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Epitypification of two bambusicolous fungi from Thailand

Rungtiwa PHOOKAMSAK[a,b,c,d], Jian-Kui LIU[c,d], Dimuthu S. MANAMGODA[c,d], Dhanushka N. WANASINGHE[a,b,c,d], Hiran ARIYAWANSA[c,d], Peter E. MORTIMER[a,b], Ekachai CHUKEATIROTE[c,d], Eric H.C. McKENZIE[e] & Kevin D. HYDE[a,b,c,d]*

[a]*Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan China*

[b]*World Agroforestry Centre, East Asia Node, Heilongtan, Kunming 650201, China*

[c]*Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[d]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[e]*Landcare Research, Private Bag 92170, Auckland, New Zealand*

Abstract – More than 1,000 species of bambusicolous fungi have been reported worldwide, although most lack modern morphological description and phylogenetic investigation. Two species of bambusicolous fungi were collected in northern Thailand. Based on morphological characters, and on a comparison with type specimens, they were determined to be *Pteridiospora javanica* and *Roussoellopsis macrospora.* In a multigene phylogenetic analysis *Pteridiospora javanica* formed a sister clade with *Astrosphaeriella stellata*, while *Roussoellopsis macrospora* clustered with *R. tosaensis* in *Roussoellaceae*. Because the current placement of *Astrosphaeriella* in Dothideomycetes cannot presently be resolved, the family placement of *Pteridiospora javanica* is also unresolved. Based on morphology and phylogeny we epitypify our strains under the names *Pteridiospora javanica* and *Roussoellopsis macrospora.* Asexual states are also described for both species.

Asexual state / *Astrosphaeriella* / bambusicolous fungi / epitypification / *Pteridiospora javanica/ Roussoellaceae / Roussoellopsis macrospora*

INTRODUCTION

Bamboo (*Bambuseae*) is the common term for woody-stemmed grasses in the family *Poaceae*, subfamily *Bambusoideae* (Hyde *et al.*, 2002; Tanaka *et al.*, 2009; Dai *et al.*, 2012). Bamboo species are found in diverse climates from tropical, subtropical and temperate regions of all continents, but are restricted in Europe (Hyde *et al.*, 2002; Tanaka *et al.*, 2009). There are an estimated 1,000-

* Corresponding author: kdhyde3@gmail.com

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1,500 bamboo species in 80-90 genera (Hyde *et al.*, 2002; Loretta *et al.*, 2008; Tanaka *et al.*, 2009). Bamboo has a wide range of uses as a building material as well as being edible and a source of medicine (Hyde *et al.*, 2002). More than 2.5 billion people, mostly in Asia, use bamboo in a commercial application (e.g. charcoal, fishing rods, food, housing, furniture, instruments, paper), worth US$ 7 billion per year (Scurlock *et al.*, 2000; Hyde *et al.*, 2002; Bystriakova *et al.*, 2003; Hameed *et al.*, 2007; Tanaka *et al.*, 2009). In addition, bamboo can be promoted for their environmental benefits and climate change mitigation, through high levels of atmospheric CO_2 capture (Campbell, 2013; Hameed *et al.*, 2007).

More than 1,100 species of bambusicolous fungi have been reported and described worldwide, including several economically important pathogenic, saprobic and endophytic species (Hyde *et al.*, 2002; Morakotkarn *et al.*, 2007, 2008; Tanaka & Tanaka, 2008; Tanaka *et al.*, 2008, 2009; Dai *et al.*, 2012). Most fungal taxa on bamboo have been reported from Asia, especially Japan (*ca.* 300 bambusicolous fungi, of which 60 species belong to Dothideomycetes were found in Japan) with fewer known from India and South America (Hyde *et al.*, 2002; Tanaka & Harada, 2004; Tanaka *et al.*, 2009; Dai *et al.*, 2012). Bamboo is an interesting substrate for the study of Dothideomycetes diversity, as it contains a high number of genera which are widespread in tropical and subtropical Asia. Furthermore, bamboo hosts a large species richness of Dothideomycetes (Ascomycetes), as indicated by previous studies of the biodiversity of bamboo fungi (Hyde *et al.*, 2002; Tanaka *et al.*, 2009). However, few studies have investigated the diversity and phylogeny of Dothideomycetes on bamboo in Thailand (Morakotkarn *et al.*, 2007; Tanaka *et al.*, 2009; Dai *et al.*, 2012).

Contributing to the knowledge base of bambusicolous Dothideomycetes from Thailand, we describe two poorly known fungal species *Pteridiospora javanica* Penz. & Sacc. and *Roussoellopsis macrospora* (I. Hino & Katum.) I. Hino & Katum. These two species were originally collected and described from bamboo. Taxonomic details, illustrations and an account of their sexual and asexual states are reported in this paper, based on recent collections from Thailand. In addition the two species are epitypified.

MATERIAL AND METHODS

Collection, isolation and identification

Two species of Dothideomycetes (two isolates were identified as *Pteridiospora javanica* and one isolate was identified as *Roussoellopsis macrospora*) were collected in Chiang Rai Province, Thailand, one from a dead branch and the other from a living stem of bamboo. The specimens were taken to the laboratory, examined and pure cultures obtained by single spore isolation following the method of Chomnunti *et al.* (2011, 2014) and Phookamsak *et al.* (2013). The fungi were transferred to standard media of malt extract agar (MEA; 33.6 g/l sterile distilled water, Difco malt extract) and potato dextrose agar (PDA; 39 g/l sterile distilled water, Difco potato dextrose). The colony characters and growth rates were determined after 7 days to 4 weeks. Asexual states were produced on sterile bamboo pieces on water agar (WA; 15 g/l sterile distilled water) after 4-8 weeks. The pure cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and duplicated in the International

Collection of Microorganisms from Plants (ICMP), Landcare Research, New Zealand. Dried herbarium specimens are deposited in Mae Fah Luang University (MFLU) herbarium, Chiang Rai, Thailand.

DNA extraction, PCR amplification and sequencing

Fungal genomic DNA was extracted from fresh fungal mycelium grown on PDA media at 25-30°C for 4 weeks. The fungal DNA extractions were done by Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's instructions (Hangzhou, P.R. China) (Phookamsak *et al.*, 2013). The forward and reverse primer pair ITS5 and ITS4 (White *et al.*, 1990) were used to amplify the internal transcribed spacers (ITS1-5.8S- ITS2), LROR and LR5 (Vilgalys & Hester, 1990) for the partial large subunit nuclear rDNA (28S, LSU); NS1 and NS4 (White *et al.*, 1990) for the small subunit nuclear rDNA (18S, SSU), fRPB2-5F and fRPB2-7cR (Liu *et al.*, 1999) for the partial RNA polymerase second largest subunit (RPB2), and EF1-983F and EF1-2218R (Rehner, 2001) for the translation elongation factor 1-alpha gene (*TEF1α*). The procedure and thermal cycle of DNA amplification followed the methodology described in Phookamsak *et al.* (2013). The quality of PCR products were checked by using 1% agarose gel electrophoresis stained with ethidium bromide. The DNA sequencing was carried out in Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China).

Phylogenetic analysis

The newly generated sequences were analyzed with sequences obtained from GenBank (Table 1), including highly similar sequences returned from a standard BLAST search. Phylogenetic analysis of combined genes of partial LSU,

Table 1. Isolates used in this study and their GenBank accession numbers. The newly generated sequences and extype strains are indicated in bold

Taxon	*Culture/voucher*	*GenBank Accession*		
		LSU	*SSU*	*TEF1α*
Aigialus grandis	BCC 18419	GU479774	GU479738	GU479838
Aigialus parvus	BCC 32558	GU479779	GU479743	GU479843
Astrosphaeriella africana	MFLUCC 10-0553	JN846721	JN846731	
Astrosphaeriella bakeriana	CBS 115556	GU301801		GU349015
Astrosphaeriella bakeriana	MFLUCC 11-0027	JN846730	JN846740	
Astrosphaeriella stellata	KT998	AB524592	AB524451	
Astrosphaeriella stellata	MFLUCC 10-0555	JN846723	JN846733	
Astrosphaeriella stellata	MFLUCC 10-0095	JN846720	JN846741	
Bambusicola bambusae	**MFLUCC 11-0614^{T}**	**JX442035**	**JX442039**	
Bambusicola massarinia	**MFLUCC 11-0389^{T}**	**JX442037**	**JX442041**	
Byssothecium circinans	CBS 675.92	AY016357	AY016339	GU349061
Dothidea insculpta	CBS 189.58	DQ247802	DQ247810	DQ471081
Falciformispora lignatitis	BCC 21117	GU371826	GU371834	GU371819

Table 1. Isolates used in this study and their GenBank accession numbers. The newly generated sequences and extype strains are indicated in bold *(continued)*

Taxon	*Culture/voucher*	*GenBank Accession*		
		LSU	*SSU*	*TEF1α*
Fissuroma aggregata	MAFF 239486/ KT 984	AB524591	AB524450	AB539105
Fissuroma maculans	**MFLUCC 10-0886^{T}**	**JN846724**	**JN846734**	
Kalmusia brevispora	KT 2313	AB524601	AB524460	AB539113
Katumotoa bambusicola	KT 1517a	AB524595	AB524454	AB539108
Lentithecium aquaticum	**CBS 123099^{T}**	**GU301823**	**GU296156**	**GU349068**
Lentithecium fluviatile	CBS 122367	GU301825	GU296158	GU349074
Lindgomyces breviappendiculatus	**MAFF 239292/ KT 1399^{T}**	**AB521749**	**AB521734**	
Lindgomyces cinctosporae	**R56-1^{T}**	**AB522431**	**AB522430**	
Lindgomyces ingoldianus	**ATCC 200398^{T}**	**AB521736**	**AB521719**	
Massaria anomia	CBS 591.78	GU301839	GU296169	
Massaria inquinans	M 19/WU 30527	HQ599402	HQ599444	HQ599342
Massarina eburnea	CBS 473.64	GU301840	GU296170	GU349040
Montagnula opulenta	CBS 168.34	DQ678086	NG_013127	
Neoastrosphaeriella krabiensis	**MFLUCC 11-0025^{T}**	**JN846729**	**JN846739**	
Neoroussoella bambusae	**MFLUCC 11-0124^{T}**	**KJ474839**	**KJ474848**	**–**
Paraphaeosphaeria michotii	CBS 591.73	GU456326	GU456305	GU456267
Preussia funiculata	CBS 659.74	GU301864	GU296187	GU349032
Pteridiospora javanica	**MFLUCC 11-0159^{T}**	**KJ742940**	**KJ739607**	**KJ739605**
Pteridiospora javanica	**MFLUCC 11-0195**	**KJ742941**	**–**	**KJ739606**
Roussoella chiangraina	**MFLUCC 10-0556^{T}**	**KJ474840**	**–**	**KJ474849**
Roussoella hysterioides	CBS 125434	AB524622	AB524481	AB539115
Roussoella neopustulans	**MFLUCC 11-0609^{T}**	**KJ474841**	**–**	**KJ474850**
Roussoella nitidula	**MFLUCC 11-0182^{T}**	**KJ474843**	**–**	**KJ474852**
Roussoella nitidula	**MFLUCC 11-0634^{T}**	**KJ474842**	**–**	**KJ474851**
Roussoella pustulans	MAFF 239637/ KT 1709	AB524623	AB524482	AB539116
Roussoella scabrispora	**MFLUCC 11-0624^{T}**	**KJ474844**		**KJ474853**
Roussoella siamensis	**MFLUCC 11-0149^{T}**	**KJ474845**		**KJ474854**
Roussoella thailandica	**MFLUCC 11-0621^{T}**	**KJ474846**		
Roussoellopsis macrospora	**MFLUCC 12-0005^{T}**	**KJ474847**	**KJ739608**	**KJ474855**
Roussoellopsis tosaensis	MAFF 239638/ KT 1659	AB524625		AB539117
Sporormiella minima	CBS 524.50	DQ678056	DQ678003	DQ677897
Trematosphaeria pertusa	**CBS 122368^{T}**	**FJ201990**	**FJ201991**	**GU456276**

Abbreviations: **ATCC**: American Type Culture Collection, Virginia, U.S.A.; **BCC**: BIOTEC Culture Collection, Bangkok, Thailand; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. Culture and specimen abbreviations: **KT**: K. Tanaka; **R**: H.A. Raja; **T** ex-type/ex-epitype isolates.

SSU and *TEF1α* were performed and related families in the *Pleosporales* were also included in the analysis. *Dothidea insculpta* was used as the outgroup taxon. The sequences were assembled and aligned by Mega 6.0.5 (Tamura *et al.*, 2013) and MAFFT v. 7.036 (Katoh & Standley, 2013). The sequence alignments were improved manually where necessary in BioEdit v. 7.2 (Hall, 1999) and formatted to nexus file by using ClustalX2 v. 1.83 (Thompson *et al.*, 1997). A maximum-parsimony (MP) analysis was obtained with stepwise additions of sequences by using PAUP v. 4.0b10 (Swofford, 2002). The heuristic search option with 1000 random sequences addition and tree-bisection reconnection (TBR) of branch-swapping algorithm were performed. Maxtrees were setup at 5000 with a zero of maximum branch length was collapsed and gaps were treated as missing data. The calculating of consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were included in the analysis. The robustness of the most parsimonious tree was estimated based on 1000 bootstrap replications with each 100 replicates of random stepwise addition of taxa (Liu *et al.*, 2011, 2012; Phookamsak *et al.*, 2013). A maximum likelihood analysis (ML) was conducted by using RaxmlGUI v.1.0 (Silvestro & Michalak, 2011). The available substitution models comprised a generalized time reversible (GTR) for nucleotides, whereas symmetric, Markovian, and ordered models for morphological (Silvestro & Michalak, 2012). A discrete GAMMA (Yang, 1994) is complemented for each substitution model. Rapid bootstrap analysis (Stamatakis *et al.*, 2008) and search for a best-scoring ML tree were applied (Silvestro & Michalak, 2012). The phylogram was visualized in Treeview (Page, 1996) with bootstrap values above the branches (Fig. 1). All the sequences generated in this study have been deposited in GenBank (Liu *et al*, 2011, 2012; Phookamsak *et al.*, 2013).

RESULTS AND DISCUSSION

Phylogenetic analysis

The LSU, SSU and *TEF1α* combined gene datasets were analyzed using maximum parsimony (MP) and maximum likelihood (ML) analysis. The combined dataset comprised 45 taxa with *Dothidea insculpta* as the outgroup taxon. The dataset consists of 2829 aligned nucleotide characters, of which 2028 characters are constant, 265 variable characters are parsimony-uninformative and 536 (26.43%) parsimony-informative characters. Four equally parsimonious trees were generated (CI = 0.451, RI = 0.598, RC = 0.270 and HI = 0.549) and one of the MP trees is presented (Fig. 1). The phylogenetic trees derived from MP and ML analysis give a similar topology. Bootstrap support (BS) values of ML and MP (equal to or greater than 60% based on 1000 replicates) are shown above the nodes.

The phylograms obtained from ML and MP analyses gave similar results relating to the families in *Pleosporales*. The strain of *Roussoellopsis macrospora* (MFLUCC 12-0005) forms a well-supported clade (99% ML and 99% MP) with *R. tosaensis* (KT 1659) in *Roussoellaceae*. The two strains of *Pteridiospora javanica* formed a sister clade with *Astrosphaeriella stellata*.

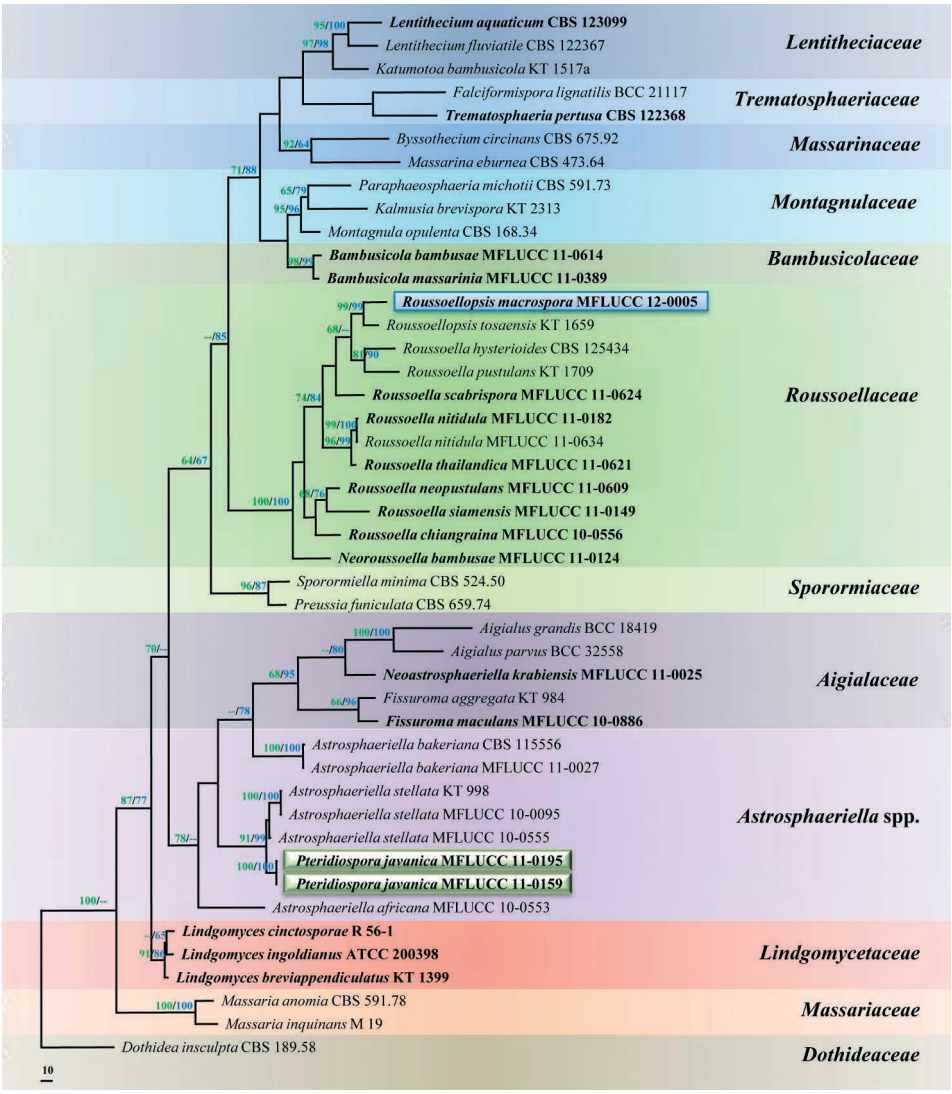

Fig. 1. The best scoring of the MP trees based on a combined dataset of LSU, SSU and *TEF1α* gene sequence data. Bootstrap support values for maximum parsimony (green) and maximum likelihood (blue) greater than 60% are given above the nodes. The tree is rooted to *Dothidea insculpta* (CBS 189.58).

Astrosphaeriella africana, with striate ascospores and coriaceous ascoma, often clusters as a single clade basal to *Astrosphaeriella*. *Pteridiospora javanica* is similar to *Astrosphaeriella* species and in the phylogenetic analysis it groups with *Astrosphaeriella stellata*, but is probably not congeneric. The current placement of *Astrosphaeriella* at the family level, however, cannot be confirmed (Figs 2, 3, 4).

Taxonomy

Pteridiospora javanica Penz. & Sacc., *Malpighia* 11(9-10): 399 (1897) Figs 2-4

= *Apiospora carbonacea* Rehm, Leafl. of Philipp. Bot. 8: 2945 (1916)

MycoBank: MB 200770

Epitypification identifier: IF550nnn

Facesoffungi number: FoF 000022

Saprobic on bamboo, visible as darkened areas on the host surface with several raised, cone-shaped, gregarious, easily broken fruiting bodies.

Sexual state: *Ascostromata* 300-400 µm high, 500-700 µm diam., erumpent through host tissue, becoming superficial, conical, dark brown to black, solitary to gregarious, uniloculate, flattened at the base with ruptured reflexed tooth-like host remnants around the base, central ostiole, with small papilla. *Peridium* 30-70 µm wide, carbonaceous, composed of opaque dark cells, poorly developed at the base. *Hamathecium* comprising numerous 1-2 µm wide, narrow, trabeculate, filiform, branched, anastomosing, pseudoparaphyses, embedded in gelatinous matrix. *Asci* (150-)160-190(-210) × (13-)16-18 µm ($\bar{x}$ = 181.5 × 16.5 µm, n = 25), 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, apically rounded with ocular chamber or surrounded by a faint ring, arising from base of ascoma. *Ascospores* (31-)33-36(-39) × 8-9(-10) µm ($\bar{x}$ = 34.2 × 8.3 µm, n = 30), overlapping, uni to bi-seriate, oblong to sub-fusoid narrowing towards apex, hyaline, 1-septate, constricted at the septum, upper cell longer than lower cell, smooth-walled, guttulate, surrounded by a large appendage in the lower cell, with wide mucilaginous sheath.

Asexual state: produced on sterilized bamboo pieces on WA or immersed in culture media. *Conidiomata* 130-260 µm high, 140-320 µm diam., black, globose on bamboo pieces, or forming conidiomata radiating outwards on cultures colony, erumpent to superficial, covered by vegetative hyphae, uniloculate, solitary to gregarious. *Conidiomata wall* 20-30 µm wide, composed of two layers of cells of equal thickness, outer layer of brown to dark brown *textura intricata*, inner layer of dark brown to black *textura angularis*. *Conidiophores* (2-)3-5(-8) × 1-2 µm, arising from the basal cavity around conidiomata, oblong to cylindrical or ampulliform, straight or slightly curved, hyaline, unbranched, aseptate. *Conidiogenous cells* integrated, phialidic. *Conidia* 2-3 × 2-3 µm ($\bar{x}$ = 2.4 × 2.1 µm, n=45), globose to subglobose, forming chains, hyaline, aseptate, smooth-walled.

Cultural characters: Colonies on PDA 51-53 mm diam. after 4 weeks at 25-30°C, white at the edge, with fluffy radiating, grey to dark grey with small to large hyaline or black water drops and yellowish white, fluffy in the centre; reverse white to pale yellowish at the edges, becoming grey to brownish grey or brownish orange in the middle with strongly radiating outwards colony, dense, circular to irregular shape, umbonate or raised with droplets in the centre, dull with entire edge, floccose, slightly radiated in the upper with strongly radiating in the lower, forming small black conidiomata radiating outwards after 8 weeks, non-pigmented.

Material examined: INDONESIA. Java: Tjibodas, on dead culms of *Bambusae* (*Poaceae*), 4 March 1896, no. 132 (PAD, holotype of *Pteridiospora javanica*); PHILIPPINES. Laguna, Los Baños, summit of Mt. Maquiling, on dead *Schizostachyum* [*Bambuseae*] (*Poaceae*), June 1914, Baker 3427a (S, F7823, type of *Apiospora carbonacea* Rehm); THAILAND. Chiang Rai Province: Muang District, Khun Korn Waterfall, on dead stem of bamboo (*Poaceae*), 5 September

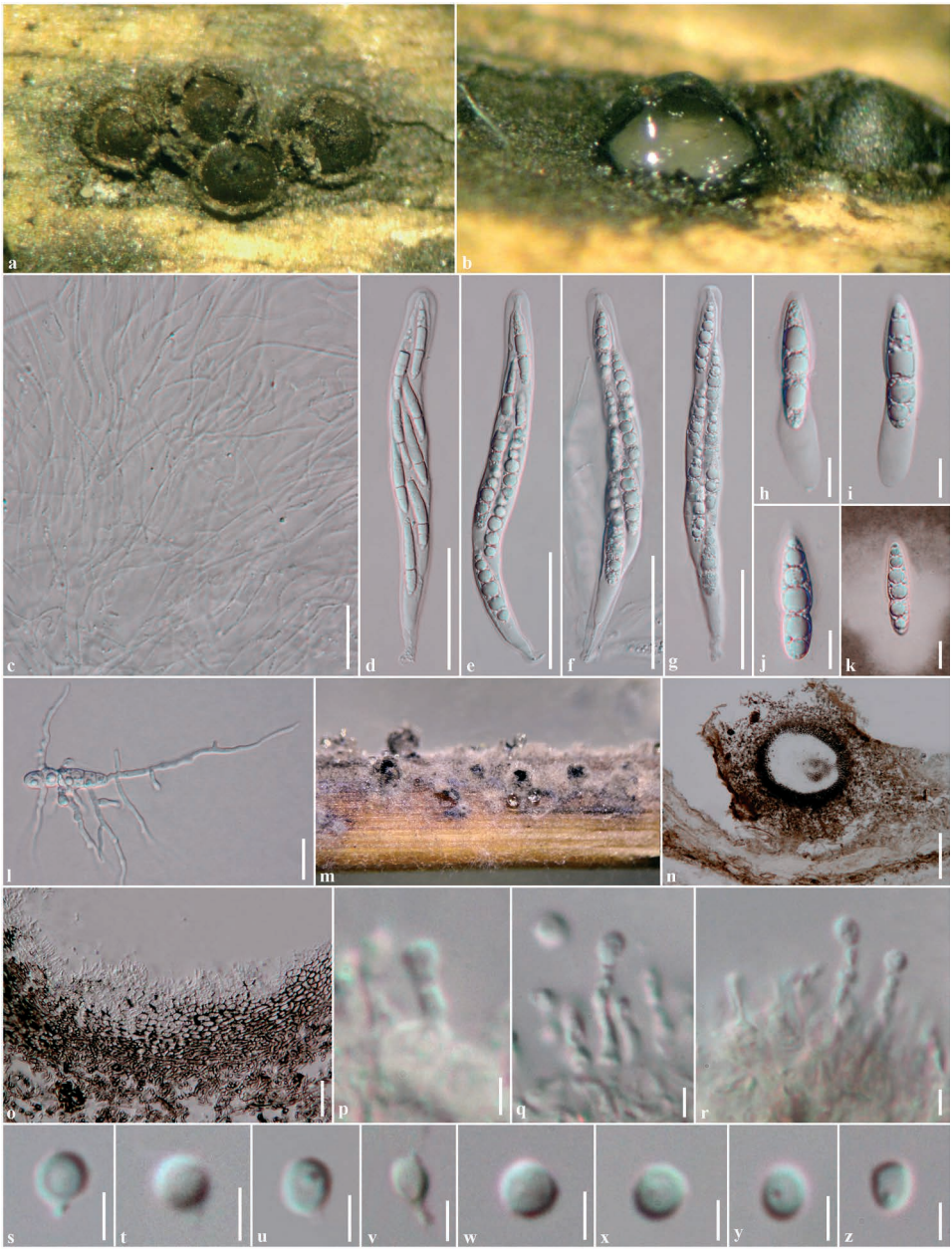

Fig. 2. *Pteridiospora javanica* (MFLUCC11-0159) **a.** Ascostromata on host surface. **b.** Section through ascostroma. **c.** Pseudoparaphyses. **d-g.** Asci. **h-j.** Ascospores. **k.** Ascospore stained in Indian ink to show sheath. **l.** Germinating ascospore. **m.** Conidiomata forming on bamboo pieces on WA after 8 weeks. **n.** Section through conidioma. **o.** Section through conidioma wall. **p-r.** Conidiophores and conidiogenous cells. **s-z.** Conidia. Scale bars: n = 100 μm, p = 100 μm, d-g = 50 μm, c, l, o = 20 μm, h-k = 10 μm, p-z = 2 μm.

Fig. 3. Iconotype of *Pteridiospora javanica* from PAD herbarium.

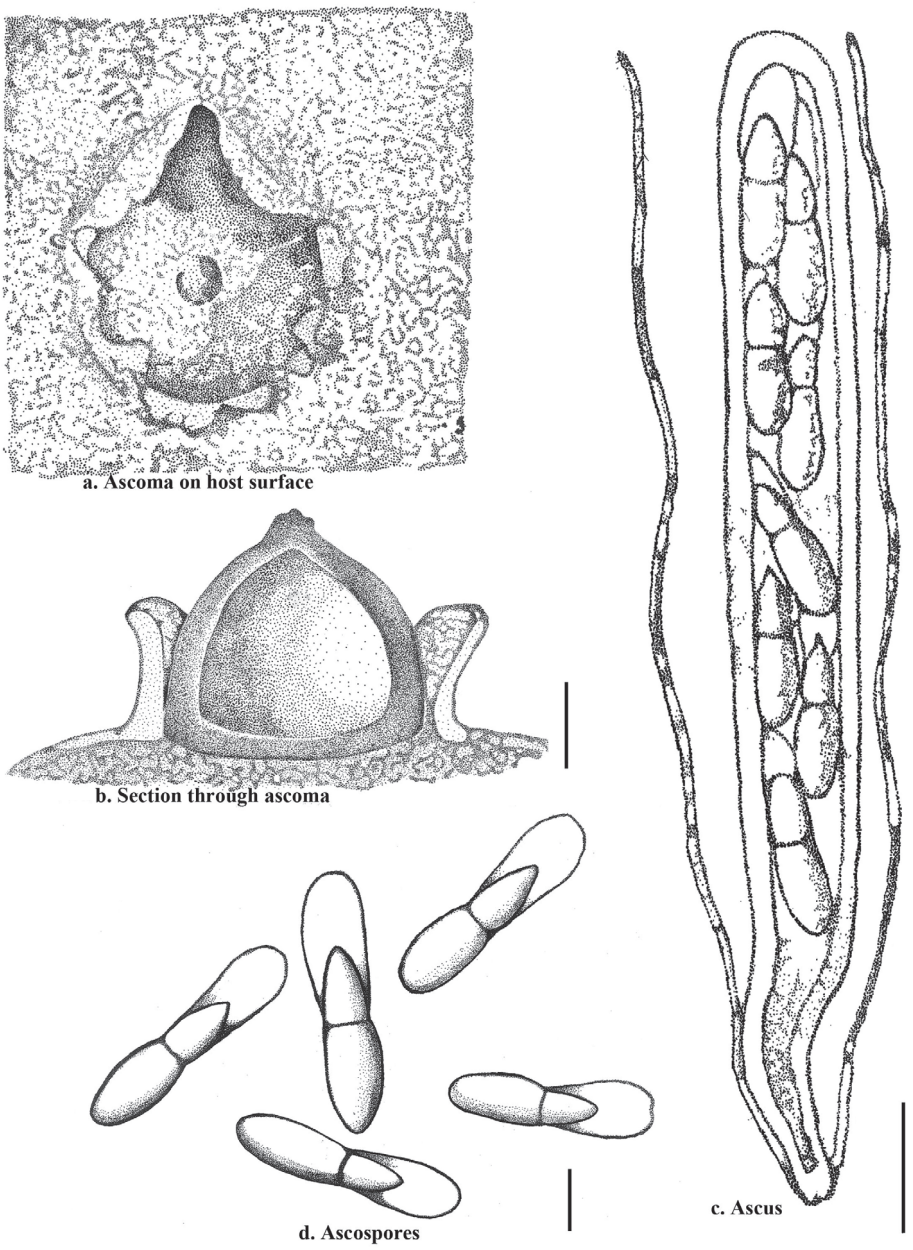

Fig. 4. *Pteridiospora javanica*, redrawn from Penzig & Saccardo (1904). **a.** Ascostroma on host surface. **b.** Section through conical ascostroma. **c.** Ascus and pseudoparaphyses. **d.** Ascospores. Scale bars: a = 200 µm, b = 20 µm, c = 10 µm.

2010, *R. Phookamsak* RP0075, (MFLU 11-0195, *epitype of Pteridiospora javanica designated here*, isoepitype in PDD); ex-epitype living culture = MFLUCC 11-0159 = ICMP; *ibid*., 17 December 2010, *R. Phookamsak* (MFLU 11-0231); living culture = MFLUCC 11-0195 = ICMP.

Notes: *Pteridiospora* was introduced by Penzig & Saccardo (1897) as typified by *P. javanica*; it was later placed in the *Pleosporaceae* (Filer, 1969). Lumbsch & Huhndorf (2010) accommodated *Pteridiospora* in the Dothideomycetes genera *incertae sedis*. *Pteridiospora javanica* is a poorly known species lacking details of morphology and phylogeny. There are six species epithets for *Pteridiospora* in Index Fungorum (2014) and four widespread species are reported in Kirk *et al.* (2008), however, no sequence data are available in GenBank.

Pteridiospora javanica was collected on dead culms of *Bambusae* from Tjibodas, Java. The species was originally described as "ascomata 0.5-0.7 mm diam., superficial, globose to conical, with a flattened base, glabrous, black, acute papillate; asci 180 × 15 µm, cylindrical with truncate apical, short pedicellate; ascospores 30 × 6-7 µm, uni to bi-seriate, oblong to fusoid, one cell is large, longer and obtuse, hyaline, constrict at the septum, surrounded by thin sheath with broad wing" (Penzig & Saccardo, 1897, 1904). Based on the morphological characters, *Pteridiospora javanica* is similar to *Astrosphaeriella* species, which form superficial, cone-shaped, carbonaceous ascomata, with star-like ruptured host tissue around the base, and trabeculate pseudoparaphyses (Hyde & Fröhlich, 1998; Hyde *et al*., 2000; Tanaka *et al.*, 2009). *Pteridiospora javanica* however, differs from *Astrosphaeriella sensu stricto* species in its asymmetrical ascospores and irregular sheath. *Astrosphaeriella* species have brown, fusiform, symmetrical ascospores and apical rounded with ocular chamber, while *P. javanica* has hyaline, oblong-fusoid ascospores, apically rounded, with ocular chamber or wedge-shaped subapical ring (Penzig & Saccardo, 1897, 1904; Hyde & Fröhlich, 1998; Hyde *et al.*, 2000; Tanaka *et al.*, 2009). Our collection shares similar characters to the type of *P. javanica* in the cone-shaped, carbonaceous ascostromata, trabeculate pseudoparaphyses, asci (181.5 × 16.5 µm) and asymmetrical ascospores (34.2 × 8.3 µm) with a distinct sheath or appendage. Phylogenetic analyses show that two strains of *Pteridiospora javanica* form a sister clade with *Astrosphaeriella stellata*. Details of the asexual state are provided below. The genera may yet prove to be congeneric, but a greater sampling of *Astrosphaeriella* species is needed.

Apiospora carbonacea was introduced by Rehm (1916) with "ascomata 1 mm high, 1-1.2 mm diam, erumpent though host tissue with broadly flattened base, conical, carbonaceous, gregarious; asci 130 × 12 µm, 8-spored, pseudoparaphyses; ascospores 27 × 5 µm, cylindrical, with rounded ends, hyaline, inaequilateral, 1-septate, non-constrict at the septum" (Saccardo, 1926). Eriksson (1994) synonymized this species under *Pteridiospora javanica*. However, this is not recorded in Index Fungorum or MycoBank. We observe the type specimen of this species and compare the morphological characters with our strains and protologue. *Apiospora carbonacea* has similar morphological characters with *Pteridiospora javanica*, although it has smaller but overlapping ascomata, asci and ascospores. Thus we support Eriksson's comment in S database and Eriksson (1994) and treat *Apiospora carbonacea* as a synonym of *Pteridiospora javanica*.

Roussoellopsis macrospora (I. Hino & Katum.) I. Hino & Katum., *J. Jap. Bot*. 40: 87 (1965) Figs 5, 6

≡ *Didymosphaeria macrospora* I. Hino & Katum., Bull. Faculty of Agriculture, Yamaguchi University 10: 1193 (1959)

MycoBank: MB 338655

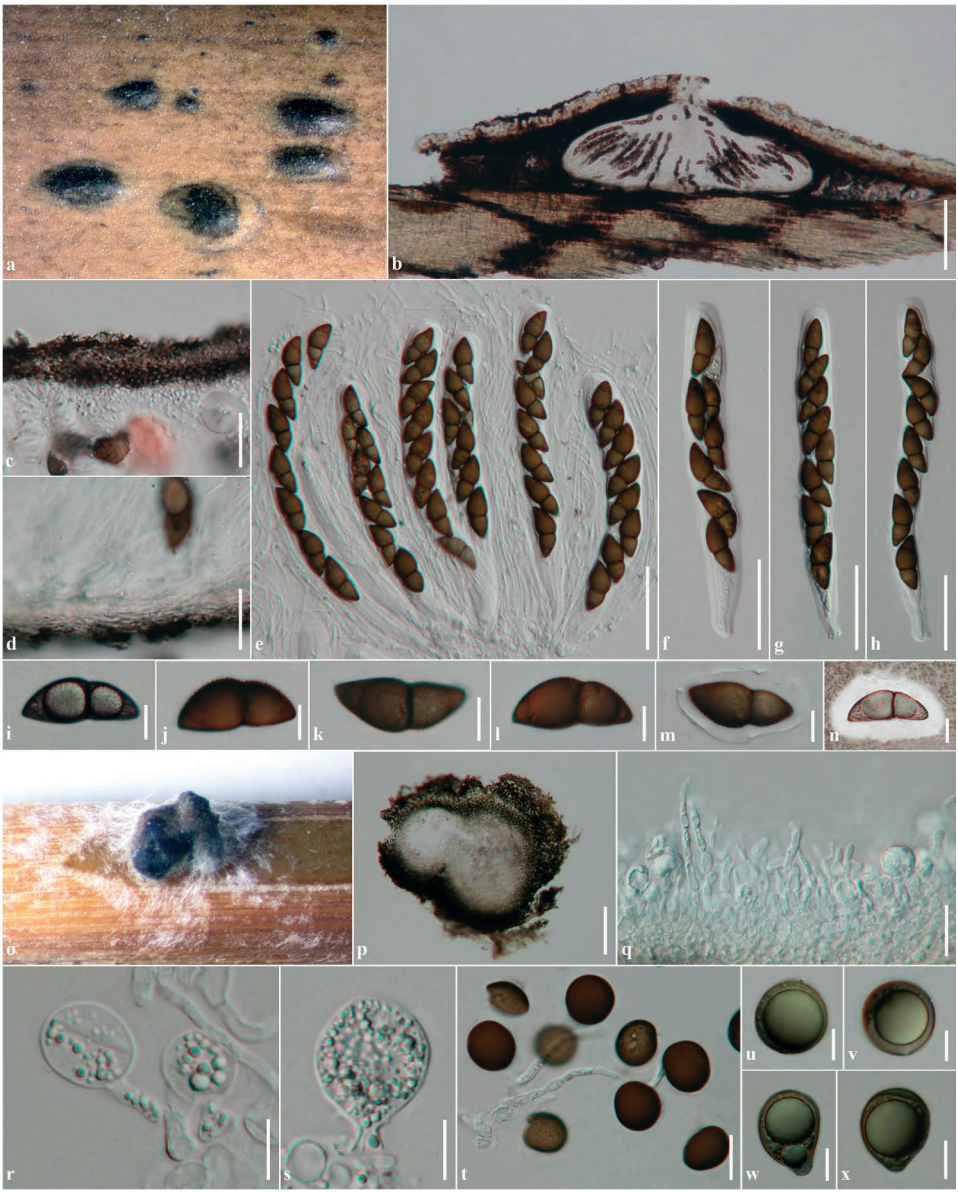

Fig. 5. *Roussoellopsis macrospora*. **a.** Ascostromata on host surface. **b.** Section through dome-shaped ascostroma. **c, d.** Section through peridium. **e.** Asci embedded in pseudoparaphyses. **f-h.** Asci. **i-m.** Ascospores. **n.** Ascospore stained by Indian ink. **o.** Conidiomata forming on bamboo pieces on WA after 4 weeks. **p.** Section through conidioma. **q.** Conidiophores. **r-s.** Conidiogenous cells. **t-x.** Conidia. Scale bars: b = 200 µm, p = 100 µm, e-h = 50 µm, c, d, q, t = 20 µm, i-n, r, s, u-x = 10 µm.

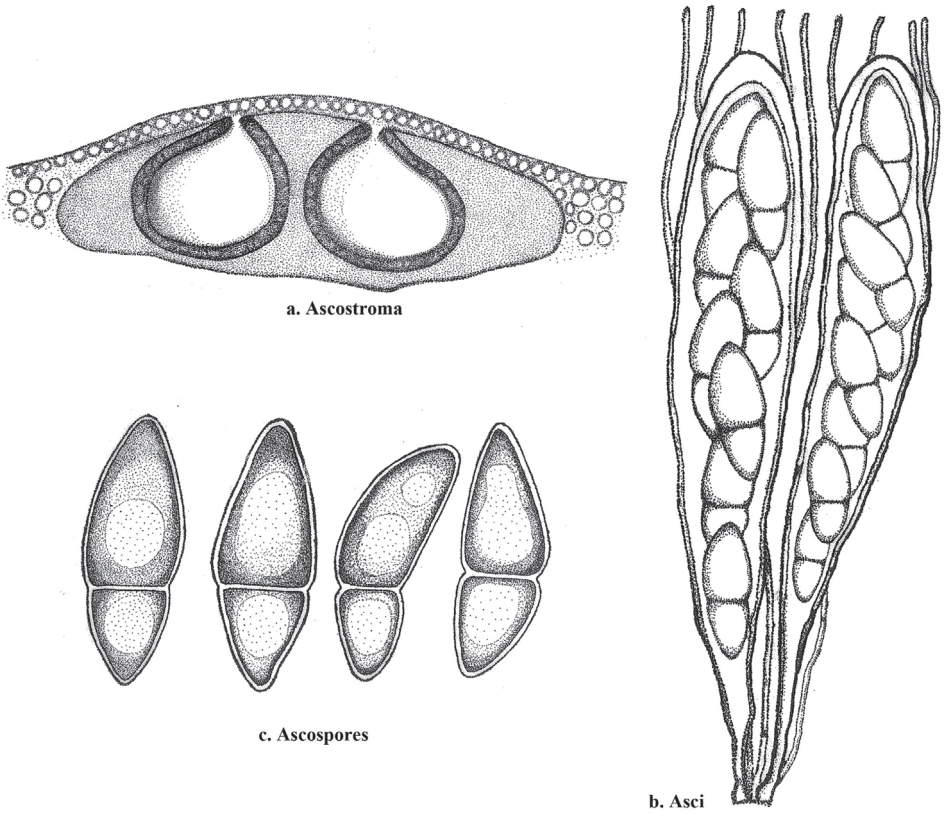

Fig. 6. *Roussoellopsis macrospora*, re-drawn from Hino & Katumoto (1961). **a.** Section through ascostroma. **b.** Asci and pseudoparaphyses. **c.** Ascospores. Scale bars: a = 200 µm, b = 20 µm, c = 10 µm.

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Pathogenic on living bamboo stems with other fungi.

Sexual state: *Ascostromata* 0.3-0.4 mm high, 1.2-2.3 mm diam., raised, black, low convex or dome-shaped, solitary, uni- to multi-loculate. *Locules* 220-290 µm high, 540-720 µm diam., immersed under ascostromata, convex to elongate with a flattened base, centrally ostiolate. *Peridium* 7.5-20 µm wide, composed of two types of pseudoparenchymatous cells, forming *textura angularis* towards apex with *textura prismatica* at the base, covered by wedge-shaped as besides the stromatic region, unequal thickness, with slightly thin at the base of ascomata. *Hamathecium* comprising numerous, 1-2 µm wide, narrow, trabeculate, branched, anastomosing, pseudoparaphyses, embedded in a gelatinous matrix. *Asci* (160-)180-200(-220) × (17-)18-20(-25) µm ($\bar{x}$ = 192.2 × 22.2 µm, n = 30), 8-spored, bitunicate, fissitunicate, clavate to cylindric-clavate, short pedicellate, apically rounded with distinct ocular chamber. *Ascospores* (28-)32-35.5(-37) × (10-)12-15 µm ($\bar{x}$ = 32.2 × 12.4 µm, n = 30), uni-seriate or overlapping, rarely bi-seriate,

broadly fusiform, brown to dark brown, 1-septate, constricted at the septum, rough-walled, echinulate, upper cell larger than lower cell, surrounded by distinct mucilaginous sheath.

Asexual state: produced on sterilized bamboo pieces on WA. *Conidiomata* 250-380 µm high, 300-420 µm diam., visible as black dome-shaped on bamboo pieces, superficial, to embedded in agar, covered by vegetative hyphae, uni- to multi-loculate, solitary to gregarious. *Conidiomata wall* 30-70 µm wide, composed of dark brown cells of *textura angularis*, unequal thickness. *Conidiophores* (2-)6-20(-45) × (2-)3-5 µm, oblong to cylindrical, straight or curved, uni- to multi-septate, constricted at the septa, branching. *Conidiogenous cells* integrated, phialidic. *Conidia* (17-)20-25(-26) × (18-)19-24 µm ($\bar{x}$ = 22.4 × 20.4 µm, n = 30), globose to subglobose, truncate at the base, hyaline when immature, with several small guttules, becoming dark brown with a large guttulate when mature, smooth-walled.

Cultural characters: Colonies on MEA 70-75 mm diam. after 4 weeks at 25-30°C, white at the edge, yellowish white with small water drops in the centre; reverse white to pale yellowish at the edges, becoming dark greenish in the centre with slightly discontinue radiating outwards colony; dense, circular to irregular in shape, raised with flattened in the centre, smooth to slightly dull with entire edge, floccose to fluffy, slightly radiated in the upper part with strongly discontinue radiating in the lower part, non-pigmented.

Material examined: JAPAN. Tikugo Province: Dazaichu-tyô, Mt. Hôman, on dead culms of *Phyllostachys bambusoides* Sieb.& Zucc. (*Poaceae*), 9 September 1956, T. Hino (YAM, holotype); THAILAND. Chiang Rai Province: Muang District, Khun Korn Waterfall, on living stem of bamboo (*Poaceae*), 21 June 2011, *R. Phookamsak*_RP0126 (MFLU 11-0244, ***epitype*** *designated here,* isoepitype in PDD), ex-type living cultures = MFLUCC 12-0005 = ICMP.

Notes: *Roussoellopsis macrospora* was introduced by Hino & Katumoto (1965) from dead stems of *Phyllostachys bambusoides* in Japan. The species was originally described as "ascostromata semi-immersed under the blackish clypeus, multiloculate; locules 400-600 µm diam, immersed in stroma, globose, black, carbonaceous; asci 130.2-162.8 × 15.6-21.2 µm, 8-spored, clavate, apically rounded with slightly thickened, pedicellate; ascospores 27.7-42.3 × 12.4-14.6 µm, fusiform, 1-septate, constrict at the septum, often curved, with obtuse ends, dark brown with guttules" (Hino & Katumoto (1961). Hino & Katumoto (1965) transferred three species, *Didymosphaeria japonica* I. Hino & Katum. (1955), *D. macrospora* I. Hino & Katum. (1959) and *D. tosaensis* I. Hino & Katum. (1955) to the new genus *Roussoellopsis* (*Pleosporaceae*). *Roussoellopsis* differs from *Didymosphaeria* in its ascostromata and ascospore characters (Hino & Katumoto, 1965). *Didymosphaeria* often forms a superficial blackish clypeus with oblong to fusoid, and non-inaequilateral ascospores, while *Roussoellopsis* usually has fusiform ascospores (Hino & Katumoto, 1965). The species was considered under both *Astrosphaeriella* and *Roussoella* based on its original descriptions (Aptroot, 1995a,b; Tanaka *et al.*, 2009). Tanaka *et al.* (2009) re-circumscribed the bambusicolous fungi and included two strains *Roussoellopsis tosaensis* and *Roussoellopsis* sp. in their phylogenetic analysis. *Roussoellopsis* species formed a sister clade with *Roussoella*, close to *Arthopyrenia salicis* (Tanaka *et al.*, 2009; Schoch *et al.*, 2009; Hyde *et al.*, 2013). Tanaka *et al.* (2009) proposed to transfer *Roussoellopsis* to the older genus *Roussoella* after careful consideration. *Roussoellopsis* is similar to *Roussoella* and phylogenetically often cluster together (Schoch *et al.*, 2009; Tanaka *et al.*, 2009; Hyde *et al.*, 2013). However, *Roussoellopsis* differs from *Roussoella* in the size of ascospores, asci shape and

asexual morph (Tanaka *et al.*, 2009; Liu *et al.*, 2011; Hyde *et al.*, 2013). *Roussoellopsis* has large fusiform ascospores and clavate asci and *Melanconiopsis* or *Neomelanconium*-like asexual morphs, while *Roussoella* forms *Cytoplea* asexual morphs (Hyde *et al*., 1996; Tanaka *et al.*, 2009; Liu *et al.*, 2011; Hyde *et al.*, 2013). Based on the unique morphology and the phylogenetic support, Liu *et al.* (2014, in press) proposed to introduce the new family *Roussoellaceae* to accommodate *Roussoella* and *Roussoellopsis*. Further details and an illustration of *Roussoellopsis* are provided.

Based on morphology, our stain is most similar to *Roussoellopsis macrospora* and the phylogenetic analyses shows that our strain forms a strongly support clade (99% ML and 99% MP) with *Roussoellopsis tosaensis,* but is not well-resolved from other *Roussoella* species within *Roussoellaceae.* The type specimen of *Roussoellopsis macrospora* was observed, but despite extensive examination of many ascostromata we could not find any typical of those in the protologue of *R. macrospora* (Hino & Katumoto, 1965). We did however find ascostromata with a typical *Roussoella* species. We therefore suggest that either the wrong material was deposited as the holotype or that very few *Roussoellopsis* ascostromata were present on the specimen. Because of the lack of *Roussoellopsis* ascostromata on the type specimen, we therefore use our fresh strain, which is identical to the illustration in the protologue to epitypify *Roussoellopsis macrospora.* The sexual and asexual morphs of our epitype are described and illustrated, isolates are deposited in MFLUCC and ICMP, while sequence data is deposited in GenBank.

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