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First report of the sexual morph of *Pseudofusicoccum adansoniae* Pavlic, T.I.Burgess & M.J.Wingf. on Para rubber

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KEY WORDS
Botryosphaeriales, morphology, new sexual record, phylogeny, Thailand.

ABSTRACT

Pseudofusicoccum adansoniae Pavlic, T.I.Burgess & M.J.Wingf. and *P. ardesiacum* Pavlic, T.I.Burgess & M.J.Wingf. were collected from branches and twigs of *Hevea brasiliensis* Müell. Arg. in Phayao Province, northern Thailand. *Pseudofusicoccum* Mohali, Slippers & M.J.Wingf. is known only by its asexual morph. Here, we report the sexual morph of *P. adansoniae* based on phylogenetic analyses

of a combined ITS, LSU, TEF1 and β-tubulin dataset. The sexual morph is characterized by immersed, uniloculate ascomata, cylindric-clavate asci with a long pedicel, a well-developed ocular chamber and short clavate, hyaline, aseptate ascospores, surrounded by a mucilaginous sheath. Descriptions, illustrations and molecular data are provided for the taxa.

RÉSUMÉ

Premier signalement de la morphologie sexuelle de Pseudofusicoccum adansoniae *Pavlic, T.I.Burgess & M.J.Wingf. sur le Para rubber.*

Pseudofusicoccum adansoniae Pavlic, T.I.Burgess & M.J.Wingf. et *P. ardesiacum* Pavlic, T.I.Burgess & M.J.Wingf. ont été prélevés sur des branches et des rameaux de *Hevea brasiliensis* Müell. Arg. dans la province de Phayao, au nord de la Thaïlande. Le *Pseudofusicoccum* Mohali, Slippers & M.J.Wingf. n'est connu que par sa morphologie asexuée. Nous présentons ici la morphologie sexuelle de *P. adansoniae* sur la base d'analyses phylogénétiques d'un ensemble de données combinées ITS, LSU, TEF1 et β-tubuline. La morphologie sexuelle est caractérisée par des ascomates immergés, uniloculés, des asques cylindriques-clavés avec un long pédicelle, une chambre oculaire bien développée et des ascospores courtes, hyalines et aseptisées, entourées d'une gaine mucilagineuse. Des descriptions, des illustrations et des données moléculaires sont fournies pour ces taxons.

MOTS CLÉS
Botryosphaeriales, morphologie, nouveau record sexuel, phylogénie, Thaïlande.

INTRODUCTION

The order Botryosphaeriales (Dothideomycetes) has a worldwide distribution, particularly in tropical and temperate regions (Phillips *et al*. 2013, 2019; Slippers *et al*. 2017) and comprises the families *Aplosporellaceae*, *Botryosphaeriaceae*, *Melanopsaceae*, *Phyllostictaceae*, *Planistromellaceae* and *Saccharataceae* (Phillips *et al*. 2019). Taxa of Botryosphaeriales occur as endophytes, saprobes or plant pathogens on a wide range of angiosperm and gymnosperm hosts. Species are associated with diseases such as fruit and stem rots, shoot blights, cankers, die-back and leaf spots (Slippers & Wingfield 2007; Doilom *et al*. 2015; Trakunyingcharoen *et al*. 2015a, b; Dissanayake *et al*. 2016, 2017; Slippers *et al*. 2017; Wijayawardene *et al*. 2017).

Pseudofusicoccum Mohali, Slippers & M.J.Wingf., is currently accommodated in *Phyllostictaceae* (Phillips *et al*. 2019) and eight species are known (Dissanayake *et al*. 2016; Jami *et al*. 2018). *Pseudofusicoccum* has been reported from Australia, South Africa, Thailand and Venezuela on various hosts as endophytes, saprobes or plant pathogens associated with diseases on stems, twigs, branches and leaves (Mohali *et al*. 2006; Trakunyingcharoen *et al*. 2014, 2015a, b; Doilom *et al*. 2015; Jami *et al*. 2018). This genus is characterized by immersed to superficial pycnidial conidiomata, and hyaline, aseptate, cylindrical to ellipsoid conidia surrounded by a mucilaginous sheath (Pavlic *et al*. 2008; Yang *et al*. 2017; Phillips *et al*. 2019). Many sexual and asexual genera have been introduced in Botryosphaeriales, however, the sexual morphs of *Pseudofusicoccum* species has never been reported (Pavlic *et al*. 2008; Mehl *et al*. 2011; Doilom *et al*. 2015; Trakunyingcharoen *et al*. 2015a; Dissanayake *et al*. 2016; Wijayawardene *et al*. 2017; Yang *et al*. 2017; Jami *et al*. 2018; Phillips *et al*. 2019).

Para rubber (*Hevea brasiliensis* Müell. Arg.) is a tropical crop from the Amazon forest of South America and the primary source of natural rubber (Rao 1975; Priyadarshan *et al*. 2009; Ahrends *et al*. 2015). Currently, Para rubber plantations have been expanding rapidly throughout Southeast Asia and especially in Thailand (Herrmann *et al*. 2016; Vongkhamheng *et al*. 2016). The tree is the host of numerous fungi including endophytes, saprobes and plant pathogens (Jayasinghe 2001; Gazis & Chaverri 2010; Seephueak *et al*. 2011; Trakunyingcharoen *et al*. 2015a, b). During our investigation of fungi associated with Para rubber in Thailand, we made seven collections of *Pseudofusicoccum* which we identified as two species, *P. adansoniae* Pavlic, T.I.Burgess & M.J.Wingf. and *P. ardesiacum* Pavlic, T.I.Burgess & M.J.Wingf.

We are carrying out surveys of fungi in the Greater Mekong Subregion and are finding that the fungi are extremely diverse (Hyde *et al.* 2018; Tibpromma *et al.* 2018). In this study we have identified species of *Pseudofusicoccum* occurring on Para rubber in Phayao Province, northern Thailand. Identifications are confirmed based on morphological studies and multigene sequence analyses. In addition, the sexual morph of *Pseudofuicoccum adansoniae* is reported herein for the first time. The identity of sexual morph was confirmed by phylogenetic analyses of a combined ITS, LSU, TEF1 and β-tubulin sequence data.

MATERIAL AND METHODS

Collection, isolations and taxonomy

A survey was conducted during 2016 in Para rubber plantations from Phayao Province, northern Thailand. Collected specimens were examined with a Motic SMZ 168 Series microscope. Images were captured with an Axio camera on a Zeiss Discovery V8 stereo microscope. Sections of micro-morphological structures were cut by hand and images recorded with a Canon 600D digital camera mounted on a Nikon ECLIPSE 80i compound microscope. Fungal structures were measured using Tarosoft® Image

Framework program (v.0.9.0.7). Photographic plates were prepared with Adobe Photoshop CS6 version 13.0. Pure cultures were obtained by single spore isolation with spore suspension technique. Fruiting bodies were removed from the plant substrate using a sterilized needle and placed in a few drops of sterilized water on a sterilized cavity slide. The spore suspension was dropped on the surface of malt extract agar (MEA; 33.6 g/l sterile distilled water, Difco malt extract media) and the droplets were spread over the agar surface with a sterile spreader. The MEA agar plate containing a spore suspension was incubated at 25-30°C for 6-24 hours. Germinating ascospores/conidia were checked by the Motic SMZ 168 stereo microscope after six hours and thereafter at 24 hours intervals and images captured with a Canon 600D camera on a Nikon ECLIPSE 80i microscope. Germinated ascospores/conidia were aseptically transferred to fresh MEA plates and colony diameters and culture characteristics were recorded after 1-4 weeks of incubation at 25-30°C. Cultures were stored in screw cap tubes at 4°C and –20°C for molecular work and incorporation into the culture collection. All cultures obtained in this study were deposited in Mae Fah Luang University Culture Collection (MFLUCC) in Thailand. The herbarium specimens were deposited in the Mae Fah Luang University Herbarium, Thailand (MFLU). Faces of fungi numbers were obtained as explained in Jayasiri *et al*. (2015).

DNA extraction and PCR

Genomic DNA was extracted from fungal mycelium grown on MEA, using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, P. R. China), and from fungal fruiting bodies, using the BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416), according to the manufacture's protocol. DNA preparations were stored at –20°C until used for the rDNA amplification. Amplification of rDNA was performed by polymerase chain reaction (PCR) using the primer pairs as follows: ITS5 and ITS4 (White *et al*. 1990) to amplify the partial gene regions of internal transcribed spacers (ITS); LR0R and LR5 (Vilgalys & Hester 1990) to amplify the 28S large subunit (LSU); EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998) to amplify the protein coding region for the translation elongation factor 1-alpha gene (TEF1), and Bt2a and Bt2b (Glass & Donaldson 1995) to amplify a fragment of the β-tubulin gene. The final volume of the PCR reaction was 25 µl, containing 1 µl of DNA template, 1 µl of each forward and reverse primer, 12.5 µl of *Taq* PCR Master Mix (2 ×, blue dye) (Sangon Biotech Co., Shanghai, China), 9.5 µl of sterilized ddH_2O. The PCR thermal cycling program for ITS and LSU gene amplifications were provided as initially 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 50 s, elongation at 72°C for 30 s, and final extension at 72°C for 10 min. The PCR thermal cycling program for TEF1 gene amplifications was provided as initially 94°C for 4 min, followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 50 s, elongation at 72°C for 1.30 min, and final extension at 72°C for 10 min. The PCR thermal cycling program for β-tubulin gene amplifications was provided as initially 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 1 min, and final extension at 72°C for 10 min. PCR products were sequenced by Sino Geno Max, Beijing, China. Sequence quality was checked, and sequences were condensed with the SeqMan II (DNASTAR software version 5.0; DNASTAR Inc., Madison, WI). Nucleotide sequences derived in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

BLASTn search were used to identify the closet matches of our nucleotide sequences, obtained from GenBank (NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Others used in the analyses were obtained from GenBank and recently published data (Phillips *et al*. 2013; Trakunyingcharoen *et al*. 2015b; Jami *et al*. 2018). Combined sequence data of ITS, LSU, TEF1 and β-tubulin were initially aligned by using MAFFT version 7 (Katoh *et al*. 2017) and manually adjusted using BioEdit v.7.0.9.1 (Hall 1999). Maximum likelihood analysis was performed by RAxML (Stamatakis *et al.* 2008) implemented in RAxMLGUI 1.3 (Silvestro & Michalak 2011). The search strategy was set to rapid bootstrapping at 1000 replicates. Maximum parsimony analysis was performed using PAUP v 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search function with 1000 random stepwise addition replicates and tree bisection-reconnection (TBR) as the branch-swapping algorithm. All informative characters were unordered and with equal weight. Statistical supports for branches of the most parsimonious tree were estimated using maximum parsimony bootstrap (BS) analysis with 1000 bootstrap replicates. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI]) were calculated.

The best fit model of evolution for phylogenetic analyses was determined with MrModeltest 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC). Bayesian posterior probabilities (BY) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) using MrBayes v3.2.2 (Huelsenbeck & Ronquist 2001). The phylogenetic tree was visualized in FigTree V.1.4.3 (Rambaut 2016) and drawn and converted to tiff file in Microsoft PowerPoint 2013 and Adobe Photoshop CS6 version 13.0 (Adobe Systems United States). The final alignment and tree were deposited in TreeBASE (http://www.treebase.org/) under the submission ID. 23953, 25548 and 25547 (The analysis results of ML, MP and Bayesian inference, respectively).

RESULTS

Phylogenetic analyses

Sequence data of ITS, LSU, TEF1 and β-tubulin were used for the phylogenetic analyses. The topologies of the obtained

TABLE 1. — Details and GenBank accession numbers of Botryosphaeriales used in the phylogenetic analyses in this study. New sequence data obtained in this study are indicated in **bold**. Notes: (T) represents ex-type or ex-epitype isolates; **ATCC**, American Type Culture Collection, Virginia, United States; **CBS**, Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; **CMW**, Tree Pathology Cooperative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; **CPC**, Culture collection of Pedro Crous, housed at CBS-KNAW; **MFLU**, Mae Fah Luang University Herbarium, Chiang Rai, Thailand; **MFLUCC**, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **MUCC**, Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie prefecture, Japan; **WAC**, Department of Agriculture Western Australia Plant Pathogen Collection, South Perth, Western Australia.

				GenBank Accession numbers			
Species	**Isolate/strain no.**	**Host**	**Locality**	**ITS**	**LSU**	**TEF1**	**β-tubulin**
Aplosporella artocarpi	CPC 22791 (T)	*Artocarpus heterophyllus*	Thailand	KM006450	–	KM006481	–
Aplosporella yalgorensis	MUCC 511 (T)	*Acacia cochlearis*	Australia	EF591926	EF591943	EF591977	EF591960
Bagnisiella examinas	CBS 551.66	–	–	EU167562	KF766316	GU349056	KF766126
Bagnisiella sp.	CBS 513.84	–	India	KX464084	KX464240	KX464554	KX464779
Botryosphaeria corticis	ATCC 22927	*Vaccinium* sp.	United States	DQ299247	EU673245	EU673291	EU673108
Botryosphaeria corticis	CBS 119047 (T)	*Vaccinium corymbosum*	United States	DQ299245	EU673244	EU017539	EU673107
Botryosphaeria dothidea	CBS 115476 (T)	*Prunus* sp.	Switzerland	AY236949	AY928047	AY236898	AY236927
Diplodia mutila	CBS 112553 (T)	*Vitis vinifera*	Portugal	AY259093	AY928049	AY573219	DQ458850
Diplodia seriata	CBS 112555 (T)	*Vitis vinifera*	Portugal	AY259094	AY928050	AY573220	DQ458856
Endomelanconiopsis endophytica	CBS 120397 (T)	*Theobroma cacao*	Panama	EU683656	EU683629	EU683637	KF766131
Endomelanconiopsis endophytica	CBS 122550	*Theobroma cacao*	Panama	EU683664	EU683634	EU683645	–
Kellermania anomala	CBS 132218 (T)	*Yucca brevifolia*	United States	KF766173	KF766343	KF766404	KF766133
Kellermania yuccigena	CBS 131727	–	–	KF766186	KF766356	KF766417	–
Melanops tulasnei	CBS 116805	*Quercus robur*	Germany	FJ824769	KF766365	KF766423	–
Melanops tulasnei	CBS 116806	*Quercus robur*	Germany	FJ824770	KX464370	KX464644	KX464919
Lasiodiplodia pseudotheobromae	CPC 22758	*Hevea brasiliensis*	Thailand	KJ607141	–	KJ607151	–
Lasiodiplodia theobromae	MFLUCC 12-0293	*Tectona grandis*	Thailand	KM396896	–	KM409634	KM510354
Neofusicoccum parvum	CBS 110301	*Vitis vinifera*	Portugal	AY259098	AY928046	AY573221	EU673095
Neofusicoccum parvum	CMW 9081 (T)	*Pinus nigra*	New Zealand	AY236943	KF766371	AY236888	AY236917
Phyllosticta citriasiana	CBS 120486	*Citrus maxima*	Thailand	FJ538360	KF766379	FJ538418	–
Phyllosticta hypoglossi	CBS 101.72	*Ruscus aculeatus*	Italy	FJ538365	KF766381	FJ538423	–
Pseudofusicoccum adansoniae	CBS 122055 (T)	*Adansonia gibbosa*	Australia	EF585523	KF766386	EF585571	–
Pseudofusicoccum adansoniae	CPC 22763	*Hevea brasiliensis*	Thailand	KJ607148	–	KJ607158	–
Pseudofusicoccum adansoniae	CPC 22764	*Hevea brasiliensis*	Thailand	KJ607149	–	KJ607159	–
Pseudofusicoccum adansoniae	CPC 22765	*Hevea brasiliensis*	Thailand	KJ607150	–	KJ607160	–
Pseudofusicoccum adansoniae	MFLUCC 13-0705	*Tectona grandis*	Thailand	KM396904	–	KM396908	KM510362
Pseudofusicoccum adansoniae	MFLUCC 14-0516	*Tectona grandis*	Thailand	KM396905	–	KM409642	KM510363
Pseudofusicoccum adansoniae	MFLUCC 14-0517	*Tectona grandis*	Thailand	KM396906	–	KM409643	KM510364
Pseudofusicoccum adansoniae	**MFLUCC 17-0327**	***Hevea brasiliensis***	**Thailand**	**MK480509**	**MK478920**	**MK495814**	**MK495807**
Pseudofusicoccum adansoniae	**MFLUCC 17-0333**	***Hevea brasiliensis***	**Thailand**	**MK480510**	**MK478921**	**MK495815**	**MK495808**
Pseudofusicoccum adansoniae	**MFLUCC 17-0334**	***Hevea brasiliensis***	**Thailand**	**MK480511**	**MK478922**	**MK495816**	**MK495809**
Pseudofusicoccum adansoniae	**MFLUCC 17-0339**	***Hevea brasiliensis***	**Thailand**	**MK480513**	**MK478924**	**MK495818**	**MK495811**
Pseudofusicoccum adansoniae	**MFLUCC 17-0359**	***Hevea brasiliensis***	**Thailand**	**MK480514**	**MK478925**	**MK495819**	**MK495812**
Pseudofusicoccum adansoniae	**MFLU 19-0242**	***Hevea brasiliensis***	**Thailand**	**MK480512**	**MK478923**	**MK495817**	**MK495810**
Pseudofusicoccum africanum	CMW 48028 (T)	*Mimusops caffra*	South Africa	MH558614	–	MH576590	–
Pseudofusicoccum africanum	CMW 48029	*Mimusops caffra*	South Africa	MH558617	–	MH576592	–
Pseudofusicoccum ardesiacum	CBS 122062 (T)	*Adansonia gibbosa*	Australia	EU144060	KF766387	EU144075	KX465069
Pseudofusicoccum ardesiacum	CBS 122063	*Adansonia gibbosa*	Australia	EU144061	–	EU144076	KX465070
Pseudofusicoccum ardesiacum	CBS 122064	*Eucalyptus* sp.	Australia	EU144062	–	EU144077	–

Table 1. — Continuation.

Species	Isolate/strain no.	Host	Locality	GenBank Accession numbers ITS	LSU	TEF1	β-tubulin
Pseudofusicoccum ardesiacum	WAC 13294	*Mangifera indica*	Australia	GU172405	–	GU172437	–
Pseudofusicoccum ardesiacum	**MFLUCC 17-0323**	***Hevea brasiliensis***	**Thailand**	**MK480508**	**MK478919**	**MK495813**	**MK495806**
Pseudofusicoccum artocarpi	CPC 22796 (T)	*Artocarpus heterophyllus*	Thailand	KM006452	–	KM006483	–
Pseudofusicoccum kimberleyense	CBS 122058	*Acacia synchronicia*	Australia	EU144057	KF766388	EU144072	–
Pseudofusicoccum kimberleyense	CBS 122061	*Adansonia gibbosa*	Australia	EU144059	–	EU144074	KX465072
Pseudofusicoccum olivaceum	CBS 124939 (T)	*Pterocarpus angolensis*	South Africa	FJ888459	–	FJ888437	–
Pseudofusicoccum olivaceum	CBS 124940	*Pterocarpus angolensis*	South Africa	FJ888462	MH874936	FJ888438	–
Pseudofusicoccum stromaticum	CBS 117448 (T)	*Eucalyptus hybrid*	Venezuela	KF766223	KF766389	KF766437	EU673094
Pseudofusicoccum stromaticum	CBS 117449	*Eucalyptus hybrid*	Venezuela	DQ436935	–	DQ436936	EU673093
Pseudofusicoccum vilaceum	CBS 124936 (T)	*Pterocarpus angolensis*	South Africa	FJ888474	–	FJ888442	–
Pseudofusicoccum vilaceum	CBS 124938	*Pterocarpus angolensis*	South Africa	FJ888472	–	FJ888441	–
Phaeosphaeria ammophilae	CBS 114595	–	–	KF766146	KF766314	KF766394	–
Saccharata capensis	CBS 122693 (T)	*Proteaceae*	South Africa	KF766224	KF766390	EU552095	–
Saccharata hawaiiensis	CBS 111787 (T)	*Protea laurifolia*	United States	KX464233	KX464543	KX464767	KX465074
Saccharata kirstenboschensis	CBS 123537 (T)	*Encephalartos princeps*	South Africa	FJ372392	FJ372409	KX464770	–
Septorioides pini-thunbergii	CBS 473.91 (T)	*Pinus thunbergii*	Japan	KF251243	MH873946	–	KF252727
Septorioides strobi	CBS 141443	*Pinus strobus*	United States	KT884699	KT884685	KT884713	KT884727
Umthunziomyces hagahagensis	CBS 142084 (T)	*Mimusops caffra*	South Africa	KY173472	KY173561	–	–

trees from each gene were compared manually to verify that the overall tree topology of the individual datasets was similar with the tree obtained from the combined alignment. The combined gene analysis representing the genera of Botryosphaeriales is shown in Figure 1, which consisted of 57 strains including our *Pseudofusicoccum* strains. *Phaeosphaeria ammophilae* (Lasch) Kohlm. & E. Kohlm. (CBS 114595) was used as the outgroup taxon. The combined dataset including 2482 total characters, were analyzed based on Bayesian inference, maximum parsimony and maximum likelihood analyses.

The combined dataset consisted of 1523 constant characters, 229 variable parsimony-uninformative characters and 730 parsimony-informative characters. The most parsimonious tree had TL = 2548, CI = 0.606, RI = 0.793, RC = 0.481, HI = 0.394. Bayesian posterior probabilities (BY) from MCMC were evaluated with the final average standard deviation of split frequencies = 0.007217. The final RAxML tree is shown in Figure 1, with a final ML optimisation likelihood value of –14889.898786. Phylogenetic trees derived from Bayesian, RAxML and MP analyses gave similar overall topologies at the family relationships. Phylogenetic analyses based on the combined ITS, LSU, TEF1 and β-tubulin sequence datasets showed that *Pseudofusicoccum* species grouped with *Phyllosticta* species within *Phyllostictaceae* (Botryosphaeriales) with high support (81% ML, 100% MP, 0.98 BY). The asexual morph of *Pseudofusicoccum adansoniae* (MFLUCC 17-0333, MFLUCC 17-0339 and MFLUCC 17-0359) and sexual morph of our strains (MFLUCC 17-0327, MFLUCC 17-0334 and MFLU 19-0242) clustered with other strains of *P. adansoniae* with high bootstrap support (95% ML, 99% MP, 1.00 BY, Fig. 1), we therefore identified them as *P. adansoniae*. The new isolate (MFLUCC 17-0323) clustered with *P. ardesiacum* (WAC 13294) (65% ML, 65% MP, 0.91 BY, Fig. 1), thus this strain was identified as *P. ardesiacum*.

Isolations

Isolates obtained from symptomatic branches and twigs of Para rubber (without causing apparent damage to trees) were recognized belonging to the genus *Pseudofusicoccum*. Isolates MFLUCC 17-0327, MFLUCC 17-0333, MFLUCC 17-0334, MFLUCC 17-0339 and MFLUCC 17-0359 were identified as *P. adansoniae*. Of these, isolates MFLUCC 17-0333, MFLUCC 17-0339 and MFLUCC 17-0359 produced an asexual morph forming brown pycnidia, immersed, solitary into plant substrate, producing hyaline, ellipsoid conidia, cover with a mucilaginous sheath. Isolates MFLUCC 17-0327 and MFLUCC 17-0334 produced a sexual morph forming globose, immersed, uniloculate, ascomata into the plant substrate, cylindric-clavate asci with a long pedicel, hyaline, aseptate ascospores, surrounded by a mucilaginous sheath. In addition, isolates MFLUCC 17-0323 was identified as *P. ardesiacum* which distinct from *P. adansoniae* by its larger conidia.

TAXONOMY

Family Phyllostictaceae Fr.
Genus *Pseudofusicoccum*
Mohali, Slippers & M.J.Wingf.

Pseudofusicoccum adansoniae
Pavlic, T.I.Burgess & M.J.Wingf.
(Figs 2; 3)

Mycologia 100: 855 (2008).

Index Fungorum number. — IF512048.

Facesoffungi number. — FoF 00168.

Culture characteristics. — Ascospores and conidia germinating on MEA within 24 hours at room temperature (25-30°C) and germ tube was produced from the ends of the ascospores and conidia. Colonies from ascospores and conidia germinating on MEA reaching 2 cm after 2 days at room temperature. Colony from ascospores: initially aerial mycelium white, circular, cottony to fluffy with sparse aspects, after 10 days become light brown to whitish grey at the center, white at the edge, slight raise, fluffy, dense, undulate, after 20-25 days of incubation, colonies become iron grey to black, hyphae septate, branched, smooth, forming conidiomata at the colony margin after 30-40 days and after 4 months of incubation, no asexual-morph produced on culture. Colony from single conidia: initially aerial mycelium white, circular, cottony with sparse aspects, after 10 days become whitish grey at the center, white at the edge, slight raise, fluffy, moderately dense, after 15-20 days of incubation, colonies become iron grey to black, hyphae septate, branched, smooth, forming conidiomata at the colony margin after 30-40 days and after 4 months of incubation, no asexual-morph produced on culture.

Material examined. — **Thailand**. Phayao Province, Muang District, associated with canker disease on branches of *Hevea brasiliensis* (Euphorbiaceae), 2.X.2016, *C. Senwanna*, RBPY08, MFLU 19-0239, living culture MFLUCC 17-0327; *ibid*. RBPY12, MFLU 19-0241, living culture MFLUCC 17-0333; *ibid*. RBPY13, MFLU 19-0242, living culture MFLUCC 17-0334; Phayao Province, Muang District, associated with canker disease on twigs of *Hevea brasiliensis* (Euphorbiaceae), 5.XII.2016, *C. Senwanna*, RBPY20, MFLU 19-0246, living culture MFLUCC 17-0339; Phayao Province, Muang District, associated with canker disease on branches of *Hevea brasiliensis* (Euphorbiaceae), 29.I.2017, *C. Senwanna*, RBPY41, MFLU 19-0251, living culture MFLUCC 17-0359.

Additional GenBank numbers. — SSU = MK503146 (MFLUCC 17-0327); MK503147 (MFLUCC 17-0334), MK503148 (MFLU 19-0242); MK503149 (MFLUCC 17-0339); MK503150 (MFLUCC 17-0359).

Description

Associated with canker disease on branches and twigs of *Hevea brasiliensis*.

Sexual morph

Ascomata. (115-)140-205(-220) diam × (140-)190-240 (-285) µm high ($\bar{x}$ = 178 × 219 µm, *n* = 15), appearing as spots on host surface, globose to subglobose, gregarious, scattered to clustered, immersed to semi-immersed, uniloculate, with a central ostiole, when cut horizontally the locular contents appear brilliantly white.

Peridium. 12-34 µm wide, outer layers dark brown to black-walled cell, inner layers thin-walled, hyaline, composed of cells of *textura angularis*.

Pseudoparaphyses. Intermixed with asci, hyaline, septate, constricted at septa.

Asci. (54.5-)60-81.5(-93) × (15-)16-19.5(-22.5) µm ($\bar{x}$ = 70 × 18 µm, *n* = 22), 8-spored, bitunicate, fissitunicate, cylindro-clavate or clavate, long-pedicellate, apically rounded with well-developed ocular chamber.

Ascospore. (10-)12-22(-27) × (4-)5-8(-9) µm ($\bar{x}$ = 16.6 × 6.3 µm, *n* = 42), overlapping uni- to bi-seriate, hyaline, aseptate, short clavate, straight, smooth-walled, with fine granular content, surrounded by a mucilaginous sheath.

Asexual morph

Conidiomata. (110-)130-230(-280) diam × (80-)105-180(-260) µm high ($\bar{x}$ = 170 × 142 µm, *n* = 20), pycnidial, solitary to scattered, immersed to semi-immersed, globose to subglobose, uni- to multilocular, with a central ostiole.

Ostiole. Periphysate, necks 28-64 long, 18-65 µm diam.

Conidiomata wall. 17-48 µm wide, outer layers dark brown to black, inner layers thin-walled, hyaline, composed of cell of *textura angularis*.

Paraphyses. Absent.

Conidiophores. Reduced to conidiogenous cells.

Conidiogenous cells. (6-)7-13(-16) × (2-)2.5-4(-4.5) µm ($\bar{x}$ = 9 × 3 µm, *n* = 30), holoblastic, cylindrical to subcylindrical, hyaline, smooth-walled.

Conidia. (14-)15-20(-21) × (4-)5-7(-8) µm ($\bar{x}$ = 18.5 × 6.6 µm, *n* = 80), ellipsoid to rod-shaped, hyaline, aseptate, straight or slightly bent, smooth-walled, with fine granular content, surrounded by a thin mucilaginous sheath.

Notes

The asexual morph of *Pseudofusicoccum adansoniae* was introduced by Pavlic *et al*. (2008) for isolates from *Adansonia gibbosa* (A.Cunn.) Guymer ex D.A.Baum, *Acacia synchronica* Maslin, *Eucalyptus* sp. and *Ficus opposita* Miq. in Western Australia. The morphology of our collections (MFLU 19-0239 and MFLU 19-0246, Figs 2; 3) are similar to that of the holotype of *P. adansoniae* (Pavlic *et al*. 2008) and collections from Para rubber (*Hevea brasiliensis*) (Trakunyingcharoen *et al*. 2015a) in having ellipsoid, slightly bent or irregularly shaped, hyaline conidia, which is also supported by the combined multi-gene phylogeny. However, the conidia of *P. adansoniae* in this study are shorter than reported by Pavlic *et al*. (2008) (14-21 µm vs 19-26 µm) and Trakunyingcharoen *et al*. (2015a) (14-21 µm vs 16-25 µm).

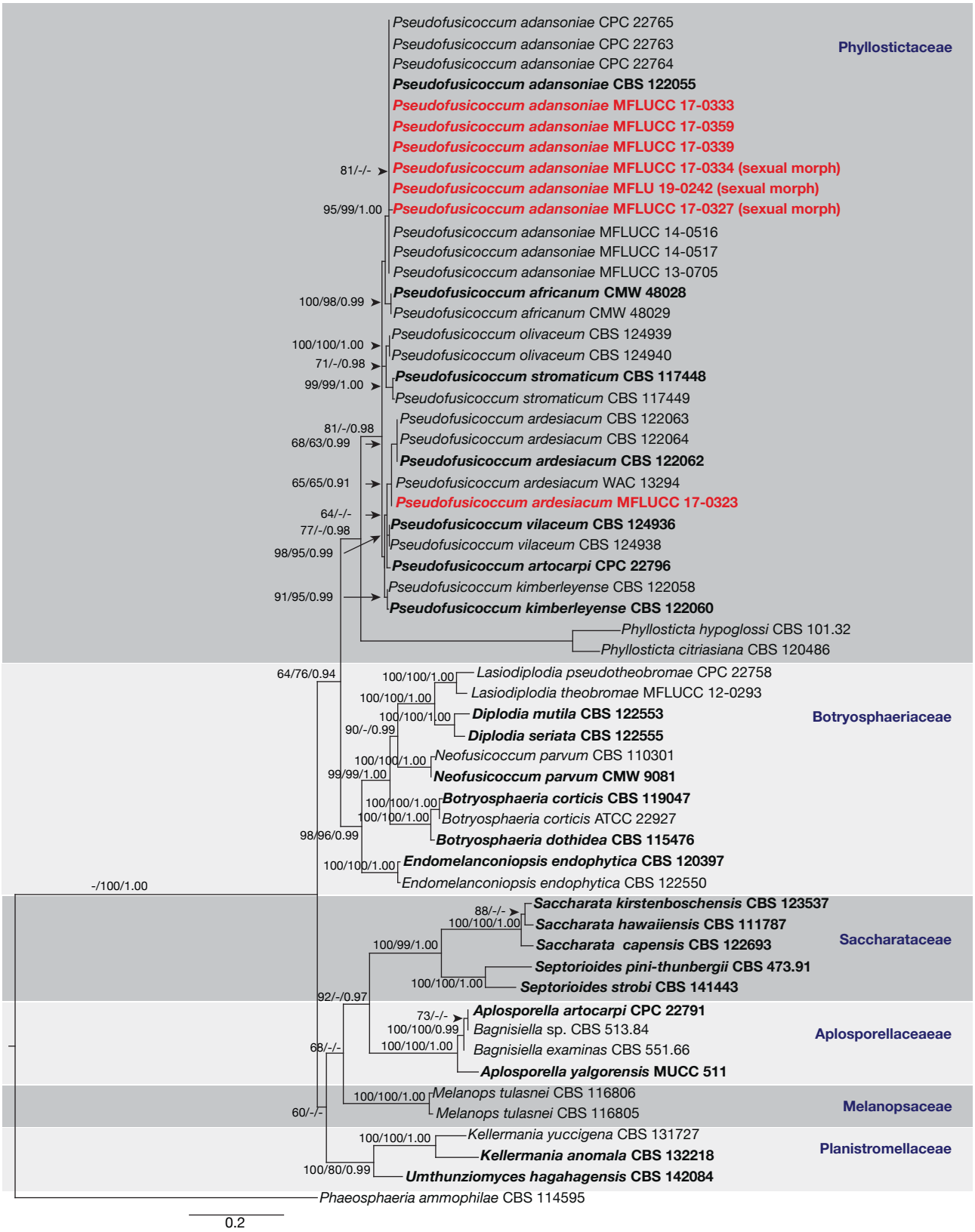

Fig. 1. — RAxML tree of Botryosphaeriales based on analyses of a combined ITS, LSU, TEF1 and β-tubulin sequence data. Bootstrap support values for ML and MP equal to or greater than 60% and Bayesian posterior probabilities (BY) equal to or greater than 0.90 are defined as ML/MP/BY above the nodes. The tree is rooted to *Phaeosphaeria ammophilae* (Lasch) Kohlm. & E.Kohlm. (CBS 114595). The type strain is noted in black **bold**. New sequence data are indicated in red **bold**.

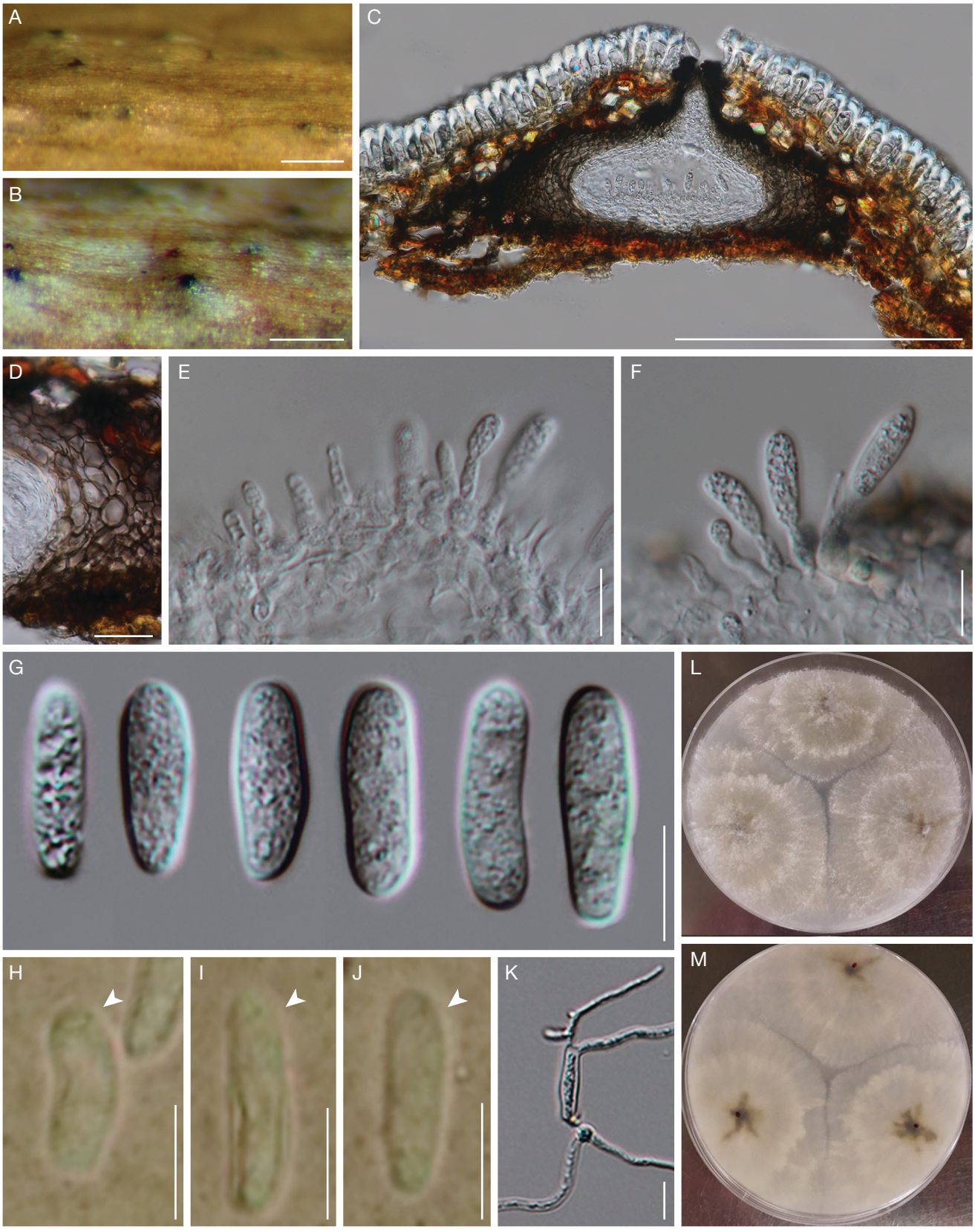

Fig. 2. — *Pseudofusicoccum adansoniae* Pavlic, T.I.Burgess & M.J.Wingf. (MFLU 19-0246): **A**, **B**, habit on host substrate; **C**, section through the conidiomata; **D**, peridium; **E**, **F**, conidiophore and conidiogenous cells; **G**, conidia (mounted in double-distilled water); **H-J**, conidia with mucilaginous sheath (**arrows**) (stained in Indian ink); **K**, germinated spores; **L**, **M**, upper and reverse view of the culture after ten days. Scale bars: A-C, 200 µm; D, 20 µm; E-K, 10 µm.

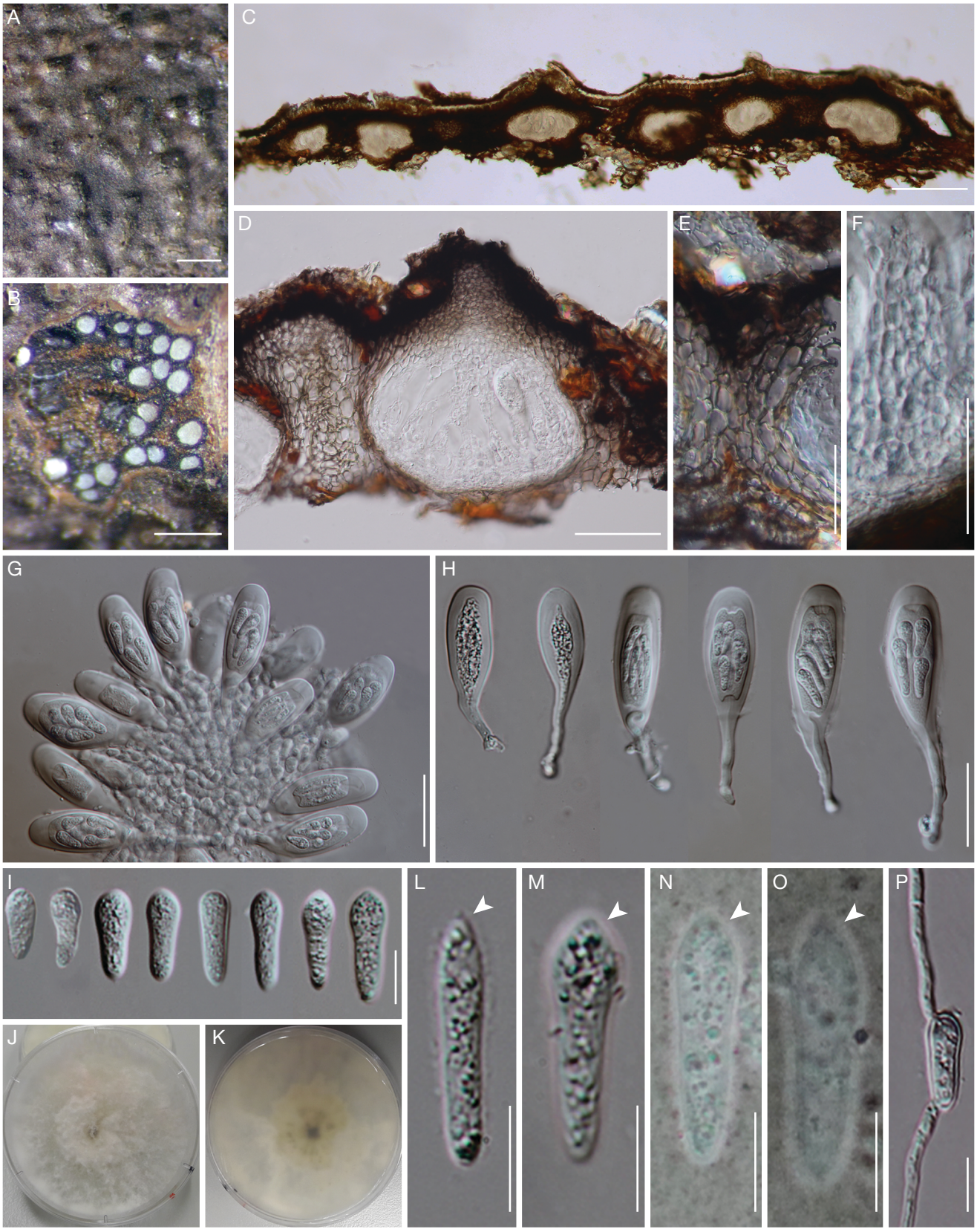

Fig. 3. — *Pseudofusicoccum adansoniae* Pavlic, T.I.Burgess & M.J.Wingf. (MFLU 19-0239): **A**, habit of ascoma in bark; **B**, transverse sections through ascomata; **C**, **D**, section through the ascoma; **E**, peridium; **F**, pseudoparaphyses; **G**, **H**, asci; **I**, ascospores (mounted in double-distilled water); **J**, **K**, upper and reverse view of the culture after ten days; **L-O**, ascospores with mucilaginous sheath (**arrows**) (stained in Indian ink); **P**, germinated spores. Scale bars: A, B, 500 µm; C, 200 µm; D, 50 µm; E-H, 30 µm; I, L-P, 10 µm.

This may be due to distribution and morphological variability within the species. In this study, isolates of the sexual morph of *P. adansoniae* associated with canker and were observed directly on branches and twigs of *Hevea brasiliensis* (Fig. 2). Phylogenetically, isolates from the sexual morph of *P. adansoniae* (MFLUCC 17-0327, MFLUCC 17-0334 and MFLU 19-0242) group with the ex-type strain CBS 122550 (Fig. 1). Therefore, this is the first report of the sexual morph of *P. adansoniae*.

Pseudofusicoccum ardesiacum
Pavlic, T.I.Burgess & M.J.Wingf.
(Fig. 4)

Mycologia 100: 858 (2008).

Index Fungorum number. — IF512051.

Facesoffungi number. — FoF 05799.

Culture characteristics. — Conidia germinating on MEA within 24 hours at room temperature (25-30°C) and germ tube was produced from the ends of the conidia. Initially aerial mycelium white, circular, fairly fluffy with sparse aspects, after 10 days become whitish grey, velvety, raise, dense, after 20-25 days of incubation, colonies become iron grey to black, hyphae septate, branched, smooth, forming conidiomata at the colony margin after 30-40 days and after 4 months of incubation, no asexual-morph produced on culture.

Material examined. — **Thailand**. Phayao Province, Muang District, associated with canker disease on branches of *Hevea brasiliensis* (Euphorbiaceae), 2.X.2016, *C. Senwanna*, RBPY04, MFLU 19-0235, living culture MFLUCC 17-0323.

Additional GenBank number. — SSU = MK503145.

Description
Associated with canker disease on branches of *Hevea brasiliensis*.

Sexual morph
Undetermined.

Asexual morph
Conidiomata. (140-)175-255(-260) diam × (120-)160-215(-220) µm high ($\bar{x}$ = 201 × 189 µm, *n* = 10), pycnidial, solitary to scattered, immersed to semi-immersed, globose to subglobose, uni- to multiloculate, with a central ostiole.

Conidiomata wall. 17-46 µm wide, outer layers dark brown to black, inner layers thin-walled, hyaline, composed of cell of *textura angularis*.

Paraphyses. Absent.

Conidiophores. Reduced to conidiogenous cells.

Conidiogenous cells. (8-)9-13(-15) × (2-)2-3.5(-4) µm ($\bar{x}$ = 10.4 × 2.8 µm, *n* = 25), holoblastic, cylindrical to subcylindrical, hyaline, smooth-walled.

Conidia. (20-)22-27(-29) × (5.5-)6-8.5(-9) µm ($\bar{x}$ = 24.8 × 7.6 µm, *n* = 70), ellipsoid to rod-shaped, hyaline, aseptate, straight or slightly bent or irregularly shaped, smooth-walled, with fine granular content, surrounded by a thin mucilaginous sheath.

Notes
Asexual morphology of our fresh collection resembles to *P. ardesiacum* (ex-type) in Pavlic *et al*. (2008) in having ellipsoid to rod-shaped, hyaline, aseptate, straight or irregularly shaped conidia. In a phylogenetic analysis of combined of ITS, LSU, TEF1 and β-tubulin gene sequence data (Figs 1; 4) our isolates clustered with *P. ardesiacum*. However, conidia of our strain are shorter (20-29 µm versus 17.5-32 µm) than the type (Pavlic *et al*. 2008). This may be due to different substrates and lifestyle of the fungi as our strain was found on *Hevea brasiliensis* and were observed directly from the host, while the type strain was found on *Adansonia gibbosa* and characters were observed in culture on pine needles (Pavlic *et al*. 2008). Based on morphological characters and phylogenetic analyses, we identified our collection as *P. ardesiacum* and report a new host record for *P. ardesiacum* from *Hevea brasiliensis* (Euphorbiaceae) in Thailand for the first time.

DISCUSSION

Two species of *Pseudofusicoccum* were identified associated with symptomatic branches and twigs of Para rubber in northern Thailand based on morphological and multigene phylogenetic analyses. We also describe the sexual morph of *Pseudofusicoccum adansoniae* for the first time based on analyses of combined ITS, LSU, TEF1 and β-tubulin dataset.

Pseudofusicoccum species play a vital role as plant pathogens, endophytes and saprobes with a worldwide distribution and host range including *Acacia synchronicia* Maslin, *Adansonia gregorii* F.Muell., *Artocarpus heterophyllus* Lam., *Caesalpinia pulcherrima* (L.) Sw., *Cassia fistula* L., *Dimocarpus longan* Lour., *Eucalyptus* sp., *Ficus opposita* Miq., *F. krishnae* C.DC, *Hevea brasiliensis*, *Mangifera indica* L., *Mimusops caffra* E.Mey. ex A.DC, *Pterocarpus angolensis* DC, *Senna siamea* (Lam.) H.S.Irwin & Barneby, *Tectona grandis* L.f. and *Veitchia merrillii* (Becc.) H.E.Moore (Pavlic *et al*. 2008; Mehl *et al*. 2011; Sakalidis *et al*. 2011; Doilom *et al*. 2015; Trakunyingcharoen *et al*. 2015a, b; Prasher & Dhanda 2017; Jami *et al*. 2018).

Pseudofusicoccum was previously placed in *Botryosphaeriaceae* based on its asexual characters which are similar to *Fusicoccum* (*Botryosphaeriaceae*), but it differs in conidia shape and conidia that are covered with a mucilaginous sheath. Yang *et al*. (2017) used evidence from molecular phylogeny to introduce the family *Pseudofusicoccumaceae* to accommodate *Pseudofusicoccum*. However, Phillips *et al*. (2019) placed *Pseudofusicoccum* in *Phyllostictaceae* based on analyses of ITS and LSU sequence data and evolutionary divergence times. This was coupled with morphology of the asexual morph which was similar to *Phyllosticta* species in having a mucilaginous sheath surrounding the conidia (Wikee *et al*. 2013). In our phylogenetic analyses (Fig. 1), taxa of Botryosphaeriales cluster in six families, which

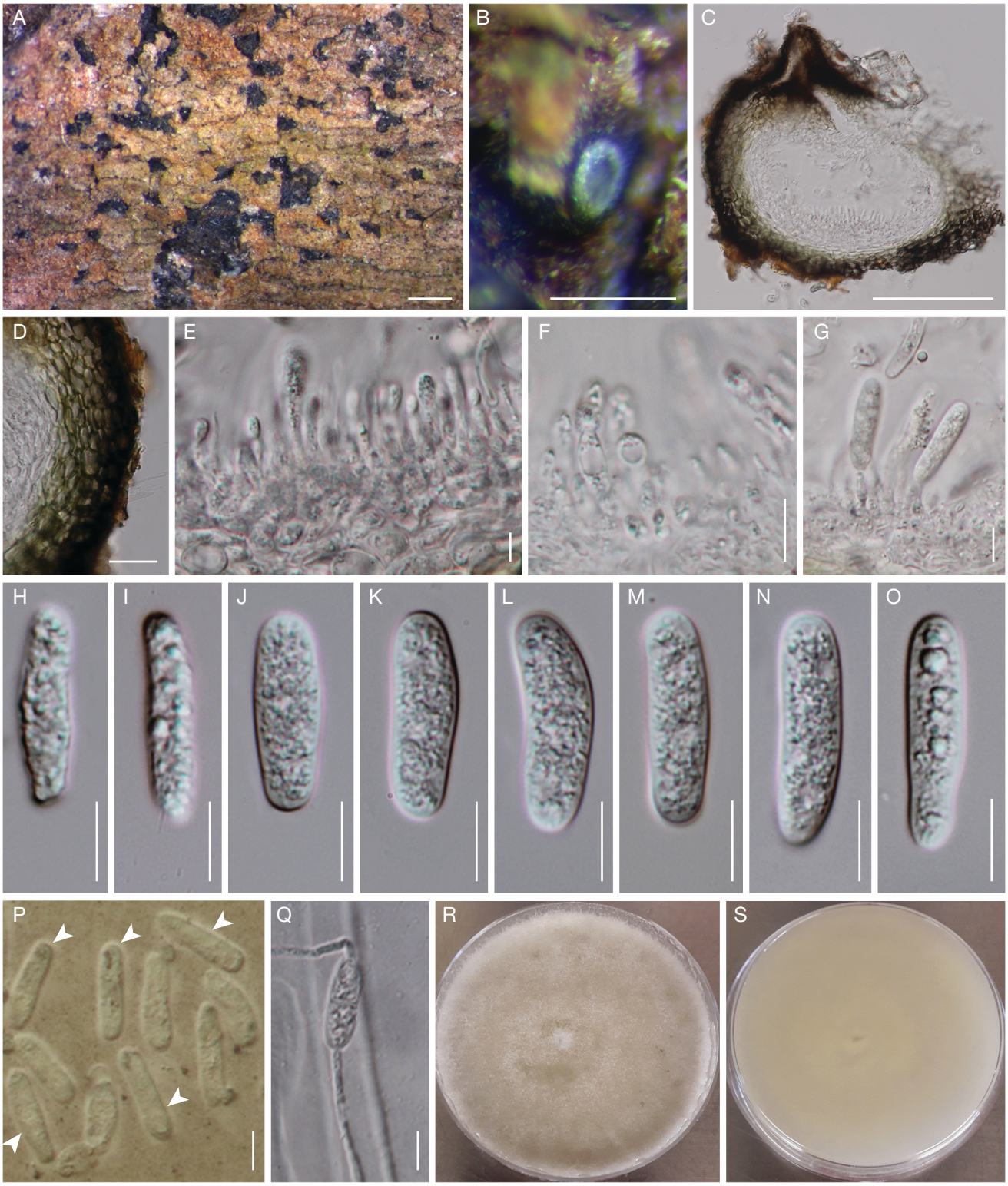

FIG. 4. — *Pseudofusicoccum ardesiacum* Pavlic, T.I.Burgess & M.J.Wingf. (MFLU 19-0235): **A**, **B**, habit on host substrate; **C**, section through the conidiomata; **D**, peridium; **E-G**, conidiophore and conidiogenous cells; **H-O**, conidia (mounted in double-distilled water); **P**, conidia with mucilaginous sheath (**arrows**) (stained in Indian ink); **Q**, germinated spores; **R**, **S**, upper and reverse view of the culture after ten days. Scale bars: A, 500 µm; B, 200 µm; C, 100 µm; D, 20 µm; E-Q, 10 µm.

concurs with Phillips *et al.* (2019). *Pseudofusicoccum* species formed a distinct lineage clustering with *Phyllosticta* species in *Phyllostictaceae* (Fig. 1), which correspond well with other species in Phyllostictaceae (Phillips *et al.* 2019).

Currently, *Pseudofusicoccum* contains *P. adansoniae*, *P. africanum* Marinc., Jami & M.J.Wingf., *P. ardesiacum*, *P. artocarpi* Trakun., L.Lombard & Crous, *P. kimberleyense* Pavlic, T.I.Burgess & M.J.Wingf, *P. olivaceum* Mehl &

Slippers, *P. stromaticum* (Mohali, Slippers & M.J.Wingf.) Mohali, Slippers & M.J.Wingf. (type species) and *P. violaceum* Mehl & Slippers (Dissanayake *et al.* 2016; Jami *et al.* 2018). *Pseudofusicoccum adansoniae*, *P. ardesiacum* and *P. artocarpi* have been recorded from Thailand, with support from molecular data. *Pseudofusicoccum adansoniae* has been recorded on *Cassia fistula*, *Dimocarpus longan*, *Hevea brasiliensis*, *Senna siamea* and *Tectona grandis*, *P. ardesiacum* has been recorded on *Caesalpinia pulcherrima*a and *Veitchia merrillii* and *P. artocarpi* has been recorded on *Artocarpus heterophyllus* (Doilom *et al.* 2015; Trakunyingcharoen *et al.* 2015b).

Pseudofusicoccum was known only from its asexual morph, however, researchers expected the sexual characters to be *Botryosphaeria*-like based on phylogenetic inference (Mehl *et al.* 2011; Phillips *et al.* 2013; Slippers *et al.* 2013). In this study, the sexual morph of *P. adansoniae* was observed directly on the host and is similar to the sexual morph of *Botryosphaeriaceae* in having clavate asci. The sexual morph of *P. adansoniae* corresponds to the sexual morph of other species in Phyllostictaceae based on uniloculate, separate to aggregated ascomata, clavate to sub-cylindrical asci with long pedicel, hyaline, aseptate ascospores surrounded by a mucilaginous sheath.

Identifications of *Pseudofusicocum* species was initially based on description of morphological characters and phylogenetic inference. We could not obtain conidia from cultures (MFLUCC 17-0327, MFLUCC 17-0333, MFLUCC 17-0334, MFLUCC 17-0339 and MFLUCC 17-0359), even after several attempts using different culture media, including sterile pine needles/host tissue on 2% water agar. The BLASTn search of LSU, ITS and TEF1 sequences showed that our strains (MFLUCC 17-0327 and MFLUCC 17-0334) is found most similar to *Pseudofusicoccum adansoniae* (CBS: 122062, type species) with 100%, 99% and 99% similar respectively. Base pair comparison of LSU, ITS, TEF1 and β-tubulin gene sequences between asexual and sexual morph of *Pseudofusicoccum adansoniae* in the dataset and found that they are identical. Therefore, we consider our strains (MFLUCC 17-0327, MFLUCC 17-0334 and MFLU 17-0242) as the sexual morph with *Pseudofusicoccum adansoniae* based on phylogenetic analysis.

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