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Muyocopron garethjonesii sp. nov. (Muyocopronales, Dothideomycetes) on *Pandanus* sp.

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Abstract

During a survey of microfungi on leaves of *Pandanus* species in the Nabanhe Valley at Xishuangbanna, Yunnan Province, China in August 2016, we collected and isolated a new saprobic taxon belonging in *Muyocopron* (*Muyocopronaceae*). Both morphological characters and phylogenetic analyses of LSU and SSU sequence data provide strong evidence to place the new taxon in *Muyocopron* where it is described as *M. garethjonesii.* A description, photoplates of the macro- and micro-characteristics, and a phylogenetic tree to resolve the placement of *M. garethjonesii* are provided.

Keywords – Carbonaceous – *Pandanaceae* – Phylogenetics – Taxonomy

Introduction

We are interested in microfungi associated with *Pandanaceae* (Whitton et al. 2012, Tibpromma et al. 2016a, b, c) as the number of fungi known from this host is rather small (Whitton et al. 2012). According to Taylor & Hyde (2003), *Muyocopron* can also be found on species of *Pandanaceae*.

The family *Muyocopronaceae* was introduced by Luttrell (1951), with *Muyocopron* Speg. assigned as the type genus (Spegazzini 1881). Eriksson (1981) placed this family in the order Hemisphaeriales as it was considered to have a pleospora-type centrum as in the majority of

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Microthyriaceae, *Hemisphaeriaceae* and *Polystomellaceae* taxa. Hyde et al. (2013) accepted *Muyocopronaceae* as a distinct family with the single genus *Muyocopron* and placed it in class Dothideomycetes.

Muyocopron corrientinum Speg. is the type species of the genus *Muyocopron*, and characteristic features include distinctive black, dull, rounded regions on the surface of host plants; dimidiate-scutate, subcarbonaceous, ostiolate ascomata, forming superficially on the substrate without mycelium; and bitunicate, 8-spored asci, containing ellipsoidal, hyaline ascospores (Spegazzini 1881, Mapook et al. 2016). Index Fungorum (2016) lists 57 epithets under *Muyocopron*, with ten species having been transferred to other genera/families (*Micropeltidaceae*, *Microthyriaceae*, *Nitschkiaceae*, *Phaeopolystomella* and *Phyllachoraceae*). *Muyocopron* has been placed in various families by different mycologists (Arx & Müller 1975, Saccardo 1883, Eriksson & Hawksworth 1993, Phipps & Rember 2004, Lumbsch & Huhndorf 2007, 2010). However, molecular phylogenetic studies indicated that *Muyocopron* species group with *Pleurotrema* species in the family *Pleurotremataceae* (Pang et al. 2013, Maharachchimbura et al. 2016). Mapook et al. (2016) used fresh collections and combined sequence datasets to show *Muyocopron* to be a distinct lineage with *Pleurotremataceae* and Acrospermales in Dothideomycetes.

In this paper, we introduce a new species *Muyocopron garethjonesii* in the family *Muyocopronaceae*. We provide an analysis of combined LSU and SSU sequence data to infer the phylogenetic placement of the new taxon. A comparison of the new taxon with *Muyocopron* species, a comprehensive description, a photoplates of the host, and micrographs of the new taxon are provided.

Materials & methods

Sample collection and specimen examination

Fresh specimens of *Pandanus* spp. (*Pandanaceae*) in the form of dead and fallen leaves were collected from Nabanhe Valley, Xishuangbanna, Yunnan Province, China. The leaves were brought to the laboratory in Zip-lock bags and examined using a JNOEC JSZ4 (ser. No. 030233) stereo microscope. Fruiting bodies were rehydrated in water before sectioning. Sections were cut using a razor blade and microscopic features observed using a Nikon ECLIPSE Ni compound microscope. Photographs were taken with a Canon 600D digital camera mounted on the microscope. All photomicrographs of microscopic fungal structures were measured using Tarosoft® Image Framework program v.0.9.0.7.

Description of cultures

Malt extract agar (MEA; 30 g/L of malt extract, 15 g/L of agar, 20 g/L of dextrose, pH 5.6±0.2) was used as the medium for culturing the isolated fungi. Single spore isolates were obtained following the method of Chomnunti et al. (2014) using MEA and incubated overnight at room temperature (20–25 ºC). Germinating ascospores were aseptically transferred to new MEA media plates after 24 hours. Sexual cultures were subcultured and transferred to water agar (WA) media containing sterile toothpicks and pine needles (Phookamsak et al. 2015) and incubated at room temperature for three months to induce the asexual morph. The cultures were incubated at room temperature (20–25 ºC) for 4–6 weeks and colonies were then observed. Herbarium specimens were dried using silica gel and deposited in Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand and Kunming Institute of Botany Academia Sinica

(HKAS). Ex-type living cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC), while duplicate cultures were deposited in Kunming Institute of Botany Culture (KMUCC). Facesoffungi numbers (FoF) and Index Fungorum (IF) numbers were registered as described in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and DNA sequencing

Isolates were grown on MEA at room temperature for two weeks, and the fungal mycelia were scraped off and transferred to 1.5 ml Eppendorf tubes. The fungal genomic DNA extraction followed the protocol of Biospin Fungal Genomic DNA extraction Kit–BSC14S1 (BioFlux, P.R. China). Polymerase chain reactions (PCR) were used to amplify partial gene regions LSU (Vilgalys & Hester 1990) and SSU (White et al. 1990) using primers and conditions as outlined in Tibpromma et al. (2016b). The total volume of PCR mixtures for amplifications were 25 µL containing 8.5 µL ddH_2O, 12.5 µL 2× Easy Taq PCR Super Mix (mixture of Easy Taq TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China), 2 µL of DNA template, 1 µL of each forward and reverse primers (10 pM). The quality of PCR products were checked on 1% agarose gel electrophoresis stained with 4S green nucleic acid (Life Science Products & Services, Cat. No: A616694). Purification and sequencing of PCR products were carried out by Sangon Biotech Co., Shanghai, China.

Phylogenetic analyses

LSU and SSU sequence data generated in this study were subjected to BLAST searches in the nucleotide database of GenBank (www http://blast.ncbi.nlm.nih.gov/) to establish the most similar taxa. Sequence data were retrieved from GenBank based on most recent publications (Mapook et al. 2016). *Lichenothelia convexa* Henssen 1987 (L1607) was used as the outgroup taxon (Table 1). All sequence alignments were done with MAFFT v.6.864b (Katoh & Standley 2016) and alignments were manually improved if necessary. The sequence datasets were combined using BioEdit v.7.2.5 (Hall 2004). The phylogenetic analyses were performed by using Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian posterior probabilities (BYPP). A maximum likelihood analysis including 1,000 bootstrap replicates was done using RAxML v. 8.2.4 (Stamatakis 2014), which is a part of RAxML-HPC BlackBox tool (Miller et al. 2010). To perform Bayesian analysis, the model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004) with the nucleotide substitution models selected for combined datasets as GTR+I+G. Posterior probabilities (PP) (Rannala & Yang 1996) were established by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v 3.0b4 (Liu et al. 2012). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generations (Cai et al. 2006). The first 2,000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8,000 (post-burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01) (Zhaxybayeva & Gogarten 2002, Ariyawansa et al. 2015). The phylogenetic trees were figured in FigTree v. 1.4 (Rambaut & Drummond 2008) and edited using Microsoft Office PowerPoint 2007 and Adobe illustrator CS3 (Adobe Systems Inc., USA). Sequences derived in this study were deposited in GenBank, and the alignments in TreeBASE (www.treebase.org) submission ID: 20286.

Results and discussion

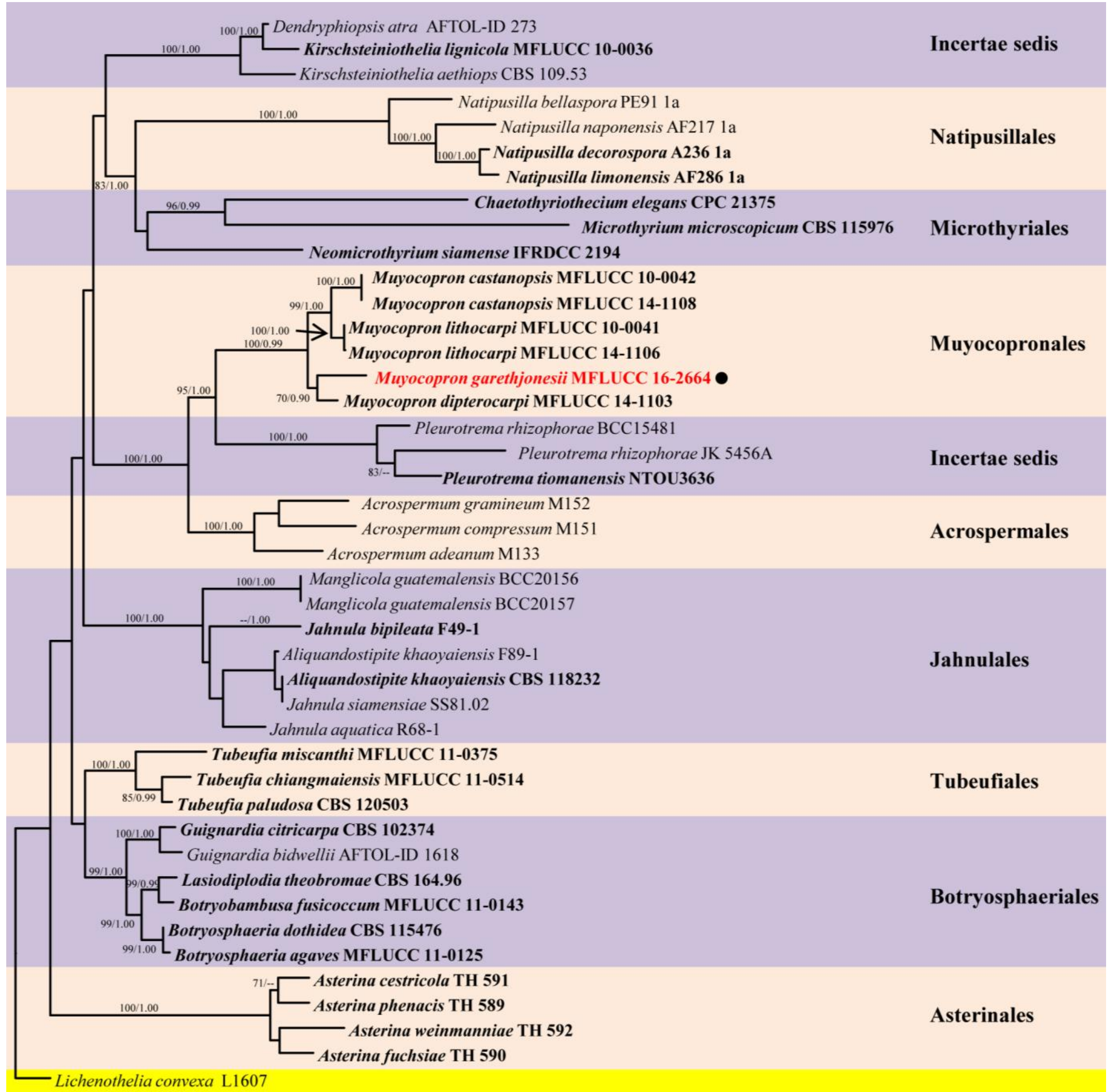

Fig. 1. Phylogram generated from RAxML based on combined LSU and SSU sequence data. Bootstrap support values for maximum likelihood (ML, left) greater than 70% and Bayesian posterior probabilities (PP, right) equal to or greater than 0.90 are indicated at the nodes. The ex-type and reference sequences are in bold. The newly generated sequences are in red with black dots.

Table 1. Taxa used in the phylogenetic analyses and their GenBank accession numbers. The new taxon is indicated with an asterisk.

Taxon	Culture Accession	GenBank Accession	
		LSU	SSU
Acrospermum adeanum	M133	EU940104	EU940031
A. compressum	M151	EU940084	EU940012
A. gramineum	M152	EU940085	EU940013
Aliquandostipite khaoyaiensis	F89-1	EF175647	EF175625
A. khaoyaiensis	CBS 118232	GU301796	-
Asterina cestricola	TH 591	GU586215	GU586209
A. fuchsiae	TH590	GU586216	GU586210
A. phenacis	TH589	GU586217	GU586211
A. weinmanniae	TH592	GU586218	GU586212
Botryobambusa fusicoccum	MFLUCC 11-0143	JX646809	JX646826
Botryosphaeria agaves	MFLUCC 11-0125	JX646808	JX646825
B. dothidea	CBS 115476	DQ377852	DQ677998
Chaetothyriothecium elegans	CPC 21375	KF268420	-
Dendryphiopsis atra	AFTOL-ID 273	DQ678046	DQ677996
Guignardia bidwellii	AFTOL-ID 1618	DQ678085	DQ678034
G. citricarpa	CBS 102374	DQ377877	GU296151
Jahnula aquatica	R68-1	EF175655	EF175633
J. bipileata	F49-1	EF175657	EF175635
J. siamensiae	SS81.02	EF175666	EF175645
Kirschsteiniothelia aethiops	CBS 109.53	AY016361	AF346547
K. lignicola	MFLUCC 10-0036	HQ441568	HQ441569
Lasiodiplodia theobromae	CBS 164.96	EU673253	EU673196
Lichenothelia convexa	L1607	KC015068	KC015083
Manglicola guatemalensis	BCC20156	KC015069	KC015084
M. guatemalensis	BCC20157	FJ743450	FJ743444
Microthyrium microscopicum	CBS 115976	GU301846	GU296175
Muyocopron castanopsis	MFLUCC 10-0042	-	JQ036225
M. castanopsis	MFLUCC 14–1108	KU726965	KU726968
M. dipterocarpi	MFLUCC 14–1103	KU726966	KU726969
*M. garethjonesii**	MFLU 16-2664	KY070274	KY070275
M. lithocarpi	MFLUCC 10-0041	JQ036230	JQ036226
M. lithocarpi	MFLUCC 14–1106	KU726967	KU726970
Natipusilla bellaspora	PE91 1a	JX474864	JX474868
N. decorospora	AF236 1a	HM196369	HM196376
N. limonensis	AF286 1a	HM196370	HM196377
N. naponensis	AF217 1a	HM196371	HM196378
Neomicrothyrium siamense	IFRDCC 2194	JQ036228	JQ036223
Pleurotrema tiomanensis	NTOU3636	KC692156	KC692155
P. rhizophorae	JK 5456A	GU479799	-
P. rhizophorae	BCC15481	-	KF160009
Tubeufia chiangmaiensis	MFLUCC 11-0514	KF301538	KF301543
T. miscanthi	MFLUCC 11-0375	KF301533	KF301541
T. paludosa	CBS 120503	GU301877	GU296203

ABBREVIATIONS: **AFTOL-ID**: Assembling the Fungal Tree of Life: **BCC**: BIOTEC Culture Collection Laboratory: **CBS**: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; **JK**: J. Kohlmeyer; **KMUCC:** Kunming Institute of Botany Culture; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

Phylogenetic analyses

LSU and SSU sequence data we obtained from 45 taxa including our new taxon and the outgroup taxon (Table 1). Bootstrap support (BS) values of ML equal to or above 70% based on 1,000 replicates are shown in Fig. 1, from a best scoring RAxML analysis based on a combined aligned dataset of LSU and SSU sequence data. Bayesian posterior probabilities more than 0.90 are also shown on the nodes of the phylogenetic tree (Fig. 1). The phylogenetic tree obtained in this study shows similar results as in previous study by Mapook et al. (2016). The phylogenetic analyses shows our new taxon groups together with *M. dipterocarpi*, but is well-separated with strong support (70% in ML, 0.90 in PP).

Table 2. Comparison of the *Muyocopron* spp. reported on *Pandanaceae*

Taxa	Ascomata (µm)	Asci (µm)	Ascospores (µm)	Host/Distribution
M. pandani Höhn. 1920	200–500 × 100–200	26–30 × 18–20	12–15 × 5–8	*Pandanus* sp., Indonesia
M. freycinetiae (F. Stevens & R.W. Ryan) G. Arnaud 1931	400–650	34–50 × 15–20	13–18 × 6–8	*Freycintia arnotti*, Hawaii
M. garethjonesii Tibpromma, Karun. & K.D. Hyde 2016	110–130 × 400–525	60–120 × 19–35	19–25 × 11–13	*Pandanus* sp., China

Taxonomy

Muyocopron garethjonesii Tibpromma, Karun. & K.D. Hyde, sp. nov.

Index Fungorum number: IF552529, Facesoffungi number: FoF02662 Figs 2, 3

Etymology: Named in honour of Professor E.B. Gareth Jones who turns 80 years old in January 2017, in recognition of his immense contribution to mycology.

Holotype: MFLU 16–2664

Saprobic on dead leaves of *Pandanus* sp. **Sexual morph**: *Colonies* on natural substrate dry, black, circular, dull, undulate, umbonate, rough. *Ascomata* 110–130 µm high × 400–525 µm diam. ($\bar{x}$ = 118 × 478 µm, n = 10), superficial, solitary or scattered, conspicuous at the host surface, appearing as circular, flattened, dark brown to black spots, carbonaceous, without a papilla, dull, with irregular margin, ostiole central. *Peridium* 32–70 µm wide, outer layer comprising dark brown to black, pseudoparenchymatous, occluded cells of *textura angularis*, inner layer comprising yellow-brown cells of *textura angularis*. *Hamathecium* comprising numerous, 1.5–2.4 µm wide, cylindrical to filiform, septate, unbranched pseudoparaphyses. *Asci* 60–120 × 19–35 µm ($\bar{x}$ = 87 × 28 µm, n = 20), 4–8-spored, bitunicate, broadly cylindrical to ovoid, with long pedicle. *Ascospores* 19–25 × 11–13 µm ($\bar{x}$ = 21 × 12 µm, n = 20), overlapping 1–2 seriate, hyaline to yellowish, 1-celled, ellipsoid to obovoid, granular, with one or two large oil guttules, lacking a mucilaginous sheath. **Asexual morph**: Undetermined. The mycelia produced pigments and hyphal coin structures (Fig. 3).

Culture characteristics: Colonies on MEA at room temperature (20–25 °C) reaching 4 cm in two weeks, circular with undulate, yellow-grey mycelium, velvety and flat on the media.

Material examined: CHINA, Yunnan Province, Xishuangbanna, Nabanhe Valley, on dead leaves of *Pandanus* sp. (*Pandanaceae*), 4 August 2016, S. Tibpromma & S.C. Karunarathna NBH15 (MFLU 16-2664, **holotype),** ex-type living cultures, MFLUCC 16-1370, KMUCC 16-0150.

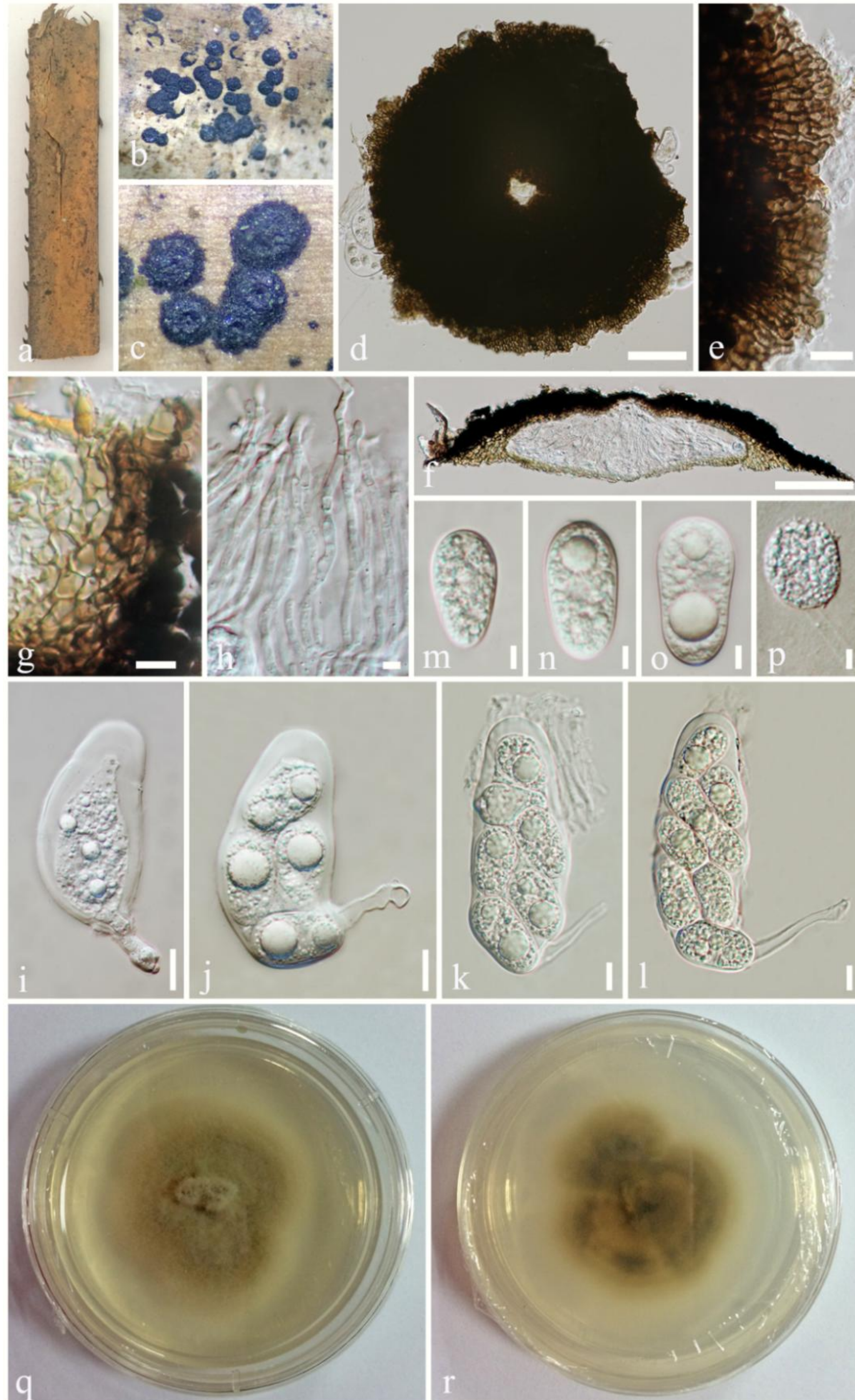

Fig. 2 *Muyocopron garethjonesii* (holotype). a–c Superficial ascomata on substrate. d, e Arrangement of cells in outer region of ascomata. f Section of ascoma. g Peridium. h Pseudoparaphyses. i–l Asci. m–o Ascospores. p Germinated ascospore. q, r Colony on PDA after 7 days. Scale bars: d = 100 µm, e = 20 µm, f = 100 µm, g = 10 µm, h = 5 µm, i–l = 10 µm, m–p = 5 µm.

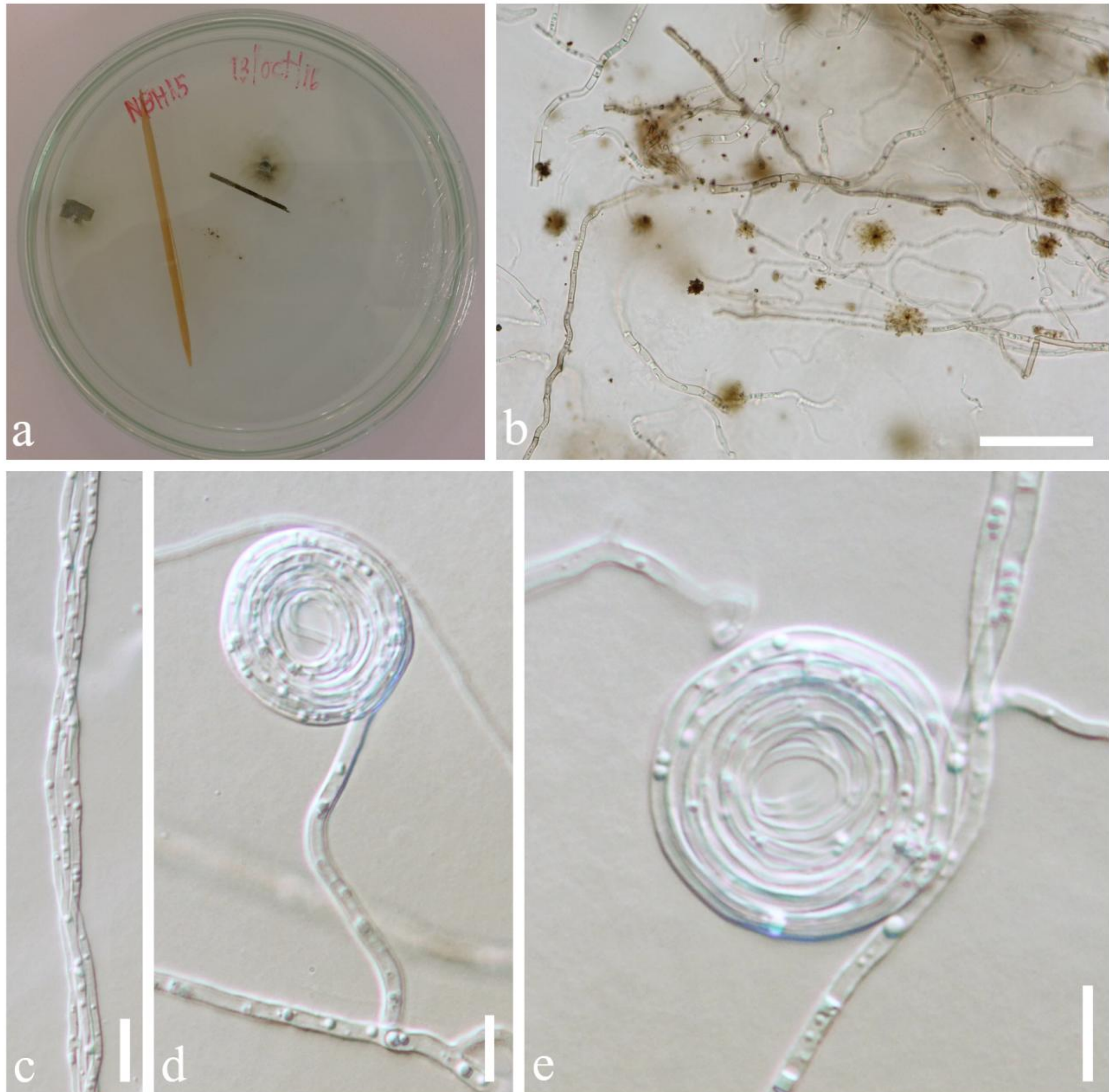

Fig. 3 *Muyocopron garethjonesii* (ex-type culture on WA media). a Growth of asexual morph on plant tissues. b Hyphae with produced pigments. c Hyphae. d, e Hyphal coil structures formed by mycelia. Scale bars: b = 50 µm, c–e = 10 µm.

Notes: *Muyocopron garethjonesii* is introduced as a new species as it has distinct morphological features. These include a thick peridium, 4–8-spored, broadly cylindrical to ovoid, long pedicellate asci and hyaline to yellowish ascospores. We compare our new species with those known from *Arecaceae* and *Pandanaceae* (Taylor & Hyde 2003). *Muyocopron hongkongense*, which was described from a palm, has saccate to clavate or obclavate asci, with granular ascospores (Taylor & Hyde 2003). There are also obvious differences when compared to two other species of *Muyocopron* found on *Pandanaceae* (Table 2) with *M. garethjonesii* having larger ascospores.

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