one large central guttule per cell, while *Setoseptoria suae* has conidia with many small guttules in each cell. We, therefore, introduce *S. suae* as a new species.

## 4. Discussion

Yunnan, located on the Yunnan–Guizhou Plateau, is one of the global biodiversity hotspots with rich biological resources [18,19,75]. In recent years, research on lignicolous freshwater fungi in Yunnan has developed rapidly, and a large number of new species have been reported from lotic freshwater habitats such as streams and rivers [10,13,76–81]. A few studies have reported lignicolous freshwater fungi from lentic habitats in Yunnan Province. For example, Cai et al. [17] and Luo et al. [2] investigated lignicolous freshwater fungi in Fuxianhu and Dianchi Lakes, respectively. However, freshwater fungi in lentic habitats have not been updated recently. In this study, we investigate the freshwater fungi in Cibihu, Luguhu, Qiluhu, Xihu, and Xingyunhu lakes in Yunnan Province, one new genus, two new species, and three new records are reported, the results indicate that high undiscovered diversity of lignicolous freshwater fungi in lentic habitats.

Zhang et al. [36] provided the first multigene phylogenetic analysis of Pleosporales and introduced the family Lentitheciaceae which accepted the genera *Lentithecium, Katumotoa,* and *Keissleriella*. Dong et al. [10] treated the family with ten genera and this was followed by Wijayawardene et al. [41]. Previous studies based on morphology and phylogenetic analyses showed that the classification of *Lentithecium, Keissleriella,* and *Setoseptoria* is confusing as the placement of several taxa was problematic and has been transferred to

different genera. For example, Suetrong et al. [82] transferred *Keissleriella rara* to *Lentithecium* as *L. rarum*; however, later studies showed that *L. rarum* clustered with *K. trichophoricola* in *Keissleriella* [14]. Similarly, Zhang et al. [35] transferred *Keissleriella linearis* to *Lentithecium* as *L. lineare*, Singtripop et al. [83] re-examined the type specimen of *K. linearis* (*L. lineare*) and transferred it to *Keissleriella* based on LSU phylogenetic analysis, and this was confirmed by subsequent phylogenetic studies [14,34,72]. The placements of *Lentithecium* species have been revised in recent years based on multigene phylogenetic studies [14,34,84]. Calabon et al. [14] transferred several *Lentithecium* species with brown and versicolored ascospores without sheaths and hyaline conidia to *Halobyssothecium*, including *L. cangshanense*, *L. carbonneanum*, *L. kunmingense*, *L. unicellulare*, and *L. voraginesporum*. Currently, 13 species are accepted in *Halobyssothecium*. In the present study, we report the sexual morph of *H. phragmitis* and provide detailed morphological descriptions for its sexual morph.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof9100962/s1.

Author Contributions: Conceptualization, Z.-L.L., S.B. and K.D.H.; methodology, H.-W.S. and X.-J.S.; formal analysis, H.-W.S.; investigation, H.-W.S., D.-F.B., X.-J.S. and X.-G.T.; resources, Z.-L.L. and S.B.; data curation, H.-W.S.; writing—original draft preparation, H.-W.S. and X.-G.T.; writing—review and editing, D.-F.B., S.B., X.-J.S., K.D.H. and Z.-L.L.; funding acquisition, Z.-L.L. All authors have read and agreed to the published version of the manuscript.

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