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Additions to the genus *Rhytidhysteron* in Hysteriaceae

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Abstract – *Rhytidhysteron* (Hysteriaceae) species are widely distributed as saprobes or weak pathogens on a wide range of woody plants. In this study, several *Rhytidhysteron* collections were made in northern Thailand and multi-gene phylogenetic analyses were used to resolve the phylogenetic boundaries of species. Two novel species, *R. thailandicum* and *R. neorufulum* are introduced, based on morphological traits and multi-gene phylogeny. The genus is revised with a key to species.

Hysteriales / Phylogeny / new species / multi-gene phylogeny / Thailand

INTRODUCTION

Hysteriaceous fungi are primarily lignicolous or corticolous and are saprobes on wide host range in temperate and tropical regions (Boehm *et al*., 2009a, b; Murillo *et al*., 2009; Hyde *et al*., 2013; de Almeida *et al*., 2014; Liu *et al*., 2015; Yacharoen *et al*., 2015). The family Hysteriaceae was introduced by Chevallier (1826) as "Hysterineae" and this family has been treated with different genera by various authors (Zogg 1962; von Arx & Müller 1975; Kirk *et al*., 2001; Eriksson 2006; Lumbsch and Huhndorf 2010). Recent molecular phylogenetic analyses placed Hysteriaceae in Hysteriales, *Pleosporomycetidae* (Boehm *et al*., 2009a, b; Hyde *et al*., 2013; Wijayawardene *et al*., 2014). Hyde *et al*. (2013) and Wijayawardene *et al*. (2014) accepted 13 genera including *Actidiographium* Lar.N. Vassiljeva, *Coniosporium* Link, *Gloniella* Sacc., *Gloniopsis* De Not., *Hysterium* Pers., *Hysterobrevium* E. Boehm & C.L. Schoch, *Hysterocarina* H. Zogg, *Hysteropycnis* Hilitzer, *Oedohysterium* E. Boehm & C.L. Schoch, *Ostreichnion* Duby, *Psiloglonium*

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Höhn. *Rhytidhysteron* Speg. and *Sphaeronaema* Fr in the family, while de Almeida *et al*., (2014) introduced a new genus *Hysterodifractum*. Hysteriaceae is characterized by carbonaceous, immersed to erumpent to entirely superficial hysterothecia, distinctly navicular in outline, bearing a pronounced, longitudinal slit, running the length of the long axis, bitunicate asci and hyaline to pigmented, 1 to multi-septate, or muriform ascospores (Boehm *et al*., 2009a, b; Hyde *et al*., 2013; de Almeida *et al*., 2014).

Rhytidhysteron was introduced by Spegazzini (1881) to accommodate *R. brasiliense* Speg. and *R. viride* Speg., without a type species being designated. Subsequently, Clements & Shear (1931) designated *R. brasiliense* as the type species (Samuels & Müller 1979; Yacharoen *et al*., 2015). Later, several species were added to the genus based on morphology (Spegazzini 1921; Sharma & Rawla 1985; Barr 1990; Magnes 1997) and currently there are 17 epithets listed in Index Fungorum (2016). Various authors have classified *Rhytidhysteron* within the family Patellariaceae (Barr 1987; Kutorga & Hawksworth 1997; Eriksson 2006; Lumbsch and Huhndorf 2010). However, recent multi-gene phylogenetic studies have shown that *Rhytidhysteron* should be placed in Hysteriaceae (Boehm *et al*., 2009a, b, de Almeida *et al*., 2014; Wijayawardene *et al*., 2014). The genus *Rhytidhysteron* is characterized by closed and navicular ascomata, later opening by a longitudinal slit to become irregularly apothecioid at maturity and heavily pigmented, and with thick-walled ascospores (Boehm *et al*., 2009b). Aposphaeria-like' or diplodia-like' asexual morphs have been reported for *Rhytidhysteron* (Samuels & Müller 1979).

In this study, we made several *Rhytidhysteron* collections on dead woody branches in northern Thailand. We used morphological characters and multi-gene molecular analyses to resolve species in the genus.

MATERIALS AND METHODS

Collection of samples, isolation and morphological examination

Fresh specimens were collected from dead wood in northern Thailand and samples were grown on potato dextrose agar (PDA). Isolates were derived via single spore isolation following the protocols of Chomnunti *et al*., (2014). Germinating spores were transferred to PDA and incubated at 25°C in the dark. Cultural characteristics, such as mycelium colour, shape, texture and growth rate were determined. Type and voucher specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, and New Zealand Fungal & Plant Disease Collection (PDD), while cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in International Collection of Microorganisms from Plants (ICMP) Landcare Research, New Zealand.

Specimens were observed and examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters of the taxon were examined with a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. Observations and photographs were made from material mounted in water and Indian ink was added to water mounts to show the presence of gelatinous sheaths around the ascospores. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3

Extended version 10.0 software. Faces of fungi numbers and Index Fungorum numbers are provided as detailed in Jayasiri *et al*. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelium was grown on PDA at 25°C for 21 days. Extraction of genomic DNA from mycelia and sequencing of PCR products were carried out following the method of Thambugala *et al*. (2015). Four partial gene portions were amplified in this study including LROR and LR5 (Vilgalys and Hester, 1990) for the nuclear ribosomal large subunit (LSU), ITS4 and ITS5 (White *et al*., 1990) for the internal transcribed spacer (ITS), EF1-983F and EF1-2218R (Carbone and Kohn 1999) for translation elongation factor 1-alpha (EF1-α) and NS1 and NS4 (White *et al*., 1990) for the nuclear ribosomal small sub unit (SSU). The amplifications were performed in 25 µL of PCR mixtures containing 9.5 µL ddH_2O, 12.5 µL 2×PCR Master Mix (TIANGEN Co., China), 1 µL of DNA template, 1 µL of each primer (10 µM). The amplification reactions were performed and analyzed as described by Thambugala *et al*., (2015).

Phylogenetic analyses

The phylogenies of the new strains were determined using two analyses: The first phylogenetic analysis was carried out based on a combined data set of LSU, SSU and EF1-α sequence data of 80 isolates belonging to *Hysteriaceae* (Table 1) in order to show the placement of the genus *Rhytidhysteron* in the family, with *Delitschia winteri* (CBS 225.62) as the outgroup taxon. The second phylogenetic analysis based on a combined data set of LSU, SSU, EF1-α and ITS sequence data were done only for the species in the genus *Rhytidhysteron*, using 19 isolates and *Gloniopsis praelonga* (CBS 112415) as the outgroup taxon. All newly generated sequences are deposited in GenBank. Isolates and GenBank accession numbers used in these analyses are listed in Table 1.

SeqMan v. 7.0.0 (DNASTAR, Madison, WI) was used to compute consensus sequences. The sequence data were aligned and combined using Bioedit (Hall 1999) and MEGA 5.0 (Tamura *et al.,* 2011). Alignments were checked and manual improved manually where necessary. The maximum likelihood analysis was performed using RAxML v 7.4.2 Black Box software (Stamatakis *et al*., 2008). The general time reversible model (GTR) including estimation of invariable sites was applied with a discrete gamma distribution with four rate classes. The best scoring trees were selected for the each analysis and illustrated with MEGA 5.0 and with Adobe Illustrator CS3.

RESULTS

Phylogenetic analyses

The first analysis was based on a combined data set of LSU, SSU and EF1-α sequence data belonging to Hysteriaceae and the best scoring RAxML tree is shown in Fig. 1. The outgroup taxon, *Delitschia winteri* (CBS 225.62) is clearly

Table 1. GenBank and culture collection accession numbers of species included in the phylogenetic study. Newly generated sequences are shown in bold

Species	*Isolate no.**	*GenBank Accession no.*			
		LSU	*SSU*	*EF*	*ITS*
Delitschia winteri	CBS 225.62	DQ678077	DQ678026	DQ677922	
Gloniopsis arciformis	GKM L166A	GU323211	GU323180	_	_
Gloniopsis praelonga	CBS 112415	FJ161173	FJ161134	FJ161090	_
	CMW 19983	FJ161193	FJ161152	_	_
	CBS 123337	FJ161195	FJ161154	FJ161103	_
Gloniopsis sp.	MFLUCC 14-0581	KU377563	KU377568	KU497491	_
Gloniopsis subrugosa	GKM 1214	GQ221895	_	GU397336	_
	CBS 123346	FJ161210	FJ161170	_	_
	GKM 1010	GQ221891	_	_	_
	SMH 557	GQ221896	_	GU397337	_
Graphyllium caracolinensis	HUEFS 42838	KF914914	_	_	_
Hysterium angustatum	GKM5211	GQ221906	_	_	_
	CMW 20409	FJ161194	FJ161153	_	_
	SMH 5216	GQ221908	_	GQ221933	_
	GKM 243A	GQ221899	_	_	_
	CBS 123334	FJ161207	FJ161167	FJ161111	_
	CBS 236.34	FJ161180	GU397359	FJ161096	_
	ANM 85	GQ221898	_	_	_
Hysterium barrianum	ANM 1495	GQ221885	GU323182	_	_
	ANM 1442	GQ221884	GU323181	_	_
Hysterium hyalinum	CBS 237.34	FJ161181	FJ161141	_	_
Hysterium pulicare	ANM 1455	GQ221904	_	GQ221932	_
Hysterium vermiforme	GKM 1234	GQ221897	_	_	_
Hysterobrevium constrictum	SMH 5211.1	GU397361	GQ221905	_	_
Hysterobrevium mori	SMH 5286	GU397345	_	_	_
	SMH 5273	GQ221910	_	GQ221936	_
	CBS 123564	FJ161198	FJ161158	FJ161106	_
	CBS 123336	FJ161204	FJ161164	_	_
	CBS 123563	FJ161196	FJ161155	FJ161104	_
	CBS 123335	FJ161202	FJ161162	_	_
	GKM 1013	GU397344	_	GU397338	_
Hysterobrevium smilacis	GKM 426N	GQ221901	_	_	_
	CMW 18053	FJ161191	FJ161150	_	_
	SMH 5280	GQ221912	GU323183	_	_
	CBS 200.34	FJ161177	FJ161138	_	_
	CBS 114601	FJ161174	FJ161135	FJ161091	_
Hysterodifractum partisporum	CCMB 252/2012	KF914916	_	_	_
Hysterographium flexuosum	GKM 1262c	GQ221886	_	GQ221935	_
Hysterographium fraxini	CBS 242.34	FJ161189	_	_	_
	CBS 109.43	FJ161171	FJ161132	FJ161088	_

Oedohysterium insidens	ANM 1443	GQ221882	GU323190	_	_
	CBS 238.34	FJ161182	FJ161142	FJ161097	_
Oedohysterium sinense	CBS 123345	FJ161209	FJ161169		_
	EB 0339	GU397348	GU397364	GU397339	_
Ostreichnion centramurum	MFLUCC 12-0802	KM272256	KM272257	KM277819	_
Ostreichnion curtisii	CBS 198.34	FJ161176	FJ161137	FJ161093	_
Ostreichnion sassafras	CBS 322.34	FJ161188	FJ161148	_	_
Psiloglonium araucanum	CMW 18760	FJ161192	FJ161151	_	_
	CBS 112412	FJ161172	FJ161133	FJ161089	_
	CMW 17941	FJ161190	FJ161149	_	_
Psiloglonium clavisporum	CBS 123340	FJ161205	FJ161165	_	_
	CBS 123341	FJ161206	FJ161166	_	_
	CBS 123338	FJ161197	FJ161156	_	_
	GKM L172A	GU323204	GU323192	_	_
	GKM 344A	GQ221889	GU397365	_	_
Psiloglonium colihuae	MFLUCC 11-0178	KP744511	_	_	_
Psiloglonium macrosporum	MFLUCC 13-0448	KU243049	_	_	KU243048
Psiloglonium multiseptatum	MFLUCC 11-0164	KP744512	KP753969	_	_
Psiloglonium sasicola	MFLUCC 10-0565	KP744513.	_	_	_
Psiloglonium simulans	CBS 206.34	FJ161178	FJ161139	FJ161094	_
	ANM 1557	GQ221873	_	GQ221920	_
Rhytidhysteron hysterinum	EB 0351	GU397350	_	GU397340	_
Rhytidhysteron neorufulum	**MFLUCC 13-0221**	**KU377567**	**KU377572**	**_**	**KU377562**
	MFLUCC 13-0216	**KU377566**	**KU377571**	**KU510400**	**KU377561**
	GKM 361A	GQ221893	GU296192	GU349031	_
	MFLUCC 12-0529	KJ526124	KJ546127	_	KJ546122
	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	**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	MFLUCC 12-0530	KJ526125	KJ546128	_	KJ546123
	MFLUCC 14-0503	**KU377564**	**KU377569**	**KU497490**	**KU377559**

*ANM: A.N. Miller; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CCMB: Bahia Culture Collection of Microorganisms; CMW: Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Republic of South Africa; E.W.A. Boehm; GKM: G.K. Mugambi; HUEFS, Herbarium of the State University of Feira de Santana; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; SMH: S.M. Huhndorf.

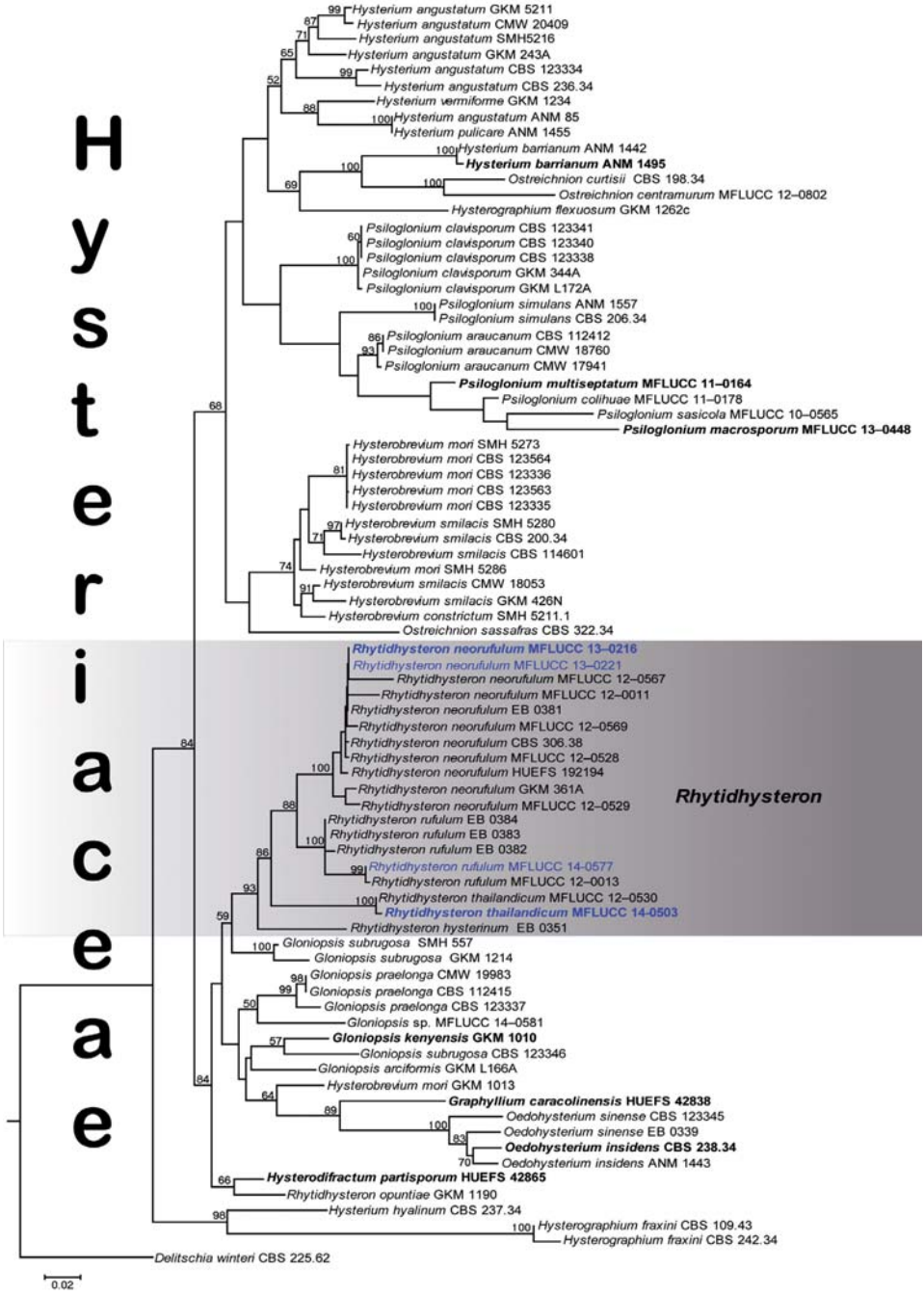

Fig. 1. Maximum Likelihood (ML) tree from analysis of combined dataset of LSU, SSU and EF1-α sequence data of *Hysteriaceae*. Bootstrap support values equal or greater than 50% are given above and below the nodes. The tree is rooted to *Delitschia winteri* (CBS 225.62). Newly generated sequences are in blue. Ex-type strains are in bold.

excluded from the family. Species residing in Hysteriaceae, Hysteriales were positioned on the tree, and represented the genera *Gloniopsis*, *Hysterium*, *Hysterobrevium*, *Hysterodifractum*, *Ostreichnion*, *Oedohysterium*, *Psiloglonium* and *Rhytidhysteron*. All *Rhytidhysteron* isolates, except *Rhytidhysteron opuntiae* (GKM 1190) clustered in a separate clade in *Hysteriaceae,* with strong bootstrap support (93%). *Rhytidhysteron opuntiae* (GKM 1190) forms a separate subclade with *Hysterodifractum partisporum* (HUEFS: 42865).

The second analysis was based on a combined data set of LSU, SSU, EF1-α and ITS sequence data belonging to the isolates in the *Rhytidhysteron* clade (Fig. 1) and the best scoring RAxML tree is shown in Fig. 2. The tree is rooted to *Gloniopsis praelonga* (CBS 112415). *Rhytidhysteron* isolates separated into four distinct subclades (A-D). MFLUCC 13-0216 and 13-0221 clustered in the Clade A (*R. neorufulum*) with eleven isolates of *R. rufulum* (CBS 306.38, EB 0381, GKM 361A, HUEFS 192194, MFLUCCC 12-0011, 12-0528, 12-0567, 12-0569, 12-0529). MFLUCC 14-0577 grouped in the Clade B (*R. rufulum*) with four isolates of *R. rufulum* (EB 0382, 0383, 0384 and MFLUCC 12-0013). MFLUCC 12-0530 formed Clade C (*R. thailandicum)* together with MFLUCC 14-0503. *Rhytidhysteron hysterinum* (EB 0351) grouped as a separate Clade D (Fig. 2).

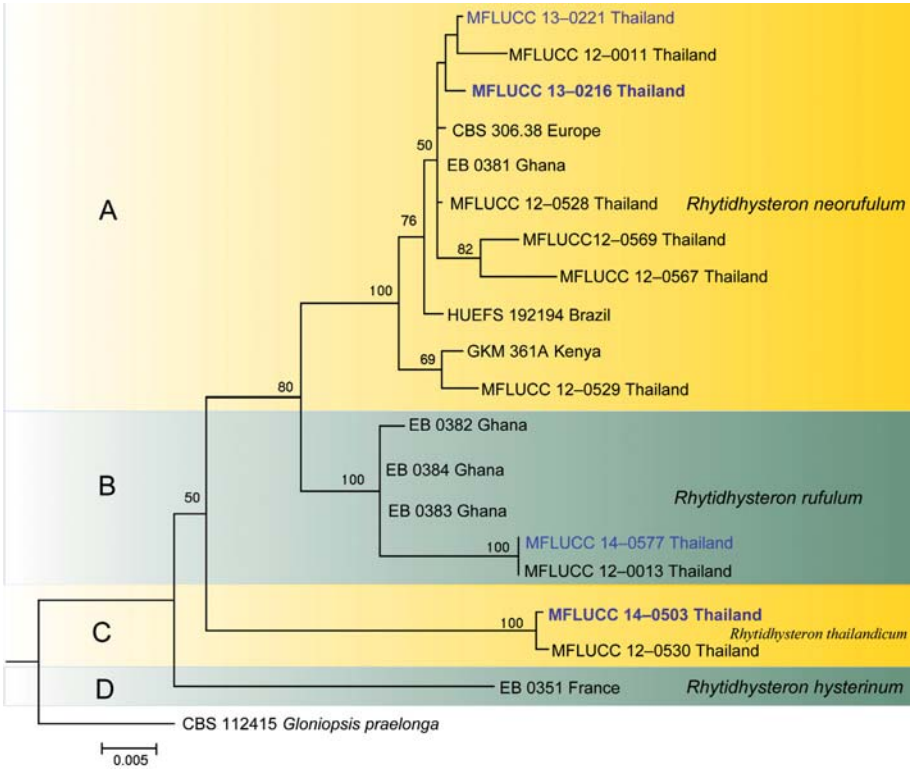

Fig. 2. Maximum Likelihood (ML) tree from analysis of combined LSU, SSU, EF1-α and ITS sequence data of *Rhytidhysteron* species. Bootstrap support values equal or greater than 50% are given above and below the nodes. Culture accession numbers are mentioned along with the country of origin. The tree is rooted to *Gloniopsis praelonga* (CBS 112415). Newly generated sequences are indicated in blue and type sequences are in bold.

Notes: *Rhytidhysteron rufulum* (Spreng.) Speg. was introduced by Spegazzini (1921) based on *Hysterium rufulum* Spreng., while Samuels & Müller (1979) synonymized *R. brasiliense*, the type species of *Rhytidhysteron* and *R. viride* Speg. under *R. rufulum* (Spreng.) Speg. and accepted only two species in the genus, *R. rufulum* and *R. hysterinum* (Dufour) Samuels & E. Müll. However, Samuels & Müller (1979) observed only the external morphology of the type specimen of *R. brasiliense* as it contained only one old ascoma, while they pointed out that the specimen of *R. viride* was immature and had no ascospores. They also did not observe the type specimen of *R. rufulum* and considered published descriptions. Later, Kutorga & Hawksworth (1997) designated a neotype for *R. rufulum,* as the original material was not extant. Kutorga & Hawksworth (1997) also observed the microscopic characters of the remaining old ascoma on the type material of *R. brasiliense*. This observation revealed that both *R. rufulum* and *R. brasiliense* have the same exciple, hypothecium and hamathecium morphology, but there is a significant difference in ascospore size, as *R. brasiliense* has significantly larger ascospores ((38-)40-44(-47) × (15-)17-19(-21) µm). Moreover, they did not discuss the striations on the surface of ascomata and considered ascomata with or without striations on the surface, as *R. rufulum*. Kutorga & Hawksworth (1997) designated a neotype for *R. rufulum* (NY 6149) and their illustrations clearly show the striations on the surface of ascomata in the neotype. We believe that *R. rufulum* and *R. brasiliense* are different species. Unfortunately none of our collections has a similar morphology as *R. brasiliense*. Therefore, re-collection of *R. brasiliense* from the same host and location and epitypification (Ariyawansa *et al*. 2014) is essential.

Morphological differences in ascomata and measurements of asci and ascospores are apparent in recent collections of *R. rufulum* (Boehm *et al*., 2009b; Murillo *et al*., 2009; Yacharoen *et al*., 2015). ITS rDNA data plus chemical and morphological analyses of Murillo *et al*., (2009) concluded that their Costa Rican collections of *R. rufulum* clustered in four lineages (clade I-IV) and they suggested further molecular and morphological studies were needed to establish their taxonomy. We did not include the isolates of Murillo *et al*., (2009) in to our combined gene phylogenetic analyses as only ITS sequence data are available in the GenBank. However, phylogenetic analysis of all available ITS sequence data (including the strains of Murillo *et al*., 2009) of *R. rufulum* showed similar results as in Murillo *et al*., (2009) and MFLUCC 14-0503 and MFLUCC 12-0530 grouped in a different lineage (data not shown). Furthermore, Clade I in the phylogenetic tree of Murillo *et al.* (2009) comprised *R. rufulum* specimens, lacking striations on the hysteriothecium. In the present study we show that striations on the surface of hysterothecia are an important character to distinguish between species.

Rhytidhysteron rufulum is widely distributed in tropical countries as a saprobe or weak pathogen on a wide range of woody plants (Murillo *et al*., 2009; Almeida *et al*., 2014a, b; Yacharoen *et al*., 2015). In the present combined phylogenetic analyses (Fig. 1), we included 14 strains of *R. rufulum* used in previous studies, from different countries (Boehm *et al*., 2009b; de Almeida *et al*., 2014; Yacharoen *et al*., 2015) together with our collections from Thailand. These strains separated in three distinct Clades (Figs 1 & 2). Strains MFLUCC 13-0216 and 13-0221 clustered in the Clade A, while, MFLUCC 14-0577 grouped in the Clade B. Strain MFLUCC 12-0530 formed a separate Clade (C), together with strain MFLUCC 14-0503 (Fig. 2). These three clades have different morphological characters and should be treated as three distinct species, based on both morphological and phylogenetic analyses. The strains in Clade B are typical of *R. rufulum* (Kutorga

& Hawksworth 1997) and therefore this clade is named *R. rufulum*. The main characteristic feature of the species in the Clade B is hysterothecia with distinct striations, perpendicular to the long axis (Boehm *et al*., 2009b), which are lacking in the species in Clades A and C. The overall morphology of MFLUCC 14-0577 fits with the neotype description in Kutorga & Hawksworth (1997). Therefore, here we designate our specimen (MFLUCC 14-0577) as a reference specimen of *R. rufulum* and Clade B is named as *R. rufulum*.

Clade A comprises 11 strains and a new epithet is required for this clade which is morphologically and phylogenetically different from *R. rufulum* Clade (B) and Clade C. Therefore, a new species *Rhytidhysteron neorufulum* is introduced here and we select MFLU 14-0609 as the holotype. *Rhytidhysteron neorufulum* mainly differs from *R. rufulum* in having smooth ascomata without striations and a one-layered exciple, comprising dark brown to brown cells of *textura angularis*. However, *R. neorufulum* Clade (A) may yet be shown to be a species complex with species other than *R. neorufulum*. Therefore a detailed morphological re-examination of other specimens with sequence data is required to confirm this.

Clade C represents MFLUCC 14-0503 and MFLUCC 12-0530 which is a new species of *Rhytidhysteron*. It differs from both *R. rufulum* and *R. neorufulum* in having smaller, semi-immersed to superficial, coriaceous hysterothecia, with the margin folded over the pseudothecium. The exciple is composed of dark brown to hyaline cells of *textura angularis*. The asexual morph reported for this species is aposphaeria-like', which is typical of the genus. Clade D consists of *R. hysterinum* (Dufour) Samuels & E. Müll. and differs from other *Rhytidhysteron* species in having 1-septate ascospores (Kutorga & Hawksworth 1997).

Rhytidhysteron Speg. [as "Rhytidhysterion"], Anal. Soc. cient. argent. 12(4): 188 (1881)

For other possible synonyms see Index Fungorum

Facesoffungi number: FoF 00369

Saprobic or weakly parasitic on living or dead wood in terrestrial habitats. Sexual morph: *Ascomata* hysterothecial, solitary to aggregated, superficial, black, carbonaceous to coriaceous, elliptic or irregular in shape, with lenticular or irregular opening when wet, perpendicularly striate or not along the long axis, black, red or yellow at the center, when dry folded at the margin, forming an elongate slit. *Exciple* composed of 1-2-layers, outer layer comprising dark brown to black, cells of *textura angularis* or *textura globosa*, inner layer of hyaline to lightly pigmented cells of *textura angularis* to *textura prismatica*. *Hamathecium* comprising dense, septate, pseudoparaphyses, branched and forming a dark epithecium above asci, fused and slightly swollen at the apex, enclosed in a gelatinous matrix. *Asci* 6-8-spored, bitunicate, cylindrical, rounded at the apex, with a distinct ocular chamber. *Ascospores* uni-seriate, slightly overlapping, ellipsoidal to fusiform, slightly rounded or pointed at both ends, 1-3-septate, constricted at the central septum, reddish-brown to brown, without a mucilaginous sheath. Asexual morph: Coelomycetous. *Conidiomata* pycnidial, solitary or aggregated, black, globose to subglobose. *Pycnidial wall* thin-walled, composed of brown to lightly pigmented, cells arranged in a *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, cylindrical to subcylindrical or ampulliform, with a truncate apex on conidial secession, short, smooth, hyaline. *Conidia* globose to subglobose, hyaline, smooth-walled.

Type species: *Rhytidhysteron brasiliense* Speg.

Rhytidhysteron brasiliense Speg., Anal. Soc. cient. argent. 12(4): 188 (1881) **Fig. 3**

Facesoffungi number: FoF 00370

Saprobic on decaying woody branches in terrestrial habitats. Sexual morph: *Ascomata* hysterothecial, scattered to gregarious, erumpent to nearly superficial, black, perpendicularly striate along the long axis. *Hamathecium* comprising dense, septate, pseudoparaphyses, branched and forming a dark epithecium above the asci, fused and slightly swollen at the apex and enclosed in a gelatinous matrix. *Asci* 230-250 × 20-30 µm (*fide* Spegazzini 1881), 8-spored, bitunicate, cylindrical, with short furcate pedicel, rounded at the apex, with a distinct ocular chamber. *Ascospores* (38-)40-44(-47) × (15-)17-19(-21) µm (*fide* Kutorga & Hawksworth 1997); 40-45 × 15-20 µm (*fide* Spegazzini 1881), uni-seriate, slightly overlapping, hyaline when immature, becoming brown, ellipsoidal to fusiform, rounded or slightly pointed at both ends, 3-septate, constricted at the central septum, smooth-walled. Asexual morph: "aposphaeria-like" or diplodia-like' (Spegazzini 1881, Kutorga & Hawksworth 1997).

Notes: *Rhytidhysteron brasiliense* shares similar external morphological characters with *R. rufulum*, but comprises significantly larger ascospores (Spegazzini 1881, Kutorga & Hawksworth 1997). There are no remaining ascomata on the holotype material, but only few slides. Therefore, we took photomicrographs from those slides to illustrate asci and ascospores. We rarely observed some ascospores with transverse septa and a brief description is given based on our observations and those of Spegazzini (1881).

Rhytidhysteron rufulum (Spreng.) Speg., Anal. Soc. cient. argent. 90(1-6): 177 (1921) [1920] **Fig. 4 g-l**

Facesoffungi number: FoF 01839

Basionym: *Hysterium rufulum* Spreng., K. svenska Vetensk-Akad. Handl. 46: 50 (1820)

Reference specimen: MFLU 14-0609

Saprobic on decaying woody stems in terrestrial habitats. Sexual morph: Ascomata 900-2350 µm long × 1134-1450 µm wide × 461-820 µm high ($\bar{x}$ = 1493 × 1298 × 619 µm, n = 8), hysterothecial, scattered to gregarious, superficial, black, carbonaceous to coriaceous, elliptic or irregular in shape, with lenticular or irregular opening when wet, perpendicularly striate along the long axis, black or red at the center, when dry folded at the margin, forming an elongate slit. *Exciple* 75-228 µm ($\bar{x}$ = 118 µm, n = 15) wide, composed of 2 layers, outer layer comprising dark brown to black, cells of *textura angularis* or *textura globosa*, inner layer of hyaline to lightly pigmented cells of *textura angularis* to *textura prismatica*. *Hamathecium* comprising 1-2 µm wide, dense, septate, pseudoparaphyses, branched and forming a dark epithecium above the asci, fused and slightly swollen at the apex and enclosed in a gelatinous matrix. *Asci* 150-250 × 11-16 µm ($\bar{x}$ = 202 × 13.5 µm, n = 15), 8-spored, bitunicate, cylindrical, with short furcate pedicel, rounded at the apex, with a distinct ocular chamber. *Ascospores* 28-36 × 9-13 µm ($\bar{x}$ = 31.9 × 11.1 µm, n = 40), uni-seriate, slightly overlapping, ellipsoidal to fusiform, slightly rounded or pointed at both ends, 1-3-septate, constricted at the central septum, hyaline to lightly pigmented when immature, reddish-brown to brown when mature, without a mucilaginous sheath. Asexual morph: "aposphaeria-like" or diplodia-like'.

Material examined: THAILAND, Chiang Mai Province, Mushroom Research Centre, Mae Taeng, on dead stem, 12 October 2014, D.A. Daranagama, KM 020 (MFLU 14-0608, **reference specimen designated here**); *ibid* (PDD), living culture MFLUCC 14-0577, ICMP 20750.

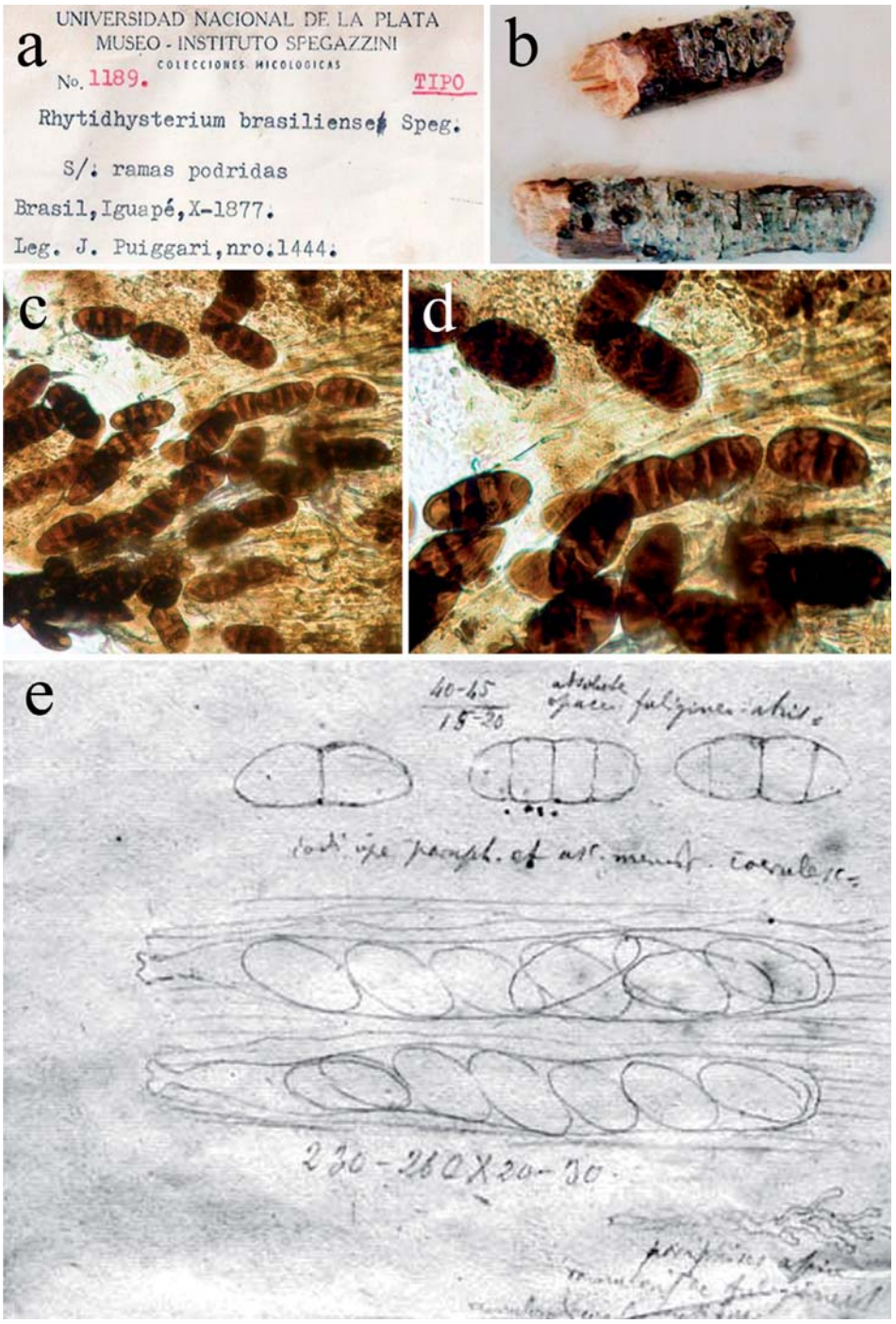

Fig. 3. *Rhytidhysteron brasiliense* (holotype LPS 1189) **a.** Material label. **b.** Holotype material. **c-d.** Asci and ascospores. **e.** Original drawing of Spegazzini (1881).

Rhytidhysteron neorufulum Thambugala & K.D. Hyde, **sp. nov.** **Fig. 4 a-f**

Index Fungorum number: IF551865; *Facesoffungi number*: FoF 01840

Etymology: The species epithet, neo (Lat., new), refers to the similarity to *Rhytidhysteron rufulum*.

Holotype: MFLU 14-0608

Saprobic on decaying woody stems and twigs. Sexual morph: *Ascomata* 835-1800 µm long × 600-1320 µm wide × 430-875 µm high ($\bar{x}$ = 1360 × 919 × 693 µm, n = 8), hysterothecial solitary to aggregated, superficial, black, coriaceous, elliptic 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* 64-160 µm ($\bar{x}$ = 118 µm, n = 15) wide, one layered, composed of dark brown to black, thick-walled cells of *textura angularis*. *Hamathecium* comprising 1.5-3 µm wide, dense, septate pseudoparaphyses, forming epithecium above the asci and enclosed in a gelatinous matrix. *Asci* 185-220 × 9.5-13 µm ($\bar{x}$ = 200 × 10.9 µm, n = 15), 8-spored, bitunicate, clavate to cylindrical, with a short, furcate pedicel, apically rounded, without a distinct ocular chamber. *Ascospores* 27-34 × (6.5-)7-10.6(-12.5) µm ($\bar{x}$ = 31.2 × 8.9 µm, n = 40), uni-seriate, slightly overlapping, ellipsoidal to fusiform, slightly rounded or pointed at both ends, 1-3-septate, constricted at the central septum, yellowish when immature, reddish-brown to brown when mature, without a mucilaginous sheath. Asexual morph: See notes.

Material examined: THAILAND, Chiang Rai Province, Chiangrai Horticultural Research Center, on dead stem, 3 November 2012, K. M. Thambugala, TL 003 (MFLU 14-0609, **holotype**); *ibid.* (PDD, **isotype**), ex-type living culture, MFLUCC 13-0216, ICMP 20754; Chiang Rai Province, Mae Fah Luang, on dead stem, 12 December 2012, K. M. Thambugala, TL 021 (MFLU 16-0026, paratype); living culture MFLUCC 13-0221; Chiang Mai Province, Mushroom Research Centre, on dead wood, 4 August 2012, Jayarama Bhat KA123/1 (MFLU 16-0027), living culture MFLUCC 12-0529; Phitsanulok Province, Sakunothayan Waterfall, on dead wood, 2 August 2012, Sinang Hongsanan KA122/1 (MFLU 16-0028, paratype), living culture MFLUCC 12-0528

Culture characteristics: Ascospores germinating on PDA within 24 h and germ tubes produced from one end or both ends. Colonies growing on PDA 3.5-4 cm diam. after 7 days at 25°C, irregular, raised, dense, surface white, reverse light brown, smooth surface with undulate edge.

Rhytidhysteron thailandicum Thambugala & K.D. Hyde **sp. nov.** **Fig. 5**

Index Fungorum number: IF551866; *Facesoffungi number*: FoF 01841

Etymology: Named after the country, where it was collected, Thailand.

Holotype: MFLU 14-0607

Saprobic on decaying wood and stems. Sexual morph: *Ascomata* 700-1200 µm long × 530-750 µm wide × 360-640 µm high ($\bar{x}$ = 885 × 645 × 527 µm, n = 5), hysterothecial, scattered or in small groups, semi-immersed to superficial, elongate and depressed, conchate, globose to subglobose, black, coriaceous, compressed at apex, opening by a longitudinal slit. *Exciple* 72-130 µm ($\bar{x}$ = 97.6 µm, n = 15) wide, one-layered, thick at the base, composed of several layers of brown to dark brown, thick-walled cells of *textura angularis*, becoming hyaline towards the inner layers and base. *Hamathecium* comprising 1-2 µm wide, dense, cellular, hyaline, septate, pseudoparaphyses, forming a dark epithecium above asci and enclosed in a gelatinous matrix. *Asci* 135-160 × 10.5-15 µm ($\bar{x}$ = 145 × 12.8 µm, n = 15), (3-)6-8-spored, bitunicate, clavate to cylindrical, short pedicellate and

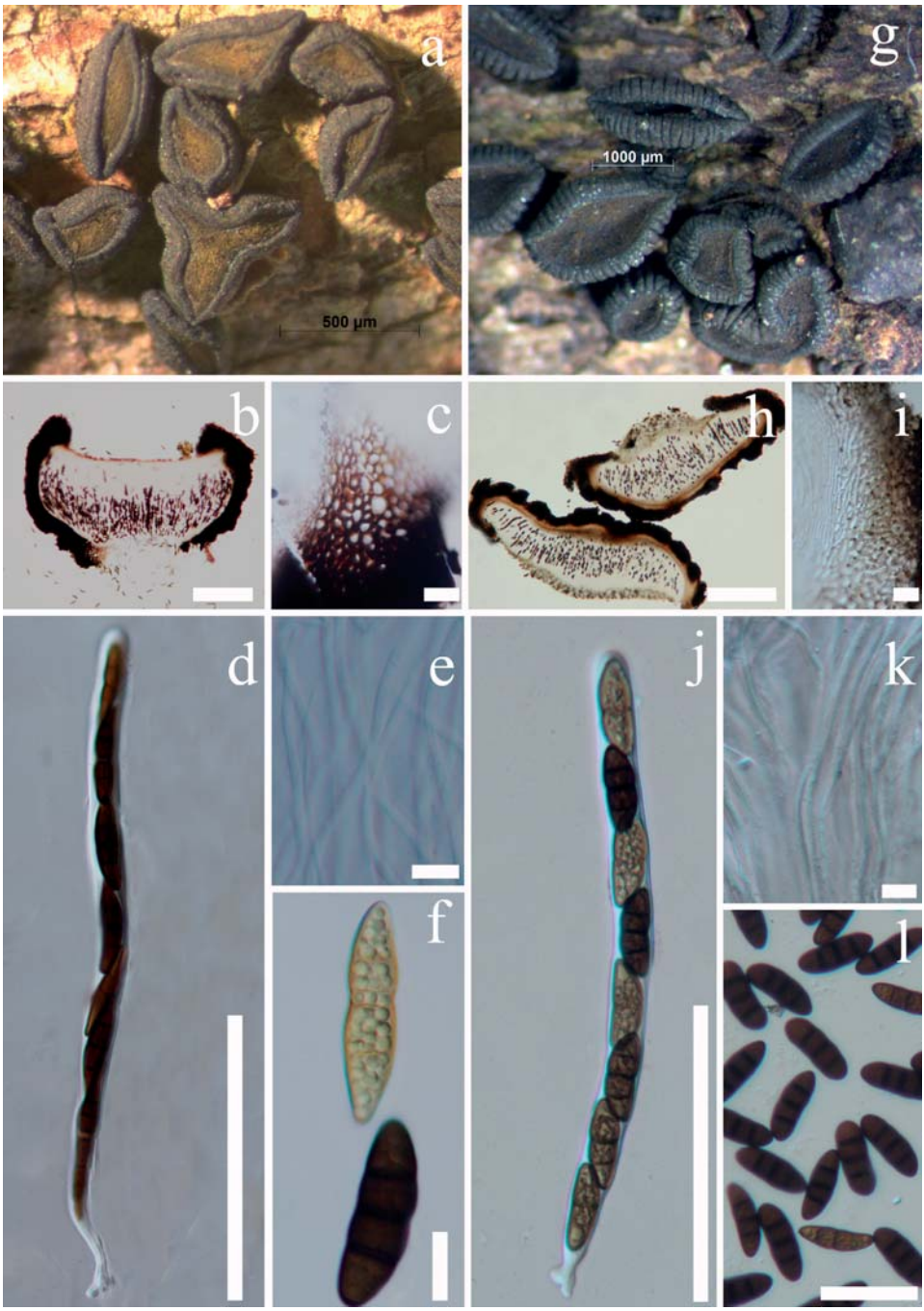

Fig. 4. **a-f.** *Rhytidhysteron neorufulum* (holotype) and **g-l.** *Rhytidhysteron rufulum* (MFLU 14-0609) **a, g.** Appearance of hysterothecia on host. b, h. Vertical sections of hysteriothecium c, i. Sections through exciple. e, k. Pseudoparaphyses. d, j. Asci. f, l. Ascospores. Scale bars: c = 250 µm, d = 25 µm, e = 400 µm, f = 20 µm, g, j = 100 µm , h, i, k = 10 µm, l = 40 µm.

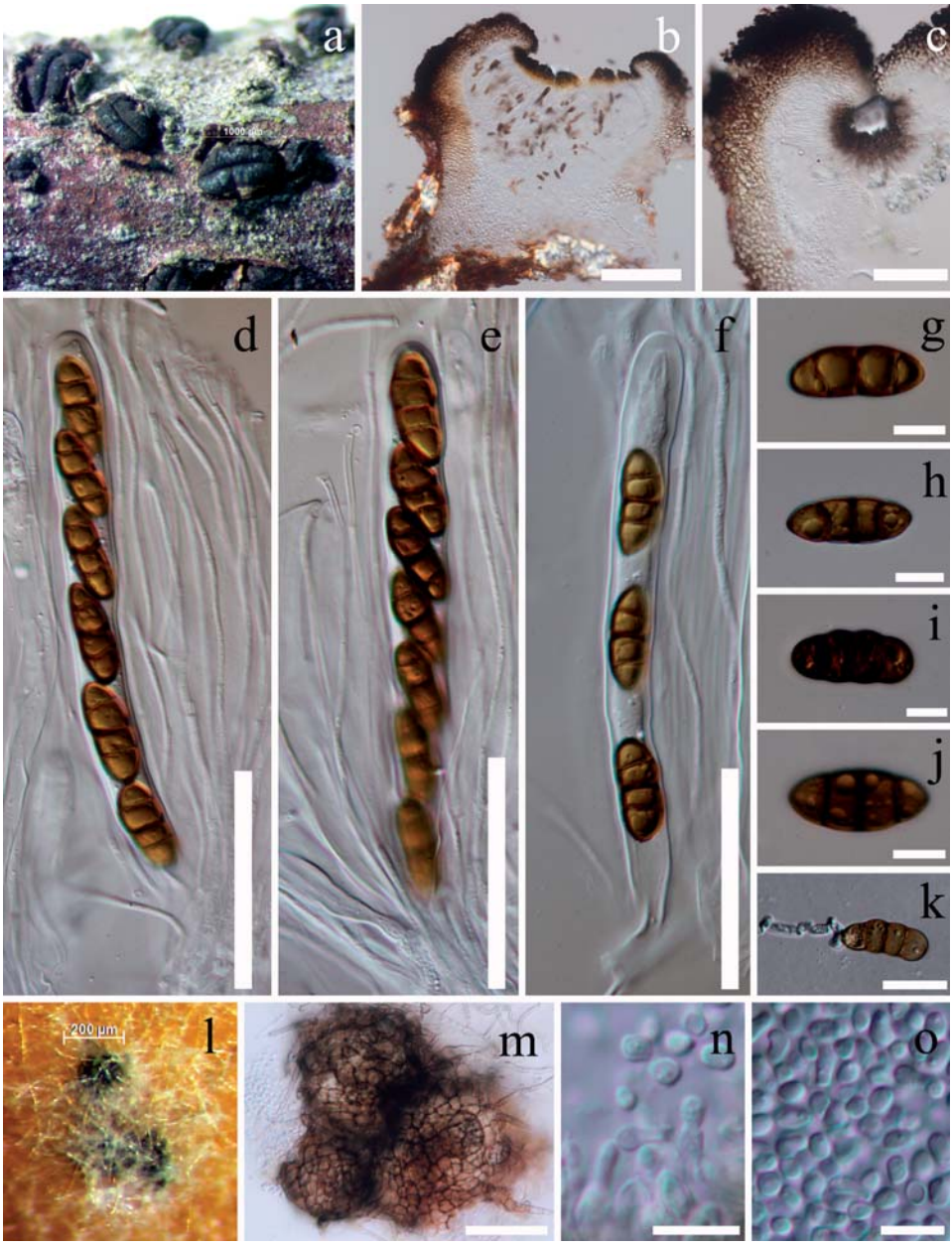

Fig. 5. *Rhytidhysteron thailandicum* (holotype) **a.** Appearance of hysterothecia on host. **b.** Vertical section through hysteriothecium **c.** Exciple. **d-f.** Pseudoparaphyses and asci. **g-j.** Ascospores. **k.** Germinating ascospore. **l.** Conidiomata on PDA. **m.** Squash mount of conidiomata. n. Conidiogenous cells. **o.** Conidia. Scale bars: b = 200 µm, c = 100 µm, d-f, m = 50 µm, g-k = 10 µm, n-o = 20 µm.

apically rounded with an ocular chamber. *Ascospores* 20-28(-31) × 7.5-12 µm ($\bar{x}$ = 24.5 × 9.5 µm, n = 20), partially overlapping, uni-seriate, 3-septate, ellipsoid or fusoid, yellowish to brown, guttulate, without a mucilaginous sheath. Asexual morph: Coelomycetous. *Conidiomata* 70-108 × 63-110 µm ($\bar{x}$ = 89 × 89.3 µm, n = 10), superficial on PDA, globose, solitary or aggregated, black, appearing in a mycelium mass, thin-walled, with a reticulate surface and wall cells arranged in a *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5-7 µm long × 2.5-3.2 µm wide ($\bar{x}$ = 5.9 × 3 µm, n = 10), phialidic, cylindrical to subcylindrical, with a truncate apex on conidial secession, short, smooth, hyaline. *Conidia* 2-3.8 µm 1.7-3, ($\bar{x}$ = 2.9 × 2.2 µm, n = 40), globose to subglobose, occasionally irregular, hyaline, finely guttulate or not, smooth-walled.

Material examined: THAILAND, Chiang Rai Province, Mae Fah Luang University Garden, on dead twig, 14 April 2014, K.M. Thambugala, KM 008 (MFLU 14-0607, **holotype**); *ibid*. (PDD, **isotype**), ex-type living culture (MFLUCC 14-0503, ICMP 20749).

Culture characteristics: Ascospores germinating on PDA within 24 h and germ tubes produced from one or both ends. Colonies growing on PDA 3.8 cm diam after 5 days at 25°C, flat, circular, initially white, becoming gray to reddish brown, smooth surface with undulate edge.

KEY TO SPECIES OF *RHYTIDHYSTERON* TREATED IN THIS STUDY

1. Ascospores 1-septate ..... *R. hysterinum*
1. Ascospores 3-septate, with or without longitudinal septa 2
    2. Ascospores 3-septate, with longitudinal septa ..... *R. opuntiae*
    2. Ascospores 3-septate, without longitudinal septa 3
3. Ascomata perpendicularly striate along the long axis 4
3. Ascomata without striations 5
    4. Ascospores 28-36 × 9-13 µm ..... *R. rufulum*
    4. Ascospores (38-)40-44-(47) × (15-)17-19(-21) µm ..... *R. brasiliense**
5. Ascomata semi-immersed to erumpent, coriaceous ..... *R. thailandicum*
5. Ascomata superficial, carbonaceous ..... *R. neorufulum*

* measurements were taken from Kutorga & Hawksworth (1997)

DISCUSSION

In this study, we have shown morphological and phylogenetic limits of the genus *Rhytidhysteron*. No sequence data is available for *Rhytidhysteron brasiliense,* the type species, while and the holotype material does not contain ascomata for morphological study. Hence recollection, epitypification and molecular analysis are required to confirm the placement of *R. brasiliense*. Some recent studies (Boehm *et al*., 2009b; Murillo *et al*., 2009; Yacharoen *et al*., 2015) have been pointed out the morphological and phylogenetic variations of *R. rufulum* collections and this study

revealed that there are several species among those collections. We introduce two new *Rhytidhysteron* species based on our *Rhytidhysteron* collections from Thailand. Other *R. rufulum*-like collections from different countries may contain new species. However, detailed morphological and phylogenetic analyses are required to confirm whether those collections are the same species as *R. rufulum*. Currently there are 17 epithets (excluding the two new species introduced in the present study) listed in Index Fungorum (2016), while only three species (*R. hysterinum*, *R. opuntiae* and *R. rufulum*) have sequence data in GenBank. Boehm *et al*., (2009b) suggested that *R. opuntiae* should be removed from the genus, based on both morphological and molecular data. In Fig. 1, *R. opuntiae* clusters in a different lineage together with the ex-type strain of *Hysterodifractum partisporum* (CCMB 252/2012). Therefore, *R. opuntiae* needs further study. "Aposphaeria-like" or diplodia-like' species have been reported as the asexual morphs of *Rhytidhysteron* (Samuels & Müller 1979). In this study we describe and illustrate the aposphaeria-like asexual morph of *R. thailandicum*. Further collections of *Rhytidhysteron* species are needed to epitypify *R. brasiliense* (Ariyawansa *et al.*, 2014) and establish species boundaries in the genus.

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