Studies in Fungi

ARTICLE

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https://doi.org/10.48130/SIF-2023-0009
Studies in Fungi **2023**, 8:9

Neotypification of *Muyocopron dipterocarpi*, a new host record on *Zanthoxylum fagara* (Rutaceae) and the potential for secondary metabolite production in Muyocopronaceae

Ausana Mapook[1] and Kevin D. Hyde[1,2,3*]

[1] *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3] *Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Haizhu District, Guangzhou 510225, China*
* Corresponding author, E-mail: kdhyde3@gmail.com

Abstract

Muyocopron is a genus with a diverse lifestyle, occurring in tropical and temperate regions and can be found on various hosts and habitats. The present study confirmed a new host record of *Muyocopron dipterocarpi* from dead twigs of *Zanthoxylum fagara* in northern Thailand, based on both morphological comparisons with multigene analyses of LSU, SSU, ITS, and TEF1 sequence data. A preliminary screening test also showed that *M. dipterocarpi* has a potential for antimicrobial activity, observable as partial inhibition, when compared with a positive control. In addition, a neotype is designated here for *Mu. dipterocarpi* due to the original material no longer existing. This will facilitate subsequent taxonomic work in stabilizing the application of a name, and to serve as a foundation for further applied research of this species.

Citation: Mapook A, Hyde KD. 2023. Neotypification of *Muyocopron dipterocarpi*, a new host record on *Zanthoxylum fagara* (Rutaceae) and the potential for secondary metabolite production in Muyocopronaceae. *Studies in Fungi* 8:9 https://doi.org/10.48130/SIF-2023-0009

Introduction

Muyocopronaceae was validly introduced by Hyde et al.[1], with a type genus *Muyocopron* Speg. The family currently comprises nine genera (*viz. Arxiella* Papendorf, *Leptodiscella* Papendorf, *Mycoleptodiscus* Ostaz., *Muyocopron* Speg., *Neocochlearomyces* Pinruan, Sommai, Suetrong, J.Z. Groenew. & Crous, *Neomycoleptodiscus* Hern.-Restr., J.D.P. Bezerra & Crous, *Paramycoleptodiscus* Crous & M.J. Wingf., *Pseudopalawania* Mapook & K.D. Hyde, and *Setoapiospora* Mapook & K.D. Hyde), based on molecular phylogeny and morphology[2−7]. Interestingly, members of Muyocopronaceae have been reported to have the potential for antimicrobial and cytotoxic activities[7−10]. *Pseudopalawania siamensis* polyketide-derived secondary metabolite produced with the potential of antimicrobial and cytotoxic activities. This comprised a new heterodimeric bistetrahydroxanthone, namely pseudopalawanone[7]. An endophytic fungus, *Muyocopron laterale* (ECN279), which was isolated from a healthy leaf of *Canavalia lineata* (Fabaceae), produced two new azaphilones, namely muyocopronones A and B with weak antibacterial activity[10]. An endophytic species, *Mycoleptodiscus indicus*, which is currently named *Muyocopron sahnii*[3], produced a new triterpenoid[9] and three new azaphilones, namely mycoleptones A, B, and C with four known polyketides. All compounds were weakly active when tested in antileishmanial and cytotoxicity assays[8]. However, the study of secondary metabolite production from Muyocopronaceae is still in the initial stages.

Muyocopron is a diverse genus which can be found in tropical and temperate regions, occurring as saprobes and pathogens on various plant parts such as dead aerial twigs, branches, stems and leaves and can also be an opportunistic pathogen on humans and animals[3,11−13]. A mycoleptodiscus-like asexual morph has been described for the genus with several additional species based on molecular analyses[3]. The asexual morphs are characterized by irregular sporodochium-like conidiomata, globose or broadly ellipsoidal to ampulliform with enteroblastic, monophialidic conidiogenous cells, and fusiform or fusoid-ellipsoid, curved, hyaline, aseptate or septate conidia with terminal and/or lateral appendages, with or without dark brown appressoria[3]. Presently, 68 species epithets are listed in Index Fungorum[14] with 56 probable species listed in Species Fungorum[15].

In this study, we provide morphological descriptions and illustrations of a new collection of *Mu. dipterocarpi* from *Zanthoxylum fagara*, in northern Thailand. The identification was confirmed using both multigene analyses and morphological comparisons. The results of preliminary screening for antimicrobial activity is also provided. The lifestyle and function with the potential for secondary metabolites production are also discussed. In addition, a neotype is designated here for *Mu. dipterocarpi* due to the original material no longer existing.

Materials and methods

Collection, examination, and isolation of fungi

Fresh material was collected from Phrae Province (India) in September 2016. The micromorphology was examined following the methodology as described by Mapook et al.[6]. Single spore isolation and culture morphology were obtained following the methods of Senanayake et al.[16]. Germinated

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spores were observed on MEA media (malt extract agar: 33.6 g/l sterile distilled water, Difco malt extract) within 24 h and transferred to new MEA media and incubated at room temperature (25 °C) in the dark. Pure cultures were used for molecular study and pre-screening tests for antimicrobial activity. The specimens with collection details and living cultures are deposited in the Herbarium of Mae Fah Luang University (Herb. MFLU) and Culture collection Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand.

Preliminary screening of antimicrobial activity

Preliminary screening of antimicrobial activity was carried out following Mapook et al.[6]. Antibacterial discs of penicillin and ciprofloxacin, with antifungal discs of nystatin were used as positive control for screening[17]. Gram-positive bacteria (*Bacillus subtilis*, DSM10), Gram-negative bacteria (*Escherichia coli*, DSM498), and filamentous fungus (*Mucor plumbeus*, MUCL 49355) were determined by the zone of inhibition using agar plug diffusion method, compared with positive control[18].

DNA extraction, PCR amplification and sequencing

DNA extraction, PCR amplification and sequencing were carried out following the methodology as described by Mapook et al.[6]. The partial large subunit nuclear rDNA (LSU) was amplified with primer pairs LROR and LR5[19]. The partial small subunit nuclear rDNA (SSU) was amplified with primer pairs NS1 and NS4[20]. The internal transcribed spacer (ITS) was amplified by using primer pairs ITS5 and ITS4[20]. The translation elongation factor 1-α (TEF1) was amplified by using primers EF1-983F and EF1-2218R[21]. The PCR products were sent for sequencing at SeqLab GmbH (Microsynth AG), Göttingen, Germany.

Phylogenetic analysis

Phylogenetic analysis was carried out following Mapook et al.[6] and Dissanayake et al.[22]. The closest relative strains were selected following Mapook et al.[6,7]. The combined aligned dataset was analyzed based on maximum likelihood (ML) and Bayesian inference (BI) *via* the CIPRES Science Gateway platform (V. 3.3, www.phylo.org)[23]. ML analysis was performed by RAxML-HPC2 on XSEDE (8.2.12) tool[24,25] using the GTR+I+G model of nucleotide substitution with 1,000 rapid bootstrap replicates. BI analysis was performed by using MrBayes on XSEDE (3.2.7a) tool with the GTR+I+G model. Six simultaneous Markov chains were performed for 5,000,000 generations, and trees were sampled every 1000th generation. Phylogenetic trees were drawn using FigTree 1.4.0[26] and edited by Microsoft Office PowerPoint 365 and Adobe Photoshop CS6. The sequences used for analyses with accession numbers are given in Table 1.

Results

Phylogenetic analyses

Seventy-seven strains are included in the combined dataset of LSU, SSU, ITS, and TEF1 sequence data, including our strain (MFLUCC 17-1464), which comprise 3969 characters with gaps. Tree topology of the ML analysis was similar to the BYPP. A best scoring RAxML tree with a final likelihood value of −25,539.739638 is presented in Fig. 1. The matrix had 1738 distinct alignment patterns, with 49.64% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.237294, C = 0.253374, G = 0.287766, T = 0.221566; substitution rates: AC = 1.187676, AG = 2.326266, AT = 1.446900, CG = 1.082572, CT = 5.256850, GT = 1.000000;

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

Taxa	Strain no.[1]	GenBank accession numbers[2]			
		LSU	SSU	ITS	TEF1
Acrospermum adeanum	M133	EU940104	EU940031	EU940180	–
Acrospermum compressum	M151	EU940084	EU940012	EU940161	–
Acrospermum gramineum	M152	EU940085	EU940013	EU940162	–
Arxiella dolichandrae	CBS 138853^T	KP004477	–	KP004449	–
Arxiella terrestris	CBS 268.65^T	MH870201	–	MH858565	–
Dyfrolomyces phetchaburiensis	MFLUCC 15-0951^T	MF615402	MF615403	–	–
Dyfrolomyces rhizophorae	BCC15481	–	KF160009	–	–
Dyfrolomyces rhizophorae	JK 5456A	GU479799	–	–	GU479860
Dyfrolomyces thailandica	MFLU 16-1173^T	KX611366	KX611367	–	–
Dyfrolomyces thamplaensis	MFLUCC 15-0635^T	KX925435	KX925436	–	KY814763
Dyfrolomyces tiomanensis	NTOU3636^T	KC692156	KC692155	–	KC692157
Leptodiscella africana	CBS 400.65^T	MH870275	–	MH858635	–
Leptodiscella brevicatenata	FMR 10885^T	FR821311	–	FR821312	–
Leptodiscella chlamydospora	MUCL 28859	FN869567	–	FR745398	–
Leptodiscella rintelii	CBS 144927^T	LR025181	–	LR025180	–
Lophium mytilinum	AFTOL-ID 1609	DQ678081	DQ678030	–	DQ677926
Melomastia maolanensis	GZCC 16-0102^T	KY111905	KY111906	–	KY814762
Muyocopron alcornii	BRIP 43897^T	MK487708	–	MK487735	MK495956
Muyocopron atromaculans	MUCL 34983^T	MK487709	–	MK487736	MK495957
Muyocopron castanopsis	MFLUCC 10-0042	–	JQ036225	–	–
Muyocopron castanopsis	MFLUCC 14-1108^T	KU726965	KU726968	MT137784	MT136753
Muyocopron chromolaenae	MFLUCC 17-1513^T	MT137876	MT137881	MT137777	MT136756
Muyocopron chromolaenicola	MFLUCC 17-1470^T	MT137877	MT137882	MT137778	MT136757
Muyocopron coloratum	CBS 720.95^T	MK487710	–	NR_160197	MK495958

(to be continued)

Table 1. (continued)

Taxa	Strain no.[1]	GenBank accession numbers[2]			
		LSU	SSU	ITS	TEF1
Muyocopron dipterocarpi	MFLU 18-2582	–	MW079363	MW063196	–
Muyocopron dipterocarpi	MFLUCC 14-1103^T	KU726966	KU726969	MT137785	MT136754
Muyocopron dipterocarpi	MFLUCC 17-0075	MH986833	MH986829	MH986837	–
Muyocopron dipterocarpi	MFLUCC 17-0354	MH986834	MH986830	MH986838	–
Muyocopron dipterocarpi	MFLUCC 17-0356	MH986835	MH986831	MH986839	–
Muyocopron dipterocarpi	**MFLUCC 17-1464^NT**	**OQ861270**	**OQ861267**	**OQ832759**	**OQ856779**
Muyocopron dipterocarpi	MFLUCC 18-0470	MK348001	MK347890	MK347783	–
Muyocopron garethjonesii	MFLU 16-2664^T	KY070274	KY070275	–	–
Muyocopron geniculatum	CBS 721.95^T	MK487711	–	MK487737	MK495959
Muyocopron heveae	MFLUCC 17-0066^T	MH986832	MH986828	MH986836	–
Muyocopron laterale	CBS 127677	MK487718	–	MK487744	MK495965
Muyocopron laterale	CBS 141029^T	MK487712	–	MK487738	MK495960
Muyocopron laterale	CBS 141033	MK487715	–	MK487741	MK495963
Muyocopron laterale	CBS 145309	MK487722	–	MK487748	MK495969
Muyocopron laterale	CBS 145310	MK487719	–	MK487745	MK495966
Muyocopron laterale	CBS 145311	MK487724	–	MK487750	–
Muyocopron laterale	CBS 145312	MK487725	–	MK487751	MK495971
Muyocopron laterale	CBS 145313	MK487721	–	MK487747	MK495968
Muyocopron laterale	CBS 145314	MK487723	–	MK487749	MK495970
Muyocopron laterale	CBS 145315	MK487720	–	MK487746	MK495967
Muyocopron laterale	CBS 145316	MK487726	–	MK487752	MK495972
Muyocopron laterale	CBS 719.95	MK487714	–	MK487740	MK495962
Muyocopron laterale	FMR 13797	MK874616	–	MK874615	MK875803
Muyocopron laterale	IMI 324533	MK487713	–	MK487739	MK495961
Muyocopron laterale	URM 7801	MK487717	–	MK487743	–
Muyocopron laterale	URM 7802	MK487716	–	MK487742	MK495964
Muyocopron lithocarpi	–	MK447738	MK447740	–	–
Muyocopron lithocarpi	MFLU 18-2087	MK347930	MK347821	MK347716	–
Muyocopron lithocarpi	MFLU 18-2088	MK347931	MK347822	MK347717	–
Muyocopron lithocarpi	MFLUCC 10-0041	JQ036230	JQ036226	–	–
Muyocopron lithocarpi	MFLUCC 14-1106^T	KU726967	KU726970	MT137786	MT136755
Muyocopron lithocarpi	MFLUCC 16-0962	MK348034	MK347923	–	–
Muyocopron lithocarpi	MFLUCC 17-1465	MT137878	MT137883	MT137779	MT136758
Muyocopron lithocarpi	MFLUCC 17-1466	MT137879	MT137884	MT137780	MT136759
Muyocopron lithocarpi	MFLUCC 17-1500	MT137880	MT137885	MT137781	MT136760
Muyocopron zamiae	CBS 203.71^T	MK487727	–	–	MK495973
Mycoleptodiscus endophytica	MFLUCC 17-0545^T	MG646946	MG646978	MG646961	MG646985
Mycoleptodiscus suttonii	CBS 141030	MK487729	–	–	MK495975
Mycoleptodiscus suttonii	CBS 276.72^T	MK487728	–	MK487753	MK495974
Mycoleptodiscus terrestris	CBS 231.53^T	MK487730	–	MK487754	MK495976
Mycoleptodiscus terrestris	IMI 159038	MK487731	–	MK487755	MK495977
Mytilinidion rhenanum	CBS 135.34	FJ161175	FJ161136	–	FJ161092
Neocochlearomyces chromolaenae	BCC 68250^T	MK047514	MK047552	MK047464	MK047573
Neocochlearomyces chromolaenae	BCC 68251	MK047515	MK047553	MK047465	MK047574
Neocochlearomyces chromolaenae	BCC 68252	MK047516	MK047554	MK047466	MK047575
Neomycoleptodiscus venezuelense	CBS 100519^T	MK487732	–	MK487756	MK495978
Palawania thailandensis	MFLU 16-1871	KY086494	–	MT137788	–
Palawania thailandensis	MFLUCC 14-1121^T	KY086493	KY086495	MT137787	–
Paramycoleptodiscus albizziae	CBS 141320	KX228330	–	KX228279	MK495979
Paramycoleptodiscus albizziae	CPC 27552^T	MH878220	–	–	–
Pseudopalawania siamensis	MFLUCC 17-1476a^T	–	MT137789	MT137782	MT136752
Pseudopalawania siamensis	MFLUCC 17-1476b	–	MT137790	MT137783	–
Setoapiospora thailandica	MFLUCC 17-1426^T	MN638847	MN638851	MN638862	MN648731

[1] AFTOL-ID: Assembling the Fungal Tree of Life; BCC: BIOTEC Culture Collection; BRIP: Biosecurity Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, the Netherlands; FMR: Facultad de Medicina, Reus, Tarragona, Spain; GZCC: Guizhou Culture Collection; IMI: The International Mycological Institute Culture Collections; JK: J. Kohlmeyer; MFLU: the Herbarium of Mae Fah Luang University; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL: Belgian Coordinated Collections of Microorganisms; URM: Universidade Federal de Pernambuco; ^T: ex-type isolates; ^NT: Neotype.
[2] LSU: 28S large subunit of the nrRNA gene; SSU: 18S small subunit of the nrRNA gene; ITS: internal transcribed spacer regions 1 and 2 including 5.8S nrRNA gene; TEF1: partial translation elongation factor 1-α gene.

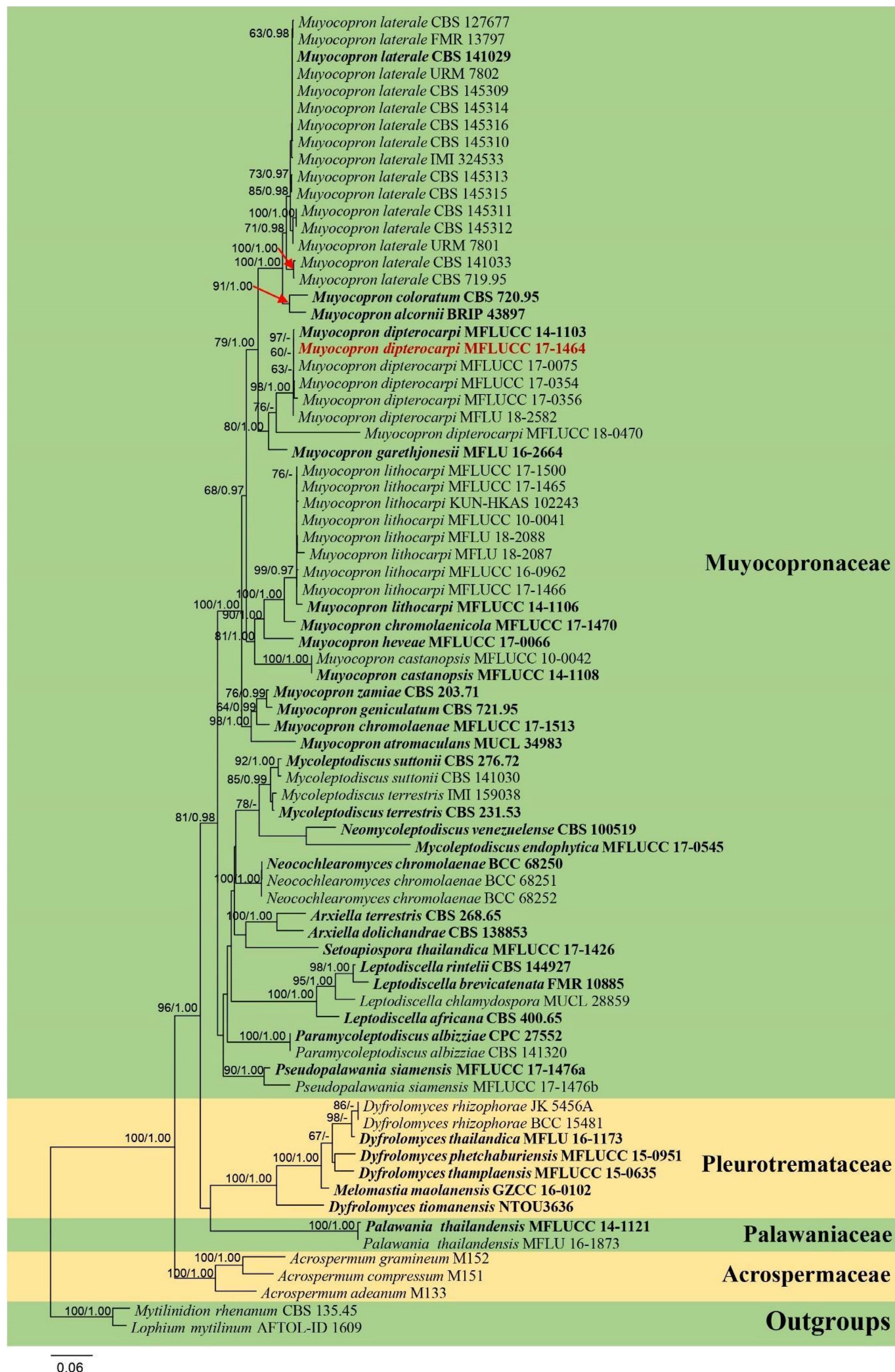

Fig. 1 Phylogram generated from maximum likelihood analysis based on combined dataset of LSU, SSU, ITS, and TEF1 sequence data. Bootstrap support values for maximum likelihood (ML) equal to or greater than 60% and Bayesian posterior probabilities (BYPP) equal to or greater than 0.97 are given above the nodes. Newly generated sequences are in dark red bold and ex-type isolates are in bold. *Lophium mytilinum* (AFTOL-ID 1609) and *Mytilinidion rhenanum* (CBS 135.45) are used as outgroup taxa.

gamma distribution shape parameter α = 0.309869. The phylogram generated from ML analysis (Fig. 1) shows that our strain grouped within *Muyocopron dipterocarpi* clade. In a BLASTn search of NCBI GenBank, the closest match of the LSU, ITS, and TEF1 sequence of MFLUCC 17-1464 is *Mu. dipterocarpi* with 100% similarity to the strain MFLUCC 14-1103 (ex-holotype), while the closest match of the SSU sequence was identical with 99.90% similarity to *Mu. dipterocarpi* (strain MFLUCC 14-1103).

Taxonomy

Muyocopron dipterocarpi Mapook, Doilom, Boonmee & K.D. Hyde, Phytotaxa 265(3): 232 (2016)

Index Fungorum number: IF551617, *Facesoffungi number*: FoF 01889; Fig. 2

Saprobic on dead twigs of *Zanthoxylum fagara* (L.) Sarg. **Sexual morph**: *Ascomata* (65–)80–110 μm high × (230–)340–395 μm diam. ($\bar{x}$ = 92 × 332 μm, n = 10), 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* 15–25 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 1–1.5 μm wide, cylindrical to filiform, septate, pseudoparaphyses. *Asci* 55–75 × 20–30 μm ($\bar{x}$ = 64 × 23.5 μm, n = 15), 8-spored, bitunicate, saccate or broadly obpyriform, pedicellate, straight or slightly curved, with a small ocular chamber. *Ascospores* 14–20 × 8.5–10 μm ($\bar{x}$ = 17 × 9.5 μm, n = 25), irregularly arranged, overlapping in the ascus, hyaline, oval or ellipsoid 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 irregular, initially aerial mycelium white, slightly raised, filiform, becoming light brown from the center, flattened on surface, pale brown to light brown in reverse from the center of the colony with white margin.

Pre-screening for antimicrobial activity: *Muyocopron dipterocarpi* (MFLUCC 14-1103, ex-holotype) showed antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*

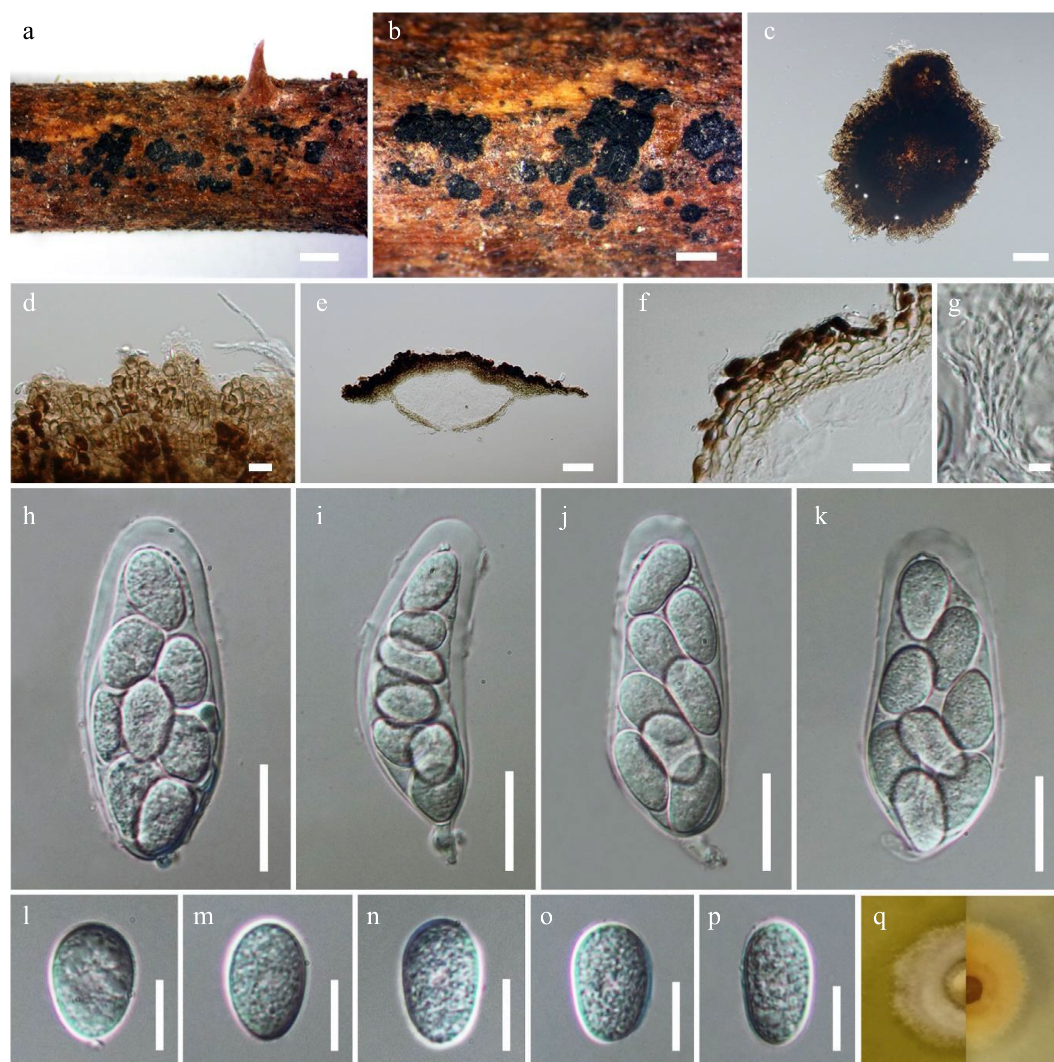

Fig. 2 *Muyocopron dipterocarpi* (neotype). (a), (b) Superficial ascomata on substrate. (c), (d) Squash mounts showing ascomata walls. (e) Section of ascoma. (f) Peridium. (g) Pseudoparaphyses. (h)–(k). Asci. (l)–(p) Unicellular ascospores. (q) Culture characteristic on MEA. Scale bars: a = 1,000 μm, b = 500 μm, c = 100 μm, e = 50 μm, f, h–k = 20 μm, d, l–p = 10 μm, g = 5 μm.

(11 and 10 mm inhibition zone, respectively), observable as partial inhibition, when compared with the positive control (26 and 9 mm, respectively), but no inhibition of *Mucor plumbeus*; *Mu. dipterocarpi* (MFLUCC 17-1464) showed antimicrobial activity against *M. plumbeus*, *B. subtilis* and *E. coli* (11, 8, and 10 mm inhibition zone, respectively), observable as partial inhibition, when compared with the positive control (17, 26 , and 9 mm, respectively).

Known hosts and distribution: On dried twigs of *Dipterocarpus tuberculatus* (Dipterocarpaceae) in Chiang Rai Province, Thailand[12]; on dried twig of *Hevea brasiliensis* (Euphorbiaceae) in Phayao Province, Thailand[27]; on dead twigs of *Mangifera indica* (Anacardiaceae) in Sukhothai Province, Thailand[28]; on decaying pod septum of *Delonix regia* (Fabaceae) in Phrae Province, Thailand[29]; on dead leaves and decaying twig of *Celtis formosana* (Cannabaceae) in Taiwan[30,31].

Material examined: THAILAND, Phrae Province, on dead twigs of *Zanthoxylum fagara* (Rutaceae), 22 September 2016, A. Mapook, (DPKP1, MFLU 23-0072, **neotype designated here**), ex-neotype culture MFLUCC 17-1464.

Note: We identify our isolate (MFLUCC 17-1464) as *Mu. dipterocarpi*, based on phylogenetic analyses, together with morphological comparison. However, our isolate was found on different host families as compared with other previously reported strains. Therefore, the isolate is introduced here as a new host record on *Zanthoxylum fagara* from Thailand.

During this study, we tried to examine the original collection of this species. Unfortunately, the type material of *Mu. dipterocarpi* could not be located in the MFLU fungarium, where the holotype specimen was deposited with a code number MFLU 15–1132[12]. A neotype for *Mu. dipterocarpi* is, therefore, needed to facilitate subsequent taxonomic work in stabilizing the application of name, and to serve as a foundation for further applied research of this species. Although the species have been reported from various hosts and is mostly distributed in Asia, especially in Thailand[6,12,27–29], there are no collections from the same locality as indicated in the protologue. Neotypes from different locations may also be considered as long as the author are confident that they are conspecific[32]. Therefore, a neotype specimen is designated here for *Mu. dipterocarpi* due to the original material no longer existing, and in accordance with Art. 9.16[33]. This specimen is recent and well-preserved, and has typical morphology suitable to the description given in the protologue, completed with illustrations, molecular data and additional evidence of a potential for antimicrobial activity based on a preliminary screening.

Discussion

Muyocopron species can be saprobic, endophytic, or pathogenic on various hosts with an ability to produce specialized infection structures such as appressoria from germinating ascospores and therefore probably has an endophytic lifestyle[3,27,34]. *Muyocopron dipterocarpi* is mostly reported from northern Thailand and probably not specific to the host, due to the species have been reported on a variety of plant families such as Anacardiaceae, Dipterocarpaceae, Euphorbiaceae, and Fabaceae[6,12,27–29]. The species have not been reported to form any specialized infection structures from the other hosts, as well as our strain in this study except the first isolate from a dried twig of *Hevea brasiliensis* (Euphorbiaceae), which was assumed to have endophytic lifestyle[12,27–31,34,35]. This suggests that *Muyocopron* species may have the ability to be endophytic or pathogenic and probably not specific to any hosts due to the genus reported on various hosts. Although fungi have the ability to change their lifestyle which can be exhibited in more than one lifestyle in a different host, the mechanism of appressoria production and the ecological lifestyle of *Muyocopron* is not well understood. In addition, *Mu. dipterocarpi* also has potential for antimicrobial activity against the tested organism based on a preliminary screening in this study.

Acknowledgments

Ausana Mapook would like to thank the Mae Fah Luang University Fund (Grant No. 651A16029), entitled 'Taxonomy, phylogeny, risk assessment, and potential impact of fungi on Siam weed in northern Thailand'. Kevin D. Hyde thanks the Basic Research Fund 2022 (Grant No. 652A01001), entitled 'Studies of fungi associated with Asteraceae and the discovery of biological properties', Princess Srinagarindra's Centenary Celebrations Foundation (Grant No. 64316001), and National Research Council of Thailand (NRCT) grant, entitled 'Total fungal diversity in a given forest area with implications towards species numbers, chemical diversity and biotechnology' (Grant No. N42A650547).

Conflict of interest

Kevin D. Hyde is the Editorial Board members of Journal *Studies in Fungi*. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and his research groups.

Dates

Received 26 January 2023; Accepted 9 April 2023; Published online 27 April 2023

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