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Discovery of a Novel *Occultibambusa* Species (Occultibambusaceae, Dothideomycetes) from Poaceae Host from Guizhou Province, China

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Abstract

In the exploration of fungal diversity in the southwest of China, four ascomycetous taxa were discovered inhabiting the culms of *Phragmites australis* (*Poaceae*) and *Bambusoideae* (*Poaceae*) near a tobacco field in Qingzhen, Guizhou Province, China. These newly discovered species morphologically resemble *Occultibambusa* (*Occultibambusaceae*) species. Phylogenetic analysis of combined small subunit rDNA (SSU), internal transcribed spacers (ITS), large subunit rDNA (LSU), translation elongation factor 1-α gene region (*tef1-α*), and RNA polymerase II second largest subunit (*rpb2*) sequence data has further identified our four strains into two species including one novel species, *Occultibambusa phragmitis* and one known species, *O. jonesii*. The discretions, illustrations, and notes of these identified taxa species are provided.

Keywords – A new taxon – Phylogeny – Taxonomy

Introduction

Occultibambusaceae was introduced by Dai et al. (2017) and it is characterized by immersed, solitary to gregarious ascomata with black ostioles, broadly cylindrical to clavate, bitunicate asci, cellular pseudo-paraphyses and broad-fusiform, hyaline to dark brown ascospores with 1–3 septa (Dai et al. 2017). *Occultibambusaceae* is phylogenetically related to *Biatriosporaceae*, but members of *Occultibambusaceae* later develop manglicolous and the dark brown ascospores usually have hyaline, rounded, swollen ends which will release mucilage (Hyde et al. 2013, Dai et al. 2017). *Occultibambusaceae* comprises five genera namely *Brunneofusispora* (Phookamsak et al. 2019), *Neooccultibambusa* (Doilom et al. 2017), *Occultibambusa* (Dai et al. 2017), *Seriascoma* (Dai et al. 2017) and *Versicolorisporium* (Hatakeyama et al. 2008). Members of *Occultibambusaceae* are usually reported on monocotyledons, but it is also possible to find them on hardwood trees (Dai et al. 2017, Doilom et al. 2017).

Occultibambusa is the type genus of *Occultibambusaceae* characterized by immersed, dark ascostromata with periphysate ostioles, broad cylindrical to clavate asci, and dark brown, fusiform,

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1-septate ascospores (Dai et al. 2017). This genus has a unique morphology as asexual morphs of *Occultibambusa* can produce black necks at the center of ascomata (Dai et al. 2017).

During our survey of fungal diversity associated with plants in Guizhou Province, which is a complex ecosystem with a warm and humid climate and rich biodiversity, four *Occultibambusa* strains were obtained near the tobacco field. The objective of this study was to use the multi loci of SSU, ITS, LSU, *tef1-α*, and *rpb2* gene regions to clarify the new strains. The taxonomic placement of these collections was revealed as two *Occultibambusa* species. Based on the morphology and molecular study, a novel species in *Occultibambusa* was introduced. An updated generic description and backbone tree of *Occultibambusaceae* are provided.

Materials & Methods

Collection and Examination of Specimens

Specimens of *Phragmites australis* and *Bambusoideae* were collected in Qingzhen, Guizhou Province, China, and subsequently preserved within envelopes. Macro morphological characters were observed using VHX-7000 (Keyence, Osaka, Japan), dissecting microscopes with fully integrated Head VHX-7100 (Keyence, Osaka, Japan) and High-Performance Camera VHX-7020 (Keyence, Osaka, Japan). Micro morphological characters were examined and photographed by a ZEISS Axioscope 5 Camera (Zeiss, Oberkochen, Germany) compound microscope fitted with a ZEISS Axiocam 208 Color Microscope Camera (Zeiss, Oberkochen, Germany). Tarosoft Image Framework software was used for measurement. Single spore isolations were made onto potato dextrose agar (PDA) and germinated spores were transferred onto PDA within 12–24 h. The collected specimens and living cultures were deposited at Guizhou University in Guiyang, China. Index Fungorum numbers were registered as mentioned in Index Fungorum (2023).

DNA Extraction, PCR Amplification, and Sequencing

Fungal mycelium was scraped off through a sterilized scalpel and then transferred to a 1.5 mL centrifuge tube to facilitate the extraction of genomic DNA. Fungi Genomic DNA Isolation Kit (BW-GD2416, Hangzhou Beiwo Medical Technology Co., Ltd, Hangzhou, China) was used to extract DNA following the manual.

Small subunit rDNA (SSU), internal transcribed spacers (ITS), large subunit rDNA (LSU), translation elongation factor 1-α gene region (*tef1-α*), and RNA polymerase II second largest subunit (*rpb2*) gene regions were amplified by polymerase chain reaction (PCR) using the primer pairs NS1/NS4, ITS5/ITS4, LR0R/LR5, 983F/2218R, and fRPB2-5F/fRPB2-7cR, respectively (Vilgalys & Hester 1990, White et al. 1990, Rehner & Samuels 1994, Liu et al. 1999). The DNA fragments were amplified in a 20 μL reaction volume containing 10 μL 2 × Master Mix (2 × Bench TopTM Taq Master Mix), 1 μL of each primer (10 μM), 1 μL template DNA, 7 μL dd H2O using the Biometra Thermal Cycler (Biometra TRIO) and followed the thermal cycle programme described by (Zhang et al. 2017). Successful PCR products were sequenced in Shanghai Sangon Biological Engineering Technology and Services Co., Shanghai, China. The raw sequences were deposited in GenBank, as shown in Table 1.

Phylogenetic Analyses

Sequences used in this study were downloaded from GenBank according to previous publications (Table 1) (Zhang et al. 2017). Consensus sequences were aligned using MAFFT (Katoh et al. 2019, https://mafft.cbrc.jp/alignment/server/). The alignments were checked and manually improved where necessary using BioEdit v. 7.0.5 (Hall et al. 2011). The combined alignment of multi-genes was carried out by SequenceMatrix v. 1.7.8 (Vaidya et al. 2011). Phylogenetic analyses were performed by maximum likelihood (ML) and Bayesian inference (BI) analyses.

Table 1 Taxa used in the phylogenetic analyses and their GenBank accession numbers. * Denotes ex-type strains. New sequences from this study are in bold.

Organism	**Strain Number**	**Host**	**Country**	**GenBank Accession Number**				
				SSU	**ITS**	**LSU**	***tef1***	***rpb2***
Brunneofusispora clematidis	MFLUCC 17-2070*	Dead branches of *Clematis subumbellata*	Thailand	MT226685	MT310615	MT214570	MT394629	MT394692
Brunneofusispora hyalina	MFLUCC 21-0008*	Decaying wood submerged in a stream	Thailand	MW485613	MW260330	MW287234	MW512606	MW512609
Brunneofusispora inclinatiostiola	CGMCC 3.20403*	Decaying petioles of palm	China	MZ964884	MZ964866	MZ964875	OK061069	OK061075
Brunneofusispora sennae-torae	BRIP 72515d*	Leaf spot of sickle seena	Australia	–	OK493236	OK493235	–	–
Brunneofusispora sinensis	KUMCC 17-0030*	Submerged dead wood	China	MH393556	MH393558	MH393557	MH395329	–
Neooccultibambusa chiangraiensis	MFLUCC 12-0559*	Dead twigs of teak	Thailand	KU712458	KU712442	KU764699	–	–
Neooccultibambusa jonesii	MFLUCC 16-0643*	Dead and aerial stem of marram grass	Italy	KY111438	–	KY111437	–	–
Neooccultibambusa kaiyangensis	CGMCC 3.20404*	Decaying petioles of palm	China	MZ964886	MZ964868	MZ964877	OK061071	OK061077
Neooccultibambusa pandanicola	KUMCC 17-0179*	Fallen dead and decaying leaves of common screwpine	China	MG298942	MG298941	MG298940	MG298943	MG298944
Neooccultibambusa thailandensis	MFLUCC 16-0274*	Dead leaf of screw pine	Thailand	MH260348	MH275074	MH260308	MH412780	MH412758
Neooccultibambusa trachycarpi	CGMCC 3.20405*	Decaying petioles of palm	China	MZ964888	MZ964870	MZ964879	OK061073	OK061079
Occultibambusa aquatica	MFLUCC 11-0006*	Submerged bamboo	Thailand	KX698112	–	KX698110	–	–
Occultibambusa bambusae	MFLUCC 11-0394	Dead culms of bamboo	Thailand	KU872117	KU940124	KU863113	KU940194	KU940171
Occultibambusa bambusae	MFLUCC 13-0855*	Dead culms of bamboo	Thailand	KU872116	KU940123	KU863112	KU940193	KU940170

Table 1 Continued

Organism	Strain Number	Host	Country	GenBank Accession Number				
				SSU	ITS	LSU	*tef1*	*rpb2*
Occultibambusa chiangraiensis	MFLUCC 16-0380*	Dead stem of *bamboo*	China	KX655551	–	KX655546	KX655561	KX655566
Occultibambusa fusispora	MFLUCC 11-0127*	Dead branch of bamboo	Thailand	–	KU940125	KU863114	KU940195	KU940172
Occultibambusa hongheensis	KUMCC 21-0020	Dead branches of bamboo	China	MZ329029	MZ329037	MZ329033	MZ325467	N/A
Occultibambusa jonesii	GZCC 16-0117*	Dead bamboo culms	China	KY628324	–	KY628322	KY814756	KY814758
Occultibambusa jonesii	**GUCC 23-0006**	**Dead bamboo culms**	**China**	**OR392459**	**OR392464**	**OR392462**	**OR396954**	**OR396951**
Occultibambusa kunmingensis	HKAS 102151*	Submerged bamboo in a stream	China	MT864342	MT627716	MN913733	MT954407	MT878453
Occultibambusa maolanensis	GZCC 16-0116*	Dead bamboo culms	China	KY628325	–	KY628323	KY814757	KY814759
Occultibambusa phragmitis	**GUCC 23-0004***	**Up-righting culms of reed**	**China**	**OR392457**	**OR392463**	**OR392460**	**OR396952**	**OR396950**
Occultibambusa phragmitis	**GUCC 23-0005**	**Up-righting culms of reed**	**China**	**OR392458**	**PQ432813**	**OR392461**	**OR396953**	**PQ441884**
Occultibambusa pustula	MFLUCC 11-0502*	Dead culm of bamboo	China	KU872118	KU940126	KU863115	–	–
Occultibambusa sichuanensis	CGMCC 3.20938*	Dead branches of bamboo	China	N/A	ON332913	ON332931	ON381181	ON383989
Ohleria modesta	CBS 141480*	Branches of tagasaste	Spain	KX650513	KX650563	KX650563	KX650534	KX650583
Ohleria modesta	MGC	Branches of tagasaste	Spain	–	KX650562	KX650562	KX650533	KX650582
Seriascoma didymospora	MFLUCC 11-0179*	Bamboo	Thailand	–	KU940127	KU863116	KU940196	KU940173
Seriascoma honghense	KUN-HKAS 112013*	Roadside bamboo	China	MZ325471	MW981351	MW981347	MZ325472	MZ325473

Table 1 Continued

Organism	Strain Number	Host	Country	GenBank Accession Number				
				SSU	ITS	LSU	*tef1*	*rpb2*
Seriascoma yunnanense	MFLU 19-0690*	Dead branches of bamboo	China	MN174694	–	MN174695	MN381858	MN210324
Versicolorisporium triseptatum	JCM 14775*	Dead culms of bamboo	Japan	AB524501	AB365596	AB330081	–	–

BRIP – Plant Pathology Herbarium, Department of Agriculture and Fisheries, Queensland; CBS – Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC – China General Microbiological Culture Collection Center, Beijing, China; GUCC – Guizhou University Culture Collection, Guizhou, China; GZCC – Guizhou Culture Collection, Guizhou, China; HKAS – Kunming Institute of Botany Academia Sinica, Kunming, China; JCM – Japan Collection of Microorganisms, RIKEN BioResource Center, Japan; KUMCC – Kunming Institute of Botany Culture Collection, Chinese Academy of Sciences, Kunming, China; KUN-HKAS – Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China; MFLU – Mae Fah Luang University Herbarium, Chiang Rai, Thailand; MFLUCC – Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MGC – MGC Herbarium of University of Malaga, Spain.

Maximum likelihood (ML) analysis was performed through the IQ-TREE web server (Jana et al. 2016) using a model selected by auto with rapid bootstrap analysis followed by 1000 bootstrap replicates. K2P+I+G4, TIM2e+G4, TNe+I+G4, TN+F+I+G4, TIM3e+I+G4 individual models were selected for SSU, ITS, LSU, *tef1-α* and *rpb2* partitions respectively. The ML analysis resulted in a best scoring RAxML tree with a final best score of -20472.240. MrModeltest2 v. 2.3 (Nylander 2008) was used to infer the appropriate substitution model which would be the fittest model of DNA evolution for the combined dataset in BI analysis. The HKY + I + G substitution model was selected for the SSU partition. GTR + I + G substitution model was selected for ITS, LSU, *tef1-α* and *rpb2* partitions. BI analysis was employed through MrBayes 3.2.6 in the CIPRES science Gateway v. 3.3 (Huelsenbeck & Ronquist 2001, Miller et al. 2010). Four simultaneous Markov chains were run for 1,000,000 generations. Trees were sampled every 1000 generations. The first 25% of trees representing the burn-in phase of the analysis were discarded. The remaining trees were used for calculating posterior probabilities of recovered branches (Larget & Simon 1999). The program ends automatically when the average standard deviation of split frequencies is below 0.01.

The generated trees were visualized using FigTree v. 1.4 and the overall layout was created using Adobe Illustrator 2019 (Adobe Systems, San Jose, CA, USA). The bootstrap values of ML were equal to or greater than 70% and Bayesian posterior probabilities were equal to or greater than 0.90 are left on the branches.

Results

Phylogenetic Analyses

The reference dataset included 26 representatives of the five accepted genera in *Occultibambusaceae*. Five gene loci SSU, ITS, LSU, *tef1-α* and *rpb2* were used to determine the phylogenetic placement of our collections. The concatenated matrix comprised 32 taxa with a total of 4,961 characters (SSU: 1–1,520; ITS: 1,521–2,150; LSU: 2,151–2,973; *tef1-α*: 2,974–3916; *rpb2*: 3,917–4,961) including gaps. *Ohleria modesta* (CBS 141480) and *O. modesta* (MGC) were selected as outgroup taxa. The best scoring RAxML tree is shown in Figure 1. The analyzed ML and Bayesian trees were similar in topology and did not conflict significantly. Our four strains nested within *Occultibambusa* representing two species. *Occultibambusa phragmitis* (GUCC 23-0004 and GUCC 23-0005) were characterized based on two strains. These strains were found to be closely related to *O. aquatica* (MFLUCC 11-0006) in the generated tree, although this relationship had moderate maximum-likelihood bootstrap support (72% MLBP/- BYPP). This could be attributed to the fact that *O. aquatica* (MFLUCC 11-0006) has available only the SSU and LSU genes for analysis. Another strain, GUCC 23-0006 and GUCC 23-0471 were clustered alongside the ex-type strain (GZCC 16-0117) of *Occultibambusa jonesii*. This grouping was based on matching SSU and *tef1-α* sequences, with only a single base pair difference observed in LSU sequences and another single base pair difference detected in *rpb2* sequences.

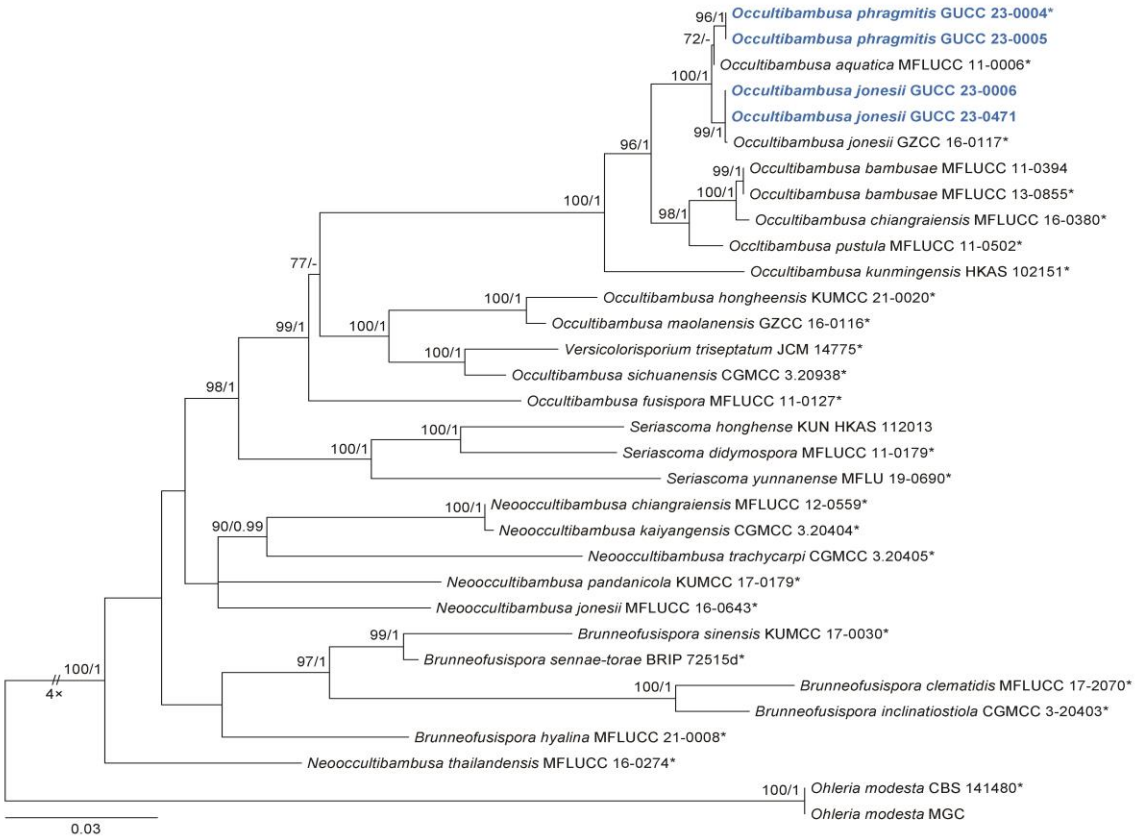

Fig. 1 – Phylogenetic tree obtained from maximum likelihood analysis (RAxML) of combined SSU, ITS, LSU, tef1 and rpb2 sequence data. Bootstrap support values for maximum likelihood equal to or greater than 70% and Bayesian Posterior Probabilities equal to greater than 0.90 are shown near the nodes as ML/PP. The tree is rooted to *Ohleria modesta* (CBS 141480) and *Ohleria modesta* (MGC). The ex-type strains are indicated with an asterisk (*) while the new isolates from this study are in bold. Bar = 0.03 which represents the estimated number of nucleotide substitutions of site per branch.

Taxonomy

Occultibambusa phragmitis G.Y. Wei & Yong Wang bis, *sp. nov.*, Fig. 2

Index Fungorum identifier: IF 900961

Etymology: Named after the host genus *Phragmites*.

Holotype: HGUP 23-0002

Saprobic on *Phragmites australis*. *Sexual* morph: *Ascomata* 79–164 μm high, 198–235 μm diam, black, scattered, immersed, with a short neck, subglobose, with minute ostiolate papilla, flattened at the base. *Ostiolar* neck black, short, with a small opening on the surface of ascomata. *Peridium* 7–79 μm thick, thin at the base and becoming wider laterally, composed of several layers of brown to dark brown, thin-walled cells of *textura angularis*. *Pseudoparaphyses* 2–3 μm wide, cellular, hypha-like, hyaline, septate. *Asci* 60–127(–138) × 13–17.5(–19) μm ($\bar{x}$ = 96.8 × 16 μm, n = 25), 8-spored, bitunicate, fissitunicate, clavate or cylindric-clavate, narrowly rounded at the apex, with a distinct ocular chamber, long pedicellate, up to 44 μm long. *Ascospores* 21–32 × 5–8 μm ($\bar{x}$ = 27.8 × 6.5 μm, n = 30), L/W 4.3, mostly 2-seriate, fusiform, straight or curved, pale brown, mostly 1-septate, minority 2–3-septa, constricted at the septa, upper cell slightly shorter and wider than lower part, guttulate, thin-walled, smooth, partly surrounded by a 1–3 μm thick sheath. *Asexual* morph not seen.

Culture characteristics – Ascospores germinating on PDA within 12–24 h. Colonies on PDA, reaching up to 80 mm diameter after 30 days at 25 °C, with dense, floccose, brown mycelium on the surface, brown to dark brown from below, pale brown margin raised, central embossing.

Material examined – China, Guizhou Province, Qingzhen city, Guang-Yu Wei, 12 March 2022, LB5 (HGUP 23-0002, holotype); ex-type culture GUCC 23-0004; *ibid.*, LB5Z (isotype), living culture GUCC 23-0005.

Occultibambusa jonesii Jin F. Zhang, Jian K. Liu, K.D. Hyde & Zi Y. Liu, in Zhang, Liu, Hyde, Yang & Liu, Mycosphere 8(4): 553 (2017); Fig. 3

Index Fungorum number: IF552743

Saprobic on decaying bamboo. *Sexual* morph *Ascomata* 158–219 μm high, 103–162 μm diam., scattered or in small groups, immersed to semi-immersed, subglobose, with a flattened base, brown to dark brown, with minute *ostiolate*, central papilla, with rounded slot. *Peridium* carbonaceous and fragile, unequal in thickness, composed of rectangular to polygonal celadon cells. *Hamathecium* comprises 1–2.5 μm wide, septate, hypha-like, numerous. *Asci* 63–116(–146) × 10–16 μm ($\bar{x}$ = 93.3 × 12.2 μm, n = 30), 8-spored, bitunicate, fissitunicate, clavate, with a short furcate pedicel, which elongates after discharge (up to 38 μm), apically rounded with a small ocular chamber. *Ascospores* 20–32.5 × 4–7.5 μm ($\bar{x}$ = 24.9 × 5.1 μm, n = 30), L/W 4.9, mostly 2-seriate, narrowly fusiform with acute ends, mostly 1-septate, minority 2–3-septa, not constricted at the septum, septum mostly median, upper cell slightly broader than lower cell, slightly swollen near the septum, straight to curved, brownish, with one large guttule in each cell, smooth-walled, thin-walled, surrounded by a 2–6 μm thick sheath. Germ tube mainly formed from both end cells. *Asexual* morph not seen.

Culture characteristics – On PDA, the colony exhibits a circular form, attaining a diameter of 45 mm within 30 days at a temperature of 25 °C. The colony's upper surface manifests a dark grey hue, while its below side presents a black shade, surface rough, with dense mycelium, dry, raised, and edge entire.

Material examined – China, Guizhou Province, Qingzhen city, on dead bamboo culms near the tobacco field, Qian Zhang, 3 February 2022, WF27-2 (HGUP 23-0003), living culture GUCC 23-0006; *ibid.*, GUCC 23-0471.

Note – Based on our phylogenetic analysis (Fig. 1), our collection GUCC 23-0006 and GUCC 23-0471 have been identified as *O. jonesii*. Notably, HGUP 23-0003 shares identical morphological traits with the ex-type of *O. jonesii* (GZCC 16-0117), except for slightly elongated and thinner asci (63–116(–146) × 10–16 μm vs. (65–)75–89(–105) × 13.5–19 μm), as well as ascospores featuring a sheath (Zhang et al. 2017). Consequently, the morphological and molecular evidances collectively

support the classification of GUCC 23-0006 as *O. jonesii*. This is the first report of this species in the middle region (temperate) of Guizhou Province, which differed from Zhang et al. (2017) in the subtropics.

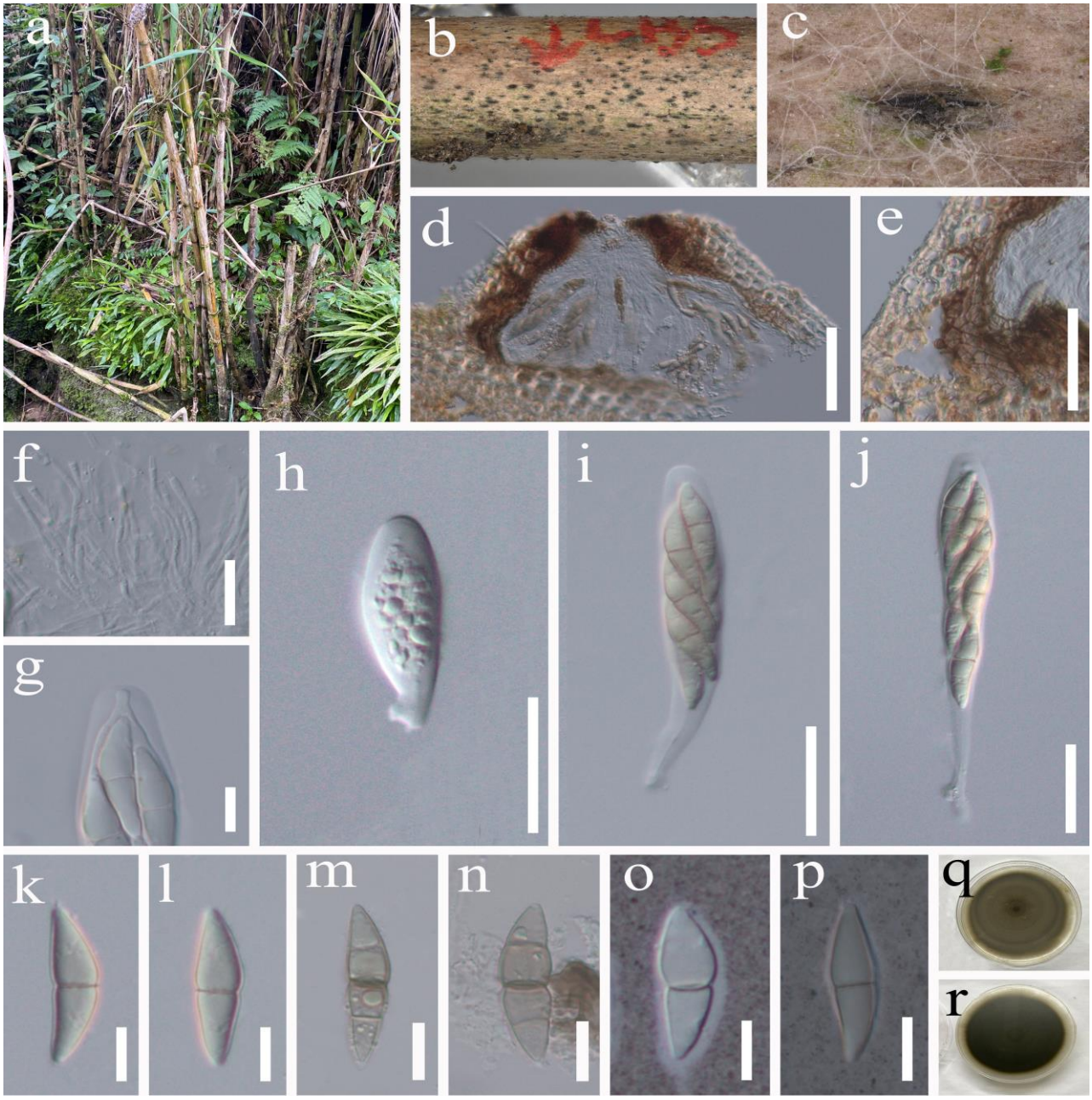

Fig. 2 – *Occultibambusa phragmitis* (HGUP 23-0002, holotype) (a) Host. (b–c) Ascomata on host substrate. (d) Vertical section of ascoma. (e) Structure of peridium. (f) Pseudoparaphyses. (g) Ocular chamber. (h–j) Asci. (k–p) Ascospores. (o–p) Ascospore in Indian Ink. (q, r) Colonies on PDA, above (q), reverse(r). Scale bars: (b) = 1000 μm, (c) = 200 μm, (d–e, i–j) = 50 μm, (f–h) = 20 μm, (k–p) = 10 μm.

Note – *Occultibambusa phragmitis* exhibits morphology resemblances to *O. aquatica* as described by Hyde et al. (2016). Both species possess clavate asci and fusiform, brownish ascospores with straight to curved shapes and guttulate. However, *O. phragmitis* stands out due to its larger asci (60–127 × 13–17.5 μm) compared to *O. aquatica* (73–86 × 9–13 μm), as well as its larger ascospores with more septa (21–32 × 5–8 μm, 1–3-septa) in contrast to *O. aquatica* (19–25 × 3.5–6.5 μm, 1-septate) and with a thinner sheath. Similarities are also observed between

O. phragmitis and *O. jonesii* as detailed by Zhang et al. (2017). Nonetheless, *O. jonesii* displays shorter asci (75–89 µm) with a shorter pedicel and relatively narrower ascospores (5.5–6.5 µm) lacking a mucilaginous sheath. The phylogenetic analyses (Fig. 1) also supported the taxonomic placement of our new taxon.

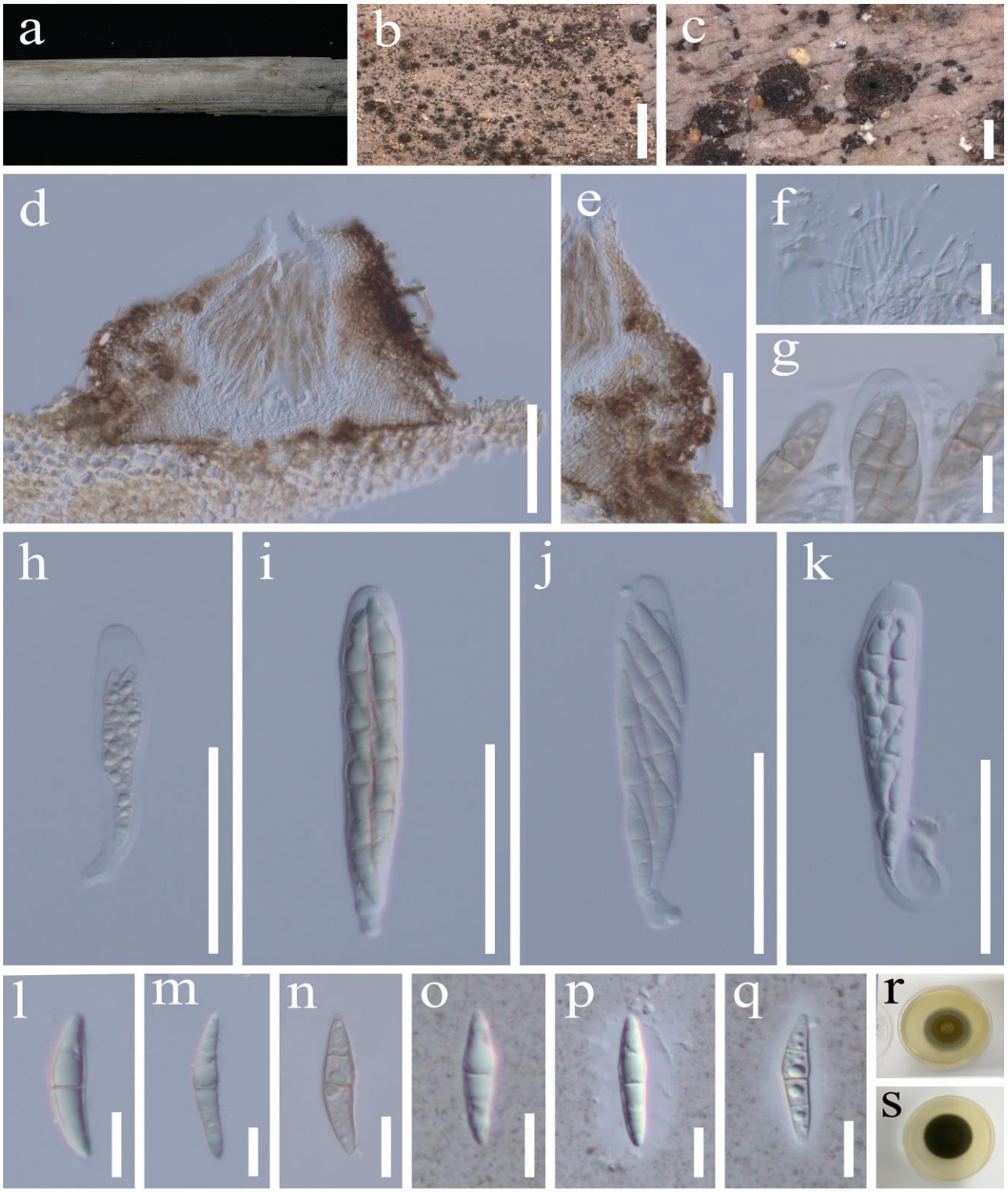

Fig. 3 – *Occultibambusa jonesii* (HGUP 23-0003). (a) Host. (b–c) Ascomata on host substrate. (d) Vertical section of ascoma. (e) Structure of peridium. (f) Pseudoparaphyses. (g) Ocular chamber. (h–k) Asci. (l–q) Ascospores. (o–q) Ascospore in Indian Ink. (r,s) Colonies on PDA, above (r), reverse(s). Scale bars: (b) = 2000 µm, (c) = 200 µm, (d–e, h–k) = 50 µm, (f–g) = 20 µm, (l–q) = 10 µm.

Discussion

Occultibambusaceae members are typically associated with monocotyledons (Dai et al. 2017). Notably, all previously documented *Occultibambusa* species were identified on bamboo hosts, as indicated by studies conducted by Hyde et al. (2016), Dai et al. (2017), Zhang et al. (2017), and Dong et al. (2020). Our strain GUCC 23-0006 was also isolated from bamboo. Interestingly, our newly identified taxon, *Occultibambusa phragmitis* (GUCC 23-0004, GUCC 23-0005) represents the first instance of its occurrence on *Phragmites australis*, a monocot plant. This notable discovery expands the known host range of this fungal genus.

In our phylogenetic tree (Fig. 1), the phylogenetic placement of *Versicolorisporium*, *Neooccultibambusa jonesii* (MFLUCC 16-0643) and *Brunneofusispora hyaline* (MFLUCC 21-0008) lacks clear resolution, rendering their placement unstable. This instability in placement is consistent with observations in previous studies, as indicated by Dong et al. (2020) and Yu et al. (2021), where the placement of *Versicolorisporium* was similarly found to be unstable. However, our new taxon demonstrates both morphological and phylogenetic alignment with the described *Occultibambusa* species. Notably, the absence of sequence data for *Versicolorisporium*, *Neooccultibambusa jonesii* (MFLUCC 16-0643), and *Brunneofusispora hyaline* (MFLUCC 21-0008) sequence data does not impact the phylogenetic relationships among *Occultibambusa* species. Therefore, to enhance the robustness of our analysis, we have excluded the molecular data associated with *Versicolorisporium*, *Neooccultibambusa jonesii* (MFLUCC 16-0643), and *Brunneofusispora hyaline* (MFLUCC 21-0008) from our phylogenetic investigation.

Dai et al. (2017) initially introduced the features of *Occultibambusa* taxa, describing their sunken, dark-colored ascostromata with periphysate ostioles, as well as their broad cylindrical to clavate asci, and dark brown, fusiform, 1-septate ascospores. However, we disagree that 1-septate ascospores exclusively define the characteristics of *Occultibambusa* fungi. In our present study, all strains investigated displayed ascospores with 1 to 3 septa. *Occultibambusa chiangraiensis* and *O. fusispora* also have 1–3-septa ascospores (Hyde et al. 2016, Dai et al. 2017). When the ascospores are young, the ascospores of *O. maolanensis* and *O. pustula* have only 1-septate, but as they mature, the ascospores of *O. maolanensis* and *O. pustula* have 3-septa (Zhang et al. 2017, Dong et al. 2020). There are 3-septa when the ascospores of *O. kunmingensis* are *germinateing* (Dong et al. 2020). Distinctive among the asexual morphs of *Occultibambusa* members is the formation of black necks at the center of ascomata, a feature not documented in other genera within the *Occultibambusaceae* family (Dai et al. 2017). Notably, the fungi in our present study showcase this characteristic.

Our strains introduced herein were found in the karst region of Guizhou, China. Additionally, *Brunneofusispora inclinatiostiola*, *Neooccultibambusa kaiyangensis*, *N. trachycarpi*, *O. jonesii*, and *O. maolanensis* have also been documented in the same karst landform of Guizhou, China, as reported by Zhang et al. (2017) and Yu et al. (2021). While the dataset may be limited, it nevertheless provides insight into the latent fungal diversity concealed within the karst formations of Guizhou, China. This observation underscores the potential richness and complexity of fungal life within this unique geographical setting.

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