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Additions to *Occultibambusaceae* (*Pleosporales*, *Dothideomycetes*): Unrevealing Palmicolous Fungi in China

Xian-Dong Yu [1,†], Sheng-Nan Zhang [1,2,†], Ratchadawan Cheewangkoon [2,*] and Jian-Kui Liu [1,2,*]

1 School of Life Science and Technology, Center for Informational Biology, University of Electronic Science and Technology of China, Chengdu 611731, China; yuxiandong@std.uestc.edu.cn (X.-D.Y.); zshengnanbio@gmail.com (S.-N.Z.)
2 Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
* Correspondence: ratchadawan.c@cmu.ac.th (R.C.); liujiankui@uestc.edu.cn (J.-K.L.); Tel.: +86-028-6183-1832 (J.-K.L.)
† These authors have contributed equally to this work and share first authorship.

Abstract: During a survey of microfungi associated with palms from karst formations, three novel ascomycetes were found from decaying petioles of *Trachycarpus fortunei* (*Arecaceae*) in Guizhou, China. Multi-gene phylogenetic analyses based on a combined SSU, ITS, LSU, RPB2 and TEF1α sequence data showed that these collections were affiliated to *Brunneofusispora* and *Neooccultibambusa* in the family *Occultibambusaceae*. A new species *Brunneofusispora inclinatiostiola* is introduced. It is phylogenetically close to *B. clematidis* but represents a distinct lineage. Morphologically, it differs from the latter in having immersed ascomata with eccentric, periphysate ostiole and smaller ascospores. Morpho-phylogenetic evidence also revealed two new *Neooccultibambusa* species, *N. kaiyangensis* and *N. trachycarpi*. Together with the generic type *N. chiangraiensis*, they formed a distinct lineage within the genus *Neooccultibambusa*. Three novel palmicolous fungi of *Occultibambusaceae* are described, illustrated and notes on their identification are provided. The ecological significance of the new taxa and the phylogenetic relationship of genera in *Occultibambusaceae* is discussed.

Keywords: three new taxa; multi-gene; phylogeny; sexual morph; taxonomy

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1. Introduction

Occultibambusaceae was established by Dai et al. [1] and typified by *Occultibambusa* D.Q. Dai & K.D. Hyde, which is represented by *Bambusicola*-like and *Massarina*-like sexual morphs and a coelomycetous asexual morph with annellidic conidiogenous cells and pale brown, oblong conidia. Five genera are accepted in the family, viz. *Brunneofusispora* S.K. Huang & K.D. Hyde, *Neooccultibambusa* Doilom & K.D. Hyde, *Occultibambusa* D.Q. Dai & K.D. Hyde, *Seriascoma* Phookamsak, D.Q. Dai & K.D. Hyde and *Versicolorisporium* Sat. Hatak., Kaz. Tanaka & Y. Harada [1–4]. *Versicolorisporium* is the genus that only has an asexual morph in the family but is clustered with *Occultibambusa* in recent phylogenetic studies [2,5,6]. The difference between *Seriascoma* and the other four genera is that its sexual morphs have stromatic ascomata under a clypeus [1,7]. *Brunneofusispora* was considered to be different because of phylogenetic distinction and the ascomata of the type species have long necks [4]. *Neooccultibambusa* resembles *Occultibambusa*, however they are phylogenetically distinct, and the former has hyphomycetous asexual morphs or produces chlamydospores in culture [3,8–10].

Most Occultibambusaceae species are saprobes and have been found on Ammophila arenaria (Poaceae), bamboo (Poaceae), Clematis subumbellata (Ranunculaceae), Magnolia denudate (Magnoliaceae), Pandanus sp. (Pandanaceae), Tectona grandis (Lamiaceae), or dead twigs of woody plants in freshwater and terrestrial habitats [1–14]. In this study, saprobic fungi on Trachycarpus fortunei (Arecaceae) from karst formations were collected

in Guizhou province, China. Morphological examination and multi-gene phylogeny based on SSU, ITS, LSU, RPB2 and TEF1α sequence data were carried out to identify these fungi, of which three new Occultibambusaceae species belong to Brunneofusispora and Neooccultibambusa were introduced, respectively.

2. Materials and Methods

2.1. Isolation and Morphological Examination

Decayed petioles of *Trachycarpus fortunei* (*Arecaceae*) were collected from Guiyang city, Guizhou province in China. The samples were brought back to the laboratory in plastic ziplock bags and stored at room temperature. Fungal fruiting bodies were observed using Motic SMZ 168-B stereo microscope and made into slides within water mounts by using a syringe needle. Morphological characters were observed using a Nikon ECLIPSE E200 stereo microscope and photographed by a Nikon ECLIPSE Ni-U compound microscope fitted with a Nikon DS-Ri2 digital camera. All the details of the morphological approaches used in this paper were based on Senanayake et al. [15]. Photographs were processed with Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA). Isolations were obtained from single spore as described in Chomnunti et al. [16]. Herbarium specimens (dry petioles with fungal material) were deposited in the herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), Kunming, China and the herbarium of Guizhou Academy of Agricultural Sciences (GZAAS), Guizhou, China. The isolates obtained in this study were deposited in China General Microbiological Culture Collection Center (CGMCC) and Guizhou Culture Collection (GZCC). The new taxa were registered in MycoBank [17].

2.2. DNA Extraction, PCR Amplification and Sequencing

A Trelief TM Plant Genomic DNA Kit (Beijing TsingKe Biotech Co., Ltd., Beijing, China) was used to extract total genomic DNA from fresh mycelia. DNA amplification was performed by a polymerase chain reaction (PCR). SSU, ITS, LSU, RPB2 and TEF1α sequences were amplified using primer pairs NS1/NS4, ITS5/ITS4, LR0R/LR5, fRPB2-5F/fRPB2-7cR and 983F/2218R, respectively [18–21]. The amplification reactions were performed in 25 μL PCR mixtures containing 22 μL PCR MasterMix (Green) (TsingKe Co., Beijing, China), 1 μL DNA template and 1 μL of each primer (10μM/L). The PCR thermal cycle program for SSU, ITS, LSU, RPB2 and TEF1α amplification were as follows: initial denaturing step of 98 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 47 °C (SSU), 56 °C (ITS, LSU, RPB2), 61.7 °C (TEF1α) for 10 s, elongation at 72 °C for 10 s, and final extension at 72 °C for 5 min. PCR products were checked on 1% agarose electrophoresis gels stained with Gel Red. The sequencing reactions were carried out with primers mentioned above by Beijing Tsingke Biotechnology Co., Ltd., Chengdu, China.

2.3. Phylogenetic Analyses

The quality of the new sequences was initially checked with Finch TV Version 1.4.0 (https://digitalworldbiology.com/FinchTV (accessed on 1 December 2020)). The BLAST was performed to find the similar sequences that match our data. A concatenated dataset of the SSU, ITS, LSU, RPB2 and TEF1α sequences was used for phylogenetic analyses with the inclusion of reference taxa from GenBank (Table 1). Sequences were aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/ (accessed on 11 June 2021)) [22] and then checked visually and manually optimized using BioEdit v.7.0.9 [23]. Each dataset was concatenated with Mesquite v. 3.11 (http://www.mesquiteproject.org/ (accessed on 11 June 2021)) for multi-gene analyses. Maximum likelihood (ML), maximum parsimony (MP) and bayesian inference (BI) were carried out as detailed in Dissanayake et al. [24]. The programs used include RAxMLGUI v. 1.0 [25], PAUP v.4.0b10 [26], MrModeltest 2.3 [27] and MrBayes v. 3.1.2 [28,29]. Phylogenetic tree was visualized

by FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 11 June 2021)), and the alignment was submitted in TreeBASE (Study ID S28856 (accessed on 7 October 2021)).

Table 1. Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers. The ex-type strains are indicated in bold and newly generated sequences are indicated in red.

Species	Voucher/Strain/ Isolate	GenBank Accession Number				
		SSU	ITS	LSU	RPB2	TEF1α
Brunneofusispora clematidis	**MFLUCC 17-2070**	**MT226685**	**MT310615**	**MT214570**	**MT394692**	**MT394629**
Brunneofusispora hyalina	**MFLUCC 21-0008**	**MW485613**	**MW260330**	**MW287234**	**MW512609**	**MW512606**
Brunneofusispora inclinatiostiola	**CGMCC 3.20403**	**MZ964884**	**MZ964866**	**MZ964875**	**OK061075**	**OK061069**
Brunneofusispora inclinatiostiola	GZCC 21-0185	MZ964885	MZ964867	MZ964876	OK061076	OK061070
Brunneofusispora sinensis	**KUMCC 17-0030**	**MH393556**	**MH393558**	**MH393557**	_	**MH395329**
Brunneofusispora sinensis	MFLUCC 20-0016	MT159636	MT159630	MT159624	MT159613	MT159607
Brunneofusispora sp.	X135	_	MK304223	_	_	_
Massarina rubi	CBS 691.95	GU456301	_	FJ795453	FJ795470	_
Massarina rubi	MUT 4323	_	KF636766	KF636772	_	_
Massarina rubi	MUT 4887	KT587318	KR014359	KP671721	_	_
Neooccultibambusa chiangraiensis	**MFLUCC 12-0559**	**KU712458**	**KU712442**	**KU764699**	_	**KU872761**
Neooccultibambusa jonesii	**MFLUCC 16-0643**	**KY111438**	_	**KY111437**	_	_
Neooccultibambusa kaiyangensis	**CGMCC 3.20404**	**MZ964886**	**MZ964868**	**MZ964877**	**OK061077**	**OK061071**
Neooccultibambusa kaiyangensis	GZCC 21-0184	MZ964887	MZ964869	MZ964878	OK061078	OK061072
Neooccultibambusa pandanicola	**KUMCC 17-0179**	**MG298942**	**MG298941**	**MG298940**	**MG298944**	**MG298943**
Neooccultibambusa thailandensis	**MFLUCC 16-0274**	**MH260348**	**MH275074**	**MH260308**	**MH412758**	**MH412780**
Neooccultibambusa trachycarpi	**CGMCC 3.20405**	**MZ964888**	**MZ964870**	**MZ964879**	**OK061079**	**OK061073**
Neooccultibambusa trachycarpi	GZCC 21-0181	MZ964889	MZ964871	MZ964880	OK061080	OK061074
Occultibambusa aquatica	**MFLUCC 11-0006**	**KX698112**	**KX698114**	**KX698110**	_	_
Occultibambusa bambusae	**MFLUCC 13-0855**	**KU872116**	**KU940123**	**KU863112**	**KU940170**	**KU940193**
Occultibambusa chiangraiensis	**MFLUCC 16-0380**	**KX655551**	_	**KX655546**	**KX655566**	**KX655561**
Occultibambusa fusispora	**MFLUCC 11-0127**	_	**KU940125**	**KU863114**	**KU940172**	**KU940195**
Occultibambusa jonesii	**GZCC 16-0117**	**KY628324**	_	**KY628322**	**KY814758**	**KY814756**
Occultibambusa kunmingensis	**HKAS 102151**	**MT864342**	**MT627716**	**MN913733**	**MT878453**	**MT954407**
Occultibambusa maolanensis	**GZCC 16-0116**	**KY628325**	_	**KY628323**	**KY814759**	**KY814757**
Occultibambusa pustula	**MFLUCC 11-0502**	**KU872118**	**KU940126**	**KU863115**	_	_
Ohleria modesta	**CBS 141480**	**KX650513**	**KX650563**	**KX650563**	**KX650583**	**KX650534**
Ohleria modesta	MGC	_	KX650562	KX650562	KX650582	KX650533
Seriascoma didymosporum	**MFLUCC 11-0179**	**KU872119**	**KU940127**	**KU863116**	**KU940173**	**KU940196**
Seriascoma yunnanense	**MFLU 19-0690**	**MN174694**	_	**MN174695**	**MN210324**	**MN381858**
Versicolorisporium triseptatum	JCM 14775	AB524501	AB365596	AB330081	_	_

3. Results

3.1. Phylogenetic Analyses

The reference dataset included representatives of the five accepted genera in *Occultibambusaceae*, as well as three *Massarina rubi*, which were detected by blastn search of our strains. Five gene loci SSU, ITS, LSU, RPB2 and TEF1α were used to determine the phylogenetic placement of the new collections. The concatenated matrix comprised of 31 taxa with a total of 4556 characters (SSU: 1053 bp; ITS: 663 bp; LSU: 881 bp; RPB2: 948 bp; TEF1α: 1011 bp) including gaps. Maximum likelihood (ML), Maximum-parsimony (MP) and Bayesian analysis of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies. The best scoring ML tree (Figure 1) was selected to represent the relationships among taxa, in which a final likelihood value of −19,906.313448 is presented. The matrix had 1442 distinct alignment patterns. Estimated base frequencies were as follows: A = 0.245814, C = 0.251328, G = 0.269846, T = 0.233012; substitution rates AC = 1.712622, AG = 3.759418, AT = 1.465059, CG = 1.312466, CT = 8.469117, GT = 1.000000. GTR+I+G is the best-fit model selected by AIC in MrModeltest based on each gene (SSU, ITS, LSU, RPB2 and TEF1α), which is used for maximum likelihood and Bayesian analysis. The maximum parsimonious dataset consists of 4,556 characters, of which 3381 characters were constant and 307 variable characters parsimony uninformative. Maximum parsimony analysis of the remaining 868 parsimony-informative characters resulted in 1000 trees with TL = 2859, CI = 0.567, RI = 0.674, RC = 0.382, and HI = 0.433. Six simultaneous Markov chains were run for 595,000 generations and trees were sampled every 1000 generation and 595 trees were obtained. The first 119 trees representing the burn-in phase of the analyses were discarded, while the remaining 476 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01).

The six isolates obtained in this study grouped with *Brunneofusispora* and *Neoocculitibambusa* based on the multi-gene phylogeny analyses. Two of them were sister to *Brunneofusispora clematidis* with strong support and can be recognized as a new species *B. inclinatiostiola*. The other four strains were closely related to the generic type *N. chiangraiensis* and formed a well-supported clade (Figure 1), of which two new species *N. kaiyangensis* and *N. trachycarpi* were recognized. *Neooccultibambusa kaiyangensis* clustered together with *N. chiangraiensis* but can be morphologically and phylogenetically distinguished, while *N. trachycarpi* showed to be sister to *N. chiangraiensis* and *N. kaiyangensis*.

3.2. Taxonomy

Brunneofusispora inclinatiostiola S.N. Zhang & Jian K. Liu, sp. nov., Figure 2.

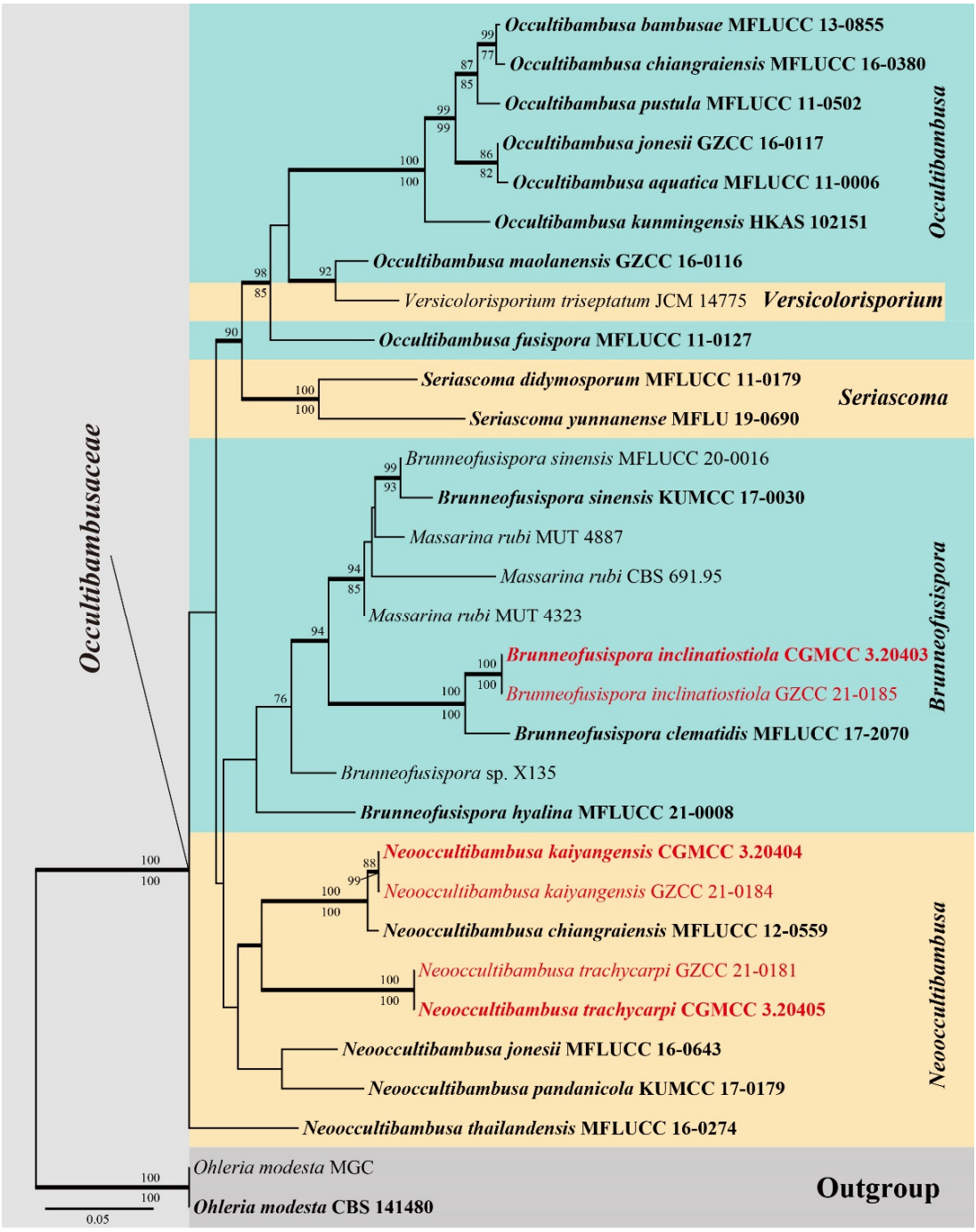

Figure 1. RAxML tree generated from combined SSU, ITS, LSU, RPB2 and TEF1α sequence data. Bootstrap values for ML and MP equal to or greater than 75% are placed above and below the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 are in bold. The tree is rooted with *Ohleria modesta* (CBS 141480) and *O. modesta* (MGC). The ex-type strains are indicated in bold and newly generated sequences are indicated in red.

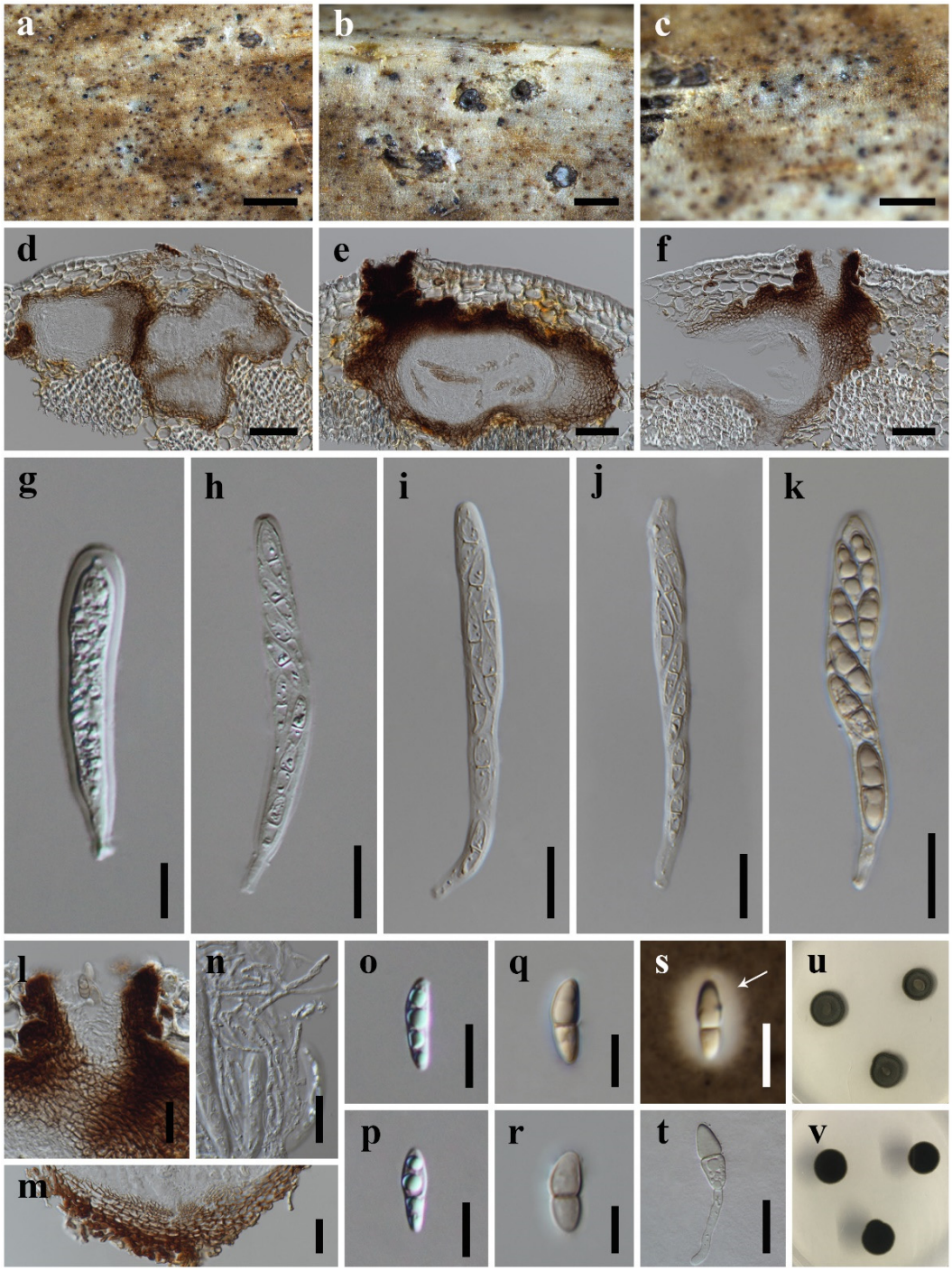

Figure 2. ***Brunneofusispora inclinatiostiola*** (HKAS 113022, **holotype**) (**a**–**c**) Ascomata on host substrate. (**d**–**f**) Vertical section of ascoma. (**g**–**k**) Asci. (**l**) Ostiole, showing periphyses. (**m**) Structure of peridium. (**n**) Pseudoparaphyses. (**o**–**s**) Ascospores (**s**) Arrow indicated mucilaginous sheath). (**t**) Germinated ascospore. (**u**,**v**) Colonies on PDA, above (**u**) and reverse (**v**). Scale bars: (**a**) = 1000 μm, (**b**,**c**,) = 500 μm, (**d**–**f**) = 50 μm, (**g**,**o**–**s**) = 10 μm, (**h**–**n**,**t**) = 20 μm.

MycoBank: MB 841009;

Etymology: The epithet "*inclinatiostiola*" in reference to the incline ostiole.

Holotype: HKAS 113022

Saprobic on decaying petioles of *Trachycarpus fortunei*. **Sexual morph**: *Ascomata* are solitary, scattered to gregarious, immersed, slightly erumpent the host epidermis, visible as whitish-grey to pale brown area with a small black dot, in vertical section 90–230 μm high, 150–340 μm diam., depressed globose or irregular subglobose, uniloculate, with

an immersed ostiolar neck. *Ostiole* are usually ecentric, brown to black and periphysate. *Peridium* 15–55 µm wide, sometimes up to 70 µm at the side, composed of several layers of hyaline to brown, thick-walled cells of *textura angularis*, darker and thicker outwardly. *Hamathecium* are 2.0–3.5 µm wide, cellular pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 60–145 × 7.5–13.5 µm ($\overline{x}$ = 100.6 × 10.3 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical or cylindric-clavate, straight or slightly curved, short pedicellate, rounded at the apex, with an ocular chamber. *Ascospores* are (13.5–)14.0–19.0(–27.0) × 4.0–6.5 µm ($\overline{x}$ = 17.4 × 4.7 µm, n = 50), uniseriate to partially biseriate, fusiform to ellipsoidal, hyaline, becoming pale brown at maturity, 1-septate, the upper cell slightly wider than the lower cell, guttulate when young, smooth-walled, surrounded by a mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA reaching 12–15 mm diam. after 21 days at 25 °C, circular, raised to umbonate, the margin entire, dark green, surface rough, dry, cottony, reverse dark green to black.

Material examined: CHINA, Guizhou Province, Guiyang City, Kaiyang County, Nanjiang Gorge Scenic Area, 26°57′24″ N, 106°59′19″ E, 2900 ft Elevation, on decaying petioles of *Trachycarpus fortunei* (*Arecaceae*), 19 March 2019, S.N. Zhang, SNC56 (HKAS 113022, holotype); ex-type living culture CGMCC 3.20403; *ibid.*, 26°57′36″ N, 106°59′34″ E, 2860 ft Elevation, on decaying petioles of *Trachycarpus fortunei* (*Arecaceae*), 19 March 2019, S.N. Zhang, SNC51 (GZAAS 21-0046, paratype); living culture GZCC 21-0185.

Notes: *Brunneofusispora inclinatiostiola* resembles *B. clematidis* in having fusiform to ellipsoidal ascospores surrounded by mucilaginous sheaths [13]. However, *B. inclinatiostiola* has smaller ascospores than that of *B. clematidis* ((13.5–)14.0–19.0(–27.0) × 4.0–6.5 µm vs. 17–35 × 5–10 µm). The phylogenetic results (Figure 1) indicated that *B. inclinatiostiola* is a phylogenetically distinct species. In addition, the comparison of nucleotide differences between *B. inclinatiostiola* and *B. clematidis* showed that there are 5.0% (23/460) differences in ITS, 2.4% (21/868) in RPB2 and 4.3% (25/577) in TEF1α gene regions, which also supports the introduction of *B. inclinatiostiola* as a new species.

Neooccultibambusa kaiyangensis X.D. Yu, S.N. Zhang & Jian K. Liu, sp. nov., Figure 3.

MycoBank: MB 841010;

Etymology: The epithet "*kaiyangensis*" in reference to Kaiyang County, where the fungus was collected.

Holotype: HKAS 113021.

Saprobic on rachides or petioles of palms. **Sexual morph**: *Ascomata* are scattered to gregarious, immersed, visible as brown or dark area with erumpent black dots on host surface, in vertical section 200–360 µm high, 110–270 µm diam., coriaceous, uniloculate, ampulliform, or globose with an ostiolar neck beneath the host surface. *Ostiole* are central, periphysate. *Peridium* are 16.5–38.0 µm wide, comprising several layers of brown, thin-walled cells of *textura intricate*, and hyaline, compressed cells of *textura angularis*. *Hamathecium* are 1.0–1.8 µm wide, cellular pseudoparaphyses, anastomosing above asci, embedded in a gelatinous matrix. *Asci* are 70–108 × 7.5–11.0 µm ($\overline{x}$ = 85.1 × 9.3 µm, n = 20), 8-spored, bitunicate, cylindrical to clavate, with short furcate pedicel, apically rounded, with an ocular chamber. *Ascospores* are 16.0–23.5 × 3.0–5.5 µm ($\overline{x}$ = 20.1 × 4.5 µm, n = 50), overlapping biseriate, hyaline to pale brown, fusoid, 1–3-septate, constricted at the center septum, guttulate when young, smooth, surrounded by a gelatinous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies on PDA and attaining a diameter about 13 mm after 21 days at 25 °C, circular, medium dense, dark olive green, reverse dark green.

Material examined: CHINA, Guizhou Province, Guiyang City, Kaiyang County, Nanjiang Gorge Scenic Area, 26°57′24″ N, 106°59′19″ E, 2900 ft Elevation, on decaying petioles of *Trachycarpus fortunei* (*Arecaceae*), 19 March 2019, S.N. Zhang, SNC53 (HKAS 113021, holotype); ex-type living culture CGMCC 3.20404; *ibid.*, 26°57′36″ N, 106°59′34″ E, 2860 ft Elevation, on decaying petioles of *Trachycarpus fortunei* (*Arecaceae*), 19 March 2019, S.N. Zhang, SNC50 (GZAAS 21-0045, paratype); living culture GZCC 21-0184.

Notes: *Neoocculitibambusa kaiyangensis* are clustered with the generic type *N. chiangraiensis* with absolute bootstrap support (100% ML/100% MP/1.00 BYPP, Figure 1). However, they can be recognized as different species. *N. kaiyangensis* differs from *N. chiangraiensis* [3] in having relatively smaller asci (70–108 × 7.5–11.0 vs. (70–)115–160(–207) × 14–21) and ascospores (16.0–23.5 × 3.0–5.5 vs. (33–)36–37(–43) × 8–13) (Table 2). The comparison of molecular data of *N. kaiyangensis* and *N. chiangraiensis* showed that there are 0.65% (3/457) nucleotide differences in ITS and 1.6% (10/613) in TEF1α gene regions. Although these differences are minimal, *N. kaiyangensis* has formed a separate branch in the multi-gene phylogeny. The morphological and phylogenetic differences support the delineation of *N. kaiyangensis* as a new species.

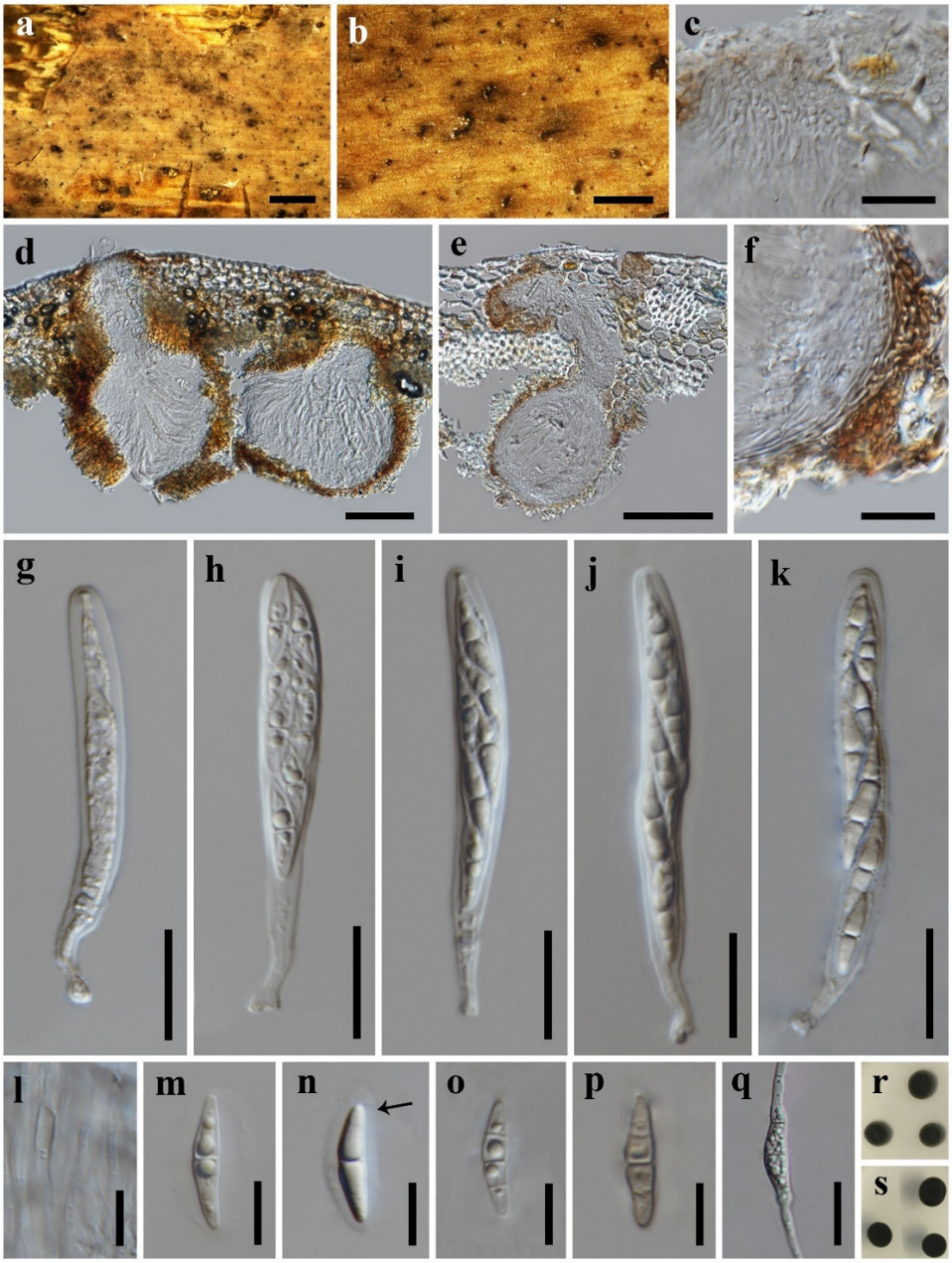

Figure 3. ***Neoocculitibambusa kaiyangensis*** (HKAS 113021, **holotype**) (**a**,**b**) Ascomata on host substrate. (**c**) Ostiole with periphyses (**d**,**e**) Vertical section of ascoma. (**f**) Structure of peridium. (**g**–**k**) Asci. (**l**) Pseudoparaphyses. (**m**–**p**) Ascospores (**n**) Arrow indicated mucilaginous sheath). (**q**) Germinated ascospore. (**r**,**s**) Colonies on PDA, above (**r**), reverse (**s**). Scale bars: (**a**) = 1000 μm, (**b**) = 500 μm, (**c**,**f**–**k**,**q**) = 20 μm, (**d**,**e**) = 100 μm, (**l**–**p**) = 10 μm.

Table 2. Comparative morphological data on species of *Neooccultibambusa*.

Taxa	Ascomata		Asci (μm)	Ascospores		References
	Morphology	Size (μm)		Morphology	Size (μm)	
N. chiangraiensis [1]	Immersed to erumpent, without a neck, globose to subglobose	(250–)345–355(–400) × (230–)245–295(–325)	(70–)115–160(–207) × 14–21	Fusoid, hyaline to pale brown, 1–3 septate, with a sheath	(33–)36–37(–43) × 8–13	[3]
N. jonesii [2]	Immersed to erumpent, without a neck, globose to subglobose	104–155 × 130–160	47–76 × 8–10	Fusiform, brown when matrure, 1-septate, submedian, without a sheath	15–20 × 2.5–4.5	[8]
N. kaiyangensis	Immersed to erumpent, without a neck, depressed globose or irregular	200–360 × 110–270	70–108 × 7.5–11.0	Fusiform, hyaline to pale brown, 1–3-septate, with a sheath	16.0–23.5 × 3.0–5.5	This study
N. thailandensis	Superficial, globose to subglobose	65–80 × 44–61	34–51 × 5–8	Fusiform, yellow–brown, 1-septate, without a sheath	6–11 × 2–3.5	[10]
N. trachycarpi	Immersed to erumpent, without a neck, depressed globose or irregular	150–240 × 140–200	48–120 × 10–15	Fusiform, hyaline, 1-septate, becoming light brown and present two pseudosepta when senescent, with a sheath	12–19 × 3.0–5.0	This study
	Conidiophores		Conidiogenous cells size (μm)	Conidia (Chlamydospores)		
	morphology	size (μm)		morphology	size (μm)	
N. pandanicola [3]	Pale-brown to brown, 3–5-septate	13–71 × 3.5–7	2.5–5.5 × 4–5.5	Obclavate, olivaceous brown to mid-brown, 7–17-euseptate	28–150 × 7–21	[9]

[1] *N. chiangraiensis*: the asexual morph was known as chlamydospores in culture, which is characterized by hyaline to brown, 1-septate, subglobose chlamydospores, (7.5–)12–14(–16) × (8.5–)12–14(–17) [3]. [2] *N. jonesii*: the asexual morph was observed in culture, which is characterized by dark brown, septate conidiophores, with pale to dark brown, unicellular, subglobose to globose conidia, chlamydospore-like, 10–14(–16) × 8–14(–17) [8]. [3] *N. pandanicola*: it is an asexual morph species found on dead leaves of *Pandanus*.

Neooccultibambusa trachycarpi X.D. Yu, S.N. Zhang & Jian K. Liu, sp. nov., Figure 4.

MycoBank: MB 841011;

Etymology: The epithet "*trachycarpi*" in reference to the host plant genus "*Trachycarpus*".

Holotype: HKAS 113020.

Saprobic on decaying petioles of *Trachycarpus fortunei*. **Sexual morph**: *Ascomata* are solitary to gregarious, scattered, immersed to erumpent, visible as small, black dots on host surface, in vertical section 150–240 μm high, 140–200 μm diam., uniloculate, depressed globose or irregular, ostiolate. *Ostiole* are circular, central, periphysate. *Peridium* are 11–38 μm wide, composed of pale brown to brown textura angularis cells, thicker outwardly. *Hamathecium* are 2.0–3.2 μm wide, hyphae-like, cellular pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 48–120 × 10–15 μm ($\overline{x}$ = 75.3 × 12.1 μm, n = 20), 8-spored, bitunicate, cylindric-clavate, with a short furcate to rounded pedicel, apically rounded, with an ocular chamber. *Ascospores* are 12–19 × 3.0–5.0 μm ($\overline{x}$ = 15.6 × 3.9 μm, n = 50), overlapping biseriate, hyaline, fusiform, tapering towards the ends, 1-septate, constricted at the center septum, becoming pale brown and present two pseudosepta when senescent (Figure 4r), guttulate when young, smooth, surrounded by mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics: Colonies growing well on PDA and attaining a diameter about 12 mm after 21 days at 25 °C, circular, medium dense, olive green, reverse dark green.

Material examined: CHINA, Guizhou Province, Guiyang City, Kaiyang County, Nanjiang Gorge Scenic Area, 26°56′28″ N, 106°58′12″ E, 2800 ft Elevation, on decaying petioles of *Trachycarpus fortunei* (*Arecaceae*), 19 March 2019, S.N. Zhang, SNC40 (HKAS 113020, holo-

type), ex-type living culture CGMCC 3.20405; *ibid.*, SNC39, (GZAAS 21-0043, paratype), living culture GZCC 21-0181.

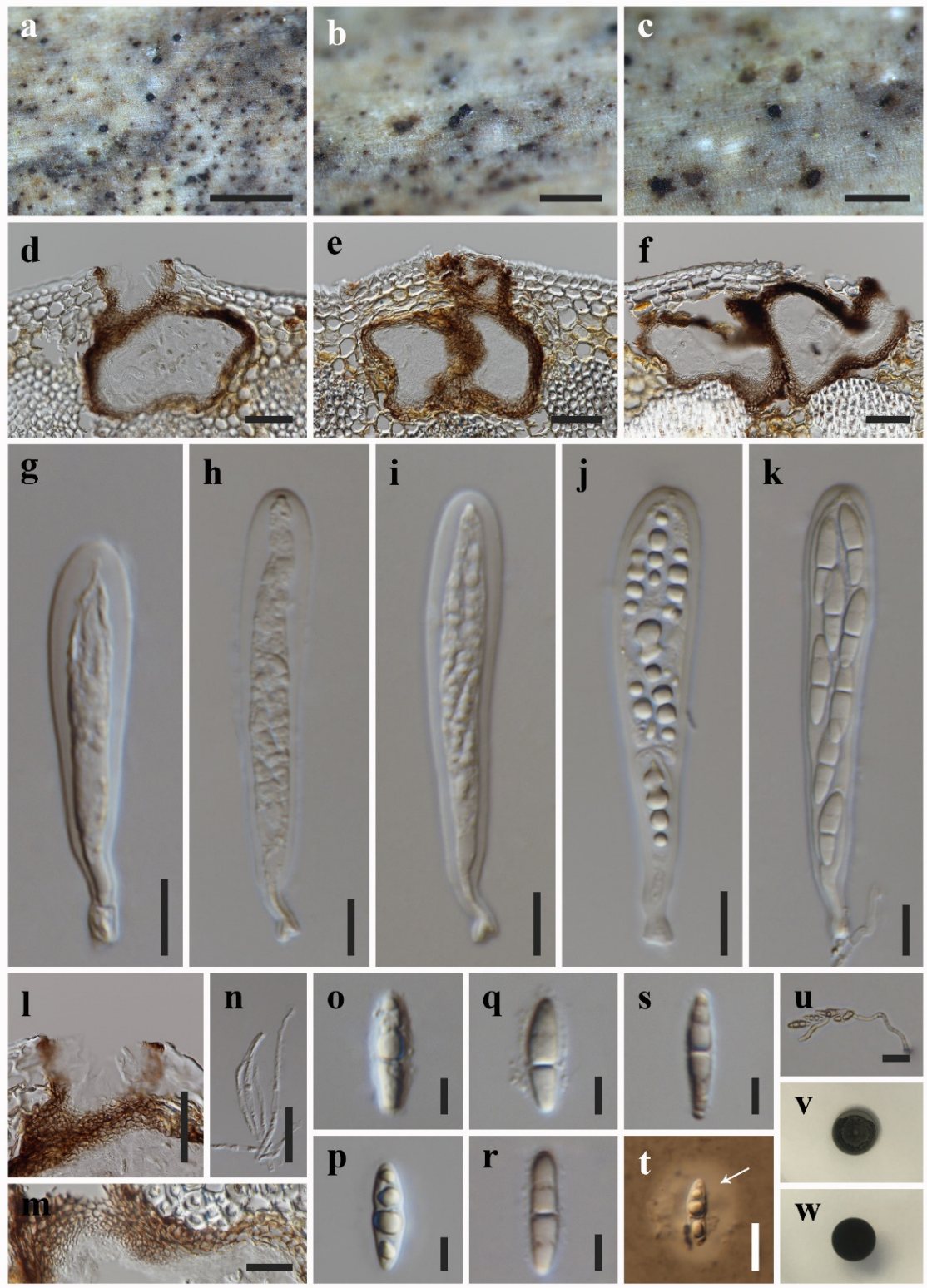

Figure 4. *Neooccultibambusa trachycarpi* (HKAS 113020, **holotype**) (**a**–**c**) Ascomata on host substrate. (**d**–**f**) Vertical section of ascoma. (**g**–**k**) Asci. (**l**) Ostiole, showing periphyses. (**m**) Structure of peridium. (**n**) Pseudoparaphyses. (**o**–**t**) Ascospores ((**t**) Arrow indicated mucilaginous sheath). (**u**) Germinated ascospores. (**v**,**w**) Colonies on PDA, above (**v**), reverse (**w**). Scale bars: (**a**,**b**) = 1000 μm, (**c**) = 500 μm, (**d**–**f**,**l**) = 50 μm, (**g**–**k**,**t**) = 10 μm, (**m**,**n**,**u**) = 20 μm, (**o**–**s**) = 5 μm.

Notes: *Neooccultibambusa trachycarpi* resembles *N. chiangraiensis* and *N. kaiyangensis* in having immersed ascomata, fusiform, hyaline to pale brown, septate ascospores with mucilaginous sheath. However, *N. trachycarpi* differs from the latter ones in having hyaline, 1-septate ascospores, which become pale brown when senescent and present two pseudosepta. The ascospores dimensions are also different (Table 2). The phylogenetic results (Figure 1) showed that *Neooccultibambusa trachycarpi* clustered with *N. chiangraiensis* and *N. kaiyangensis* and formed a distinct lineage within *Neooccultibambusa*. The establishment of the new species *Neooccultibambusa trachycarpi* is justified by morphological and phylogenetic evidence.

4. Discussions

A recent contribution to *Occultibambusaceae* is the introduction of new species for *Occultibambusa* and *Serisacoma* [30]. It also discussed that *Occultibambusa* and *Versicolorisporium* are phylogenetically related, but they are different genera due to their significant difference in asexual morphs [30]. In this study, multi-gene phylogenetic analyses showed our samples were affiliated to *Brunneofusispora* and *Neooccultibambusa*, respectively.

Based on a blastn search of LSU sequence data of *Brunneofusispora inclinatiostiola* in NCBI GenBank, the closest hits are *Massarina rubi* (MUT 4323, identities 98.11%, query cover 100%; MUT 4887, identities 97.76%, query cover 100%), *Brunneofusispora sinensis* (MFLUCC 20-0016, identities 97.96%, query cover 98%). A blastn search of LSU sequence data of *Neooccultibambusa trachycarpi* in NCBI GenBank, the closest hits are *Massarina* sp. (MUT 4863, identities 98.31%, query cover 100%; MUT 4860, identities 97.47%, query cover 100%), *Brunneofusispora hyaline* (MFLU 21-0016, identities 97.35%, query cover 100%), followed by *Massarina rubi* (MUT 4887, identities 97.35%), *Neooccultibambusa thailandensis* (MFLUCC 16-0274, identities 96.68%), *N. pandanicola* (KUMCC 17-0179, identities 96.68%). Therefore, phylogenetic analysis was performed based on different matrices with the inclusion of those *Massarina* sp. and/or *Massarina rubi* (data not shown) and the multi-gene phylogeny result (Figure 1) supports the recognition of three new species. On the other hand, the genus *Neooccultibambusa* is not monophyletic (Figure 1), of which *N. thailandensis* formed an independent lineage in *Occultibambusaceae*. However, this is consistent with recent relevant studies [6,30] and further studies are needed to provide a better understanding towards the classification of *Neooccultibambusa* with more sampling and taxa population included in the analysis.

Karst landscape is attractive to tourists but also inhabits rich biodiversity due to its typical low temperature and high humidity environment. The fan palm *Trachycarpus fortunei* (*Arecaceae*) is native to subtropical and temperate mountain forests of China and 23 fungal species have been described from this plant [31,32]. The three new species introduced herein were found on *Trachycarpus fortunei* in a karst region. Additionally, *Occultibambusa jonesii* and *O. maolanensis* were also reported from karst landforms in China [12]. It is noteworthy that they are distributed in three different genera in *Occultibambusaceae.* Although this is only a small sample of data, it reflects somehow the hidden fungal diversity of palms and the karst formations.

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