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Muyocopronales, *ord. nov.*, (Dothideomycetes, Ascomycota) and a reappraisal of *Muyocopron* species from northern Thailand

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Abstract

Muyocopron species are associated with a wide variety of plant substrates worldwide and presently 57 species epithets are listed in Index Fungorum. Species in this genus form distinctive black, dull, rounded regions on the surface of plants and the genus is probably polyphyletic. The present study clarifies the phylogenetic placement of *Muyocopron* and related species, using fresh tropical collections from northern Thailand. Three *Muyocopron* species are characterized based on analyses of combined LSU and SSU sequence datasets. Phylogenetic analyses indicate that *Muyocopron* species form a distinct lineage with the Dyfrolomycetales and Acrospermales lineages. The new order *Muyocopronales* with three new *Muyocopron* species is introduced based on its distinct phylogeny and unique morphological characteristics. The taxonomy and phylogenetic relationships of tropical *Muyocopron* species are reappraised with suggestions for future work.

Key words: *Muyocopronaceae*, Muyocopronales, new species, phylogeny, taxonomy

Introduction

The family *Muyocopronaceae* was introduced by Luttrell (1951) and included in the order Hemisphaeriales as it was considered to have a *Pleospora*-type of centrum as in the majority of *Microthyriaceae, Hemisphaeriaceae* and *Polystomellaceae* (Eriksson 1981). Hyde *et al*. (2013) accepted *Muyocopronaceae* as a distinct family with a single genus *Muyocopron*, which was placed in class Dothideomycetes, and provided with illustrated account of the genus.

Muyocopron was introduced by Spegazzini (1881) based on its black, dimidiate-scutate, subcarbonaceous, ostiolate ascomata, forming superficially on the substrate without mycelium, and bitunicate, 8-spored asci, containing ellipsioidal, hyaline ascospores. The type species, *M. corrientinum* Speg., occurred on rotting dead leaves of *Oncidium* sp. in Argentina (Spegazzini 1881). Many species have since been introduced and the genus has a fairly broad concept. Index Fungorum (2016) lists 57 species epithets but many of these have been transferred to other genera and families, with 46 species epithets in Species Fungorum. Spegazzini (1905, 1911) described most of the earlier species from Argentina, for example *M. argentinense* Speg. was described from stems of *Foeniculum piperitum*, *M. yerbae* Speg. from branches of *Ilex paraguayensis* and *M. caseariae* Speg. from leaves of *Casearia sylvestris*. Spegazzini (1889, 1909, 1917) also described *M. umbilicatum* Speg. from leaves of *Bignoniaceae amphilophium* from Brazil; *M. valdivianum* Speg. from leaves of *Eugenia* and *M. litorale* Speg. from leaves of *Rhodostachis litoralis* from Chile. The most recently described species is *M. hongkongense* Joanne E *et al.* from a dead inflorescence of *Archontophoenix alexandrae* (Taylor & Hyde 2003). This species has asci with 4–8 ascospores and is common on members of *Palmae* and *Pandanaceae*, and appears to be widespread in tropical regions (Taylor & Hyde 2003). Some species are also known from temperate regions, e.g. *Muyocopron calamagrostidis* Rostr. (Iceland), *Muyocopron eleocharidis* Grove (UK) and it would be interesting to establish if these are really characteristic of the genus (Taylor & Hyde 2003).

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Saccardo (1883) re-described *Muyocopron* and referred it to the family *Microthyriaceae*, but considered that it differed from *Microthyrium* in having aseptate ascospores (Dennis 1981), while von Arx & Müller (1954) synonymized the genus under *Ellisiodothis*. Von Arx & Müller (1975) re-described *Muyocopron* and placed it in the family *Botryosphaeriaceae*, because *Muyocopron* and *Botryosphaeria* have similar ascospores, but the scutate ascomata and granular appearance of the aseptate ascospores differ. Morphologically similar genera are *Ellisiodothis* and *Haplopeltis* that were regarded as synonyms of *Muyocopron* by Eriksson & Hawksworth (1993) and this name has been accepted until now (Phipps & Rember 2004). Lumbsch & Huhndorf (2007) placed *Muyocopron* in the family *Microthyriaceae*, and other authors have accepted this (Lumbsch & Huhndorf 2010, Index Fungorum 2016, MycoBank 2015). However in the family, *Microthyriaceae* (type *Microthyrium*), ascomata are true thyriothecia with pseudoparaphyses developing above the asci, ascospores are 1-septate and with or without ciliate appendages (Doidge 1942, Müller & von Arx 1962, Luttrell 1973, Hofmann & Piepenbring 2006, Hofmann 2010, Wu *et al*. 2011a, b, Hyde *et al*. 2013, Hongsanan *et al*. 2014, Ariyawansa *et al*. 2015), while ascomata in the family *Muyocopronaceae* (type *Muyocopron*) are pseudothyriothecia with the peridial wall comprising two layers, pseudoparaphyses are longer than the asci, and ascospores are aseptate. Molecular studies indicate that *Muyocopron* species group with *Dyfrolomyces rhizophorae*, in the family *Dyfrolomycetaceae* (Pang *et al*. 2013), in the order Dyfrolomycetales (Hyde *et al*. 2013).

Several taxonomic novelties were found during collections of *Muyocopron*-like taxa in northern Thailand. Illustrated accounts of the Thai collections of *Muyocopron* collected in this study are provided and their phylogenetic placement determined, together with a reappraisal of the genus. A new order, Muyocopronales, is introduced to accommodate this distinct lineage.

Material and methods

Collection, examination, and isolation of fungi

Fresh material was collected from different sampling sites in Chiang Rai Province at Doi Tung, Doi Pui and Mae Lao forests during the rainy season (June-October) in 2014. Samples of terrestrial decomposing leaves and woody litter were randomly collected. Samples were examined with a Motic SMZ 168 Series microscope. Images were captured with Carl Zeiss GmbH stereo microscope with an AxioCam ERC 5S camera. Sections of ascomata were made by free hand. Microscopic characters were made in water mounts and photographed with a Nikon ECLIPSE 80i compound microscope fitted with a Canon EOS 550D digital camera. All measurements were calculated using Tarosoft Image Frame Work program (IFW).

Single spore isolation and culture morphology were obtained following the methods of Chomnunti *et al*. (2014). Growth rate was measured from 7 day-old colonies and some of these cultures were used for molecular study. The specimens and living cultures are deposited in the herbaria of Mae Fah Luang University (Herb. MFLU) and Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), China and culture collection of Mae Fah Luang University (MFLUCC) and BIOTEC (BCC). Faces of fungi and Index Fungorum numbers were obtained as in Jayasiri *et al*. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and sequencing

Methods for sample preparation, DNA extraction and PCR reaction were carried out according to Telle and Thines (2008). Fungal colonies were grown on MEA at 25 °C for two weeks. Mycelium was scraped off and transferred to 1.5 ml. micro centrifuge tubes. Genomic DNA was extracted by Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's instructions (Hangzhou, P.R. China). The partial small subunit nuclear rDNA (SSU) was amplified with primer pairs NS1 and NS4 (White *et al*. 1990). The partial large subunit nuclear rDNA (LSU) was amplified with primer pairs LROR and LR5 (Vilgalys & Hester 1990). PCR amplification and sequencing were carried out according to the following protocol: The final volume of the PCR reaction was 50 µl which contained 2.0 µl of DNA template, 1.5 µl of each forward and reverse primers, 25 µl of 2× Easy Taq PCR SuperMix (mixture of EasyTaqTM DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Ltd., Chaoyang District, Beijing, PR China) and 20µl sterilized water. The PCR thermal cycle program for LSU gene amplification were: initially 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 50 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 7 min. The PCR thermal cycle program for SSU genes amplification were: initially 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 7 min. The quality of PCR products were checked on 1

% agarose gel electrophoresis stained with ethidium bromide. The PCR products were sent for sequencing at BGI Tech Solutions Co., Ltd., Konggang B-6, Shunyi, Beijing, China.

Phylogenetic analysis

Analysis of combined LSU and SSU sequence data from the closest relatives to Muyocopronales (*Muyocopronaceae*) in Acrospermales (*Acrospermaceae*), Asterinales (*Asterinaceae*), Botryosphaeriales (*Botryosphaeriaceae* and *Phyllostictaceae*) Dyfrolomycetales (*Dyfrolomycetaceae*), Jahnulales (*Aliquandostipitaceae* and *Manglicolaceae*), Microthyriales (*Microthyriaceae*), Natipusillales (*Natipusillaceae*), Tubeufiales (*Tubeufiaceae*) and Dothideomycetes families *incertae sedis* (*Kirschsteiniotheliaceae*) were used to confirm the phylogenetic placement of *Muyocopron* strains. *Lichenothelia convexa* L1606 and *Lichenothelia convexa* L1607 was selected as outgroup taxa based on their placement close to the ingroup. The closest matched taxa were determined through nucleotide blast searches in GenBank. The sequences used for analyses with accession numbers are given in Table 1. All sequence data were aligned using MAFFT (v7.110) online program (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013). The alignments were checked and uninformative gaps minimized manually where necessary in BioEdit 7.0.1 (Hall 1999). Maximum Likelihood (ML) and Bayesian Inference (BI) were used in the analyses following the methodology as described in Mapook *et al*. (2016). The nucleotide substitution models use for analyses were determined using MrModeltest v. 2.2 (Nylander *et al.* 2004). The GTR+I+G model with inverse gamma rate were selected for individual data from each partition with the combined aligned dataset.

TABLE 1. Taxa used in this study and their GenBank accession numbers. New sequences are in bold.

Taxon	Culture accession no.[1]	GenBank accession no.[2]	
		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
Dyfrolomyces tiomanensis	NTOU3636	KC692156	KC692155
D. rhizophorae	JK 5456A	GU479799	-
D. rhizophorae	BCC15481	-	KF160009
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

...Continued on next page

TABLE 1. (Continued)

Taxon	Culture accession no.[1]	GenBank accession no.[2]	
		LSU	SSU
Kirschsteiniothelia aethiops	CBS 109.53	AY016361	AF346547
K. lignicola	MFLUCC 10-0036	HQ441568	HQ441569
Lasiodiplodia theobromae	CBS 164.96	EU673253	EU673196
Lichenothelia convexa	L1606	KC015068	KC015083
L. convexa	L1607	KC015069	KC015084
Manglicola guatemalensis	BCC20156	FJ743448	FJ743442
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. 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
Tubeufia chiangmaiensis	MFLUCC 11-0514	KF301538	KF301543
T. miscanthi	MFLUCC 11-0375	KF301533	KF301541
T. paludosa	CBS 120503	GU301877	GU296203

[1]**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; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **T**: ex-type/ex-epitype isolates.

[2] **LSU**: 28S large subunit of the nrRNA gene and **SSU**: 18S small subunit of the nrRNA gene.

Phylogenetic trees were drawn using Treeview v. 1.6.6 (Page 1996). The new nucleotide sequence data are deposited in GenBank.

Results and discussion

Phylogenetic analysis of combined LSU and SSU nrDNA sequence data

The combined dataset included LSU and SSU sequence data and were analyzed by Maximum Likelihood (ML) and Bayesian analyses. All trees were similar in topology and did not differ significantly (data not shown). The combined sequence alignment comprised 43 taxa, including our new strains, with *Lichenothelia convexa* (L1606) and *Lichenothelia convexa* (L1607) as the outgroup taxa. A best scoring RAxML analysis based on a combined aligned dataset of LSU and SSU sequence data is shown in Fig. 1.

The 43 taxa analyzed in the cladogram formed ten clades representing the orders Acrospermales (*Acrospermaceae*), Asterinales (*Asterinaceae*), Botryosphaeriales (*Botryosphaeriaceae* and *Phyllostictaceae*) Dyfrolomycetales (*Dyfrolomycetaceae*), Jahnulales (*Aliquandostipitaceae* and *Manglicolaceae*), Microthyriales (*Microthyriaceae*), Muyocopronales (*Muyocopronaceae*), Natipusillales (*Natipusillaceae*), Tubeufiales (*Tubeufiaceae*) and Dothideomycetes

family *incertae sedis* (*Kirschsteiniotheliaceae*) within the Dothideomycetes. The phylogenetic tree (Fig. 1) shows *Muyocopron* taxa form a distinct lineage with the lineages of Dyfrolomycetales and Acrospermales. The orders Acrospermales and Dyfrolomycetales have characters that are very different from those of species of *Muyocopronaceae* and therefore we introduce a new order Muyocopronales to accommodate this group of unique fungi.

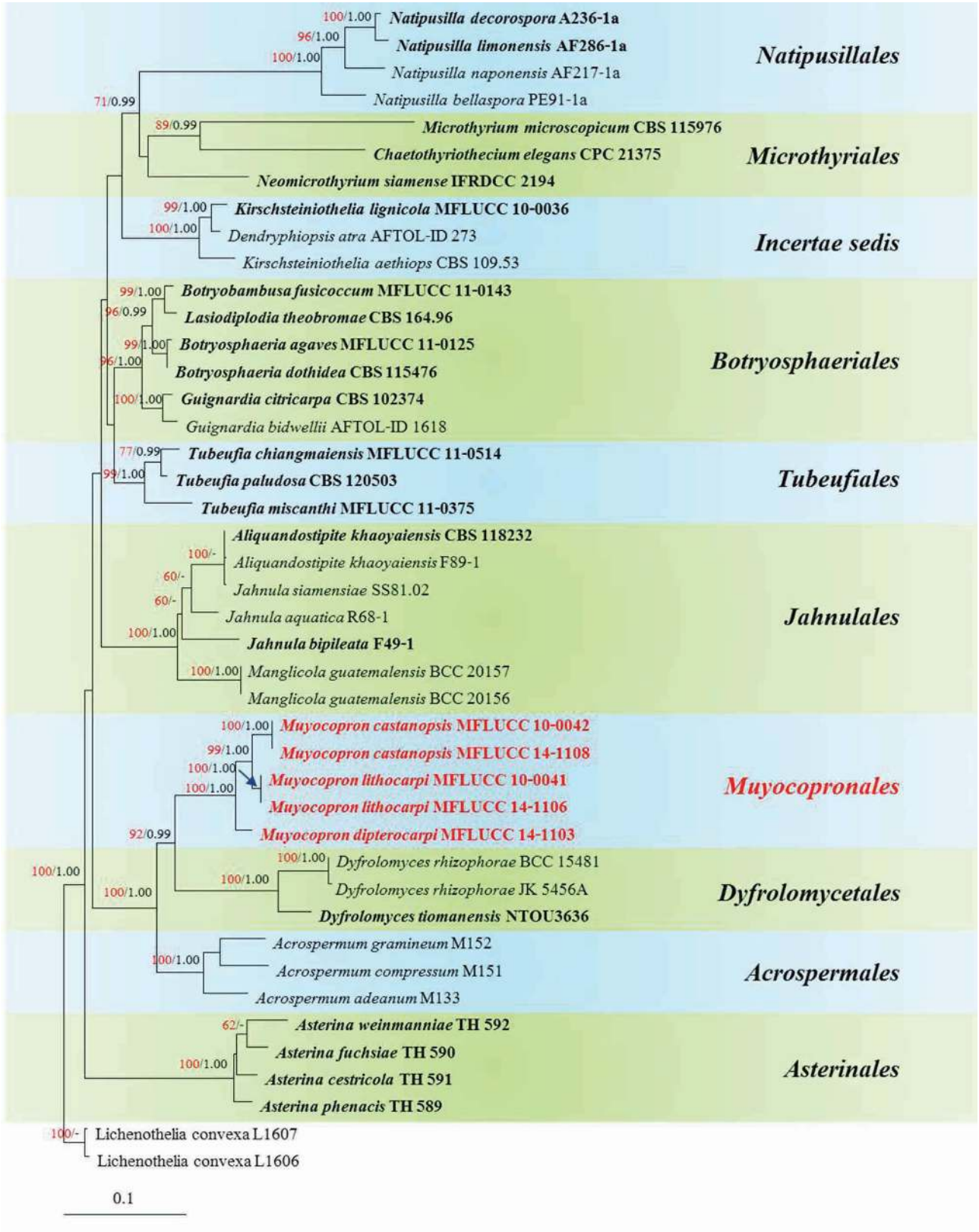

FIGURE 1. phylogram generated from RAxML based on combined LSU and SSU sequence data to establish the new order Muyocopronales. Bootstrap support values for maximum likelihood (ML, red) greater than 60% are given above the nodes and Bayesian posterior probabilities (PP, black) equal to or greater than 0.95 are indicated above the nodes. The ex-type and reference specimens are in bold.

Taxonomy

Muyocopronales Mapook, Boonmee & K.D. Hyde, *ord. nov.*
Index Fungorum number: IF551615, *Faces of fungi number*: FoF 01886
Type family: Muyocopronaceae (see Hyde *et al*. 2013 for description)

Saprobic, common on the surface of dried twigs, less common on leaves. Sexual morph: *Ascomata* superficial, coriaceous, appearing as circular, flattened, brown to dark brown spots covering the host, without a subiculum, with a poorly developed basal layer. *Ostiole* central without setose or hairy appendages. *Peridium* mostly comprising pseudoparenchymatous cells of *textura angularis*. *Hamathecium* comprising filiform, septate, branched, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate, saccate or broadly obpyriform, sometime oval to obovoid, with or without an ocular chamber. *Ascospores* multi-seriate to irregularly arranged, hyaline, oval to obovoid with obtuse ends, aseptate with or without granular appearance. Asexual morph: Undetermined.

Notes:—Muyocopronales forms a distinct order in the clade comprising Acrospermales and Dyfrolomycetales (Fig. 1). The three orders differ as follows: Acrospermales (type *Acrospermum*) has club-shaped, coriaceous ascomata, with narrow cylindrical asci, with the longest asci more than 1,000 µm, and filiform, multi-septate ascospores (Riddle 1920, Minter *et al*. 2007, Hyde *et al*. 2013); Dyfrolomycetales (type *Dyfrolomyces*) has immersed, coriaceous ascomata, with uniseriate, broadly fusiform multi-septate ascospores (Pang *et al*. 2013, Hyde *et al*. 2013). Muyocopronales (type *Muyocopron*) has superficial, flattened, carbonaceous, brittle ascomata, pseudoparaphyses that are longer than the asci and ellipsoidal to ovate, unicellular ascospores (Hyde *et al*. 2013).

Muyocopron Speg., Anales de la Sociedad Cientifica Argentina. 12: 113 (1881)
Faces of fungi number: FoF 01887
Possible synonyms (from Index Fungorum 2016)

Saprobic on the surface of dried leaves or twigs. Sexual morph: *Ascomata* superficial, solitary or scattered, coriaceous, appearing as circular, scattered, flattened, brown to dark brown spots covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiole* central. *Peridium* 10–40 µm wide, outer layer comprising dark-brown to black pseudoparenchymatous cells of *textura angularis*, inner layer comprising light brown cells of *textura angularis*. *Hamathecium* comprising 1.5–3 µm wide, cylindrical to filiform, septate pseudoparaphyses. *Asci* 8-spored, bitunicate, saccate or broadly obpyriform, pedicellate, straight or slightly curved, with small ocular chamber. *Ascospores* irregularly arranged, hyaline, oval to obovoid with obtuse ends, aseptate, with granular appearance. Asexual morph: Undetermined.

Type species:—*Muyocopron corrientinum* Speg., Anales de la Sociedad Científica Argentina 12(3): 113 (1881). See Wu *et al*. (2011b) for description and illustration.

Muyocopron castanopsis Mapook, Boonmee & K.D. Hyde, *sp. nov.*
Index Fungorum number: IF551616, *Facesoffungi number*: FoF 01888; Fig. 2
Etymology: Named after the host genus *Castanopsis*.
Holotype: MFLU 15–1131

Saprobic on dried twigs of *Castanopsis indica* (Roxb.) Rehder. Sexual morph: *Ascomata* (140–)170–250 µm high × 450–750 µm diam. ($\bar{x}$ = 196.5 × 562 µm, n = 5), superficial, solitary or scattered, coriaceous, appearing as circular, scattered, flattened, brown to dark brown spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiole* central. *Peridium* 25–35 µm wide, widest at the sides, outer layer comprising dark brown to black, pseudoparenchymatous, occluded cells of *textura angularis*, inner layer comprising light brown cells of *textura angularis*. *Hamathecium* comprising 1.5–2.5 µm wide, cylindrical to filiform, septate, pseudoparaphyses. *Asci* (85–)95–110 × 23–24(–28) µm ($\bar{x}$ = 98 × 24 µm, n = 10), 8-spored, bitunicate, saccate or broadly obpyriform to ovoid, pedicellate, straight or slightly curved, with an ocular chamber. *Ascospores* 20–26 × 10–13 µm ($\bar{x}$ = 23 × 11.5 µm, n = 20), overlapping 2–4 seriate, hyaline, ellipsoid to obovoid, with obtuse ends, aseptate, with granular appearance. Asexual morph: Undetermined.

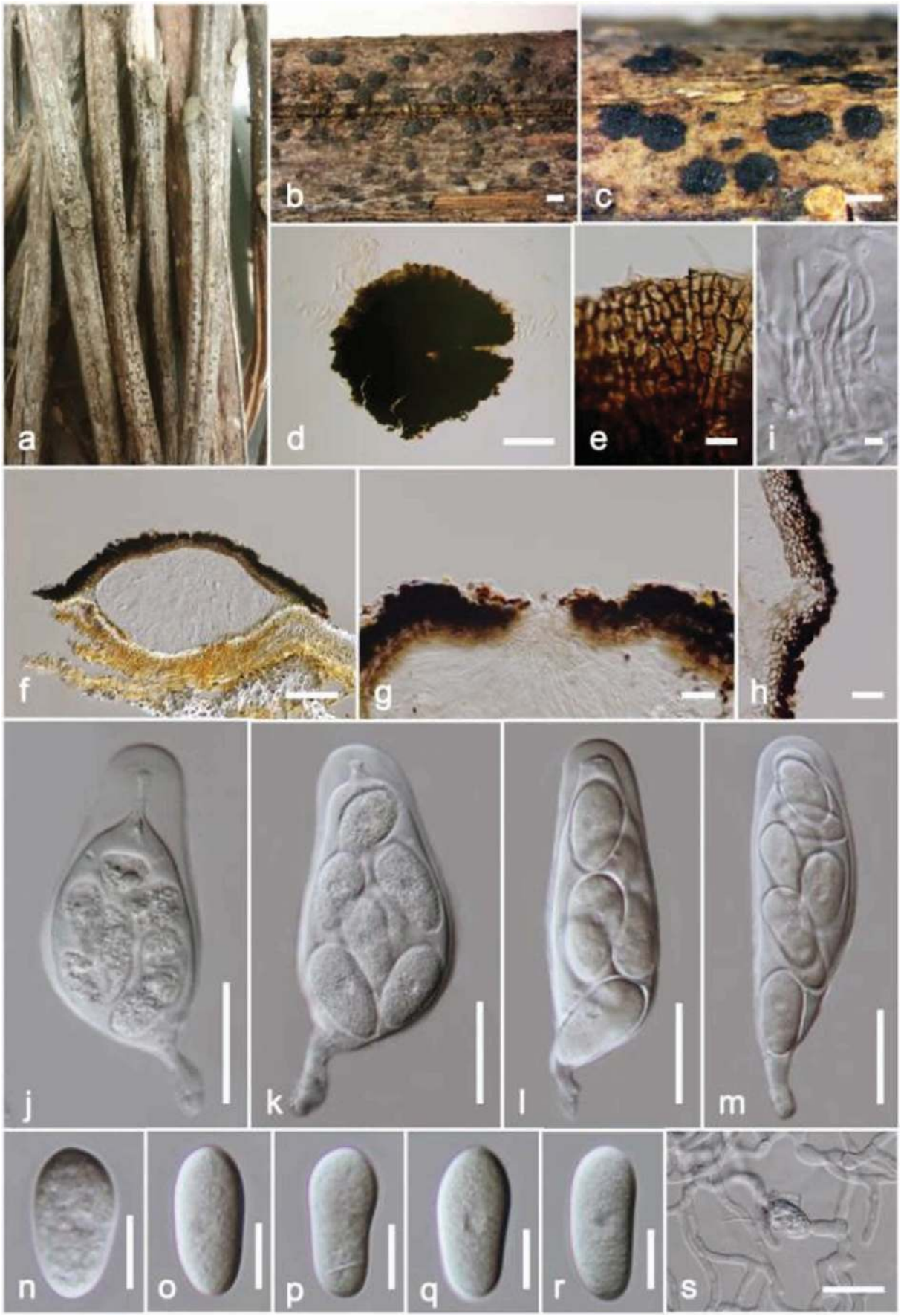

FIGURE 2. *Muyocopron castanopsis* (holotype). **a–c**. Superficial ascomata on substrate. **d–e**. Squash mounts showing ascomata walls. **f**. Section of ascoma. **g**. Ostiole **h**. Peridium. **i**. Pseudoparaphyses. **j–m**. Asci. **n–r**. Unicellular ascospores. **s**. Germination of ascospore. Scale bars: b,c = 500 µm, d = 200 µm, g = 100 µm, j–m = 20 µm, e, h, i, n–s = 10 µm, f = 5 µm.

Culture characteristics:—Ascospores germinating on MEA within 24 hr. at room temperature and germ tubes produced from both ends of the ascospores. Colonies on MEA reaching 0.8 cm diam after 1 week, at 25 °C. Aerial mycelium initially white, slightly raised, in old cultures grayish to light brown, flattened on surface, brown to dark brown from below, margin light brown to white.

Material examined:—THAILAND, Chiang Rai, on dried twigs of *Castanopsis indica* (Fagaceae), 22 July 2014, A. Mapook, (MFLU 15–1131, **holotype**), ex-type culture MFLUCC 14–1108, BCC; *ibid.* (HKAS92522, **isotype**); on dried twigs of *Castanopsis indica* (Fagaceae), 26 August 2014, A. Mapook (MFLU 16–0476, **paratype**)

Notes:—*Muyocopron castanopsis* is a saprobe on dried twigs of *Castanopsis indica* (Roxb.) Rehder. Our collection is most similar to *Muyocopron hongkongense* in size and shape of the ascospores, but it differs in having larger ascomata ((140–)170–250 × 450–750 µm vs. 220–440 × 92–160 µm) and longer ascospores (Table 2) and different host families (Fagaceae vs Arecaceae). Thus, we consider our collection to be a new species based on morphology.

TABLE 2. Synopsis of *Muyocopron* species with similar morphological features discussed in this study.

Name	Ascomata (µm)	Asci (µm)	Ascospore (µm)	Reference
Muyocopron castanopsis (MFLUCC 14–1108)	(140–)170–250 high × 450–750 diam.	(85–) 95–110 × 23–24(–28)	20–26 × 10–13	This study
Muyocopron corrientinum (LPS 1538, holotype)	77–108 high × 270–370 diam.	55–60×18–26	18–15.5×8.5–6	Wu *et al*. 2011b
Muyocopron corrientinum	300–400 diam.	70 × 20	13–16 × 7–8	Speg. 1881
Muyocopron dipterocarpi (MFLUCC 14–1103)	85–120 high × 225–270(–295) diam.	(45–)50–70 × (15–)20–25(–30)	(12–)15–18 × (6–)7–9(–11)	This study
Muyocopron hongkongense	220–440 high × 92–160 diam.	56–84 × 22–30	18–24 × 9.8–13	Joanne E. Taylor, K.D. Hyde & E.B.G. Jones 2003
Muyocopron lithocarpi (MFLUCC 14–1106)	40–70(–120) high × 220–320 diam.	45–65 × (15–)23–28	13–18 × 9–11	This study
Muyocopron umbilicatum	150–250 diam	50 × 30	18 × 11	Speg. 1919

Muyocopron dipterocarpi Mapook, Doilom, Boonmee & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551617, *Facesoffungi number*: FoF 01889; Fig. 3

Etymology: Named after the host genus *Dipterocarpus*, from which this species was collected.

Holotype: MFLU 15–1132

Saprobic on dried twigs of *Dipterocarpus tuberculatus* Roxb. Sexual morph: *Ascomata* 85–120 µm high × 225–270(–295) µm diam. ($\bar{x}$ = 110 × 256.5 µm, n = 5), superficial, coriaceous, solitary or scattered, appearing as circular, scattered, flattened, brown to dark brown spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiole* central. *Peridium* (11–)15–30 µm wide, widest at the sides, outer layer comprising dark brown to black pseudoparenchymatous, occluded cells of *textura angularis*, inner layer comprising light brown cells of *textura angularis*. *Hamathecium* comprising 1.5–3 µm wide, cylindrical to filiform, septate, pseudoparaphyses. *Asci* (45–)50–70 × (15–)20–25(–30) µm ($\bar{x}$ = 56.5 × 22 µm, n = 20), 8-spored, bitunicate, saccate or broadly obpyriform, pedicellate, straight or slightly curved, with small ocular chamber. *Ascospores* (12–)15–18 × (6–)7–9(–11) µm ($\bar{x}$ = 16.5 × 9 µm, n = 30), irregularly arranged, overlapping in the ascus, hyaline, oval to obovoid with obtuse ends, aseptate, with granular appearance. Asexual morph: Undetermined.

Culture characteristics:—Ascospores germinating on MEA within 24 hr. at room temperature and germ tubes produced from the ends of the ascospore. Colonies on MEA reaching 1 cm diam after 1 week, at 25 °C. Initially aerial mycelium white, slightly raised, in old cultures grayish to light brown, flattened on surface, dark to dark brown from below, light brown to white margin.

Material examined:—THAILAND, Chiang Rai, Mae Lao, on dried twigs of *Dipterocarpus tuberculatus* (Dipterocarpaceae), 21 June 2014, M. doilom (MFLU 15–1132, **holotype**), ex-type culture MFLUCC 14–1103, BCC; *ibid.* (HKAS92523, **isotype**).

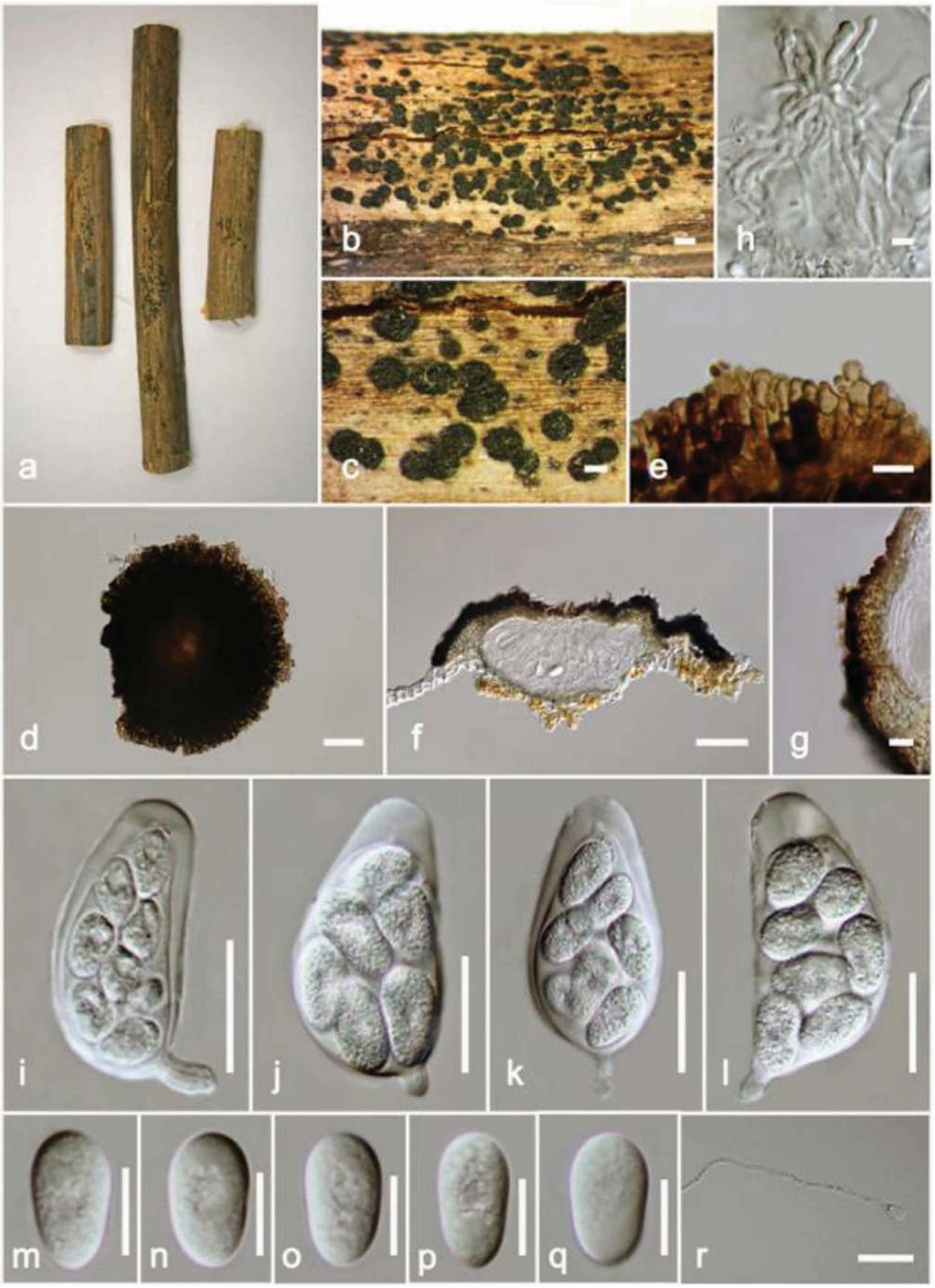

FIGURE 3. *Muyocopron dipterocarpi* (holotype) **a–c**. Superficial ascomata on substrate. **d–e**. Squash mounts showing ascomata walls. **f**. Section of ascoma. **g**. Peridium. **h**. Pseudoparaphyses. **i–l**. Asci. **m–q**. Unicellular ascospores. **r**. Germination of ascospore. Scale bars: b = 500 µm, c = 200 µm, d, f, r = 50 µm, i–l = 20 µm, e, g, m–q = 10 µm, h = 5 µm.

Notes:—*Muyocopron dipterocarpi* was collected from dried twigs of *Dipterocarpus tuberculatus* Roxb. The new species is similar to *Muyocopron corrientinum* (Spegazzini 1881), in having similar asci and ascospores (Table 2). Our collection differs from *Muyocopron corrientinum* in having wider ascospores ($\bar{x}$ = 9 vs 7.7 µm, Wu *et al*. 2011b). Furthermore, the species were associated with different host families and were collected from different continents.

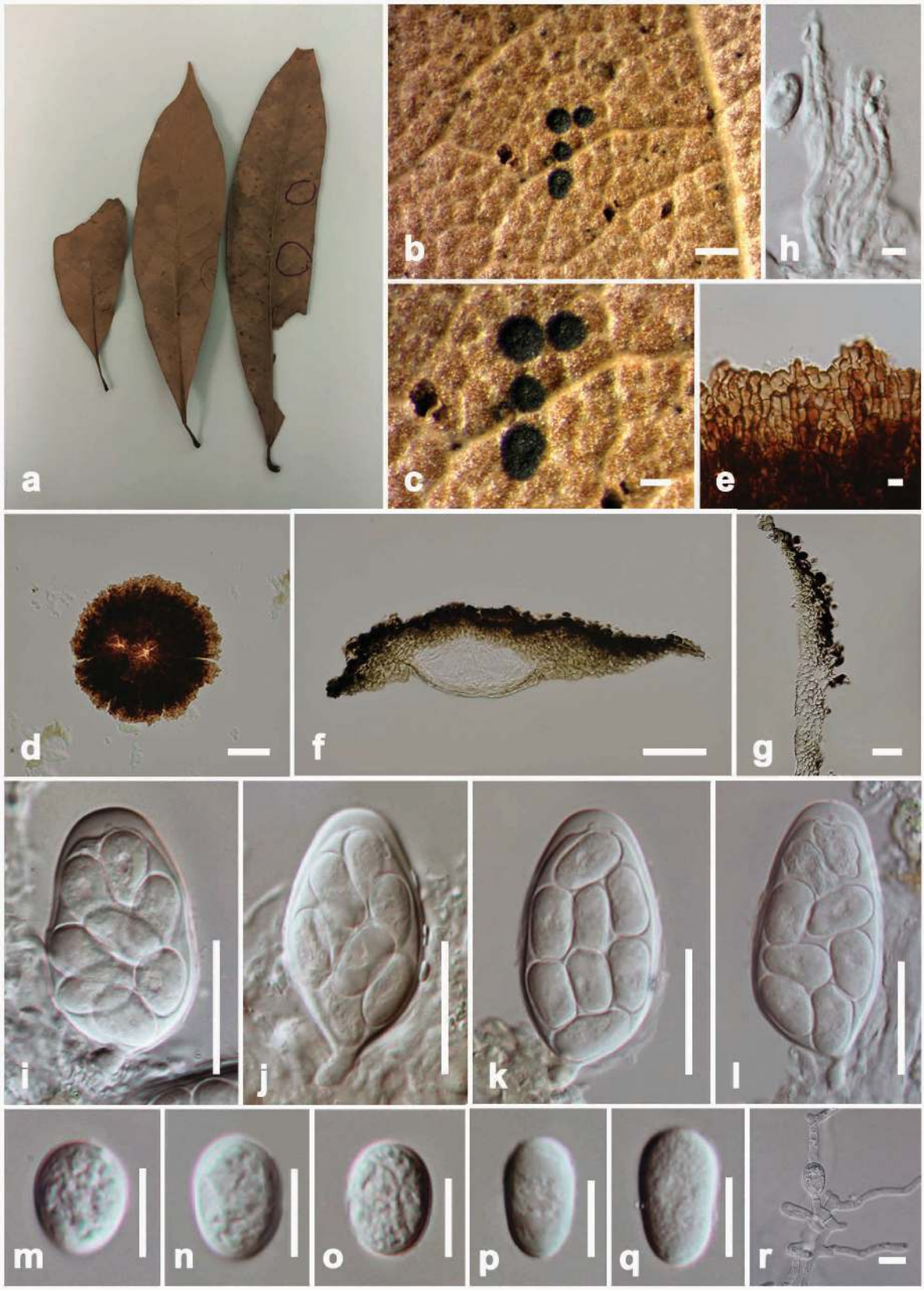

FIGURE 4. *Muyocopron lithocarpi* (holotype). **a–c**. Superficial ascomata on substrate. **d–e**. Squash mounts showing ascomata walls. **f**. Section of ascoma. **g**. Peridium. **h**. Pseudoparaphyses. **i–l**. Asci. **m–q**. Unicellular ascospores. **r**. Germinating ascospore. Scale bars: b = 500 µm, c = 200 µm, d = 100 µm, f–g = 50 µm, i–l = 20 µm, m–r = 10 µm, e, h = 5 µm.

Muyocopron lithocarpi Mapook, Boonmee & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551618, *Facesoffungi number*: FoF 01890; Fig. 4

Etymology: Named after the host genus *Lithocarpus*, from which this species was collected.

Holotype: MFLU 15–1133

Saprobic on dead leaves of *Lithocarpus lucidus* (Roxb.) Rehder. Sexual morph: *Ascomata* 40–70(–120) µm high× 220–320 µm diam. ($\bar{x}$ = 69 × 262 µm, n = 5), superficial, coriaceous, solitary or scattered, appearing as circular, scattered, flattened, brown to dark brown spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiolar* central. *Peridium* 10–20(–28) µm wide, widest at the sides, outer layer comprising dark brown to black pseudoparenchymatous, occluded cells of *textura epidermoidea*, inner layer comprising light brown cells of *textura angularis*. *Hamathecium* comprising 1.5–2.5 µm wide, cylindrical to filiform, septate, pseudoparaphyses. *Asci* 45–65 × (15–)23–28 µm ($\bar{x}$ = 54.5 × 25 µm, n = 20), 8-spored, bitunicate, saccate or broadly obpyriform, pedicellate, straight or slightly curved, with small ocular chamber. *Ascospores* 13–18 × 9–11 µm ($\bar{x}$ = 15.5 × 10 µm, n = 20), irregularly arranged, overlapping in the ascus, hyaline, oval to obovoid with obtuse ends, aseptate, with granular appearance. Asexual morph: Undetermined.

Culture characteristics:—Ascospores germinating on MEA within 24 hr. at room temperature and germ tubes produced from the ends of the ascospore. Colonies on MEA reaching 1.2 cm diam for 1 week, at 25 °C. Initially aerial mycelium white, slightly raised, in old cultures grayish to light brown, flattened on surface, brown to dark brown from below, light brown to white margin.

Material examined:—THAILAND, Chiang Rai, on fallen leaves of *Lithocarpus lucidus* (Fagaceae). 30 September 2014, A. Mapook (MFLU 15–1133, **holotype**), living culture MFLUCC 14–1106, BCC; *ibid.* (HKAS92524, **isotype**); on fallen leaves of *Lithocarpus lucidus* (Fagaceae), 30 September 2014, A. Mapook (MFLU 16-0477, **paratype**)

Notes:—*Muyocopron lithocarpi* was isolated from fallen leaves of *Lithocarpus lucidus* in Chiang Rai and the general morphology is similar to *Muyocopron umbilicatum* Speg., especially in the shape and size of asci and ascospores (Table 2) but differs in host family and continent and in the mean size of the ascospores (15.5 × 10 µm vs 18 × 11 µm). Therefore we introduce a new species based on morphology.

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