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Roussoella guttulata (Roussoellaceae, Pleosporales), a novel bambusicolous ascomycete from Thailand

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Abstract

Roussoella guttulata sp. nov. (Roussoellaceae) was found on bamboo culms collected in the northeast of Thailand. *Roussoella guttulata* differs from other species in having immersed, gregarious, black pseudoascostromata, single and central ostiole with periphyses, thin-walled peridium composed of cells of *textura angularis* to *textura prismatica* and yellowish brown ascospores. The multigene phylogenetic analysis shows that the new species forms a distinct lineage basal to *R. hysterioides*, *R. japanensis*, *R. scabrispora* and *R. verrucispora*. The new species is compared with closely related *Roussoella* species. A full description, color photo plates and a phylogenetic tree to show the placement of the new species are provided.

Keywords: 1 new taxon, Dothideomycetes, Phylogeny, Taxonomy, Thai mycobiota

Introduction

Roussoellaceae was introduced by Liu *et al.* (2014) to accommodate three genera *Neoroussoella* J.K. Liu, Phookamsak & K.D. Hyde, *Roussoella* Sacc. and *Roussoellopsis* I. Hino & Katum., which mainly occurs as saprophyte on decaying bamboo culms (Liu *et al.* 2014, Jiang *et al.* 2019). Jaklitsch & Voglmayr (2016) synonymized Roussoellaceae under Thyridariaceae based on phylogenetic analysis of limited taxa. However, Tibpromma *et al*. (2017) argued that Roussoellaceae and Thyridariaceae were separate families in Pleosporales, and this was conferred in later studies using more taxa coupled with morphology and DNA sequences (Hyde *et al.* 2018a, Jiang *et al.* 2019, Phookamsak *et al.* 2019, Mapook *et al.* 2020, Poli *et al.* 2020). Roussoellaceae is now recognized as a well-resolved family in Pleosporales (Hongsanan *et al*. 2020a). Wijayawardene *et al*. (2020) listed Roussoellaceae in the 'Outline of *Fungi* and fungus-like taxa' with 12 accepted genera, viz. *Appendispora* K.D. Hyde, *Cytoplea* Bizz. & Sacc., *Elongatopedicellata* J.F. Zhang *et al*., *Immotthia* M.E. Barr, *Neoroussoella* J.K. Liu *et al.*, *Pararoussoella* Wanas. *et al*., *Pseudoneoconiothyrium* Wanas. *et al*., *Pseudoroussoella* Mapook & K.D. Hyde, *Roussoella* Sacc., *Roussoellopsis* I. Hino & Katum., *Setoarthopyrenia* Mapook & K.D. Hyde and *Xenoroussoella* Mapook & K.D. Hyde.

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Roussoella, the type genus of Roussoellaceae, was established by Saccardo & Paoletti (1888) based on the type species *R. nitidula* Sacc. & Paol. *Roussoella* is characterized by immersed, clypeate ascostromata, pseudoparaphyses embedded in a gelatinous matrix, bitunicate asci, and ornamented, 2-celled, brown, longitudinally striate ascospores (Hyde *et al*. 1996, Hyde 1997, Tanaka *et al*. 2009, Liu *et al*. 2014, Jiang *et al*. 2019). *Rouusoella* has cytoplea-like asexual morph (Hyde *et al.* 1996, Liu *et al.* 2014, Dai *et al* 2017, Jiang *et al.* 2019). Index Fungorum (2020) have listed 49 epithets under *Roussoella*, of which eight species without molecular data and 23 species with molecular data were accepted (Hyde 1997, Hyde *et al.* 1999, Zhou *et al.* 2003, Liu *et al*. 2014, Dai *et al.* 2017, Jiang *et al.* 2019, Poli *et al.* 2020).

In this study, we aim to introduce a new species, *Roussoella guttulata,* obtained from bamboo in Thailand. The phylogenetic analysis based on a combined LSU, ITS, TEF1-α and RPB2 sequence dataset and morphological evidences support its novelty. The comparisons of the new taxon with morphologically similar taxa are also provided.

Materials and methods

Sample collection, isolation and culture and specimen depositions

The decaying bamboo culms were collected in the disturbed forest in Nakhon Phanom Province, northeastern Thailand. The samples were packed into a plastic bag with the sampling information (time, place and host) and were taken to the laboratory for fungal examination. Pseudostromata on the host surface were examined and observed with a stereomicroscope (Leica EZ4 Microsystems (Schweiz) AG, Singapore). Micro-morphological characters were captured using a Nikon ECLIPSE Ni compound microscope (Nikon, Japan) fitted with a Nikon_DS-Ri2 digital camera (Nikon, Japan). The gelatinous sheath ascospores were checked with Indian ink reagent. Tarosoft (R) Image Frame Work was used to measure the size of pseudoascostromata, asci, ascospores, pseudoparaphyses and peridium. Adobe Illustrator CS6 (Adobe Systems, USA) was used to process and edit figures.

The method described by Dai *et al.* (2017) was used to carry out single spore isolations. Germinated spores were aseptically transferred to fresh potato dextrose agar (PDA) plates and incubated at room temperature (25 °C). Cultures were grown for 1 month and morphological characteristics, such as colony size, shape, color and texture were recorded. Holotype specimen and ex-type living culture were deposited in the Herbarium of Mae Fah Luang University (Herb. MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand, respectively. Isotype specimen was deposited at the Herbarium of Guizhou Academy of Agricultural Sciences (Herb. GZAAS), Guiyang, China. Index Fungorum and Facesoffungi (Jayasiri *et al.* 2015) numbers are registered.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fungal mycelium grown on PDA at 25 °C for 4 weeks. LR0R and LR5 (Vilgalys & Hester 1990), ITS5 and ITS4 (White *et al.* 1990), EF1-983F and EF1-2218R (Rehner & Buckley 2005), and fRPB2-5F and fRPB2-7cR (Liu *et al.* 1999) primers were used for the amplification of the 28S large subunit nuclear ribosomal DNA (LSU), the internal transcribed spacer region of ribosomal DNA (ITS), the translation elongation factor 1-alpha (TEF1-α) and the RNA polymerase II large subunit (RPB2), respectively. The PCR products were purified and sequenced with the same primers. The methods described by Lu *et al.* (2017) were used to amplify LSU, ITS, TEF1-α and RPB2. The quality of PCR products was checked on 1% agarose gel electrophoresis stained with ethidium bromide. The PCR products were sent to Sangon Biotech (Shanghai, China) for sequencing.

DNA sequence alignments and phylogenetic analysis

SeqMan v. 7.0.0 (DNASTAR, Madison, WI) was used for assembled consensus of forward and reverse sequences. The new sequences were subjected to BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for preliminary determination of the possible species identification range in the GenBank database. The sequence accession numbers are listed in TABLE 1 and the sequences of closely related taxa in Roussoellaceae and representative taxa in Thyridariaceae were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). The individual gene sequence matrix was aligned using online MAFFT version 7 (Katoh *et al.* 2019) (https://mafft.cbrc.jp/alignment/server/) and manually adjusted using the BioEdit 7.1.3.0 (Hall 1999). The concatenated LSU, ITS, TEF1-α and RPB2 sequence dataset was performed using SequenceMatrix 1.7.8 for further analyses.

The aligned fasta file was converted to phylip format by using the website tool "ALTER" (http://www.sing-group.org/ALTER/) (Glez-Peña *et al.* 2010). Maximum likelihood (ML) analysis was performed via the CIPRES Science Gateway V 3.3 (Miller *et al.* 2010) (https://www.phylo.org/portal2/home.action) using RAxML-HPC v.8 tool (Stamatakis 2014). One thousand non-parametric bootstrap iterations were run with the GTRGAMMA model, and the best scoring tree was selected from suboptimal trees under the GTRGAMMA substitution model.

The aligned fasta file was also converted to nexus format for Bayesian inference (BI) analyses by using AliView. BI analysis was performed with the tool of MrBayes on XSEDE (3.2.7a) via the CIPRES (Miller *et al*. 2010) web portal. The best-fit evolutionary model was estimated by MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996) were evaluated based on Markov Chain Monte Carlo (MCMC) sampling. Six simultaneous Markov chains were performed for 10,000,000 generations, and trees were sampled trees every 1,000 generation (resulting in a total of 10,000 trees). The first 2500 trees were the analyzed burn-in phase and were discarded based on tracer analysis. The remaining 7500 trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree.

Phylogenetic trees were visualized with FigTree v. 1.4.4 (Rambaut & Drummond 2008). The layout was edited using Microsoft Office PowerPoint 2010. The final alignment and tree were deposited in TreeBASE under submission ID: 27065 (http://www.treebase.org/)

TABLE 1. Taxa used in this study and their GenBank accession numbers for LSU, ITS, TEF1-α and RPB2 sequence data.

Taxa	Strain/Voucher No.	GenBank Accession no.			
		LSU	ITS	TEF1-α	RPB2
Arthopyrenia sp.	UTHSC DI16–362	LN907505	LT796905	LT797145	LT797065
Arthopyrenia sp.	UTHSC DI16–334	LN907477	LT796887	LT797127	LT797047
Neoroussoella alishanense	**FU31016**	**MK503822**	**MK503816**	**MK336181**	**MN037756**
Neoroussoella bambusae	**MFLUCC 11–0124**	**KJ474839**	**KJ474827**	**KJ474848**	**KJ474856**
Neoroussoella entadae	MFLUCC 15–0098	MH260309	MH275075	-	-
Neoroussoella heveae	**MFLUCC 17–0338**	**MH590689**	**MH590693**	-	-
Neoroussoella lenispora	**GZCC 16-0020**	**KX791431**	-	-	-
Neoroussoella leucaenae	**MFLUCC 18–1544**	**MK347984**	**MK347767**	**MK360067**	**MK434876**
Neoroussoella solani	**CPC 26331**	**KX228312**	**KX228261**	-	-
Pararoussoella mukdahanensis	KUMCC 18-0121	MH453485	MH453489	MH453478	MH453482
Pararoussoella rosarum	**MFLUCC 17–0796**	**NG_059872**	**NR_157529**	**MG829224**	-
Parathyridaria ramulicola	**CBS 141479**	**KX650565**	**KX650565**	**KX650536**	**KX650584**
Parathyridaria robiniae	**MFLUCC 14–1119**	**KY511141**	**KY511142**	**KY549682**	-
Pseudoneoconiothyrium euonymi	**CBS 143426**	**MH107961**	**MH107915**	-	**MH108007**
Pseudoneoconiothyrium rosae	**MFLUCC 15–0052**	**NG_059868**	**NR_157523**	-	-
Pseudoroussoella chromolaenae	**MFLUCC 17–1492**	**MT214439**	**MT214345**	**MT235769**	-
Pseudoroussoella elaeicola	MFLUCC 17–1483	MT214442	MT214348	MT235772	MT235808
Pseudoroussoella elaeicola	MFLUCC 15–0276b	MH742327	MH742330	-	-
Pseudoroussoella elaeicola	**MFLUCC 15–0276a**	**MH742326**	**MH742329**	-	-
Roussoella angusta	**MFLUCC 15–0186**	**KT281979**	-	-	-
Roussoella arundinacea	**CPC 35554**	**MT223928**	**MT223838**	**-**	**MT223699**
Roussoella chiangraina	**MFLUCC 10–0556**	**KJ474840**	**KJ474828**	**KJ474849**	**KJ474857**
Roussoella doimaesalongensis	**MFLUCC 14–0584**	**KY000659**	**KY026584**	**KY651249**	**KY678394**
Roussoella guttulata	**MFLUCC 20–0102**	**MT734818**	**MT734821**	**MW022188**	**MW022187**
Roussoella hysterioides	CBS 546.94	KF443381	KF443405	KF443399	KF443392
Roussoella intermedia	NBRC 106245	AB524624	KJ474831	-	-

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TABLE 1 (Continued)

Taxa	Strain/Voucher No.	GenBank Accession no.			
		LSU	ITS	TEF1-α	RPB2
Roussoella japanensis	**MAFF 239636**	**AB524621**	**KJ474829**	**AB539114**	**AB539101**
Roussoella kunmingensis	**KUMCC 18-0128**	**MH453487**	**MH453491**	**MH453480**	**MH453484**
Roussoella magnata	**MFLUCC 15–0185**	**KT281980**	-	-	-
Roussoella margidorensis	**MUT 5329**	**MN556322**	**KU314944**	**MN605897**	**MN605917**
Roussoella mediterranea	**MUT 5369**	**MN556324**	**KU314947**	**MN605899**	**MN605919**
Roussoella mexicana	**CPC 25355**	**KT950862**	**KT950848**	-	-
Roussoella neopustulans	**MFLUCC 11–0609**	**KJ474841**	**KJ474833**	**KJ474850**	-
Roussoella neopustulans	MFLUCC 12–0003	KU863119	KU940130	-	-
Roussoella nitidula	**MFLUCC 11–0182**	**KJ474843**	**KJ474835**	**KJ474852**	**KJ474859**
Roussoella nitidula	MFLUCC 11–0634	KJ474842	KJ474834	KJ474851	KJ474858
Roussoella padinae	**MUT 5503**	**MN556327**	**KU158170**	**MN605902**	**MN605922**
Roussoella padinae	MUT<ITA>:5341	MN556325	KU158153	MN605900	MN605920
Roussoella pseudohysterioides	**MFLUCC 13–0852**	**KU863120**	**KU940131**	**KU940198**	-
Roussoella pseudohysterioides	KUMCC 18-0111	MH453486	MH453490	MH453479	MH453483
Roussoella pustulans	**MAFF 239637**	**AB524623**	**KJ474830**	**AB539116**	**AB539103**
Roussoella scabrispora	**MFLUCC 11–0624**	**KJ474844**	**KJ474836**	**KJ474853**	**KJ474860**
Roussoella scabrispora	MFLUCC 14–0582	KY000660	KY026583	-	-
Roussoella scabrispora	strain RSC	KX650566	KX650566	KX650537	
Roussoella siamensis	**MFLUCC 11–0149**	**KJ474845**	**KJ474837**	**KJ474854**	**KJ474861**
Roussoella thailandica	**MFLUCC 11–0621**	**KJ474846**	**KJ474838**	-	-
Roussoella tuberculata	**MFLUCC 13–0854**	**KU863121**	**KU940132**	**KU940199**	-
Roussoella verrucispora	**CBS 125434**	**AB524622**	**KJ474832**	**AB539115**	**AB539102**
Roussoella yunnanensis	**KUMCC 18-0115**	**MH453488**	**MH453492**	**MH453481**	-
Roussoellopsis macrospora	**MFLUCC 12–0005**	**KJ474847**	-	**KJ474855**	**KJ474862**
Roussoellopsis sp.	NBRC 106246	AB524626	-	-	-
Roussoellopsis tosaensis	MAFF 239638	AB524625	-	AB539117	AB539104
Setoarthopyrenia chromolaenae	**MFLUCC 17–1444**	**MT214438**	**MT214344**	**MT235768**	**MT235805**
Thyridaria acaciae	**CBS 138873**	**KP004497**	**KP004469**	-	-
Thyridaria broussonetiae	**CBS 141481**	**KX650568**	**NR_147658**	**KX650539**	**KX650586**
Torula herbarum	CBS 111855	KF443386	KF443409	KF443403	KF443396
Torula hollandica	**CBS 220.69**	**KF443384**	**KF443406**	**KF443401**	**KF443393**
Xenoroussoella triseptata	**MFLUCC 17–1438**	**MT214437**	**MT214343**	**MT235767**	**MT235804**

Notes:—No data in GenBank. The newly generated sequences are shown in red. The ex-type strains are in bold.

Results

Phylogenetic analysis

The combined analyses included 59 strains which comprised the new taxon, and 56 ingroup taxa, *Torula herbarum* (CBS 111855) and *T. hollandica* (CBS 220.69) as outgroup taxa. The ML and BI analyses based on a combined

LSU, ITS, TEF1-α and RPB2 sequence dataset provided similar tree topologies, and the result of ML analysis (lnL =-26065.067850) is shown in FIGURE 1. The novel species *Roussoella guttulata* formed a separate clade basal to *R. hysterioides*, *R. japanensis*, *R. scabrispora* and *R. verrucispora* in Roussoellaceae.

The phylogram generated from maximum likelihood (RAxML) analysis based on combined LSU (876 bp), ITS (587 bp), TEF1-α (928 bp) and RPB2 (1044 bp) sequence dataset. The aligned sequence matrix comprises a total of 3,435 characters after alignment including the gaps, of which 2,315 characters were constant, 219 variable characters were parsimony-uninformative and 901 characters were parsimony informative. The matrix had 1,436 distinct alignment patterns, with 29.30% undetermined characters or gaps. Estimated base frequencies were as follows: T = 0.222408, G = 0.270872, C = 0.265331, A = 0.24139, substitution rates GT = 1.000000, CT = 9.620689, CG = 1.379050, AT = 2.055149, AG = 4.981444, AC = 1.809449, and the Tree-Length = 2.943862. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with a final average standard deviation of split frequencies was 0.009948.

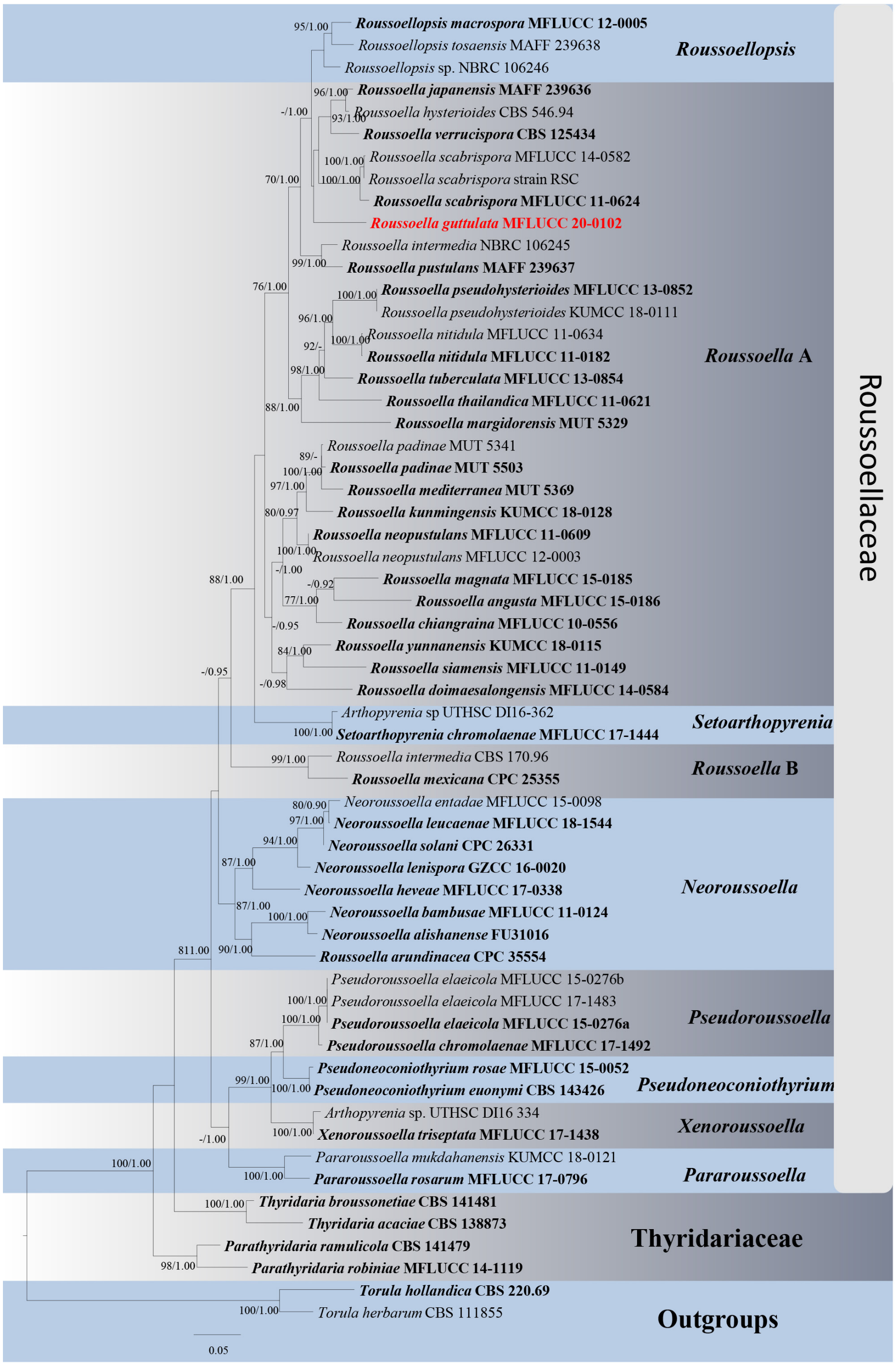

FIGURE 1. Phylogenetic tree generated from maximum likelihood (ML) analysis based on a combined LSU, ITS, TEF1-α and RPB2 sequence dataset. Bootstrap values of ML equal to or greater than 70% and Bayesian posterior probability equal to or greater than 0.95 PP are given at the nodes as ML/PP. The newly generated strain is shown in red bold and the ex-type strains are in black bold.

FIGURE 2. *Roussoella guttulata* (MFLU 20–0483, holotype). **a**, **b** Pseudoascostromata on the bamboo host. **c** Section through pseudoascostroma. **d** Peridium. **e** Ostiole **f** Trabeculate pseudoparaphyses. **g**–**k** Ascus. **l**–**q** Ascospore (**m** in Indian ink). **r** Germinating ascospores. **s**, **t** Colony on PDA from above and below. Scale bars: **a** = 1000 µm, **b** = 200 µm, **c** = 50 µm, **e**–**k**, **r** = 20 µm, **d, q** = 10 µm, **l**–**p** = 5 µm.

TABLE 2. Morphological comparison among *Roussoella guttulata*, *R. hysterioides*, *R. japanensis*, *Roussoella scabrispora* and *R. verrucispora* was discussed in this study.

Species	Ascostromata (high ×diam.)	Asci	Ascospores	References
Roussoella guttulata	**Pseudoascostromata, Locules 150–280 × 305–400 μm**	**90–110 × 6–8 μm, with indistinct ocular chamber**	**12–15 × 5–6 μm, yellowish brown to dark brown, covered with longitudinal striations, 1–3 guttules in each cell.**	**This study**
Roussoella hysterioides	Ascostromata, Locules 160–200 × 400–500 μm	140–210 × 8–11 μm, with a distinct ocular chamber	18–34 × 6–8 μm, brown, covered with longitudinal striations, aguttulate	Hyde *et al*. (1996)
Roussoella japanensis	Ascostromata 0.5–2 mm diam., Locules 190–210 × 500–560 μm	107–132 × 8–9.5 μm, with an indistinct ocular chamber	16–22 × 5.5–7 μm, brown, covered with longitudinal striations, aguttulate	Liu *et al*. (2014)
Roussoella scabrispora	Ascostromata 2−2.5 mm diam., Locules 350−500 × 1000−1500 μm diam.	(165−)197.5−217.5(−228) × 9.5−11.5(−12.5) μm, with an apical chamber.	(24−)25−29(−32) × (7−)9−10.5 μm, brown, covered with a reticulate spore wall ornamentation, aguttulate	Liu *et al*. (2014)
Roussoella verrucispora	Ascostromata 1.3–4.8 mm diam., Locules 280–320 × 400–430 μm	135–160 × 8–10 μm, with an indistinct ocular chamber	18–24.5 × 5.5–7 μm, brown, covered with irregular longitudinal short striations, aguttulate	Liu *et al*. (2014)

Taxonomy

Roussoella guttulata J.Y. Zhang, Y.Z. Lu & K.D. Hyde, *sp. nov*. FIGURE 2.

Index Fungorum number: IF557950, *Facesoffungi number*: FoF 08732

Etymology:—the epithet "*guttulata*" refers to the ascospores with small guttules.

Holotype:—MFLU 20-0483

Saprobic on decaying bamboo culms. **Sexual morph**: *Pseudoascostromata* immersed, gregarious, raised hemispherical or dome-shaped on host epidermis, visible as black, dull, scattered to clustered, coriaceous, glabrous, uni-to bi-loculate. *Ascomata* 305–400 µm diam., 150–280 µm high, solitary, immersed under a pseudostroma, scattered or clustered, elliptical to ampulliform, ostiolate beneath the host tissue, internally lined with periphyses. *Peridium* 16–25 µm thick, intermixed with host tissue, barely visible, composed of dark brown cells of *textura angularis* to *textura prismatica*, thin-walled. *Hamathecium* composed of dense, 1–1.8 µm broad ($\bar{x}$ = 1.5 µm, n = 20), hyaline, anastomosing, branched, smooth-walled, trabeculate pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 90–110 × 6–8 µm ($\bar{x}$ = 100.5 × 6.8 µm, n = 20), cylindrical, bitunicate, 8-spored, apically rounded with an indistinct ocular chamber, short or long pedicellate with slightly furcate pedicel. *Ascospores* 12–15 × 5–7 µm ($\bar{x}$ = 13.5 × 5.6 µm, n = 30), partial overlapping uni-seriate, initially hyaline, becoming yellowish brown to dark brown when mature, 2-celled, constricted at the septum, ellipsoidal to fusiform, with rounded ends, rough-walled, with longitudinal ribs, 1–3 guttules in each cell, surrounded by a large mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characteristics:—Ascospores germinated on PDA within 15 hours at room temperature. The hyaline germ tube germinates from a point of one cell of the ascospore, with a hyaline germ tube. Colonies grow on PDA at room temperature, slow growth, cultures creamy-white at center slightly raised, reaching 0.6 cm diam. in 22 days, irregular in shape with lobate edge, flat or effuse, marginal hyphae emission, brown to pale brown in reverse from the center of the colony with a creamy white margin.

Material examined:—THAILAND, Nakhon Phanom Province, Na Kae District, Nong Bo Subdistrict, dead bamboo culms in disturbed forest, 12 December 2018, 175 msl, D.P. Wei, XW509 (MFLU 20-0483, holotype; GZAAS 20-0301, isotype); ex-type living culture, MFLUCC 20-0102.

Notes:—*Roussoella guttulata* forms a distinct clade basal to *R. hysterioides*, *R. japanensis*, *R. scabrispora* and *R. verrucispora*. It is difficult to do a morphological comparison of taxa in *Roussoella* since most of the taxa in the genus share similar morphological features i.e. raised, immersed to semi-immersed ascostromata, cylindrical, bitunicate asci, anastomosed pseudoparaphyses, two-celled, longitudinally striate, brown ascospores with mucilaginous sheath. However, these species can be distinguished based on a number of locules in ascostromata and the size range of asci and ascospores as well as molecular analysis (Hyde *et al.* 1996, Liu *et al.* 2014). *Roussoella guttulata* can be distinguished from *R. hysterioides*, *R. japanensis*, *R. scabrispora* and *R. verrucispora* in uni-to bi-loculate of pseudoascostromata and the sizes of asci and ascospores (TABLE 2). A comparison of nucleotide base pairs of ITS, TEF1-α and RPB2 also shows their differences. In ITS, *R. guttulata* differs from *R. hysterioides*, *R. japanensis*, *R. scabrispora* and *R. verrucispora* in 29/468 bp (6%), 27/460 bp (6%), 31/365 bp (8%) and 38/520 bp (7%), respectively. In TEF1-α, they are different in 37/831 bp (4%), 43/934 bp (5%), 45/911 bp (5%) and 50/927 bp (5%), respectively. In RPB2, they are different in 96/994 bp (10%), 112/1054 bp (11%), 94/913 bp (10%) and 115/1055 bp (11%), respectively. Based on the guidelines provided of Jeewon & Hyde (2016) for delimitation of new species, and the morphological characteristics we introduce *R. guttulata* as a novel species.

Discussion

Most species of *Roussoella* are saprobic on terrestrial plants from monocotyledons, such as bamboo, palms and other large grasses (Hyde 1997, Liu *et al.* 2014, Ariyawansa *et al.* 2015, Dai *et al.* 2017, Jiang *et al.* 2019). Three species, namely, *R. margidorensis* Bovio, Prigione, A. Poli & Varese, *R. mediterranea*, A. Poli, E. Bovio, Prigione & Varese and *R. padinae* Prigione, E. Bovio, A. Poli & Varese have also been discovered from the marine milieu in Italy (Poli *et al.* 2020). Several *Roussoella* species have been reported from the northern part of Thailand (Liu *et al.* 2014, Dai *et al.* 2017). The present study introduces a novel taxon, *R. guttulata* from the northeast Thailand. This adds to the growing evidence of a high fungal diversity in northern Thailand (Hyde *et al.* 2018b). Introduction of *R. guttulata* also points towards *Roussoella* and its segregates being highly speciose. Moreover, while *Roussoella* species were formerly mainly grouped using morphological evidence (Hyde 1997, Hyde *et al.* 1999, Zhou *et al.* 2003). In this study, a bambusicolous taxon, *R. guttulata* is introduced as novel based on both phylogenetic evidence and morphological characteristics.

Pseudoparaphyses in *Roussoella guttulata* are often trabeculae and anastomose between the asci. This feature is often overlooked or even misinterpreted in Dothideomycetes and needs more detailed attention. The pseudoparaphyses in some *Roussoella* species are trabeculae (*sensu* Liew *et al.* 2000), for example they can clearly be seen in the plates of *R. japanensis, R. neopustulans* and *R. nitidula* which were described as cellular pseudoparaphyses by Liu *et al.* (2014). The characteristics of trabeculate pseudoparaphyses and cellular pseudoparaphyses was discussed by Liew *et al.* (2000) and Hongsanan *et al*. (2020b). Trabeculate pseudoparaphyses are distinct as they are generally 1 μm or thinner, anastomose (somewhat like a ladder) between the vertical pseudoparaphyses with remotely septate and are embedded in a gelatinous matrix and are only found in specific genera (e.g. *Astrosphaeriella* species). Cellular pseudoparaphyses, on the other hand, are often regularly septate and usually wider and do not anastomose between the vertical pseudoparaphyses between the asci. They may, however, be embedded in a gelatinous matrix and may anastomose above the asci only, which may confuse. Often this can only be confirmed by making careful sections. In this study, *R. guttulata* has narrow pseudoparaphyses (1–1.8 μm diam.) with remotely septate, branched and anastomosing among the asci, and embedded in a hyaline gel matrix. Pseudoparaphyses in *R. guttulata*, however, are not strictly trabeculate like *Astrosphaeriella* species, which are usually less than 1 μm diam.

The tree topology of maximum likelihood analysis in FIGURE 1 shows that *Roussoella* is polyphyletic, forming two distant clades indicated as *Roussoella* A and *Roussoella* B, the new species *R. guttulata* is restricted to the *Roussoella* A, which contain the type species *R. nitidula*. The phylogenetic placement of *Roussoellopsis* species group within the *Roussoella* A clade (Liu *et al.* 2014, Ariyawansa *et al.* 2015, Jiang *et al.* 2019, Karunarathna *et al.* 2019, Phookamsak *et al.* 2019, Mapook *et al.* 2020). However, they can be differentiated by morphological characteristics. *Roussoellopsis* is distinct from *Roussoella* in having clavate asci, large fusiform ascospores, melanconiopsis-like or neomelanconium-like asexual morphs with annellidic conidiogenous cells (Tanaka *et al.* 2009, Liu *et al*. 2014). Given the remark differences in morphology between *Roussoellopsis* species and *Roussoella* species, we keep *Roussoellopsis macrospora*, *Roussoellopsis sp.* (NBRC 106246) and *Ro. tosaensis* in the original genus *Roussoellopsis*.

Roussoella arundinacea Crous & R.K. Schumach. was introduced by Crous *et al.* (2020) based on a megablast search of NCBI's GenBank nucleotide database and phylogenetic tree using incomplete taxa. In this study, *R. arundinacea* formed a single clade basal to *Neoroussoella alishanense* and *N. bambusae* with high support (90%BS, 1.00 PP) within *Neoroussoella* (FIGURE 1). Morphologically, *R. arundinacea* formed an asexual morph in the culture and is similar to *N. alishanense* as well as the size of conidiophores, conidiogenous cells and conidia. Hence, *R. arundinacea* may need to be synonymized to closely related genus *Neoroussoella*.

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