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Pseudorobillarda sichuanensis sp. nov. associated with *Bambusa* sp. from Sichuan province, China

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Abstract

During an investigation of ascomycetous fungi on bamboos in Sichuan province, China, a new coelomycetous species, *Pseudorobillarda sichuanensis* was found on dead culms of *Bambusa* sp. and described. The new taxon differs from other *Pseudorobillarda* species in having paraphyses, subcylindrical conidiogenous cells, subcylindrical to naviculate, aseptate, guttulate conidia with 3–4 apical appendages. Phylogenetic analyses of a combined internal transcribed spacer (ITS), the partial 28S large subunit rDNA (LSU), the partial 18S small subunit rDNA (SSU) and the partial DNA-directed RNA polymerase II subunit (*rpb2*) sequence data showed that *P. sichuanensis* has a close phylogenetic affinity to *P. texana*. A novel bambusicolous fungi of *Pseudorobillarda* is described, illustrated and notes on its identification are provided.

Keywords: 1 new taxon, anamorph, Dothideomycetes, phylogeny, taxonomy

Introduction

Sichuan province is located between the Qinghai-Tibet Plateau and the plains of the middle and lower reaches of the Yangtze River in southwest China. Its complex landform, changeable terrain, humid climate and numerous nature reserves contain huge fungal resources (Zong *et al.* 2003; You *et al.* 2014; Yan *et al.* 2021). Bamboos are gramineous plants with economic and ornamental value. There are more than 1,300 fungi have been found in bamboos (Dai *et al.* 2018) and mainly inhabited leaves and culms (Dai *et al.* 2018; Feng *et al.* 2021; Jiang *et al.* 2021). Numerous bambusicolous fungi are reported as pathogens causing serious economic losses in China (Xu *et al.* 2006, 2007). In addition, some fungal species produce secondary metabolites (Kuhnert *et al.* 2015) and have a high value in medical treatment, such as *Engleromyces goezi*, which can secrete diterpenes to inhibit cholesterol ester transfer protein activity to decrease cholesterol biosynthesis (Wang *et al.* 2015; Dai *et al.* 2018).

Pseudorobillarda was established by Morelet (1968) to accommodate *P. phragmitis* (= *Robillarda phragmitis*) and *P. muhlenbergiae* (= *R. muhlenbergiae*). The genus is characterized by pycnidial conidiomata, presence or absence of paraphyses, phialidic or annellidic conidiogenous cells, subcylindrical to fusiform, 0–4-septate conidia with appendages at one end (Raj *et al.* 1972; Tangthirasunun *et al.* 2014; Li *et al.* 2020). Species in *Pseudorobillarda* have varied lifestyles in different habitats which are mostly found as saprobes and endophytes (Vujanovic & St-Arnaud 2003; Tangthirasunun *et al.* 2014; Li *et al.* 2020). *Pseudorobillarda texana* has been commonly isolated from soil (Kadowaki *et al.* 2014) and *P. peltigeriae* is occasionally found as lichenicolous fungi (Pieter *et al.* 1998). In addition, species of *Pseudorobillarda* are found on living or dead leaves, stems and barks of a broad host range, viz. *Asparagus*, *Bambusa*, *Bolusanthus*, *Camellia*, *Dicotyledon*, *Eucalyptus*, *Setaria* in terrestrial habitats (Nag Raj *et al.* 1972; Vujanovic *et al.* 2003; Plaingam *et al.* 2005; Tangthirasunun *et al.* 2014; Crous *et al.* 2018; Rathnayaka *et al.* 2021), and *P. aquatica* and *P. sojae* were reported from freshwater habitats (Pande 1981; Plaingam *et al.* 2005). *Pseudorobillarda* is widely distributed from temperate to tropical regions, viz., America, Argentina, Canada, Cuba, China, Germany, India, Nigeria, Thailand, the UK and Ukraine (Uecker & Raj 1994; Bianchinotti 1997; Vujanovic & St-Arnaud 2003; Plaingam *et al.* 2005; Rathnayaka *et al.* 2021) and this is the first time to report this genus on the

mainland China. Up to date, twenty species are listed under *Pseudorobillarda* in Index Fungorum (accessed 30 June 2022) and Li *et al.* (2020) accepted 16 species based on the re-examination of the type specimens of *Pseudorobillarda* species. There is no teleomorph that has been reported or linked to *Pseudorobillarda* (Wijayawardene *et al.* 2012). In addition, nine species of *Pseudorobillarda* were supported by molecular data and the taxonomic placement of *Pseudorobillarda* (Pseudorobillardaceae) was confirmed based on phylogenetic results (Crous *et al.* 2019; Li *et al.* 2020; Rathnayaka *et al.* 2021).

During investigations of microfungi in Sichuan Province, China, we found a new species, namely *Pseudorobillarda sichuanensis*, from dead culms of *Bambusa* sp. and the establishment is justified by the morphological and phylogenetic evidence. A detailed description, microphotographic illustration, morphological comparisons with all accepted *Pseudorobillarda* species and an updated phylogenetic tree (with available molecular data) are provided herein.

Materials and methods

Samples collection, morphological studies and herbarium deposition

Dead culms of *Bambusa* sp. were collected from Chengdu, Sichuan Province, China in September 2021. The samples were taken to the laboratory in paper envelopes and maintained at room temperature. The specimens were examined following the methods described in Senanayake *et al.* (2020). Morphological observations were made using a Motic SMZ 168 Series stereomicroscope and digital images were recorded with a Nikon e80i microscope-camera system. Measurements were made with the Tarosoft Image Frame Work program v. 0.9.7 following in Liu *et al.* (2010) and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems, USA). Isolations were made from single spore isolation as described by Senanayake *et al.* (2020). Single germinating conidium was transferred to 2% potato dextrose agar (PDA) media plates after 12 hours and incubated in an incubator at 25 °C for a week.

The type specimen was deposited in the herbarium of Cryptogams, Kunming Institute of Botany academia Sinica (KUN-HKAS) in Kunming, China and Herbarium, University of Electronic Science and Technology (HUEST), Chengdu, China. The cultures isolated were deposited at the China General Microbiological Culture Collection Center (CGMCC) in Beijing, China and the University of Electronic Science and Technology Culture Collection (UESTCC), Chengdu, China. The new taxon was registered in MycoBank.

DNA extraction, PCR amplification and sequencing

Genomic DNA extraction was carried out from fresh mycelium growing on PDA at 25 °C using Tsingke Fungus Genomic DNA Extraction Kit (TSINGKE Biotech, Shanghai, P.R. China) according to the manufacturer's instructions. The primer pairs ITS5/ITS4 (White *et al.* 1990), LR0R (Rehner & Samuels 1994) /LR5 (Vilgalys & Hester 1990), NS1/NS4 (White *et al.* 1990), *fRPB2-5F/fRPB2-7cR* (Liu *et al.* 1999) were used to amplify for the internal transcribed spacer region (ITS), the large subunit of ribosomal rDNA (LSU), the small subunit of ribosomal rDNA (SSU) and RNA polymerase II subunit (*rpb2*), respectively. The amplification was performed in a 25 µL reaction volume containing 12.5 µL Master Mix (Sangon Biotech, Shanghai, P.R. China), 1 µL of each primer (10 µM), 1 µL template DNA and 9.5 µL deionized water. The PCR thermal cycles for four genes were performed under the following reaction conditions: an initial denaturing step for 94 °C 4 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, elongation at 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were verified on 1 % agarose electrophoresis gels stained with ethidium bromide. PCR products were sequenced by Beijing Tsingke Biological Engineering Technology and Services Co., Ltd (Beijing, P.R. China). Sequences were deposited in GenBank.

Phylogenetic analysis

The quality of the raw Sanger sequencing results was initially checked, and the leading region and the tail region with bad quality were trimmed using BioEdit v.7.0.9.0 (Hall 1999). Related sequences (TABLE 1) were downloaded from GenBank based on BLAST search results. *Speirospis pedatospora* (CBS 397.59) was selected as the outgroup taxon and aligned with the sequences obtained in this study using MAFFT v.7 (Katoh *et al.* 2019) and manually edited in BioEdit v.7.0.9.0 (Hall 1999) when necessary.

Phylogenetic analyses were performed by maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) as detailed in Dissanayake *et al.* (2020). Best fit models for ML and BI analyses were revealed by PartitionFinder v2.2.1 (Lanfear *et al.* 2017), running under the Akaike Information Criterion (AIC) implemented in PAUP v. 4.0a. For the *Pseudorobillardaceae* dataset, SYM+I+G was selected as the best model for the ITS region, GTR+I+G was selected for LSU, GTR+G was selected for *rpb2*, and GTR+I+G was selected for SSU. The ML analysis

was performed using RAxML-NG (v. 0.9.0) tool (Kozlov *et al.* 2019) and run 1,000 bootstrap replicates. MrBayes v.3.2.7 (Ronquist *et al.* 2012) was used to run the BI analysis and posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (MCMC). The phylogenetic tree was visualized by FigTree v.1.4.4 (Rambaut 2014) and edited using Adobe Illustrator (Adobe Systems, USA).

TABLE 1. Taxa used in this study and their GenBank accession numbers. Newly generated sequences are in red and the ex-type strains are in bold, the “NA” sequence is unavailable.

Species	Strain no.	GenBank accession numbers			
		ITS	LSU	SSU	<i>rpb2</i>
<i>Pseudorobillarda bolusanthi</i>	CBS 145072	MK047441	MK047491	NA	NA
<i>P. Camelliae-sinensis</i>	NCYUCC 19-0408	MW478596	MW478592	MW478590	MW478492
<i>P. eucalypti</i>	MFLUCC 12-0417	NR137846	NG059497	NG063556	NA
<i>P. magna</i>	HKAS 93638	MT185551	MT183516	MT214985	MT432246
<i>P. parasiamensis</i>	MFLUCC 12-0414	KF827448	KF827454	KF827460	KF827493
<i>P. phragmitis</i>	CBS 398.61	MH858101	MH869670	EU754104	NA
<i>P. phragmitis</i>	CBS 842.84	MH861840	MH873528	EU754103	NA
<i>P. phragmitis</i>	IA04	KM246187	KM246104	NA	NA
<i>P. phragmitis</i>	IA10	KM246192	KM246109	NA	NA
<i>P. sojae</i>	MFLUCC 12-0422	KF827451	KF827457	KF827463	KF827496
<i>P. sojae</i>	MFLUCC 12-0423	KF827452	KF827458	KF827464	KF827497
<i>P. sojae</i>	MFLUCC 12-0316	KF827447	KF827453	KF827459	KF827492
<i>P. sojae</i>	BCC 20495	FJ825371	FJ825376	FJ825366	NA
<i>P. siamensis</i>	BCC 12531	FJ825370	FJ825375	FJ825365	NA
<i>Pseudorobillarda</i> sp.	MFLU 19-2895	MT185552	MT183517	MT214986	NA
<i>Pseudorobillarda</i> sp.	G fon3	NA	MF337012	NA	NA
<i>P. sichuanensis</i>	CGMCC 3.20951	ON614098	ON614139	ON614099	ON639624
<i>P. texana</i>	BCC 12535	FJ825372	FJ825377	FJ825367	NA
<i>Speiropsis pedatospora</i>	CBS 397.59	MH857901	MH869443	NA	NA

Acronyms of culture collections: BCC: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Klong Luang, Pathumthani, Thailand; CBS: CBS Filamentous fungi and Yeast Collection, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; HKAS: Cryptogams Kunming Institute of Botany Academia Sinica, Kunming, China; MFLU: Mae Fah Luang University Herbarium Collection, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NCYUCC: the National Chiayi University Culture Collection, Taiwan, China.

Results

Phylogenetic analysis

The combined LSU-SSU-ITS-*rpb2* sequence matrix comprised 19 taxa with *Speiropsis pedatospora* (CBS 397.59) as the outgroup taxon. The concatenated alignment comprised 3,234 total characters including gaps (LSU: 1–836 bp; SSU: 837–1,868 bp; ITS: 1,869–2,455 bp; *rpb2*: 2,456–3,234 bp) with 2,359 constant, 320 parsimony uninformative and 555 parsimony informative characters. The MP analysis resulted a single most parsimonious tree (CI = 0.710, RI = 0.700, RC = 0.497). The best ML phylogram (FIGURE 1) with a final likelihood value of -12409.797399. The matrix had 815 distinct alignment patterns, with 30.18% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.264599, C = 0.213158, G = 0.258622, T = 0.263621; substitution rates AC = 1.259141, AG = 3.094544, AT = 1.710917, CG = 0.847543, CT = 7.743777, GT = 1.000000; gamma distribution shape parameter α

= 0.185184. Single gene analyses (ITS, LSU, SSU) are also carried out and the topology of the trees and clades are basically stable. The MP and BI phylogenetic analyses produced trees with similar topologies to ML. The phylogenetic analysis indicated two major clades in *Pseudorobillarda* (FIGURE 1), one clade included the species *P. siamensis*, *P. parasiamensis*, *P. eucalypti*, *P. camelliae-sinensis* and *P. sojae*; and the other one had *P. magna*, *P. phragmitis*, *P. texana*, *P. sichuanensis* (*sp. nov.*) and *P. bolusanthi*. The isolate (CGMCC 3.20951) of *Pseudorobillarda sichuanensis* formed a sister affinity to *P. texana*.

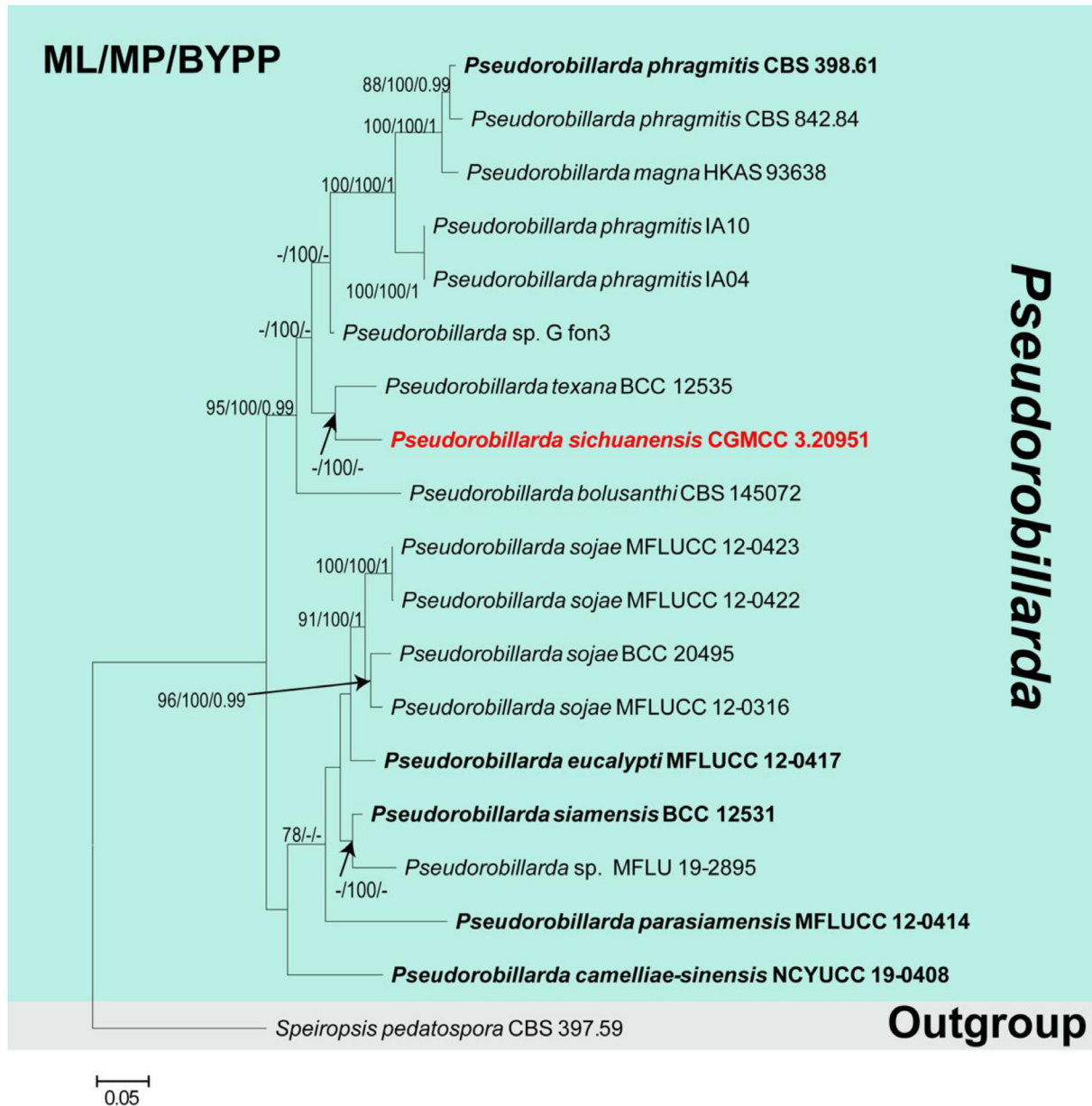


FIGURE 1. Phylogenetic tree generated from ML analyses of a combined ITS, LSU, SSU and *rpb2* dataset. The tree was rooted to *Speiropsis pedatospora* (CBS 397.59). Bootstrap support values for MP, ML ($\geq 75\%$) and Bayesian posterior probabilities (≥ 0.95 PP) are given above or below the branches respectively. The new taxon is indicated in red and ex-type strains are in bold.

Taxonomy

Pseudorobillarda sichuanensis J.Y. Song & Jian K. Liu, *sp. nov.*

Mycobank: MB844119; FIGURE 2

Etymology: In reference to the location where the fungus was collected, Sichuan Province, China.

Holotype: HKAS 124018

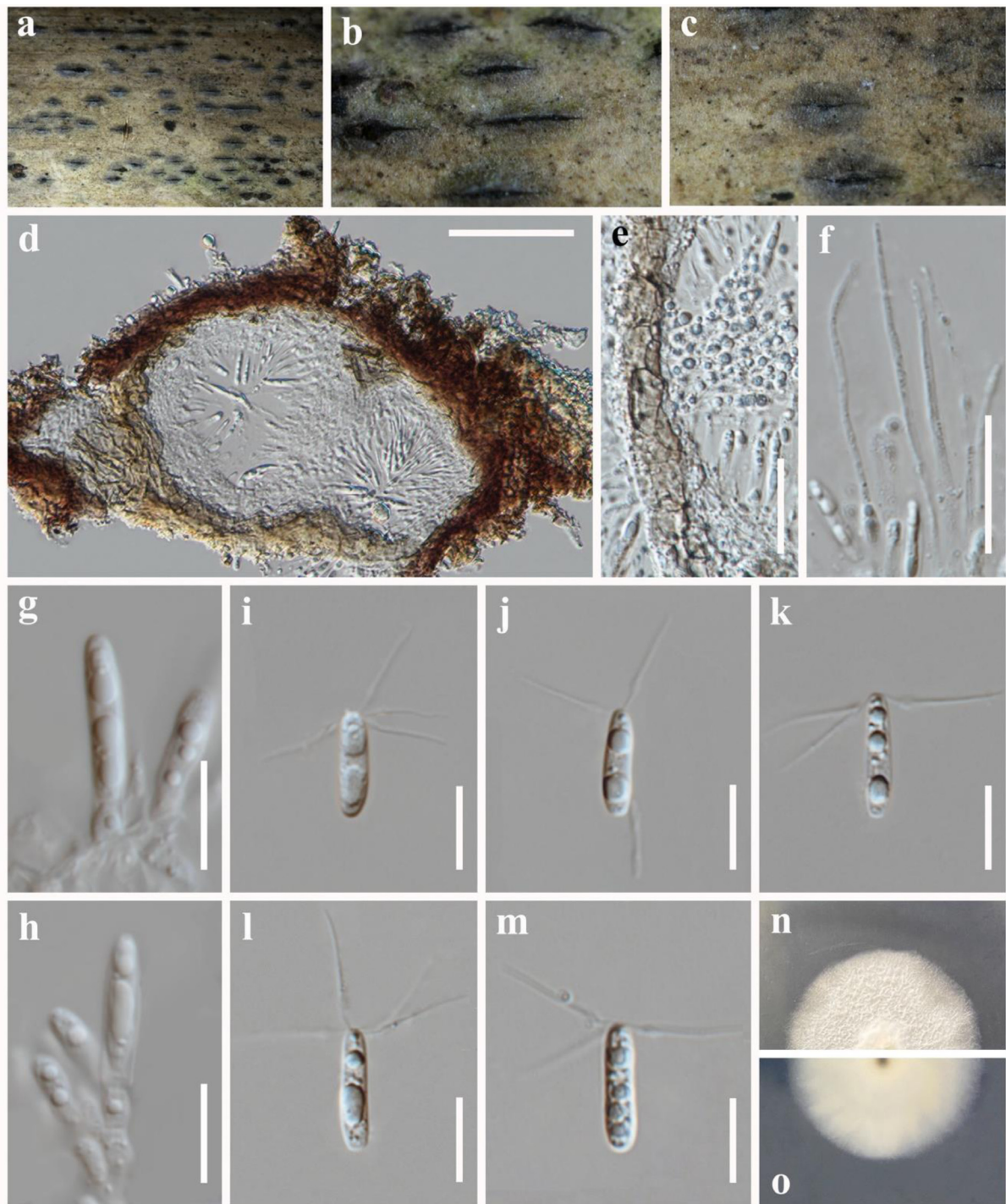


FIGURE 2. *Pseudorobillarda sichuanensis* (HKAS 124018, holotype). **a** Appearance of *Conidiomata* on the host surface. **b–c** Close-up of *Conidiomata*. **d** Vertical section of conidioma. **e** Vertical section through *Conidiomatal* wall. **f** *Paraphyses*. **g, h** *Conidiogenous* cells with developing conidia. **i–m** Conidia with appendages. **n** Colony from above **o** Colony from below. Scale bars: d = 50 μm , e, f = 20 μm , g–m = 10 μm .

Saprobic on a dead culm of *Bambusa* sp. in terrestrial habitat. Forming black, lenticular spots on the host surface, with *Conidiomata* breaking through raised cracks with a black center. teleomorph: Undetermined. anamorph: *Conidiomata* 170–320 μm diam. \times 100–180 μm high (\bar{x} = 237 \times 128 μm ; n = 10), pycnidial, scattered, immersed to slightly erumpent, multilocular or unilocular, forming a slit-like opening at the apex, black, glabrous, ostiolate. *Ostiole* in the center of the pileus, circular, dark brown, ostiolar canal filled with hyaline cells, non-papillate. *Conidiomatal* wall 9–16 μm diam. (\bar{x} = 12.5 μm ; n = 10), multi-layered, composed of thick-walled, hyaline to brown cells of *textura angularis* or *textura prismatica*. *Conidiophores* arising all around the cavity of the conidiomata, reduced to conidiogenous cells, mixed with paraphyses and embedded in a mucilage matrix. *Paraphyses* 24.5–66 μm long \times 1.5–2.5 μm wide (\bar{x} =

39.5 × 2 µm; n = 20), hyaline, unbranched, narrowly cylindrical to filiform, 1-septate, smooth-walled. *Conidiogenous cells* 3–4.5 × 2–3.5 µm (\bar{x} = 4 × 2.6 µm; n = 20), enteroblastic, phialidic, subcylindrical, hyaline, straight, smooth-walled. *Conidia* 12–17 × 2.5–4 µm (\bar{x} = 14.5 × 3.5 µm; n = 30), subcylindrical to naviculate, hyaline, rounded at apex, slightly truncated at the base, aseptate, 1–4-guttulate, smooth-walled, bearing 3–4 unbranched, filiform, attenuated, flexuous apical appendages (11–16 µm long). Mean conidial length/width (L/W) ratio = 4.5 (n = 30).

Culture characteristics: Conidia germinated on PDA in 24 hours. Germ tube produced from two-end of the conidia, without sporulation. Colonies on PDA, reaching 30 mm diam. after seven days at 25 °C, rough, dry, opaque, felty, flat, initially white, gradually becoming light pink in the middle and white at the margin, with regular margin and reverse white.

Material examined: CHINA, Sichuan Province, Chengdu, Science City Mountain Park (30°40.99' N, 104°08.24' E), on dead culms of *Bambusa* sp. (Poaceae), 10 September 2021, Jingyi Song (HKAS 124018, holotype; HUEST 22.0027, isotype); ex-type living culture CGMCC 3.20951 (= UESTCC 22.0029).

Notes: *Pseudorobillarda sichuanensis* fits well with the generic concept of *Pseudorobillarda* by its pycnidial conidiomata, paraphyses, phialidic conidiogenous cells, and subcylindrical conidia with appendages at one end. However, it differs from other species in having subcylindrical to fusiform, aseptate, guttulate conidia with 3–4 apical appendages. A detailed morphological comparison of *Pseudorobillarda* species is provided in TABLE 2. *Pseudorobillarda sichuanensis* (sp. nov.) resembles to *P. aquatica*, *P. bolusanthi*, *P. camelliae-sinensis*, *P. eucalypti*, *P. siamensis*, *P. sojae* and *P. texana* in having aseptate conidia. While *P. eucalypti*, *P. siamensis* and *P. sojae* are distinguished from *P. sichuanensis* by the absent of paraphyses. *Pseudorobillarda aquatica* has larger (25–35 µm), eguttulate conidia, while *P. sichuanensis* has smaller, guttulate conidia (12–17 µm). *Pseudorobillarda sichuanensis* resembles *P. texana* in having similar conidiogenous cells and conidia. However, *P. sichuanensis* has conidiomata with a circular, non-papillate ostiole, conidia with a rounded apex and slightly truncated base, and shorter apical appendages. While *P. texana* has conidiomata with oval, beaked ostiole and conidia with both ends rounded (Plaingam *et al.* 2005). In addition, the Mean conidial L/W ratio of *P. sichuanensis* (4.6) is significantly less than *P. texana* (5.5). The multi-gene (LSU-SSU-ITS-*rpb2*) phylogenetic analysis suggested that *Pseudorobillarda sichuanensis* clustered together with *P. texana* and formed a distinct lineage. In addition, a comparison of the ITS region reveals a 62 bp (base pair) difference (without gaps) between *P. sichuanensis* and *P. texana* which provides further evidence to support the establishment of new species.

Discussion

Species in *Pseudorobillarda* are distinguished mainly by the absence or presence of paraphyses, conidial features including origin and position of conidial appendages and the mean conidial length/width (L/W) ratio of conidia (Nag Raj *et al.* 1972; Bianchinotti 1997; Vujanovic & St-Arnaud 2003; Tangthirasunun *et al.* 2014; Li *et al.* 2020; Rathnayaka *et al.* 2021). Bianchinotti (1997) proposed that the origin and position of appendages should be considered essential characteristics to identify species in *Pseudorobillarda*. However, it is difficult to observe the position of the appendages in developed conidia and it was roughly described as conidia 'bearing appendages at one end' in several previous studies (Cunnell 1958; Nag Raj *et al.* 1972; Pande 1981; Uecker & Kulik 1986; Vujanovic & St-Arnaud 2003; Li *et al.* 2020).

The classification status of *Pseudorobillarda* remains unresolved (Rungjindamai *et al.* 2012; Tangthirasunun *et al.* 2014; Crous *et al.* 2014, 2018; Li *et al.* 2020; Rathnayaka *et al.* 2021). Tangthirasunun *et al.* (2014) placed *Pseudorobillarda* in Dothideomycetes *incertae sedis* on basis of LSU sequence data. Subsequently, a new family *Pseudorobillardaceae* was established to accommodate *Pseudorobillarda* in Minutisphaerales (Crous *et al.* 2018, 2019). The recent studies placed the family in Dothideomycetes families *incertae sedis* (Dong *et al.* 2020; Hongsanan *et al.* 2020; Li *et al.* 2020) as the phylogenetic analysis did not support the placement of assigning *Pseudorobillardaceae* to Minutisphaerales, and its treatment needs further studies to resolve. *Pseudorobillarda* taxa are widely distributed in temperate to tropical countries and they have been reported as saprobes, pathogens, endophytes as well as lichenicolous and humicolous fungi (Uecker & Raj 1994; Bianchinotti 1997; Vujanovic & St-Arnaud 2003; Kadowaki *et al.* 2014). This study contributes the diversity of bamboo fungi in Sichuan province, China, and a new species *Pseudorobillarda sichuanensis* was isolated, identified and well justified. With more molecular data available (taxa population and species diversity) in this group and it will provide the opportunity to address better understanding towards a natural classification of *Pseudorobillarda*.

TABLE 2. *Anamorph* morphology comparison among *Pseudorobillarda* species.

Species	Conidiomata		Paraphyses		Conidiogenous cells		Conidia				Reference
	Size (µm)		Size (µm)		Size (µm)	Shape	Septation	Size (µm)	Guttulation	No.	
<i>Pseudorobillarda agrostidis</i>	260–360 × 140–230	present	2–5 × 1.5–2.5	fusiform	1-septate	13–20 × 2–3	guttulate	3–4	10–16.5		Nag Raj <i>et al.</i> (1972)
<i>P. aquatica</i>	90–140 (diam.)	present	not report	cylindrical, allantoids	aseptate, rarely 1-septate	25–35 × 3.5–4.2	eguttulate	4	not report		Plaingam <i>et al.</i> (2005)
<i>P. asparagis</i>	100–500 (diam.)	present	5–8 × 2–5	fusiform	1-septate	10–14 × 2–2.5	not report	3–4	15–33		Plaingam <i>et al.</i> (2005)
<i>P. Bambusae</i>	100–340 × 120–250	present	4–8 × 2–4	subcylindrical to fusiform	1-septate	16–20 × 3–5	eguttulate	2–3	14–20		Li <i>et al.</i> (2020)
<i>P. bolusanthi*</i> (Macroconidia)	200–250 (diam.)	present	4–7 × 3–4	not report	(1–)3-septate	26–28 × 6	guttulate	3–5	up to 30		Crous <i>et al.</i> (2018)
<i>P. bolusanthi*</i> (Microconidia)			10–15 × 4–5	subcylindrical	aseptate	4–8 × 2–4	guttulate	3–5	up to 10		
<i>P. camelliae-sinensis*</i>	106–120 × 120–155	present	5–15 × 2–4	subcylindrical	aseptate	13–14 × 2–4	guttulate	2–4	10–16		Rathnayaka <i>et al.</i> (2021)
<i>P. eucalypti</i>	255–330 × 350–500	absent	6–17 × 2–5	subcylindrical to fusiform	aseptate	15–18 × 5–8	guttulate	2–3	10–19		Li <i>et al.</i> (2020)
<i>P. indica</i>	150–300 × 200–350	present	6–9 × 3–5	subcylindrical to fusiform	1-septate, occasionally 3-septate	16–24 × 2.4–4	guttulate	2–4	11–23		Li <i>et al.</i> (2020)
<i>P. jaczewskii</i>	90–200 × 60–110	present	4–7 × 3–3.5	ellipsoidal	1-septate, occasionally 3-septate	15–21 × 3–3.5	guttulate	2–5	10–19		Plaingam <i>et al.</i> (2005)
<i>P. magna*</i>	160–220 × 130–200	present	3–7 × 2–2.5	not report	3-septate	22–28 × 3–5	guttulate	5–6	17–26		Li <i>et al.</i> (2020)

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TABLE 2. (Continued)

Species	Conidiomata			Paraphyses			Conidiogenous cells			Conidia			Appendages		Reference
	Size (µm)	Size (µm)	Shape	Septation	Size (µm)	Guttulation	No.	Length (µm)	Size (µm)	Guttulation	No.	Length (µm)	Appendages		
<i>P. monica</i>	150–400 (diam.)	present	2–10 × 2–2.5	subcylindrical to fusiform	1-septate, occasionally 3-septate	10–12 × 2.5–3	guttulate	2–4	15–25	Vujanovic & St-Arnaud (2003)					
<i>P. muhlbergiae</i>	60–200 (diam.)	not report	not report	cylindrical	1-septate	12–16 × 3.4–3.8	not report	3–4	8–13	Sprague <i>et al.</i> (1951)					
<i>P. parasiamensis</i>	140–160 × 100–130	absent	5–10 × 2–4	fusiform to ellipsoidal	septate	15–23 × 6–8	guttulate	2–4	12–23	Li <i>et al.</i> (2020)					
<i>P. peltigerae</i>	100–280 (diam.)	present	26–31 × 1–1.5	subcylindrical	1-septate	16–20 × 2.5–3	not report	2–3	16–20	Plaingam <i>et al.</i> (2005)					
<i>P. phragmitis</i> *	150–250 × 110–250	present	5–10 × 2–4	fusiform	1-septate	14.5–20 × 2–4	guttulate	2–5	10–20	Li <i>et al.</i> (2020)					
<i>P. siamensis</i> *	99–123 × 135–153	absent	2.5–5 × 1.5–2	cylindrical to fusiform	aseptate	16–21 × 6–8	not report	4–5	17–26.5	Tangthirasunun <i>et al.</i> (2014)					
<i>P. sojae</i> *	100–250 × 100–300	absent	4–7 × 3–5	subcylindrical to fusiform	aseptate	13.5–19 × 3–5	guttulate	3–4	10–21	Li <i>et al.</i> (2020)					
<i>P. subfusca</i>	65–100 × 90	absent	3–3.5 × 3	subcylindrical to ellipsoid	1-septate	9.1–12.2 × 3–3.6	eguttulate	2–4	15–25	Brackel (2019)					
<i>P. Setariae</i>	150 (diam.)	present	5.5–6.5 × 2.5–3	subcylindrical to ellipsoid	1-septate	14–21 × 3–4	guttulate	2–4	9–21	Plaingam <i>et al.</i> (2005)					
<i>P. sichuanensis</i> *	170–320 × 100–180	present	3–4.5 × 2–3.5	subcylindrical to naviculate	aseptate	12–17 × 2.5–4	guttulate	3–4	11–16	This study					
<i>P. texana</i> *	110–190 × 100–140	present	3–6 × 2–3.5	cylindrical to naviculate	aseptate	12.5–18.5 × 2.5–3.5	guttulate	3–4	12.5–20.5	Plaingam <i>et al.</i> (2005)					

*Species with molecular data

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