Asian Journal of Mycology 3(1): 295–306 (2019) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/3/1/4

Morphology and multigene phylogeny reveal a new species and a new record of *Rhytidhysteron* (Dothideomycetes, Ascomycota) from China

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De Silva NI, Tennakoon DS, Thambugala KM, Karunarathna SC, Lumyong S, Hyde KD 2020 – Morphology and multigene phylogeny reveal a new species and a new record of *Rhytidhysteron* (Dothideomycetes, Ascomycota) from China. Asian Journal of Mycology 3(1), 295–306, Doi 10.5943/ajom/3/1/4

Abstract

Rhytidhysteron is an ecologically diverse group of Dothideomycetes occurring as endophytes, saprobes and weak pathogens on woody plants in terrestrial and intertidal habitats. During our field surveys in China, we collected *Rhytidhysteron* species on dead twigs of *Magnolia grandiflora* and decaying wood of *Morus australis*. Both morphology and multigene phylogenetic analyses showed one taxon to be a new species, while the other is a new record of *Rhytidhysteron thailandicum*. Combined LSU, SSU, ITS and *tef1* sequence data were used for the phylogenetic analyses. Descriptions, micrographs and a phylogenetic tree to show the placement of the two species in *Rhytidhysteron* (Hysteriaceae) are provided.

Key words – 1 new taxon – Hysteriaceae – Hysteriales – *Magnolia grandiflora* – *Morus australis* – *Rhytidhysteron magnoliae*

Introduction

Dothideomycetes comprise a highly diverse range of fungi occurring on various hosts and substrates in different types of ecosystems worldwide (Liu et al. 2017). The class is mainly characterized by asci with two wall layers (bitunicate asci) often with fissitunicate dehiscence (Hyde et al. 2013, Wijayawardene et al. 2017, 2018). Currently, with an estimated 19,000^{+} species, the Dothideomycetes is considered as the largest class in Ascomycota (Liu et al. 2017, Ariyawansa et al. 2018, Hongsanan et al. 2020).

Submitted 10 March 2020, Accepted 13 April 2020, Published 5 May 2020
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Hysteriaceous fungi are a group of Dothideomycetes that are characterized by carbonaceous, semi-immersed to superficial hysterithecioid or apothecioid ascomata, which distinctly navicular in outline, bearing a pronounced, longitudinal slit, running the entire length of the long axis. Asci are bitunicate and ascospores are hyaline to pigmented, one to multi-septate, or muriform (Boehm et al. 2009a, b, Hyde et al. 2013, de Almeida et al. 2014, Thambugala et al. 2016, Kumar et al. 2019). Species of the family are mainly saprobes and rarely weak pathogens on a wide range of woody plants in temperate and tropical regions (Boehm et al. 2009a, b, de Almeida et al. 2014, Thambugala et al. 2016). Molecular analyses place Hysteriaceous fungal taxa in Hysteriaceae, Hysteriales, Pleosporomycetidae and currently, 14 genera are accepted (Boehm et al. 2009a, b, Hyde et al. 2013, Jayasiri et al. 2017, Wijayawardene et al. 2017, 2018).

Rhytidhysteron Speg. is a species-rich genus in Hysteriaceae Chevall. and is characterized by its rather large, conspicuous closed and navicular ascomata, which open by a longitudinal slit that becomes irregularly apothecioid when wet and pigmented, sparsely septate to submuriform ascospores (Boehm et al. 2009b, Thambugala et al. 2016, Soto & Lucking 2017). Members of *Rhytidhysteron* have a worldwide distribution and play a vital role as endophytes, saprobes, weak pathogens on woody plants in terrestrial habitats and rarely as human pathogens (Thambugala et al. 2016, Soto & Lucking 2017, Kumar et al. 2019). Currently, there are 21 epithets listed in Index Fungorum (2020).

The present study reports a novel taxon of *Rhytidhysteron* on *Magnolia grandiflora* L. and a new host record of *Rhytidhysteron thailandicum* Thambug. & K.D. Hyde on *Morus australis* Poir. in China.

Materials & Methods

Fresh plant specimens were collected from a subtropical rain forest at the Xishuangbanna Tropical Botanical Garden in Yunnan Province and the Dahu Forest, Fanlu Township area, Chiayi, Taiwan region. Fungi were isolated from dead twigs attached to the *Magnolia grandiflora* and decaying wood of *Morus australis*. The collections were brought to the laboratory in Ziplock plastic bags and observed with a JNOEC JSZ4 stereomicroscope. Micro-morphological characteristics were examined with an OLYMPUS SZ61 compound microscope and images were recorded with a Canon EOS 600D digital camera mounted on a Nikon ECLIPSE 80i compound microscope. All microscopic measurements were made with the Tarosoft (R) image framework v. 0.9.0.7 and images were further processed with Adobe Photoshop CS3 Extended version. Pure cultures were obtained by single spore isolation as outlined by Chomnunti et al. (2014). Germinating ascospores were transferred aseptically to potato dextrose agar (PDA) and culture characteristics, such as growth rate and colony characteristics, were determined from cultures grown on PDA at room temperature (25°C) for one week.

The specimens cited in this paper are lodged at the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and Kunming Institute of Botany herbarium (HKAS), Kunming, China. The living fungal cultures obtained in this study were deposited at the Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi numbers and Index Fungorum numbers were registered as described in Jayasiri et al. (2015) and Index Fungorum (2020), respectively.

DNA extraction, PCR amplification and sequencing

Isolates of fungi were grown on PDA for one week at 25°C under normal light conditions and scraped mycelia were used for DNA extraction. DNA extraction was carried out using a Biospin fungus genomic DNA kit (BioFlux®, P.R. China) following the manufacturer's protocol.

PCR amplifications were carried out for the partial 28S large subunits of the nuclear ribosomal RNA genes (LSU) using primer pair LROR and LR5 (Vilgalys & Hester 1990); the partial 18S small subunit nuclear rDNA (SSU) using primer pair NS1 and NS4 (White et al. 1990); the internal transcribed spacers (ITS) using primer pair ITS5 and ITS4 (White et al. 1990) and the translation elongation factor 1-alpha gene (*tef1*) using primer pair EF1-983F and EF1-2218R (Rehner 2001).

Table 1 GenBank accession numbers and culture accession numbers of the taxa included in the present phylogenetic analysis. The newly generated sequences are shown in black bold.

Taxon	Culture Accession Number	GenBank Accession Number			
		LSU	SSU	*tef1*	ITS
Gloniopsis praelonga	CBS 112415	FJ161173	FJ161134	FJ161090	-
Rhytidhysteron bruguierae [T]	MFLUCC 18-0398	MN017833	MN017901	MN077056	-
Rhytidhysteron hysterinum	EB 0351	GU397350	-	GU397340	-
Rhytidhysteron magnoliae [T]	**MFLUCC 18-0719**	**MN989384**	**MN989382**	**MN997309**	**MN989383**
Rhytidhysteron mangrovei [T]	MFLUCC 18-1113	MK357777	-	MK450030	MK425188
Rhytidhysteron neorufulum [T]	MFLUCC 13-0216	KU377566	KU377571	KU510400	KU377561
	GKM 361A	GQ221893	GU296192	GU349031	-
	HUEFS 192194	KF914915	-	-	-
	MFLUCC 12-0528	KJ418117	KJ418119	-	KJ418118
	CBS 306.38	FJ469672	AF164375	GU349031	-
	MFLUCC 12-0011	KJ418109	KJ418110	-	KJ206287
	MFLUCC 12-0567	KJ526126	KJ546129	-	KJ546124
	MFLUCC 12-0569	KJ526128	KJ546131	-	KJ546126
	EB 0381	GU397351	GU397366	-	-
Rhytidhysteron opuntiae	GKM 1190	GQ221892	-	GU397341	-
Rhytidhysteron rufulum [T]	MFLUCC 14-0577	KU377565	KU377570	KU510399	KU377560
	EB 0384	GU397354	GU397368	-	-
	EB 0382	GU397352	-	-	-
	EB 0383	GU397353	GU397367	-	-
	MFLUCC 12-0013	KJ418111	KJ418113	-	KJ418112
Rhytidhysteron thailandicum [T]	MFLUCC 14-0503	KU377564	KU377569	KU497490	KU377559
Rhytidhysteron thailandicum	MFLUCC 12-0530	KJ526125	KJ546128	-	KJ546123
Rhytidhysteron thailandicum	**MFLU17-0788**	**MT093472**	**MT093495**	-	**MT093733**
Rhytidhysteron tectonae [T]	MFLUCC 13-0710	KU764698	KU712457	KU872760	KU144936

[T] Ex-type strains

The new species and the new record are in black bold.

The final volume of the PCR reaction was 25 µl, containing 1 µl of DNA template, 1 µl of each forward and reward primer, 12.5 µl of 2×Easy Taq PCR SuperMix (a mixture of *EasyTaq* TM DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Ltd., Beijing, P.R. China) and 9.5 µl of ddH_2O. The PCR amplification was performed for LSU, SSU, ITS and *tef1* with an initial denaturing step of 94°C for 3 min., followed by 40 amplification cycles of 94°C for 45 s, 55°C for 50 s and 72°C for 1 min. and a final extension step of 72°C for 10 min. PCR purification and sequencing of amplified PCR products were carried out at Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, P.R. China.

Phylogenetic analyses

The BLAST search engine of the National Centre for Biotechnology Information (NCBI) was used for the preliminary identification of DNA sequences of the new isolates. The LSU, SSU, ITS and *tef1* sequences of the closely related taxa to our isolates were retrieved from GenBank based on the BLAST search results and recent publications (Thambugala et al. 2016, Doilom et al. 2017, Kumar et al. 2019).

Maximum likelihood trees were generated using RAxMl GUI v. 1.3 (Silvestro & Michalak 2012) and parameters were set to rapid bootstrapping and the analysis carried out using 1000 replicates and GTRGAMMAI model of nucleotide substitution. An evolutionary model for phylogenetic analyses was selected using MrModeltest v. 3.7 (Posada & Crandall 1998) under the Akaike Information Criterion (AIC). Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001). The GTR+I+G model was used for the Bayesian analysis. Parameters of Bayesian analysis in MrBayes v. 3.2; markov chains were run for 1 000 000 generations and trees were sampled every 100th generations (printfreq = 100) and 10 000 trees were obtained. Initial trees were discarded (20% burn-in value) and remaining trees were used to

evaluate posterior probabilities (PP) in the majority rule consensus tree. Maximum parsimony analysis was conducted with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Parameters of maximum parsimony in PAUP were set-up as a heuristic search option, random stepwise addition, and 1000 random sequence additions, with 1000 maxtrees. Gaps were treated as missing data. Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for the maximum parsimonious tree. Phylograms were visualized with FigTree v1.4.0 (Rambaut & Drummond 2008) and annotated in Microsoft Power Point (2010).

Results

The combined dataset of LSU, SSU, ITS and *tef1* comprises 23 strains of *Rhytidhysteron*, and the outgroup taxon *Gloniopsis praelonga* (Schwein.) Underw. & Earle (CBS 112415). The maximum likelihood analysis resulted in trees largely with similar topology and clades as in the maximum parsimony and Bayesian analyses. The RAxML analysis yielded the best-scoring tree with a final ML optimization likelihood value of – 8082.346462 (ln). The maximum parsimonious dataset consisted of 3459 characters, of which 3051 were conserved and 200 variable characters. The parsimony analysis resulted in 1000 equally parsimonious trees and the first tree (length = 568 steps) with CI = 0.824, RI = 0.826, RC = 0.681 and HI = 0.176. Estimated base frequencies were as follows; A = 0.245603, C = 0.236491, G= 0.279689, T = 0.238217; substitution rates AC = 1.477459, AG = 2.203910, AT = 1.391008, CG = 1.013275, CT = 7.644469, GT = 1.000000; gamma distribution shape parameter α = 0.737155 (Fig. 1). The new species *Rhytidhysteron magnoliae* formed a sister clade to *R. tectonae* (MFLUCC13-0710). The new host record of *R. thailandicum* clustered with the type of *R. thailandicum* (MFLUCC 14-0503) with strong statistical support.

Rhytidhysteron magnoliae N.I. de Silva, Lumyong S & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF 557220; Facesoffungi number: FoF 07369

Etymology – The specific epithet reflects the host plant genus *Magnolia grandiflora*

Holotype – MFLU 18–1021

Saprobic on dead twigs of *Magnolia grandiflora*. Sexual morph: *Hysterothecia* 1200–2300 µm long, 430–550 µm high, 540–600 µm diameter ($\overline{x}$ = 1750 × 490 × 570 µm, n = 15), apothecioid, with a longitudinal slit, sometimes elliptic or irregular in shape, with lenticular or irregular opening, dark brown to black, coriaceous, solitary to aggregated, semi-immersed to superficial, with striations perpendicular to the long axis, dark brown at the center. *Exciple* 80–100 µm, comprising of two cell layers; outer layer comprising black to dark brown, thick-walled cells of *textura angularis*, inner layer comprising hyaline, thin-walled, somewhat flattened cells of *textura angularis* to *textura prismatica*. *Hamathecium* comprising 2–3 µm wide, cylindrical to filiform, septate, hyaline pseudoparaphyses, slightly swollen at the apex and enclosed in a gelatinous matrix. *Asci* (148–)160–200 (–210) × (11–)13–15(–16) µm ($\overline{x}$ = 176 × 14 µm, n = 30), 8-spored, bitunicate, fissitunicate, clavate to cylindrical, with a short pedicel. *Ascospores* (25–)28–30(–32) × (8–)10–11(–12) µm ($\overline{x}$ = 29 × 10 µm, n = 30), uniseriate, slightly overlapping, pale brown to dark brown, initially ellipsoidal, hyaline, aseptate, becoming fusiform, 1–3-septate, slightly rounded or pointed at both ends, constricted at the central septum, with large guttules and without a mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Colonies on PDA reaching 30 mm diameter after 7 days at 25°C in the dark, colonies circular, flat, margin wavy, fairly, fluffy appearance in margins, colony from above: light brown and; reverse: pale brown centre and dark brown margin.

Material examined – China, Yunnan Province, Xishuangbanna, dead twigs (attached to the tree) of *Magnolia grandiflora* (Magnoliaceae), 26 April 2017, N. I. de Silva, NI154 (MFLU 18-1021 holotype, HKAS100657 isotype), ex-type living culture, MFLUCC 18-0719 = KUMCC 17-0189.

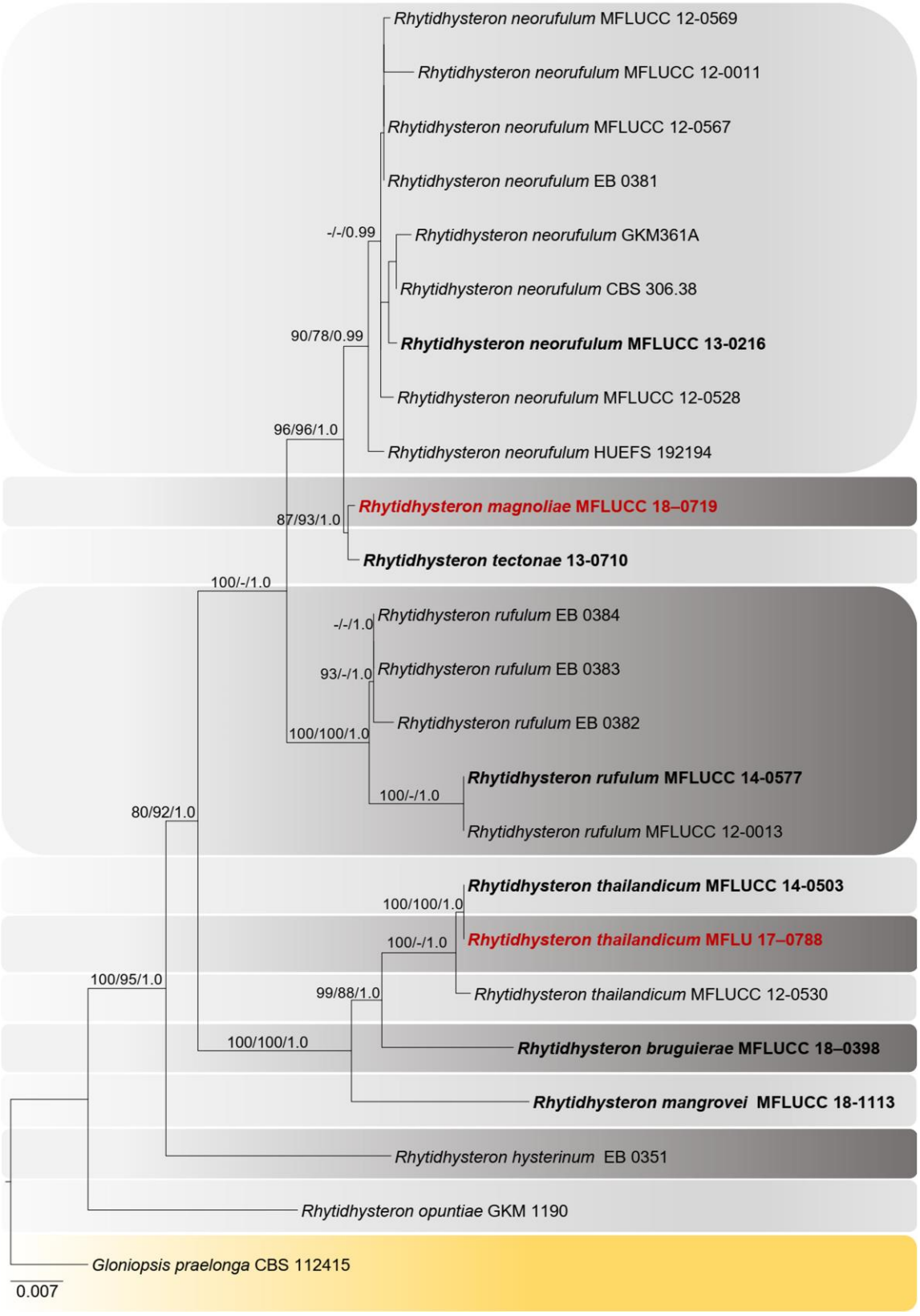

Fig. 1 – Phylogram generated from RAxML based on combined LSU, ITS, SSU and *tef1* sequence data. Maximum likelihood bootstrap support values (ML), maximum parsimony bootstrap support values (MP) and Bayesian posterior probabilities (PP) are indicated on the nodes as (ML/MP/PP). Bootstrap support values greater than 75% and Bayesian posterior probabilities greater than 0.90 are indicated at the nodes. The tree is rooted with *Gloniopsis praelonga* (CBS 112415). The new species and the new record are in red bold. Ex-type strains are in black bold.

Table 2 Synopsis of *Rhytidhysteron magnoliae* and related species.

Names	Ascomata	Exciple	Asci	Ascospores	References
R. bruguierae	(400–950 × 548–570) µm, perpendicularly striate	Dark brown to black, thick-walled cells of *textura angularis*	(128–148 × 10–14) µm, 6–8-spored	1–3-septate, (14–26 × 6.2–9) µm	Dayarathne et al. (2020)
R. brasiliense	(1087–1715 × 340–447) µm, rough-without striations	Not given	(230–250 × 20–30) µm, 8-spored	1–3-septate, (40–45 × 12–20) µm	Thambugala et al. (2016)
R. columbiense	(1500–3000 × 1200–1800) µm, boat-shaped, striate, brown-black disc	Dark brown to black, thick-walled cells of *textura angularis*	(175–190 × 14–18) µm, 6–8-spored	(1–)3-septate, 38–52 × 13–18 µm	Soto & Lucking (2017)
R. hysterinum	(1000–3000 × 500) µm, smooth-striate, black disc	Not given	(185–220 × 15–17) 4-8-spored	1-septate, (20–32 × 12–15) µm	Samuels & Müller (1979)
R. magnoliae	(1200–2300 × 540–600) µm elliptic or irregular shape ascomata with distinct striation, dark brown at the center	2 layers; black to dark brown, thick-walled cells of *textura angularis* outer layer and hyaline, thin-walled, somewhat flattened cells of *textura angularis* to *textura prismatica* inner layer	(176 × 14) µm, 8-spored	1–3-septate, (25–32 × 8–12) µm	This study
R. mangrovei	(930–1980 ×785–91) µm, lenticular to irregular, rough-striate, brown-black disc	Dark brown to black, thin-walled cells of *textura angularis*	(146 × 9.5) µm, (2–6–) 8-spored	1–3-septate, (21–28 × 7.5–8.5) µm	Kumar et al. (2019)
R. neorufulum	(835–1800 × 600–1320) µm, elliptic or irregular, rough-without striations, black or yellow center	Dark brown to black, thick-walled cells of *textura angularis*	(200 × 10.9) µm, 8-spored	1–3-septate, (27–34 × 6.5–12.5) µm	Thambugala et al. (2016)
R. rufulum	(900–2350 × 1134–1450) µm, elliptic or irregular, striate, black or red center	2 layers; outer layer dark brown to black, cells of *textura angularis* or *textura globosa*, inner layer of hyaline cells of *textura angularis* to *textura prismatica*	(202 × 13.5) µm, 8-spored	1–3-septate, (21–36 × 9–13) µm	Thambugala et al. (2016)

Table 2 Continued.

Names	Ascomata	Exciple	Asci	Ascospores	References
R. tectonae	(1225–3365 × 370−835) µm, striate, yellow center	2 layers; outer layer black to dark reddish, thick-walled cells of *textura angularis* and inner layer hyaline, thin-walled cells of *textura angularis*	(155× 13) µm, 8-spored	1-septate, (19–31 × 8−13) µm	Doilom et al. (2017)
R. thailandicum	(700–1200 × 530–750) µm, globose to subglobose, rough-without striations	Brown to dark brown, thick-walled cells of *textura angularis*	(145 × 12.8) µm, (3-)6-8-spored	3-septate, (20–31 × 7.5–12) µm	Thambugala et al. (2016)

Notes – The morphological features of *Rhytidhysteron magnoliae* are in accordance with the generic concept of *Rhytidhysteron* as they have large, conspicuously navicular hysterothecia, which open by a longitudinal slit and become irregularly apothecioid when wet. Ascospores are pigmented, sparsely septate (Boehm et al. 2009b, Thambugala et al. 2016, Soto & Lucking 2017). In the present multi-gene analyses (LSU, ITS, SSU and *tef1*), *R. magnoliae* (MFLU 18-1021) nested with other *Rhytidhysteron* species (Fig. 1), in particular, it was closely related to *R. tectonae* Doilom & K.D. Hyde and formed a well- supported clade (87% ML, 93% MP, 1.00 BYPP). However, *R. magnoliae* is distinct from *R. tectonae* in having elliptic or irregular ascomata with distinct striations. Further details are given in Table 2.

Rhytidhysteron magnoliae also differs from *R. tectonae* in having slightly larger asci (176 × 14 µm) and ascospores (25–32 × 8–12; $\overline{x}$ = 29 × 10 µm), whereas *R. tectonae* has slightly smaller asci (155 × 13 µm) and ascospores (19–31 × 8−13; $\overline{x}$ = 27 × 10 µm). In contrast to *R. tectonae*, *R. magnoliae* initially has ellipsoidal, hyaline ascospores becoming fusiform, pale brown to dark brown when mature with large guttules, whereas *R. tectonae* initially has subglobose, hyaline to pale brown ascospores becoming ellipsoidal to fusiform, pale brown to dark brown when mature and without guttules (Doilom et al. 2017). *Rhytidhysteron magnoliae* also differs from *R. tectonae* in terms of host association and locality, as the latter has been reported from the dead branches of *Tectona grandis* in Thailand (Doilom et al. 2017).

Rhytidhysteron thailandicum Thambug. & K.D. Hyde, Cryptog. Mycol. 37(1): 110 (2016)

Fig. 3

Index Fungorum Number: IF 551866; Facesoffungi number: FoF 01841

Saprobic on decaying wood of *Morus australis*. Sexual morph: *Hysterothecia* 750–900 µm long, 480–600 µm high, 600–750 µm diameter ($\overline{x}$ = 850 × 550 × 650, n = 5), scattered to gregarious, semi immersed to superficial, apothecioid, elongate and depressed, globose to subglobose, dark brown to black, coriaceous, compressed at apex, opening by a longitudinal slit. *Exciple* 70–110 µm wide ($\overline{x}$ = 95, n = 10), composed of dark brown to black, thin-walled cells of *textura angularis*. *Hamathecium* comprising 1–2 µm wide, dense, cellular, hyaline, septate, pseudoparaphyses, forming a dark epithecium above asci and enclosed in a gelatinous matrix. *Asci* 130–160 × 9–14 µm ($\overline{x}$ = 147 × 11.5, n = 20), 8-spored, bitunicate, cylindrical, with short pedicel, rounded at the apex, with an ocular chamber. *Ascospores* 22–25 × 9–10 µm ($\overline{x}$ = 23.8 × 9.3, n = 30), uniseriate, hyaline to lightly pigmented when immature, becoming reddish brown to brown when mature, ellipsoidal to fusoid, straight or curved, rounded to slightly pointed at both ends, (1–)3-septate, guttulate, smooth-walled. Asexual morph: Undetermined.

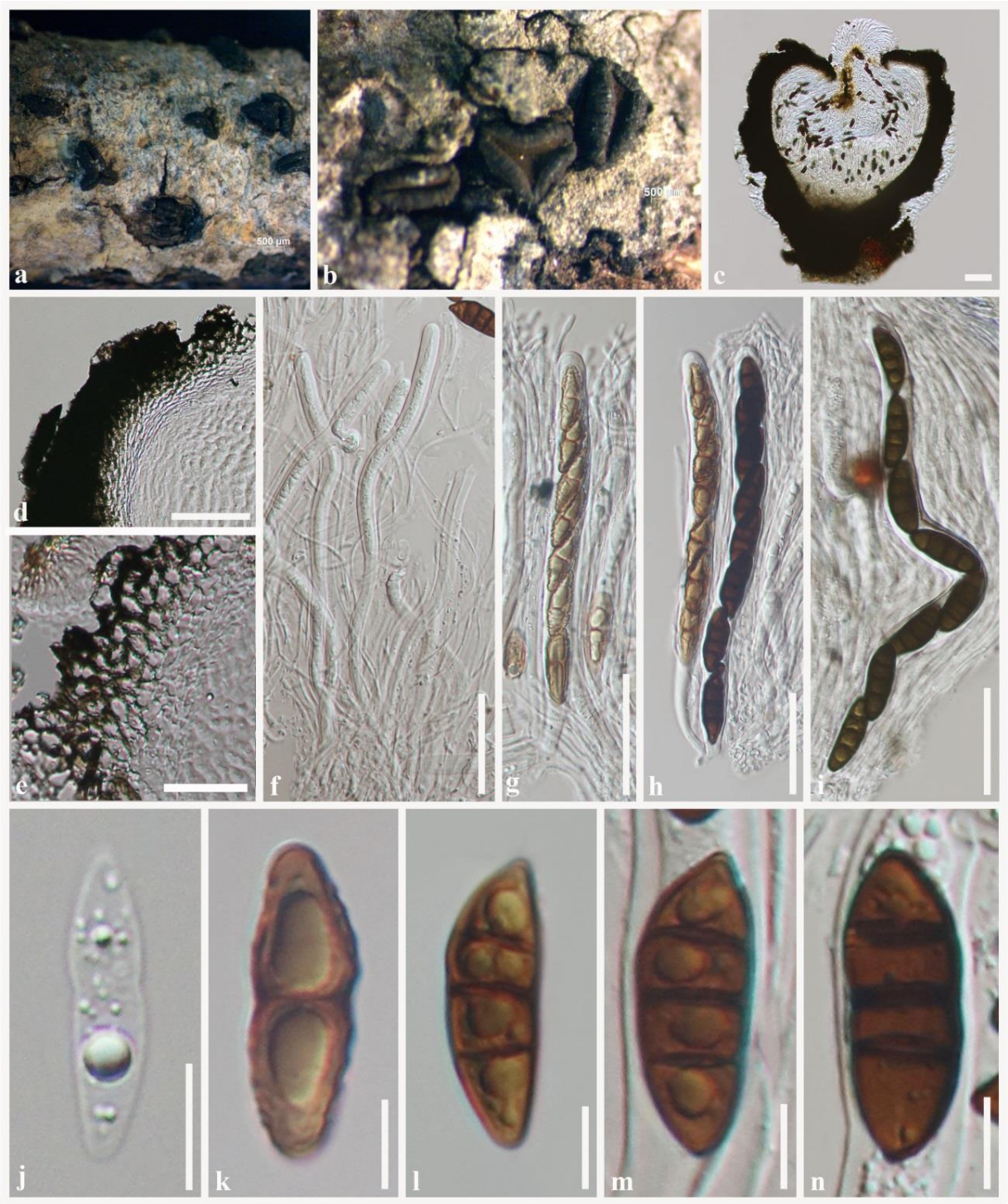

Fig. 2 – *Rhytidhysteron magnoliae* (MFLU 18-1021, holotype). a, b Appearance of hysterothecia on the host surface. c Vertical section through hysterothecium. d, e Exciple. f Pseudoparaphyses with immature asci. g–i Asci. j–n Ascospores. Scale bars: c = 80 µm, d = 50 µm, e = 20 µm, f–i = 50 µm, j–n = 10 µm.

Material examined – China, Taiwan region, Chiayi, Fanlu Township area, Dahu forest, decaying wood of *Morus australis* Poir. (Moraceae), 18 September 2017, D.S. Tennakoon, SL001 (MFLU 17-0788, new host record).

Notes – *Rhytidhysteron thailandicum* was introduced by Thambugala et al. (2016) based on both morphology and phylogenetic analyses. As both morphological characteristics and molecular data examined largely overlap with those of *R. thailandicum* (MFLUCC 14-0503), we report our collection (MFLU 17-0788) as a new host record of *R. thailandicum* from decaying wood of *Morus*

australis (Moraceae) in Taiwan region of China. Our collection shares similar morphological characteristics with *R. thailandicum* (MFLU 14-0607) in having semi-immersed to superficial, coriaceous, apothecioid hysterothecia, cylindrical, short pedicellate asci and ellipsoidal to fusoid, (1–)3-septate, reddish-brown ascospores (Thambugala et al. 2016, Hyde et al. 2017). According to our combined multi-gene (LSU, ITS, SSU and *tef1*) phylogenetic analyses, our collection nested together with *R. thailandicum* (MFLUCC 14-0503). This is the first record of *R. thailandicum* on *Morus australis*.

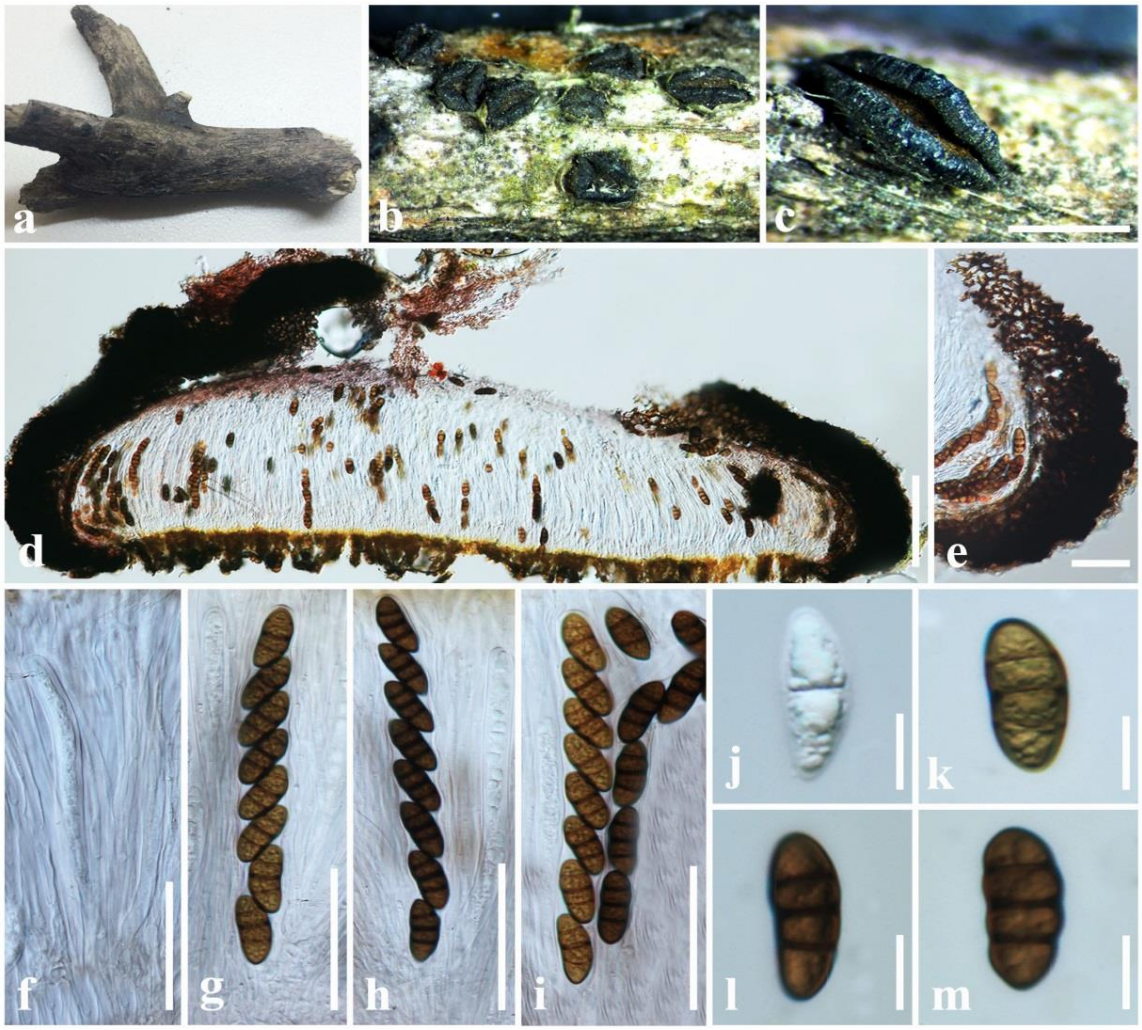

Fig. 3 – *Rhytidhysteron thailandicum* (MFLU 17-0788, a new host record). a Decaying wood specimen of *Morus australis*. b Appearance of hysterothecia on the host surface. c Close-up of hysterothecium. d Vertical section through hysterothecium. e Exciple. f Pseudoparaphyses with immature asci. g–i Asci. j–m Ascospores. Scale bars: c = 500 µm, d = 200 µm, e–i = 50 µm, j–m = 10 µm.

Discussion

Hysteriaceous ascomycetes are an interesting group of fungi, mainly occurring on twigs or bark of various woody and herbaceous plants in terrestrial and aquatic environments worldwide. Hysteriaceae is one of the most diverse families in the order Hysteriales. Currently, 13 genera are accepted in Hysteriaceae, viz. *Actidiographium* Lar.N. Vassiljeva, *Gloniella* Sacc., *Gloniopsis* De Not., *Hysterium* Tode, *Hysterobrevium* E. Boehm & C.L. Schoch, *Hysterocarina* H. Zogg 1949, *Hysterodifractum* D.A.C. Almeida, Gusmão & A.N. Mill., *Hysteroglonium* Rehm ex Lindau, *Oedohysterium* E. Boehm & C.L. Schoch, *Ostreichnion* Duby, *Pseudoscypha* J. Reid & Piroz., *Psiloglonium* Höhn. and *Rhytidhysteron* (Hongsanan et al. 2020).

Rhytidhysteron species seem to have a diverse distribution since they have been recorded from both temperate and tropical regions in Australia, Brazil, Colombia, Cuba, India, New Zealand, Taiwan, Thailand and Venezuela (Thambugala et al. 2016, Doilom et al. 2017, Kumar et al. 2019, Farr & Rossman 2020). This study provides the first geographical records of *Rhytidhysteron* species in China. Apart from being cosmopolitan, it appears that this genus is phylogenetically highly diverse given that recent studies have revealed a number of new species. For instance, *Rhytidhysteron neorufulum* Thambug. & K.D. Hyde and *R. thailandicum* were introduced by Thambugala et al. (2016) and Doilom et al. (2017) introduced *R. tectonae* Doilom & K.D. Hyde. Subsequently, *R. columbiense* Soto-Medina & Lücking was introduced by Soto-Medina and Lücking (2017) only based on morphological studies and *R. mangrovei* Vin. Kumar & K.D. Hyde was introduced by Kumar et al. (2019), based on morphological studies and molecular data. In this study, we provide morphological characteristics and phylogenetic data for another new species of *Rhytidhysteron*; *R. magnoliae* collected from *Magnolia grandiflora* (Magnoliaceae).

The host specificity of *Rhytidhysteron* species has yet to be studied, despite having been collected from various plant families (i. e. Acanthaceae, Annonaceae, Apocynaceae, Bignoniaceae, Capparaceae, Fabaceae, Lamiaceae, Rutaceae) (Thambugala et al. 2016, Doilom et al. 2017, Kumar et al. 2019, Farr & Rossman 2020). Interestingly, we found *R. thailandicum* from *Morus australis* is the first *Rhytidhysteron* record from the plant family Moraceae. This study incorporates both morphological and multigene phylogenetic approaches (LSU, ITS, SSU and *tef1*) and provides insights into the taxonomic novelties of the genus *Rhytidhysteron*. It is also noted that phylogeny recovered herein, agreed with previously established taxa in that *Rhytidhysteron* should be placed within the Hysteriales (Thambugala et al. 2016, Doilom et al. 2017, Kumar et al. 2019, Dayarathne et al. 2020). The findings of this study expand the *Rhytidhysteron* taxa up to 22 species. However, only nine *Rhytidhysteron* species including the new species introduced in the present study have molecular data. Therefore, it is essential to use sequence data for clarifying the phylogenetic affinity of *Rhytidhysteron* species in future studies (especially for the type species, *R. brasiliense* Speg.). Thus, it is necessary to collect more species of *Rhytidhysteron* and similar taxa in different geographic regions and hosts, isolate them into cultures, describe their morphology, analyze their DNA sequences and investigate their phylogenetic relationships for better identification and classification.

Acknowledgements

This work was supported by grants from Chiang Mai University and TRF Research-Team Association Grant (RTA5880006). Samantha C. Karunarathna thanks CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2018PC0006) and National Science Foundation of China (NSFC) for funding his research work under project code 31851110759. We wish to thank the Key Research Program of Frontier Sciences, CAS (grant no. QYZDY-SSWSMC014" and "973 key project of the National Natural Science Foundation of China (grant no. 2014CB954101). Kevin D. Hyde thanks the grant Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion (grant no: RDG6130001). Yunnan Provincial Department of Human Resources and Social Security (grant no. Y836181261) and the National Nature Science Foundation of China (NSFC; grant no. 31850410489) are also acknowledged. Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Science, Kunming is thanked for supporting molecular phylogenetic experiments of this study. Dr. Shaun Pennycook is thanked for nomenclatural clarification of the new species.

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