

***Bambusicola*, a new genus from bamboo with asexual and sexual morphs**

Dongqin DAI^{a,b,c,d}, D. Jayarama BHAT^e, Jiankui LIU^{b,c},
Ekachai CHUKEATIROTE^c, Ruilin ZHAO^{*,a} & Kevin D. HYDE^{b,c,d}

^a Key Laboratory of Forest Disaster Warning and Control in Yunnan Province,
Faculty of Conservation Biology, Southwest Forestry University,
Kunming 650224, China

^b Institute of Excellence in Fungal Research, Mae Fah Luang University,
Chiang Rai 57100, Thailand, e-mail: cicidaidongqin@gmail.com

^c School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100,
Thailand

^d International Fungal Research and Development Centre, the Research Institute
of Resource Insects, Chinese Academy of Forestry, Kunming 650224, China

^e Department of Botany, Goa University, Goa 403206, India

Abstract – *Bambusicola*, gen. nov., is introduced for four new saprobic taxa from culms of bamboos in northern Thailand. One species, chosen as the generic type, has both sexual and asexual morphs, one species has the sexual morph only and two species have the asexual morph only. The four new species, *Bambusicola massarinia*, *B. bambusae*, *B. irregulispota* and *B. splendida* are illustrated and described. Isolates of each species were sequenced using LSU, SSU and ITS genes and phylogenetic analysis shows the genus to be a distinct clade in the suborder “Massarineae”. Morphological differences between *Bambusicola* and some related genera are noted. *Bambusicola* is characterized by small, cone-shaped ascomata, slightly broad and fusiform ascospores and a coelomycete asexual morph with light brown conidia. *Bambusicola* clusters in Trematosphaeriaceae, but the closeness of this relationship cannot be resolved.

***Bambusicola* / anamorph / coelomycete / phylogeny / taxonomy**

INTRODUCTION

Bamboos, members of the grass family Poaceae and subfamily Bambusoideae, are widely distributed in Thailand, in several forest types, especially mixed deciduous forest and in regenerated bamboo forests (Choeyklin *et al.*, 2009). A review of literature on bamboo-associated fungi reveals that nearly 1100 species have so far been described or recorded worldwide (Hyde *et al.*, 2002). According to Tanaka *et al.* (2009), most of the recorded bambusicolous fungi in Asia are

* Corresponding author: Ruilin Zhao, email address: zhaoruilin@gmail.com

from Japan. Only a few of bamboo-associated fungi have so far been reported from Thailand (Morakotkarn *et al.*, 2007). We are documenting the fungi diversity on bamboo of Thailand and in this paper reports on four new taxa with sexual and/or coelomycetous asexual states in the Dothideomycetes.

The coelomycetes are asexual morphs of a large number of taxa belonging to mostly Ascomycota (Sutton, 1980). These are normally found and recovered from cultivated and wild plant habitats, especially on decaying plant litter and other organic debris, from marine, freshwater and lichens and as parasites of insects (Sutton, 1980). There are more than 1000 genera and 7000 species of coelomycetes (Kirk *et al.*, 2008). Phylogenetic studies of coelomycetes are relatively poor and of the 992 recognized genera, only 256 can be fitted into the taxonomic framework for ascomycetes (Hyde *et al.*, 2011).

There are a number of reports of ascomycetes from bamboo (Hino & Katumoto, 1954-1966; Eriksson & Yue, 1998). However, there is much less data on the asexual morphs and few reports linking asexual and sexual morphs. However, Tanaka *et al.* (2009) described five new genera in Tetraplosphaeriaceae linking *Tetralpoa* and *Tetralpoa*-like asexual morphs with *Massarina*-like sexual morphs. During our studies on the diversity of bambusicolous fungi of northern Thailand, we have encountered numerous taxa of fungi. In this paper, we introduce a genus with four new species. The genus includes sexual and asexual morphs and is introduced on morphological and analysis of molecular sequence data.

MATERIALS AND METHODS

Collection and isolation of fungi: Fallen and decomposing bamboo culms were collected from various localities in Chiang Rai Province, Thailand, during May to September 2011. The samples were placed in plastic Zip lock bags and returned to laboratory for examination. The specimens were incubated in sterile moist chambers and examined at regular intervals until the resident fungi attained maturity and sporulated. The fungi were examined under dissecting and compound microscopes to establish if they required further study. Specimens were isolated by single spore following the method of Chomnunti *et al.* (2011). The colonies were transferred to 2% potato-dextrose agar (PDA) and incubated at room temperature to promote sporulation. Observations and photographs were made as described in Liu *et al.* (2010) and Boonmee *et al.* (2011). Herbarium material is deposited at MFLU herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). The cultures are maintained at Mae Fah Luang University Culture Collection (MFLUCC) and Research Institute of Resource Insects, Chinese Academy of Forestry (IFRD), the latter under Material Transfer Agreement (No.C29/2011).

DNA extraction, PCR amplification and sequencing: In this study, fungal isolates were grown on PDA for 30 to 45 d at 25-32°C in light. Genomic DNA was extracted from fresh mycelia, following the specification of Biospin Fungus Genomic DNA Extraction Kit (BioFlux®). The primers ITS5 and ITS4, NS1 and NS4 (White *et al.*, 1990), LROR and LR5 (Vilgalys & Hester 1990) were used to amplify the regions internal transcribed spacers (ITS), large subunit rDNA (LSU) and small subunit rDNA (SSU). Polymerase chain reaction (PCR) amplification was carried out following Phillips *et al.* (2008). Amplified PCR fragments were sequenced by Kunming Shuo Yang Technology Company (P.R. China).

DNA sequence analyses. Sequences were aligned using Bioedit (Hall, 2004) and ClustalX (Kohli & Bachhawat, 2003). Alignments were checked and manual adjustments were made wherever necessary. Phylogenetic analyses were performed by using PAUP v. 4.0b10 (Swofford, 2003) for Maximum-parsimony (MP). Trees were visualized with TreeView (Page, 1996).

A blast search was performed to reveal the closest matches with taxa in the Pleosporales. For the phylogenetic analyses, DNA sequences of LSU region in this study, together with the reference various taxa of different families of Pleosporales obtained from GenBank (Table 1) were aligned by using Bioedit (Hall, 1999). The whole ambiguously aligned regions within each dataset were excluded from the analyses (Roux *et al.*, 2010). In the analyses, gaps were treated as missing data, and all characters were unordered and of equal weight (Liu *et al.*, 2011).

Table 1. The list of species used in this study

<i>Species</i>	<i>Source</i>	<i>Host</i>	<i>Collector</i>	<i>Location</i>	<i>LSU</i>	<i>SSU</i>	<i>ITS</i>
<i>Aigialus grandis</i>	BCC 18419	Mangrove wood	E.B.G. Jones	Malaysia, Morib	GU479774		
<i>Aigialus grandis</i>	JK 5244A	Mangrove wood	J. Kohlmeyer	Bahamas, Mores Island	GU301793		
<i>Bambusicola bambusae</i>	MFLUCC 11-0614	Bamboo	D.Q. Dai	Thailand, Chiang Rai	JX442035	JX442039	JX442031
<i>Bambusicola irregulispora</i>	MFLUCC 11-0437	Bamboo	D.Q. Dai	Thailand, Chiang Rai	JX442036	JX442040	JX442032
<i>Bambusicola massarina</i>	MFLUCC 11-0389	Bamboo	D.Q. Dai	Thailand, Chiang Rai	JX442037	JX442041	JX442033
<i>Bambusicola splendida</i>	MFLUCC 11-0439	Bamboo	D.Q. Dai	Thailand, Chiang Rai	JX442038	JX442042	JX442034
<i>Didymella bryoniae</i>	CBS 133.96				GU301863		
<i>Falciformispora lignatilis</i>	BCC 21117	Mangrove wood (<i>Elaeis guineensis</i>)	U. Pinruan	Thailand, Ban Bang Sak	GU371826		
<i>Falciformispora lignatilis</i>	BCC 21118	Mangrove wood (<i>Elaeis guineensis</i>)	U. Pinruan	Thailand, Ban Bang Sak	GU371827		
<i>Halomassarina thalassiae</i>	BCC17054	Mangrove wood	E.B.G. Jones	U.S.A., Florida	GQ925849		
<i>Halomassarina thalassiae</i>	BCC 17055	Mangrove wood	E.B.G. Jones	U.S.A., Florida	GQ925850		
<i>Halomassarina thalassiae</i>	JK5385B	Mangrove wood	J. Kohlmeyer	Fiji, Viti Levu, Suva	GU479804		
<i>Halomassarina thalassiae</i>	JK 5262D	Mangrove wood	J. Kohlmeyer	Belize, Tobacco	GU301816		
<i>Lentithecium fluviatile</i>	CBS 123090			Range	FJ795450		
<i>Lentithecium fluviatile</i>	CBS 122367				GU301825		

Table 1. The list of species used in this study (*continued*)

<i>Species</i>	<i>Source</i>	<i>Host</i>	<i>Collector</i>	<i>Location</i>	<i>LSU</i>	<i>SSU</i>	<i>ITS</i>
<i>Massarina eburnea</i>	CBS 473.64				GU301840		
<i>Morosphaeria ramunculicola</i>	BCC 18405	Mangrove wood	E.B.G. Jones	Malaysia, Morib	GQ925854		
<i>Morosphaeria ramunculicola</i>	JK 5304B				GU479794		
<i>Morosphaeria velataspora</i>	BCC 17059	Mangrove wood	E.B.G. Jones	U.S.A., Florida	GQ925852		
<i>Morosphaeria velataspora</i>	BCC 17058	Mangrove wood	E.B.G. Jones	U.S.A., Florida	GQ925851		
<i>Myriangium hispanicum</i>	CBS 247.33				GU301854		
<i>Neottiosporina paspali</i>	CBS 331.37				EU754172		
<i>Phaeosphaeria avenaria</i>	DAOM 226215				AY544684		
<i>Phaeosphaeriopsis musae</i>	CBS 120026				GU301862		
<i>Phoma betae</i>	CBS 109410				EU754178		
<i>Phoma exigua</i>	CBS 431.74				EU754183		
<i>Pleospora herbarum</i>	CBS 191.86				DQ247804		
<i>Polyposphaeria fusca</i>	KT 1043				AB524603		
<i>Pseudotetraploa curviappendiculata</i>	MAFF 239495	<i>Sasa kurilensis</i>	Y. Harada	Japan, Aomori	AB524608		
<i>Quadricrura septentrionalis</i>	HHUF 28781	<i>Sasa kurilensis</i>	N. Asama	Japan, Aomori	AB524615		
<i>Stagonospora macropycnidia</i>	CBS 114202				GU301873		
<i>Trematosphaeria pertusa</i>	CBS 122368				FJ201990		
<i>Trematosphaeria pertusa</i>	CBS 122371				GU301876		
<i>Triplosphaeria maxima</i>	KT 870				AB524637		

Abbreviations of isolates and culture collections: BCC = BIOTEC Culture Collection, Bangkok, Thailand; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; DAOM Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; KJ = Jacobs and Rehner 1998; MFUCC = Mae Fah Luang University Culture Collection; MAFF Ministry of Agriculture, Forestry and Fisheries, Japan; JK J. Kohlmeyer; KT K. Tanaka.

Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Max trees were unlimited,

branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis and Bull 1993).

RESULTS AND DISCUSSION

Phylogenetic analyses

Partial nucleotide sequences of the LSU ribosomal DNA (870 bp) were determined for four fresh specimens isolates. The other sequences used in the analyses were obtained from GenBank (Table 1). The single gene 28S (LSU) rRNA data comprised 35 sequences of 26 taxa, including the outgroup taxon, were aligned and analysed separately. New sequences including LSU, ITS, SSU regions are deposited in GenBank (Table 1).

Single LSU gene sequences of *Bambusicola* isolates were aligned with a set of sequences retrieved from GenBank (Table 1) representing all genera (*Halomassarina*, *Trematosphaeria* - type, *Falciformispora*) in Trematosphaeriaceae (Suetrong *et al.*, 2011; Zhang *et al.*, 2012) and three families in suborder “Mas-sarineae”, and a further five families in Pleosporales (Zhang *et al.*, 2012), as well as Dothideomycetidae sequences that were selected as the outgroup taxa (*Myriangium hispanicum*) (Schooch *et al.*, 2009). The LSU alignment contained 840 characters including coded alignment gaps. Of the remaining 827 characters, 282 were constant, while 333 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 212 parsimony informative characters resulted in a single tree is shown Fig. 1 with bootstrap support at the branches.

The phylogenetic trees obtained from Maximum-parsimony analyses showed that the new isolates in this study regarding to the genera relationships of Trematosphaeriaceae. All the strains of *Bambusicola* were shown to be a distinguishable genus in the Trematosphaeriaceae with high bootstrap support (97%), and were distinct from the *Halomassarina*, *Trematosphaeria*, and *Falciformispora* clades. Therefore, a new genus is described to accommodate these taxa.

Taxonomy

Bambusicola D. Q. Dai & K.D. Hyde, **gen. nov.**

Mycobank: MB 801041

Etymology: From the Latin *cola* meaning dwelling on and *Bambusa* in reference to the first record of this genus on bamboo.

Habit saprobic on decaying bamboo culms. *Ascomata* small, solitary, scattered, immersed, conical, ostiolate, coriaceous. *Peridium* thin composed of brown and thick-walled cells *textura angularis*, with the basal part composed of thinner, hyaline, smaller cells. *Hamathecium* dense, anastomosing and branching, with pseudoparaphyses above the asci. *Asci* 8-spored, bitunicate, cylindrical, with a short furcate pedicel, with a shallow apical chamber. *Ascospores* 2-3-seriate above, slightly broad fusiform, 1-septate, narrowly rounded at both ends, hyaline, surrounded by a gelatinous sheath. *Conidiomata* small, pycnothyrial, acerose or

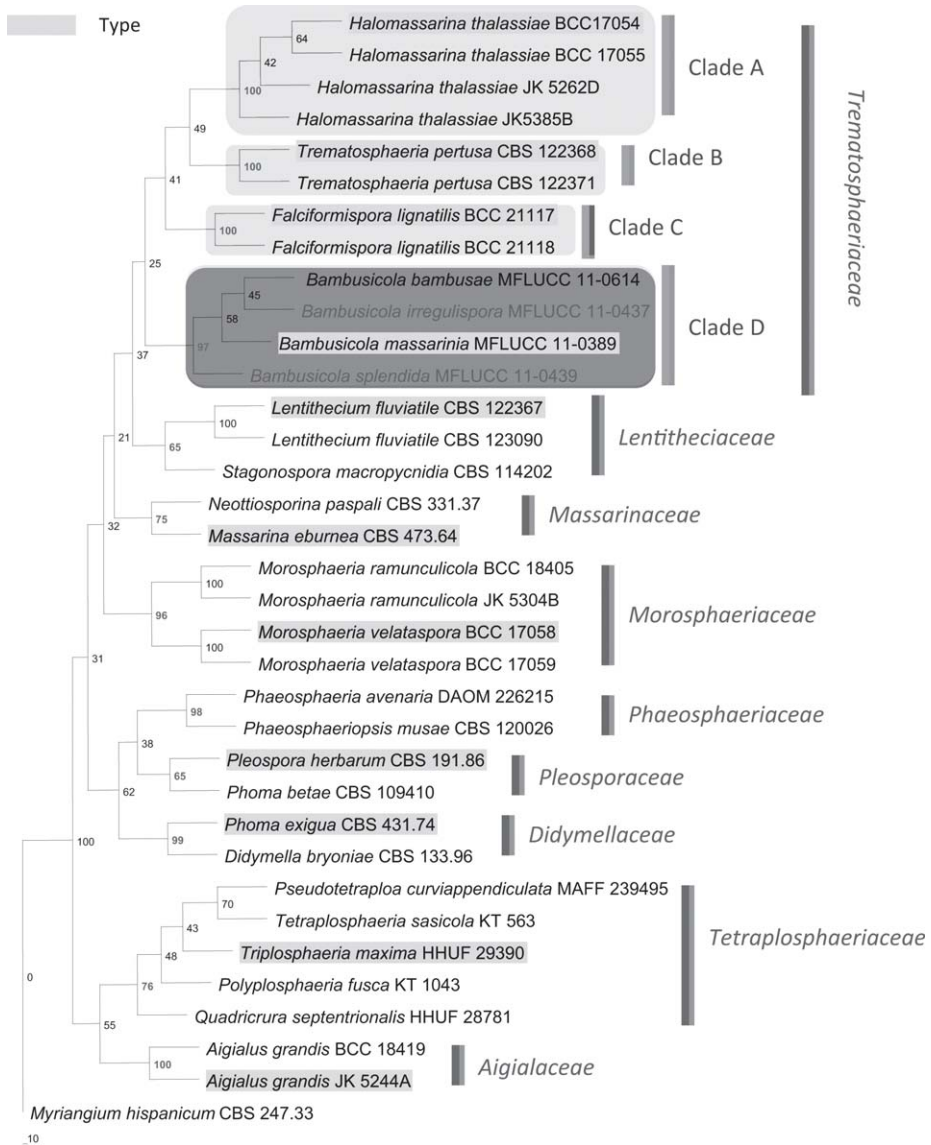


Fig. 1. Phylogenetic tree generated from parsimony analysis based on LSU nrDNA sequences. Data were analyzed with random addition sequence and treating gaps as missing data. Maximum parsimony bootstrap support values are shown at nodes based on 1000 replicates. The tree is rooted with *Myriangiium hispanicum*.

subglobose, solitary, scattered, immersed to half-immersed. *Conidiophores* indistinct. *Conidiogenous cells* holoblastic, annelidic, discrete, cylindrical, and smooth, *Conidia* pale brown to dark brown, 1-3-septate, cylindrical, straight or slightly curved, obtuse at the apex, with many guttules.

Generic type: *Bambusicola massarinia* D. Q. Dai & K.D. Hyde

Notes: The morphology of *Bambusicola* species is similar to that of some species of *Massarina sensu lato* (Hirayama *et al.*, 2010). This includes immersed to erumpent ascomata with a peridium incorporating hosts tissues, cylindrical asci with an ocular chamber and hyaline, fusiform ascospores with a mucilaginous sheath (Zhang *et al.*, 2009b). The genus *Massarina* has now been shown to be polyphyletic (Hirayama *et al.*, 2010). *Bambusicola* species are also similar to genera in Tetraplosphaeriaceae (Tanaka *et al.*, 2009). *Tetraplosphaeria*, *Triplo-sphaeria* and *Polyplosphaeria* have immersed to superficial, globose to subglobose ascomata, cylindrical to clavate asci and hyaline, fusiform ascospores surrounding by a mucilaginous sheath. *Triplosphaeria* and *B. bambusae* especially have similar rim-like side walls (Tanaka *et al.*, 2009). Tetraplosphaeriaceae however, differs in having hyphomycetous anamorphs in the genera *Tetraplosphaeria*, *Triplosphaeria* and *Polyplosphaeria*. In the phylogenetic tree based on Maximum-parsimony analysis of LSU genes (Fig. 1), *Bambusicola* clusters close to Trematosphaeriaceae (97% MP support). Trematosphaeriaceae includes the genera *Falciformispora*, *Halomassarina* and *Trematosphaeria* (Suetrong *et al.*, 2011), however, the morphology of species in these genera are unlike those in *Bambusicola*. No asexual morphs are also known for this family although *Trematosphaeria pertusa* produced hyphopodia-like structures (or conidia) in culture (Suetrong *et al.*, 2011).

Bambusicola species also form a sister group to species in Lentitheciaceae (Fig. 1). *Lentithecium* has lenticular, globose to subglobose or irregularly globose ascomata and clavate to oblong-clavate asci, and broadly fusoid ascospores (Zhang *et al.*, 2009a; b). In *Bambusicola* however, the ascomata are conical in section, asci are cylindrical and ascospores are slightly broadly fusiform. The ascomata in *Keissleriella* have setae around the papilla (Zhang *et al.*, 2012); such setae are lacking in *Bambusicola*. *Katumotoa*, a genus only known from bamboo, differs in having asymmetric, 2-4-septate ascospores, with a reddish brown echinate central cell at maturity and long gelatinous terminal appendages (Tanaka & Harada, 2005). All genera differ from *Bambusicola* as the latter has coelomycetous asexual states. The anamorphs of species in Lentitheciaceae are *Stagonospora* species (Wijayawardene *et al.*, 2012).

The asexual morph of *Bambusicola* can be compared with *Asteromidium*, *Chaetoseptoria*, *Cytostagnospora*, *Phaeoseptoria*, *Phloeosporella*, *Sclerostagnospora* and *Stagonospora*. In *Asteromidium*, *Chaetoseptoria*, *Cytostagnospora* and *Stagonospora* however the conidia are hyaline and distinct from *Bambusicola* (Nag Raj, 1993; Pomella *et al.*, 1997; Sutton, 1980; Tehon, 1937). *Phaeoseptoria*, *Phloeosporella* and *Sclerostagnospora* have brown conidia, but in *Phaeoseptoria*, the pycnidia are globose, and conidiogenous cells are small and without annulations (Dianese *et al.*, 1993; Tehon, 1937; Walker *et al.*, 1992). In *Phloeosporella*, conidiomata are acervular, conidiogenous cells lack annulations and conidia have obtuse ends (Dianese *et al.*, 1993). In *Sclerostagnospora* conidiogenous cells are small, ampulliform to irregular and conidia have a truncate base (Sutton, 1980). Another genus warranting reference here is *Septoriella* as this genus is isolated from grasses belonging to the family Poaceae, the same as that of bamboo. In *Septoriella*, the relatively long cylindrical conidia have an apical gelatinous cap and truncate base and the conidiogenous cells are not annelidic (Sutton, 1980). Molecular data also indicate that these genera are unrelated (data not shown).

Key to species of *Bambusicola*

- 1. Fruiting bodies ascomata 2
- 1. Fruiting bodies conidiomata 3

2. Ascomata forming dark round erumpent spots on host surface with a black hallow around ostiolar opening *Bambusicola massarinia*
 2. Ascomata forming dark brown spots on the host surface without black hallow at ostiolar opening *B. bambusae*
 3. Conidiomata acerose, conidia $15-18 \times 1.5-3 \mu\text{m}$ *B. irregulispora*
 3. Conidiomata subglobose, conidia $20-30 \times 3.5-5 \mu\text{m}$ *B. splendida*

***Bambusicola massarinia* D.Q. Dai & K.D. Hyde, sp. nov.**

Figs 2, 3

Mycobank: MB 801045

Etymology: With reference to its broad similarity to *Massarina* species

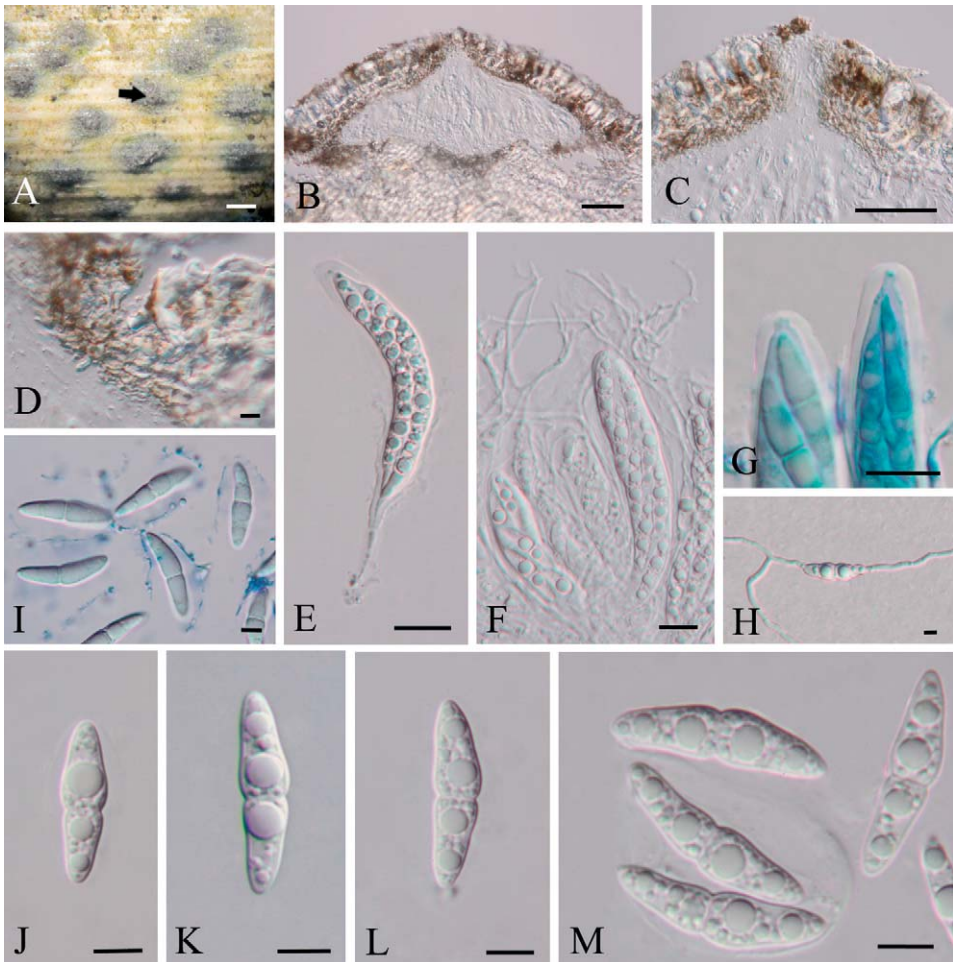


Fig. 2. *Bambusicola massarinia* (holotype). **A.** Habit of immersed ascomata on host. **B.** Section of ascoma **C.** Section through ostiole with periphyses. **D.** Peridium. **E.** Asci with ascospores. **F.** Pseudoparaphyses above asci. **G.** Apex of asci. **H.** Germinating ascospore. **I.** Ascospore with gelatinous sheath. **J-M.** Hyaline and 1-septate ascospores. Scale Bars: A = 300 μm B, C = 50 μm , D-G = 10 μm , H-M = 5 μm .

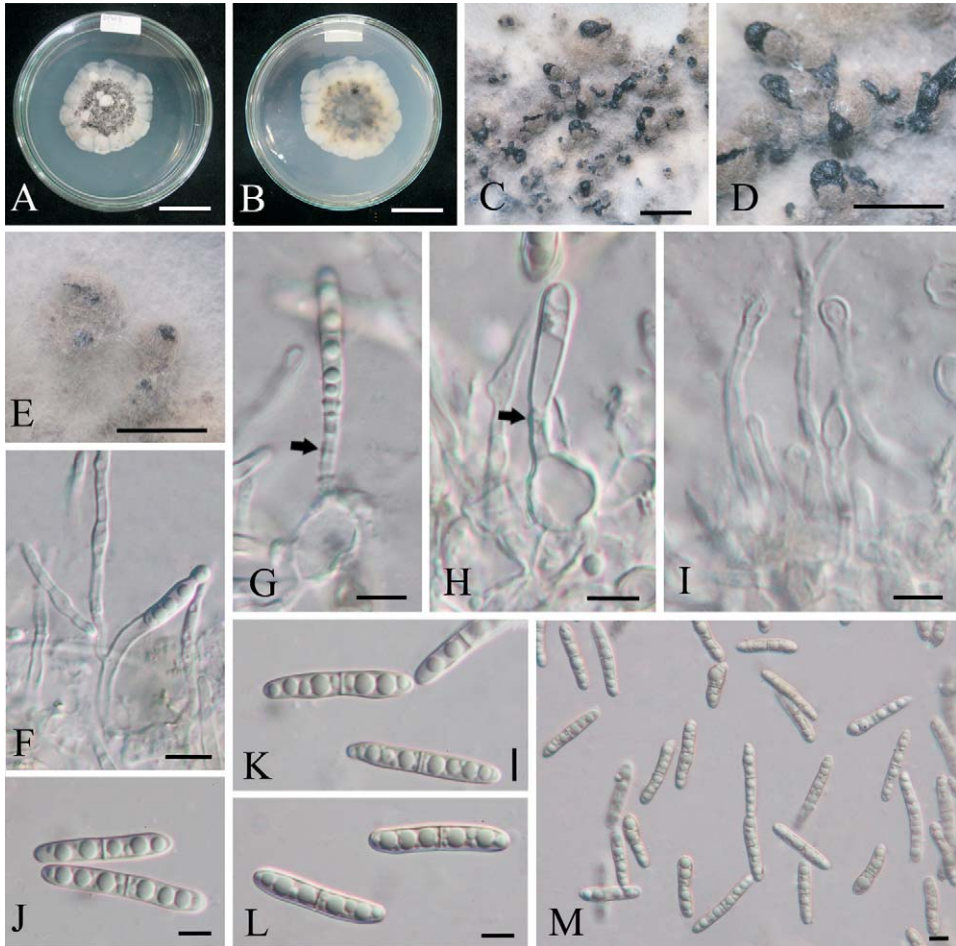


Fig. 3. *Bambusicola massarinia*. Anamorph coelomycete. **A-B**. Colony on PDA. **C-E**. Conidiomata surrounded by mycelium. **F-I**. Conidiophores and conidiogenous cells producing conidia. **G-H**. Conidiogenous cells with 1-5 annellations. **K-M**. 1-sepate conidia. Scale bars: A, B = 25 mm, C-E = 100 μ m, F-M = 5 μ m.

Habit saprobic on decaying bamboo culms, forming dark round erumpent spots on host surface with ascocarps on raised areas. *Ascوماتa* 190-290 μ m high, 75-130 μ m diam., solitary, scattered, remaining immersed under host tissue at maturity, conical in section, black at ostiolar opening, coriaceous, with centrally located ostiole lined with periphyses (Fig. 2A). *Peridium* comprising host and fungal tissues, laterally 5-15 μ m thick, composed of brown and thick-walled cells of *textura angularis*, with the basal part composed of thinner, hyaline, smaller cells (Fig. 2 BD). *Hamathecium* of dense, with long and 0.7-1.8 μ m wide, anastomosing and branching pseudoparaphyses above the asci. *Asci* 44-94 \times 7-14 μ m (\bar{x} = 70.1 \times 12 μ m, n = 20), 8-spored, bitunicate, cylindrical, with a short furcate pedicel, with a shallow apical chamber (Fig. 2 E-G). *Ascospores* 16-22 \times 4-6.5 μ m (\bar{x} = 21.5 \times 5 μ m, n = 20), 2-3-seriate, slightly broad fusiform, 1-septate, with large upper

cell, narrowly rounded at both ends, hyaline, surrounded by a gelatinous sheath (Fig. 2 I-M).

Culture on PDA: Colonies circular, with uneven margin, brown at the centre, light-colored at the periphery, floccose, slow growing, 48 mm diam. in 45 days at 25-32 C (Fig. 3 A, B). *Conidiophores* 5-20 μm long, 1-2 μm wide, indistinct, hyaline, branched, septate, smooth. *Conidiomata* on culture, 40-80 μm diam., subglobose, pale brown, carbonaceous (Fig. 3 C-E). *Conidiogenous cells* holoblastic, annelidic, cylindrical, discrete, and smooth, with 1-5 barely discernible annellations (Fig. 3 G, H). *Conidia* 14-20 \times 2-3 μm (\bar{x} = 16 \times 2.5 μm , n = 20), pale brown, 1-septate, cylindrical, straight or slightly curved, obtuse at the apex, with many guttules (Fig. 3 J-M).

Material examined: THAILAND, Chiang Rai Province, Doi Fung, on decaying culm of bamboo, 4 May 2011, Dongqin Dai DDQ0003 (MFLU12-0405, holotype); ex-type living culture at MFLUCC 11-0389, IFRDCC 2452.

Notes: *Bambusicola massarinia* is selected as the type of *Bambusicola* as it has both sexual and asexual morphs. This taxon is characterized by ascomata forming dark round erumpent spots on the host surface and with black, conspicuous ostioles. Conidiogenous cells are holoblastic, annelidic and pale brown, and conidia are 1-septate and cylindrical. The asexual morph of *B. massarinia* has distinct conidiophores and 1-septate conidia, whereas in *B. irregulispora* and *B. splendida* conidiophores are absent and conidia remain 3-septate.

***Bambusicola bambusae* D.Q. Dai & K.D. Hyde, sp. nov.**

Fig. 4

Mycobank: MB 801046

Etymology: With reference to its occurrence on *Bambusa* sp.

Habit saprobic on decaying bamboo culms, forming dark brown spots on the host surface with ascomata breaking through slightly raised cracks at the centre. *Ascomata* 80-125 μm high, 450-700 μm diam., solitary, scattered or in groups of 2-5, immersed under the host tissue, conical in section, slight brown, coriaceous, ostiolate at the centre, black at the tip of ostiolar opening (Fig. 4A). *Peridium* comprising host and fungal tissues, laterally 30-45 μm thick, composed of brown and thick-walled cells *textura angularis* intermingled with host cells, with basal part 15-20 μm thick, with 100-150 μm thick sides, composed of small to big, hyaline, rim-like cells (Fig. 4B-C). *Hamathecium* composed of dense, long, 0.5-1 μm broad, septate, branched pseudoparaphyses between and above the asci (Fig. 4F). *Asci* 40-70 \times 5-7.5 μm (\bar{x} = 56.6 \times 6.6 μm , n = 10), 8-spored, bitunicate, fissitunicate, cylindrical, with a shallow apical chamber and a short furcate pedicel (Fig. 4D-E). *Ascospores* 15-20 \times 2-4.5 μm (\bar{x} = 17.6 \times 3.4 μm , n = 20), 2-3-seriate, 1-septate, slightly broad fusiform, occasionally with large upper cell, with narrowly rounded ends, hyaline, surrounded by a thick gelatinous sheath 1.5-2 μm wide (Fig. 4H-K).

Culture on PDA: Colonies slow growing, attaining 34 mm diam. after 45 days at room temp. (25-32C), circular, drift white on the periphery and yellow in center from front view, dark yellow to black from back view (Fig. 4 L, M).

Material examined: THAILAND, Chiang Rai Prov., Mae Fah Luang University, on dead culm on Bamboo, 1 August 2011, Dongqin Dai, DDQ0099 (MFLU12-0406, holotype), living culture at MFLUCC 11-0614, IFRDCC 2581.

Note: *Bambusicola bambusae* is characted by immersed ascomata, which are conical in section and have rim-like side walls. Asci are cylindrical and ascospores are 1-septate, slightly broadly fusiform, hyaline and surrounded by a thick gelatinous sheath. Such characters are simialr to some species in *Triplosphaeria* (Tanaka *et al.*, 2009). However species in *Triplosphaeria* differ in

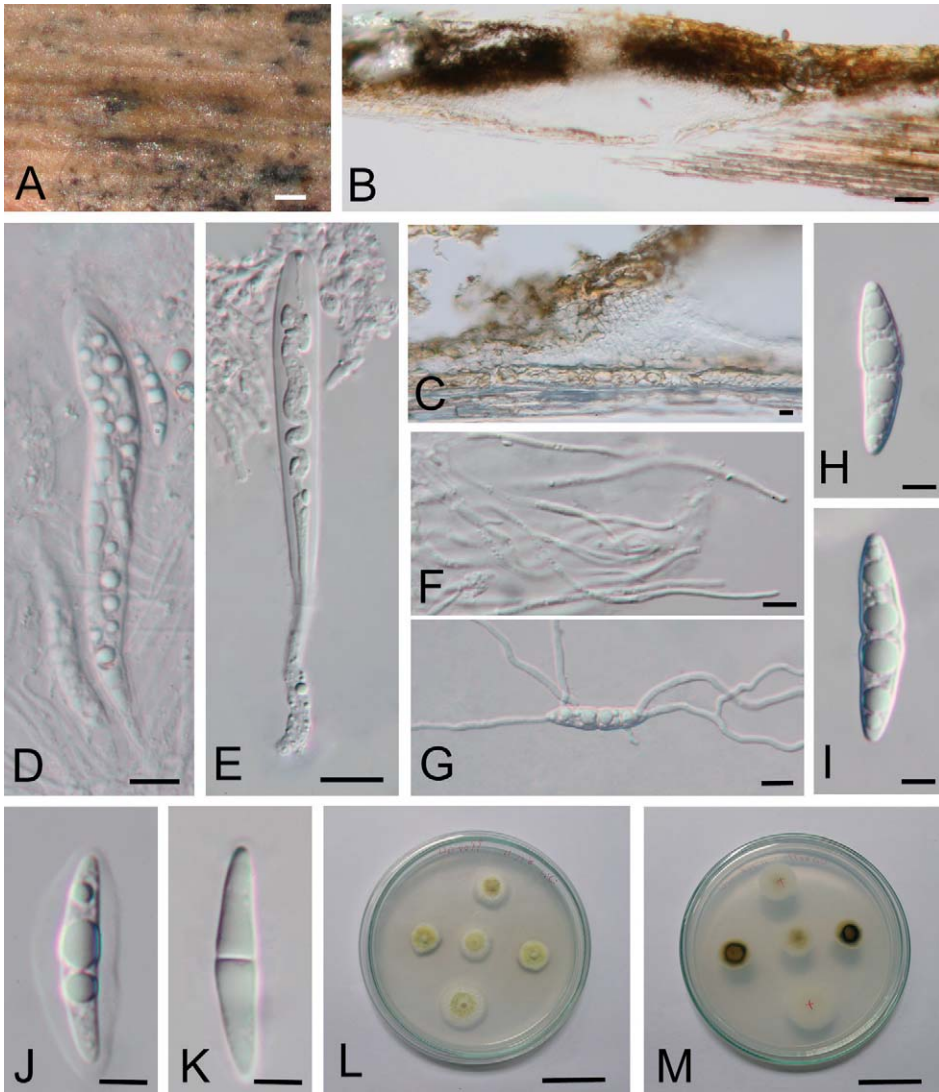


Fig. 4. *Bambusicola bambusae* (holotype). Sexual morph. **A.** Habit of immersed ascomata on host. **B.** Section of ascoma. **C.** Peridium at the base composed of hyaline cells. **D.** Ascus with ascospores. **E.** Immature ascus. **F.** Pseudoparaphyses. **G.** Germinating ascospore. **H-K.** Hyaline, 2-celled ascospores. **J.** Spore surrounded by thick gelatinous sheath. **L-M.** Colony on PDA 30 d. **B-K.** Photograph of slides with water mount, L, M. Photograph on white paper. Scale bars: A = 500 μ m, B = 50 μ m, C-E = 10 μ m, F-K = 5 μ m, L, M = 25 mm.

having *Tetraploa*-like asexual morphs with 3 setose appendages anamorph and in molecular data (Tanaka *et al.*, 2009).

***Bambusicola irregulispora* D.Q. Dai & K.D. Hyde, sp. nov.**

Mycobank: MB 801047

Etymology: With reference to irregular septation of the conidia.

Fig. 5

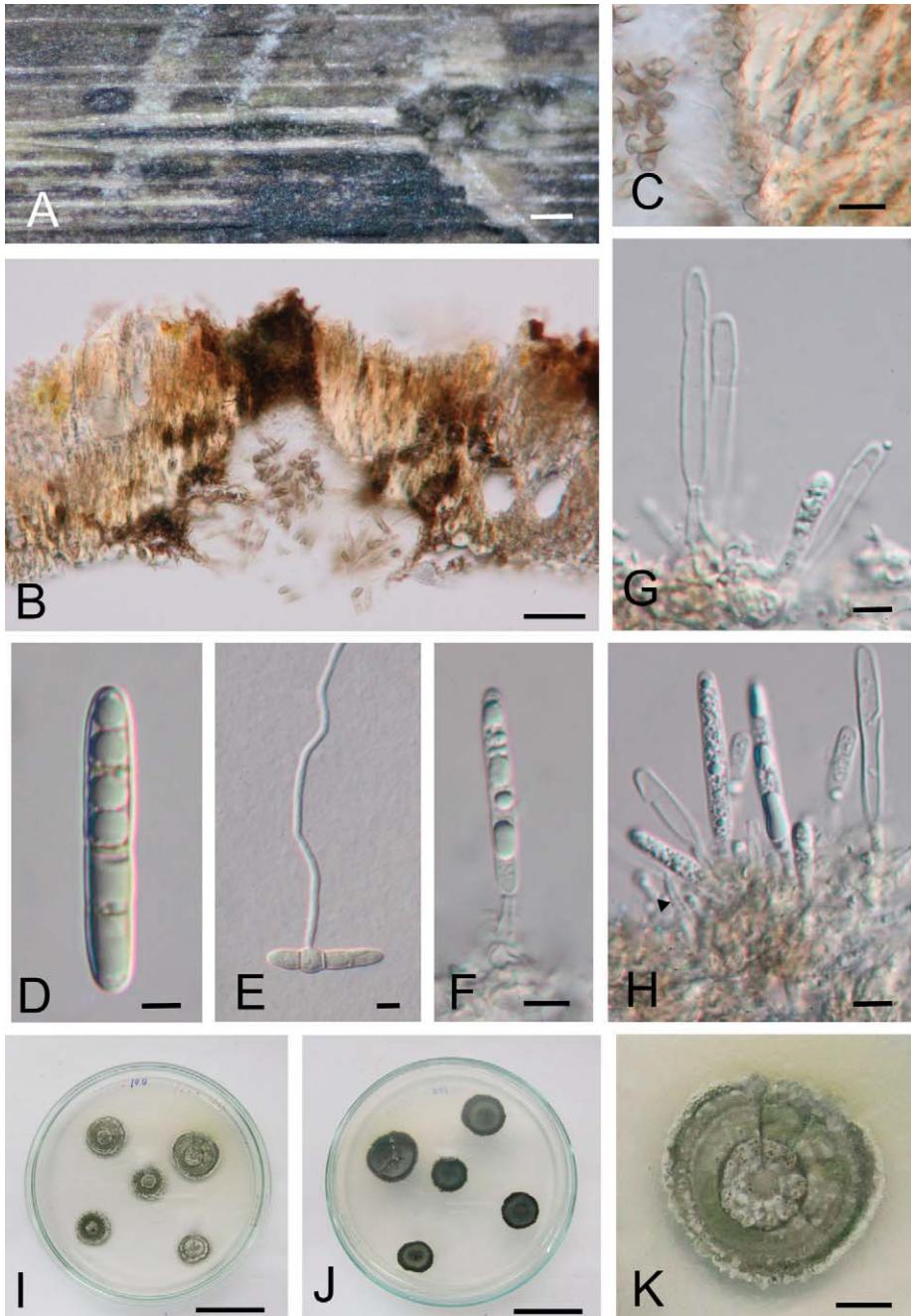


Fig. 5. *Bambusicola irregulispora* (holotype). Asexual morph. **A.** Fruiting body on bamboo host. **B.** Section of pycnothyrium. **C.** Wall of pycnothyrium showing the cell's shape. **D.** Pale brown conidia with 3 septa. **E.** Geminating conidia. **F-H.** Conidiogenous cells and developing conidia. **I-K.** Cultures on PDA in 45 days. Scale Bars: A = 100 μ m, B = 100 μ m, C = 10 μ m, D-F = 5 μ m, I, J = 25 mm, K = 5 mm.

Habit saprobic on bamboo culms, forming slightly darkened areas on the host surface with conidiomata breaking through slightly raised areas. *Conidiomata* pycnothyrial partly immersed under the host tissue, slightly erumpent when mature, solitary, scattered, 400-750 µm long, 80-165 µm high, 80-150 µm in diam., acerose, pyriform in section, coriaceous, with 60-80 µm long dark, ostiolar neck (Fig. 5A). *Conidiomatal wall* moderately thick, 3.5-10 µm wide, comprising 2-3 layers of cells *textura angularis*, with dark brown outer layers and hyaline conidiogenous inner layer (Fig. 5B-C). *Conidiogenous cells* 3-5 × 0.5-1.5 µm (\bar{x} = 3.8 × 1.1 µm, n = 15), holoblastic, annelidic, cylindrical, integrated to discrete, smooth, hyaline, each with 1-5 barely discernible annellations (Fig. 5C, H). *Conidia* 15-18 × 1.5-3 µm (\bar{x} = 17.7 × 2.4 µm, n = 15), initially hyaline to pale brown and aseptate, on maturity becoming pale brown to dark brown, 3-septate, cylindrical, obtuse at both ends, and smooth, straight with many guttules (Fig. 5D, F).

Culture on PDA: Colonies slow growing, 19 mm diam. after 45 d at 25-32°C, circular, with uneven margin, floccose at center, drift white from front view and light brown at the centre from back view after 10 d. Mature colonies in 30 d show concentric and alternating grey and dark zones from front view (Fig. 5I-K).

Material examined: THAILAND, Chiang Rai Prov., Jiew Santonkok, on dead stem on Bamboo, 11 August 2011, Dongqin Dai, DDQ00100 (MFLU12-0407, holotype), living culture at MFLUCC 11-0437, IFRDCC 2610.

Note: *Bambusicola irregulispora* is similar to *Ciferriella* in conidial shape. However, the conidia of *Ciferriella* are hyaline (Sutton 1980). *B. irregulispora* is characterized by conidiomata which are acerose and pyriform in section, and is distinct from *B. splendida* which has subglobose conidiomata. In *B. irregulispora* conidia are smaller (15-18 × 1.5-3 µm), while in *B. splendida* they are larger (20-30 × 3.5-5 µm).

***Bambusicola splendida* D.Q. Dai & K.D. Hyde, sp. nov.**

Fig. 6

Mycobank: MB 801048

Etymology: In reference to magnificent form of the conidia.

Habit saprobic on bamboo culms, with conidiomata star-like, breaking through raised cracked areas. *Conidiomata* pycnothyrial, 75-150 µm high, 160-210 µm diam., subglobose, partly immersed under host tissue, partly erumpent, solitary, in groups of 3-7, subglobose, dark brown, coriaceous, ostiolate in the centre (Fig. 6 A). *Conidiomatal wall* 5-15 µm wide, comprising 3-4 layers, with cells *textura angularis*, with brown to dark brown outer layers and hyaline innermost conidiogenous layer (Fig. 6 B). *Conidiogenous cells* 3.5-9 × 1-2 µm (\bar{x} = 6.2 × 1.5 µm, n = 10), holoblastic, cylindrical, integrated, smooth (Fig. 6C, D). *Conidia* 20-30 × 3.5-5 µm (\bar{x} = 24.6 × 4.5 µm, n = 20), initially hyaline and aseptate, at maturity becoming pale to moderately dark brown, cylindrical, straight to slightly curved, obtuse at both ends, 3-septate, slightly constricted at septa, smooth, occasionally slightly narrower at the lower end, with many guttules (Fig. 6E-H).

Culture on PDA: Colonies slow growing, 33 mm diam. after 30 d at 25-32°C, circular, smooth margin, cottony. Young, 7 day old colonies drift white; mature 30 d old colonies becoming yellowish to dark green in the center and yellowish in the outer region (Fig. 6J-L).

Material examined: THAILAND, Chiang Rai Prov., Doi Pui, on dead stem of Bamboo, 1 September 2011, Dongqin Dai, DDQ00117 (MFLU12-0408, holotype), living culture at MFLUCC 11-0439, IFRDCC 2564.

Notes: *Bambusicola splendida* is established herein for its star-like conidiomata, breaking through raised cracked host wall, partly immersed under host tissue to partly erumpent. *B. splendida* has larger conidia than the other species in this genus.

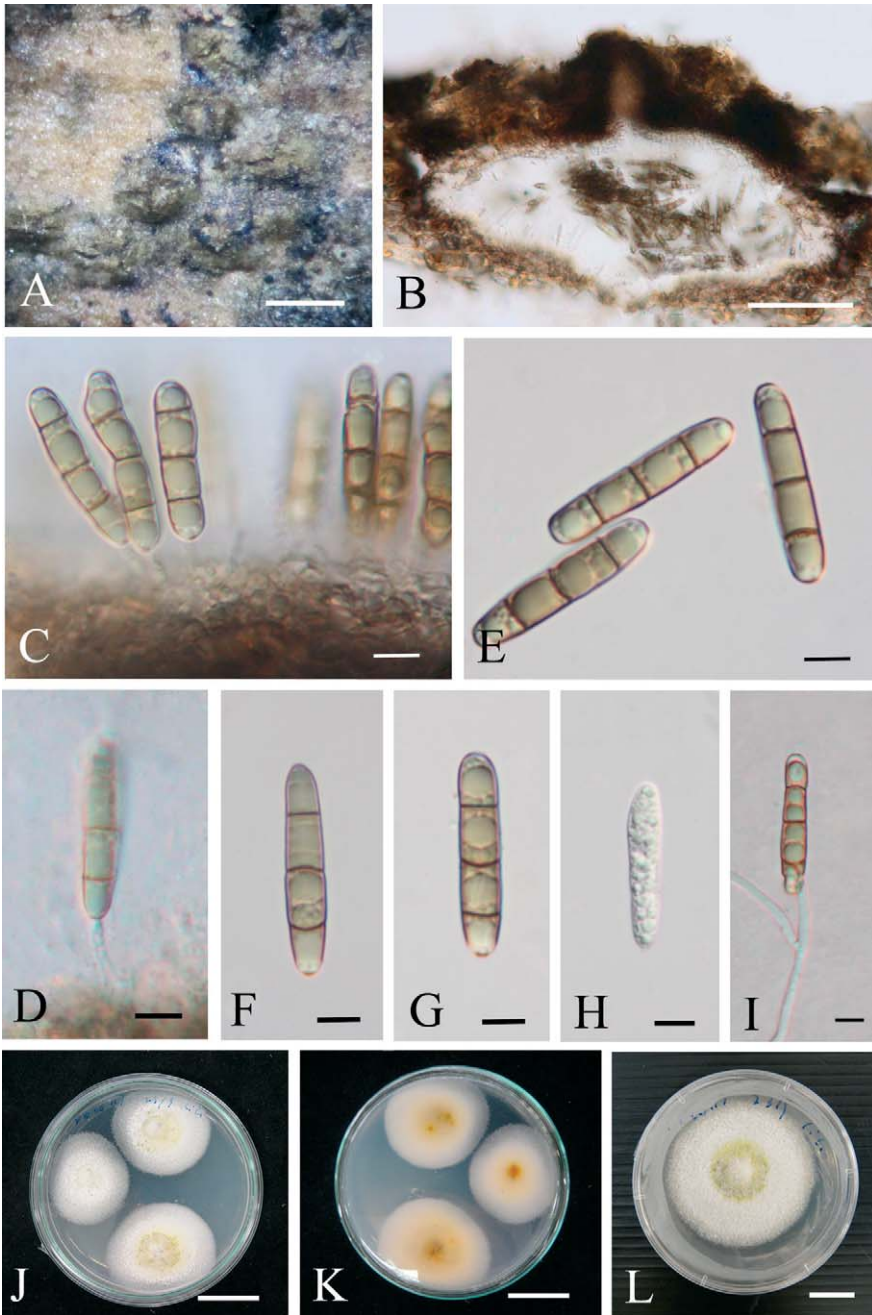


Fig. 6. *Bambusicola splendida* (holotype). Asexual morph. **A.** Fruiting bodies on bamboo host. **B.** Section of pycnothyrium. **C-D.** Conidiogenous cells with conidia. **E-H.** Conidia. **E-G.** Brown conidia with 3-septate. **H.** Initially hyaline and aseptate conidia. **I.** Gminating conidia. **J-L.** Cultures on PDA in 30 days. Scale Bars: A = 200 μ m, B = 50 μ m, C-I = 5 μ m, J, K = 25 mm, L = 10 mm.

DISCUSSION

Naming unusual coelomycetes is extremely difficult despite the excellent books such as *Fungi Imperfecti with Pycnidia Acervuli and Stromata* by Sutton (1980) and *Icones Generum Coelomycetum* series by Nag Raj (1971-1987) as well as *Coelomycetous anamorphs with appendage-bearing conidia* by Nag Raj (1993). Thus we believe that there are numerous taxa of pycnidial fungi awaiting recognition and description. These taxa need to be named, while the type species of existing genera needed to be recollected, epitypified, sequenced and sequence data deposited in GenBank. This is only this way that we will be able to untangle the large numbers of coelomycetous asexual morphs and link them to their sexual states or at least classify them in the ascomycete taxonomic framework.

We have found both sexual and asexual morphs of this new genus and named it as a single biological entity *Bambusicola*. The generic type produced the sexual morph on bamboo and the asexual morph in culture. The International Code of Nomenclature for algae, fungi and plants no longer accepts naming separate species for sexual and asexual morphs (Hawksworth, 2012) and therefore we opt for one name here which represents the entire fungus.

Bambusicola is a distinct genus from the other three genera, namely *Halomassarina*, *Trematosphaeria* and *Falciformispora* in Trematosphaeriaceae based on our molecular phylogenetic tree and is also phylogenetically close to the Lentitheciaceae, which is one of the families within the suborder “Massarineae” (Zhang et al., 2012). However, the LSU gene cannot resolve the taxonomic placement of this new genus within or outside Trematosphaeriaceae. Further research using TEF, RPB1 and RPB2 analysis (Suetrong et al., 2011) of all genera in Dothideomycetes is needed.

Acknowledgements. The Mushroom Research Foundation, Bandoo, Chiang Rai Province, Thailand is acknowledged for providing postgraduate scholarship support. The Thailand Research Fund (BRG 5280002) and the National Natural Science Foundation of China (Project ID: 31000013) and West Light Foundation of the Chinese Academy of sciences to Ruilin Zhao are also thanked for support. The authors thank Wenjing Li (Mae Fah Luang University, Thailand, MFU) for assistance in molecular work. Dongqin Dai thanks Rungtiwa Phookamsak and Dhanushka Udayanga (Mae Fah Luang University, Thailand, MFU) for finding literature and also thanks Feng Wen (International Fungal Research and Development Centre, the Research Institute of Resource Insects, Chinese Academy of Forestry, China,) for depositing cultures in IFRD.

REFERENCES

- PHILLIPS A.J.L., ALVES A., PENNYCOOK S.R., JOHNSTON P.R., RAMALEY A., AKULOV A., CROUS P.W., 2008 — Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* 21: 29-55.
- CHOEYKLIN R., HATTORI T., JARITKHUAN S. & JONES E.B.G., 2009 — *Bambusicolous* polypores collected in Central Thailand. *Fungal Diversity* 36: 121-128.
- CHOMNUNTI P., SCHOCH C.L., AGUIRRE-HUDSON B., KO-KO T.W., HONGSANAN S., JONES E.B.G., KODSUEB R., PHOOKAMASAK R., CHUKEATIROTE E. & BAHKALL A.H., 2011 — Capnodiaceae. *Fungal Diversity* 51: 103-134.
- DIANESE J.C., SUTTON B.C. & TESSMANN D.J., 1993 — Two deuteromycetes, *Phloeosporella flavio-moralis* sp. nov. and *Pseudocercospora punctata* comb. nov., causing leaf lesions on *Eugenia* spp. *Mycological Research* 97(1): 123-126.
- ERIKSSON O. E. & YUE J. Z., 1998 — *Bambusicolous* pyrenomycetes, an annotated check-list. *Myconet* 1: 25-78.

- HALL T., 2004 [INTERNET] — BIOEDIT VERSION 6.0.7. [CITED 2010 NOV17]; AVAILABLEFROM: HTTP://WWW.MBIO.NCSU.EDU/BIOEDIT/BIOEDIT.HTML
- HILLIS D.M. & BULL J.J., 1993 — An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42(2): 182-192.
- HINO 1. & KATUMOTO K., 1954 — Illustrationes fungorum bambusicolorum. n. Bulletin of the Faculty of Agriculture, Yamaguti University 5: 213-234.
- HINO 1. & KATUMOTO K., 1955 — Illustrationes fungorum bambusicolorum III. Bulletin of the Faculty of Agriculture, Yamaguti University 6: 29-68.
- HINO 1. & KATUMOTO K., 1956 — Illustrationes fungorum bambusicolorum IV. Bulletin of the Faculty of Agriculture, Yamaguti University 7: 267-274.
- HINO 1. & KATUMOTO K., 1957 — Illustrationes fungorum bambusicolorum V. Bulletin of the Faculty of Agriculture, Yamaguti University 8: 649-658.
- HINO 1. & KATUMOTO K., 1958 — Illustrationes fungorum bambusicolorum VI. Bulletin of the Faculty of Agriculture, Yamaguti University 9: 877-908.
- HINO 1. & KATUMOTO K., 1959 — Illustrationes fungorum bambusicolorum VII. Bulletin of the Faculty of Agriculture, Yamaguti University 10: 1175-1194.
- HINO 1. & KATUMOTO K., 1960 — Illustrationes fungorum bambusicolorum VIII. Bulletin of the Faculty of Agriculture, Yamaguti University J I: 9-34.
- HINO 1. & KATUMOTO K., 1961 — Illustrationes fungorum bambusicolorum IX. Bulletin of the Faculty of Agriculture, Yamaguti University 12: 151-162.
- HINO 1. & KATUMOTO K., 1965 — Notes on bambusicolous fungi (1). *Journal of Japanese Botany* 40: 81-89.
- HIRAYAMA K., TANAKA K., RAJA H.A., MILLER A.N. & SHEARER C.A., 2010 — A molecular phylogenetic assessment of *Massarina ingoldiana* sensu lato. *Mycologia* 102(3): 729-746.
- HYDE K.D., ZHOU D. & DALISAYL T., 2002 — Bambusicolous fungi: a review. *Fungal Diversity* 9:1-14.
- HYDE K.D., MCKENZIE E.H.C. & KOKO T.W., 2011 — Towards incorporating anamorphic fungi in a natural classification-checklist and notes for 2010. *Mycosphere* 2(1):1-88.
- KOHLI D.K. & BACHHAWAT A.K., 2003 — CLOURE: Clustal Output Reformatter, a program for reformatting ClustalX/ClustalW outputs for SNP analysis and molecular systematics. *Nucleic acids research* 31(13): 3501-3502.
- LIU J.K., PHOOKAMSAK R., JONES E.B.G., ZHANG Y., Ko-Ko T.W., HU H.L., BOONMEE S., Doilom, M., CHUKEATIROTE E. & BAHKALI A.H., 2011 — *Astrosphaeriella* is polyphyletic, with species in *Fissuroma* gen. nov., and *Neoastrrosphaeriella* gen. nov. *Fungal Diversity* 51: 135-154.
- LIU J.K., CHOMNUNTI P., Cai L., PHOOKAMSAK R., CHUKEATIROTE E., JONES E.B.G., MOSLEM M. & HYDE D.K., 2011—Phylogeny and morphology of *Neodeightonia palmicola* sp. nov. from palms. *Sydowia* 62: 260-276.
- MORAKOTKARN D., KAWASAKI H. & SEKI T., 2007 — Molecular diversity of bamboo - associated fungi isolated from Japan. *FEMS microbiology letters* 266(1): 10-19.
- NAG RAJ T.R., 1993 — Coelomycetous anamorphs with appendage bearing conidia. *Mycologue Publications*. Waterloo, Canada.
- NAG RAJ T.R., 1971-1987 — Icones generum coelomycetum I-XIII. University of Waterloo Biology Series.
- PAGE R.D.M., 1996 — TreeView. An application to display phylogenetic trees on personal computer. *Computer Applications in the Biosciences* 12: 357-358.
- PAUL M. K., PAUL F. C., DAVID W. M. & JOOST A. S., 2008 — Dictionary of the Fungi 10th Edition, p. 155.
- POMELLA A.W.V., BARRETO R.W., ALFENAS A.C. & MESQUITA J.B., 1997 — *Asteromidium tabebuiae-impetiginosae* sp. nov. causing a leaf spot disease of *Tabebuia impetiginosa* in Brazil. *Mycotaxon* 64: 83-89.
- ROUX B. A., DIDIER B., BERNARD S., MICHAEL J., WINGFIELD & JOLANDA, 2010 — Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. *Mycological Progress* 9: 101-123.
- SCHOCH C.L., CROUS P.W., GROENEWALD J.Z., BOEHM E.W.A., BURGESS T.I., DE GRUYTER J., De HOOG G.S., DIXON L.J., GRUBE M. & GUEIDAN C., 2009 — A class-wide phylogenetic assessment of *Dothideomycetes*. *Studies in Mycology* 64(1): 1-15.
- SUETRONG S., HYDE K.D., ZHANG Y., BAHKALI A.H. & GARETH JONES E.B., 2011 — Trematosphaeriaceae fam. nov. (Dothideomycetes, Ascomycota). *Cryptogamie-Mycologie* 32(4): 343.
- SUTTON B.C., 1980 —The Coelomycetes- Fungi imperfecti with Pycnidia, Acervuli and Stromata. *Commonwealth Mycological Institute*, Kew, UK.

- SWOFFORD D.L., 2003 — PAUP*: phylogenetic analysis using parsimony, version 4.0 b10. Sinauer Associates, Sunderland.
- TANAKA K. & HARADA Y., 2005 — Bambusicolous fungi in Japan (6): Katumotoa, a new genus of phaeosphaeriaceous ascomycetes. *Mycoscience* 46(5): 313-318.
- TANAKA K., HIRAYAMA K., YONEZAWA H., HATAKEYMA S., HARADA Y., SANO T., SHIROUZU T. & HOSOYA T., 2009 — Molecular taxonomy of bambusicolous fungi: *Tetraplophaeriaceae*, a new pleosporalean family with *Tetraploa*-like anamorphs. *Studies in Mycology* 64(1): 175-209.
- TEHON L.R., 1937 — Notes on the Parasitic Fungi of Illinois: VI. *Mycologia* 29(4): 434-446.
- VILGALYS R. & HESTER M., 1990 — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172(8): 4238-4246.
- WALKER J., SUTTON B.C. & PASCOE I.G., 1992 — *Phaeoseptoria eucalypti* and similar fungi on *Eucalyptus*, with description of *Kirramyces* gen. nov. (Coelomycetes). *Mycological Research* 96(11): 911-924.
- WHITE T.J., BRUNS T., LEE S. & TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods and applications: 315-322. Academic Press, San Diego, USA.
- WIJAYAWARDENE D.N.N., MCKENZIE E.H.C. & HYDE K.D., 2012 — Towards incorporating anamorphic fungi in a natural classification—checklist and notes for 2011. *Mycosphere* 3(2): 157-228.
- ZHANG Y., CROUS P.W., SCHOCH C.L. & HYDE K.D., 2012 — *Pleosporales*. *Fungal Diversity* 53: 1-221.
- ZHANG Y., SCHOCH C.L., FOURNIER J., CROUS P.W., DE GRUYTER J., WOUDEMBERG J.H.C., HIRAYAMA K., TANAKA K., POINTING S.B. & SPATAFORA J.W., 2009a — Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re-evaluation. *Studies in Mycology* 64(1): 85-102.
- ZHANG Y., WANG H.K., FOURNIER J., CROUS P.W., JEEWON R., POINTING S.B. & HYDE K.D., 2009b — Towards a phylogenetic clarification of *Lophiostoma/Massarina* and morphologically similar genera in the *Pleosporales*. *Fungal Diversity* 38: 225-251.