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New host records for three saprobic Dothideomycetes in Thailand

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Abstract

Three dothideomycetous saprobic species, *Clavatispora thailandica*, *Muyocopron dipterocarpi* and *Rhytidhysteron neorufulum* were collected from dead twigs in Thailand. Multigene phylogenetic analyses confirmed their taxonomic placement. *Clavatispora thailandica* and *Rhytidhysteron 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 – *Rhytidhysteron*

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

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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-1α (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).

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).

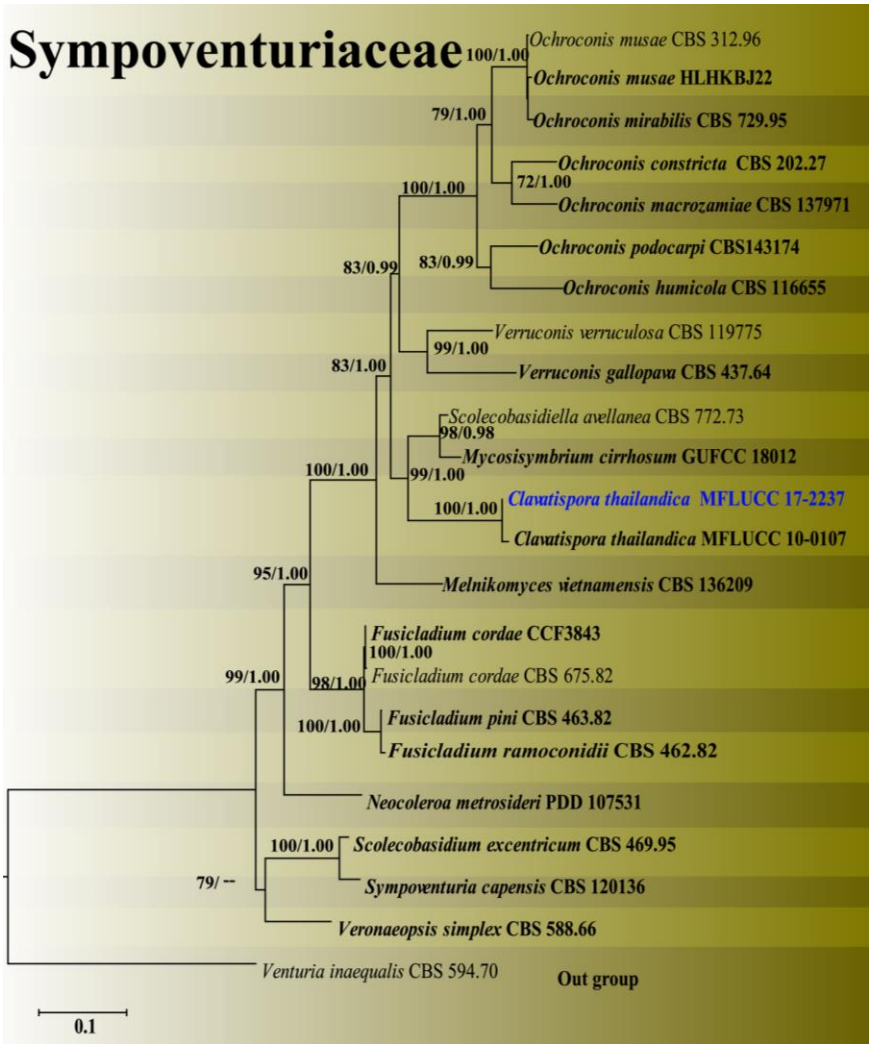

Fig. 1 – Phylogram generated from maximum likelihood tree from analysis of combined LSU SSU and ITS sequence data of species in Sympoventuriaceae. Bootstrap (ML) support values greater

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 *Clavatisspora 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).

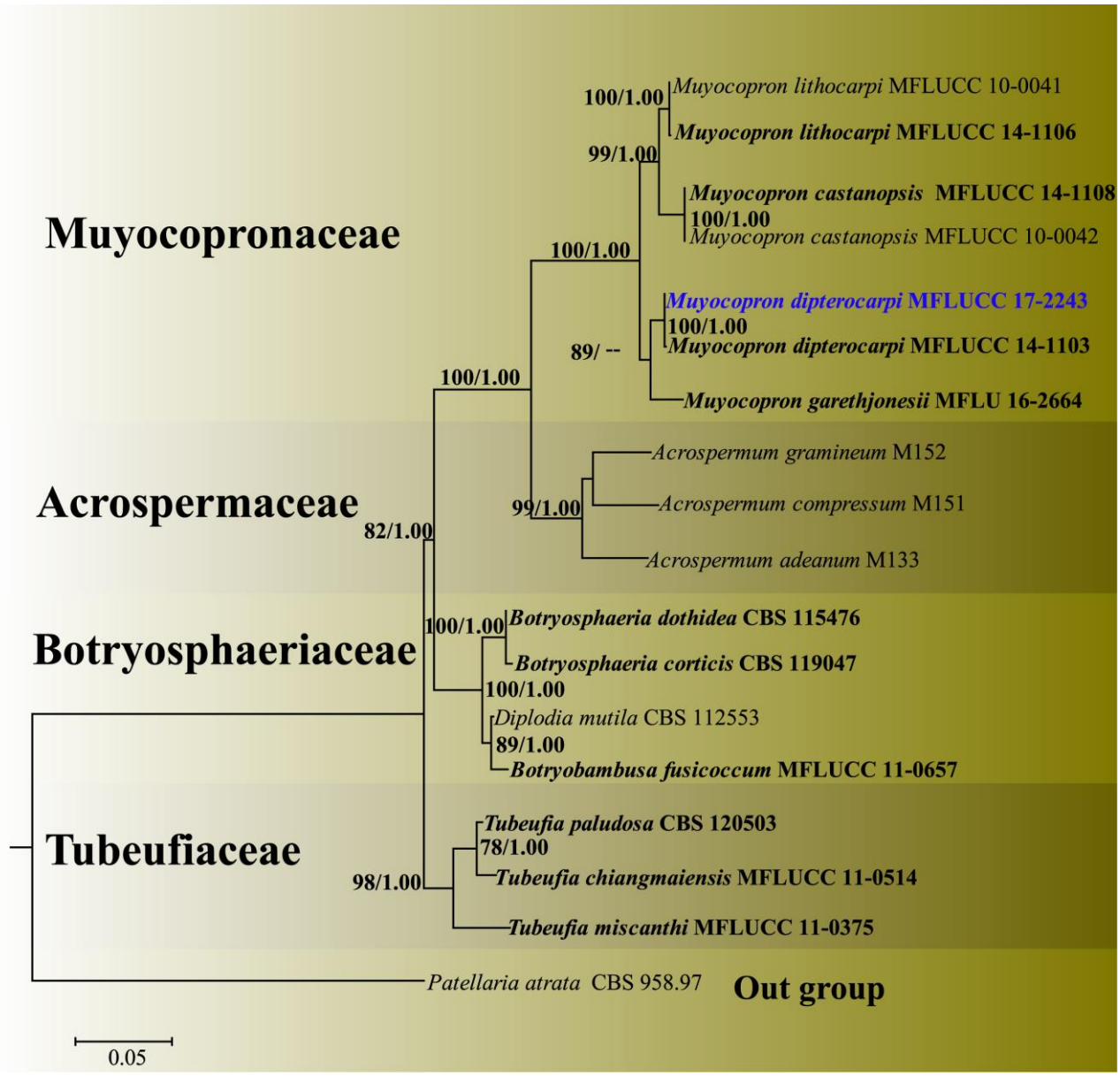

Fig. 2 – Phylogram generated from maximum likelihood tree from analysis of combined LSU SSU and ITS sequence data of species in Acrospermaceae, Botryosphaeriaceae, Muyocopronaceae and Tubeufiaceae. Bootstrap (ML) support values greater than 60% and BYPP greater than 0.95 are given above or below the 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) clusters sister to *R. neorufulum* (MFLUCC 12–0011) and its ex-type strain (MFLUCC 13-0216).

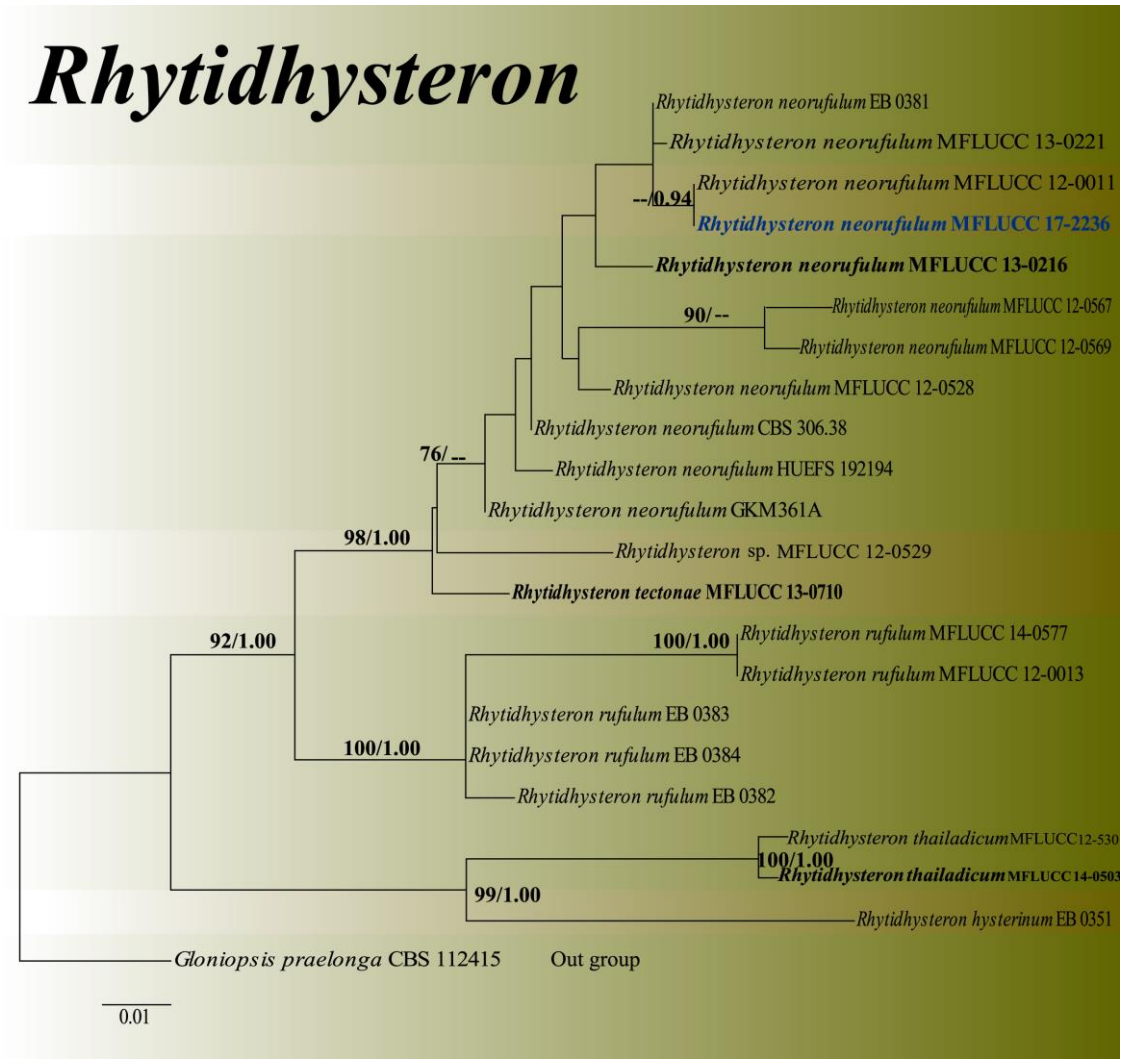

Fig. 3 – Phylogram generated from maximum likelihood tree from analysis of combined LSU, SSU and ITS sequence data of species in *Rhytidhysteron*. Bootstrap (ML) support values greater than 60% and BYPP greater than 0.95. Culture accession numbers are given after the species name and the tree is rooted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

Venturiales Y. Zhang ter, 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 ter, 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*, *Veronaeopsis* 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 only one species 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).

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).

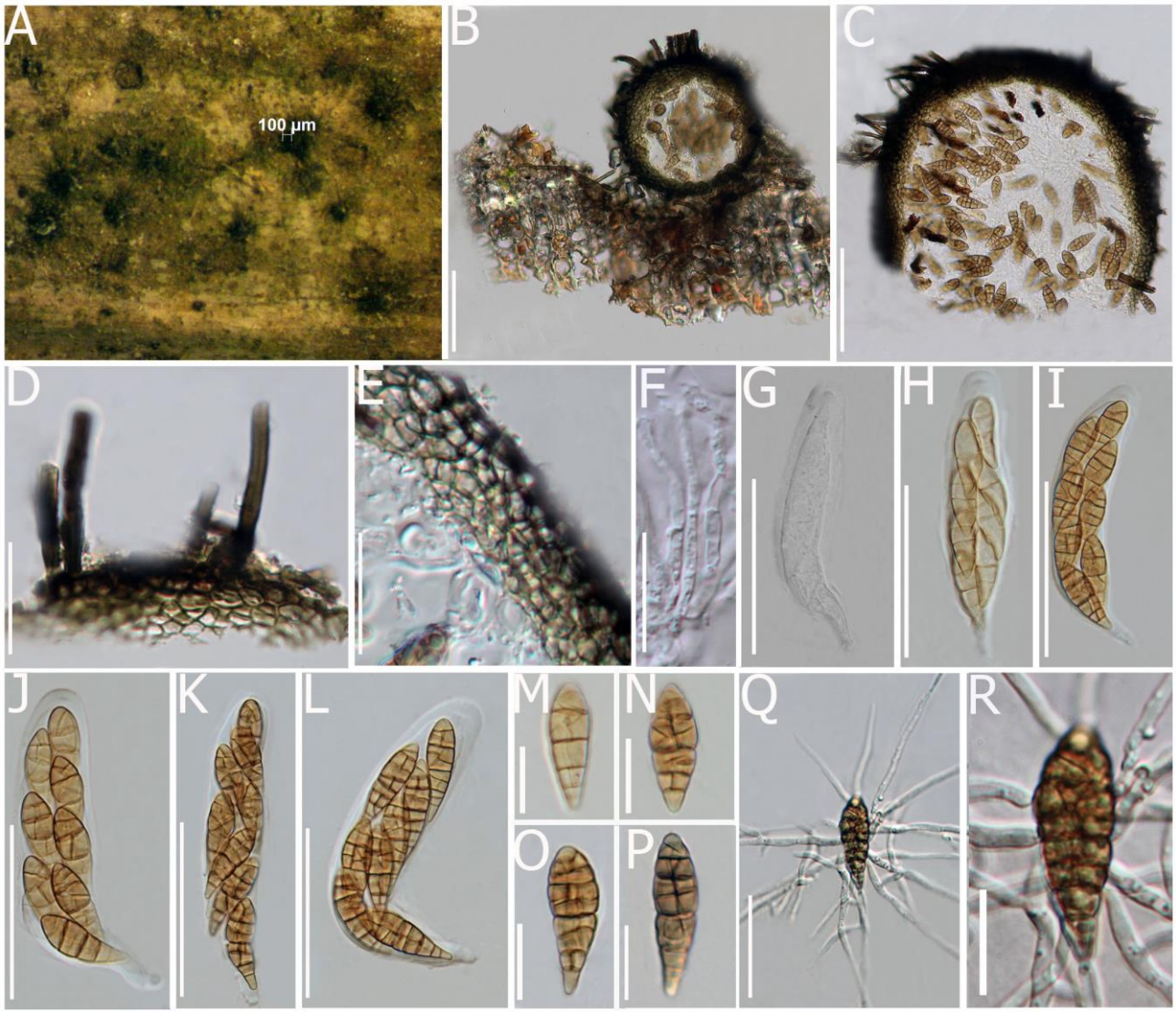

Fig. 4 – *Clavatispora thailandica* (MFLU 18–0710, sexual morph). A Appearance of ascomata on host surface. B, C Vertical sections through ascomata. D Setae. E Peridium. F Pseudoparaphyses. G–L Immature and mature asci. M–P Ascospores. Q, R Germinated ascospores. Scale bars: B, C = 50 µm, D–E = 20 µm,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

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 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 *Muyocopron* 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. 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.

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 × 279µm, 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 ($\overline{x}$ = 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 ($\overline{x}$ =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.

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 ($\overline{x}$ = 110 × 256.5 µm) 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, 2009b, 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., *Graphyllium* 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 *Rhytidhysteron* Speg. However, based on morphology alone, *Actidiographium* Lar.N. Vassiljeva., *Gloniella* Sacc., *Hysterocarina* H. Zogg. and *Hysteropycnis* Hilitzer. also belong to Hysteriaceae (Boehm et al. 2009a, b, Wijayawardene et al. 2018, Jayasiri et al. 2018).

***Rhytidhysteron* Speg.**

Thambugala et al. (2016) revised the genus *Rhytidhysteron*, 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 *Rhytidhysteron* are often thought of as hysterothecial as the genus belongs in *Hysteriales* in Dothideomycetes. Thambugala et al. (2016) mentioned that the ascomata of *Rhytidhysteron* species were hysterothecial, however, the ascomata of *Rhytidhysteron* 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).

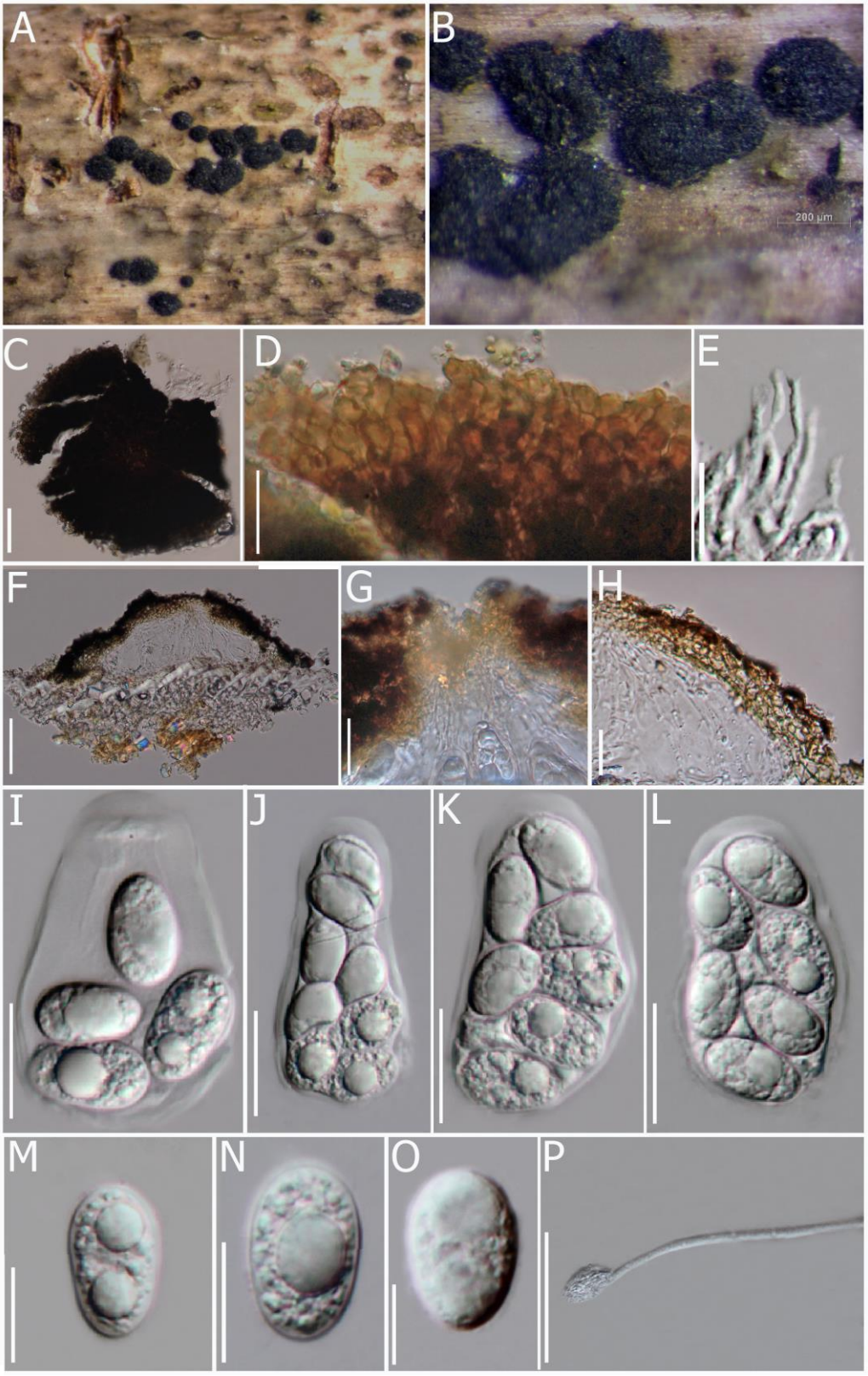

Fig. 6 – *Muyocopron dipterocarpi* (MFLU 18–0711): A, B Appearance of ascomata on host, C Squash mount of ascoma, D Ascomata wall, E Pseudoparaphyses, F Vertical section through ascoma, G Apex of ascoma, H Peridium, I–L Asci; M–O. Ascospores, P Germinating ascospore. Scale bars: C = 100 µm, D–H I–L = 20 µm, F = 70 µm, M–O = 10 µm, P = 50 µm.

Rhytidhysteron 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 × 310–464 diam. ($\overline{x}$ = 311 × 400 μm, 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 × 10–15 μm ($\overline{x}$ = 185 × 12.5 μm, n = 15), 8-spored, bitunicate, clavate to cylindrical, with a short, furcate pedicel, apically rounded, without a distinct ocular chamber. *Ascospores* 25–29 × 8–11 μm ($\overline{x}$ = 26 × 9.2 μm, 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.

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 – *Rhytidhysteron 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 *Rhytidhysteron* 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 the 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*.

Rhytidhysteron 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.

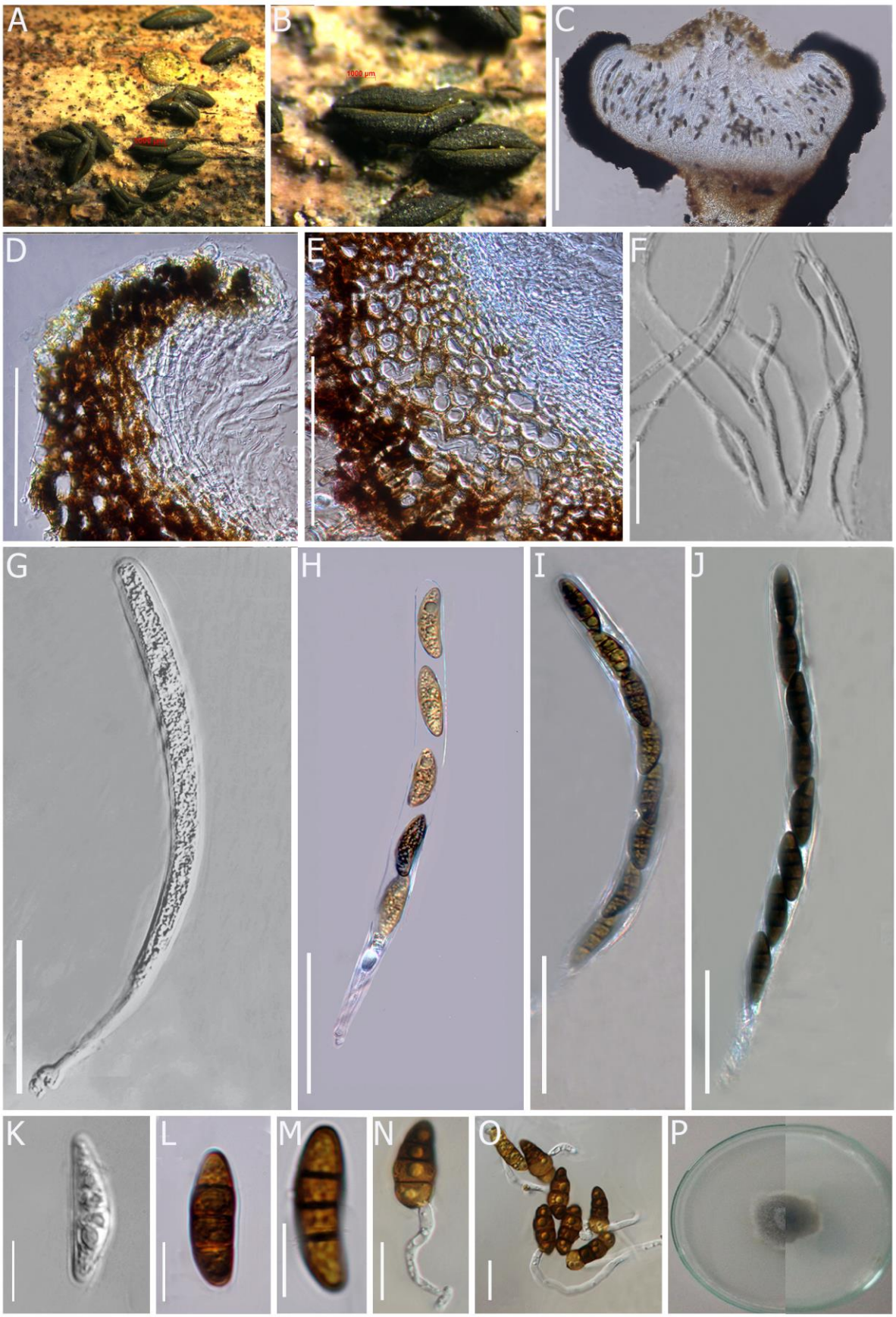

Fig. 7 – *Rhytidhysteron neorufulum* (MFLU 18–0641): A–B Appearance of ascomata on host, C Vertical section through ascoma, D, E Exciple, F Pseudoparaphyses, G, H Immature asci, I–J. mature asci, K–M. Ascospores, N–O Germinating ascospore, P Colony on PDA. Scale bars: C = 400 µm, D–E, H–J = 50 µm, F, G, N, O = 20 µm, K–M = 10 µm.

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
Acrospermum adeanum	M133	EU940104	EU940256	–
Acrospermum compressum	M151	EU940084	EU940012	EU940161
Acrospermum gramineum	M152	EU940085	EU940013	EU940162
Botryosphaeria corticis	**CBS 119047**	**EU673244**	**KF766232**	**DQ299245**
Botryosphaeria dothidea	**CBS 115476**	**DQ377852**	**EU673173**	**KF766151**
Botryobambusa fusicoccum	**MFLUCC 11–0657**	**–**	**JX646827**	**–**
Clavatispora thailandica	**MFLUCC 10–0107**	**KF770458**	**KF770457**	**–**
Clavatispora thailandica	**MFLUCC 17–2237**	**MH062960**	**MH062967**	**MH065721**
Diplodia mutila	CBS 112553	AY928049	EU673213	AY259093
Fusicladium cordae	**CCF 3843**	**FN377748**	**–**	**–**
Fusicladium cordae	CBS 675.82	MH873281	–	MH861540
Fusicladium pini	**CBS 463.82**	**–**	**–**	**MH861517**
Fusicladium ramoconidii	**CBS 462.82**	**MH873263**	**–**	**MH861516**
Gloniopsis praelongea	CBS 112415	FJ161173	FJ161134	–
Melnikdium vietnamensis	**CBS 136209**	**MH877613**	**–**	**KJ869156**
Muyocopron castanopsis	MFLUCC 10–0042	–	JQ036225	–
Muyocopron castanopsis	**MFLUCC 14–1108**	**KU726965**	**KU726968**	**–**
Muyocopron dipterocarpi	**MFLUCC 14–1103**	**KU726966**	**KU726969**	**–**
Muyocopron dipterocarpi	**MFLUCC 17–2243**	**MH062986**	**MH062971**	**MH065723**
Muyocopron garethjones	**MFLU 16–2664**	**KY070274**	**KY070275**	**–**
Muyocopron lithocarpi	MFLUCC 10–0041	JQ036230	JQ036226	–
Muyocopron lithocarpi	**MFLUCC 14–1106**	**KU726967**	**KU726970**	**–**
Mycosisymbrium cirrhosum	**GUFCC 18012**	**KR259884**	**KR259885**	**KR259883**
Neocoleroa metrosideri	**PDD 107531**	**KU131677**	**–**	**KU131678**
Ochroconis constricta	**CBS 202.27**	**MH866423**	**KF156072**	**MH854929**
Ochroconis humicola	**CBS 116655**	**KF156124**	**KF156068**	**–**
Ochroconis macrozamiae	**CBS 1379771**	**KJ869180**	**KJ869123**	**–**
Ochroconis mirabilis	**CBS 729.95**	**KF282661**	**KF282676**	**–**
Ochroconis musae	CBS 312.96	KT272083	KT272093	KT272078
Ochroconis musae	**HLHKBJ22**	**JQ364739**	**–**	**JQ364738**
Ochroconis podocarpi	**CBS 143174**	**MG386085**	**–**	**MG386032**
Patellaria atrata	CBS 958.97	GU301855	GU296181	–
Rhytidhysteron hysterinum	EB 0351	GU397350	–	–
Rhytidhysteron neorufulum	CBS 306.38	–	GU296191	–
Rhytidhysteron neorufulum	EB 0381	GU397351	GU397366	–
Rhytidhysteron neorufulum	GKM 361A	–	–	GU397342
Rhytidhysteron neorufulum	HUEFS 192194	KF914915	–	–
Rhytidhysteron neorufulum	MFLUCC 12–0011	KJ418109	KJ418110	KJ206287
Rhytidhysteron neorufulum	MFLUCC 12–0528	KJ418117	KJ418119	KJ418118
Rhytidhysteron neorufulum	MFLUCC 12–0567	KJ526126	KJ546129	KJ546124
Rhytidhysteron neorufulum	MFLUCC 12–0569	KJ526128	KJ546131	KJ546126
Rhytidhysteron neorufulum	**MFLUCC 13–0216**	**KU377566**	**KU377571**	**KU377561**
Rhytidhysteron neorufulum	MFLUCC 13–0221	KU377567	KU377572	KU377562

Table 1 Continued.

Species	Culture number	GenBank accession numbers		
		LSU	SSU	ITS
Rhytidhysteron neorufulum	**MFLUCC 17–2236**	**MH063266**	**MH062969**	**MH062956**
Rhytidhysteron rufulum	EB 0382	GU397352	–	–
Rhytidhysteron rufulum	EB 0383	GU397353	GU397367	–
Rhytidhysteron rufulum	EB 0384	GU397354	GU397368	–
Rhytidhysteron rufulum	MFLUCC 12–0013	KJ418111	KJ418113	KJ418112
Rhytidhysteron rufulum	MFLUCC 14–0577	KU377565	KU377570	KU377560
Rhytidhysteron sp.	MFLUCC 12–0529	KJ526124	KJ546127	KJ546122
Rhytidhysteron tectonae	**MFLUCC 13–0710**	**–**	**KU712457**	**KU144936**
Rhytidhysteron thailandicum	MFLUCC 12–0530	KJ526125	KJ546128	KJ546123
Rhytidhysteron thailandicum	**MFLUCC 14–0503**	**KU377564**	**KU377569**	**KU377559**
Scolecobasidiella avellanea	CBS 772.73	EF204505	EF204520	–
Scolecobasidium excentricum	**CBS 469.95**	**MH874174**	**KF282683**	**MH862538**
Sympoventuria capensis	**CBS 120136**	**KF156104**	**KF156094**	**KF156039**
Tubeufia chiangmaiensis	**MFLUCC 11–0514**	**KF301538**	**KF301543**	**KF301530**
Tubeufia miscanthi	**MFLUCC 11–0375**	**KF301533**	**KF301541**	**KF301525**
Tubeufia paludosa	**CBS 120503**	**GU301877**	**GU296203**	**–**
Venturia inaequalis	CBS 594.70	MH87164	KF156093	KF156040
Veronaeopsis simplex	**CBS 588.66**	**KF156103**	**KF156095**	**EU041820**
Verruconis gallopava	**CBS 437.64**	**KF282656**	**KF282636**	**HQ667553**
Verruconis verruculosa	CBS 119775	KF156106	KF156055	KF156014

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