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Introducing Seriascoma yunnanense sp. nov. (Occultibambusaceae, Pleosporales) based on evidence from morphology and phylogeny

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Abstract

In this study, we introduce a new fungal species from dead branches of bamboo (*Poaceae*) in Yunnan Province, China. Combined LSU, SSU, *RPB2* and *TEF1-α* gene sequence data analysis showed it belongs to the family *Occultibambusaceae* (Pleosporales). Maximum likelihood and Bayesian analyses were performed to clarify the phylogenetic affinities of the taxon. The novel species is typical to *Seriascoma* in its elongated, gregarious, ostiolate ascostromata, immersed beneath the clypeus and clavate to fusiform, didymosporous, hyaline ascospores, but differs from *S. didymospora* in having uni-loculate ascostromata, short pedicellate asci and ascospores with gelatinous sheath and relatively large guttules. The new taxon is compared with known *Seriascoma* species and a comprehensive description and micrographs are provided.

Key words – 1 new taxon – Dothideomycetes – Multi-gene – Phylogenetic analysis – Taxonomy

Introduction

Occultibambusaceae D.Q. Dai & K.D. Hyde was introduced by Dai et al. (2017) and is typified by Occultibambusa D.Q. Dai & K.D. Hyde (with the type species O. bambusae D.Q. Dai & K.D. Hyde). The family comprises the genera Neooccultibambusa Doilom & K.D. Hyde, Occultibambusa, Seriascoma Phook., D.Q. Dai & K.D. Hyde and Versicolorisporium Sat. Hatak., Kaz. Tanaka & Y. Harada (Hatakeyama et al. 2008, Doilom et al. 2017, Wijayawardene et al. 2018). Members of Occultibambusaceae usually occur on the monocotyledons such as bamboo and have also been found on teak (Tectona grandis) (Dai et al. 2017, Doilom et al. 2017). Species of Occultibambusaceae have immersed, solitary to gregarious ascomata with black ostioles, bitunicate, broadly cylindrical to clavate asci, cellular pseudoparaphyses and broad-fusiform, hyaline to dark brown, 1–3 septate ascospores (Dai et al. 2017). The asci and ascospore characters of Occultibambusaceae are similar to some other members in Pleosporales, viz. Bambusicola D.Q. Dai & K.D. Hyde (Bambusicolaceae), Lophiostoma Ces. & De Not. (Lophiostomataceae) and

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Massarina Sacc., (Massarinaceae) (Zhang et al. 2009, Dai et al. 2012, 2015, Tibpromma et al. 2018). Asexual morphs of *Occultibambusaceae* are diverse in their morphology, which often comprises eustromatic conidiomata, phialidic conidiogenous cells with enteroblastic conidiogenesis (Dai et al. 2017).

The monotypic genus *Seriascoma*, typified by *S. didymospora* Phookamsak, D.Q. Dai & K.D. Hyde was introduced by Dai et al. (2017) and found on bamboo (Dai et al. 2017). *Seriascoma* species are characterized by elongated, gregarious, coriaceous, multiloculate, ostiolate ascostromata, immersed beneath a clypeus, which are erumpent with a slit-like opening, a thick-walled peridium composed of dark brown cell layers of *textura angularis* and clavate to fusiform, didymosporous, hyaline ascospores arranged in bitunicate asci (Dai et al. 2017). *Seriascoma* can be distinguished from other genera in *Occultibambusaceae* by having ascostromata occurring under a clypeus (Dai et al. 2017). The asexual morphs of *Seriascoma* comprise solitary to gregarious, conical, uniloculate, black, eustromatic conidiomata (Dai et al. 2017). The conidiomatal wall consists of pseudoparenchymatous cells, arranged in a *textura angularis* and produce oblong, aseptate, hyaline conidia (Dai et al. 2017).

The purpose of this study is to introduce a novel taxon, *Seriascoma yunnanense*, which was collected from Yunnan Province, China. Morphological comparison and Maximum likelihood (ML), Bayesian inference (BI) analyses of combined LSU, SSU, *RBP2* and *TEF1-* α sequence data confirmed the phylogenetic placement of the novel species within the family *Occultibambusaceae*.

Materials & Methods

Sample collection and morphological studies

Dead branches of bamboo samples were collected from Yunnan Province, China on 24 May 2018 and brought to the laboratory in Zip lock plastic bags. Slides were prepared by hand-sectioning of ascostromata and examined using the Motic SMZ-171 stereomicroscope. Indian ink was used to observe the gelatinous sheath of the ascospores. Morphological characters were observed using Nikon Eclipse 80i compound microscope and photomicrography recorded with a Canon 600D digital camera fitted to the microscope. Measurements of morphological characters were taken using Tarosoft (R) Image Frame Work version 0.9.7. Adobe Photoshop CS3 Extended version 10.0 was used to prepare the photo plates. The herbarium material was deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and the Herbarium of Kunming Institute of Botany (HKAS), China. Faces of fungi number was obtained as explained in Jayasiri et al. (2015) and Index Fungorum number was registered in Index Fungorum (2019).

DNA extraction, PCR amplification and sequencing

Genomic DNA was directly extracted from the ascostromata using a DNA extraction kit (E.Z.N.A.® Forensic DNA kit, D3591-01, Omega Bio-Tek) following the manufacturer's instructions. Large subunit rDNA (LSU), Small subunit rDNA (SSU), RNA polymerase II second largest subunit gene (RPB2) and Translation elongation factor $1-\alpha$ gene (TEF1- α) gene were amplified with LR0R/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994), NS1/NS4 (White et al. 1990), RPB2-5f/fRPB2-7cr (Liu et al. 1999, Sung et al. 2007) and EF1-983F/EF1-1567R (Rehner 2001, Rehner & Buckley 2005) primers in respectively. PCR amplification reactions were done using the following PCR components. The final volume of the PCR mixture was 25 µl containing 11 μl ddH₂O, 11 μl of 2 × PCR Super Master Mix (TIANGEN Co., China), 1 μl of DNA template, 1 µl of each forward and reverse primers (10 µl). The temperature profile for the amplification of LSU, SSU and $TEF1-\alpha$ gene regions were performed with initially denaturing step at 94 °C for 3 min, followed by 35 amplification cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, extension at 72 °C for 1 min and final extension step of 72 °C for 10 min. Thermal cycle for RBP2 gene amplification was initially denaturing at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 2 min, extension at 72 °C for 90 s and final extension step of 72 °C for 10 min. The PCR products were observed on 2% agarose

electrophoresis gels stained with ethidium bromide. Purification and DNA sequencing were performed at Sunbiotech Company, Beijing, China.

Phylogenetic analysis

Sequences generated from reverse and forward primers were combined using SeqMan software version: 7.1.0 (44.1). Sequences of the family Occultibambusaceae were obtained from the GenBank following recent publications and literature (Dai et al. 2017, Zhang et al. 2017). initially aligned 6.864b Sequencing data were using **MAFFT** software (http://mafft.cbrc.jp/alignment/server/index.html). The single-gene alignment of each region was refined manually with Bioedit 7 v. 7.0.9.0 (Hall 1999) and improved using Trimmal (Phylemon 2.0) (http://phylemon.bioinfo.cipf.es/alignment.html). Phylogenetic analysis was carried out using ML and BI analyses. ML trees for each single gene region and combined gene regions were generated using the RAxML-HPC2 on XSEDE (v. 8.2.10) (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010). In this analysis nonparametric bootstrap iterations (Stamatakis et al. 2008) was run in 1,000 replicates with the GTR model and discrete gamma distribution (Liu et al. 2011).

MrBayes v.3.0b4 (Ronquist & Huelsenbeck 2003) was used to perform the BI for each single gene region and combined gene data sets. Best-fit models for BI and ML analyses were selected using MrModeltest v. 2.2 (Nylander 2004). For all four data sets, a dirichlet state frequency was predicted and the GTR+I+G model was selected as the best model. The heating parameter was set to 0.2 and trees were saved every 1,000 generations (Ronquist et al. 2012). Posterior probabilities (PP) (Rannala et al. 1998, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov Chain Monte Carlo chains were run for 1,000,000 generations and trees were sampled every 1000th generation resulting in 1000 total trees. The first 100 trees were represented the burnin-phase of the analysis and they were discarded and 900 trees were used to calculate PP in the majority rule consensus tree. The resulting phylogenetic trees were visualized in FigTree v.1.4.0 (Rambaut 2012) and with Microsoft PowerPoint (2016).

Table 1 Taxa used in the phylogenetic analysis and their GenBank accession numbers. New taxon generated in this study is in blue and type strains are in bold.

Species name	Strain No	GenBank accessions				
		LSU	SSU	RPB2	TEF1-α	
Biatriospora mackinnonii	CBS 674.75	KF015612	GQ387552	KF015703	KF407986	
B. marina	CY 1228	GQ925848	GQ925835	GU479823	GU479848	
B. peruviensis	CCF4485	LN626683	LN626677	LN626665	LN626671	
Neooccultibambusa chiangraiensis	MFLUCC 12-0559	_	NG061230	_	_	
N. jonesii	MFLUCC 16-0643	NG059741	NG062422	_	_	
N. pandanicola	KMUCC 17-0179	MG298940	MG298942	MG298944	MG298943	
N. thailandensis	MFLUCC 16-0274	MH260308	MH260348	MH412758	MH412780	
Neoroussoella bambusae	MFLUCC 11-0124	KJ474839	_	KJ474856	KJ474848	
Occultibambusa aquatica	MFLUCC 11-0006	KX698110	KX698112	_	_	
O. bambusae	MFLUCC 11-0394	KU863113	_	KU940171	KU940194	
O. bambusae	MFLU 15-1212	-	NG061237	_	_	
O. bambusae	MFLUCC 13-0855	KU863112	_	KU940170	KU940193	

Table 1 Continued.

Species name	Strain No	GenBank accessions				
		LSU	SSU	RPB2	TEF1-α	
O. bambusae	MFLU 16-0880	-	KU872117	-	_	
O. chiangraiensis	MFLUCC 16-0380	KX655546	NG062421	KX655566	KX655561	
O. fusispora	MFLUCC 11-0127	NG059669	_	KU940172	KU940195	
O. jonesii	GZCC 16-0117	KY628322	KY628324	KY814758	KY814756	
O. maolanensis	GZCC 16-0116	KY628323	KY628325	KY814759	KY814757	
O. pustula	MFLU 15-1185	_	NG062419	_	_	
O. pustula	MFLUCC 11-0502	KU863115	_	_	_	
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP753960	KX437764	_	
P. tectonicola	MFLUCC 13-0465	NG059558	KP753961	KX437763	_	
Roussoella hysterioides	HH 26988	AB524622	AB524481	AB539102	AB539115	
R. nitidula	MFLUCC 11-0634	KJ474842	_	KJ474858	KJ474851	
R. nitidula	MFLUCC 11-0182	KJ474843	_	KJ474859	KJ474852	
R. pustulans	KT 1709	AB524623	AB524482	AB539103	AB539116	
Seriascoma didymospora	MFLUCC 11-0179	NG059670	_	KU940173	KU940196	
S. didymospora	MFLUCC 11-0194	KU863117	_	KU940174	KU940197	
S. yunnanense	MFLU 19-0690	MN174695	MN174694	MN210324	IF556782	
Torula herbarum	CBS 379.58	KF443383	KF443388	GU456362	KF443400	
T. herbarum	CBS 111855	KF443386	KF443391	KF443396	KF443403	
T. pluriseptata	MFLUCC 14-0437	KY197855	KY197862	KY197869	KY197875	
Westerdykella ornata	CBS 379.55	NG057861	NG061101	GU371803	GU349021	

Results

Phylogenetic analyses

The combined LSU, SSU, *RPB2* and *TEF1-* α data set comprises 32 taxa with *Paradictyoarthrinium diffractum* (MFLUCC 13-0466), *P. tectonicola* (MFLUCC 13-0465) and *Westerdykella ornata* (CBS 379.55) as the outgroup taxa. The dataset comprises 3,781 characters (LSU: 874, SSU: 992, *RBP2*: 1027, *TEF1-* α : 888) after alignments, including gaps. Seventeen sequences of *Occultibambusaceae* were obtained from GenBank, which represent three genera in this family. Single gene analyses were performed and each tree has similar topology. Tree topologies of the ML and BI analyses were similar to each other. The best RAxML tree with a final likelihood value of -18435.756238 is presented (Fig. 1). The matrix had 1135 distinct alignment patterns, with 27.98% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.250266, C = 0.247992, G = 0.270472, T = 0.231270; substitution rates AC = 1.372546, AG = 4.220271, AT = 1.312711, CG = 1.127677, CT = 8.919304, GT = 1.000000; gamma distribution shape parameter α = 0.151881. According to the results of BI, the average standard deviation of split frequencies was 0.0108. The new species *Seriascoma yunnanense* (MFLU 19-0690) formed a well separated (99% ML, 0.95 PP) clade to *S. didymospora* (MFLUCC 11-0179 and MFLUCC 11-0194) within the genus *Seriascoma*.

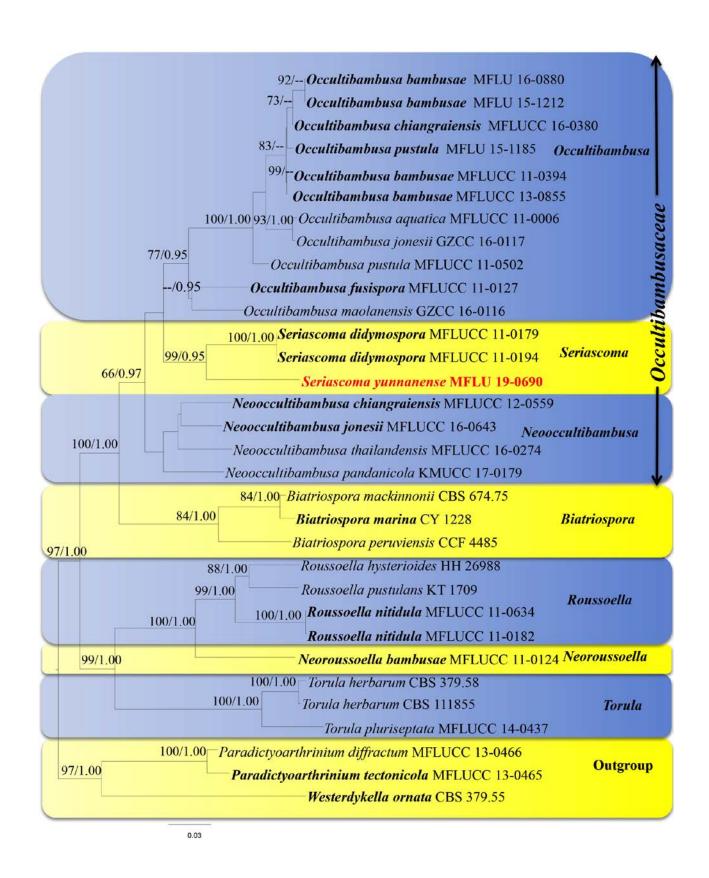


Fig. 1 – Phylogram generated from ML analysis based on combined LSU, SSU, RBP2 and $TEF1-\alpha$ sequence data. ML values ≥ 65 % and Bayesian posterior probabilities (PP) ≥ 0.95 are mentioned at the nodes. The newly generated sequence is in red bold and the type strains are in bold. The isolates numbers are noted after the species names. The tree is rooted to *Paradictyoarthrinium diffractum* (MFLUCC 13-0466), *P. tectonicola* (MFLUCC 13-0465) and *Westerdykella ornata* (CBS 379.55).

Taxonomy

Seriascoma yunnanense Rathnayaka & K.D. Hyde, sp. nov.

Fig. 2

Index Fungorum number: IF556782; Facesoffungi number: FoF 01681

Etymology – In reference to the Yunnan Province, China where the fungus was collected

Holotype – MFLU 19-0690

Saprobic on dead branches of bamboo. Sexual morph: Ascostromata 175–205 μm high, 275–400 μm diam., solitary or gregarious, immersed beneath clypeus, coriaceous, raised, erumpent in host, with a central ostiole uni-loculate, dark brown to black, globose to subglobose, papillate. Peridium 26–63.7 μm wide, thin- to thick-walled, sometimes unequal in thickness, thicker at the sides towards apex, thinner at the base, composed of several layers of small, brown to dark brown cells of textura angularis. Hamathecium 1.4–2.8 μm wide, comprising numerous, branched, cellular pseudoparaphyses. Asci 44–83 × 10–20 μm (\bar{x} = 62 × 14 μm, n = 30), 8-spored, bitunicate, fissitunicate, clavate, short pedicellate, with furcate ends, apically rounded, with well-developed ocular chamber. Ascospores 22–30 × 5–7.2 μm (\bar{x} = 24.4 × 6.5 μm, n = 30), overlapping, uni- to triseriate, didymosporous, slightly broad and fusiform, with rounded ends, hyaline, slightly constricted at the septum, straight to curved, with upper cell larger than lower cell, smooth-walled, surrounded by a gelatinous sheath, with guttules. Asexual morph: Undetermined.

Material examined – CHINA, Yunnan Province, on dead branches of bamboo, 24 May 2018, H. Ekanayaka (MFLU 19-0690, holotype), (HKAS102114, isotype).

Notes – The morphological characters of *Seriascoma yunnanense* fit with the generic concept of *Seriascoma* in having ascostromata that are immersed beneath a clypeus and didymosporous ascospores (Dai et al. 2017). The sexual morph of *S. yunnanense* is morphologically similar to *S. didymospora* but distinctly differs from ascostromatal characters. The ascostromata of *S. didymospora* are erumpent in linear rows and multi-loculate with slit-like openings (Dai et al. 2017), whereas *S. yunnanense* has scattered and uni-loculate ascostromata. *Seriascoma didymospora* also has larger ascostromata (1000–1900 µm) than those in *S. yunnanense* (275–400 µm) (Dai et al. 2017). In addition, *S. yunnanense* has larger ascospores ($\bar{x} = 24.4 \times 6.5 \mu m$) with a gelatinous sheath and larger guttules, but *S. didymospora* has smaller ascospores ($\bar{x} = 11.9 \times 3.9 \mu m$) with comparatively smaller guttules and lacks a gelatinous sheath (Dai et al. 2017). A multigene phylogeny generated herein (Fig. 1) indicates that these species are phylogenetically distinct and *S. yunnanense* can be recognized as a new species.

Discussion

More than 1300 fungal species are recorded on bamboo and microfungi are abundant in culms and leaves (Dai et al. 2018). Ascomycetes are the most abundant species in bamboo, with around 1150 taxa recorded (Dai et al. 2018).

Seriascoma is a relatively poorly known genus and comprises only the type species, S. didymospora. In this study, we provide taxonomic details for another new species; S. yunnanense collected from dead branches of bamboo and expands the genus to include two species. According to the phylogenetic investigations, S. yunnanense clusters with S. didymospora with high bootstrap support (99% ML, and 0.95 PP). In the comparison of the LSU, RBP2 and TEF1-α DNA sequence data between S. yunnanense and S. didymospora also revealed 2.3%, 15% and 7% nucleotide differences respectively that supports our establishment of the new taxon as the guidelines of Jeewon & Hyde (2016).

Seriascoma formed a robust clade close to genus Neooccultibambusa in our phylogenetic analysis which is similar to the study by Doilom et al. (2017). Seriascoma and Neooccultibambusa share similar characteristics, with both genera having uniloculate, globose to subglobose ascostromata and ascospores with gelatinous sheaths and large guttules (Doilom et al. 2017). However, these genera are distinguished by ascospore morphology.

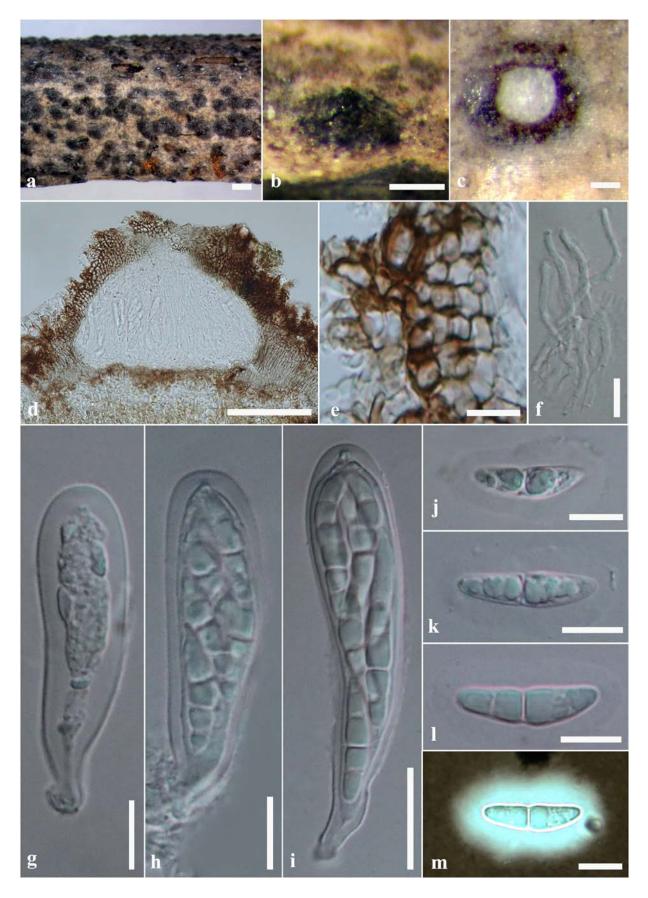


Fig. 2 – *Seriascoma yunnanense* (MFLU 19-0690, holotype). a, b Appearance of ascostromata on host surface. c Transverse section of ascostroma. d Vertical section through locule of ascostroma. e Section through peridium. f Pseudoparaphyses. g–i Immature to mature asci. j–l Immature to mature ascospores. m Ascospore showing gelatinous sheath stained with Indian ink. Scale bars: $a = 500 \, \mu m$, $b = 200 \, \mu m$, $c = 100 \, \mu m$, $d = 100 \, \mu m$, e-h, $j-m = 10 \, \mu m$, $i = 20 \, \mu m$.

The ascospores of *Neooccultibambusa* are initially hyaline and become pale brown when mature (Doilom et al. 2017). However, the ascospores of *Seriascoma* are hyaline when mature (Dai et al. 2017). *Seriascoma* and *Occultibambusa* also share somewhat similar morphological characters and both genera have asci with short furcate pedicels and guttulate ascospores with larger upper cells and surrounded by a gelatinous sheath (Dai et al. 2017). However, the genus *Occultibambusa* has dark brown ascospores (Dai et al. 2017), while *S. yunnanense* has hyaline ascospores.

Versicolorisporium, which belongs to family Occultibambusaceae, was excluded in our phylogenetic analysis. Because the phylogenetic placement of Versicolorisporium is not well-resolved (Zhang et al. 2017).

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