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Multigene phylogenetic support for novel *Rhytidhysteron* Speg. species (Hysteriaceae) from Sichuan Province, China

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ABSTRACT
We have been studying *Rhytidhysteron* Speg. species on different native trees of economic or landscape importance from different geographical regions in China. In this study, we describe three new species, *Rhytidhysteron ligustrum* X.-L. Xu & C.-L. Yang, sp. nov., *R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov., *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. and a new host record for *R. hongheense* Wanas based on combined ITS, LSU, SSU and *tef*-1α phylogenetic analysis and morphological characters. The new species are distinct from the closely related species in *tef*-1α and ITS sequence data, and distinguished from other species of the genus by exciple, ascus and ascospore characters. Descriptions, photographs and notes are provided for the new taxa.

KEY WORDS
China,
morphology,
multigene analysis,
new species.

RÉSUMÉ

La phylogénétique multigénique permet d'établir l'existence d'espèces nouvelles de Rhytidhysteron *Speg. dans la province du Sichuan, Chine.*

Nous avons étudié les espèces de *Rhytidhysteron* Speg. associées aux arbres indigènes d'un intérêt économique ou paysager dans différentes régions de Chine. Dans cette étude, nous décrivons trois espèces nouvelles, *Rhytidhysteron ligustrum* X.-L. Xu & C.-L. Yang, sp. nov., *R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov., *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. et un nouvel hôte pour *R. hongheense* Wanas, sur la base d'une analyse phylogénétique combinée ITS, LSU, SSU et *tef*-1*α*, et des caractères morphologiques. Les espèces nouvelles sont distinctes des espèces étroitement apparentées dans les données de séquence *tef*-1*α* et ITS, et distinguées des autres espèces du genre par les caractères d'exciple, d'ascus et d'ascospore. Des descriptions, des photographies et des notes sont fournies pour les nouveaux taxons.

MOTS CLÉS
Chine,
morphologie,
analyse multigénique,
espèces nouvelles.

INTRODUCTION

Rhytidhysteron Speg. (Hysteriaceae, Hysteriales), introduced by Spegazzini (1881) to accommodate *R. brasiliense* Speg. and *R. viride* Speg. without designating a type species, is characterised by ascomata that are closed and navicular but gradually opening by a longitudinal sulcus, often with incurved margins to become irregularly apothecioid at maturity (Boehm *et al*. 2009a). Subsequently, *R. brasiliense* was designated as the type species by Clements & Shear (1931). The concept of the genus has been broadened by some newly added species, viz. *R. bruguierae* Dayarathne, *R. camporesii* Ekanayaka & K.D.Hyde, *R. chromolaenae* Mapook & K.D.Hyde, *R. columbiense* Soto-Medina & Lücking, *R. cozumelense* Cobos-Villagrán, R.Valenz, Hern.-Rodr., Calvillo-Medina & Raymund, *R. erioi* Ekanayaka & K.D.Hyde, *R. esperanzae* Cobos-Villagrán, R.Valenz. & Raymundo, *R. magnoliae* N.I.de Silva, Lumyong & K.D.Hyde, *R. mangrovei* Vinit & K.D.Hyde, *R. mesophilum* Cobos-Villagrán, R.Valenz, Hern.-Rodr., Calvillo-Medina & Raymundo, *R. neohysterinum* Cobos-Villagran, Hern.-Rodr., R.Valenz. & Raymundo, *R. neorufulum* Thambug. & K.D.Hyde, *R. tectonae* Doilom & K.D.Hyde, *R. thailandicum* Thambug. & K.D.Hyde, and *R. xiaokongense* G.C.Ren & K.D.Hyde (Thambugala *et al*. 2016; Soto-Medina & Lücking 2017; Doilom *et al*. 2017; Kumar *et al*. 2019; Cobos-Villagrán *et al*. 2020; Dayarathne *et al*. 2020; de Silva *et al*. 2020; Hyde *et al*. 2020b; Mapook *et al*. 2020; Cobos-Villagrán *et al*. 2021; Ren *et al*. 2022). Researchers have proposed a combination of morphology and multigene phylogenetic analysis to distinguish various species (Boehm *et al*. 2009b; Thambugala *et al*. 2016; Kumar *et al*. 2019).

Rhytidhysteron has long been considered to be a member of the Patellariaceae Corda (Bezerra & Kimbrough 1982), although with some divergent taxonomic opinions (Samuels & Müller 1979). Boehm *et al*. (2009a, b) were the first to provide DNA sequence data indicating that genus *Rhytidhysteron* does not lie within the Patellariaceae, but within the Hysteriaceae Chevall. Subsequently, multigene phylogenetic studies place Hysteriaceae in Hysteriales Lindau, Pleosporomycetidae C.L.Schoch, Spatafora, Crous & Shoemaker, Dothideomycetes O.E.Erikss. & Winka (Hyde *et al*. 2013; Wijayawardene *et al*. 2014). Hysteriaceae was introduced by Chevallier (1826) with *Hysterium* Pers. as the type genus. Currently this family comprises 13 genera, except for controversy on *Graphyllium* Clem. as genus *incertae sedis* in Hysteriales (Dayarathne *et al*. 2020; Hongsanan *et al*. 2020; Wijayawardene *et al*. 2020). Since some of the genera lack molecular data, the classification of this family requires further investigation (Jayasiri *et al*. 2018; Wijayawardene *et al*. 2018).

In the investigations of fungi saprobic on native economic and landscape trees in Sichuan Province, China, we collected *Rhytidhysteron* species on decaying twigs of various plants, viz. Oleaceae Hoffmanns. & Link, Rutaceae Juss., Juglandaceae DC. ex Perleb, Moraceae Gaudich., Fabaceae Lindl. and Lythraceae J.St.-Hil. The objective of this study is to characterise the novel species and new record of *Rhytidhysteron* based on morphological differences and analyses of a combined ITS, LSU, SSU and *tef*-1*α* sequences.

MATERIAL AND METHODS

Collection, isolation and morphological observations

The specimens were collected from dead branches and twigs on woody plants in Sichuan Province, China. Mature stromata were selected for isolation and morphological observations. Single ascospore isolations were carried out following the method described by Chomnunti *et al*. (2014) and the germinating spores were transferred to PDA, incubated at 25°C in the dark and cultural characteristics were determined. External shape, size, and the colour of apothecia were observed and photographed using a dissecting microscope NVT-GG (Shanghai Advanced Photoelectric Technology Co. Ltd, China) matched to a VS-800C micro-digital camera (Shenzhen Weishen Times Technology Co. Ltd., China). The anatomical details of apothecia were visualised using an OLYMPUS BX43 microscope matched to an OLYMPUS DP22 microscope digital Camera. Iodine reaction of the ascus wall was tested in Melzer's reagent (MLZ). Type specimens were deposited at the Herbarium of Sichuan Agricultural University, Chengdu, China (SICAU).

The living cultures are deposited at the Culture Collection in Sichuan Agricultural University (SICAUCC). Index Fungorum (http://www.indexfungorum.org/Names/Names.asp) numbers are registered and provided.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from mycelia growing on PDA for 14 days at 25°C with Plant Genomic DNA extraction kitTM (Tiangen, China). The primers pairs LR0R and LR5 (Vilgalys & Hester 1990), NS1 and NS4, ITS5 and ITS4 (White *et al*. 1990), EF1-983F and EF1-2218R (Rehner & Buckley 2005), fRPB2-5F and fRPB2-7cR (Liu *et al*. 1999), T1 and Bt2b (O'Donnell & Cigelnik 1997) were used for the amplification of the nuclear large subunit rDNA (LSU), the nuclear small subunit rDNA (SSU), internal transcribed spacers (ITS), translation elongation factor 1-alpha (*tef*-1*α*), the RNA polymerase II second largest subunit (*rpb*2), and beta-tubulin (*tub*2) genes, respectively.

Polymerase chain reaction (PCR) amplification was carried out in 25 µl of PCR mixtures with 22 µl Master Mix (Beijing TsingKe Biotech Co., Ltd.) and 1 µl of DNA template, 1 µl of each primer (10 µm). The amplification reactions were performed as described by Dai *et al*. (2016), Hyde *et al*. (2016) and Wang *et al*. (2018). PCR products were sequenced with above mentioned primers at TsingKe Biological Technology Co., Ltd, Chengdu, China. The newly generated sequences from the ITS, LSU, SSU, *tef*-1*α*, *rpb*2, *tub*2 regions were deposited in GenBank.

Phylogenetic analyses

Phylogenetic analyses were performed with a combined dataset of LSU, SSU, ITS and *tef*-1*α* among members of the *Rhytidhysteron*. Sequence data for phylogenetic analyses were mainly from GenBank (http://www.ncbi.nlm.nih.gov/) and recent publications (Mugambi & Huhndorf 2009; Ruibal *et al*. 2009; Almeida *et al*. 2014a; Thambugala *et al*. 2016; Jayasiri *et al*. 2018, 2019; Kumar *et al*. 2019; Cobos-Villagrán *et al*. 2021; Ren *et al*. 2022). *Hysterographium fraxini* (Pers.) De Not. (MFLU 15-3681; CBS 109.43) were chosen as outgroup taxa. DNA alignments were performed by using MAFFT v.7.429 online service (Katoh *et al*. 2019) and ambiguous regions were excluded with BioEdit version 7.0.5.3 (Hall 1999). Multigene sequences were concatenated by Mesquite software (Maddison & Maddison 2019). Multigene phylogenetic analyses were obtained from maximum likelihood (ML) and Bayesian inference (BI) analyses. The best nucleotide substitution model was determined by MrModeltest v. 2.2 (Nylander 2004).

Maximum likelihood analysis and Bayesian inference analysis were generated by using the CIPRES Science Gateway web server (Miller *et al*. 2010). RAxML-HPC2 on XSEDE (8.2.10) (Stamatakis 2014) with GTR+GAMMA substitution model with 1000 bootstrap iterations was chosen for Maximum likelihood analysis. For BI analyses, the best-fit models HKY+I+G for ITS, GTR+I+G for LSU, HKY+I+G for SSU, and GTR+G for *TEF*1-*α*, were selected in MrModeltest 2.2. The analyses were computed with six simultaneous Markov Chain Monte Carlo (MCMC) Chains, with 8 000 000 generations and a sampling frequency of 100 generation. The burn-in fraction was set to 0.25, and the run automatically ended when the average standard deviation of split frequencies reached below 0.01.

Phylogenetic trees were visualized with FigTree v.1.4.3 (Rambaut & Drummond 2016) and edited using Adobe Illustrator CS6 (Adobe Systems Inc., United States). Maximum likelihood bootstrap values (ML) equal or greater than 70 % and Bayesian Posterior Probabilities (BYPP) equal or greater than 0.95 were provided (Fig. 1). The finalized alignment was deposited in TreeBASE (http://www.treebase.org), submission ID: 25684.

RESULTS

Phylogenetic analyses

In the phylogenetic analysis, 49 strains of *Rhytidhysteron* were included in the combined LSU, SSU, ITS and *tef*-1*α* gene dataset. This combined 4-gene dataset comprised 3645 characters (LSU = 938, SSU = 1043, ITS = 684, *tef*-1*α* = 980). The RAxML analysis of the combined dataset provided the best scoring tree (Fig. 1) with a final ML optimization likelihood value of –11800.897201. The matrix had 916 distinct alignment patterns with 22.46 % of gaps or undetermined characters. The gamma distribution shape parameter (*α*) was 0.155379, while the Tree-Length was 0.573157. The estimated base frequencies were as follow: A = 0.240544, C = 0.249313, G = 0.274474, T = 0.235669, with substitution rates AC = 1.369510, AG = 2.739595, AT = 1.189816, CG = 1.115164, CT = 6.343590, GT = 1.000000. The Bayesian analysis resulted in 13 002 trees after 8 000 000 generations. The first 25 % of trees (3250 trees), which representing the burn-in phase of the analyses were discarded, while the remaining 9752 trees were used for calculating posterior probabilities.

The genus *Rhytidhysteron* currently includes 32 species and only 18 species have DNA sequence data in GenBank (Table 1), excluding the new species described in this study. Our new isolates clustered within the genus *Rhytidhysteron* (Fig. 1) and the topology structures of two phylogeny trees are similar. *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. groups in the clade comprising *R. esperanzae* and *R. rufulum* (Spreng.) Speg. with high bootstrap values (100 % ML, 1.00 BYPP), and is closer to *R. esperanzae* with 95 % ML and 0.88 BYPP bootstrap values. *R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov. is closely related to *R. thailandicum* with high ML support (100 %) and strong BYPP support (1.00). *R. ligustrum* X.-L. Xu & C.-L. Yang, sp. nov. is sister to *R. camporesii* with 73 % ML and 0.99 BYPP bootstrap values. And our collection (SICAUCC 19-0007) clusters with the strains of *R. hongheense* as a monophyletic clade (100 % ML, 1.00 BYPP). In this paper, we have analyzed the LSU, SSU, ITS and *tef*-1*α* sequences, because of the unavailable of *rpb*2 and *tub*2 sequences for other species in GenBank.

TABLE 1. — GenBank and culture accession numbers of isolates included in this study. Notes: the newly generated sequences are highlighted in **bold**; ex type strains are given superscript T. Abbreviations: **CBS**, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; **EB**, collection of E.W.A. Boehm; **GKM**, collection of G.K. Mugambi; **KUMCC**, Kunming Institute of Botany Culture Collection, Kunming, China; **KUN-HKAS**, herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica, Yunnan, China; **MFLU**, herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **SICAUCC**, Sichuan Agricultural University Culture Collection, Sichuan, China; **A. Cobos Villagrán**, collection of A. Cobos Villagrán; **A. Trejo**, collection of A. Trejo; **T. Raymundo**, collection of T. Raymundo; **R. Valenzuela**, collection of R. Valenzuela.

Species	Strain/Voucher no.	GenBank accession no.				References
		LSU	SSU	tef-1α	ITS	
Hysterographium fraxini (Pers.) De Not.	MFLU 15-3681	MH535901	MH535890	MH535883	–	Jayasiri *et al*. 2018
*Hysterographium fraxini*T	CBS 109.43	FJ161171	FJ161132	FJ161088	–	Boehm *et al*. 2009a
*Rhytidhysteron bruguierae*T Dayarathne	MFLUCC 18-0398	MN017833	MN017901	MN077056	–	Dayarathne *et al*. 2020
Rhytidhysteron bruguierae	MFLUCC 17-1502	MN632453	MN632464	MN635662	MN632458	Mapook *et al*. 2020
Rhytidhysteron bruguierae	MFLUCC 17-1515	MN632452	MN632463	MN635661	MN632457	Mapook *et al*. 2020
*Rhytidhysteron camporesii*T Ekanayaka & K.D.Hyde	KUN-HKAS 104277	MN429072	–	MN442087	MN429069	Hyde *et al*. 2020b
*Rhytidhysteron chromolaenae*T Mapook & K.D.Hyde	MFLUCC 17-1516	MN632456	MN632467	MN635663	MN632461	Mapook *et al*. 2020
*Rhytidhysteron cozumelense*T Cobos-Villagrán, R.Valenz, Hern.-Rodr., Calvillo-Medina & Raymund	A. Cobos-Villagrán 951	MW939459	–	MZ457338	MZ056797	Cobos-Villagrán *et al*. 2021
Rhytidhysteron cozumelense	T. Raymundo 7321	MW939460	–	MZ457339	MZ056798	Cobos-Villagrán *et al*. 2021
*Rhytidhysteron erioi*T Ekanayaka & K.D.Hyde	MFLU 16-0584	MN429071	–	MN442086	MN429068	Hyde *et al*. 2020b
*Rhytidhysteron esperanzae*T Cobos-Villagrán, R.Valenz. & Raymundo	T. Raymundo 6579	MZ477203	–	MZ457336	–	Cobos-Villagrán *et al*. 2021
Rhytidhysteron esperanzae	R. Valenzuela 17206	MZ477204	–	MZ457337	–	Cobos-Villagrán *et al*. 2021
*Rhytidhysteron hongheense*T Wanas	KUMCC 20-0222	MW264193	MW264223	MW256815	MW264214	Wanasinghe *et al*. 2021
Rhytidhysteron hongheense	HKAS112348	MW541820	MW541831	MW556132	MW541824	Wanasinghe *et al*. 2021
Rhytidhysteron hongheense	HKAS112349	MW541821	MW541832	MW556133	MW541825	Wanasinghe *et al*. 2021
Rhytidhysteron hongheense	**SICAUCC 19-0007**	**MN956789**	**MN956798**	**MT027603**	**MN956777**	**This study**
Rhytidhysteron hysterinum (Dufour) Samuels & E.Müll.	EB 0351	GU397350	–	GU397340	–	Boehm *et al*. 2009b
***Rhytidhysteron ligustrum*T X.-L. Xu & C.-L. Yang, sp. nov.**	**SICAUCC 20-0004**	**MT062446**	**MT062451**	**MT075600**	**MT062850**	**This study**
*Rhytidhysteron mangrovei*T Vinit & K.D.Hyde	MFLUCC 18-1113	MK357777	–	MK450030	MK425188	Kumar *et al*. 2019
*Rhytidhysteron magnoliae*T N.I.de Silva, Lumyong & K.D.Hyde	MFLUCC 18-0719	MN989384	MN989382	MN997309	MN989383	de Silva *et al*. 2020
*Rhytidhysteron mesophilum*T Cobos-Villagrán, R.Valenz, Hern.-Rodr., Calvillo-Medina & Raymundo	A. Trejo 74	MW939461	–	MZ457340	MZ056799	Cobos-Villagrán *et al*. 2021
Rhytidhysteron mesophilum	A. Cobos-Villagrán 1800	MW939462	–	MZ457341	MZ056800	Cobos-Villagrán *et al*. 2021
Rhytidhysteron neorufulum Thambug. & K.D.Hyde	MFLUCC 13-0221	KU377567	KU377572	–	KU377562	Thambugala *et al*. 2016
*Rhytidhysteron neorufulum*T	MFLUCC 13-0216	KU377566	KU377571	KU510400	KU377561	Thambugala *et al*. 2016
Rhytidhysteron neorufulum	MFLUCC 12-0011	KJ418109	KJ418110	–	KJ206287	Thambugala *et al*. 2016
Rhytidhysteron neorufulum	CBS 306.38	FJ469672	AF164375	GU349031	–	Boehm *et al*. 2009b
Rhytidhysteron neorufulum	EB 0381	GU397351	GU397366	–	–	Boehm *et al*. 2009b
Rhytidhysteron neorufulum	MFLUCC 21-0035	MZ346015	MZ346020	MZ356249	MZ346025	Ren *et al*. 2022
*Rhytidhysteron opuntiae*T (J.G.Br.) M.E.Barr	GKM1190	GQ221892	GU397341	–	–	Mugambi & Huhndorf 2009
*Rhytidhysteron rufulum*T (Spreng.) Speg.	MFLUCC 14-0577	KU377565	KU377570	KU510399	KU377560	Thambugala *et al*. 2016
Rhytidhysteron rufulum	MFLUCC 12-0013	KJ418111	KJ418113	–	KJ418112	de Silva *et al*. 2020
***Rhytidhysteron sichuanensis*T X.-L. Xu & C.-L. Yang,sp. nov.**	**SICAUCC 19-0005**	**MN956787**	**MN956796**	**MT027601**	**MN956775**	**This study**
Rhytidhysteron sichuanensis	**SICAUCC 19-0006**	**MN956788**	**MN956797**	**MT027602**	**MN956776**	**This study**
Rhytidhysteron sichuanensis	**SICAUCC 19-0008**	**MN956790**	**MN956799**	**MT027604**	**MN956778**	**This study**
Rhytidhysteron sichuanensis	**SICAUCC 19-0009**	**MN956791**	**MN956800**	**MT027605**	**MN956779**	**This study**
Rhytidhysteron sichuanensis	**SICAUCC 20-0002**	**MT062444**	**MT062449**	**MT075598**	**MT062848**	**This study**
Rhytidhysteron sichuanensis	**SICAUCC 20-0005**	**MT062447**	**MT062452**	**MT075601**	**MT062851**	**This study**
Rhytidhysteron subrufulum **X.-L. Xu & C.-L. Yang, sp. nov.**	**SICAUCC 19-0010**	**MN956792**	**MN956801**	**MT027606**	**MN956780**	**This study**
***Rhytidhysteron subrufulum*T**	**SICAUCC 19-0011**	**MN956793**	**MN956802**	**MT027607**	**MN956781**	**This study**
Rhytidhysteron subrufulum	**SICAUCC 20-0003**	**MT062445**	**MT062450**	**MT075599**	**MT062849**	**This study**

TABLE 1. — Continuation

Species	Strain/Voucher no.	GenBank accession no. LSU	SSU	tef-1α	ITS	References
Rhytidhysteron subrufulum	**SICAUCC 20-0006**	**MT062448**	**MT062453**	**MT075602**	**MT062852**	**This study**
Rhytidhysteron subrufulum	**SICAUCC 20-0011**	**MW216920**	**MW216921**	**MW219740**	**MW219534**	**This study**
Rhytidhysteron subrufulum	**SICAUCC 22-0001**	**OM327628**	**OM327627**	**OM371082**	**OM333896**	**This study**
*Rhytidhysteron tectonae*ᵀ Doilom & K.D.Hyde	MFLUCC 13-0710	KU764698	KU712457	KU872760	KU144936	Doilom *et al*. 2017
Rhytidhysteron tectonae	MFLUCC 21-0037	MZ346013	MZ346018	MZ356247	MZ346023	Ren *et al*. 2022
Rhytidhysteron tectonae	MFLUCC 21-0034	MZ346014	MZ346019	MZ356248	MZ346024	Ren *et al*. 2022
Rhytidhysteron thailandicum Thambug. & K.D.Hyde	MFLUCC 13-0051	MN509434	–	MN509435	MN509433	Cobos-Villagrán *et al*. 2021
Rhytidhysteron thailandicum	MFLUCC 12-0530	KJ526125	KJ546128	–	KJ546123	Thambugala *et al*. 2016
*Rhytidhysteron thailandicum*ᵀ	MFLUCC 14-0503	KU377564	KU377569	KU497490	KU377559	Thambugala *et al*. 2016
Rhytidhysteron xiaokongense G.C.Ren & K.D.Hyde	KUMCC 20-0158	MZ346011	MZ346016	MZ356245	MZ346021	Ren *et al*. 2022
*Rhytidhysteron xiaokongense*ᵀ	KUMCC 20-0160	MZ346012	MZ346017	MZ356246	MZ346022	Ren *et al*. 2022

TAXONOMY

Family PATELLARIACEAE Corda
Genus *Rhytidhysteron* Speg.

Rhytidhysteron hongheense Wanas
(Fig. 2)

SPECIMEN EXAMINED. — **China**. Sichuan Province, Chongzhou city, 30°33'11.89"N, 103°39'25.55"E, alt. 485 m, on dead twigs of *Juglans regia* L. (Juglandaceae), 20.VI.2019, collected by C.-L. Yang, *YCL201906005* (holo-, SICAU 19-0006), living culture (SICAUCC 19-0007).

ADDITIONAL GENBANK NUMBER. — *tub*2, MT075594; *rpb*2, MT027610.

DESCRIPTION
Saprobic on decaying twigs of trees of *Juglans regia* L.

Sexual morphology
Ascomata 1350-2390 µm long × 620-1870 µm wide × 570-1070 µm high ($\bar{x}$ = 1899 × 1068 × 718 µm, n = 25), apothecioid, coriaceous, scattered to gregarious, black, elliptic or irregular in shape, perpendicularly striate along the long axis, reddish brown or brown to black on the disc. Exciple 76-131 µm wide ($\bar{x}$ = 94, n = 15), two-layerd, outer layer comprising of dark brown to brown cells of textura angularis and textura globulosa, inner layer of hyaline cells of textura angularis and textura prismatica. Hamathecium composed of 1.1 to 2.3 µm wide at the base, 2.8 to 4.9 µm wide at swollen tips (n = 20), dense, hyaline, septate pseudoparaphyses, branched and forming a reddish brown epithecium above the asci, swollen with dense septa at the apex. Hymenium turns blue in Melzer's reagent, J+. Asci 169-220 × 10-15 µm ($\bar{x}$ = 194 × 12 µm, n = 40), 6-(7-)8-spored, bitunicate, cylindrical, short pedicellate and apically rounded with an ocular chamber, J- in Melzer's reagent. Ascospores 23-32 × 8-13 µm ($\bar{x}$ = 27 × 11 µm, n = 40), uniseriate, partially overlapping, 1-2-3-septate, occasionally 1-septate, frequently 3-septate, ellipsoid or fusoid, straight or slightly curved, constricted septum, light brown to dark brown, without a mucilaginous sheath.

Asexual morphology
Undetermined.

Culture characteristics
Ascospores germinating on PDA within 24 hours and germ tubes produced from any cell. Colonies growing on PDA reach 5 cm diam after four days at 25°C, thinaerial hyphae, flat, circular, initially white, becoming gray to deep gray.

NOTES
The type strain of *Rhytidhysteron hongheense* Wanas was recently known on *Dodonaea* Mill. from Yunnan, China (Wanasinghe *et al*. 2021). The similar morphological characteristics and multigene phylogenetic analysis based on combined LSU, SSU, ITS, and *tef*-1*α* sequence data showed our collection is a new host record of *R. hongheense* on *Juglans regia*. But compared with the type description of *R. hongheense*, our collection has larger ascomata (1590 × 410 × 840 µm vs 1899 × 718 × 1068 µm) and longer asci (169-220 vs 140-180 µm). The asci in *R. hongheense* are mainly 6- or 8-spored, but 7-spored observed in this paper, and this feature has only been observed in *R. chromolaenae* (Mapook *et al*. 2020).

Rhytidhysteron ligustrum X.-L. Xu & C.-L. Yang, sp. nov.
(Fig. 3)

HOLOTYPE. — **China**. Sichuan Province, Chengdu City, Wenjiang District, 30°42'19.41"N, 103°51'27.6"E, alt. 526 m, on dead twigs of *Ligustrum quihoui* Carr. (Oleaceae), 26.XII.2019, collected by X.-L. Xu, *XXL201912001* (holo-, SICAU 20-0004), ex-type living culture (SICAUCC 20-0004).

ETYMOLOGY. — The epithet refers to the host genus *Ligustrum* L.

INDEX FUNGORUM. — IF558047.

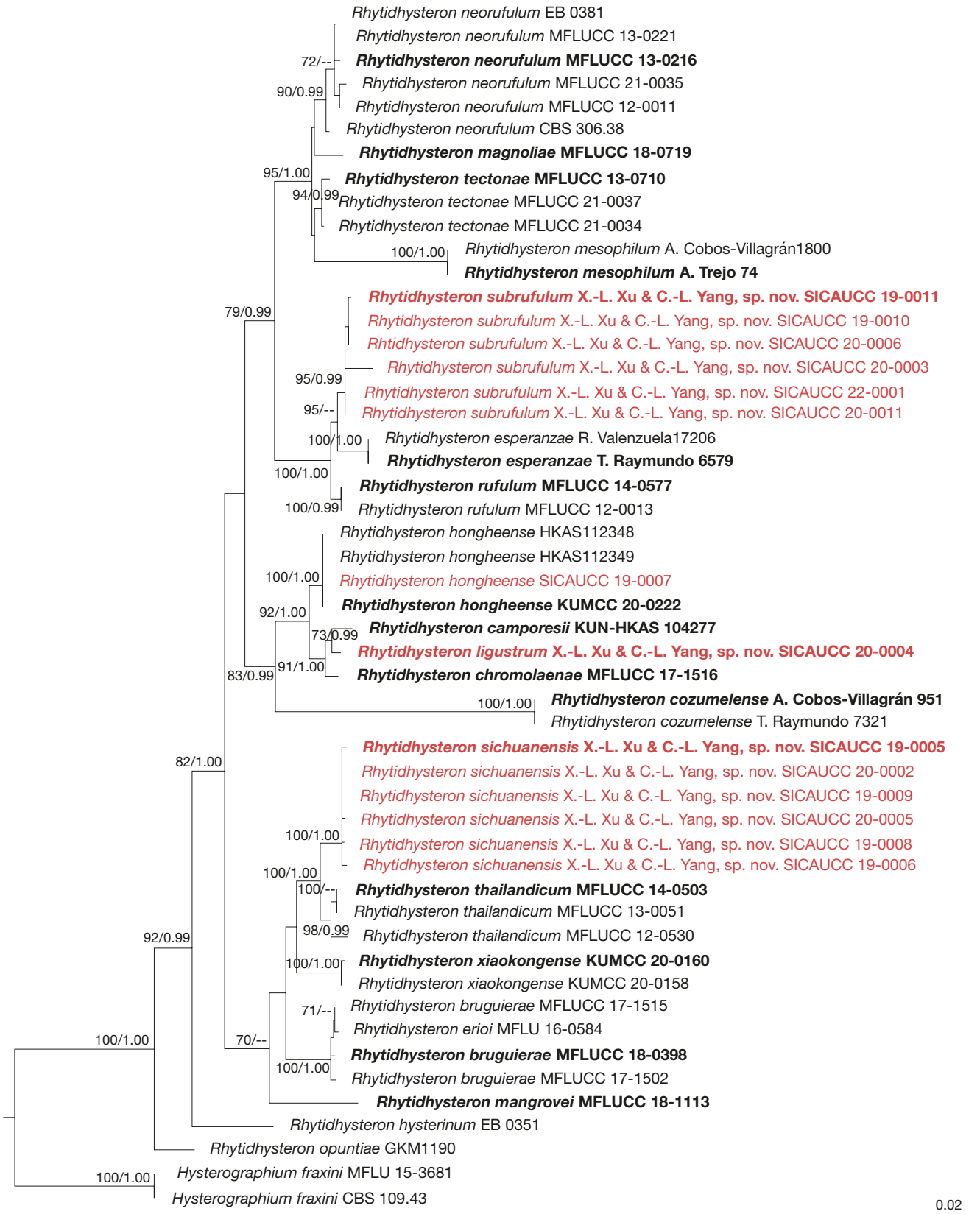

Fig. 1. — Phylogram generated from RAxML analyses based on combined LSU, SSU, ITS and *tef*-1*α* sequence dataset within the genus *Rhytidhysteron* Speg. The tree is rooted to *Hysterographium fraxini* (Pers.) De Not. (CBS 109.43 and MFLU 15-3681). ML ≥70% and BYPP ≥0.95 are defined as ML/BYPP above or below the nodes. The type strains are in **bold** and the newly generated sequences are highlighted in **red**.

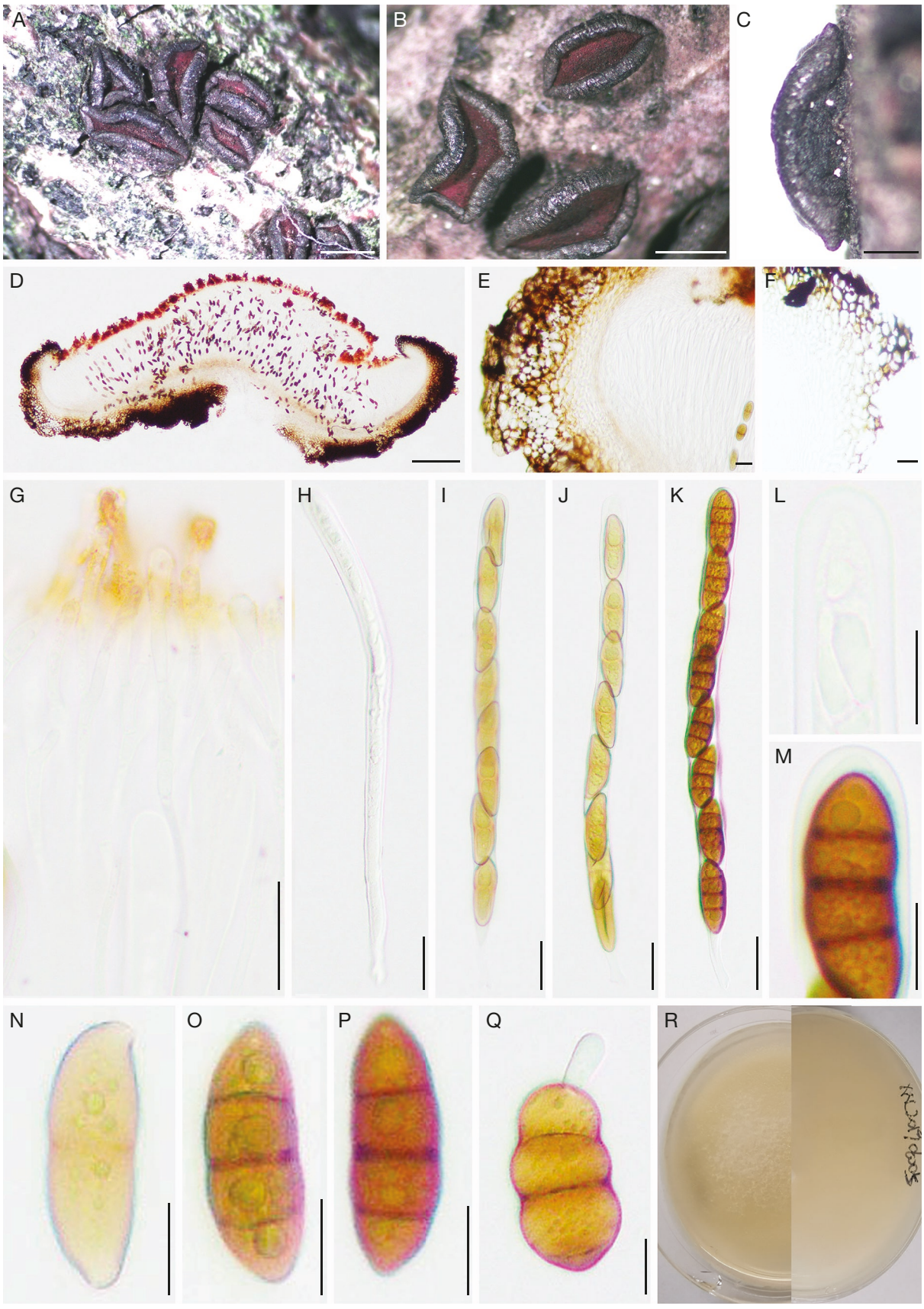

FIG. 2. — *Rhytidhysteron hongheense* Wanas (SICAU 19-0006): **A**, appearance of apothecia on host; **B**, **C**, ascomata; **D**, vertical section of hysteriothecium; **E**, **F**, exciple; **G**, pseudoparaphyses; **H**-**K**, asci; **L**, **M**, ocular chamber; **N**-**P**, ascospores; **Q**, germinated ascospores; **R**, colonies on PDA for four days. Scale bars: A, B, 1 mm; C, 0.5 mm; D, 200 µm; E-K, 20 µm; L-Q, 10 µm.

Additional GenBank number. — *tub*2, MT075589; *rpb*2, MT075605.

Description

Saprobic on decaying twigs of *Ligustrum quihoui* Carr.

Sexual morphology

Ascomata 849-2345 µm long × 508-1490 wide × 452-711 high ($\bar{x}$ = 1390 × 747 × 565 µm, n = 30), apothecioid, carbonaceous, scattered to gregarious, black, labiates and elliptic or irregular in shape, perpendicularly striate along the long axis, reddish brown to dark brown on the disc. Exciple 50-120 µm wide ($\bar{x}$ = 78, n = 15), two-layered, outer layer is composed of several layers of thick-walled, brown to dark brown cells of textura angularis, inner layer is composed several layers of narrow, long, thin-walled, hyaline to brown cells of textura prismatica. Hamathecium composed of 1.7-2.7 µm wide at the base, 3.2-6.9 µm wide at swollen tips (n = 20), dense, septate, pseudoparaphyses, branched and forming yellowish-brown to reddish brown epithecium above the asci, obviously swollen at the apex, hymenium turns blue in Melzer's reagent, J+. Asci 134-196 × 10-18 µm ($\bar{x}$ = 166 × 13 µm, n = 25), 6-8-spored, bitunicate, clavate to cylindrical, with short pedicel and apically rounded with an ocular chamber, J- in Melzer's reagent. Ascospores 18-32 × 8.9-13 µm ($\bar{x}$ = 27 × 11 µm, n = 30), ellipsoidal or fusiform, straight or slightly curved, slightly pointed at both ends, partially overlapping, uniseriate, 1-2-3-septate, and both frequently, constricted septum, hyaline to brown, without a mucilaginous sheath.

Asexual morphology

Undetermined.

Culture characteristics

Ascospores germinating on PDA within 24 hours and germ tubes produced from any cell. Colonies growing on PDA reach 5 cm diam after five days at 25°C, flat, circular, initially white, gradually becoming to brown.

Notes

The new species *Rhytidhysteron ligustrum* X.-L. Xu & C.-L. Yang, sp. nov. is morphologically similar to our collection of *R. hongheense*, but they are different in having coriaceous rim and longer asci (194 µm vs 166 µm) in *R. hongheense*. In the comparison of ITS, *tef*-1*α*, *rpb*2 and *tub*2 sequences, our new strain shows 8.68 %, 1.16 %, 1.44 %, 2.57 % differences to our collection of *R. hongheense*, respectively. Our strain is phylogenetically close to *R. camporesii* and *R. chromolaenae*. Furthermore, a pairwise comparison of *tef*-1*α* sequence data showed 1.52 % differences between *R. ligustrum* X.-L. Xu & C.-L. Yang, sp. nov. and *R. camporesii*, as well as 1.86 % differences between *R. ligustrum* X.-L. Xu & C.-L. Yang, sp. nov. and *R. chromolaenae*. *R. ligustrum* X.-L. Xu & C.-L. Yang, sp. nov. has longer ascomata (1390 µm vs 1002 µm vs 780 µm), 6-8-spored asci, and 1-2-3-septate ascospores when mature, while *R. camporesii* and *R. chromolaenae* have 8-spored and 7-8-spored asci, respectively, and both 3-septate ascospores at maturity (Hyde *et al*. 2020b; Mapook *et al*. 2020).

Rhytidhysteron sichuanensis
X.-L. Xu & C.-L. Yang, sp. nov.
(Fig. 4)

Holotype. — **China**. Sichuan Province, Zizhong county, 29°46'28.29"N, 104°49'19.66"E, alt. 316 m, on dead twigs of *Citrus maxima* (Burm.) Merr. (Rutaceae), 8.VI.2019, collected by X.-L. Xu & C.-L. Yang, *XXL201906001* (holo-, SICAU 19-0004), ex-type living culture (SICAUCC 19-0005).

Etymology. — The specific epithet refers to the place where the fungus was collected.

Index Fungorum. — IF557217.

Additional specimens examined. — **China**. Sichuan Province, Zizhong county, 29°46'28.29"N, 104°49'19.66"E, alt. 316 m, on dead twigs of *Citrus reticulata* Blanco (Rutaceae), 20.III.2019, collected by X.-L. Xu & C.-L. Yang, *YCL201903006* (SICAU 19-0008), living culture (SICAUCC 19-0009); on dead twigs of *Citrus reticulata* Blanco 'Ponkan' (Rutaceae), 8.VI.2019, *XXL201906003* (SICAU 19-0005), living culture (SICAUCC 19-0006); 5.IV.2019, *YCL201904001* (SICAU 19-0007), living culture (SICAUCC 19-0008); 29°46'22.97"N, 104°49'12.20"E, alt. 303 m, on dead twigs of *Broussonetia papyrifera* (Linnaeus) L'Heritier ex Ventenat (Moraceae), 19.X.2019, collected by X.-L. Xu & C.-L. Yang, *XXL201910003* (SICAU 20-0002), living culture (SICAUCC 20-0002); Ya'an City, Yucheng District, 30°8'7.84"N, 103°3'21.29"E, alt. 907 m, on dead twigs of *Lagerstroemia indica* L. (Lythraceae), 28.XI.2019, collected by C.-L. Yang, *XXL201911005* (SICAU 20-0005), living culture (SICAUCC 20-0005).

Additional GenBank number. — SICAUCC 19-0005: *tub*2, MT075592; *rpb*2, MT027608. SICAUCC 19-0006: *tub*2, MT075593; *rpb*2, MT027609. SICAUCC 19-0008: *tub*2, MT075595; *rpb*2, MT027611. SICAUCC 19-0009: *rpb*2, MT027612. SICAUCC 20-0002: *tub*2, MT075587; *rpb*2, MT075603. SICAUCC 20-0005: *tub*2, MT075590; *rpb*2, MT075606.

Description

Saprobic on decaying woody branches and twigs.

Sexual morphology

Ascomata 930-2370 µm long × 760-1930 µm wide × 280-820 µm high ($\bar{x}$ = 1318 × 1249 × 479 µm, n = 20), apothecioid, carbonaceous, scattered to gregarious, semi-immersed, black, exposed with irregular, oval to circular in outline, reddish brown, or brown to black disc, folded along the margins with perpendicularly striate along the long axis, compressed at the apex. Exciple 60-107 µm wide ($\bar{x}$ = 80 µm, n = 15), two-layered, outer layer is composed of thick-walled, dark brown to brown cells of textura globulosa, inner layer is composed of thin-walled, hyaline to light brown cells of textura angularis. Hamathecium composed of 1.3-3.2 µm wide at the base, 3.1-4.9 µm wide at swollen tips (n = 20), dense, hyaline, septate pseudoparaphyses, branched and forming a yellow epithecium above the asci, slightly swollen with dense septa at the apex. Hymenium turns blue in Melzer's reagent, J+. Asci 143-196 × 9.9-13 µm ($\bar{x}$ = 169 × 12 µm, n = 30),

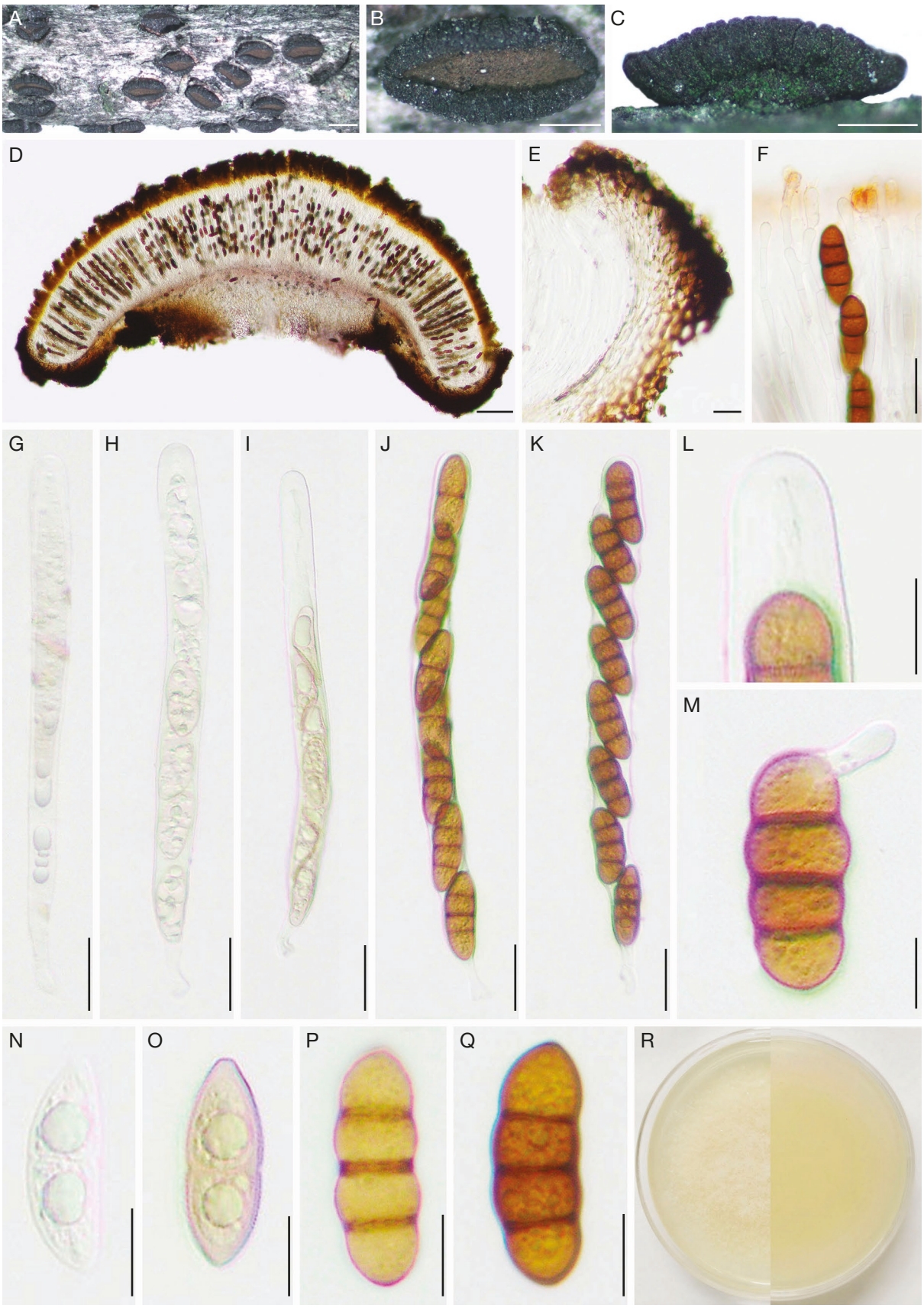

FIG. 3. — *Rhytidhysteron ligustrum* X.-L. Xu & C.-L. Yang, sp. nov. (holo-, SICAU 20-0004): **A**, appearance of apothecia on host; **B**, **C**, ascomata; **D**, vertical section of hysteriothecium; **E**, exciple; **F**, pseudoparaphyses; **G**-**K**, asci; **L**, ocular chamber; **M**, germinated ascospores; **N**-**Q**, ascospores; **R**, colonies on PDA for five days. Scale bars: A, 1 mm; B, C, 0.5 mm; D, 100 µm; E-K, 20 µm; L-Q, 10 µm.

6-8-spored, bitunicate, clavate to cylindrical, short pedicellate and apically rounded with an ocular chamber, J- in Melzer's reagent. Ascospores 18-30 × 8-11 µm ($\bar{x}$ = 24 × 9.6 µm, n = 40), partially overlapping, uniseriate, 1-2-3-septate, frequently 3-septate, ellipsoid or fusoid, straight or slightly curved, constricted septum, olive-green to brown or dark brown, without a mucilaginous sheath.

Asexual morphology
Undetermined.

Culture characteristics
Ascospores germinating on PDA within 24 hours and germ tubes produced from any cell. Colonies growing on PDA reach 3 cm diam after six days at 25°C, flat, circular, initially white, becoming gray to dark gray or reddish brown.

NOTES
The new species *Rhytidhysteron sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov. differs from *R. thailandium* in size of ascomata (930-2370 × 760-1930 × 280-820 µm vs 700-1200 × 530-750 × 360-640 µm), the width of exciple (60-107 µm, vs 72-130 µm), the length of asci (143-196 µm vs 135-160 µm), the ascospore septation (1-2-3-septate vs 3-septate), and the color of ascospore. *R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov. is phylogenetically close to *R. thailandicum*.We compared the nucleotides of LSU, SSU, *tef*-1α and ITS gene regions to *R. thailandicum* (holo-, MFLUCC 14-0503). *R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov. has 20 (*tef*-1α, 2.15%), 11 (ITS, 2.16%) base-pair differences to *R. thailandicum*.

Rhytidhysteron subrufulum
X.-L. Xu & C.-L. Yang, sp. nov.
(Fig. 5)

HOLOTYPE. — **China**. Sichuan Province, Chengdu City, Wenjiang District, 30°42'18.89"N, 103°51'30.42"E, alt. 545 m, on dead twigs of *Osmanthus fragrans* (Thunb.) Loureiro (Oleaceae), 12.X.2019, collected by C.-L. Yang, *YCL201910001* (holo-, SICAU 19-0010), ex-type living culture (SICAUCC 19-0011).

ETYMOLOGY. — The specific epithet refers to the allied species of *R. rufulum*.

INDEX FUNGORUM. — IF557216.

ADDITIONAL SPECIMENS EXAMINED. — **China**. Sichuan Province, Zizhong County, 29°46'28.29"N, 104°49'19.66"E, alt. 316 m, on dead twigs of *Citrus reticulata* Blanco (Rutaceae), 8.III.2019, collected by X.-L. Xu & C.-L. Yang, *YCL201903011* (SICAU 19-0009), living culture (SICAUCC 19-0010); 29°46'22.97"N, 104°49'12.20"E, alt. 303 m, on dead twigs of *Broussonetia papyrifera* (Linnaeus) L'Heritier ex Ventenat (Moraceae), 19.X.2019, collected by X.-L. Xu and C.-L. Yang, *XXL201910004* (SICAU 20-0003), living culture (SICAUCC 20-0003); Chongzhou City, 30°33'25.37"N, 103°39'30.67"E, alt. 511 m, on dead twigs of *Robinia pseudoacacia* L. (Fabaceae), 29.XI.2019, collected by C.-L. Yang, *XXL201911012* (SICAU 20-0006), living culture (SICAUCC 20-0006); on dead twigs of *Carya illinoinensis* (Wangenheim) K. Koch (Juglandaceae), 18.IX.2020, collected by X.-L. Xu, *XXL202009001* (SICAU 20-0011), living culture (SICAUCC 20-0011); Chengdu City, Wenjiang District, 30°42'18.89"N, 103°51'30.42"E, alt. 545 m, on dead wood of *Chimonanthus praecox* (L.) Link (Calycanthaceae), 6.XI.2020, collected by X.-L. Xu, *XXL202011001* (SICAU 22-0001), living culture (SICAUCC 22-0001).

ADDITIONAL GENBANK NUMBER. — SICAUCC 19-0011: *tub*2, MT075597; *rpb*2, MT027614. SICAUCC 19-0010: *tub*2, MT075596; *rpb*2, MT027613. SICAUCC 20-0003: *tub*2, MT075588; *rpb*2, MT075604. SICAUCC 20-0006: *tub*2, MT075591; *rpb*2, MT075607. SICAUCC 20-0011: *tub*2, MW219742; *rpb*2, MW219741. SICAUCC 22-0001: *tub*2, OM371084; *rpb*2, OM371083.

DESCRIPTION
Saprobic on decaying woody branches and twigs.

Sexual morphology
Ascomata 900-2870 µm long × 900-1720 wide × 470-660 high ($\bar{x}$ = 1909 × 1220 × 546 µm, n = 20), apothecioid, carbonaceous, scattered to gregarious, black, labiates and elliptic or irregular in shape, perpendicularly striate along the long axis, reddish brown to black on the disc. Exciple 36-83 µm wide ($\bar{x}$ = 62, n = 15), two-layered, outer layer comprising thick- walled, brown to hyaline cells of textura angularis and textura globulosa, inner layer comprising thin-walled, light brown to hyaline cells of textura angularis and textura prismatica. Hamathecium composed of 1.6-2.4 µm wide at the base, 2.5-4.0 µm wide at swollen tips (n = 20), dense, septate, pseudoparaphyses, branched and forming brown epithecium above the asci, slightly swollen at the apex, hymenium turns blue in Melzer's reagent, J+. Asci 183-214 × 13-20 µm ($\bar{x}$ = 202 × 16 µm, n = 15), (5-)8-spored, bitunicate, clavate to cylindrical, with short pedicel and apically rounded with an ocular chamber, J- in Melzer's reagent. Ascospores 29-41 × 10-15 µm ($\bar{x}$ = 33 × 13 µm, n = 30), ellipsoidal or fusiform, straight or slightly curved, slightly pointed at both ends, partially overlapping, uniseriate, (2-)3-septate, constricted septum, light brown to dark brown, without a mucilaginous sheath.

Asexual morphology
Undetermined.

Culture characteristics
Ascospores germinating on PDA within 24 hours and germ tubes produced from any cell. Colonies growing on PDA reach 4 cm diam after five days at 25°C, flat, circular, initially white, gradually becoming yellow to gray.

NOTES
Morphological comparison shows *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. with similar perpendicular striations as typical *R. rufulum* and *R. esperanzae*. But the exciple of *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. (36-83 µm) is thinner than *R. rufulum* (75-228 µm) and *R. esperanzae* (60-220 µm), and the asci are wider than *R. rufulum* (16 µm vs 13.5 µm), and shorter than *R. esper-*

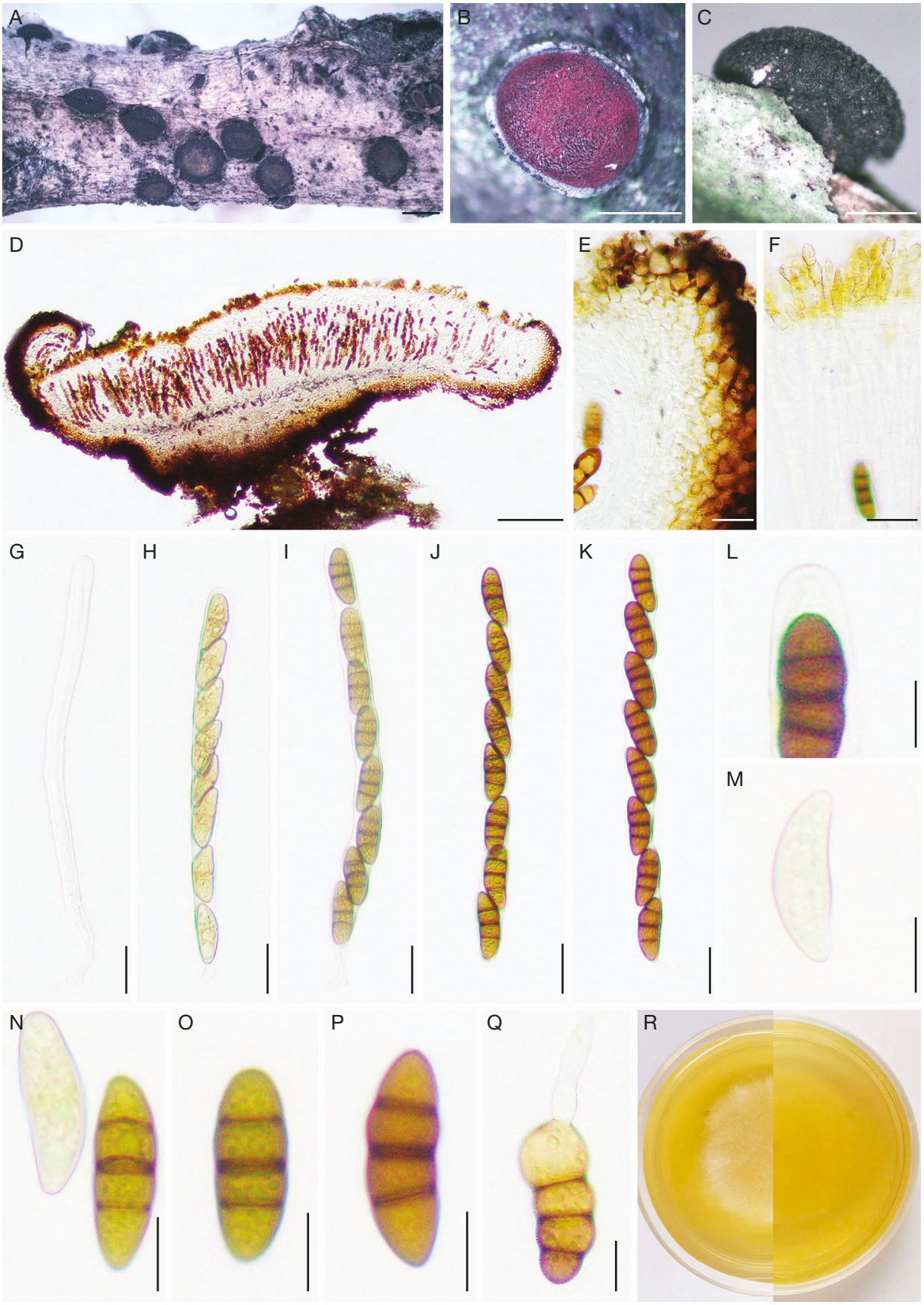

FIG. 4. — *Rhytidhysteron sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov. (holo-, SICAU 19-0004): **A**, appearance of apothecia on host; **B**, **C**, ascomata; **D**, vertical section of hysteriothecium; **E**, exciple; **F**, pseudoparaphyses; **G**-**K**, asci; **L**, ocular chamber; **M**-**P**, ascospores; **Q**, germinated ascospores; **R**, colonies on PDA for six days. Scale bars: A, B, 1 mm; C, 0.5 mm; D, 200 µm; E-K, 20 µm; L-Q, 10 µm.

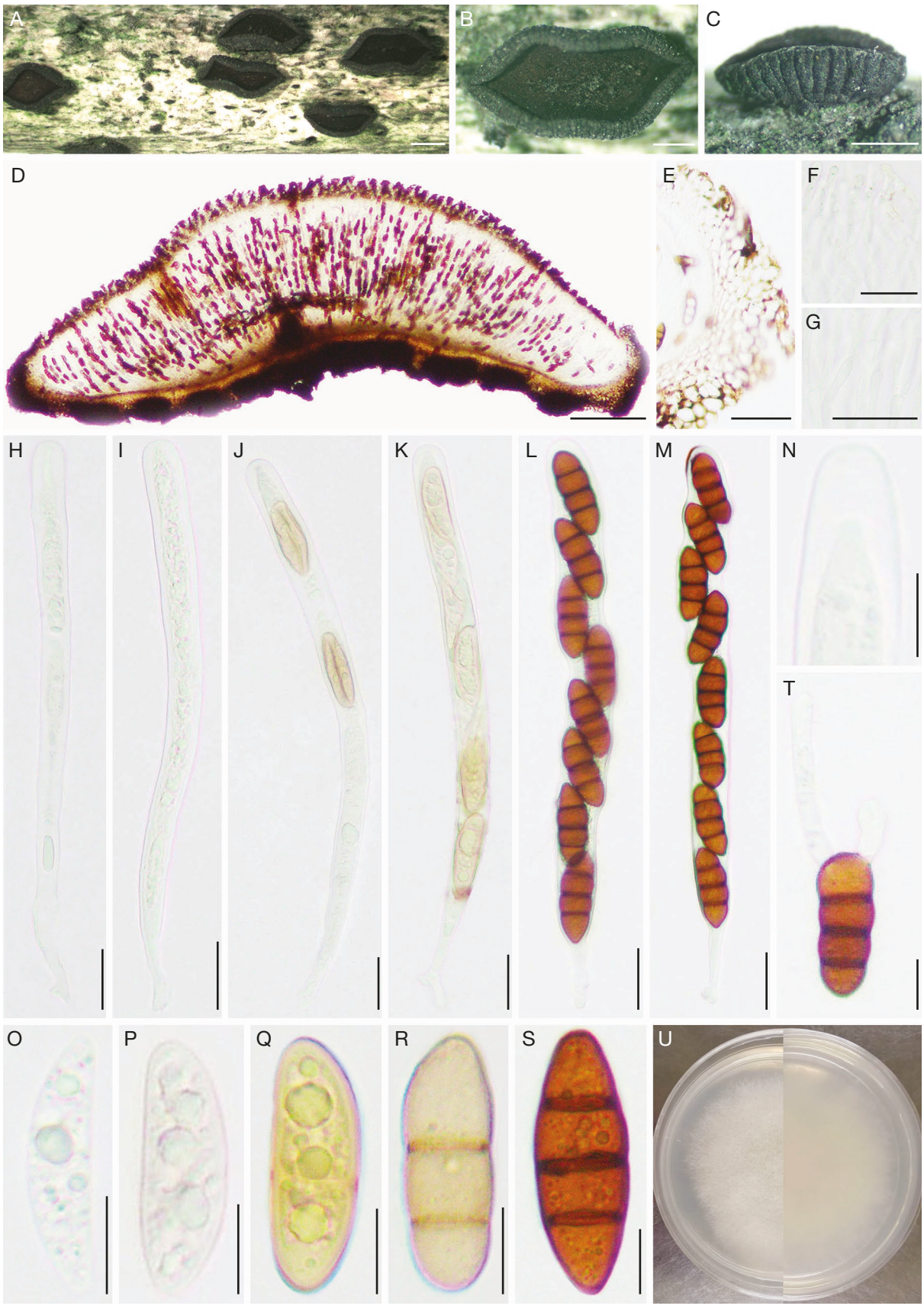

Fig. 5. — *Rhytidhysteron subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. (holo-, SICAU19-0010): **A**, appearance of apothecia on host; **B**, **C**, ascomata; **D**, vertical section of hysteriothecium; **E**, exciple; **F**, **G**, pseudoparaphyses; **H**-**M**, asci; **N**, ocular chamber; **O**-**S**, ascospores; **T**, germinated ascospores; **U**, colonies on PDA for five days. Scale bars: A, 1 mm; B, C, 0.5 mm; D, 200 µm; E, 50 µm; F-M, 20 µm; N-T, 10 µm.

TABLE 2. — Morphological differences of *Rhytidhysteron* Speg. species compared in this paper (new taxa are highlighted in **bold**).

Species	Ascoma	Asci (µm)	Ascospores (µm)	References
Rhytidhysteron brasiliense Speg.	rough without striations, red-yellow disc	220-245 × 13-18, 6-8-spored	28-33 × 10-13, 1-3-septate	Spegazzini 1881; Yacharoen *et al*. 2015; Thambugala *et al*. 2016
R. bruguierae Dayarathne	striate, brown-black disc	128-148 × 10-14, 6-8-spored	14-26 × 6.2-9, 1-3-septate	Dayarathne *et al*. 2020
R. camporesii Ekanayaka & K.D.Hyde	slightly dentate	165-175 × 13-15, 8-spored	25-28 × 9-11, 3-septate	Hyde *et al*. 2020b
R. chromolaenae Mapook & K.D.Hyde	no striation, orange or dark brown to black disc	130-155 × (8-)11-14, 7-8-spored	23-28 × 8-11, 3-septate	Mapook *et al*. 2020
R. columbiense Soto-Medina & Lücking	striate, brown-blackdisc	175-190 × 14-18, 6-8-spored	38-52 × 13-18, 1-3-septate	Soto-Medina & Lücking 2017
R. cozumelense Cobos-Villagrán, R.Valenz, Hern.-Rodr., Calvillo-Medina & Raymund	smooth -slightly striate	182-191 × 12-13	26-29(-31) × 9-11 (-13), 3-septate	Cobos-Villagrán *et al*. 2021
R. erioi Ekanayaka & K.D.Hyde	dentate, orange disc	140-200 × 9-16, 8-spored	22-28 × 9-11, 3-septate	Hyde *et al*. 2020b
R. esperanzae Cobos-Villagrán, R.Valenz. & Raymundo	striate	(250-)265-270 × (18-)19-20	(42-)45-47(-49) × (15-)17-19(-23), 3-septate	Cobos-Villagrán *et al*. 2021
R. hongheense Wanas	slightly dentate	140-180 × 12-16, 8-spored	20-33 × 9-13, 3-septate	Wanasinghe *et al*. 2021
R. hysterinum (Dufour) Samuels & E.Müll.	smooth-striate, orange-black disc	170-235 × 13-20, (4-)8-spored	21-32 × 8-14, 1-septate	Samuels & Müller 1979
***R. ligustrum* X.-L. Xu & C.-L. Yang, sp. nov.**	**striate, reddish brown to dark brown disc**	**134-196 × 10-18, 6-8-spored**	**18-32 × 8.9-13, 1-2-3-septate**	**This study**
R. mangrovei Vinit & K.D.Hyde	striate, brown-black disc	110-150 × 9.4-10, (2-6-)8-spored	21-28 × 7.5-8.5, 1-3-septate	Kumar *et al*. 2019
R. magnoliae N.I.de Silva, Lumyong & K.D.Hyde	striate, dark brown disc	(148-)160-200 (-210) × (11-)13-15(-16), 8-spored	(25-)28-30(-32) × (8-)10-11(-12), 1-3-septate	de Silva *et al*. 2020
R. mesophilum Cobos-Villagrán, R.Valenz, Hern.-Rodr., Calvillo-Medina & Raymundo	striate	267-282 × 15.5-16	(38-)40-44(-46) × 12-14, 3-septate	Cobos-Villagrán *et al*. 2021
R. neohysterinum Cobos-Villagran, Hern.-Rodr., R.Valenz. & Raymundo	striate, black disc	160-185 × 12-13, 8-spored	(23-)24.8-29(-31) × (8.5-)8.8-10(-11.2), 1-septate	Cobos-Villagrán *et al*. 2020
R. neorufulum Thambug. & K.D.Hyde	sough without striation, black-yellow disc	185-220 × 9.5-13, 8-spored	27-34 × 6.5-12.5, 1-3-septate	Thambugala *et al*. 2016
R. rufulum (Spreng.) Speg.	striate, black-red disc	150-250 × 11-16, 8-spored	28-36 × 9-13, 1-3-septate	Thambugala *et al*. 2016
***R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov.**	**striate, reddish brown-black disc**	**143-196 × 9.9-13, 6-8-spored**	**18-30 × 8-11, 1-2-3-septate**	**This study**
***R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov.**	**striate, reddish brown-black disc**	**183-214 × 13-20, (5-)8-spored**	**29-41 × 10-15, 2-3-septate**	**This study**
R. tectonae Doilom & K.D.Hyde	smooth without striation, yellow disc	135-176 × 10-15, 8-spored	19-31 × 8-13, 1-2-3-septate	Doilom *et al*. 2017
R. thailandicum Thambug. & K.D.Hyde	rough without striations	135-160 × 10.5-15, 8-spored	20-31 × 7.5-12, 3-septate	Thambugala *et al*. 2016
R. xiaokongense G.C.Ren & K.D.Hyde	–	–	–	Ren *et al*. 2022

anzae (183-214 µm vs 250-270 µm). Furthermore, the asci are (5-)8-spored in *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov., 8-spored in *R. rufulum* and undescribed in *R. esperanzae*. And ascospores of *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. are larger than *R. rufulum*, but smaller than *R. esperanzae* (33 × 13 µm vs 31 × 11 µm vs 45 × 17 µm) (Thambugala *et al*. 2016; Cobos-Villagrán *et al*. 2021). It is worth mentioning that the specimen SICAU 19-0009 had a large number of fusiform and 1-septate ascospores obviously pointed at both ends, but those ascospores did not germinate at room temperature for a week. The LSU and SSU DNA sequences between *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. (SICAUCC 19-0011) and *R. rufulum* (MFLUCC 14-0577, MFLUCC 12-0013) are almost identical, but there are sufficient base-pair differences in *tef*-1α (1.66 %, MFLUCC 14-0577) (no data, MFLUCC 12-0013) and ITS (1.47 %, MFLUCC 14-0577) (1.52 %, MFLUCC 12-0013) gene. In the comparison of *tef*-1α sequence, it shows 1.68 % differences between *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. and *R. esperanzae*, whereas no data on ITS sequence. With these morphological and DNA sequence differences, this species is identified as a new species named *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. A table summarizing major morphological differences among *Rhytidhysteron* species is shown in Table 2.

DISCUSSION

In this paper, the analyses refer to those species with morphological and molecular data, on account of the lack of sequence data in GenBank for some species. Three new species *Rhytidhysteron ligustrum* X.-L. Xu & C.-L. Yang, sp. nov., *R. sichuanensis* X.-L. Xu & C.-L. Yang, sp. nov. and *R. subrufulum* X.-L. Xu & C.-L. Yang, sp. nov. are distinguished from the extant species based on morphological comparison of ascomata, exciple, asci and ascospores, as well as molecular data, as described in the notes. We establish the new species based on differences in sequence data following the recommendations of Jeewon & Hyde (2016).

Rhytidhysteron rufulum (Spreng.) Speg. was introduced by Spegazzini (1921) based on *Hysterium rufulum* Spreng. Thambugala *et al.* (2016) revised several isolates of *R. rufulum* into two species based on multigene analysis, namely *R. rufulum* with distinct striations as a neotype (NY 6149) designated by Kutorga & Hawksworth (1997), and *R. neorufulum* without striations, showing that striations on the surface of apothecia are an important character to distinguish species. In addition, exciple, asci and ascospores are basis for morphological identification. However, there are overlaps in morphology between different species (Table 2). Moreover, strains within the same species from different hosts or distribution have differences in morphological size, as *R. fululum* described in those literatures (Samuels & Müller 1979; Thambugala *et al.* 2016; Jayasiri *et al.* 2018), as well as our strains from different hosts. Currently, it is difficult to distinguish the species completely depending on morphological differences. Thus, the phylogenetic analysis based on multigene sequences are indispensable for identification. In addition, recollection, epitypification and molecular analysis are essential for those species lacking type specimen to establish species boundaries in the genus (Ariyawansa *et al.* 2014; Thambugala *et al.* 2016). In accordance with previous studies (Boehm *et al.* 2009b; Almeida *et al.* 2014a; Thambugala *et al.* 2016; Jayasiri *et al.* 2018; Kumar *et al.* 2019), *R. opuntiae* (J.G.Br.) M.E.Barr does not cluster within *Rhytidhysteron*. Both the morphological and molecular data suggest that *R. opuntiae* should be removed from the genus *Rhytidhysteron* (Almeida *et al.* 2014a).

Rhytidhysteron includes saprobic, weakly pathogenic and endophytic species (Yacharoen *et al.* 2015; Thambugala *et al.* 2016; Rashmi *et al.* 2019), as well as a rare human pathogen (Mishra *et al.* 2014; Chander *et al.* 2017; Fraser 2020). Most species have been found in Thailand and isolated from decaying woody twigs and fruit pericarps, or herbaceous stems in terrestrial habitats, as well as marine and submerged branches (Thambugala *et al.* 2016; Doilom *et al.* 2017; Jayasiri *et al.* 2019; Kumar *et al.* 2019; Dayarathne *et al.* 2020; Hyde *et al.* 2020b; Mapook *et al.* 2020). The genus has been found to have a wide range of hosts, viz. *Citrus jambhiri* Lush., *C. sinensis* (L.) Osbeck (Rutaceae), *Swietenia mahagoni* (L.) Jacq. (Meliaceae), *Tectona grandis* L. f. (Lamiaceae), *Bruguiera* sp. (Rhizophoraceae), *Chromolaena odorata* (Linnaeus) R.M.King & H.Robinson (Asteraceae), *Acacia cochliacantha* Willd (Fabaceae), *Bursera* sp. (Burseraceae), *Bougainvillea glabra* Choisy (Nyctaginaceae), *Celtis pallida* Torr. (Cannabaceae) and *Helietta parvifolia* (A.Gray) Benth. (Rutaceae), as well as an extensive geographical distribution (i.e., Australia, Brazil, Colombia, Costa Rica, China, Cuba, Ghana, Kenya, Mexico, India, New Zealand, Thailand and Venezuela) (Bezerra & Kimbrough 1982; Boehm *et al.* 2009b; Murillo *et al.* 2009; Doilom *et al.* 2017; Jayasiri *et al.* 2019; Kumar *et al.* 2019; Cobos-Villagrán *et al.* 2020; Dayarathne *et al.* 2020; Hyde *et al.* 2020b; Mapook *et al.* 2020; Cobos-Villagrán *et al.* 2021).

Hyde *et al.* (2020a) hypothesized that if any genus is studied in a new region or from unstudied hosts, it is likely that new species will be discovered. This is borne out in the present study, where three new species and new record were determined in China, indicating that there is a high diversity of *Rhytidhysteron* species in this country. Previous records of *Rhytidhysteron* in China are *Rhytidhysteron camporesii* from an undetermined woody plant, *R. magnoliae* form *Magnolia grandiflora* L. (Magnoliaceae), *R. hongheense* from dead twigs of *Dodonaea* sp. (Sapindaceae), *R. xiaokongense* from dead wood of *Prunus* sp. (Rosaceae), *R. thailandicum* from decaying wood of *Morus australis* Poir. (Moraceae) and *R. rufulum* (Almeida *et al.* 2014b; de Silva *et al.* 2020; Hyde *et al.* 2020b; Wanasinghe *et al.* 2021; Ren *et al.* 2022).

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