



New host records for three saprobic Dothideomycetes in Thailand

Huanraluek N¹, Jayawardena RS^{1,2*}, Thambugala KM³ and Tian Q¹

¹Center of Excellence in Fungal Research, Mae Fah Lunag University, Chiang Rai 57100, Thailand

²School of Science, Mae Fah Lunag University, Chiang Rai 57100, Thailand

³Genetics and Molecular Biology Unit, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

Huanraluek N, Jayawardena RS, Thambugala KM, Tian Q 2020 – New host records for three saprobic Dothideomycetes in Thailand. Asian Journal of Mycology 3(1), 345–361, Doi 10.5943/ajom/3/1/9

Abstract

Three dothideomycetous saprobic species, *Clavatispora thailandica*, *Muyocopron dipterocarpi* and *Rhytidhysterion neorufulum* were collected from dead twigs in Thailand. Multigene phylogenetic analyses confirmed their taxonomic placement. *Clavatispora thailandica* and *Rhytidhysterion neorufulum* are reported on *Hevea brasiliensis* (rubber), while *Muyocopron dipterocarpi* is described from *Mangifera indica* (mango) in Thailand for the first time. Newly collected species are compared with other similar species and comprehensive descriptions and micrographs are provided.

Key words – *Clavatispora* – morphology – *Muyocopron* – phylogeny – *Rhytidhysterion*

Introduction

Plant saprobic fungi are specifically adapted to inhabit and utilize dead host plant tissues, and they play a vital role in decomposition, especially as they may produce various wood-decaying enzymes as only a limited group of fungi possess enzymatic capabilities to digest wood. However, some aquatic fungi also produce a rich array of enzymes that are able to degrade the major leaf polysaccharides and some can decay lignin and cause root rot. (Wong et al. 1998, Pointing 2001, Bucher et al. 2004, Cai et al. 2006, Osono 2006). Species of Dothideomycetes often occur as saprobes, mostly on leaves, stems or woods of dicotyledonous plants. Many species are plant pathogens and occur on a wide range of host plants worldwide, they can also be endophytes, epiphytes, fungicolous, lichenized, or lichenicolous fungi. (Zhang et al. 2011, Hyde et al. 2013). Some species can be found on several hosts in different habitats (Hyde et al. 2013, Phillips et al. 2013, Phookamsak et al. 2014, Thambugala et al. 2017a, b). During a survey of saprobic Dothideomycetes in Thailand, we found three dothideomycetous species associated with mango and rubber plants. The current paper presents three new host records from Thailand.

Mango and rubber are agriculturally and economically important plants widespread in tropical and subtropical areas (Jedele et al. 2003). Mango (*Mangifera indica* L., Anacardiaceae) is native to South Asia, particularly eastern India, Myanmar and Andaman Islands. These trees are distributed throughout the tropics and approximately 50% of all tropical fruits produced worldwide are mangoes (Morton 1987, Jedele et al. 2003). The rubber plant (*Hevea brasiliensis* Müll. Arg., Euphorbiaceae) is economically important as the milky latex extracted from this tree is the primary

source of natural rubber, which is an important raw material with many industrial uses (Ko et al. 2003).

The aim of this paper is to describe some poorly known species, which have been newly collected in Thailand. The descriptions and species identifications are based on morphological characters and DNA sequence data.

Materials & Methods

Sample collection, fungal isolation, and morphological study

Dead twigs of *Hevea brasiliensis* were collected from Chiang Rai and dead twigs of *Mangifera indica* were collected from Sukhothai provinces, Thailand. Fungi were isolated by single spore isolation method following Phookamsak et al. (2014). Colony characteristics of the cultures on 2% malt extract agar (MEA), were observed following growth at room temperature (25°C). Morphological characters and photomicrographs were recorded using material mounted in water following the methods of Thambugala et al. (2015), Mapook et al. (2016) and Phukhamsakda et al. (2016). Digital images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. Derived isolates were deposited in Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in International Collection of Microorganisms from Plants (ICMP), New Zealand. Dried specimens were deposited in the Herbarium of Mae Fah Luang University (MFLU), Thailand. Facesoffungi numbers and Index Fungorum numbers were obtained following Jayasiri et al. (2015) and Index Fungorum (2020).

DNA extraction, PCR amplification and sequencing

DNA extraction was carried out with an extraction kit (Biospin Fungus Genomic DNA Extraction Kit, BioFlux®, China) using fresh mycelia grown on PDA following the manufacturer's instructions (Hangzhou, P.R. China). Polymerase chain reaction (PCR) amplifications were performed for all the strains with internal transcribed spacers (ITS5/ITS4, White et al. 1990); nuclear ribosomal 28S RNA gene (LR0R/LR5, Vilgalys & Hester 1990) and nuclear ribosomal 18S RNA gene (NS1/NS4, White et al. 1990) regions; an additional gene region translation elongation factor-1 α (EF1-983F/EF1-2218R, Rehner 2001) was amplified for strain MFLUCC 15–0440, following the conditions and primers mentioned in Thambugala et al. (2017a). The PCR products were visualized under UV light on 1% agarose electrophoresis gels stained with ethidium bromide. The PCR products were purified and sequenced at Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China). All the newly generated sequences were deposited in GenBank (Table 1).

Table 1 Taxa included in the phylogenetic study. The generated in this study are in blue and Ex-type and ex-epitype in bold.

Species	Culture number	GenBank accession numbers		
		LSU	SSU	ITS
<i>Acrospermum adeanum</i>	M133	EU940104	EU940256	–
<i>Acrospermum compressum</i>	M151	EU940084	EU940012	EU940161
<i>Acrospermum gramineum</i>	M152	EU940085	EU940013	EU940162
<i>Botryosphaeria corticis</i>	CBS 119047	EU673244	KF766232	DQ299245
<i>Botryosphaeria dothidea</i>	CBS 115476	DQ377852	EU673173	KF766151
<i>Botryobambusa fusicoccum</i>	MFLUCC 11–0657	–	JX646827	–
<i>Clavatispora thailandica</i>	MFLUCC 10–0107	KF770458	KF770457	–
<i>Clavatispora thailandica</i>	MFLUCC 17–2237	MH062960	MH062967	MH065721
<i>Diplodia mutila</i>	CBS 112553	AY928049	EU673213	AY259093

Table 1 Continued.

Species	Culture number	GenBank accession numbers		
		LSU	SSU	ITS
<i>Fusicladium cordae</i>	CCF 3843	FN377748	–	–
<i>Fusicladium cordae</i>	CBS 675.82	MH873281	–	MH861540
<i>Fusicladium pini</i>	CBS 463.82	–	–	MH861517
<i>Fusicladium ramoconidii</i>	CBS 462.82	MH873263	–	MH861516
<i>Gloniopsis praelongea</i>	CBS 112415	FJ161173	FJ161134	–
<i>Melnikidium vietnamensis</i>	CBS 136209	MH877613	–	KJ869156
<i>Muyocopron castanopsis</i>	MFLUCC 10–0042	–	JQ036225	–
<i>Muyocopron castanopsis</i>	MFLUCC 14–1108	KU726965	KU726968	–
<i>Muyocopron dipterocarpi</i>	MFLUCC 14–1103	KU726966	KU726969	–
<i>Muyocopron dipterocarpi</i>	MFLUCC 17–2243	MH062986	MH062971	MH065723
<i>Muyocopron garethjones</i>	MFLU 16–2664	KY070274	KY070275	–
<i>Muyocopron lithocarpi</i>	MFLUCC 10–0041	JQ036230	JQ036226	–
<i>Muyocopron lithocarpi</i>	MFLUCC 14–1106	KU726967	KU726970	–
<i>Mycosisymbrium cirrhosum</i>	GUFCC 18012	KR259884	KR259885	KR259883
<i>Neocoleroa metrosideri</i>	PDD 107531	KU131677	–	KU131678
<i>Ochroconis constricta</i>	CBS 202.27	MH866423	KF156072	MH854929
<i>Ochroconis humicola</i>	CBS 116655	KF156124	KF156068	–
<i>Ochroconis macrozamiaae</i>	CBS 1379771	KJ869180	KJ869123	–
<i>Ochroconis mirabilis</i>	CBS 729.95	KF282661	KF282676	–
<i>Ochroconis musae</i>	CBS 312.96	KT272083	KT272093	KT272078
<i>Ochroconis musae</i>	HLHKBJ22	JQ364739	–	JQ364738
<i>Ochroconis podocarpi</i>	CBS 143174	MG386085	–	MG386032
<i>Patellaria atrata</i>	CBS 958.97	GU301855	GU296181	–
<i>Rhytidhysterion hysterinum</i>	EB 0351	GU397350	–	–
<i>Rhytidhysterion neorufulum</i>	CBS 306.38	–	GU296191	–
<i>Rhytidhysterion neorufulum</i>	EB 0381	GU397351	GU397366	–
<i>Rhytidhysterion neorufulum</i>	GKM 361A	–	–	GU397342
<i>Rhytidhysterion neorufulum</i>	HUEFS 192194	KF914915	–	–
<i>Rhytidhysterion neorufulum</i>	MFLUCC 12–0011	KJ418109	KJ418110	KJ206287
<i>Rhytidhysterion neorufulum</i>	MFLUCC 12–0528	KJ418117	KJ418119	KJ418118
<i>Rhytidhysterion neorufulum</i>	MFLUCC 12–0567	KJ526126	KJ546129	KJ546124
<i>Rhytidhysterion neorufulum</i>	MFLUCC 12–0569	KJ526128	KJ546131	KJ546126
<i>Rhytidhysterion neorufulum</i>	MFLUCC 13–0216	KU377566	KU377571	KU377561
<i>Rhytidhysterion neorufulum</i>	MFLUCC 13–0221	KU377567	KU377572	KU377562
<i>Rhytidhysterion neorufulum</i>	MFLUCC 17–2236	MH063266	MH062969	MH062956
<i>Rhytidhysterion rufulum</i>	EB 0382	GU397352	–	–
<i>Rhytidhysterion rufulum</i>	EB 0383	GU397353	GU397367	–
<i>Rhytidhysterion rufulum</i>	EB 0384	GU397354	GU397368	–
<i>Rhytidhysterion rufulum</i>	MFLUCC 12–0013	KJ418111	KJ418113	KJ418112
<i>Rhytidhysterion rufulum</i>	MFLUCC 14–0577	KU377565	KU377570	KU377560
<i>Rhytidhysterion</i> sp.	MFLUCC 12–0529	KJ526124	KJ546127	KJ546122
<i>Rhytidhysterion tectonae</i>	MFLUCC 13–0710	–	KU712457	KU144936
<i>Rhytidhysterion thailandicum</i>	MFLUCC 12–0530	KJ526125	KJ546128	KJ546123

Table 1 Continued.

Species	Culture number	GenBank accession numbers		
		LSU	SSU	ITS
<i>Rhytidhysteron thailandicum</i>	MFLUCC 14–0503	KU377564	KU377569	KU377559
<i>Scolecobasidiella avellanea</i>	CBS 772.73	EF204505	EF204520	–
<i>Scolecobasidium excentricum</i>	CBS 469.95	MH874174	KF282683	MH862538
<i>Sympoventuria capensis</i>	CBS 120136	KF156104	KF156094	KF156039
<i>Tubeufia chiangmaiensis</i>	MFLUCC 11–0514	KF301538	KF301543	KF301530
<i>Tubeufia miscanthi</i>	MFLUCC 11–0375	KF301533	KF301541	KF301525
<i>Tubeufia paludosa</i>	CBS 120503	GU301877	GU296203	–
<i>Venturia inaequalis</i>	CBS 594.70	MH87164	KF156093	KF156040
<i>Veronaeopsis simplex</i>	CBS 588.66	KF156103	KF156095	EU041820
<i>Verruconis gallopava</i>	CBS 437.64	KF282656	KF282636	HQ667553
<i>Verruconis verruculosa</i>	CBS 119775	KF156106	KF156055	KF156014

Phylogenetic analyses

Related sequences were obtained from GenBank following recently published papers (Boonmee et al. 2014, Mapook et al. 2016, Thambugala et al. 2016, 2017b). Multi-gene and single gene phylogenetic analyses based on ITS, LSU and SSU sequence data were done to establish the phylogenetic placement of each isolated taxon. Single gene data sets were aligned with BioEdit 7.1.3.0 (Hall 1999) and the consensus sequences were further improved with MUSCLE implemented in MEGA 5v (Tamura et al. 2011). Alignments were checked and optimized manually when necessary. Phylogenetic analyses were based on maximum likelihood (ML) criterion using RAxML-HPC BlackBox (8.2.10) (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES portal (Miller et al. 2010). The general time reversible model of evolution including estimation of invariable sites with GTRGAMMA + I substitution model (assuming a discrete gamma distribution with four rate categories) was used for the ML analysis. The model for Bayesian inference analysis (BYPP) was determined by using MrBayes 3.2 on XSEDE (Ronquist et al. 2011) in the CIPRES portal (Miller et al. 2010), Simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. The first 1,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 9,000 trees were used for calculating posterior probabilities in the majority rule consensus tree. The best scoring tree was selected and visualized with MEGA v. 5 (Tamura et al. 2011) and improved using Adobe Illustrator CS3 software. ML and BYPP bootstrap support (BS) (greater than 60% ML/0.95 BYPP) are shown above or below each branch. The alignment and trees are deposited in TreeBASE (S23454).

Results

Phylogenetic analysis

Three dothideomycetous species, *Clavatispora thailandica*, *Muyocopron dipterocarpi* and *Rhytidhysteron neorufulum* were isolated and sequenced. The data for the aligned sequence matrices for the trees obtained in the different analyses are provided below. Alignments of multi-genes were involved, the topologies of the trees for each gene were compared visually to confirm that the overall tree topology of the individual datasets were similar to each other and to that of the tree obtained from the combined alignment.

Clavatispora thailandica (MFLUCC 17–2237)

The concatenated and single LSU, SSU and ITS datasets comprised 23 strains of species in

Sympoventuriaceae. The best scoring tree with a final likelihood value of -7252.737610 is presented in Fig. 1. The new isolate of *Clavatispora thailandica* forms a well-supported (100% ML/1.00 BYPP) clade with its ex-type strain (MFLUCC 10-0107).

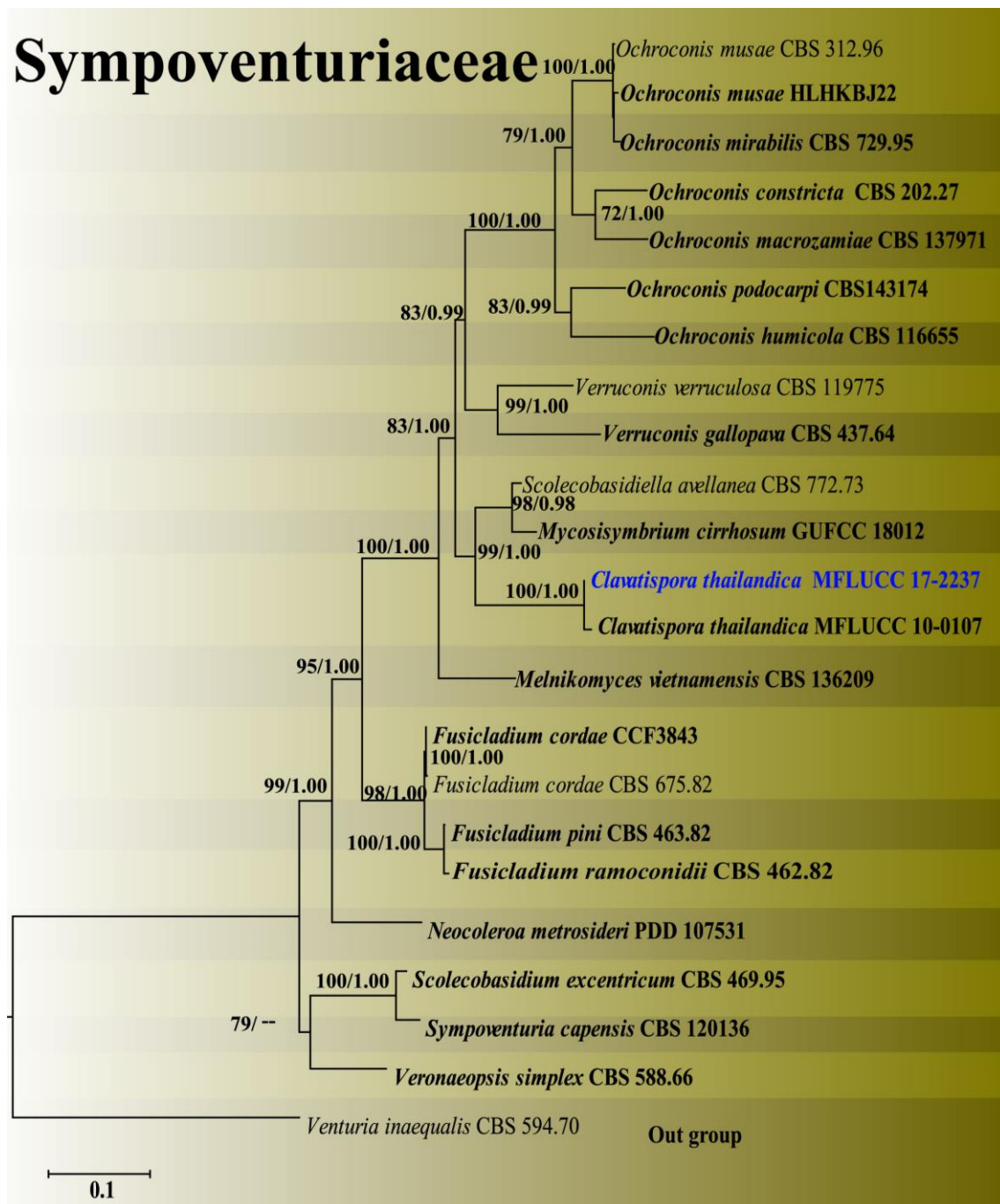


Fig. 1 – Phylogram generated from maximum likelihood tree from analysis of combined LSU SSU and ITS sequence data of species in Sympoventuriaceae. Bootstrap (ML) support values greater than 60% and BYPP greater than 0.95 are given above or below the nodes. Culture accession numbers are placed after the species name and the tree is rooted to *Venturia inaequalis*. Ex-type and ex-epitype cultures are in bold and the newly generated *Clavatispora thailandica* (MFLUCC 17-2237) is in blue.

***Muyocopron dipterocarpi* (MFLUCC 17-2243)**

The concatenated and single LSU, SSU and ITS sequence data comprised 18 strains of Acrospermaceae, Botryosphaeriaceae, Muyocopronaceae and Tubeufiaceae species. The best

scoring tree with a final likelihood value of -6888.564120 is presented in Fig. 2. *Muyocopron dipterocarpi* (MFLUCC 17–2243) clustered together with its ex-type strain (MFLUCC 14–1103) with good support (100% ML/1.00 BYPP).

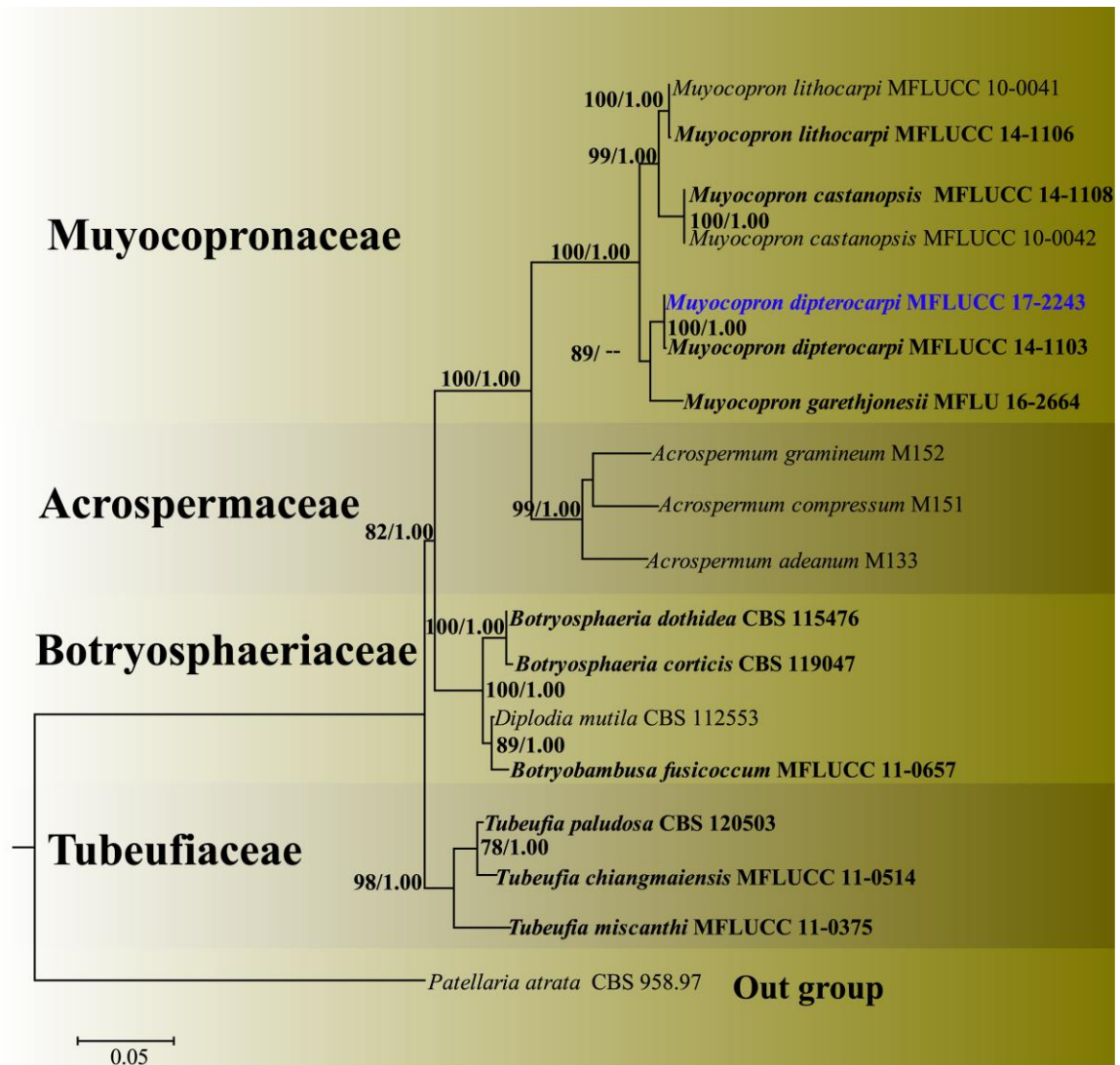


Fig. 2 – Phylogram generated from maximum likelihood tree from analysis of combined LSU SSU and ITS sequence data of species in AcrospERMACEAE, Botryosphaeriaceae, MuyocopRONACEAE and TubeufiACEAE. Bootstrap (ML) support values greater than 60% and BYPP greater than 0.95 are given above or below the nodes. Accession numbers are placed after the species name and the tree is rooted to *Patellaria atrata* (CBS 958.97). Ex-type and ex-epitype strains are in bold and the newly generated *Muyocopron dipterocarpi* (MFLUCC 17–2243) is in blue.

***RhytidhysterON neorufulum* (MFLUCC 17–2236)**

The concatenated dataset comprised 22 strains of *RhytidhysterON* species. The best scoring tree with a final likelihood value of -5562.132998 is presented in Fig. 3. In the resulting phylogenetic analysis, *RhytidhysterON neorufulum* (MFLUCC 17–2236) forms a well-supported (0.94 BYPP) clade, sister to *R. neorufulum* (MFLUCC 12–0011) and its ex-type strain (MFLUCC 13-0216).

Venturiales Y. Zhang et al., C.L. Schoch & K.D. Hyde

Venturiales was introduced by Zhang et al. (2011) based on morphological, ecological and phylogenetic approaches. Some species belonging to this order are plant pathogens and others are saprobes (Hyde et al. 2013, Tibpromma et al. 2018).

Sympoventuriaceae Y. Zhang et al., C.L. Schoch & K.D. Hyde

Zhang et al. (2011) erected Sympoventuriaceae with *Sympoventuria* Crous & Seifert as the type genus. This family is characterized by immersed, subglobose ascomata, hyaline septate pseudoparaphyses, bitunicate asci and hyaline, brown to dark brown, oblong, ascospores (Zhang et al. 2011) and also found this family contains the asexual genus as hyphomycetes (Hyde et al. 2013, Wijayawardene et al. 2018), which seven genera *Clavatispora* Boonmee & K.D. Hyde., *Mycosisymbrium* Carris., *Ochroconis* de Hoog., *Sympoventuria*, *Veronaepsis* Arzanlou & Crous., *Verruconis* Samerp., *Yunnanomyces* Tibpromma & K.D. Hyde., as well as species from *Fusicladium* Bonord., *Neocoleroa* Petr. and *Scolecobasidium* E.V. Abbott., are referred to the family Sympoventuriaceae based on multi-gene phylogeny (Zhang et al. 2019).

Clavatispora Boonmee & K.D. Hyde

Boonmee et al. (2014) introduced *Clavatispora* Boonmee & K.D. Hyde in Sympoventuriaceae with *C. thailandica* Boonmee & K.D. Hyde as the type species and have accepted in Index Fungorum (2020). *Clavatispora* is characterized by its setiferous, black ascomata, bitunicate asci, with a shrunken ectotunica, endotunica and coloured plasmalemma layers, and clavate, brown to dark brown, muriform ascospores (Boonmee et al. 2014).

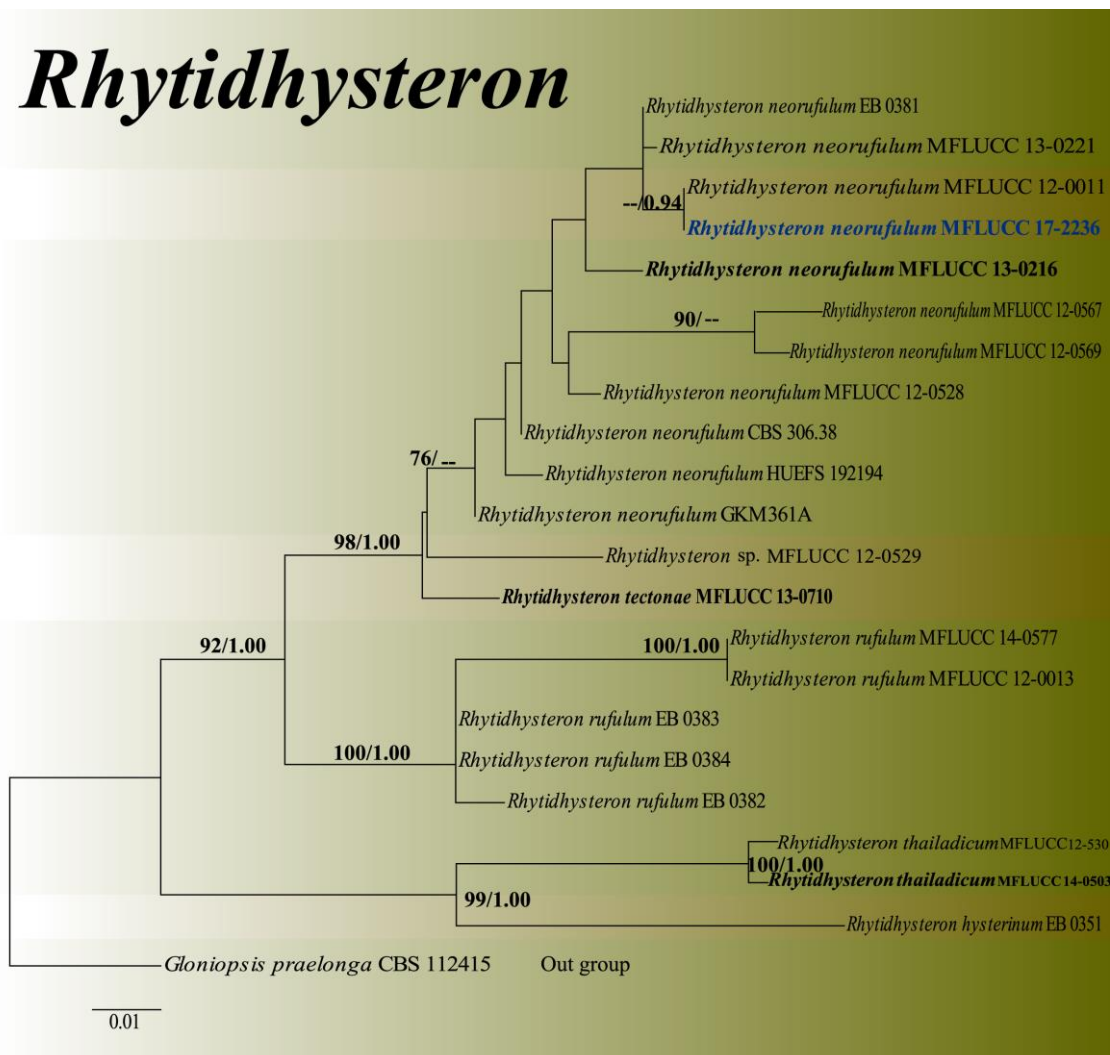


Fig. 3 – Phylogram generated from maximum likelihood tree from analysis of combined LSU, SSU and ITS sequence data of species in *Rhytidhysterion*. Bootstrap (ML) support values greater than 60% and BYPP greater than 0.95. Culture accession numbers are given after the species name and the tree is rooted to *Gloniopsis praelonga* CBS 112415. Ex-type and ex-epitype strains are in bold and the newly generated *Rhytidhysterion neorufulum* (MFLUCC 17-2236) is in blue.

Clavatispora thailandica Boonmee & K.D. Hyde

Figs 4–5

Index Fungorum number: IF805924; Facesoffungi number: FoF05124

Saprobic on dead twigs of *Hevea brasiliensis*. Sexual morph: *Ascomata* 110–235 µm diam. × 100–250 µm high, (\bar{x} = 147.3 × 160.8 µm, n = 10) superficial, solitary, scattered, developing on subiculum of brown hyphae, globose to subglobose, dark brown to black, with a bright ostiole covered with 2–3 µm wide, dark brown, thick-walled, septate, strands of radiating setae. *Peridium* 15–30 µm wide, comprising several layers of dark brown, thick-walled cells of *textura angularis*, becoming lightly pigmented towards the inner region. *Hamathecium* comprising 1–2 µm wide, anastomosing, septate, rarely branched pseudoparaphyses, embedded in gelatinous matrix. *Asci* 60–100 × 16–21 µm (\bar{x} = 83 × 18 µm, n = 20), 8-spored, bitunicate, fissitunicate, to broadly obovoid, with a short pedicel, apically rounded, with an ocular chamber. *Ascospores* (19–)22–32(–34) × 7–10 µm (\bar{x} = 27 × 8.4 µm, n = 45), overlapping biseriate, ellipsoidal to fusiform, muriform subclavate, slightly curved, asymmetrical, yellowish when young, becoming reddish brown to dark brown at maturity, 4–7(–8) transversely septate, with 1–2 vertical septa in some cells, deeply constricted at the medium septum, tapering towards a subacute base, smooth-walled. Asexual morph: *Hyphomycetous*, mycelium slightly raised, hyaline to pale brown, composed of septate, branched, smooth-walled, 1–3 µm wide hyphae. *Conidiophores* (4–)9–12 µm long (\bar{x} = 8 µm, n = 8), erect, developing on hyphae, brown or light brown, septate, smooth, sometimes branched. *Conidiogenous cells* holoblastic, pale brown, enteroblastic, annelidic, cylindrical, integrated or discrete, determinate, smooth-walled. *Conidia* (8–)10–13(–14) × 3–4(–5) µm (\bar{x} = 11 × 4 µm, n = 20), ellipsoidal to ellipsoidal-cylindrical, hyaline, 0–1-septate when young, becoming pale brown to brown and 3-septate at maturity, with a large guttule in each cell, rounded at apex, sub-acute at base, slightly constricted at the septa, smooth-walled.

Culture characteristics – Ascospores germinating on PDA within 24 h, germ tubes produced from one end or both ends. Colonies growing on MEA 15 mm diam. after 11 days at 25°C, low convex, slightly effuse hairy, edge entire, dark brown smooth, reverse brown, aerial mycelium, radiating outwards, superficial, septate.

Material examined – THAILAND, Chiang rai Province, Mueang District, Weng Chiang, on dead twigs of *Hevea brasiliensis*, 28 January 2017, Naruemon Huanraluek Rb003 (MFLU 18–0710; living culture MFLUCC 17–2237, ICMP 22456; GenBank LSU: MH062960, SSU: MH062967, ITS: MH065721).

Known distribution – Thailand (Boonmee et al. 2014) on dead stems, of an unidentified host.

Notes – In the phylogenetic analyses, the new strain (MFLUCC 17–2237) clustered with the ex-type strain of *C. thailandica* (MFLUCC 10–0107, Boonmee et al. 2014) and there is no evidence to suggest that these two strains are phylogenetically different. Nevertheless, a significant difference in ascospore measurements between the two collections were observed *Clavatispora thailandica* (MFLUCC 10–0107) has larger ascospores (\bar{x} = 37 × 11 µm) than *C. thailandica* (MFLUCC 17–2237) (\bar{x} = 27 × 8.4 µm). This is the first time any *Clavatispora* species is recorded from *Hevea brasiliensis* (Farr & Rossman 2020).

Muyocopronales Mapook, Boonmee & K.D. Hyde

Muyocopronales was introduced by Mapook et al. (2016) and has been placed in the Dothideomycetes. Members of this order are saprobes. Muyocopronales has superficial, flattened, carbonaceous, brittle ascomata, pseudoparaphyses that are longer than the asci and ellipsoidal to ovate, unicellular ascospores.

Muyocopronaceae K.D. Hyde

Muyocopronaceae was introduced by Luttrell (1951) and included in the order Hemisphaeriales as it has a pleospora-type of centrum similar to most Hemisphaeriaceae, Microthyriaceae and Polystomellaceae (Eriksson 1981). Hyde et al. (2013) accepted Muyocopronaceae as a distinct family with only *Muyocopro* Speg. in Dothideomycetes. Later Mapook et al. (2016) placed this family in Muyocopronales. Members of this family are saprobic

on a wide range of host plants and cosmopolitan in distribution (Mapook et al. 2016). In a recent study, a new genus *Pseudopalawania* Mapook & K.D. Hyde. was added to *Muyocopronaceae*, which was found on dead rachis of *Arecaceae* in Thailand (Mapook et al. 2020).



Fig. 4 – *Clavatispora thailandica* (MFLU 18–0710, sexual morph). A Appearance of ascomata on host surface. B, C Vertical sections through ascomata. D Setae. E Peridium. F Pseudoparaphyses. G–L Immature and mature asci. M–P Ascospores. Q, R Germinated ascospores. Scale bars: B, C = 50 µm, D–E = 20 µm, F–L = 50 µm, M–P = 15 µm, Q–R = 30 µm.

Muyocopron Speg

Muyocopron was introduced by Spegazzini (1881) in *Muyocopronaceae* (Hyde et al. 2013, Mapook et al. 2016, Wijayawardene et al. 2018). *Muyocopron* species are saprobic on a wide range of host plants and are cosmopolitan. More than 60 epithets are listed in this genus, but DNA sequence data are available for only a few species (Hyde et al. 2013, Mapook et al. 2016).

Muyocopron dipterocarpi Mapook, Doilom, Boonmee & K.D. Hyde

Fig. 6

Index Fungorum number: IF 551617; Facesoffungi number: FoF01889

Saprobic on dead twigs of *Mangifera indica*. Sexual morph: *Ascomata* 85–180 µm high × 230–310 µm diam. (\bar{x} = 121.5 × 279 µm, n = 10), superficial, coriaceous, solitary to scattered or aggregated, appearing as circular, flattened, black spots, covering the host surface, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiole* central without setose or hairy appendages, filled with hyaline cells. *Peridium* 12–40 µm wide, widest at the sides, comprising two cell layers, outer layer consisting of dark brown to black, thick-walled cells of *textura angularis*; inner layer composed of pale brown cells of *textura angularis*. *Hamathecium* comprising 1–3 µm wide, cylindrical to filiform, septate pseudoparaphyses, extending above asci.

Asci 43–60 × 19–29 μm (\bar{x} = 52 × 24 μm, n = 25), 8-spored, bitunicate, saccate or broadly obpyriform, short pedicellate to sessile, straight or slightly curved, with an indistinct ocular chamber. *Ascospores* 14–18(–21) × 8–12 μm (\bar{x} = 16.3 × 9.7 μm, n = 40), multi-seriate or irregularly arranged, partially overlapping, hyaline, oval to obovoid with obtuse ends, aseptate, with or without 1–2 large guttules. Asexual morph: undetermined.

Culture characteristics – Ascospores germinating on PDA within 24 h and germ tubes produced from one end or both ends. Colonies growing on MEA 20 mm diam. after 11 days 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, Sukhothai Province, Si Satchanalai District, on dead twigs of *Mangifera indica*, 2 January 2017, Naruemon Huanraluek M1 (MFLU 18–0711; living culture, MFLUCC 17–2243; ICMP 22493; GenBank LSU: MH062986, SSU: MH062971, ITS: MH065723.



Fig. 5 – *Clavatispora thailandica* (MFLUCC 17–2237, asexual morph). A Germinating conidium. B–C Culture morphology on MEA, 15 mm after 11 days (note C reverse). D Vegetative hyphae

formed in culture. E–I Conidiophores and developing conidia. J–L Conidia. Scale bars: A = 30 μm , D = 20 μm , E–I = 15 μm , J–L = 10 μm .

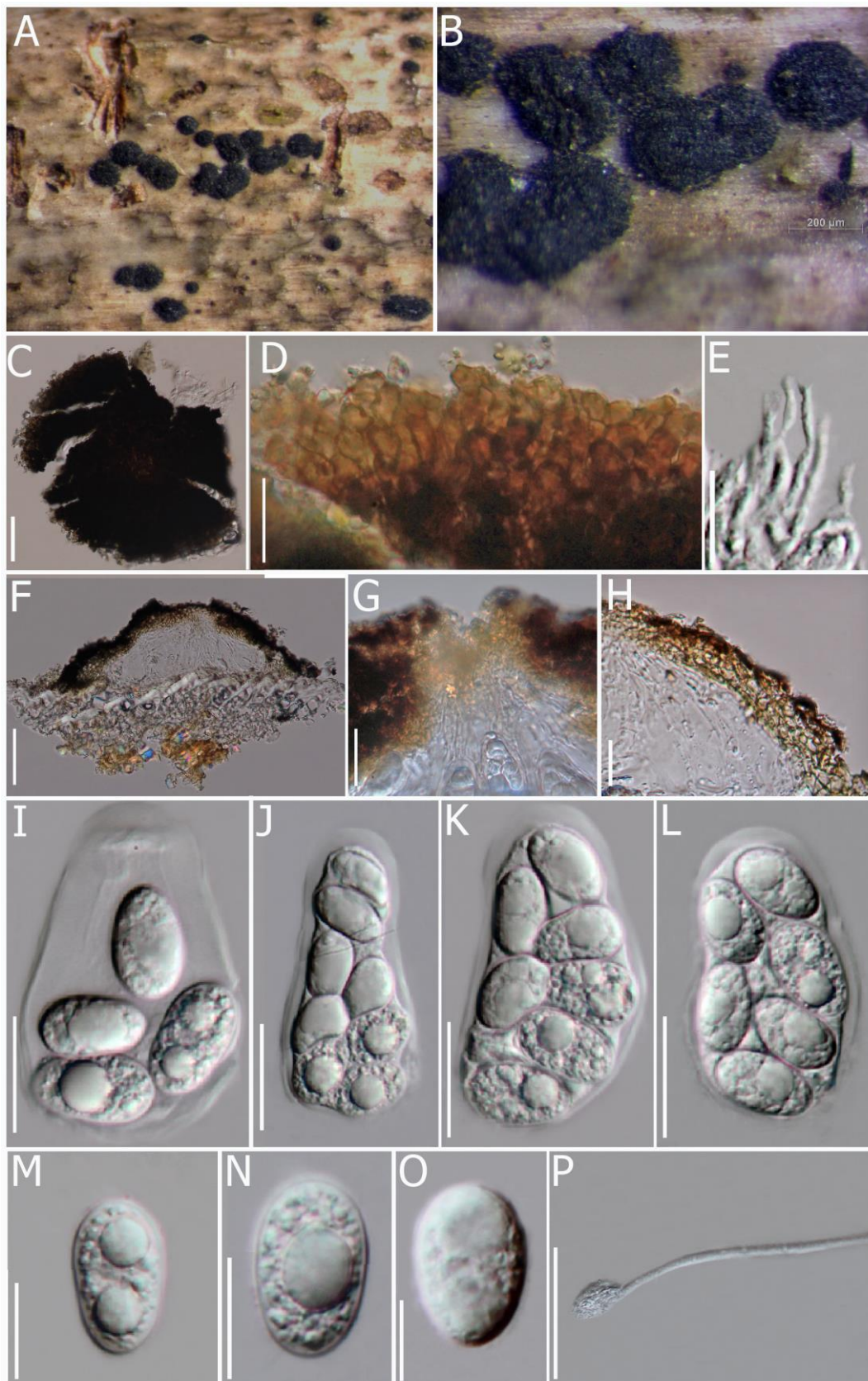


Fig. 6 – *Myocopron diptercarpi* (MFLU 18-0711). A, B Appearance of ascomata on host. C Squash mount of ascoma. D Ascomata wall. E Pseudoparaphyses. F Vertical section through

ascoma. G Apex of ascoma. H Peridium. I–L Asci. M–O Ascospores. P Germinating ascospore. Scale bars: C = 100 µm, D–H, I–L = 20 µm, F = 70 µm, M–O = 10 µm, P = 50 µm.

Known distribution – Thailand, on hosts *Dipterocarpus tuberculatus* (Mapook et al. 2016), *Hevea brasiliensis* (Senwanna et al. 2019).

Notes – *Muyocopron dipterocarpi* was introduced from dried twigs of *Dipterocarpus tuberculatus* (Dipterocarpaceae) in Thailand. The new collection on dead twigs of *Mangifera indica* fits well with the protologue (Mapook et al. 2016). In the phylogenetic analyses, the new strain clusters with the type strain of *M. dipterocarpi* (MFLUCC 14–1103) and together they form a well-supported clade (100% ML/1.00 BYPP). However, *M. dipterocarpi* (MFLUCC 14–1103) has larger ascomata ($\bar{x} = 110 \times 256.5 \mu\text{m}$) than the type strain (MFLUCC 17–2243). This is the first time a *Muyocopron* species has been recorded from *Mangifera indica* (Farr & Rossman 2020)

Hysteriales Lindau

Hysteriales was introduced by Lindau (1897) and this order has been placed among the Pyrenomycetes and the Discomycetes at different times by various authors (Rehm 1896). However, molecular data places Hysteriales in Dothideomycetes (Boehm et al. 2009a, b, Shearer et al. 2009, Suetrong et al. 2009, Hyde et al. 2013, Thambugala et al. 2016, Jayasiri et al. 2018).

Hysteriaceae Chevall.

Hysteriaceae was introduced by Chevallier (1826) in *Hysteriales* (Boehm et al. 2009a, b, Hyde et al. 2013, De Almeida et al. 2014, Wijayawardene et al. 2014). Based on morphological and phylogenetic data, this family comprises nine genera: *Gloniopsis* De Not., *Graphyllum* Clem., *Hysterium* Pers., *Hysterobrevium* E. Boehm & C.L. Schoch., *Hysterodifractum* D.A.C. Almeida, Gusmão & A.N. Mill., *Oedohysterium* E. Boehm & C.L. Schoch., *Ostreichnion* Duby., *Psiloglonium* Höhn. and *Rhytidhysterion* Speg. However, based on morphology alone, *Actidiographium* Lar.N. Vassiljeva., *Gloniella* Sacc., *Hysterocarina* H. Zogg. and *Hysteropycnis* Hiltzer. also belong to Hysteriaceae (Boehm et al. 2009a, b, Wijayawardene et al. 2018, Jayasiri et al. 2018).

Rhytidhysterion Speg.

Thambugala et al. (2016) revised the genus *Rhytidhysterion*, introduced two new species and showed the presence of striations on the surface of ascomata as a distinct character to delimit species in this genus. The ascomata of *Rhytidhysterion* are often thought of as hysterothecial as the genus belongs in *Hysteriales* in Dothideomycetes. Thambugala et al. (2016) mentioned that the ascomata of *Rhytidhysterion* species were hysterothecial, however, the ascomata of *Rhytidhysterion* species are hysterothecium-like when young or dry, having their margin incurved, but they are completely open, revealing the hymenium, at maturity (or when moist). Twenty-two epithets are listed in Index Fungorum (2020).

Rhytidhysterion neorufulum Thambugala. & K.D. Hyde

Fig. 7

Index Fungorum number: IF 551617; Facesoffungi number: FoF01840

Saprobic on dead twigs of *Hevea brasiliensis*. Sexual morph: *Ascomata* 271–364 long \times 310–464 diam. ($\bar{x} = 311 \times 400 \mu\text{m}$, $n = 4$), apothecioid, solitary to aggregated, superficial, black, carbonaceous to coriaceous, elliptic, compressed at apex or irregular in shape, with lenticular or irregular opening when wet, not striate, black or yellow at the center, when dry folded at the margin, forming an elongate slit. *Exciple* 75–190 µm wide, comprising several layers of dark brown to black, thick-walled cells of *textura angularis* becoming somewhat flattened and lightly pigmented towards the inner region. *Hamathecium* comprising 2–3 µm wide, dense, septate pseudoparaphyses, forming epithecium above the asci and enclosed in a gelatinous matrix turning blue when stained with Melzer's reagent. *Asci* 160–210 \times 10–15 µm ($\bar{x} = 185 \times 12.5 \mu\text{m}$, $n = 15$), 8-spored, bitunicate, clavate to cylindrical, with a short, furcate pedicel, apically rounded, without a

distinct ocular chamber. *Ascospores* $25\text{--}29 \times 8\text{--}11 \mu\text{m}$ ($\bar{x} = 26 \times 9.2 \mu\text{m}$, $n = 40$), uni-seriate, slightly overlapping, ellipsoidal to fusiform, slightly rounded or pointed at both ends, 1-septate and hyaline to yellowish when young, becoming 3-septate and reddish brown to brown at maturity, smooth-walled, guttulate, without a mucilaginous sheath. Asexual morph: undetermined.

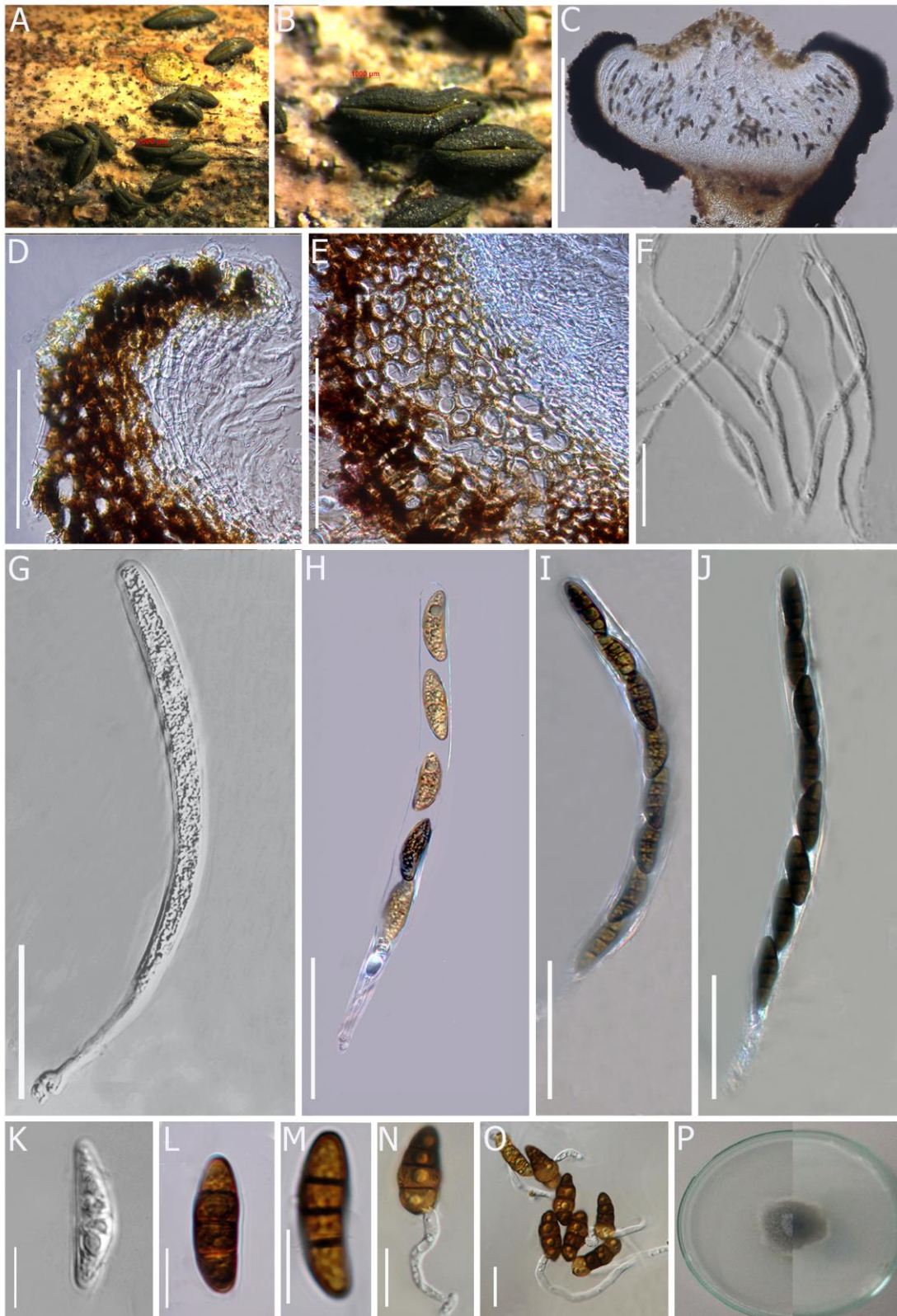


Fig. 7 – *Rhytidhysterium neorufulum* (MFLU 18-0641). A–B Appearance of ascomata on host. C Vertical section through ascoma. D, E Exciple. F Pseudoparaphyses. G, H Immature asci. I, J Mature asci. K–N Ascospores. O Cluster of mature ascospores. P Petri dish with culture.

I–J mature asci. K–M Ascospores. N–O Germinating ascospore. P Colony on PDA. Scale bars: C = 400 µm, D–E, H–J = 50 µm, F, G, N, O = 20 µm, K–M = 10 µm.

Culture characteristics – Ascospores germinating on MEA within 24 h and germ tubes produced from one end or both ends. Colonies growing on MEA 20 mm diam. after 10 days at 25°C, irregular, raised, dense, surface white, reverse saffron to reddish brown, margin yellowish, smooth surface with undulate edge.

Material examined – THAILAND, Chiang Rai Province, Mueang District, on dead twigs of *Hevea brasiliensis*, 26 December 2016, Naruemon Huanraluek, Rb002 (MFLU 18–0641); living culture MFLUCC 17–2236; ICMP 22179; GenBank LSU: MH063266, SSU: MH062969, ITS: MH062956.

Known distribution – Thailand, Chiang Rai Province, on dead stem and Chiang Mai Province, on dead wood and in Phitsanulok Province, on dead wood. (Thambugala et al. 2016)

Notes – *Rhytidhysterion neorufulum* was introduced by Thambugala et al. (2016) and found on twigs and dead wood from Chiang Rai, Chaing Mai and Phitsanulok. It is characterized by superficial apothecioid carbonaceous to coriaceous ascomata without striations (Thambugala et al. 2016, Hyde et al. 2017). The new strain clusters with the strain of *R. neorufulum* (MFLUCC 12–0011) well-supported clade (0.94 BYPP). However, *R. neorufulum* (MFLUCC 12–0011) has larger ascomata than the *R. neorufulum* MFLUCC 17–2236 strain (Thambugala et al. 2016). This is the first record of a *Rhytidhysterion* species from *Hevea brasiliensis* (Farr & Rossman 2020).

Discussion

Fungal saprobes play a major role in the decomposition of organic matter in nature (Wong et al. 1998, Cai et al. 2006), which helps to maintain ecological balance. We made new collections of three saprobic fungi. *Clavatispora thailandica* is morphology identical to the type species and in the phylogenetic analyses it clustered with the ex-type strain of *C. thailandica* (Fig. 1).

Muyocopron dipterocarpi was collected from dead twigs of *Mangifera indica* from Sukhothai and have similar morphology to the ex-type strain of *M. dipterocarp* (Mapook et al. 2016) and in the phylogenetic analyses, our strain clustered with the type strain of *M. dipterocarpi*.

Rhytidhysterion neorufulum found on *Hevea brasiliensis* in Chiang Rai, showed similar morphology and in the phylogenetic analyses, it clustered with the type strain of *R. neorufulum* (Thambugala et al. 2016).

The above fungi were reported on different host species, which resulted in new host records from Thailand. Expanding collections of saprobic micro-fungi on different hosts may lead to the identification of new host and geographical records for these fungi.

Acknowledgements

Authors would like to thank Mae Fah Luang University and Kevin D. Hyde.

References

- Boehm EWA, Schoch CL, Spatafora JW. 2009a – On the evolution of the Hysteriaceae and Mytilindiaceae (*Pleosporomycetidae*, Dothideomycetes, Ascomycota) using four nuclear genes. *Mycological Research* 113, 461–479.
- Boehm EWA, Mugambi GK, Miller AN, Huhndorf SM et al. 2009b – A molecular phylogenetic reappraisal of the Hysteriaceae, Mytilindiaceae and Gloniaceae (*Pleosporomycetidae*, Dothideomycetes) with keys to world species. *Studies in Mycology* 64, 49–83.
- Boonmee S, Bhat JD, Maharachchikumbura SS, Hyde KD. 2014 – *Clavatispora thailandica* gen. et sp. nov., a novel taxon of *Venturiales* (Dothideomycetes) from Thailand. *Phytotaxa* 176, 92–101.
- Bucher VVC, Hyde KD, Pointing SB, Reddy CA. 2004 – Production of wood decay enzymes, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs. *Fungal*

- Diversity 15, 1–14.
- Cai L, Ji KF, Hyde KD. 2006 – Variation between freshwater and terrestrial fungal communities on decaying bamboo culms. *Antonie van Leeuwenhoek* 89, 293–301.
- Chevallier FF. 1826 – Flore générale des environs de Paris, vol I. Ferra Librairie-Editeur, Paris.
- De Almeida DAC, Gusmão LFP, Miller AN. 2014 – A new genus and three new species of hysteriaceous ascomycetes from the semiarid region of Brazil. *Phytotaxa* 176, 298–308.
- Eriksson OE. 1981 – The families of bitunicate Ascomycetes. *Opera Botanica* 60, 1–209.
- Farr DF, Rossman AY. 2020 – Fungal Databases, U.S. National Fungus Collections Fungus-Host Database, ARS, USDA. (Retrieved on April 3, 2020)
- Hall TA, 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95e98
- Hyde KD, Jones EBG, Liu JK, Ariyawansa H et al. 2013 – Families of Dothideomycetes. *Fungal Diversity* 63, 1–313.
- Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A et al. 2017 – Fungal Diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Diversity* 87, 1–235.
- Index Fungorum. 2020 – <http://www.indexfungorum.org/Names/Names.asp>
- Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity* 74, 3–8.
- Jayasiri SC, Hyde KD, Jones EBG, Peršoh D et al. 2018 – Taxonomic novelties of hysteriform Dothideomycetes. *Mycosphere* 9, 803–837.
- Jedele S, Hau AM, von Oppen M. 2003 – An analysis of the world market for mangos and its importance for developing countries. In: Conference on International Agricultural Research for Development (Vol. 1). October 8–10, Göttingen, Germany.
- Ko JH S, Han KH. 2003 – Transcriptome analysis reveals novel features of the molecular events occurring in the laticifers of *Hevea brasiliensis* (para rubber tree). *Plant Molecular Biology* 53, 479–492.
- Lindau G. 1897 – Hysteriineae. In: Engler & Prantl, *Natürliche Pflanzenfamilien*. I. Teil, I. Abteilung. 1, 265–278.
- Luttrell ES. 1951 – Taxonomy of Pyrenomycetes. *University of Missouri Studies* 24, 1–120.
- Mapook A, Hyde KD, Dai DQ, Li J et al. 2016 – *Muyocoprionales*, ord. nov., (Dothideomycetes, Ascomycota) and a reappraisal of *Muyocopron* species from northern Thailand. *Phytotaxa* 265, 225–237.
- Mapook A, Macabeo APG, Thongbai B, Hyde KD. 2020 – Polyketide-Derived Secondary Metabolites from a Dothideomycetes Fungus, *Pseudopalawania siamensis* gen. et sp. nov., (Muyocoprionales) with Antimicrobial and Cytotoxic Activities. *Biomolecules* 10, 569.
- Miller MA, Pfeiffer W, Schwartz T. 2010 – Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 November. 2010, New Orleans, Los Angeles, pp, 1–8.
- Morton JF. 1987 – Fruits of warm climates. *New CROP*, New Crop Resource Online Program, Center for New Crops & Plant Products, Purdue University. pp. 221–239.
- Osono T. 2006 – Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology* 52, 701–716.
- Phillips AJL, Alves A, Abdollahzadeh J, Slippers B Wingfield MJ et al. 2013 – The Botryosphaeriaceae: genera and species known from culture. *Studies in Mycology* 76, 51–167.
- Phookamsak R, Liu JK, McKenzie EHC, Manamgoda DS Ariyawansa A et al. 2014 – Revision of Phaeosphaeriaceae. *Fungal Diversity* 68, 159–238.
- Phukhamsakda C, Ariyawansa HA, Phillips AJL, Wanasinghe DN et al. 2016 – Additions to Sporormiaceae: Introducing two novel genera, *Sparticola* and *Forliomyces*, from *Spartium*. *Cryptogamie, Mycologie* 37, 75–97.

- Pointing SB. 2001 – Exploiting of versatile ligninolytic system of white-rot fungi. *Fungal Diversity* 6, 253–290.
- Rehm H. 1896 – *Ascomyceten: Hysteriaceen* and *Discomyceten*, In L. Rabernhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. 2nd Ed, Eduard Kummer, Leipzig 3, 1–56.
- Rehner S. 2001 – Primers for elongation factor 1- α (EF1- α).
<http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf>
- Ronquist F, Huelsenbeck J, Teslenko M. 2011 – Draft MrBayes version 3.2. Manual: tutorials and model summaries. *Bioinformatics* 85–131.
- Senwana C, Hongsanan S, Phookamsak R, Tibpromma S et al. 2019 – *Muyocopron heveae* sp. nov. and *M. dipterocarpi* appears to have host-jumped to rubber. *Mycological Progress* 18, 741–752.
- Shearer CA, Raja HA, Miller AN, Nelson P et al. 2009 – The molecular phylogeny of freshwater Dothideomycetes. *Studies in Mycology* 64, 145–153.
- Spezzazzini CL. 1881 – Fungi argentini additis nonnullis brasiliensibus montevidensibusque. *Pugillus quartus (Continuacion)* *Anales de la Sociedad Científica Argentina* 12, 97–117.
- Stamatakis A. 2006 – RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stamatakis A, Hoover P, Rougemont J. 2008 – A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57, 758–771.
- Suetrong S, Schoch CL, Spatafora JW, Kohlmeyer J et al. 2009 – Molecular systematics of the marine Dothideomycetes. *Studies in Mycology* 64, 155–173.
- Tamura K, Peterson D, Peterson N, Stecher G et al. 2011 – MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.
- Thambugala KM, Hyde KD, Tanaka K, Tian Q et al. 2015 – Towards a natural classification and backbone tree for Lophiostomataceae, Floricolaceae, and Amorosiaceae fam. nov. *Fungal Diversity* 74, 199–266.
- Thambugala KM, Hyde KD, Eungwanichayapant PD, Romero AI, Liu ZY. 2016 – Additions to the genus *Rhytidhysterion* in Hysteriaceae. *Cryptogamie Mycologie* 37, 99–116.
- Thambugala KM, Daranagama DA, Phillips AJL, Bulgakov TS et al. 2017a – Microfungi on Tamarix. *Fungal Diversity* 82, 239–306.
- Thambugala KM, Wanasinghe DN, Phillips AJL, Camporesi E et al. 2017b – Mycosphere notes 1–50: grass (Poaceae) inhabiting Dothideomycetes. *Mycosphere* 8, 697–796.
- Tibpromma S, Hyde KD, McKenzie EHC, Bhat DJ. 2018 – Fungal diversity notes 840–928: microfungi associated with Pandanaceae. *Fungal Diversity* 93, 1–160.
- Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172, 4238–4246.
- White T, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18, 315–322.
- Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL et al. 2014 – Naming and outline of Dothideomycetes-2014 including proposals for the protection or suppression of generic names. *Fungal Diversity* 69, 1–55.
- Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK et al. 2018 – Outline of Ascomycota: 2017. *Fungal Diversity* 88, 167–263.
- Wong MK, Goh TK, Hodgkiss IJ, Hyde KD et al. 1998 – Role of fungi in freshwater ecosystems. *Biodiversity & Conservation*. 7, 1187–1206.
- Zhang SN, Liu JK, Jones EBG, Cheewangkoon R. 2019 – Morphology and phylogeny of *Yunnanomyces phoenicis* sp. nov. (Symptoventuriaceae) from Thailand. *Asian Journal of Mycology* 2, 213–221.

Zhang Y, Crous PW, Schoch CL, Bahkali AH et al. 2011 – A molecular, morphological and ecological reappraisal of *Venturiales* – a new order of Dothideomycetes. *Fungal Diversity* 51, 249–277.