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# The phylogenetic placement of *Eriosporella bambusicola* sp. nov. in *Capnodiales*

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**Abstract** – A new species of *Eriosporella, E. bambusicola*, is described from bamboo in Northern Thailand. *Eriosporella* is monotypic genus of coelomycetous fungi known from palms and bamboo and characterized by hyaline conidia which have a short basal cell and three slender divergent apical arms. Maximum-likelihood and maximum parsimony analyses of combined LSU and SSU rDNA sequence data set show *E. bambusicola* to belong to the *Capnodiales* where it forms a cluster with *Pseudoramichloridium*. Its relationships with other genera are unresolved and therefore considered as *Capnodiales incertae sedis*. The tree needs to be better populated with sequence data from more related species to clarify the familial placement of this genus.

#### Asexual morphs / coelomycetous fungi / multi-gene analyses / taxonomy

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# **INTRODUCTION**

Coelomycetous fungi are characterized by producing conidia inside the conidiomata *viz*. acervuli, pycnidia or stromata (Sutton, 1980; Nag Raj, 1993; Wijayawardene *et al.*, 2012b). Most of these described coelomycetous fungi have not been linked to sexual forms or placed in natural classification system (Wijayawardene *et al.*, 2012a). Recognition of mere co-occurrence of both morphs on same host material (Spooner & Kirk, 1982) and culture-based methods (Hyde *et al.*, 1996) were the most common practices used by taxonomists for establishing links of sexual and asexual states, before the introduction of molecular based methods (Hyde *et al.*, 2013a, b). Recent taxonomic studies of conidial fungi and their phylogenetic placements were entirely based on DNA sequence analyses (Boonmee *et al.*, 2011; Dai *et al.*, 2012; Zhang *et al.*, 2012; Wijayawardene *et al.*, 2013, 2014).

The genus *Eriosporella* was introduced by Höhnel (1916) with *E. calami* ( $\equiv$  *Cryptosporium calami* Niessl.) as the type species. The genus is presently placed in *Pezizomycotina incertae sedis* (Kirk *et al.*, 2008). However, Wijayawardene *et al.* (2012a) stated that this genus might have a sexual state in *Laetinaevia* Nannf. (*Dermateaceae, Helotiales*). Currently gene sequences for this genus are lacking in GenBank hence its taxonomic placement is uncertain.

During studies on the diversity and taxonomy of microfungi occurring on bamboo (*Poaceae, Bambusoideae*) in Northern Thailand (Dai *et al.*, 2012), we have collected a coelomycetous species which has the main morphological features of *Eriosporella*. It is described as *Eriosporella bambusicola* on the basis of its morphology. Further, we have carried out maximum-likelihood (ML) and maximum-parsimony (MP) analyses of combined data set of LSU and SSU rDNA to show the ordinal placement of this genus.

# **MATERIALS AND METHODS**

**Collection and isolation of fungi:** Living bamboo leaves were collected from Chiang Rai Province, Thailand. The samples were placed in plastic zip lock bags and brought to laboratory. The specimens were incubated in a sterile moist chamber and examined at regular intervals until the resident fungi attained maturity and sporulated. The collection was isolated from single spores following the method of Chomnunti *et al.* (2011). Colonies were transferred to 1.5 ml. microcentrifuge tubes with 2% potato-dextrose agar (PDA) and 2.0 ml screw cap microcentrifuge tubes with 10-15% glycerol for depositing at 4°C and –20°C respectively. Microscopic observation and photomicrographs were made as described in Liu *et al.* (2011) and Boonmee *et al.* (2011). Herbarium materials are deposited at MFLU herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). The living cultures are maintained at Mae Fah Luang University Culture Collection (MFLUCC), Research Institute of Resource Insects, Chinese Academy of Forestry (IFRD), Landcare Research, New Zealand (ICPM) and Microbial diversity for science and industry, the latter under Material Transfer Agreement (No.C29/2011).

**DNA extraction, PCR amplification and sequencing:** In our study, the fungal isolates were grown on PDA for 30 d at 27°C in dark. Genomic DNA was extracted from fresh mycelia, following the specification of Biospin Fungus Genomic

DNA Extraction Kit (BioFlux<sup>®</sup>). The primer pairs ITS5 (GGAAGTAAAAGTC GTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC), and NS1 (GTAGTCATATGCTTGTCTC) and NS4 (CTTCCGTCAATTCCTTTAAG) (White *et al.*, 1990) and LROR (GTACCCGCTGAACTTAAGC) and LRS (ATCCTGAGGGAAACTTC) (Vilgalys & Hester, 1990) were used to amplify the internal transcribed spacers (ITS) small subunit rDNA (SSU) and large subunit rDNA (LSU) respectively. Polymerase chain reaction (PCR) amplification was carried out following Phillips *et al.* (2008). Amplified PCR fragments were sequenced at Kunming Shuo Yang Technology Company (P.R. China).

**DNA sequence analyses:** Blast searches at GenBank were carried out both LSU and SSU rDNA sequences in order to reveal the closest relatives of our strain and their sequences were downloaded (Table 1). The downloaded sequences represent *Capnodiaceae*, *Cladosporiaceae* (*Davidiellaceae*), *Dissoconiaceae*, *Mycosphaerellaceae*, *Schizothyriaceae* and *Teratosphaeriaceae*. Sequences were aligned by using Bioedit (Hall, 2001) and ClustalX (Kohli & Bachhawat, 2003). Alignments were checked and manual adjustments were made wherever necessary. Phylogenetic analyses were performed using PAUP v. 4.0b10 (Swofford, 2003) for maximum-parsimony (MP) and raxmlGUI v.0.9b2 (Silvestro & Michalak, 2010) for maximum-likelihood (ML). Trees were visualized with TreeView (Page, 1996). The whole ambiguously aligned regions within each dataset were excluded from the analyses (Begoude 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).

Maximum-parsimony analysis was 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 & Bull, 1993).

# RESULTS

# **Phylogenetic analyses**

Partial nucleotide sequences of the LSU and SSU ribosomal DNA (around 800 bp) were determined for the fresh isolate. LSU and SSU regions were used in the phylogenetic analyses for the species placement (Fig. 1). The ITS gene sequences are not included in the phylogenetic analyses of this study, but are deposited in GenBank (accession number KF548664) as it is preferred loci for use in fungal phylogenetics (Schoch *et al.*, 2011; Liu *et al.*, 2012).

The data set contained 33 sequences of 32 taxa including two outgroups (i.e. *Myriangium duriaei* and *M. hispanicum*). Of the 1821 characters used in the phylogenetic analyses, 329 were parsimony-informative, 88 variable and parsimony-uninformative and 1404 constant. Maximum-parsimony and maximum-likelihood analyses resulted in the same tree as shown in Fig. 1 with bootstrap support on the branches.

Species name	Source	GenBank accession numbers		
		LSU	SSU	ITS
Capnodium coartatum	MFLUCC10-0069	JN832614	JN832599	
Capnodium coffeae	CBS 147.52	GU214400	DQ247808	
Catenulostroma germanicum	CBS 539.88	EU019253	GU214518	
Cladosporium bruhnei	CBS 115683; ATCC66670	GU214408	AY251096	
Davidiella tassiana	CBS 723.79; ATCC 201090	GU214410	HQ871897	
Devriesia staurophora	CPC 3687; CBS 375.81	GU214416	EF137359	
Dissoconium aciculare	CBS 204.89	GU214419	GU214522	
Dothidea insculpta	CBS 189.58	NG_027643	DQ247810	
Dothidea sambuci	DAOM 231303	NG_027611	AY544722	
Eriosporella bambusicola	MFLUCC 11-0436	KF548665	KF548666	KF548664
Graphiopsis chlorocephala	CBS 121523; CPC 11969	EU009458	GU214534	
Leptoxyphium cacuminum	MFLUCC10-0059	JN832603	JN832588	
Mycosphaerella graminicola	CBS 110744	EU019298	AY251117	
Mycosphaerella punctiformis	CBS 113265	NG_027571	AY490775	
Myriangium duriaei	CBS 260.36	NG_027579	NG_013129	
Myriangium hispanicum	CBS 247.33	GU301854	GU296180	
Passalora vaginae	CBS 140.34; DSM 1148	GQ852624	GU214561	
Pleospora chenopodii	CBS 344.78	EU754132	EU754033	
Pleospora herbarum	CBS 191.86	GU238160	GU238232	
Pseudoramichloridium brasilianum	CBS 283.92	EU041854		
Pseudoramichloridium henryi	CBS:124775	GQ303320		
Ramichloridium apiculatum	CPC 12310	GU214687	GU214687	
Ramularia nagornyi	CBS 120253	EU019257	GU214579	
Schizothyrium pomi	CBS 406.61	EF134949	EF134949	
Schizothyrium pomi	CBS 486.50	EF134948	EF134948	
Teratosphaeria fibrillosa	CBS 121707	GU323213	GU296199	
Teratosphaeria jonkershoekensis	CBS 112224	GU301874	GU296200	
Toxicocladosporium irritans	CBS 185.58	EU040243	GU214619	
Uwebraunia commune	CBS:110747	GU214420	NG_016521	
Uwebraunia dekkeri	CBS 110748; CMW14906	GU214422	GU214528	

#### Table 1. The list of species used in this study

Abbreviations of isolates and culture collections: ATCC: American Type Culture Collection, Virginia, USA; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; DSM: Deutsche Sammlung von Mikrorrganismen und Zellkulturen GmbH, Braunschweig, Germany; MFUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand.

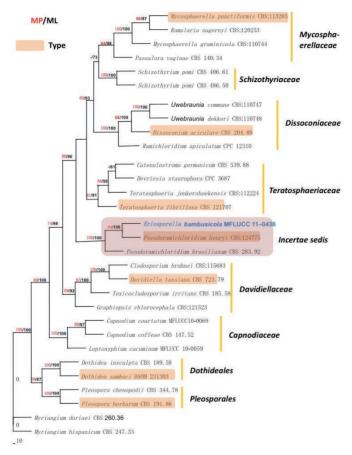


Fig. 1. RAxML tree based on a combined dataset of SSU and LSU sequences. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) greater than 50% are given above the nodes. The original culture numbers are given after the species names. The tree is rooted with *Myriangium duriaei* (CBS 260.36) and *M. hispanicum* (CBS 247.33).

Our *Eriosporella* species nested in a well-supported clade together with two species of *Pseudoramichloridium* (*P. henryi* (CBS: 124775) and *P. braziliensis* (CBS 283.92) (Cheewangkoon *et al.*, 2009) with 94/100% (MP/ML) bootstrap values.

# Taxonomy

Eriosporella bambusicola D. Q. Dai, N.N. Wijayawardene & K.D. Hyde sp. nov.

Fig. 2

MycoBank: MB 805309 Holotype: MFLU 13-0107 Etymology: with reference to its occurrence on *Bambusa* sp.

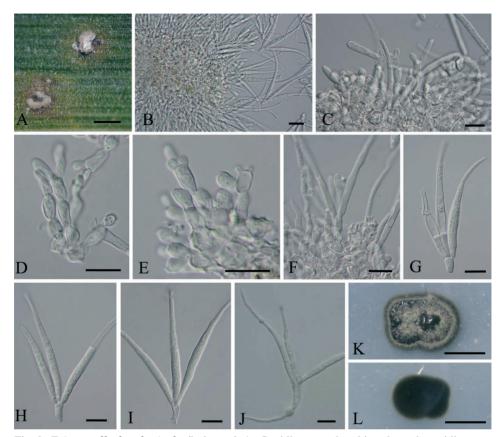


Fig. 2. *Eriosporella bambusicola* (holotype) A. Conidia group breaking through conidiomata. B-F. Conidiophores, conidiogenous cells and conidia. G-I. Hyaline conidium. J. Germinating conidium. K. Cultures on PDA from above after 15 days. L. Cultures on PDA from reverse after 15 days. Scale bars: A = 3 mm,  $B-J = 10 \mu \text{m}$ , K, L = 1 cm.

Growing on living bamboo leaves, forming brown to olive-green areas on leaf surface with conidial mass breaking through slightly raised areas. Sexual state: not observed. Asexual state: *Conidiomata* eustromatic, immersed, solitary, flattened to subglobose, with a concave base with loose, sub-hyaline, pseudoparenchymatous tissue. *Ostiole* not present. *Conidiophores* 10-13 × 4.5-7 µm ( $\bar{x} = 11.6 \times 6.2$  µm, n = 20), arising from basal tissue in a palisade layer, hyaline, septate and branched at the base, smooth, cylindrical to irregular, formed from the inner cells of the locular wall. *Conidiogenous cells* 4-10.5 × 2.5-5 µm ( $\bar{x} = 8.6 \times 4.5$  µm, n = 20), indeterminate, discrete, cylindrical to ellipsoidal, hyaline, smooth-walled, with several conidiogenous loci, phialidic, enteroblastic, polyblastic. *Conidia* complex, consisting of a short basal cell with 3 developing divergent arms; basal cell truncate to obtuse at the point of secession, sometimes carrying part of conidiogenous cell (Fig. 2, I) 4.5-8.5 × 2.5-5 µm ( $\bar{x} = 6.6 \times 3.9$  µm, n = 20), aseptate, hyaline, smooth-walled; arms, cylindrical, tapering at the tip, constricted at the point of attachment to basal cell, 42.5-60 × 3-5 µm ( $\bar{x} = 49.4 \times$ 3.9 µm, n = 20), 0-3-septate, hyaline, smooth-walled. Culture on PDA: Colonies slow growing, reaching 10 mm diam. after 15 d at 25-32°C, circular, with even margin, dark brown, viscous, with brown floccose on mucus surface. Dark brown from reverse.

Material examined: THAILAND, Chiang Rai Province, Mae Fah Luang University, on living leaves of bamboo, 14 October 2011, Dong-Qin Dai, DDQ00214 (MFLU13-0107, holotype); ex-type living cultures = MFLUCC 11-0436 = IFRDCC 2582.

# Key to *Eriosporella* species

#### DISCUSSION

*Eriosporella* was introduced by Höhnel (1916) with one species, *E. calami* ( $\equiv$  *Cryptosporium calami* Niessl.). This genus is characterized by immersed conidiomata with a thin lower wall and thick lateral wall, without an ostiole, hyaline, branched and septate conidiophores, blastic-type conidiogenesis, discrete conidiogenous cells and hyaline conidia with one basal cell (occasionally one septate) and three apical divergent, septate arms (Sutton, 1980). Sutton (1980) described the conidiogenesis in *Eriosporella* as enteroblastic and phialidic. Nag Raj & DiCosmo (1981) considered this genus has blastic and annelidic conidiogenesis. However, no annelations were observed in conidiogenesis of *Eriosporella bambusicola*.

*Eriosporella bambusicola* differs from *E. calami* in having wider basal cells (2.5-5  $\mu$ m versus 1.5-2  $\mu$ m respectively) and fewer septa in the arms (0-3 septa versus 3-5 septa respectively). *Eriosporella* is similar to *Suttoniella* Ahmad, typified by *S. gaubae* (Petr.) S. Ahmad, in having conidia with apical divergent arms. However, *Eriosporella* differs from *Suttoniella* in having multiseptate conidia with a short basal cell and three slender divergent arms developing from the tip of the basal cell. This character is not seen in *Suttoniella* which has one-celled, Y-shape conidia. *Eriosporella* are found on monocotyledons (Sutton, 1980), while *Suttoniella* are so far found on dicotyledons (Hoyo & Gomez-Bolea, 2004, Sutton, 1980). *Eriosporella* also can be compared with *Crucellisporium* Farr and *Belaina* Bat & Peres which has longer (more than 15  $\mu$ m long) basal cells in the conidia (Nag Raj & DiCosmo, 1981; Sutton, 1980). In *Crucellisporium*, the conidia have 2-3 shorter conidia arms usually without septa (Nag Raj & DiCosmo, 1981). However, in *Belaina* the conidia have 2-4 conidial arms with one septum (Sutton, 1980).

The closest relative to *Eriosporella* (as deduced from our phylogenetic inferences) is the hyphomycetous *Pseudoramichloridium* (Fig. 1). Moreover, *Pseudoramichloridium* is characterized by unbranched conidiophores, polyblastic, pale brown conidiogenous cells and obovoid to ellipsoidal conidia without septa (Cheewangkoon *et al.*, 2009). For the time being, we place *Eriosporella* along with *Pseudoramichloridium* in *Capnodiales incertae sedis* (Cheewangkoon *et al.*, 2009).

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