



## *Pseudokeissleriella bambusicola* gen. et sp. nov. (Lentitheciaceae, Pleosporales) from bamboos in Sichuan province, China

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### Abstract

During an investigation of ascomycetous fungi on bamboos in Sichuan province, China, a monotypic genus, *Pseudokeissleriella*, collected from dead culms of bamboos is introduced to accommodate *P. bambusicola*. *Pseudokeissleriella bambusicola* is characterized by having subglobose to globose, glabrous ascomata, and hyaline, septate, fusiform ascospores with subobtusate ends and a swollen upper cell, surrounded by a mucilaginous sheath with center depression. The phylogenetic analyses based on multi-gene matrix of SSU, ITS, LSU, *tef-1a* sequences showed that *P. bambusicola* presented a distinct lineage sister to *Katumotoa* and *Neophiosphaerella* in Lentitheciaceae. The establishment of new taxa were justified by morphological and phylogenetic evidences. Morpho-phylogenetic differences between *Pseudokeissleriella* and some related genera *Katumotoa*, *Keissleriella*, and *Neophiosphaerella* are discussed. Descriptions, illustrations, and notes for the new taxa are provided.

**Keywords:** 2 new taxa, Dothideomycetes, multi-gene, phylogeny, taxonomy

### Introduction

Lentitheciaceae Y. Zhang ter, C.L. Schoch, J. Fourn., Crous & K.D. Hyde was introduced by Zhang *et al.* (2009) to accommodate *Lentithecium* K.D. Hyde, J. Fourn. & Ying Zhang, *Katumotoa* Kaz. Tanaka & Y. Harada., and *Keissleriella* Höhn. (tentatively). Additions to Lentitheciaceae were subsequently contributed based on morpho-phylogenetic evidence (Hirayama *et al.* 2010, Quaedvlieg *et al.* 2013, Wanasinghe *et al.* 2014, Knapp *et al.* 2015, Phookamsak *et al.* 2015, Tanaka *et al.* 2015, Li *et al.* 2016, Dayarathne *et al.* 2018, Hyde *et al.* 2020, Liu *et al.* 2022). The sexual morph of the family is characterized by globose to lenticular ascomata with glabrous or brown setae, 8-spored, bitunicate asci, and hyaline or brown, aseptate, mostly 1–3-septate (muriform in some species), ellipsoid, filiform, or narrowly fusiform to broadly cylindrical ascospores, surrounded by an entire or elongated appendage-like mucilaginous sheath (Tanaka & Harada 2005, Singtripop *et al.* 2015, Su *et al.* 2016, Dayarathne *et al.* 2018). The coelomycetous asexual morphs have been found in *Halobyssothecium*, *Keissleriella*, *Murilentithecium*, *Phragmocamarosporium*, *Setoseptoria* and *Towyspora* (Quaedvlieg *et al.* 2013, Wanasinghe *et al.* 2014, Tanaka *et al.* 2015, Wijayawardene *et al.* 2015, Li *et al.* 2016, Calabon *et al.* 2021). Up to date, Lentitheciaceae accommodates 15 genera with more than 110 species (Hongsanan *et al.* 2020, Liu *et al.* 2022). Members of Lentitheciaceae widely distribute in terrestrial, aquatic, marine, as well as semiarid sandy grasslands, and most of them were found on dicotyledonous plants, of which more than 30 species (i.e., *Poaceascoma aquaticum*, *Keissleriella dactylidis*) have been reported from Poaceae (Tanaka & Harada 2005, Knapp *et al.* 2015, Wanasinghe *et al.* 2018, Calabon *et al.* 2021).

Bamboos (Poaceae) are fast-growing perennial plants and richly colonize by saprobic microfungi on their culms and leaves (Dai *et al.* 2018, Rathnayaka *et al.* 2019, Sun *et al.* 2020, Yu *et al.* 2022). There are more than 1300 fungi, including 150 basidiomycetes, 800 ascomycetous species with 240 hyphomycetous and 110 coelomycetous taxa reported from bamboos (Dai *et al.* 2018). Several genera within Lentitheciaceae contain species found on bamboos, such as *Keissleriella*, *Katumotoa*, *Neophiosphaerella* and *Poaceascoma* (Tanaka & Harada 2005, Tanaka *et al.* 2015,

Luo *et al.* 2016, Devadatha *et al.* 2020). *Katumotoa* and *Neophiosphaerella* are monotypic bambusicolous genera and they presented as sister groups in previous phylogenetic studies, and their ascospore morphology (apiosporous in *Katumotoa* vs. multi-septate scolecosporous in *Neophiosphaerella*) distinguish them as different genera (Suetrong *et al.* 2009; Zhang *et al.* 2009; Zhang *et al.* 2012; Tanaka *et al.* 2015).

During investigations of microfungi on bamboos in Sichuan Province, China, two collections are identified as a new species *Pseudokeissleriella bambusicola*, and the new genus *Pseudokeissleriella* is introduced to accommodate this species. The multi-gene phylogenetic analyses indicated that two isolates of *P. bambusicola* formed a distinct clade within Lentitheciaceae and showed close phylogenetic relationship to *Katumotoa* and *Neophiosphaerella*.

## Materials and methods

### Sample collection, examination, and isolation

Specimens were collected from dead culms of bamboos in Chengdu City, Sichuan Province, China. Samples were taken to the laboratory in paper envelopes and then were examined following the methods as described in Senanayake *et al.* (2020). Fungal fruiting bodies were examined by using stereomicroscope Motic SMZ 168 series and the micro-morphological characteristics were photographed by using a Nikon E80i microscope-camera system. Measurements were made with the Tarosoft Image Frame Work program v. 0.9.7 (Liu *et al.* 2010) and the photo plates were processed with Adobe Photoshop CS6 software. Single spores were isolated following the method in Chomnunti *et al.* (2014) to obtain the pure cultures.

Herbarium materials containing the fungal species were deposited in the herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), Kunming, China and Herbarium, University of Electronic Science and Technology (HUEST), Chengdu, China. The living cultures were deposited in China General Microbiological Culture Collection Center (CGMCC) in Beijing, China and the University of Electronic Science and Technology Culture Collection (UESTCC) in Chengdu, China. The new taxa were registered in MycoBank.

### DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fungal mycelium by using a Tsingke Fungus Genomic DNA Extraction Kit (TSINGKE Biotech, Shanghai, P.R. China) referring to the manufacturer's instructions. DNA amplification procedure was performed by Polymerase Chain Reaction (PCR) in a 25 µl reaction volume, which contained 9.5 µl distilled deionized water, 12.5 µl PCR Master Mix (Sangon Biotech, Shanghai, P.R. China), 1 µl DNA template, and 1 µl of each forward and reverse primers. Four partial gene regions, the large subunit of ribosomal rDNA (LSU), internal transcribed spacer region (ITS), small subunit of ribosomal rDNA (SSU) and translated elongated factor 1-alpha (*tef-1α*), were used in this study. The primers used were LR0R/LR5 (Vilgalys & Hester 1990) for LSU, ITS9mun/ITS4 (Toju *et al.* 2012) for ITS, NS1/NS4 (White *et al.* 1990) for SSU and TEF1-983F/TEF1-2218R for *tef-1α* (Rehner & Buckley 2005). PCR thermal cycles of the 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 (SSU, ITS, LSU), 61 °C (*tef-1α*) for 30 s, elongation at 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were observed on 1% agarose electrophoresis gels stained with ethidium bromide, and then were purified and sequenced with primers mentioned above by Beijing Tsingke Biological Engineering Technology and Services Co. Ltd. (Beijing, P.R. China).

### Phylogenetic analyses

The quality of the new sequences was initially checked with BioEdit v. 7.0.9.0 (Hall 1999). BLAST searches in GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) were performed to find the closely related sequences that match our data. A concatenated dataset of the SSU, ITS, LSU and *tef-1α* sequences of selected strains in Lentitheciaceae were used for phylogenetic analyses (Table 1). Sequences were aligned by MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley 2013) and manually adjusted in BioEdit v. 7.0.9.0 (Hall 1999) where necessary. Each dataset was concatenated with Mesquite v. 3.01 (Maddison & Maddison 2014).

Phylogenetic analyses of maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) were carried out as detailed in Dissanayake *et al.* (2020). Maximum likelihood analysis was performed by using RAxML-HPC2 on XSEDE 8.2.12 (Stamatakis *et al.* 2008) in CIPRES Science Gateway (Miller *et al.* 2010). The MP and BI analyses were performed by using PAUP v. 4.0b (Swofford 2002) and MrBayes v.3.2.7 (Ronquist *et al.* 2012).

The best evolution model for different gene partitions was determined by Partition Finder v2.2.1 (Lanfear *et al.* 2017), of which GTR+I+G is the best-fit model of SSU, ITS, LSU and *tef-1a*. The phylogenetic tree was visualized by FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/> (accessed on 18 April 2022)) and edited with Adobe Illustrator (Adobe Systems, USA).

**TABLE 1.** Taxa used in this study and their GenBank accession numbers. Newly generated sequences were indicated in bold and the ex-type strains were indicated with a superscripted “T”.

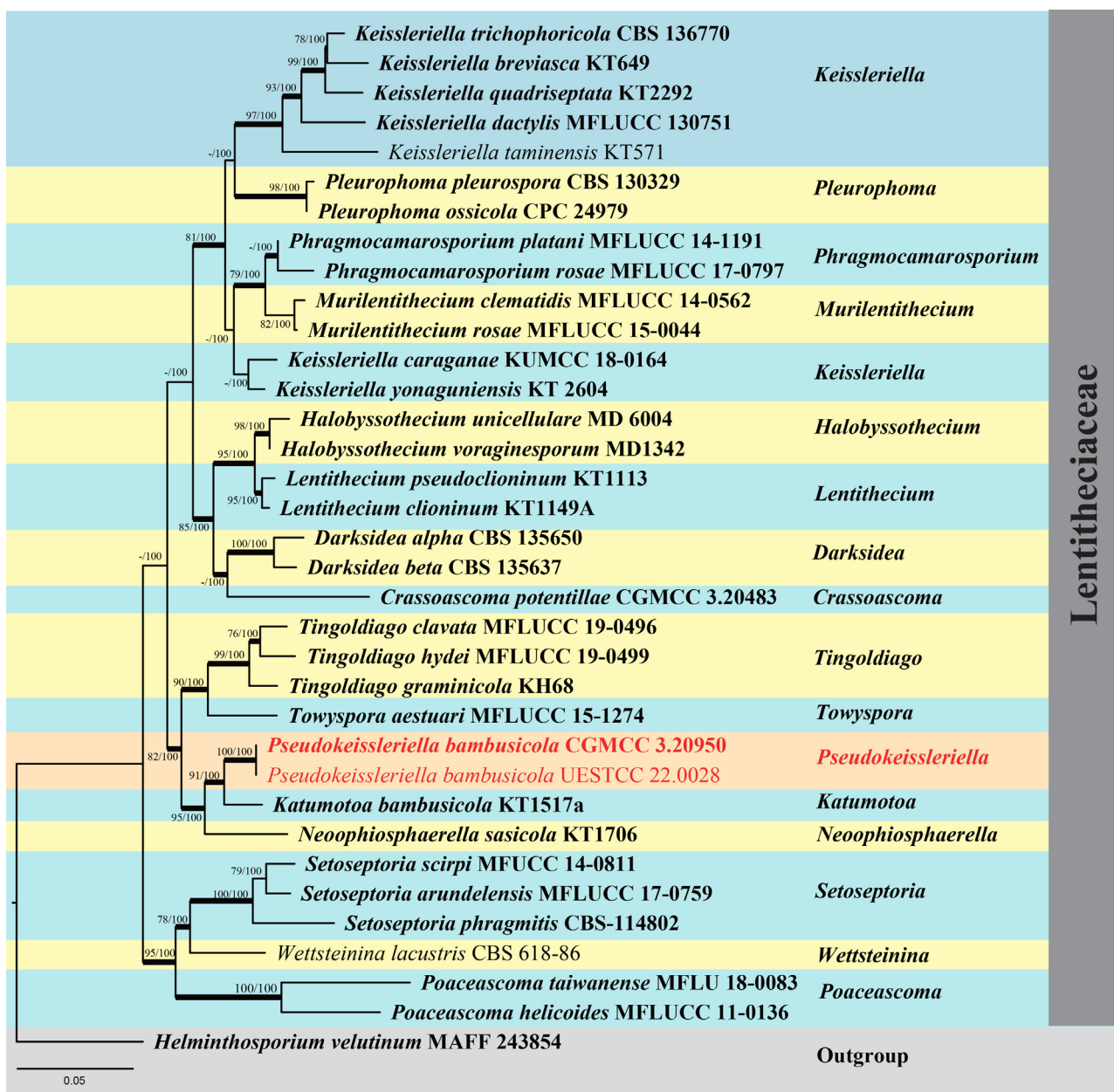
Taxa	Vouchers/Cultures	GenBank accession numbers			
		SSU	ITS	LSU	<i>tef-1a</i>
<i>Crassoascoma potentillae</i>	CGMCC 3.20483 <sup>T</sup>	OK161236	OK161240	OK161257	OK181168
<i>Darksidea alpha</i>	CBS 135650 <sup>T</sup>	KP184049	KP183998	KP184019	KP184166
<i>Darksidea beta</i>	CBS 135637 <sup>T</sup>	KP184074	KP183978	KP184023	KP184189
<i>Halobyssothecium unicellulare</i>	MD 6004 <sup>T</sup>	KX505374	–	KX505376	–
<i>Halobyssothecium voragineporum</i>	MD1342 <sup>T</sup>	KX499519	–	KX499520	–
<i>Katumotoa bambusicola</i>	KT1517a <sup>T</sup>	AB524454	LC014560	AB524595	AB539108
<i>Keissleriella breviasca</i>	KT649 <sup>T</sup>	AB797298	AB811455	AB807588	AB808567
<i>Keissleriella dactylis</i>	MFLUCC 130751 <sup>T</sup>	KP197666	NR_155219	NG_059541	KP197669
<i>Keissleriella caraganae</i>	KUMCC 18-0164 <sup>T</sup>	MK359444	MK359434	MK359439	MK359073
<i>Keissleriella quadriseptata</i>	KT2292 <sup>T</sup>	AB797303	AB811456	AB807593	AB808572
<i>Keissleriella taminensis</i>	KT571	AB797305	LC014564	AB807595	AB808574
<i>Keissleriella trichophoricola</i>	CBS 136770 <sup>T</sup>	–	KJ869113	KJ869171	–
<i>Keissleriella yonaguniensis</i>	KT 2604 <sup>T</sup>	AB797304	AB811457	AB807594	AB808573
<i>Lentithecium clioninum</i>	KT1149A <sup>T</sup>	AB797250	LC014566	AB807540	AB808515
<i>Lentithecium pseudoclioninum</i>	KT1113 <sup>T</sup>	AB797255	AB809633	AB807545	AB808521
<i>Murilentithecium clematidis</i>	MFLUCC 14-0562 <sup>T</sup>	KM408761	KM408757	KM408759	KM454445
<i>Murilentithecium rosae</i>	MFLUCC 15-0044 <sup>T</sup>	MG829137	MG828920	MG829030	–
<i>Neophiosphaerella sasicola</i>	KT1706 <sup>T</sup>	AB524458	LC014577	AB524599	AB539111
<i>Phragmocamarosporium platani</i>	MFLUCC 14-1191 <sup>T</sup>	KP842919	–	KP842916	–
<i>Phragmocamarosporium rosae</i>	MFLUCC 17-0797 <sup>T</sup>	MG829156	–	MG829051	MG829225
<i>Pleurophoma ossicola</i>	CPC 24979 <sup>T</sup>	–	KR476736	KR476769	–
<i>Pleurophoma pleurospora</i>	CBS 130329 <sup>T</sup>	–	–	JF740327	–
<i>Poaceascoma helicoides</i>	MFLUCC 11-0136 <sup>T</sup>	KP998463	KP998459	KP998462	KP998461
<i>Poaceascoma taiwanense</i>	MFLU 18-0083 <sup>T</sup>	MG831568	MG831569	MG831567	–
<b><i>Pseudokeissleriella bambusicola</i></b>	<b>CGMCC 3.20950<sup>T</sup></b>	<b>ON614135</b>	<b>ON614096</b>	<b>ON614138</b>	<b>ON639623</b>
<b><i>Pseudokeissleriella bambusicola</i></b>	<b>UESTCC 22.0028</b>	<b>ON614134</b>	<b>ON614095</b>	<b>ON614137</b>	<b>ON639622</b>
<i>Setoseptoria arundelensis</i>	MFLUCC 17-0759 <sup>T</sup>	MG829173	MG828962	MG829073	–
<i>Setoseptoria phragmitis</i>	CBS 114802 <sup>T</sup>	–	KF251249	KF251752	–
<i>Setoseptoria scirpi</i>	MFUCC 14-0811 <sup>T</sup>	KY770980	MF939637	KY770982	KY770981
<i>Tingoldiagio clavata</i>	MFLUCC 19-0496 <sup>T</sup>	MN857186	MN857182	MN857178	–
<i>Tingoldiagio graminicola</i>	KH68 <sup>T</sup>	AB521726	LC014598	AB521743	AB808561
<i>Tingoldiagio hydei</i>	MFLUCC 19-0499 <sup>T</sup>	–	MN857181	MN857177	–
<i>Towyspora aestuari</i>	MFLUCC 15-1274 <sup>T</sup>	KU248853	KU248851	KU248852	–
<i>Wettsteinina lacustris</i>	CBS 618.86	DQ678023	AF250831	–	DQ677919
<i>Helminthosporium velutinum</i>	MAFF 243854	AB797240	LC014556	AB807530	AB808505

Abbreviation: **CGMCC**: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **KT**: Kazuaki Tanaka; **UESTCC**: University of Electronic Science and Technology Culture Collection.

## Results

### Phylogenetic analyses

The combined SSU, ITS, LSU, and *tef-1a* sequence matrix comprised 35 taxa with *Helminthosporium velutinum* (MAFF 243854) as the outgroup taxon. The dataset comprised 3,972 characters (SSU: 1,242bp; ITS: 650bp; LSU: 1,100bp; *tef-1a*: 980bp) after alignment, including gaps. Maximum parsimony analysis indicated that 3,195 characters were constant, 328 variable characters were parsimony-uninformative and 449 characters were parsimony-informative. The MP analysis resulted a single most parsimonious tree (CI = 0.580, RI = 0.565, RC = 0.328). The RAxML analysis resulted in a best scoring likelihood tree (FIGURE 1) with a final optimization likelihood value of -15239.234466. The matrix composed of 991 distinct alignment patterns, with 31.82% undetermined characters or gaps. Estimated base frequencies were: A = 0.237429, C = 0.251738, G = 0.27158, T = 0.239254; substitution rates AC = 1.400824, AG = 2.205205, AT = 1.383724, CG = 1.321812, CT = 7.911238, GT = 1.000000; gamma distribution shape parameter (alpha) = 0.150022; proportion of invariable sites = 0.804381. The ML, MP and BI analyses were resulted in generally congruent topologies, which was also in line with previous works (Dayarathne *et al.* 2018; Liu *et al.* 2022).



**FIGURE 1.** RAxML analysis of Lentitheciaceae based on a concatenated dataset of SSU, ITS, LSU and *tef-1a* sequence data. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) equal to or greater than 75% were placed above the branches (ML/MP). Bayesian posterior probabilities (BYPP) equal to or greater than 0.95 were shown as bold branches. Ex-type strains were in bold and new strains generated in this study were indicated in red.

Representatives of the sequenced genera (except for *Pseudomurilentithecium*) of Lentitheciaceae are included in our phylogenetic analysis (FIGURE 1). The two newly generated isolates formed a well-supported monotypic clade and can be recognized as a new genus (namely as *Pseudokeissleriella*) in Lentitheciaceae, and it showed close relationship to *Katumotoa* and *Neophiosphaerella*. *Crassoascoma*, *Darksidea*, *Halobyssothecium* and *Lentithecium* showed stable phylogenetic positions and relationships. *Katumotoa*, *Neophiosphaerella*, *Pseudokeissleriella*, *Tingoldiagio*, *Towyspora* showed to be closely related group.

## Taxonomy

*Pseudokeissleriella* Jian K. Liu, *gen. nov.*

*Mycobank*: MB 844117; *Facesoffungi* number: FoF 12707

*Etymology*:—The prefix “pseudo-” means “lying, false”, and the name “*Pseudokeissleriella*” refers to its morphological similarity to the genus “*Keissleriella*”.

*Saprobic* on decaying woody substrates. **Sexual morph**: *Ascomata* immersed to erumpent, subglobose, dark brown to black, ostiolate, glabrous, unilocular, coriaceous. *Ostiole* dark brown, periphysate. *Peridium* with multi-layers, comprising hyaline to brown cells of *textura angularis*. *Hamathecium* pseudoparaphyses, remotely septate. *Asci* 8-spored, bitunicate, cylindrical to cylindrical-clavate, short pedicellate, with an ocular chamber. *Ascospores* overlapping bi-seriate, fusiform, tapering to subobtuse ends, the upper cell is swollen towards the median septum, hyaline, septate, guttulate and with or without a mucilaginous sheath. **Asexual morph**: Undetermined.

**Type species**: *Pseudokeissleriella bambusicola* Yi Yang, S.N. Zhang & Jian K. Liu

*Notes*:—The phylogenetic analysis showed that two isolates of *Pseudokeissleriella* formed a monophyletic clade in Lentitheciaceae and is closely related to the monotypic genera *Katumotoa* (type: *Ka. bambusicola*) and *Neophiosphaerella* (type: *N. sasicola*) (FIGURE 1). *Pseudokeissleriella* resembles *Katumotoa* in having immersed, subglobose *ascomata*, but they can be distinguished by the *ascospores*. *Pseudokeissleriella* has fusiform *ascospores* tapering to subobtuse ends, with a swollen upper cell near the median septum and mucilaginous sheath, while *Katumotoa* has apiosporous *ascospores* with distinctive elongated bipolar mucilaginous sheath (Tanaka & Harada 2005). *Pseudokeissleriella* differs from *Neophiosphaerella* in having immersed, globose *ascomata* and fusiform, 1-septate *ascospores*, while the latter has superficial, hemispherical, with clypei *ascomata* and filiform, multi-septate *ascospores* (Tanaka *et al.* 2015). In addition, referring to the recommendations of Jeewon & Hyde (2016) for the establishment of species boundaries among fungi, *Pseudokeissleriella* can be distinguished from *Ka. bambusicola* and *N. sasicola* based on sequences data comparison from LSU (14/831; 11/831) and ITS (25/548; 36/548). The *ascospores* of *Pseudokeissleriella* are somewhat similar to *Keissleriella*. However, *Keissleriella* is thought to have a striking feature that most of their species have brown or black setae inside or around the *ostiole*. *Pseudokeissleriella* can be easily distinguished from the latter by the absence of setae around the *ostiole*, as well as the distance in molecular phylogeny.

*Pseudokeissleriella bambusicola* Yi Yang, S.N. Zhang and Jian K. Liu, *sp. nov.*

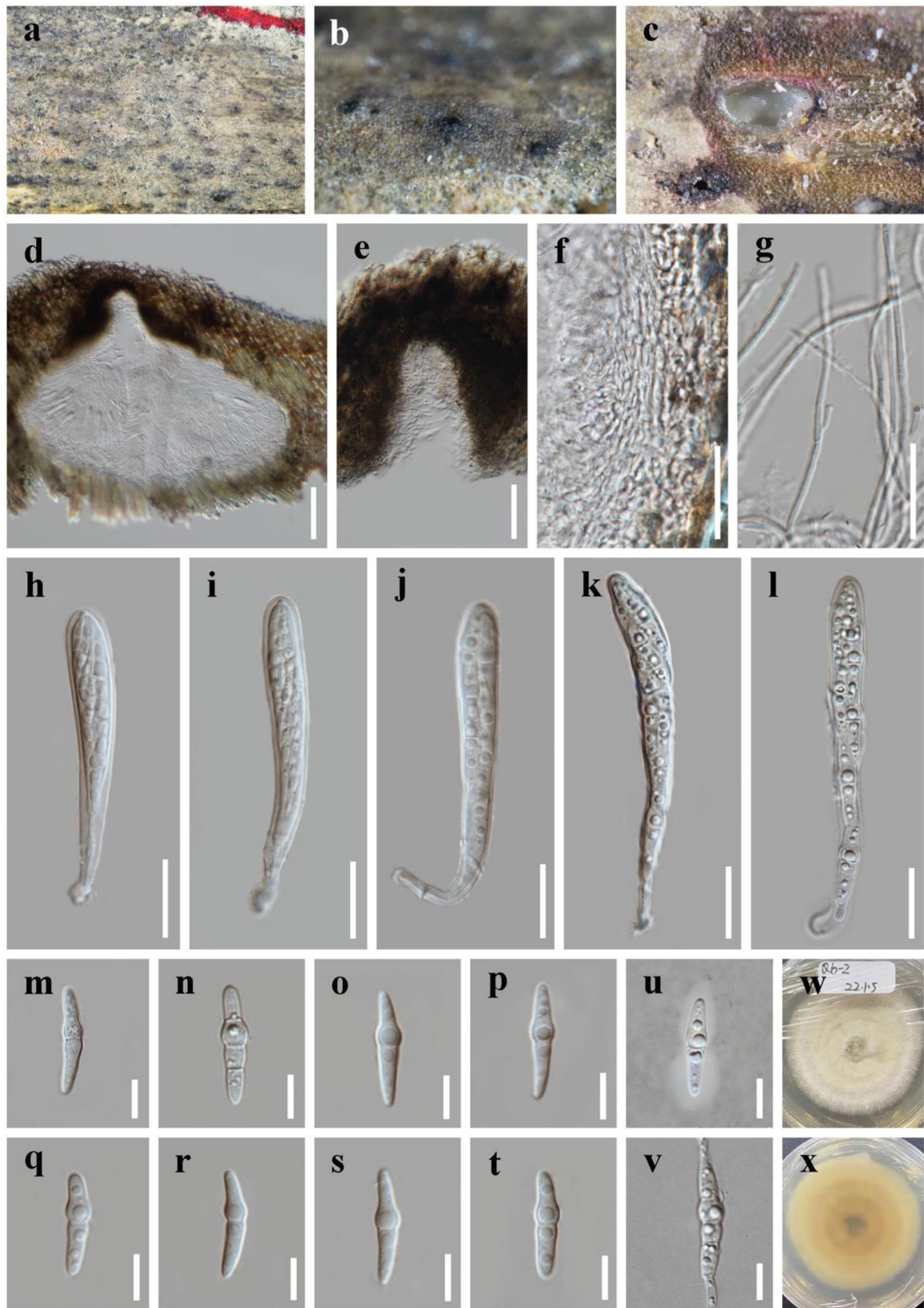
*Mycobank*: MB 888118; *Facesoffungi* number: FoF 12708; FIGURE 2

*Etymology*:—The suffix “-cola” means “inhabitant”, and the epithet “*bambusicola*” refers to the subfamily of plant host “Bambusoideae”, from which the fungus was collected.

*Holotype*:—HKAS 124020

*Saprobic* on dead culms of bamboo in a terrestrial habitat. **Sexual morph**: *Ascomata* 310–660 µm high, 400–520 µm diam ( $\bar{x}$  = 480 × 450 µm, n = 10), scattered, immersed to slightly erumpent, visible as dark brown to black, circular or lenticular areas with a central black dot, glabrous, in vertical section subglobose, base flattened, unilocular, coriaceous, with a central *ostiole*. *Ostiole* 85–160 µm long, 40–85 µm diam. ( $\bar{x}$  = 105 × 55 µm, n = 10), dark brown, periphysate. *Peridium* 15–45 µm diam, relatively thick, multi-layers, comprising hyaline to brown cells of *textura angularis*. *Hamathecium* 1.4–2.3 µm wide, comprising numerous, filiform, branched, septate, pseudoparaphyses. *Asci* 70–120 × 9–13 µm ( $\bar{x}$  = 95.1 × 11.1 µm, n = 30), 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, with a short pedicel, apically rounded with an ocular chamber. *Ascospores* 21–28.5 × 4.2–6.5 µm ( $\bar{x}$  = 25.5 × 5.5 µm, n = 30), overlapping bi-seriate, usually uniseriate in the lower half, fusiform, tapering to subobtuse ends, hyaline,

1(–3)-septate, the upper cell swollen towards the median septum, straight or slightly curved, smooth-walled, guttulate, surrounded by a mucilaginous sheath with depression in the middle. **Asexual morph:** Undetermined.



**FIGURE 2.** *Pseudokeissleriella bambusicola* (HKAS 124020, holotype) **a–b** Ascomata on host surface. **c** Horizontal section of an ascoma. **d** Vertical section of ascomata. **e** Ostiole. **f** Peridium. **g** Hamathecium. **h–l** Asci, **m–t** Ascospores. **u** Ascospore stained with India ink showing the mucilaginous sheath. **v** Germinating ascospore. **w, x** Colony on PDA (four weeks). Scale bars: **d** = 100  $\mu$ m, **e** = 50  $\mu$ m, **f–l** = 20  $\mu$ m, **m–v** = 10  $\mu$ m.

*Culture characteristics*:—Spores germinated on PDA within 24h. Colonies growing on PDA, reaching a diameter of 25 mm after 24 d at 25 °C, circular, surface slightly rough, white to faint yellow, reverse yellowish.

*Material Examined*:—CHINA. Sichuan province, Dujiang weir, Qingcheng Mountain, 30° 55' 7" N, 103° 29' 39" E, on dead culms of Bamboo, 2 December 2021, Yi Yang, Q6-2 (HKAS 124020, holotype); ex-type culture CGMCC 3.20950; *ibid.*, Xian-Dong Yu, HUEST 22.0030, paratype; ex-paratype culture UESTCC 22.0028.

## Discussion

This study contributes the diversity of bamboo fungi in Sichuan province, China, and a monotypic genus *Pseudokeissleriella* belong to Lentitheciaceae was isolated, identified and well-described. It is worth noting that three monotypic bambusicolous genera *Katumotoa*, *Neoophiosphaerella* and *Pseudokeissleriella* formed a well-supported clade in Lentitheciaceae. Nevertheless, the ascospore morphology and phylogenetic evidence justify their identification as distinct genera.

In the preliminary analysis based on a blast search in the NCBI GenBank nucleotide database, the LSU sequence of *Pseudokeissleriella bambusicola* was closest to *Keissleriella* (*Ke. dactylis* and *Ke. camporesiana*). However, the morphology and molecular phylogeny did not reveal any close associations between them. The previous studies of *Keissleriella* have agreed that the appearance of dark setae filled in and over the papilla is a distinguishing feature for the genus (Zhang *et al.* 2009, 2012, Crous *et al.* 2014, Ariyawansa *et al.* 2015, Liu *et al.* 2015, Singtripop *et al.* 2015, Tanaka *et al.* 2015, Tibpromma *et al.* 2017, Wanasinghe *et al.* 2018, Jiang *et al.* 2019, Phookamsak *et al.* 2019, Hyde *et al.* 2020) which is absent in *Pseudokeissleriella* and it is morphologically inconsistent with the traditional concept of *Keissleriella*. In addition, *Pseudokeissleriella* and *Keissleriella* species (those have sequence data) are phylogenetically distinct in the phylogenetic analysis, which also supports the establishment of *Pseudokeissleriella* as a new genus.

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