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Towards a natural classification of *Dothidotthia* and *Thyrostroma* in Dothidotthiaceae (Pleosporineae, Pleosporales)

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Abstract

Dothidotthia and *Thyrostroma* (Dothidotthiaceae, Pleosporineae, Pleosporales) species are plant pathogens causing canker, dieback and leaf spots on a wide range of hosts. However, the naming species is difficult, due to insufficient protologues, poor phylogenetic understanding due to the lack of sequence data from type species and low-quality illustrations. Moreover, the connections between asexual and sexual morphs of these genera are unclear. In this study, fresh samples of *Dothidotthia* and *Thyrostroma* were collected from symptomatic twigs and branches in southern European Russia. Multi-gene phylogenetic analyses based on a concatenated LSU, SSU, ITS and TEF1-α sequence dataset were used to investigate the phylogenetic position and confirm relationships of the asexual and sexual morphs in these genera of Dothidotthiaceae. In this study, *Dothidotthia* can easily be distinguished from *Thyrostroma* based on multi-gene phylogenetic analyses coupled with morphological characters. The new species, *Dothidotthia robiniae*, *Thyrostroma celtidis*, *T. lycii*, *T. moricola*, *T. robiniae*, *T. styphnolobii*, *T. tiliae*, *T. ulmicola* and *T. ulmigenum* are introduced. In addition, *Neodothidotthia negundinicola* clusters with species of *Dothidotthia* and hence *Neodothidotthia* is synonymized under *Dothidotthia*. Two new combinations, *D. negundinicola* and *D. negundinis*, are introduced.

Key words – 9 new species – Ascomycota – Dothideomycetes – Holomorph – Phylogeny – Taxonomy

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Introduction

Pleosporineae M.E. Barr is a suborder of Pleosporales Luttr. ex M.E. Barr which includes 22 families, *viz*. Acrocalymmaceae Crous & Trakun., Ascocylindricaceae Abdel-Wahab, Bahkali, E.B.G. Jones, Ariyaw. & K.D. Hyde, Camarosporiaceae Wanas., Wijayaw., K.D. Hyde & Crous, Camarosporidiellaceae Wanas., Wijayaw., Crous & K.D. Hyde, Coniothyriaceae W.B. Cooke, Cucurbitariaceae G. Winter, Didymellaceae Gruyter, Aveskamp & Verkley, Dothidotthiaceae Crous & A.J.L. Phillips, Halojulellaceae Suetrong, K.D. Hyde & E.B.G. Jones, Leptosphaeriaceae M.E. Barr, Libertasomycetaceae Crous, Microsphaeropsidaceae Qian Chen, L. Cai & Crous, Neocamarosporiaceae Wanas., Wijayaw., Crous & K.D. Hyde, Neophaeosphaeriaceae Ariyaw. & K.D. Hyde, Neopyrenochaetaceae Valenz.-Lopez, Crous, Cano, Guarro & Stchigel, Parapyrenochaetaceae Valenz.-Lopez, Crous, Stchigel, Guarro & Cano, Phaeosphaeriaceae M.E. Barr, Pleosporaceae Nitschke, Pseudopyrenochaetaceae Valenz.-Lopez, Crous, Stchigel, Guarro & Cano, Pyrenochaetopsidaceae Valenz.-Lopez, Crous, Cano, Guarro & Stchigel, Shiraiaceae Y.X. Liu, Zi Y. Liu & K.D. Hyde and Tzeananiaceae Ariyaw., A.J.L. Phillips & Chuang (Zhang et al. 2012, Hyde et al. 2013, Wanasinghe et al. 2017a, Ariyawansa et al. 2018). Many genera and families of Pleosporineae are well-resolved based upon their morphological characteristics coupled with phylogenetic affinities i.e. Camarosporidiellaceae, Cucurbitariaceae, Didymellaceae, Leptosphaeriaceae, Phaeosphaeriaceae and Pleosporaceae (Phookamsak et al. 2014, 2017, Ariyawansa et al. 2015a, b, Chen et al. 2017, Wanasinghe et al. 2017a, b, Jaklitsch et al. 2018). In contrast, some genera and families are not well-resolved. This is because of limited taxon sampling and brief protologues (Liu et al. 2016, 2017).

Barr (1989) accepted *Dothidotthia* as a member of Botryosphaeriaceae based on its morphological characters, such as immersed ascomata becoming erumpent to nearly superficial on woody twigs, broad and bitunicate asci. Nevertheless, multi-gene phylogenetic analyses showed that *Dothidotthia* resides in Pleosporales following which Phillips et al. (2008) introduced Dothidotthiaceae. Subsequent studies by Zhang et al. (2012), Hyde et al. (2013), Wijayawardene et al. (2014, 2018) and Liu et al. (2017) accepted this taxonomic arrangement. Species of Dothidotthiaceae have been commonly reported as parasitic on living plants, as well as saprobes on wood or branches of dead or decaying plants in terrestrial habitats (Hyde et al. 2013, Marin-Felix et al. 2017). Currently, *Dothidotthia* Höhn, *Neodothidotthia* Crous, *Mycocentrospora* Deighton, *Phaeomycocentrospora* Crous, H.D. Shin & U. Braun, *Pleiochaeta* (Sacc.) S. Hughes, *Thyrostroma* Höhn. and *Wilsonomyces* Adask., J.M. Ogawa & E.E. Butler are recognized in this family (Marin-Felix et al. 2017, Wijayawardene et al. 2018, Crous et al. 2019). However, *Mycocentrospora*, *Phaeomycocentrospora* and *Pleiochaeta* exhibit different morphological characters from the type genus (especially in conidia shape and appendages) but were accommodated in Dothidotthiaceae.

The genus *Thyrostroma* was introduced by Höhnel (1911), with *T. compactum* (Sacc.) Höhn. as the type species. The generic synonym of *Thyrostroma* has been reported as *Coryneum* Sacc., *Stegonsporium* Corda, *Stigmina* Sacc., and *Thyrococcum* Höhn, *Thyrostromella* Höhnel and *Wilsonomyces* (Höhnel 1911, Morgan-Jones 1971, Sutton & Pascoe 1989, Sutton 1997, Index Fungorum 2019). However, *Thyrostromella* is still treated in Ascomycota genera *incertae sedis* (Wijayawardene et al. 2018) and *Coryneum*, *Stigmina*, *Stegonsporium* and *Wilsonomyces* were accepted as distinct genera (Ramaley 2005, Marin-Felix et al. 2017, Wijayawardene et al. 2018). *Thyrostroma* species have been reported as plant pathogens causing stem canker and dieback on various hosts, with a cosmopolitan distribution (Yuan & Old 1990, Kolemasova 1999, Kuz'michev et al. 2001, Sokolova 2003, Sokolova et al. 2006, Phillips et al. 2008, Kolganikhina & Sokolova 2012, Stravinskienė et al. 2015, Crous et al. 2016, Marin-Felix et al. 2017, Farr & Rossman 2019). However, based on morphology, Slippers et al. (2013) placed *Thyrostroma* in Botryosphaeriaceae. *Dothidotthia* has been reported as a sexual morph of *Thyrostroma* based on the production of hyphomycetes in culture from asci (Ramaley 2005). There is, however, no phylogenetic evidence for this link between these two genera. Wijayawardene et al. (2014) suggested that the usage of both names is desirable until the above-mentioned links are proven. Phylogenetic analyses based on LSU sequence data performed by Marin-Felix et al. (2017) showed that *Thyrostroma* clustered in

the Dothidotthiaceae as originally proposed by Phillips et al. (2008). Furthermore, the type species of *Thyrostroma, T. compactum* formed a distinct clade from *Dothidotthia* in Dothidotthiaceae. It was therefore assumed that *Thyrostroma* and *Dothidotthia* are not congeneric (Marin-Felix et al. 2017). Although *Thyrostroma* is an old genus of Dothideomycetes (Wijayawardene et al. 2014), the taxonomic concepts and phylogenetic analyses of this genus remain unclear.

The objective of the present study was to clarify the taxonomic placement of *Dothidotthia* and *Thyrostroma* in Dothidotthiaceae and identify taxa based on morphology coupled with multi-gene phylogeny. Thirty-one thyrostroma-like taxa collected from Russia were examined and their DNA sequence data were obtained for use in multi-gene phylogenetic analyses.

Materials & Methods

Collections, isolation and identification

Symptomatic specimens (Fig. 1) were collected from many hosts in different Provinces of Russia during 2015–2016. Collected specimens were brought to the laboratory in small paper bags. Specimens were examined with a Motic SMZ 168 series and the appearance of fruiting structures on the host surface was captured using a ZEISS STEREO Discovery.V8 stereomicroscope fitted with AxioCamERc5s camera. Squash mounts of fungal microscopic structures and free-hand sections of ascomata and sporodochia on glass slides in double-distilled water (ddH_2O) were prepared for photography. Micro characteristics were examined and captured using a Nikon ECLIPSE 80i compound microscope, connected with a Cannon 600D digital camera and illustrated with DIC microscopy. Fungal structures were measured using Tarosoft® Image Framework program v.0.9.0.7. Photographic plates were made using Adobe Photoshop CS6 version 13.0.

Pure cultures were obtained by single spore isolation with spore suspension technique. The spore suspension was prepared by aseptically removing ascospores/conidia from the ascostroma/sporodochium and soaking them in a drop of sterilized water. 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 4–24 hours. Germinating ascospores/conidia were checked under the Motic SMZ 168 stereomicroscope after 4 hours and thereafter at 12-hour 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 4ºC and -20ºC for molecular work and incorporation into the culture collection. Herbarium specimens were deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU). Cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC). The new species were justified based on the guidelines of Jeewon & Hyde (2016). Faces of Fungi and Index Fungorum numbers were obtained based on the guidelines described in Jayasiri et al. (2015) and Index Fungorum (2019).

DNA extraction, PCR amplification and DNA sequencing

Cultures were grown on MEA at 25–30°C for two weeks and mycelia were scraped off and kept in 1.5 ml sterilized tubes. Genomic DNA was extracted by Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, P. R. China) following the manufacturer's protocol. In addition, DNA extraction of some samples was made directly from dry fungal fruiting bodies to obtain sequence data. Extraction was started by placing individual sporodochia in 1.5 ml sterilized tubes and leaving overnight at -20°C. Genomic DNA was extracted by using OMEGA E.Z.N.A.® Forensic DNA Kit following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify partial gene regions (ITS, LSU, SSU, TEF1-α, RPB2 and TUB2) using primers and conditions as shown in Table 1. The amplification reactions were performed in 25 µl final volumes contained 8.5 µl of sterilized water, 12.5 µl of 2 × Easy Taq PCR SuperMix (a mixture of Easy Taq TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang

District, Beijing, PR China), 1 µl of each forward and reverse primer (10 pM), and 2 µl of DNA template. PCR fragments were purified and sequenced by Sangon Biotech Co., Shanghai, China and Sino Geno Max, Beijing, China.

Figure 1 – Symptoms on different host caused by species of *Dothidotthia* and *Thyrostroma*. a, b *Robinia pseudoacacia* (*D. robiniae*). c, d *Robinia pseudoacacia* (*T. robiniae*). e–h *Morus alba* (*T. moricola*). i *Tilia cordata* (*T. tiliae*). j *Ulmus pumila* (*T. tiliae*). k, l *Ulmus pumila* (*T. ulmicola*). m *Ulmus pumila* (*T. ulmigenum*). n–q *Styphnolobium japonicum* (*T. styphnolobii*).

Table 1 Details of genes/loci, primers used and PCR thermal cycles

Gene/loci [a]	PCR primers (forward/reverse)	PCR conditions	References
ITS	ITS5/ITS4	1. Initialization = 94ºC for 3 minutes 2. Denaturation = 94ºC (1 minute) 3. Annealing = 55ºC (50 seconds) 4. Extension = 72ºC (1 minute) 5. Final elongation = 72ºC (10 minutes) 6. Final hold at 4°C (Step 2–4 = 35 cycles)	White et al. 1990
LSU	LR0R/ LR5		Vilgalys & Hester 1990
SSU	NS1/NS4		White et al. 1990
TEF1-α	EF1-983F/EF1-2218R	1. Initialization = 95ºC for 3 minutes 2. Denaturation = 94ºC (2 minutes) 3. Annealing = 56ºC (1 minute) 4. Extension = 72ºC (1 minute) 5. Final elongation = 72ºC (10 minutes) 6. Final hold at 4°C (Step 2–4 = 35 cycles)	Rehner 2001
RPB2	fRPB2-5F/fRPB2-7cR	1. Initialization = 95ºC for 5 minutes 2. Denaturation = 95ºC (1 minute) 3. Annealing = 52ºC (2 minutes) 4. Extension = 72ºC (90 seconds) 5. Final elongation = 72ºC (10 minutes) 6. Final hold at 4°C (Step 2–4 = 40 cycles)	Liu et al. 1999
TUB2	Bt2a/Bt2b	1. Initialization = 94ºC for 5 minutes 2. Denaturation = 94ºC (1 minute) 3. Annealing = 58ºC (30 seconds) 4. Extension = 72ºC (90 seconds) 5. Final elongation = 72ºC (10 minutes) 6. Final hold at 4°C (Step 2–4 = 37 cycles)	Glass & Donaldson 1995

[a] ITS: Part of rDNA 18S (3' end), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2), and part of the 28S rRNA (5' end); LSU: 28S large subunit rDNA; SSU: 18S small subunit rDNA; TEF1-α: translation elongation factor 1-alpha gene; RPB2: RNA polymerase II second largest subunit; TUB2: β-tubulin

Phylogenetic analyses

The new LSU and ITS sequences generated in this study were subjected to BLASTn searches of the NCBI nucleotide database (http://blast.ncbi.nlm.nih.gov/) to determine their most probable closely related taxa. The sequences of representative taxa in Pleosporineae used in our phylogenetic analyses were selected from GenBank based on the BLASTn searches and recently published data (Marin-Felix et al. 2017, Wanasinghe et al. 2017a, Valenzuela-Lopez et al. 2018). Phylogenetic analyses in this study are represented by two phylogenetic trees. The first tree was generated from a combined LSU, SSU, ITS and TEF1-α gene dataset. The dataset comprises 131 sequence strains from 21 representative families in Pleosporineae, including the new taxa proposed in this study. *Cyclothyriella rubronotata* (CBS 121892 and CBS 141486) was selected as the outgroup taxon (Table 2).

The second phylogenetic tree was obtained from a combined ITS, LSU, SSU and TEF1-α gene dataset. The dataset consists of 55 sequence strains from six genera within Dothidotthiaceae. *Didymella exigua* (CBS 183.55) and *Phoma herbarum* (CBS 615.75) were selected as the outgroup taxa (Tables 2, 3). The individual gene alignments were initially aligned by MAFFT version 7 (Katoh et al. 2017; http://mafft.cbrc.jp/alignment/server/) and improved manually where necessary in BioEdit v.7.0.9.1 (Hall 1999) and MEGA7 (Kumar et al. 2015). The final alignments of the

combined multi-gene dataset were analyzed and inferred the phylogenetic trees based on maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses.

Maximum likelihood)ML(analyses of the Pleosporineae and Dothidotthiaceae were performed by using the RAxML-HPC2 on XSEDE (v. 8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) via the CIPRES Science Gateway platform (Miller et al. 2010) with the GTR+I+G model of nucleotide substitution.

Maximum Parsimony (MP) analysis was carried out with PAUP v 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search function with 1,000 random stepwise addition replicates and tree bisection–reconnection (TBR) as the branch-swapping algorithm. All informative characters were unordered and of equal weight. The consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Statistical supports for branches of the most parsimonious tree were estimated using maximum parsimony bootstrap (BS) analysis with 1,000 bootstrap replicates (Felsenstein 1985).

Bayesian inference (BI) analysis was performed by MrBayes on XSEDE, MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001) via the CIPRES Science Gateway platform (Miller et al. 2010). Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were evaluated by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted using the default settings, but with the following adjustments: Six simultaneous Markov chains were set up at 7,000,000 generations for Pleosporineae (Analyses 1) and 2,000,000 generations for Dothidotthiaceae (Analyses 2). Trees were sampled every 100th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence, using Tracer v. 1.6 program (Rambaut et al. 2013). The first 10% of generated trees representing the burn-in phase were discarded and the remaining trees were used to calculate posterior probabilities of the majority rule consensus tree.

The phylogenetic tree was visualized in FigTree v.1.4.3 (Rambaut 2016) and edited in Adobe Photoshop CS6 version 13.0. (Adobe Systems. U.S.A.). The newly generated sequences in this study were deposited in GenBank (Table 3). The final alignment and tree were deposited in TreeBASE (http://www.treebase.org/) under the submission ID. 24625, 25013 and 25016 (The analysis results of Pleosporineae of ML, MP and BI, respectively) and ID. 24626, 25019 and 25017 (The analysis results of Dothidotthiaceae of ML, MP and BI, respectively).

Results

Phylogenetic analyses

Analysis for familial placement in Pleosporineae (Analyses 1)

Phylogenetic analyses of the Pleosporineae based on the concatenated LSU, SSU, ITS and TEF1-α sequence dataset comprised 131 taxa and including 3197 bp (LSU: 1–808; SSU: 809–1731; ITS: 1732–2332; TEF1-α: 2333–3197). The RAxML analysis of the combined gene dataset resulted in the best scoring likelihood tree selected with a final ML optimization likelihood value of -21537.730459 which is represented in Fig. 2. The dataset comprised 992 distinct alignment patterns, with 31.42% of undetermined characters or gaps. The MP analysis results: 2366 characters were constant, 84 variable characters were parsimony-uninformative, and 747 were (included) parsimony-informative characters. The most parsimonious tree is shown where TL = 3529, CI = 0.364, RI = 0.788, RC = 0.287, HI = 0.636. Bayesian posterior probabilities from MCMC were evaluated with a final average standard deviation of split frequency = 0.009530. It is noteworthy that we received a low consistency index (CI) for this character dataset in the MP analysis. We believe that the introns of ITS1 and ITS2 may provide a homoplasious nature to the dataset. Similar phylogenetic scenarios are reported with the inclusion of ITS in order/ suborder level, resulted in

low CI values (Wanasinghe et al. 2017a, 2018). Phylogenies concerining to the placement of families in Pleosporineae were largely similar to those of Wanasinghe et al. (2017a), Ariyawansa et al. (2018) and Valenzuela-Lopez et al. (2018). However, some taxa were resolved differently in the internal node relationships among BI, ML and MP trees.

Twenty-five sequenced strains of *Dothidotthia* and *Thyrostroma* generated in this study are included in the analysis. Phylogenetic relationships of these taxa in Dothidotthiaceae and their relationships with other families in Pleosporineae are shown in Fig. 2. Phylogenetic results of this study show that Dothidotthiaceae forms a well-supported clade in Pleosporineae (100% ML, 99% MP, 1.00 PP). Families in Pleosporineae are well-resolved, except Neophaeosphaeriaceae which clusters with Neopyrenochaetaceae. Genera in Dothidotthiaceae form clearly distinct subclades and is confirmed by analyses 2 (Fig. 3). *Dothidotthia* is distinct from *Thyrostroma* and this corroborates previous studies (Crous et al. 2016, Marin-Felix et al. 2017) suggesting that they are not congeneric.

Analysis for genera in Dothidotthiaceae (Analyses 2)

Phylogenetic analyses of the Dothidotthiaceae based on the concatenated LSU, SSU, ITS and TEF1-α sequence data of 55 taxa included 3102 bp [LSU: 1–806; SSU: 807–1729; ITS: 1730–2236; TEF1-α: 2237–3102]. The RAxML analysis of the combined gene dataset had 295 distinct alignment patterns with 29.90% of undetermined characters or gaps. The best scoring of RAxML analysis is shown in Fig. 3, with the final ML optimization likelihood value of -6956.613576. In the MP analysis: 2827 characters were constant, 59 variable characters were parsimony-uninformative, and 216 were (included) parsimony-informative characters. The most parsimonious tree is shown where TL = 453, CI = 0.682, RI = 0.902, RC = 0.615, HI = 0.318. Bayesian posterior probabilities were evaluated by MCMC with a final average standard deviation of split frequencies = 0.007819. ML, MP and BI analyses of a combined multi-gene phylogeny were similar in the overall tree topologies (data not shown) and concurred with Marin-Felix et al. (2017) and Crous et al. (2019).

To discuss phylogenetic analyses of taxa in Dothidotthiaceae (Fig. 3), we divided the ingroup taxa in Dothidotthiaceae into six subclades (A–F). Newly generated sequences from 19 isolates of thyrostroma-like taxa grouped with *Thyrostroma compactum*, *T. cornicola*, *T. ephedricola*, *T. fransiriae* and *T. jaczewskii*. These taxa formed a separate subclade (clade A) from other genera in Dothidotthiaceae, with moderate support in ML analysis and well-supported in MP and BI analyses (69% ML, 95% MP, 1.00 PP, Fig. 3). Eight *Thyrostroma* species *viz. T. celtidis*, *T. lycii*, *T. moricola*, *T. robiniae*, *T. styphnolobii*, *T. tiliae*, *T. ulmicola* and *T. ulmigenum* are introduced to accommodate these 19 isolates.

Four newly generated strains of a new species *Dothidotthia robiniae* (MFLUCC 16-1175, MFLUCC 16-1177, MFLUCC 16-1185, MFLUCC 18-0692) and two strains of *D. negundinicola* (MFLUCC 16-1157, MFLUCC 16-1183) grouped with *D. negundinis* (CPC 12928, CPC 12930, CPC 12932, CPC 12933), *D. negundinicola* (CBS145039) and *D. symphoricarpi* (CPC12929; type species). These taxa form a well-resolved clade in Dothidotthiaceae (Fig. 3; clade E). *Dothidotthia* has a close relationship with *Mycocentrospora*, *Wilsonomyces* and *Pleiochaeta* in analyses 1, however, the genus formed a distinct clade in analyses 2 (Fig. 3).

Wilsonomyces (clade B) is sister to *Mycocentrospora* (clade C) in analyses 2 (Fig. 3; clade B and C). *Pleiochaeta* (Fig. 3; clade D) forms a sister clade with *Wilsonomyces* and *Mycocentrospora* with significant support (90% ML, 76% MP, 1.00 PP, Fig. 3; clades B, C and D). *Phaeomycocentrospora* (Fig. 3; clade F) is represented by a putative species *P. cantuariensis* (E.S. Salmon & Wormald) Crous, H.D. Shin & U. Braun, the genus forms a well-resolved clade basal to Dothidotthiaceae (100% ML, 100% MP, 1.00 PP).

Table 2 GenBank and culture collection accession numbers of taxa in Pleosporineae used in this study.

Taxon	Culture no.	GenBank accession number			
		LSU	**SSU**	**ITS**	**TEF1-α**
Acrocalymma aquatica	MFLUCC 11-0208^{T}	JX276952	JX276953	NR_121544	–
Acrocalymma ficus	CBS 317.76^{T}	KP170712	–	NR_137953	KP170663
Acrocalymma medicaginis	CPC 24340^{T}	KP170718	–	KP170625	–
Acrocalymma medicaginis	CPC 24345	KP170713	–	KP170620	–
Alternaria alternata	MFLUCC 14-1184	KP334701	KP334721	KP334711	KP334735
Alternaria eureka	CBS 193.86^{T}	KC584331	KC584589	–	–
Alternariaster helianthi	CBS 327.69^{T}	KC584369	KC584627	KC609335	–
Ascochyta pisi	CBS 126.54^{T}	DQ678070	DQ678018	_	DQ677913
Ascocylindrica marina	MD6011	KT252905	KT252907	_	_
Ascocylindrica marina	MD6012	KT252906	–	–	–
Boeremia exigua var. *exigua*	CBS 431.74^{T}	EU754183	EU754084	FJ427001	GU349080
Camarosporidiella caraganicola	MFLUCC 14-0605^{T}	KP711381	KP711382	KP711380	–
Camarosporidiella celtidis	MFLUCC 14-0904	MF434217	MF434305	MF434129	MF434392
Camarosporidiella elaeagnicola	MFLUCC 14-0908^{T}	MF434225	MF434313	MF434137	MF434400
Camarosporidiella halimodendri	MFLUCC 14-0901^{T}	MF434234	MF434322	MF434146	MF434409
Camarosporidiella laburni	MFLUCC 14-0885	MF434241	MF434329	MF434153	MF434416
Camarosporidiella schulzeri	MFLUCC 14-0897^{T}	MF434273	MF434361	MF434185	MF434448
Camarosporium quaternatum	CPC 23216	KY929170	KY929122	KY929135	KY929200
Camarosporium quaternatum	CPC 31081^{T}	KY929171	KY929123	KY929136	KY929201
Camarosporium quaternatum	CPC 31518	KY929172	KY929124	KY929137	KY929202
Cucurbitaria berberidis	CBS 363.93	GQ387606	GQ387545	JF740191	–
Cucurbitaria berberidis	MFLUCC 11-0386	KC506795	KC506799	–	–
Cucurbitaria ephedricola	HA 42^{T}	KT313007	KT313005	–	–
Cyclothyriella rubronotata	CBS 121892	–	–	KX650541	KX650516
Cyclothyriella rubronotata	CBS 141486^{T}	KX650544	KX650507	KX650544	KX650519
Didymella exigua	CBS 183.55^{T}	EU754155	EU754056	GU237794	–
Dothidotthia negundinis	CPC 12928	EU673272	EU673225	MK442598	–
Dothidotthia negundinis	CPC 12930	EU673274	EU673226	MK442599	–
Dothidotthia negundinis	CPC 12932	EU673275	EU673227	MK442600	–
Dothidotthia negundinis	CPC 12933	EU673276	EU673228	MK442601	–
Dothidotthia symphoricarpi	CPC 12929^{T}	EU673273	EU673224	–	–
Dothidotthia symphoricarpi	CBS 119687^{T}	MH874618	–	MH863064	–
Dothidotthia negundinicola	CBS 145039^{T}	MK442537	–	MK442597	–
Foliophoma fallens	CBS 161.78	GU238074	GU238215	KY929147	–
Foliophoma fallens	CBS 284.70	GU238078	GU238218	KY929148	–
Halojulella avicenniae	BCC 18422	GU371822	GU371830	–	GU371815
Halojulella avicenniae	BCC 20173	GU371823	GU371831	–	GU371816

Table 2 Continued.

Taxon	Culture no.	GenBank accession number			
		LSU	**SSU**	**ITS**	**TEF1-α**
Halojulella avicenniae	JK5326A	GU479790	GU479756	–	–
Leptosphaeria maculans	CBS 260.94	JF740307	–	JF740235	–
Leptosphaerulina australis	CBS 317.83	GU301830	GU296160	GU237829	GU349070
Libertasomyces myopori	CPC 27354[T]	KX228332	–	NR_145200	_
Libertasomyces platani	CPC 29609[T]	KY173507	–	KY173416	_
Libertasomyces quercus	CBS 134.97[T]	DQ377883	–	KY929152	KY929197
Microsphaeropsis olivacea	CBS 233.77	N712563	–	JN712497	–
Microsphaeropsis proteae	CBS 111319	GU237988	–	GU237803	–
Mycocentrospora acerina	CBS 148.52	MH868490	–	MH856968	–
Mycocentrospora acerina	CBS 113.24	MH866268	–	MH854764	–
Neocamarosporium lamiacearum	MFLUCC 17-0560[T]	MF434279	MF434367	MF434191	MF434454
Neocamarosporium lamiacearum	MFLUCC 17-0750	MF434280	MF434368	MF434192	MF434455
Neocamarosporium salicorniicola	MFLUCC 15-0957[T]	MF434281	MF434369	MF434192	–
Neocamarosporium salsolae	MFLUCC 17-0827[T]	MF434283	MF434371	MF434195	MF434457
Neophaeosphaeria agaves	CPC 21264[T]	KF777227	–	KF777174	_
Neophaeosphaeria filamentosa	CBS 102202	GQ387577	GQ387516	JF740259	GU349084
Neopyrenochaeta acicola	CBS 812.95[T]	GQ387602	GQ387541	LT623218	–
Neopyrenochaeta fragariae	CBS 101634[T]	GQ387603	GQ387542	LT623217	–
Paraleptosphaeria dryadis	CBS 643.86 [T]	KC584632	GU301828	JF740213	GU349009
Paraleptosphaeria rubi	MFLUCC 14-0211[T]	KT454718	KT454733	KT454726	–
Parapyrenochaeta acaciae	CBS 141291[T]	KX228316	–	KX228265	–
Parapyrenochaeta protearum	CBS 131315[T]	JQ044453	–	JQ044434	–
Parapyrenochaeta protearum	CBS 137997	KJ869209	–	KJ869152	–
Phaeomycocentrospora cantuariensis	CPC 10157	GU253712	–	GU269664	GU384381
Phaeomycocentrospora cantuariensis	CPC 10762	GU253713	–	GU269665	GU384382
Phaeomycocentrospora cantuariensis	CPC 11646	GU253715	–	GU269667	GU384384
Phaeomycocentrospora cantuariensis	CPC 11694	GU253716	–	GU269668	–
Phaeosphaeria chiangraina	MFLUCC 13-0231[T]	KM434280	KM434289	KM434270	KM434298
Phaeosphaeria musae	MFLUCC 11-0133	KM434277	KM434287	KM434267	KM434296
Phaeosphaeria thysanolaenicola	MFLUCC 10-0563[T]	KM434276	KM434286	KM434266	KM434295
Phaeosphaeriopsis dracaenicola	MFLUCC 11-0157[T]	KM434283	KM434292	KM434273	KM434301
Phaeosphaeriopsis dracaenicola	MFLUCC 11-0193	KM434284	KM434293	KM434274	KM434302
Pleiochaeta carotae	CBS 142644[T]	KY905663	–	KY905669	–
Pleiochaeta ghindensis	CBS 552.92[T]	–	–	EU167561	–
Pleiochaeta setosa	CBS 496.63	–	–	EU167563	–
Pleiochaeta setosa	DB50112	–	–	JQ358708	–
Pleiochaeta setosa	487630	–	–	KR536610	–

Table 2 Continued.

Taxon	Culture no.	GenBank accession number			
		LSU	**SSU**	**ITS**	**TEF1-α**
Plenodomus guttulatus	MFLUCC 15-1876^{T}	KT454713	KT454729	KT454721	–
Plenodomus salviae	MFLUCC 13-0219^{T}	KT454717	KT454732	KT454725	–
Pleospora herbarum	CBS 191.86	JX681120	–	JX681120	KC584471
Phoma herbarum	CBS 615.75	KF251715	EU754087	KF251212	KR184186
Pseudopyrenochaeta lycopersici	CBS 306.65^{T}	EU754205	EU754106	NR_103581	–
Pseudopyrenochaeta terrestris	CBS 282.72^{T}	LT623216	–	LT623228	–
Pyrenochaetopsis confluens	CBS 142459^{T}	LN907446	–	LT592950	–
Pyrenochaetopsis decipiens	CBS 343.85^{T}	GQ387624	–	LT623223	–
Pyrenochaetopsis indica	CBS 124454^{T}	GQ387626	–	LT623224	–
Shiraia bambusicola	NBRC 30753	AB354968	–	AB354987	–
Shiraia bambusicola	NBRC 30754	AB354969	–	AB354988	–
Shiraia bambusicola	NBRC 30771	AB354971	–	AB354990	–
Shiraia bambusicola	NBRC 30772	AB354972	–	AB354991	–
Staurosphaeria aloes	CPC 21572^{T}	KF777198	–	KF777142	–
Staurosphaeria lycii	MFLUCC 17-0210^{T}	MF434284	MF434372	MF434196	MF434458
Staurosphaeria lycii	MFLUCC 17-0211	MF434285	MF434373	MF434197	MF434459
Staurosphaeria lycii	MFLUCC 17-0720	MF434286	MF434374	MF434198	MF434460
Staurosphaeria rhamnicola	MFLUCC 17-0814	MF434289	MF434377	MF434201	MF434463
Subplenodomus valerianae	CBS 630.68	GU238150	GU238229	JF740251	–
Subplenodomus violicola	CBS 306.68	GU238156	GU238231	FJ427083	–
Thyrostroma compactum	CBS 335.37	KY905664	–	KY905670	KY905681
Thyrostroma cornicola	CBS 141280^{T}	KX228300	–	KX228248	KX228372
Thyrostroma ephedricola	MFLUCC 18-1125	MK765854	MK765853	MK765855	–
Thyrostroma franseriae	CBS 487.71^{T}	KX228301	–	KX228249	KY905680
Thyrostroma franseriae	CBS 700.70	KX228302	–	KX228250	KY905682
Thyrostroma jaczewskii	MFLUCC 18-0787	MK765857	MK765858	MK765856	–
Tzeanania taiwanensis	NTUCC 17-005	MH461120	MH461126	MH461123	MH461130
Tzeanania taiwanensis	NTUCC 17-006	MH461121	MH461127	MH461124	MH461131
Wilsonomyces carpophilus	CBS 147.36	KY905667	–	–	–
Wilsonomyces carpophilus	CBS 159.51	KY905665	–	KY905671	KY905683
Wilsonomyces carpophilus	CBS 231.89	KY905666	–	KY905672	–
Wilsonomyces carpophilus	SF4	–	–	MF055699	–

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, the Netherlands; MFLUCC: Mae Fah Luang University Culture Collection, Thailand; NBRC: NITE Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan; NTUCC: Department of Plant Pathology and Microbiology, National Taiwan University Culture Collection.

ITS: internal transcribed spacers and intervening 5.8S nrRNA; LSU: partial 28S large subunit RNA gene; SSU: partial 18S small subunit RNA gene; TEF1-α: partial translation elongation factor 1–alpha gene.

The ex-type strains are noted with superscripted T.

Table 3 GenBank and culture collection accession numbers of taxa in Dothidotthiaceae obtained in this study.

Taxon	Original no.	Strain	Specimens no.	GenBank accession number					
				LSU	SSU	ITS	TEF1-α	RPB2	TUB2
Dothidotthia negundinicola	T-1288	MFLUCC 16-1157	MFLU 16-1582	MK751815	MK751760	MK751725	MK908015	MK920235	MK933784
	T-1465	MFLUCC 16-1183	MFLU 16-1759	MK751816	MK751761	MK751726	MK908016	MK920236	MK933785
Dothidotthia robiniae	**T-1369**	**MFLUCC 16-1175**	**MFLU 16-1663**	MK751817	MK751762	MK751727	MK908017	MK920237	MK933786
	T-1410	MFLUCC 16-1177	MFLU 16-1704	MK751818	MK751763	MK751728	MK908018	–	MK933787
	T-1504	MFLUCC 16-1185	MFLU 16-1798	MK751819	MK751764	MK751729	MK908019	MK920238	MK933788
		MFLU 16-1798	MFLU 16-1798	MK751820	MK751765	MK751730	MK908020	MK920239	MK933789
	T-1509	MFLUCC 18-0692	MFLU 16-1803	MK751821	MK751766	MK751731	MK908021	MK920240	MK933790
Thyrostroma celtidis	**T-1506**	**MFLUCC 16-1186**	**MFLU 16-1800**	MK751822	MK751767	MK751732	MK908022	–	MK933791
Thyrostroma moricola	**T-1501**	**MFLU 16-1795**	**MFLU 16-1795**	MK751823	MK751768	MK751733	MK908023	–	MK933792
Thyrostroma lycii	**T-1348**	**MFLUCC 16-1170**	**MFLU 16-1642**	MK751824	MK751769	MK751734	MK908024	MK920241	MK933793
Thyrostroma robiniae	**T-1504B**	**MFLUCC 18-1191**	**MFLU 18-0631**	MK751825	MK751770	MK751735	MK908025	MK920242	MK933794
Thyrostroma styphnolobii	**T-1325**	**MFLUCC 16-1160**	**MFLU 16-1619**	MK751826	MK751771	MK751736	MK908026	MK920243	MK933795
Thyrostroma tiliae	T-1387	MFLUCC 16-1176	MFLU 16-1681	MK751827	MK751772	MK751737	MK908027	MK920244	MK933796
	T-1446	**MFLUCC 16-1178**	**MFLU 16-1740**	MK751828	MK751773	MK751738	MK908028	MK920245	MK933797
	T-1454B	MFLUCC 16-1180	MFLU 18-0628	MK751829	MK751774	MK751739	MK908029	–	MK933798
	T-1519	MFLUCC 16-1188	MFLU 16-1813	MK751830	MK751775	MK751740	MK908030	MK920246	MK933799
Thyrostroma ulmicola	T-1295	MFLUCC 16-1158	MFLU 16-1589	MK751831	MK751776	MK751741	MK908031	MK920247	MK933800
	T-1326	MFLUCC 16-1161	MFLU 16-1620	MK751832	MK751777	MK751742	MK908032	MK920248	MK933801
	T-1327	MFLUCC 16-1162	MFLU 16-1621	MK751833	MK751778	MK751743	MK908033	MK920249	MK933802
	T-1328A	MFLUCC 16-1163	MFLU 16-1622	MK751834	MK751779	MK751744	MK908034	MK920250	MK933803
	T-1329	MFLUCC 16-1165	MFLU 16-1623	MK751835	MK751780	MK751745	MK908035	MK920251	MK933804
	T-1330B	MFLUCC 16-1167	MFLU 18-0627	MK751836	MK751781	MK751746	MK908036	MK920252	MK933805
	T-1331	MFLUCC 16-1168	MFLU 16-1625	MK751837	MK751782	MK751747	MK908037	MK920253	MK933806
		MFLUCC 16-1169	MFLU 16-1625	MK751838	MK751783	MK751748	MK908038	MK920254	MK933807
	T-1357	MFLUCC 16-1711	MFLU 16-1651	MK751839	MK751784	MK751749	MK908039	MK920255	MK933808
	T-1358	**MFLUCC 16-1172**	**MFLU 16-1652**	MK751840	MK751785	MK751750	MK908040	MK920256	–
		MFLUCC 16-1173*	**MFLU 16-1652**	MK751841	MK751786	MK751751	MK908041	MK920257	MK933809
	T-1454A	MFLUCC 16-1179	MFLU 16-1748	MK751842	MK751787	MK751752	MK908042	MK920258	MK933810
	T-1455	MFLUCC 16-1181	MFLU 16-1749	MK751843	MK751788	MK751753	MK908043	MK920259	MK933811
	T-1459	MFLUCC 16-1182	MFLU 16-1753	MK751844	MK751789	MK751754	MK908044	MK920260	MK933812
Thyrostroma ulmigenum	T-1328B	MFLUCC 16-1164	MFLU 18-0629	MK751845	MK751790	MK751755	MK908045	MK920261	MK933813
	T-1330A	**MFLUCC 16-1166**	**MFLU 16-1624**	MK751846	MK751791	MK751756	MK908046	MK920262	MK933814

Ex-type strains are in bold. The isolation from single ascospore is indicated with an asterisk.

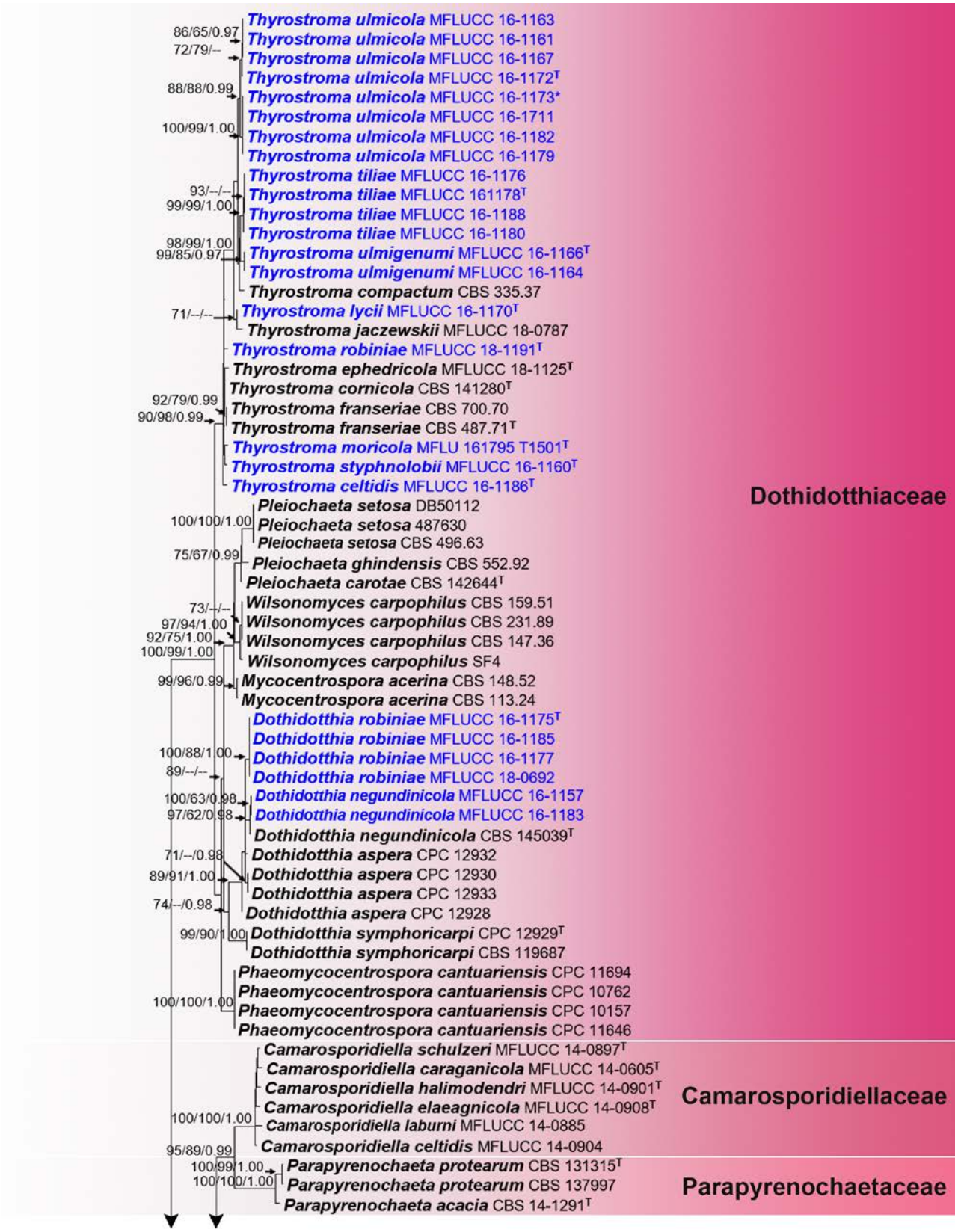

Figure 2 – Phylogram generated from RAxML analysis of the families in Pleosporineae based on a combined LSU, SSU, ITS and TEF1-α sequence dataset. Bootstrap support values for ML and MP equal to or greater than 60%, and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are defined as ML/MP/PP above the nodes. The tree is rooted to *Cyclothyriella rubronotata* (CBS 121892 and CBS 141486). The new isolates are in blue. Asterisk marks show the origin of the *Dothidotthia* and *Thyrostroma* isolates from ascospores. Ex-type strains are noted with superscript T.

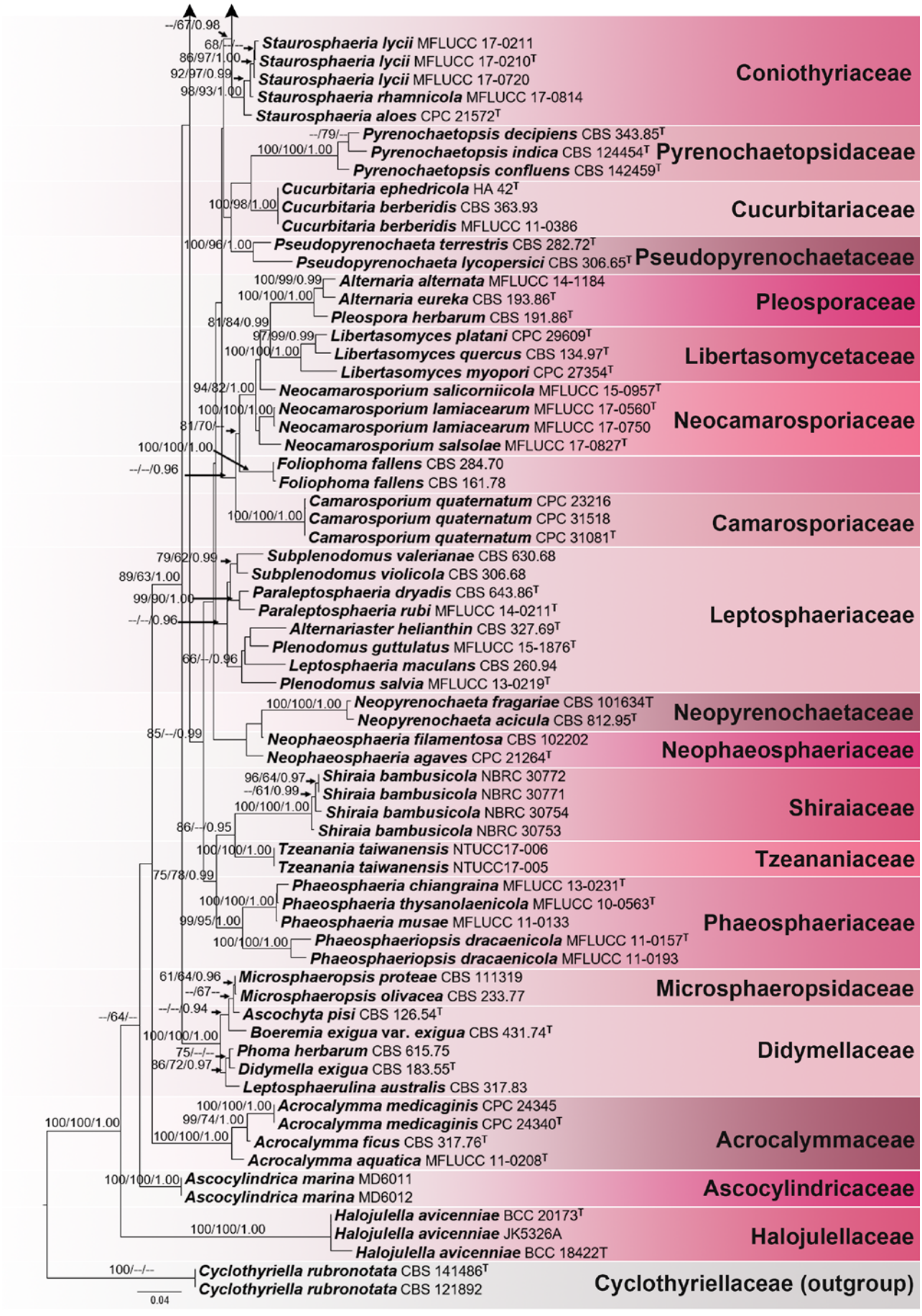

Figure 2 – Continued.

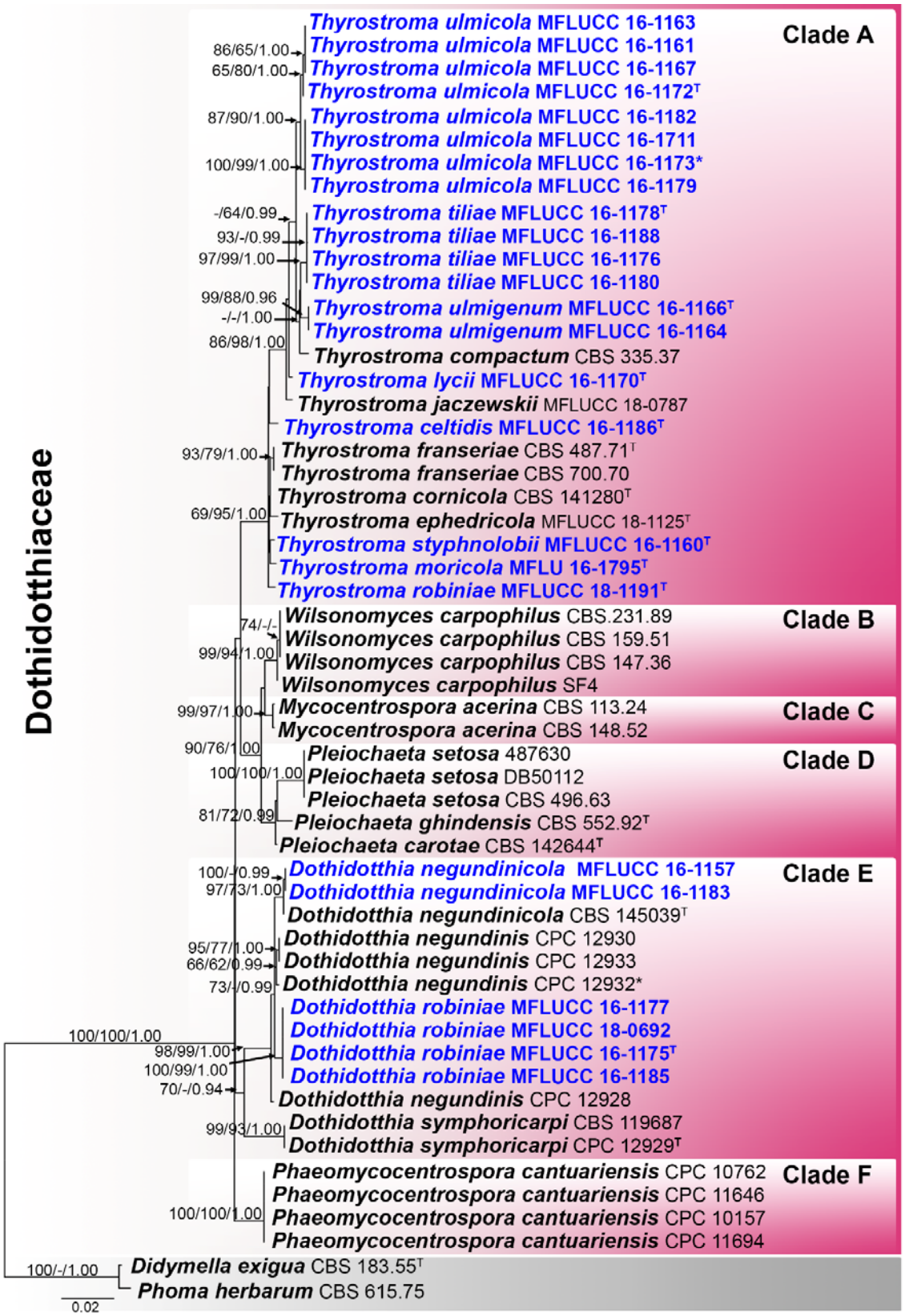

Figure 3 – Phylogram generated for Dothidotthiaceae from RAxML analysis based on a combined dataset of LSU, SSU, ITS and TEF1-α sequence dataset. Bootstrap support values for ML and MP equal to or greater than 60%, and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are defined as ML/MP/PP above the nodes. The tree is rooted to *Didymella exigua* (CBS 183.55) and *Phoma herbarum* (CBS 615.75). The new isolates are in blue. Asterisk marks show the origin of the *Dothidotthia* and *Thyrostroma* isolates from ascospores. Ex-type strains are noted with superscript T.

Taxonomy

Dothidotthiaceae Crous & A.J.L. Phillips, Persoonia 21: 35 (2008)

Saprobic, pathogenic on leaves, branches, twigs and wood in terrestrial habitats. Sexual morph: *Ascostromata* solitary to gregarious, raised to erumpent, breaking through host surface, ruptured, rounded, elongate, lenticular, or irregular in shape, multi-loculate, glabrous, ostiolate. *Ascomata* coriaceous, globose to subglobose, dark brown to black, ostiolate with apex somewhat papillate to depress. *Peridium* consists of dark brown to black, scleroplectenchymatous cells of *textura angularis* to *globulosa*, or *textura prismatica* in between the locules, basal region giving rise, dark brown, thick-walled hyphae extending from the base of the ascostroma into the host substrate. *Hamathecium* composed of dense, broad, septate, branched, anastomosed pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, clavate, with a short pedicel, rounded at the apex with an ocular chamber. *Ascospores* fusiform to ellipsoidal, pale to medium brown, 1-septate, slightly constricted at the septum, smooth-walled, thin-walled, with or without a gelatinous sheath. Asexual morph: *Colonies* punctiform, brown to black, stromatic, visible as raised, erumpent or nearly superficial, brown to dark brown, pulvinate sporodochia. *Conidiophores* macronematous, mononematous, short and packed, usually unbranched, septate or aseptate, straight or flexuous, hyaline to brown, or olivaceous brown, smooth or verrucose. *Conidiogenous cells* monoblastic, holoblatic, polyblastic, annelledic, integrated, terminal, or intercalary, or conidiophores reduced to conidiogenous cells, percurrent, cylindrical to subcylindrical. *Conidia* varied in shape, clavate to obclavate, cylindrical, ellipsoid or fusiform, filiform, subhyaline to dark brown, euseptate, phragmospores or muriform, smooth or rough, verrucose or echinulate, with or without appendages around the apical cell (Phillips et al. 2008, Hyde et al. 2013, Marin-Felix et al. 2017).

Type genus – *Dothidotthia* Höhn.

Notes – Barr (1989) included *Dothidotthia* in Botryosphaeriaceae with its coelomycetous asexual morph (as *Dothiorella*; *fide* Crous et al. 2006), and the characteristic of peridium, pseudoparaphyses and asci. However, Ramaley (2005) reported the asexual morph of *Dothidotthia aspera* as a hyphomycete in *Thyrostroma*. Considering the asexual morph differences together with molecular support, Phillips et al. (2008) established Dothidotthiaceae to accommodate *Dothidotthia* and proposed *Thyrostroma* as the asexual morph of *Dothidotthia*. Marin-Felix et al. (2017), however, showed that *Dothidotthia* is phylogenetically distinct from *Thyrostroma* and proposed that these two genera are not congeneric. Our phylogenetic results concur with Marin-Felix et al. (2017) and thus, we treat that *Dothidotthia* and *Thyrostroma* are two distinct genera.

Dothidotthia Höhn., Berichte der Deutschen Botanischen Gesellschaft 36: 312 (1918)

Saprobic or *pathogenic* on leaves and wood in terrestrial habitats (Fig. 1). Sexual morph: *Ascostromata* solitary to clustered, gregarious, raised to erumpent, breaking through host surface, rounded, elongate, lenticular or irregular in shape, multi-loculate, glabrous, ostiolate. *Ascomata* coriaceous, globose to subglobose, dark brown to black, ostiolate, apapillate. *Peridium* composed of 3–6 layers of dark brown to black cells of *textura angularis*, thick-walled, the basal region extending into the host substrate. *Hamathecium* composed of dense, broad, septate, branched pseudoparaphyses, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, clavate, short pedicellate, apically rounded with a well-developed ocular chamber. *Ascospores* ellipsoid, pale brown to medium brown, obtuse at ends or somewhat acute, transversely 1-septate, constricted at the septum (Phillips et al. 2008). Asexual morph: *Colonies* partly immersed, stromatic, effuse, sporodochial, with basal pseudoparenchymatous stroma, erumpent, dark brown to black. *Conidiophores* macronematous, septate, branched, subhyaline, smooth, arising from basal colonies. *Conidiogenous cells* enteroblastic, annellidic, integrated, terminal. *Conidia* acrogenous, fusiform to obclavate or obpyriform, pale brown to brown, rounded at apex, truncate at base, with a protruding hilum, septate, 0–3-transversely septate, constricted at the septa, rough-walled, echinulate.

Type species – *Dothidotthia symphoricarpi* (Rehm) Höhn.

Notes – According to Ramaley (2005), *Dothidotthia* was the sexual morph of *Thyrostroma* based on a cultural examination, however, there is no further evidence to confirm this link. There are very few molecular data-based studies of *Dothidotthia* compared to morphological studies. It is problematic to confirm the link between sexual and asexual morphs of *Dothidotthia* only based on morphological characteristics. Currently, there are 11 epithets listed in this genus (Index Fungorum 2019) and 106 sequences (including our sequences in this study) are available in GenBank (accessed on 15th August 2019). In this study, *Dothidotthia* forms a single clade, separate from *Thyrostroma*. Therefore, *Dothidotthia* and *Thyrostroma* are considered as distinct genera based on the sexual and asexual morph differences coupled with phylogenetic support.

Neodothidotthia Crous was introduced by Crous et al. (2019) with *N. negundinicola* Crous & Akulov as the type species. The genus was introduced to accommodate *N. negundinicola* and *N. negundinis* (Berk. & M.A. Curtis) Crous (≡ *Coryneum negundinis* Berk. & M.A. Curtis) based only on LSU sequences. In this study (based on a concatenated LSU, SSU, ITS and TEF1-α sequence dataset), *Neodothidotthia* forms a well-resolved clade in *Dothidotthia* (73% ML, 0.99 PP, Fig. 3). We could not find any significant morphological differences between these two genera and based on the phylogenetic results obtained herein, it is wise to consider both of them as one genus. We, therefore, synonymize *Neodothidotthia* under *Dothidotthia*. Thus, a novel species *Dothidotthia robiniae* and two new combinations, *D. negundinicola* and *D. negundinis* are proposed.

Dothidotthia negundinicola (Crous & Akulov) Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, comb. nov. Fig. 4

Index Fungorum number: IF556640; Facesoffungi number: FoF06139

Basionym: *Neodothidotthia negundinicola* Crous & Akulov, in Crous et al., Fungal Systematics and Evolution 3: 93 (2019)

Holotype – UKRAINE, Kharkiv region, Zolochiv District, on dead aerially attached branches of *Acer negundo* (Sapindaceae), 28 May 2017, A. Akulov & R.K. Schumacher, CWU AS 6293 = HPC 2127 = RKS 116 (holotype CBS H-23832, ex-type culture CPC 34071 = CBS 145039).

Associated with canker on twigs of *Acer negundo* (Sapindaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* up to 300 µm diam., stromatic, effuse, sporodochial, with partly immersed, basal pseudoparenchymatous stroma, erumpent, black, velvety. *Conidiophores* (16–)21–40(–51) × 5–12 µm ($\overline{x}$ = 31.1 × 7.6 µm, *n* = 70), semi-macronematous, septate, branched, subhyaline, smooth, arising from basal stroma. *Conidiogenous cells* 3–28 µm long, enteroblastic, annellidic, with 1–2 annellations, integrated, terminal. *Conidia* (29–)31–36(–42) × 11–16.5 µm)$\overline{x}$ = 33.5 × 13.9 µm, *n* = 70), acrogenous, fusiform to obclavate or obpyriform, septate, pale to brown, truncate at base, (2–)3–5(–6) µm diam., with a protruding hilum, rounded at apex, 2-transversely septate, constricted at the septa, minutely echinulate.

Culture characteristics – Colonies on MEA, reaching 2.5 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, circular, velvety to woolly, rough margin, white to creamy-grey, with aerial mycelium.

Material examined – RUSSIA, Rostov region, Krasnosulinsky District, Donskoye forestry, artificial forest, on dead and dying attached twigs of *Acer negundo* (Sapindaceae), 6 April 2016, T.S. Bulgakov, T-1465 (MFLU 16-1759), living culture MFLUCC 16-1183; *ibid*., Shakhty City, ravine grove near Atukhta river, 5 March 2016, T-1288 (MFLU 16-1582), living culture MFLUCC 16-1157.

Host and distribution – *Acer negundo* (Russia, Ukraine).

Notes – The BLASTn search of ITS and LSU sequences showed that our strains (MFLUCC 16-1157 and MFLUCC 16-1183) are 99% similar (ITS = 478/478 bp, LSU = 804/805 bp) to *Neodothidotthia negundinicola* (CBS 145039). Crous et al. (2019) introduced *N. negundinicola*, collected from *Acer negundo* in Ukraine. The morphology of our fresh collections resembles *N. negundinicola* (ex-type: CBS 145039) (Crous et al. 2019) based on its conidial shape and dimension (Table 4). The phylogenetic analyses based on the combined LSU, SSU, ITS and TEF1-

α sequence dataset show that the strains MFLUCC16-1183 and MFLUCC 16-1157 group with the ex-type strain of *N. negundinicola* (CBS 145039) and has a close relationship with *D. negundinis* and *D. robiniae* in *Dothidotthia* (Figs 2, 3; clade E). We, hence, designate *Neodothidotthia* as a synonym of *Dothidotthia* and introduce a new combination for the species, *Dothidotthia negundinicola*. Our new collections are the first records from Russia.

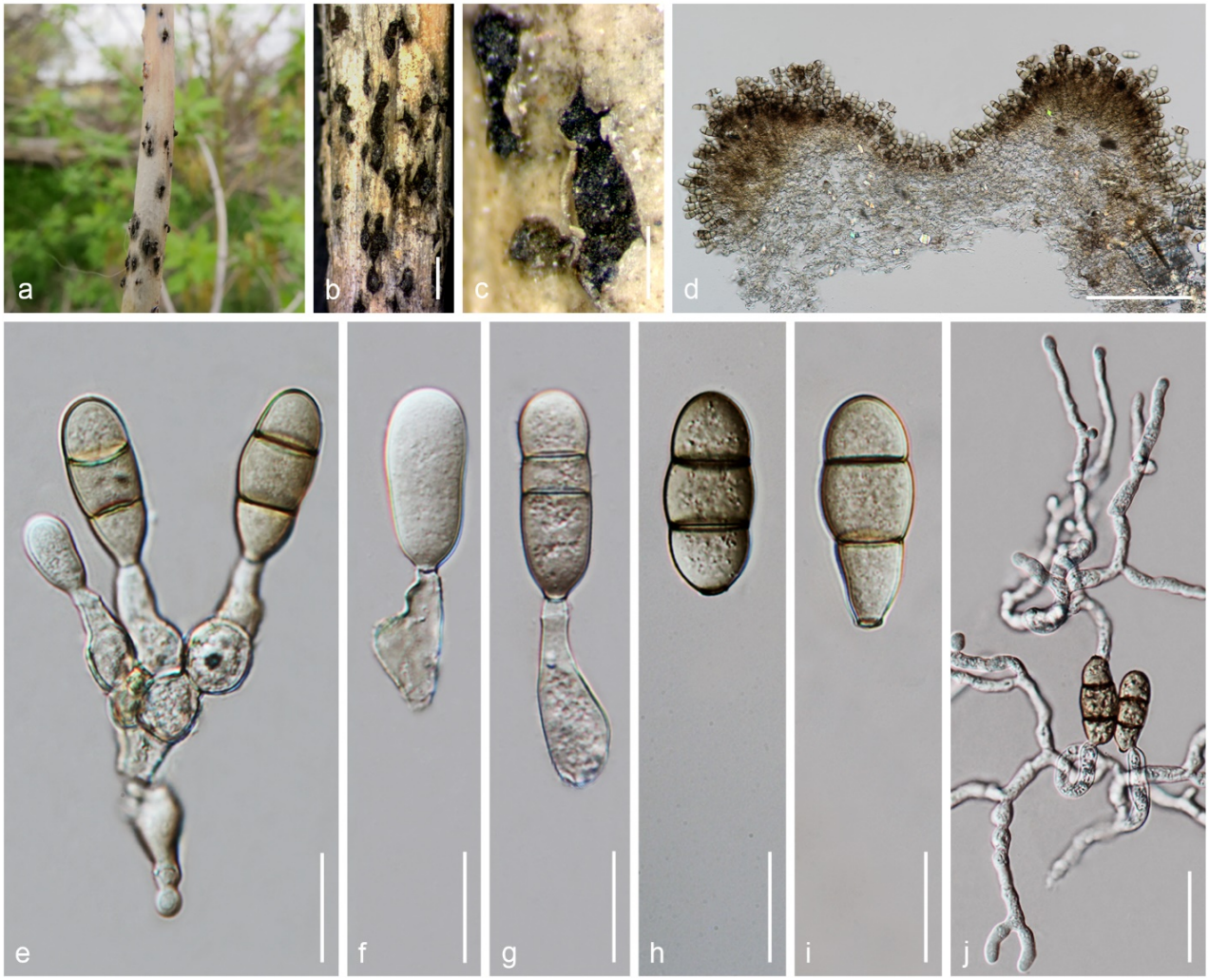

Figure 4 – *Dothidotthia negundinicola* (MFLU 16-1759). a–c Sporodochia on host surface. d Vertical section of sporodochium. e–g Conidia attached to the conidiogenous cells. h, i Conidia. j Germinated conidia. Scale bars: b = 1000 µm, c = 500 µm, d = 200 µm, e–i = 20 µm, j = 40 µm.

Dothidothia negundinis (Crous) Senwanna, Phookamsak & K.D. Hyde, comb. nov.

Index Fungorum number: IF556646

Basionym: *Thyrostroma negundinis* (Berk. & M.A. Curtis) A.W. Ramaley, Mycotaxon 94: 131 (2006) [2005]

Synonym: *Neodothidotthia negundinis* (Berk. & M.A. Curtis) Crous, in Crous et al., Fungal Systematics and Evolution 3: 94 (2019)

Description – See Phillips et al. (2008).

Host and distribution – *Acer negundo*, *Euonymus alatus*, *Fendlera rupicola* (USA).

Table 4 Morphological comparison of the asexual morph of *Thyrostroma, Dothidotthia* and related species discussed in this study.

Taxon	Conidia		Conidiophore size (µm)	Conidiogenous cells (length, µm)	Host/substrate/Locality	Reference
	Size (µm), conidial base (µm diam.)	Type & Septation*				
Dothidotthia negundinis	18–47 × 13.5–21.5	Phragmospores, (1–)3; mostly 2-septate/0	–	–	*Euonymus alatus* (Celastraceae), USA; *Acer negundo* (Sapindaceae), USA; *Fendlera rupicola* (Hydrangeaceae), USA	Barr 1989, Ramaley 2005
D. negundinicola	(29–)31–36(–42) × 11–16.5, (2–)3–5(–6)	Phragmospores, 2/0	(16–)21–40(–51) × 5–12	3–28	*Acer negundo* (Sapindaceae), Russia	This study
D. negundinicola	(25–)30–35(–37) × (12–)13–15(–16), 4–5	Phragmospores, 2/0	60–150 × 7–12	8–15 × 5–7	*Acer negundo* (Sapindaceae), Ukraine	Crous et al. 2019
D. robiniae	(26–)30–40(–46) × (10–)13–16(–18), (2–)3–5	Phragmospores, 2–3 (mostly 2-septate)/0	18–)22–49(–54) × 6–9(–11)	7–28	*Robinia pseudoacacia* and and *R. neomexicana* (Fabaceae), Russia	This study
Thyrostroma celtidis	(24–)27–48(–59) × 12–19(–21), 4–7	Dictyospores, 3–7/2–5	(21–)25–51(–57) × 3–8	9–18	*Celtis occidentalis* (Cannabaceae), Russia	This study
T. cornicola	(25–)30–36(–40) × (12–)14–17(–26), 5–6	Dictyospores, 1–3/0–3	10–50 × 7–10	7–20	*Cornus officinalis* (Cornaceae), Korea	Crous et al. 2016
T. ephedricola	25–34 × 14–22	Dictyospores, 1–3/0–4	25–30 × 4–5	–	*Ephedra equisetina* (Ephedraceae)	Pem et al. 2019
T. franseriae	(25–)28–33(–35) × (18–)20–25, 8–9	Dictyospores; 1–3/2–4	10–18 × 6–11	5–10	*Franseria* sp. (Asteraceae), USA	Marin-Felix et al. 2017
T. jaczewskii	28–42 × 13–17	Dictyospores; 2–6/0–1	13–16 × 4–9	–	*Elaeagnus angustifolia* (Elaeagnaceae)	Pem et al. 2019
T. lycii	(36–)39–49(–55) × 10–17, 5–7	Phragmospores, 1–3 (mostly 3-septate)/0	(20–)34–55(–74) × 6–9	8–26	*Lycium barbarum* (Solanaceae), Russia	This study
T. moricola	(30–)33–60 (–73) × (10–)12–21(–25), 6–9	Dictyospores, 3–8/3–6	(28–)33–50 × 5–8	9–20	*Morus alba* (Moraceae), Russia	This study
T. robiniae	(33–)38–50(–53) × (11–)13–20, (4–)5–8	Dictyospores, 3–4/0–3	(18–)23–35(–43) × 4–9	8–16	*Robinia pseudoacacia* (Fabaceae), Russia	This study
T. styphnolobii	(26–)30–38 × (11–)13–18(–22), (4–)5–7	Dictyospores, 2–3 (mostly 3-septate)/0–2	(14–)18–33(–35) × 4–9	5–25	*Styphnolobium japonicum* (Ulmaceae), Russia	This study
T. tiliae	(41–)50–77(–88) × (12–)15–21(–23), (3–)5–6(–9)	Dictyospores, 3–7/0–5	(24–)27–54(–69) × (3–)5–9	9–29	*Tilia cordata* (Malvaceae), Russia; *Ulmus pumila* (Ulmaceae), Russia	This study
T. ulmicola	(30–)35–50(–59) × (12–)15–20(–26), (3–)4–5(–7)	Dictyospores, 3–7/0–5	(12–)23–56(–73) × (3–)4–8(–10)	(6–)10–13	*Ulmus pumila* (Ulmaceae), Russia	This study
T. ulmigenum	(39–)42–67(–90) × (11–)13–17(–20), (3–)4–5(–6)	Dictyospores, 3–7/0–3	(15–)25–67(–77) × 3–9	3–29	*Ulmus pumila* (Ulmaceae), Russia	This study

* Number of septation (transverse septa / longitudinal septa)

Notes – Crous et al. (2019) proposed a new combination under *Neodothidotthia negundinis* based on collections of Ramaley (2005) which were identified as *Thyrostroma negundinis* (Berk. & M.A. Curtis) A.W. Ramaley (collection no. A.W. Ramaley 0403, 0411 and 0414). Ramaley (2005) introduced a new combination as *Thyrostroma negundinis* and treated *Coryneum negundinis* Berk. & M.A. Curtis as a basionym of *T. negundinis* without studying the type. She also proposed *Dothidotthia aspera* (Ellis & Everh.) M.E. Barr as the sexual morph of *Thyrostroma negundinis* based on cultural characteristics. However, the connection between *Thyrostroma negundinis* and *Dothidotthia aspera* has not yet been proven. Phillips et al. (2008) followed Ramaley (2005) and treated Ramaley's collections (A.W. Ramaley 0403, 0411 and 0414) as *Dothidotthia aspera* based on phylogenetic analysis of a combined SSU and LSU sequence dataset. However, Crous et al. (2019) compared the morphology of these specimens with the holotype of *Amphisphaeria aspera* Ellis & Everh. (basionym of *Dothidotthia aspera*) and mentioned that these collections are not conspecific with *Dothidotthia aspera*. Based on phylogenetic analysis coupled with morphological distinctiveness, Crous et al. (2019) treated these collections as *Neodothidotthia negundinis* in the new genus *Neodothidotthia*. In this study, *N. negundinis* isolates (CPC 12928, CPC 12930, CPC 12932 and CPC 12933), obtained from Ramaley's collection (no. 0403, 0411 and 0414), form a sister subclade with *Dothidotthia negundinicola* and cluster with other known *Dothidotthia* species in Dothidotthiaceae. We, therefore, synonymized *Neodothidotthia negundinis* under *Dothidotthia* as *D. negundinis* based on Ramaley's collections. However, we do not treat *Coryneum negundinis* as the basionym of this species and do not reinstate these collections as *Dothidotthia aspera* due to lack of their type studies. Further studies of type specimens, as well as their epitypes are needed to clarify their taxonomic boundaries.

Dothidotthia robiniae Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 5

Index Fungorum number: IF556526; Facesoffungi number: FoF06140

Etymology – Named after the host genus *Robinia*, from which this species was isolated.

Holotype – MFLU 16-1663

Associated with canker on branches and twigs of *Robinia neomexicana* and *R. pseudoacacia* (Fabaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* 110–710 µm diam., partly immersed, stromatic, sporodochial, effuse, with partly immersed to erumpent, basal pseudoparenchymatous stroma, black, velvety. *Conidiophores* (18–)22–49(–54) × 6–9(–11) µm ($\bar{x}$ = 34.5 × 8 µm, *n* = 60), semi-macronematous, short, compactly packed, septate, subhyaline, smooth, arising from the basal stroma. *Conidiogenous cells* 7–28 µm long, enteroblastic, annellidic, integrated, terminal. *Conidia* (26–)30–40(–46) × (10–)13–16(–18) µm ($\bar{x}$ = 34.3 × 14.6 µm, *n* = 120), acrogenous, fusiform, obclavate or obpyriform, pale to golden brown, truncate at base, (2–)3–5 µm diam., rounded at apex, 2–3-septate (mostly 2-septate), constricted at the septa, rough-walled, minutely echinulate.

Culture characteristics – Colonies on MEA, reaching 2.5 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, circular, velvety, rough margin, greenish-brown, lacking aerial mycelium. Colonies on PDA, slow-growing, reaching 1 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, rough margin, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov region, Shakhty City, near the spoil tip of former coal mine "Proletarian Dictature", on dead twigs of *Robinia pseudoacacia* (Fabaceae), 24 March 2016, T.S. Bulgakov, T-1369 (MFLU 16-1663, holotype), ex-type living culture MFLUCC 16-1175; Rostov region, Krasnosulinsky District, Donskoye forestry, artificial forest, on dead attached twigs of *Robinia neomexicana* (Fabaceae), 6 April 2016, T.S. Bulgakov, T-1410 (MFLU 16-1704), living culture MFLUCC 16-1177; Rostov region, Shakhty City, artificial forest near Grushevka river, on dead twigs of *Robinia pseudoacacia* (Fabaceae), 14 May 2016, T.S. Bulgakov, T-1504 (MFLU 16-1798), living culture MFLUCC 16-1185; Rostov region, Shakhty City, Solyonaya balka (Salty gully), artificial forest, on dead twigs of *Robinia*

pseudoacacia (Fabaceae), 14 May 2016, T.S. Bulgakov, T-1509 (MFLU 16-1803), living culture MFLUCC 18-0692.

Host and distribution – *Robinia neomexicana* and *R. pseudoacacia* (Russia).

Notes – Based on the NCBI BLASTn search of ITS sequence data, the closest match of *Dothidotthia robiniae* is *D. aspera* (CPC 12933; 99% similarity). Multi-gene phylogenetic analyses (Figs 2, 3) show that four isolates of *D. robiniae* (MFLUCC 16-1175, MFLUCC 16-1177, MFLUCC 16-1185 and MFLUCC 18-0692) form a distinct lineage (clade E), sister to *D. negundinis* and *D. negundinicola*. *Dothidotthia robiniae* is morphologically similar to *D. negundinis* (CPC 12930 and CPC12933) and *D. negundinicola* (MFLUCC 16-1157 and MFLUCC 16-1183) in conidial shape. However, these species differ in their conidial dimensions (Table 4). A comparison of ITS nucleotides shows that *D. robiniae* is not significantly different from *D. negundinis* (7/478 bp (1.46%)) and *D. negundinicola* (5/478 bp (1.05%)). However, a comparison of RPB2 sequence data shows that *D. robiniae* differs from *D. negundinicola* in 31/1057 bp (2.93%). Thus, we introduce *D. robiniae* as a new species in *Dothidotthia*.

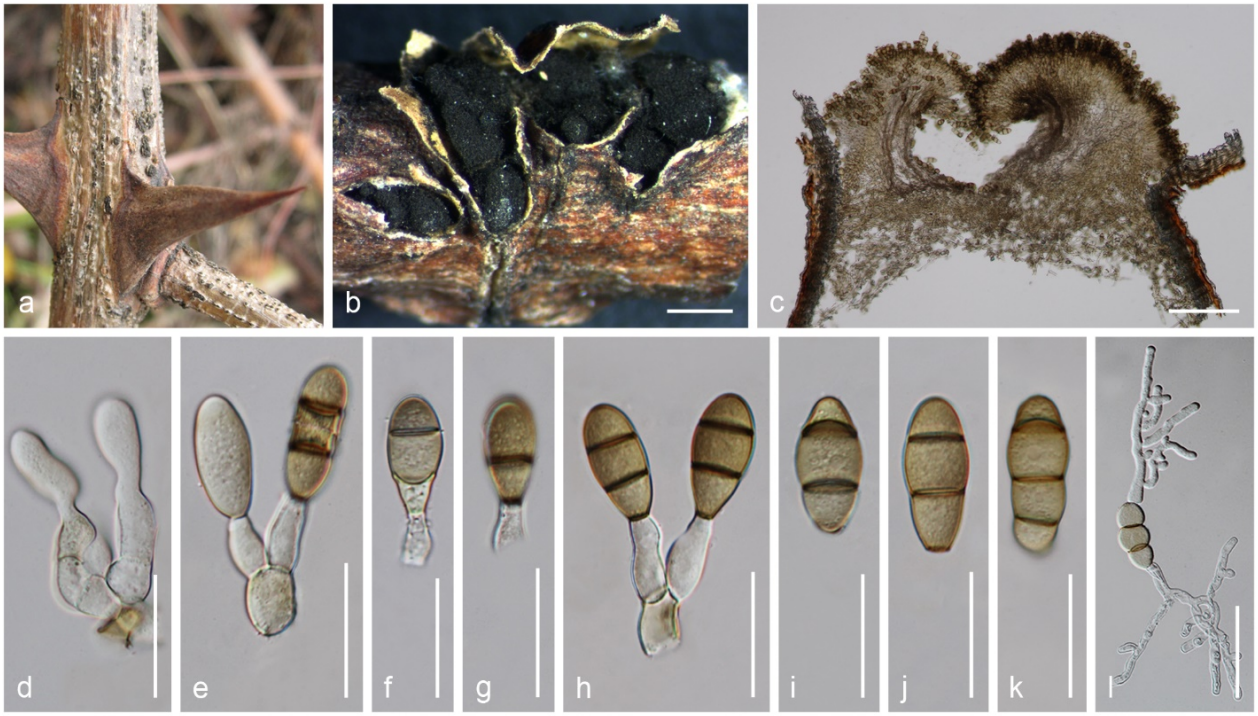

Figure 5 – *Dothidotthia robiniae* (MFLU 16-1663, holotype). a, b Sporodochia on host surface. c Vertical section of sporodochium. d Conidiogenesis. e–h Conidia attach to conidiogenous cells. i–k Conidia. l Germinated conidia. Scale bars: b = 1000 µm, c = 200 µm, d–l = 30 µm.

Thyrostroma Höhn., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt. I 120: 472 (1911)

Saprobic, pathogenic on leaves and wood in terrestrial habitats (Fig. 1). Sexual morph: *Ascostromata* pseudothecial, immersed, raised, erumpent to superficial, breaking through host surface, ruptured, rounded, elongate, lenticular, or irregular in shape, uni- to multi-loculate, glabrous, ostiolate, apapillate. *Ascomata* immersed in ascostroma, dark brown to black, clustered, gregarious, rarely solitary, globose to subglobose, ostiole central, apapillate. *Peridium* thin- to thick-walled of unequal thickness, thicker at the apex, thinner at the base, with several cell layers of *textura angularis*, outer layer comprising brown to black, inner layer comprising hyaline to pale brown cells. *Hamathecium* composed of dense, hyaline, septate pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, clavate, short pedicellate, apically

rounded, with well-developed ocular chamber. *Ascospores*, pale brown, fusiform to ellipsoidal, with rounded ends, 1-septate, constricted at the septum. Asexual morph: *Colonies* stromatic, sporodochial, immersed to erumpent, breaking through host surface, ruptured, convex to applanate, or pulvinate, lenticular, or irregularly dehiscent, dark brown to black. *Conidiophores* macronematous, cylindrical to subcylindrical, septate, branched, hyaline to brown, arising from basal pseudoparenchymatous sporodochia. *Conidiogenous cells* holoblastic, monoblastic, polyblastic, integrated, terminal. *Conidia* acrogenous, straight or curved, variable in shape, clavate, ellipsoidal, obpyriform, subglobose, or oblong to subcylindric-clavate, phragmosporous to muriform, rounded at the apex, tapered or truncate at the base, with 1–8 transverse septa, and 0–6 longitudinal septa, pale to dark brown, constricted at the septa, rough-walled, echinulate.

Type species – *Thyrostroma compactum* (Sacc.) Höhn.

Notes – *Thyrostroma* was previously considered as the asexual morph of *Dothidotthia* (Ramaley 2005, Phillips et al. 2008). Phylogenetic analysis of LSU sequence data indicated that *Thyrostroma* and *Dothidotthia* are not congeneric (Crous et al. 2016, Marin-Felix et al. 2017). Currently, there are 15 epithets listed in this genus (Index Fungorum 2019). However, there are 165 sequences available in GenBank, comprising *T. compactum*, *T. cornicola*, *T. franseriae* and our sequences in this study. In our phylogenetic analyses, *Thyrostroma* species form a strongly supported clade (77% ML, 97% MP, 1.00 PP; Fig. 2) distinct from *Dothidotthia* in Dothidotthiaceae. Further, we introduce eight novel species, *viz. T. celtidis*, *T. lycii*, *T. moricola*, *T. robiniae*, *T. styphnolobii*, *T. tiliae*, *T. ulmigenum* and *T. ulmicola*. We also report the sexual morph of *T. ulmicola* and it is the first record of sexual morph in *Thyrostroma*.

Thyrostroma celtidis Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 6

Index Fungorum number: IF556527; Facesoffungi number: FoF06141

Etymology – Named after the host genus on which it occurs, *Celtis*.

Holotype – MFLU 16-1800

Associated with canker on twigs of *Celtis occidentalis* (Cannabaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* stromatic, sporodochial, comprising pseudoparenchymatous cells at basal stroma, erumpent through host surface, ruptured, applanate, circular to elliptical, or lenticular dehiscent, black, velvety. *Conidiophores* (21–)25–51(–57) × 3–8 µm ($\bar{x}$ = 35.7 × 5.3 µm, *n* = 25), macronematous, septate, branched, hyaline, smooth, arising from the basal stroma. *Conidiogenous cells* 9–18 µm long, holoblastic, integrated, terminal. *Conidia* (24–)27–48(–59) × 12–19(–21) µm ($\bar{x}$ = 35.8 × 15.9 µm, *n* = 75), acrogenous, straight or curved, variable in shape, usually clavate to obpyriform, tapered to the base, truncate at base, 4–7 µm diam., rounded at apex, muriform, (3–)5–6 transverse septa, with 2–5 longitudinal septa, constricted at the septa, pale to dark brown, rough-walled, minutely echinulate.

Culture characteristics – Colonies on PDA, slow growing, reaching 1 cm diam. after 3 weeks at 25–30°C, producing dense mycelium, raised to pulvinate, rough margin, dark brown, lacking aerial mycelium.

Material examined – RUSSIA, Rostov region, Shakhty City, Solyonaya balka (Salty gully), artificial forest, on dead and dying attached twigs of *Celtis occidentalis*, 21 May 2015, T.S. Bulgakov, T-1506 (MFLU 16-1800, holotype), ex-type living culture MFLUCC 16-1186.

Host and distribution – *Celtis occidentalis* (Russia).

Notes – In the NCBI BLASTn search of ITS sequences, *Thyrostroma celtidis* most closely matches *T. cornicola* Crous & H.D. Shin and *T. compactum* with 99% similarity. Phylogenetic analyses of multi-gene sequence dataset show that *T. celtidis* forms a separate lineage with *T. cornicola* and *T. compactum* and other *Thyrostroma* species (Figs 2, 3). A comparison of ITS nucleotides shows that *T. celtidis* differs from *T. compactum* in 19/481 bp (3.95%), and also different from *T. cornicola* in 6/479 bp (1.25%). They are different in conidial dimension and conidial morphology (Table 4). *Thyrostroma celtidis* has a close phylogenetic relationship and similar morphological characters with *T. jaczewskii* (B. Suttton) D. Pem, Bulgakov, Jeewon &

K.D. Hyde (Pem et al. 2019). However, these two species occurred on different hosts and countries and their conidial dimension and septation were different. A comparison of ITS nucleotides shows that *T. celtidis* differs from *T. jaczewskii* in 17/479 bp (3.52%). The conidial morphology of *T. celtidis* is similar to *T. ulmicola* but *T. celtidis* differs from *T. ulmicola* in having shorter conidia and a different number of transverse and longitudinal septa (Table 4).

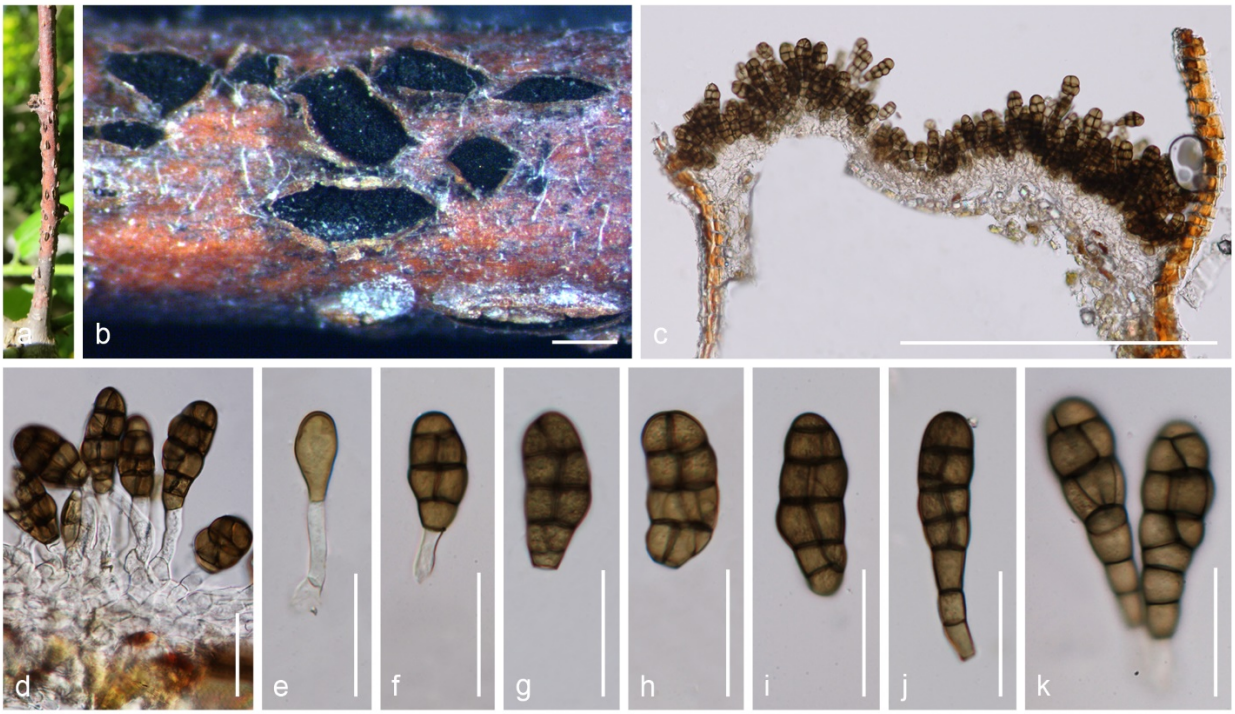

Figure 6 – *Thyrostroma celtidis* (MFLU 16-1800, holotype). a, b Sporodochia on host surface. c Vertical section of sporodochium. d–f Conidia attached to conidiogenous cells. g–k Conidia with variable shape. Scale bars: b = 500 µm, c = 300 µm, d–k = 30 µm.

Thyrostroma lycii Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 7

Index Fungorum number: IF556528; Facesoffungi number: FoF06142

Etymology – Named after the host genus on which it occurs, *Lycium*.

Holotype – MFLU 16-1642

Associated with canker on twigs of *Lycium barbarum* (Solanaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* 260–370 µm diam. sporodochia, stromatic, sporodochial, comprising pseudoparenchymatous cells at basal stroma, partly immersed, becoming erumpent through host epidermis, ruptured, applanate, black, velvety. *Conidiophores* (20–)34–55(–74) × 6–9 µm ($\bar{x}$ = 45.2 × 7.8 µm, *n* = 10), compactly arranged, macronematous, septate, branched, hyaline, smooth. *Conidiogenous cells* 8–26 µm long, holoblastic, monoblastic, integrated, terminal. *Conidia* (36–)39–49(–55) × 10–17 µm ($\bar{x}$ = 43 × 13.7 µm, *n* = 50), acrogenous, slightly curved, ellipsoidal to clavate, pale to golden brown, truncate at base, 5–7 µm diam., rounded at apex, 1–4-septate (mostly 3-septate), constricted at the septa, minutely echinulate.

Culture characteristics – Colonies on PDA, reaching 2.5 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, rough margin, white at the margin, greenish brown at the centre, lacking aerial mycelium.

Material examined – RUSSIA, Rostov region, Shakhty City, near spoil tip of a former coal mine “Proletarian Dictature”, on dead and dying twigs of *Lycium barbarum* (Solanaceae), 24 March 2016, T.S. Bulgakov, T-1348 (MFLU 16-1642, holotype), ex-type living culture MFLUCC 16-1170.

Host and distribution – *Lycium barbarum* (Russia).

Notes – In the NCBI BLASTn search of ITS sequence, *Thyrostroma lycii* has the closest match with *T. compactum* (CBS 335.37) with 99% similarity and is also similar to *T. compactum* (D5/5c; fungal endophyte) and *T. cornicola* (CPC 25427) with 97% similarity. *Thyrostroma lycii* is introduced as a new species based on morphological characters and phylogenetic evidence. Phylogenetic analyses of the combined LSU, SSU, ITS and TEF1-α sequence dataset show that *T. lycii* forms a distinct lineage closely related to *T. compactum*, *T. ulmigenum*, *T. tiliae* and *T. ulmicola* (Figs 2, 3; clade A). *Thyrostroma lycii* is different from the other *Thyrostroma* in having phragmosporous conidia, while others have muriform conidia.

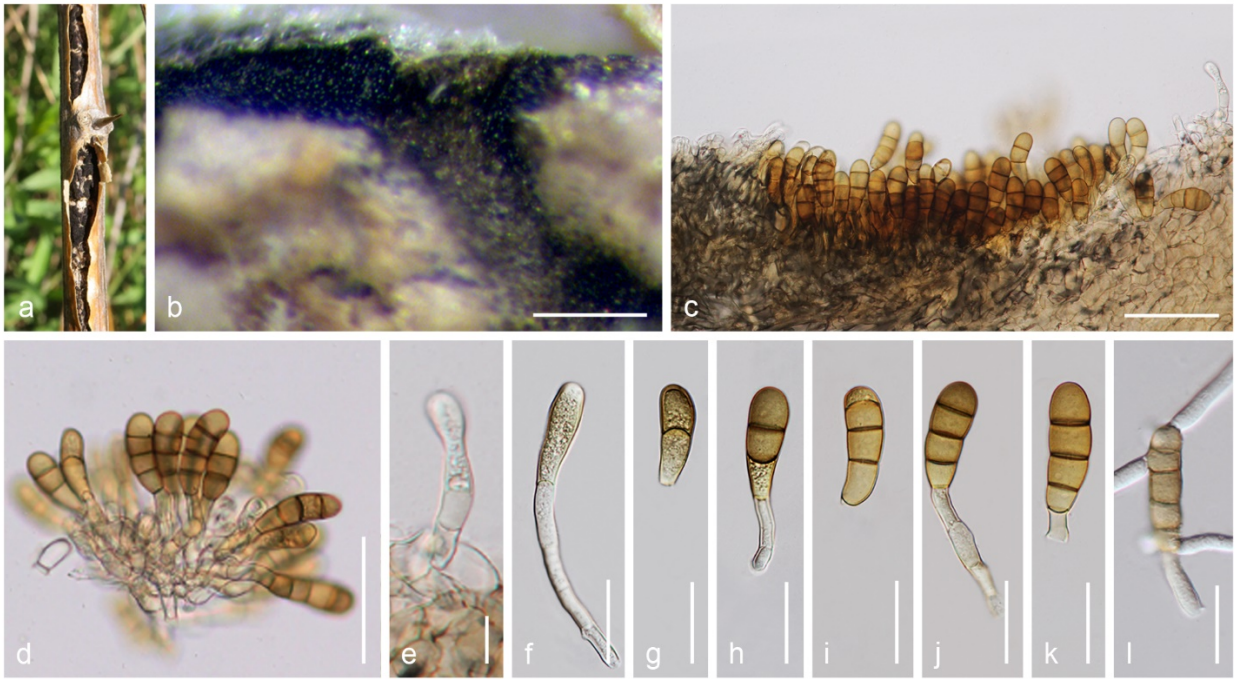

Figure 7 – *Thyrostroma lycii* (MFLU 16-1642, holotype). a, b Sporodochia on host surface. c Vertical section of sporodochium. d Conidia attached with conidiogenous cells. e Conidiogenous cell. f–k Different stages of conidial development and conidia. l Germinated conidium. Scale bars: a = 1000 µm, b, c = 200 µm, d–m = 30 µm.

Thyrostroma moricola Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 8

Index Fungorum number: IF556529; Facesoffungi number: FoF06143

Etymology – Named after the host genus *Morus*, from which this species was isolated.

Holotype – MFLU 16-1795

Associated with canker on twigs of *Morus alba* (Moraceae). Sexual morph: Undetermined. Asexual morph: *Colonies* 285–1295 µm diam., stromatic, sporodochial, with pseudoparenchymatous cells at basal stroma, partly immersed, becoming erumpent through host epidermis, ruptured, lenticular or irregular in shape, applanate, black, velvety. *Conidiophores* (28–)33–50 × 5–8 µm ($\bar{x}$ = 35.6 × 6.4 µm, *n* = 6), compactly arranged, macronematous, septate, pale yellowish to pale brown, smooth. *Conidiogenous cells* 9–20 µm long, holoblastic, polyblastic, sympodial (Figs 8e, f), integrated, terminal. *Conidia* (30–)33–60(–73) × (10–)12–21(–25) µm ($\bar{x}$ = 45 × 17.2 µm, *n* = 80), acrogenous, muriform, with several sectors, straight or slightly curved, varied in shape but commonly broadly subglobose to clavate, brown to dark brown, 3–8 transverse septa, with 3–6 longitudinal septa, rounded at the apex, truncate at the base, 6–9 µm diam., smooth.

Material examined – RUSSIA, Rostov region, Shakhty City, Solyonaya balka (salty gully), artificial forest, on dead twigs of *Morus alba* (Moraceae), 21 May 2015, T.S. Bulgakov, T-1501 (MFLU 16-1795, holotype).

Host and distribution – *Morus alba* (Russia).

Figure 8 – *Thyrostroma moricola* (MFLU 16-1795, holotype). a–c Sporodochia on host surface. d Vertical section of sporodochium. e, f Polyblastic conidiogenesis. g, k, m Conidia with conidiogenous cells. h, i, j, n Conidia. Scale bars: b, c = 1000 µm, d = 200 µm, e–n = 20 µm.

Notes – *Thyrostroma moricola* is associated with canker on twigs of *Morus alba* in Russia. The species can be distinguished from other *Thyrostroma* species based on phylogenetic analyses and characteristics of conidial development, size and septation. The species is similar to *T. celtilis* in conidial shape but differs in size of conidiophores and conidia and numbers of conidial septation (Table 4). Unlike in the others, the conidial development of this species is polyblastic and sympodial (Figs 8e, f). In phylogenetic analyses, *T. moricola* has a close relationship with *T. styphnolobii* (Figs 2, 3; clade A). However, these taxa are different in conidial shape and size (Table 4). In the NCBI BLASTn search of LSU and ITS sequences, *T. moricola* most closely

matches *T. cornicola* (99% similarity). A comparison of ITS and TEF1-α nucleotide base pairs shows that *T. moricola* is not significantly different from *T. celtidis* and *T. styphnolobii*. However, the species is significantly different from *T. celtidis* and *T. styphnolobii* based on comparison of TUB2 gene (9/346 bp (2.60%) and 15/346 bp (4.35%), respectively). We, therefore, introduce *T. moricola* as a new species.

Thyrostroma robiniae Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 9

Index Fungorum number: IF556530; Facesoffungi number: FoF06144

Etymology – Named after the host genus *Robinia*, from which this species was isolated.

Holotype – MFLU 18-0631

Associated with canker on twigs of *Robinia pseudoacacia* (Fabaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* stromatic, with pseudoparenchymatous cells at basal stroma, partly immersed, erumpent through host epidermis, black, velvety, lenticular or triangular dehiscent. *Conidiophores* (18–)23–35(–43) × 4–9 µm ($\bar{x}$ = 28.7 × 6.5 µm, *n* = 10), compactly arranged, macronematous, septate, pale brown, smooth, reduced to conidiogenous cells. *Conidiogenous cells* 8–16 µm long, holoblastic, monoblastic, integrated, terminal. *Conidia* (33–)38–50(–53) × (11–)13–20 µm ($\bar{x}$ = 44 × 15.7 µm, *n* = 30), acrogenous, slightly curved, oblong to clavate, pale to dark brown, truncate at the base, (4–)5–8 µm diam., rounded at the apex, muriform, 3–4 transverse septa (mostly 3-septate), with 0–3 longitudinal septa, constricted at the septa, rough-walled, minutely echinulate.

Culture characteristics – Colonies on PDA, slow-growing, reaching 1 cm diam. after 3 weeks at 25–30°C, producing dense mycelium, raised to pulvinate, rough margin, light brown, lacking aerial mycelium.

Figure 9 – *Thyrostroma robiniae* (MFLU 18-0631, holotype). a, b Sporodochia on host surface. c Vertical section of sporodochium. d Conidia attached with conidiogenous cells. e–j Variable in shape of conidia. Scale bars: b = 1000 µm, c = 200 µm, d–j = 30 µm.

Material examined – RUSSIA, Rostov region, Shakhty City, artificial forest near Grushevka River, on dead twigs of young trees of *Robinia pseudoacacia* (Fabaceae), 14 May

2015, T.S. Bulgakov, T-1504B (MFLU 18-0631, holotype), ex-type living culture MFLUCC 18-1191.

Host and distribution – *Robinia pseudoacacia* (Russia).

Notes – In the NCBI BLASTn search of LSU and ITS sequences, *Thyrostroma robiniae* most closely matches *T. cornicola* with 99% similarity. Phylogenetic analyses of multi-gene sequence dataset, *T. robiniae* nested with *T. celtidis*, *T. moricola* and *T. styphnolobii* (Figs 2, 3; clade A). *Thyrostroma robiniae* is morphologically similar to *T. styphnolobii* in conidial shape but differs in size of conidia, conidiophores and conidiogenous cells. Whereas, *T. robiniae* differs from *T. celtidis* and *T. moricola* in conidial size and different numbers of septation (Table 4). A comparison of nucleotides of ITS and TEF1-α regions shows that *T. robiniae* is not significantly different from *T. moricola* and *T. styphnolobii*. However, *T. robiniae* is distinct from *T. moricola* and *T. styphnolobii* in RPB2 and TUB2 regions (180/1057 bp (17.03%) of RPB2 between *T. robiniae* and *T. styphnolobii*, and 17/347 bp (4.9%) and 13/346 bp (3.76%) of TUB2, respectively).

Thyrostroma styphnolobii Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 10

Index Fungorum number: IF556531; Facesoffungi number: FoF06145

Etymology – Named after the host genus *Styphnolobium*, from which this species was isolated.

Holotype – MFLU 16-1619

Associated with canker and necrosis on branches and twigs of *Styphnolobium japonicum* (Fabaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* 230–540 µm diam. sporodochia, effuse, stromatic, sporodochial, with pseudoparenchymatous at the base of stroma, arranged in *textura angularis* to *textura prismatica*, partly immersed to erumpent, black, velvety, lenticular, trapezoid or triangular dehiscent. *Conidiophores* (14–)18–33(–35) × 4–9 µm ($\bar{x}$ = 28.2 × 6.5 µm, *n* = 20), semi-macronematous, septate, branched, hyaline to pale brown, smooth, compactly arranged. *Conidiogenous cells* 5–25 µm long, holoblastic, monoblastic, integrated, terminal. *Conidia* (26–)30–38 × (11–)13–18(–22) µm ($\bar{x}$ = 33.5 × 14.7 µm, *n* = 25), acrogenous, subglobose to oblong, subclavate, pale to dark brown, truncate at the base, (4–)5–7 µm diam., rounded at the apex, muriform, with 2–3 transverse septa (mostly 3-septate), 0–2 longitudinal septa, slightly constricted at the septa, rough-walled, minutely echinulate.

Culture characteristics – Colonies on MEA, reaching 3 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, surface dull and smooth, velvety, rough margin, light brown to greyish, lacking aerial mycelium. Colonies on PDA, reaching 1.5 cm diam. after 3 weeks at 25–30°C, producing dense mycelium, flat, slightly raised, velvety, rough margin, light brown at the centre, dark brown to black at the margin, with aerial mycelium.

Material examined – RUSSIA, Rostov region, Shakhty City, Alexandrosky Park, on dying and dead twigs of *Styphnolobium japonicum* (Fabaceae), 14 March 2016, T.S. Bulgakov, T-1325 (MFLU 16-1619, holotype), ex-type living culture MFLUCC 16-1160.

Host and distribution – *Styphnolobium japonicum* (Russia).

Notes – In the NCBI BLASTn search, *Thyrostroma styphnolobii* closely matches *T. cornicola* (100% and 99% similarity in ITS and LSU sequences, respectively) and *T. compactum* (99% similarity in both ITS and LSU sequences). Based on the multi-gene phylogenetic analyses, *T. styphnolobii* forms a stable lineage, sister to *T. moricola* in analyses 1 and 2 (Figs 2, 3; clade A) and closely related to *T. cornicola*, *T. franseriae* and *T. ephedricola*. A comparison of ITS nucleotides shows that *T. styphnolobii* differs from *T. cornicola*, *T. franseriae*, *T. moricola* and *T. ephedricola* in 2/452 bp (0.44%), 3/469 bp (0.64%), 2/479 bp (0.42%) and 5/479 bp (1.04%), respectively. Our attempts to obtain RPB2 for *T. styphnolobii* was unsuccessful. However, *T. styphnolobii* and *T. moricola* are different in a comparison of TEF1-α and TUB2 nucleotides (9/780 bp (1.15%) of TEF1-α and 15/346 bp (4.33%) of TUB2). Therefore, we

introduce *T. styphnolobii* as a new species, based on different type of conidiogenesis, its comparatively smaller conidia and different numbers of transverse and longitudinal septa from *T. cornicola*, *T. franseriae* and *T. moricola* (Table 4), and its phylogenetic position.

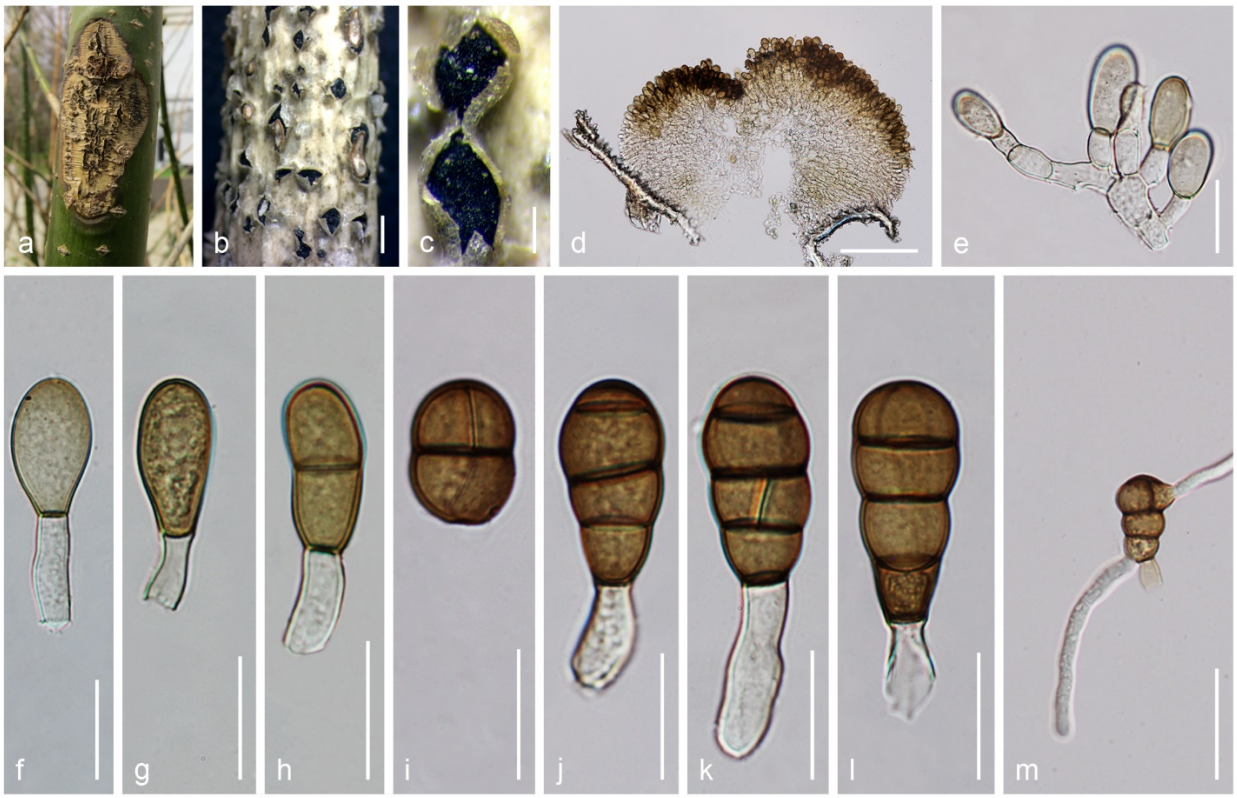

Figure 10 – *Thyrostroma styphnolobii* (MFLU 16-1619, holotype). a–c Sporodochia on host surface. d Vertical section of sporodochium. e Conidiogenesis. f–i Variable in shape of conidia attached with conidiogenous cells. m, n Germinated conidium. Scale bars: b = 1000 µm, c, d = 200 µm, e–l = 30 µm, m, n = 60 µm.

Thyrostroma tiliae Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov. Fig. 11

Index Fungorum number: IF556532; Facesoffungi number: FoF06146

Etymology – Named after the host genus on which it occurs, *Tilia*.

Holotype – MFLU 16-1740

Associated with canker on barks, branches and twigs of *Tilia cordata* (Malvaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* 205–780 µm diam. sporodochia, stromatic, sporodochial, pseudoparenchymatous at the base of stroma, partly immersed to erumpent through host epidermis, ruptured, pulvinate; with rounded to lenticular, or irregularly dehiscent, black, velvety, gnarled. *Conidiophores* (24–)27–54(–69) × (3–)5–9 µm ($\bar{x}$ = 40 × 6.7 µm, *n* = 40), semi-macronematous, septate, branched, hyaline to pale brown, smooth, arising from pseudoparenchymatous stroma. *Conidiogenous cells* 9–29 µm long, holoblastic, annellidic, integrated, terminal. *Conidia* (41–)50–77(–88) × (12–)15–21(–23) µm ($\bar{x}$ = 62.9 × 18.1 µm, *n* = 120), acrogenous, cylindrical to ellipsoidal, or subclavate to vermiform, pale to brown, truncate at base, (3–)5–6(–9) µm diam., rounded at apex, muriform, 3–7-transversely septate, with 0–5-longitudinally septate at the 2nd to the 4th cells from above, constricted at the septa, rough-walled, echinulate.

Culture characteristics – Colonies on MEA, reaching 3 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, flat, surface dull and smooth, rough margin, white to greyish becoming dark brown or greenish-brown when mature, reverse dark brown to black at

centre, white at the margin, with aerial mycelium.

Figure 11 – *Thyrostroma tiliae* (MFLU 16-1740, holotype). a, b Sporodochia on host surface. c Vertical section of sporodochium. d, e, h Conidia attached with conidiogenous cells. f–g Conidia. i Germinated conidia. Scale bars: b = 500 µm, c = 200 µm, d–h = 30 µm, i = 60 µm.

Material examined – RUSSIA, Rostov region, Krasnosulinsky District, Donskoye forestry, artificial forest, on dead and dying twigs of *Tilia cordata* (Malvaceae), 6 April 2016, T.S. Bulgakov, T-1446 (MFLU 16-1740, holotype), ex-type living culture, MFLUCC 16-1178; *ibids*., on dead and dying twigs of *Tilia cordata* (Malvaceae), 6 April 2016, T.S. Bulgakov, T-1387 (MFLU 16-1681), living culture, MFLUCC 16-1176; Rostov region, Krasnosulinsky District, Donskoye forestry, artificial forest, on dead twigs and bark of *Ulmus pumila* (Ulmaceae), 6 April 2016, T.S. Bulgakov, T-1454B (MFLU 18-0628), living culture MFLUCC 16-1180; Rostov region, Shakhty City, Alexandrovsky Park, on dead and dying twigs of *Tilia cordata* (Malvaceae), 1 May 2015, T.S. Bulgakov, T-1519, (MFLU 16-1813), living culture, MFLUCC 16-1188.

Host and distribution – *Tilia cordata*, *Ulmus pumila* (Russia).

Notes – In the BLASTn search of the ITS sequence, *Thyrostroma tiliae* has a 98% (469/480) similarity with *T. compactum* (CBS 335.37; GenBank KY905670) and a 96% (462/479) similarity with *T. compactum* (isolate D5/5C; GenBank MG020345) and *T. cornicola* (CBS 141280; GenBank KX228248). In this study, we refer four strains to *T. tiliae viz.* MFLUCC 16-1180, MFLUCC 16-1176, MFLUCC 16-1178 and MFLUCC 16-1188. These four strains form a single lineage with high support (97% ML, 99% MP, 1.00 PP, Fig. 3; clade A),

and is sister to *T. ulmigenum*. *Thyrostroma tiliae* morphologically resembles *T. ulmigenum* in its conidial shape. However, their sporodochia and size of the conidia are different (Table 4). A comparison of ITS and TEF1-α nucleotides shows that *T. tiliae* is different from *T. ulmigenum* (5/480 bp (1.04%) of ITS and 10/787 bp (1.27%) of TEF1-α). The species is also different from *T. ulmigenum* in RPB2 and TUB2 regions (55/1062 bp (5.18%) and 15/342 bp (4.39%), respectively). In addition, *T. tiliae* differs from *T. compactum* in 11/481 bp (2.28%) of ITS region. *Thyrostroma compactum* var. *tiliae* (Sacc.) Höhn. associated with linden canker has been also reported in Russia and Eastern Europe from the last decades (Kolemasova 1999, Kuz'michev et al. 2001, Sokolova et al. 2006, Mel'nik et al. 2007, Kolganikhina & Sokolova 2012, Bulgakov et al. 2014, Stravinskienė et al. 2015). However, the species differs from *T. tiliae* in having shorter and narrower conidia (50–55 × 15–16 µm, Potebnia 1907). Thus, we introduce *Thyrostroma tiliae* as a new species.

Thyrostroma ulmicola Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov.

Fig. 12

Index Fungorum number: IF556533; Facesoffungi number: FoF06283

Etymology – Named after the host genus on which it occurs, *Ulmus pumila*.

Holotype – MFLU 16-1652

Associated with canker on twigs and bark of *Ulmus pumila* (Ulmaceae). Sexual morph: *Ascostromata* pseudothecial, immersed in host epidermis, visible as raised, becoming erumpent to superficial, ruptured, reflexed, stellate, host remnants around the base, ostiolate, apapillate. *Ascomata* 180–365 µm diam., 135–310 µm high, immersed in ascostroma, dark brown to black, clustered, gregarious, rarely solitary, globose to subglobose, uni- to multi-loculate, ostiole central, apapillate. *Peridium* thin- to thick-walled, of unequal thickness, thicker at the apex, composed of 9–11 layers of pale brown to dark brown, or black, pseudoparenchymatous cells, arranged in *textura angularis*, 95–100 µm; thinner at the base, 45–60 µm, composed of 5–6 layers of brown to black cells, of *textura angularis*. *Hamathecium* composed of dense, filamentous, 3–5 µm wide, hyaline, septate pseudoparaphyses. *Asci* (71–)113–190(–200) × (13–)19–25(–27) µm ($\overline{x}$ = 148 × 21.8 µm, *n* = 25), 8-spored, bitunicate, fissitunicate, clavate, short pedicellate, with knob-like to truncate pedicel, apically rounded, with a well-developed ocular chamber. *Ascospores* (22–)25–33(–36) × (7–)9–16 µm ($\overline{x}$ = 29.8 × 13.2 µm, *n* = 28), overlapping 1–2-seriate, pale brown, fusiform to ellipsoidal with rounded ends, 1-septate, constricted at the septum, widest at above cell, rough-walled, finely verruculose. Asexual morph: *Colonies* 150–900 µm diam., sporodochia, partly immersed, or effuse, stromatic, with pseudoparenchymatous basal stroma, erumpent through host epidermis, pulvinate to applanate, black, velvety, with lenticular or irregularly dehiscent. *Conidiophores* (12–)23–56(–73) × (3–)4–8(–10) µm ($\overline{x}$ = 40.3 × 6.1 µm, *n* = 300), macronematous, erect, compactly packed, septate, branched, hyaline to pale brown, smooth. *Conidiogenous cells* (6–)10–13 µm long, holoblastic, monoblastic, annellidic, with 1–2 annellations, integrated, terminal. *Conidia* (30–)35–50(–59) × (12–)15–20(–26) µm ($\overline{x}$ = 42 × 18.1 µm, *n* = 420), acrogenous, ellipsoidal to obovoid, subclavate, muriform, pale to dark brown, truncate at base, (3–)4–5(–7) µm diam., rounded at apex, 3–7-transverse septate, with longitudinally 0–5-septate, constricted at the septa, rough-walled, minutely echinulate.

Culture characteristics – Colonies on MEA, reaching 2.5 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, circular, velvety to woolly, rough margin, white to grey becoming greenish grey when mature, with aerial mycelium. Colonies on PDA reaching 1 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, rough margin, white to dark brown to black, with aerial mycelium.

Material examined – RUSSIA, Rostov region, Shakhty City, shrubs near a former coal mine "Proletarian Dictature", on dead twigs of *Ulmus pumila* (Ulmaceae), 24 March 2016, T.S. Bulgakov, T-1358 (MFLU 16-1652, holotype), ex-type living culture MFLUCC 16-1172

(asexual morph), ex-type living culture MFLUCC 16-1173 (sexual morph); *ibids.*, T-1295 (MFLU 16-1589), living culture MFLUCC 16-1158; T-1357 (MFLU 16-1651), living culture MFLUCC 16-1171; Rostov region, Shakhty City, Alexandrovsky Park, on dead twigs of *Ulmus pumila* (Ulmaceae), 14 March 2016, T.S. Bulgakov, T-1326 (MFLU 16-1620), living culture MFLUCC 16-1161; *ibids.*, T-1327 (MFLU 16-1621), living culture MFLUCC 16-1162; T-1328 (MFLU 16-1622), living culture MFLUCC 16-1163; T-1329 (MFLU 16-1623), living culture MFLUCC 16-1165; T-1330 (MFLU 18-0627), living culture MFLUCC 16-1167; T-1331 (MFLU 16-1625), living culture MFLUCC 16-1168, MFLUCC 16-1169; Rostov region, Krasnosulinsky District, Donskoye forestry, artificial forest, on dead twigs of *Ulmus pumila* (Ulmaceae), 6 April 2016, T.S. Bulgakov, T-1454 (MFLU 16-1748), living culture MFLUCC 16-1179; *ibids.*, T-1455 (MFLU 16-1749), living culture MFLUCC 16-1181; T-1459 (MFLU 16-1749), living culture MFLUCC 16-1182.

Host and distribution – *Ulmus pumila* (Russia).

Notes – In the NCBI BLASTn search of LSU and ITS sequences, *Thyrostroma ulmicola* is most similar to *T. compactum* and *T. conicola* with 99% and 98% similarities, respectively. The asexual morph of *T. ulmicola* is most similar to *T. celtidis*, but they are different in the length and width of conidia (*T. ulmicola*, (30–)35–50(–59) × (12–)15–20(–26) µm versus (24–)27–39(–42) × 12–19 µm, *T. celtidis*). Phylogenetic analyses of a combined LSU, SSU, ITS and TEF1-α sequence dataset indicated that *T. ulmicola* forms an independent subclade in *Thyrostroma* (Figs 2, 3; clade A). In a comparison of ITS and TEF1-α nucleotides, *T. ulmicola* differs from *T. celtidis* in 11/480 bp (2.29%) and 20/822 bp (2.43%), respectively.

In this study, the sexual morph of *Thyrostroma ulmicola* is morphologically similar to *Dothidotthia* species in the shape of asci and ascospores (Ramaley 2005, Phillips et al. 2008, Hyde et al. 2013). However, the peridium structure of *T. ulmicola* is different from *Dothidotthia* species. *Dothidotthia* species have a thin peridium with only 3–6 pigmented cell layers and lack hyaline cell layers, while *Thyrostroma* has a thick peridium with several hyaline and pigmented cell layers. Furthermore, the ascospores of *T. ulmicola* are constricted at the septum, whereas ascospores of *Dothidotthia* species are not constricted or slightly constricted at the septum (Barr 1989).

Thyrostroma ulmigenum Senwanna, Wanas., Bulgakov, Phookamsak & K.D. Hyde, sp. nov.
Fig. 13

Index Fungorum number: IF556534; Facesoffungi number: FoF06147

Etymology – Named after the host genus *Ulmus*, from which this species was isolated.

Holotype – MFLU 16-1624

Associated with canker on bark, branches and twigs of *Ulmus pumila* (Ulmaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* 100–750 µm diam. sporodochia, stromatic, with pseudoparenchymatous basal stroma, partly immersed to erumpent, rounded to irregularly dehiscent, applanate, black, velvety. *Conidiophores* (15–)25–67(–77) × 3–9 µm ($\bar{x}$ = 47 × 5.7 µm, n = 70), compactly arranged, macronematous, septate, branched, hyaline to pale brown, smooth. *Conidiogenous cells* 3–29 µm long, monoblastic, annellidic, with 1–2 annellations, integrated, terminal. *Conidia* (39–)42–67(–90) × (10–)13–18 µm ($\bar{x}$ = 52 × 14.8 µm, n = 80), acrogenous, oblong to subcylindric-clavate, or clavate, phragmosporous to muriform, pale to dark brown, truncate at the base, (3–)4–5(–6) µm diam., rounded at the apex, 3–7 transverse septa, with 0–3 longitudinal septa, constricted at the septa, rough-walled, minutely echinulate.

Culture characteristics – Colonies on MEA, reaching 3 cm diam. after 2 weeks at 25–30°C, producing dense mycelium, rough margin, light greenish brown at the centre, dark greenish brown and white at the margin, lacking aerial mycelium. Colonies on PDA, reaching 2 cm diam. after 3 weeks at 25–30°C, producing dense mycelium, flat, surface dull and smooth, velvety, rough margin, dark greenish-brown at the centre, white at the margin, lacking aerial mycelium.

Material examined – RUSSIA, Rostov region, Shakhty Park, Alexandrovsky Park, on fallen and attached twigs of *Ulmus pumila* (Ulmaceae), 14 March 2016, T.S. Bulgakov, T-1330A (MFLU 16-1624, holotype), ex-type living culture MFLUCC 16-1166; *ibid.*, T-1328B (MFLU 18-0629), living culture, MFLUCC 16-1164.

Host and distribution – *Ulmus pumila* (Russia).

Notes – In the NCBI BLASTn search of LSU sequences, *Thyrostroma ulmigenum* is found most similar to *T. conicola* and *T. compactum* with 99% similarity, whereas the closest hits of the ITS sequences were 98% similar to *T. compactum* and 97% similar to *T. conicola*. In this study, three novel *Thyrostroma* species, *viz. T. tiliae*, *T. ulmigenum* and *T. ulmicola* were collected from *Ulmus pumila*. However, these species can be distinguished based on their conidial characters and phylogenetic analyses. *Thyrostroma ulmigenum* is morphologically similar to *T. tiliae*, however, these taxa are different in the width of conidia (Table 4). In multi-gene phylogenetic analyses, *T. ulmigenum* is sister to *T. tiliae* (Fig. 3; clade A). In a comparison of ITS and TEF1-α nucleotides, *T. ulmigenum* and *T. celtidis* are not significantly different from each other (5/479 bp (1.04 %(of ITS and 10/787 bp (1.27%) of TEF1-α). These two species are different in a comparison of RPB2 and TUB2 nucleotides (55/1060 bp (5.19%) of RPB2 and 15/342 bp (4.39%(of TUB2). Therefore, we introduce *T. ulmigenum* as a novel species in *Thyrostroma* based on morphological characters and the guidelines of Jeewon & Hyde (2016).

Discussion

Phylogenetic analyses of the combined LSU, SSU, ITS and TEF1-α gene dataset in the present study (Figs 2, 3) show that *Thyrostroma* is distinct from *Dothidotthia*, and concurs with the taxonomic scheme from a previous study (Marin-Felix et al. 2017). Hence, we segregate *Thyrostroma* from *Dothidotthia* based upon multi-gene phylogenetic analyses coupled with morphological characteristics. The status of *Thyrostroma* is resolved and shows that the asexual morph of *Thyrostroma* is not related to *Dothidotthia*. Six genera are accepted in Dothidotthiaceae, *viz. Dothidotthia*, *Mycocentrospora*, *Phaeomycocentrospora*, *Pleiochaeta*, *Thyrostroma* and *Wilsonomyces*.

Neodothidotthia was introduced to accommodate *N. negundinicola* and *N. negundinis* by Crous et al. (2019). Crous et al. (2019) analyzed the phylogenetic relationship of *Dothidotthia* and *Neodothidotthia* based on LSU sequence dataset. They treated *Neodothidotthia* as a distinct genus from *Dothidotthia* and also synonymized Ramaley's collections [described as *Thyrostroma negundinis* in Ramaley (2005) as well as *Dothidotthia aspera* in Phillips et al. (2008)] as a synonym of *Neodothidotthia negundinis*. The combined LSU, SSU, ITS and TEF1-α gene analyses in the present study shows that *Neodothidotthia negundinicola* and *N. negundinis* nest with *D. robiniae* and *D. symphoricarpi* (Figs 2, 3). In addition, a comparison of the conidial morphology also reveals that these genera are congeneric. Therefore, we synonymize *Neodothidotthia* under *Dothidotthia*. In our phylogenetic analyses (analyses 2, Fig. 3), *Dothidotthia negundinis* strain CPC 12928 forms an unstable lineage, separated from other strains of *D. negundinis*. This isolate (A.W. Ramaley 0403, BPI 871821) was also collected from a different host. However, there are only ITS, LSU and SSU sequences obtained for this strain which are not appropriate to distinguish species. Therefore, this strain needs to be revisited based on both reliable genes phylogenetic analysis and morphological characteristics.

Dothidotthia and *Thyrostroma* species have been reported as plant pathogens causing canker, dieback and leaf spot diseases on twig, branch, bark and leaf in both temperate and tropical countries (i.e. Australia, Italy, Korea, Russia, USA) (Yuan & Old 1990, Kuz'michev et al. 2001, Mel'nik et al. 2007, Phillips et al. 2008, Bulgakov et al. 2014, Marin-Felix et al. 2017). Host-specificity of these taxa have not yet been sorted out and they have been recorded from various plant families, i.e. Asteraceae, Caprifoliaceae, Cannabaceae, Celastraceae, Cornaceae, Compositae, Fabaceae, Hydrangeaceae, Moraceae, Myrtaceae, Oleaceae, Rosaceae, Sapindaceae, Solanaceae and Ulmaceae (Yuan & Old 1990, Ramaley 2005, Crous et al. 2016,

Marin-Felix et al. 2017, Farr & Rossman 2019). More taxon-sampling from other hosts and regions is required for a better understanding of their host-specificity and pathogenicity.

In this study, most species in *Dothidotthia* and *Thyrostroma* are quite different from one another based on a comparison of ITS and TEF1-α gene regions. We witnessed that concatenating RPB2 and TUB2 (in primary analyses of our new isolates) provided a good resolution regarding relationships. Therefore, we suggest that RPB2 and TUB2 are reliable genes for distinguishing species within *Dothidotthia* and *Thyrostroma*. However, there are mostly ITS, LSU and TEF1-α sequences available in GenBank for *Dothidotthia* and *Thyrostroma* from previous studies. Hence, more reliable genes (i.e. RPB2 and TUB2) from further collections, as well as the re-sequencing of the generic types of genera in Dothidotthiaceae are required.

Thyrostroma ulmicola (MFLU 16-1652) forms a holomorph on *Ulmus pumila* (Fig. 11). Single spore isolation for both isolates (MFLUCC 16-1172 and MFLUCC 16-1173) was obtained from the molecular data. Phylogenetic analyses based on multi-gene sequence data indicated these isolates from sexual and asexual morphs are conspecific and are distinct from *Dothidotthia* (Figs 2, 3; clade A and D). This is the first record of the sexual morph of *Thyrostroma*. *Thyrostroma ulmicola* was collected from symptomatic twigs in European Russia. Recent molecular analyses indicate that eight strains of *Thyrostroma ulmicola* are separated into two subclades with good support (88% ML, 88% MP, 0.99 PP (Fig. 2) and 87% ML, 90% MP, 1.00 PP (Fig. 3; clade A), although the conidial characters of these isolates are the same. These isolates have very few morphologically differences and narrow in the host range. Thus, we compared the ITS, RPB2, TEF1-α and TUB2 alignments of these eight strains and found that they are not different. Therefore, we consider these eight strains as the same species based on the morphological similarity of the asexual morph characters. *Thyrostroma* species are well-known as plant pathogens occurring on twigs and branches (Yuan & Old 1990, Kuz'michev et al. 2001, Phillips et al. 2008, Crous et al. 2016, Marin-Felix et al. 2017). More collections from different hosts may further clarify their taxonomy and their possibility of occurring as a species complex. The data of *Thyrostroma* and *Dothidotthia* provided in this study are a preliminary and more exhaustive sampling of other hosts from other regions will assist in clarifying the taxonomy and the sexual and asexual relationships, as well as, host range and distribution of these taxa.

The genera *Mycocentrospora*, *Pleiochaeta* and *Wilsonomyces* form well-resolved clades in Dothidotthiaceae (Figs 2, 3; clade B, C and D). *Mycocentrospora* and *Pleiochaeta* possess unique characteristics derived from *Wilsonomyces*, due to its appendage at the conidial apex (Pollack & Ellett 1974, Crous et al. 2013, Marin-Filix et al. 2017). Currently *Pleiochaeta* comprises six species and only three species are supported by molecular data (Marin-Filix et al. 2017). In our preliminary analyses, *Pleiochaeta* sp. (B17_3; FJ378717) forms a separate lineage, away from *P. carotae* (type species), however, the strains were obtained from ectomycorrhizal root tips and identified by blast searching of GenBank using only ITS sequence data (Gao & Yang 2010). We, therefore, did not include this sequence data in our analyses as we cannot verify these data using morphology. Further study of the taxon is needed to resolve its natural placement.

Sutton (1997) treated *Wilsonomyces* as a synonym of *Thyrostroma*. However, our phylogenetic analyses showed that *W. carpophilus* (clade B) forms a robust clade distinct from *T. compactum* (type species of *Thyrostroma*). Hence, the generic synonymy of *Wilsonomyces* with *Thyrostroma* seems unlikely. In addition, *Wilsonomyces* morphologically resembles *Dothidotthia* in having similar conidial character, however, its conidia differ in being round to acute at the apex. The genus is phylogenetically distinct from *Dothidotthia symphoricarpi*, the type species of *Dothidotthia*. Thus, it is necessary to include more taxa in future studies.

Figure 12 – *Thyrostroma ulmicola* (MFLU 16-1652, holotype). a–c Ascostromata on bark. d Vertical section of ascoma. e Peridium. f Pseudoparaphyses. g, h Germinated ascospores. i–n Ascospores. o–r Asci. s, t Sporodochia on host surface. u Vertical section of sporodochium. v, w Conidiogenesis and conidiogenous cells. x–ae Stages of developing conidia. af Germinated conidium. Scale bars: b, c = 500 µm, d, u = 100 µm, e = 50 µm, f, i–r = 30 µm, g, h = 60 µm, t = 1000 µm, v–af = 20 µm.

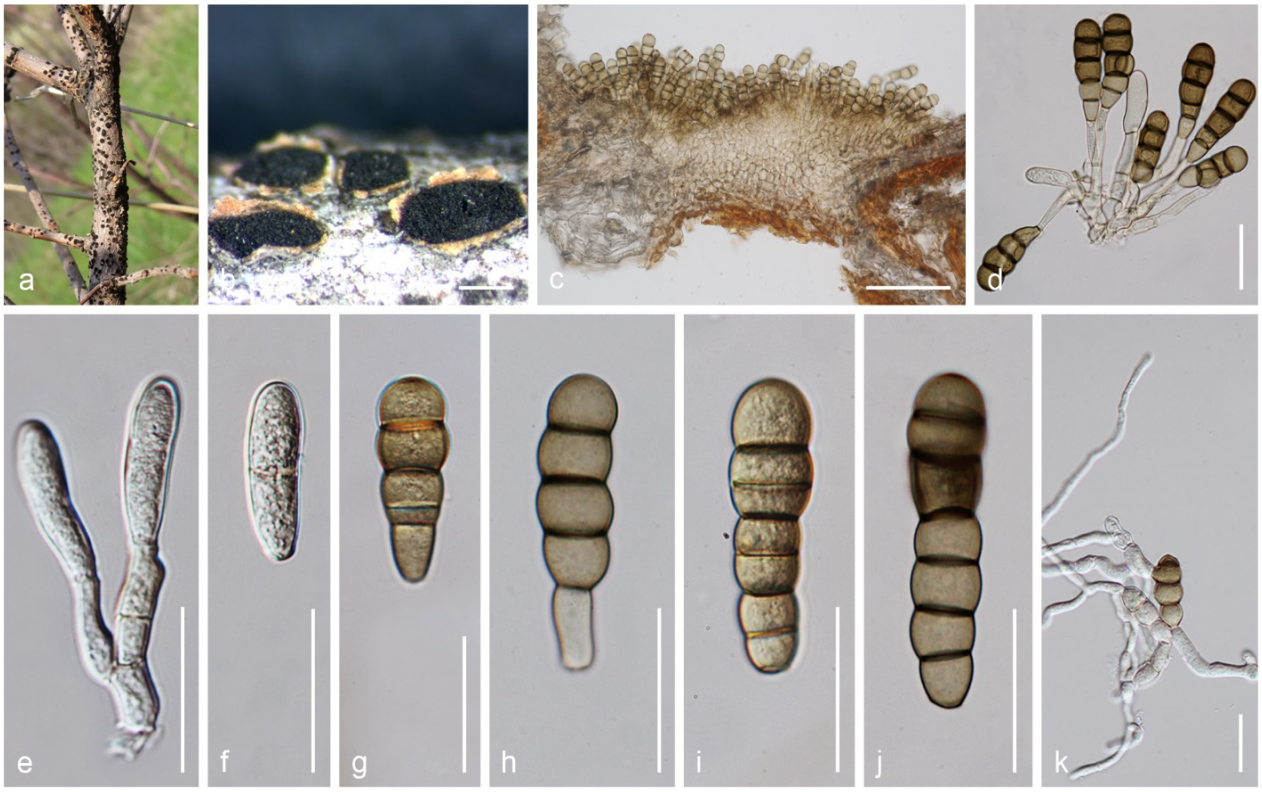

Figure 13 – *Thyrostroma ulmigenum* (MFLU 18-0629, holotype). a, b Sporodochia on host surface. c Vertical section of sporodochium. d Conidia attached with conidiogenous cells. e Immature conidia attach to conidiogenous cells. f–g, i–j Stages of developing conidia. h conidia attach with conidiogenous cell. k Germinated conidium. Scale bars: b = 1000 µm, c = 100 µm, d–k = 30 µm.

Mycocentrospora was established by Deighton (1972) with *M. acerina* (R. Hartig) Deighton as the type species and classified in Pleosporales genera *incertae sedis* by Wijayawardene et al. (2018). Phylogenetic analysis based on the LSU sequence data of Dothideomycetes by Crous et al. (2019) shows that *M. acerina* (MH868490) clustered between *Pleiochaeta* and *Wilsonomyces* and similar results are obtained in our study (Figs 1, 2). *Mycocentrospora* is morphologically similar to *Pleiochaeta* in having conidia with appendages (Braun 1993, Crous et al. 2013) and is similar to *Phaeomycocentrospora* in conidial shape (Crous et al. 2013). Species of this genus can be pathogens on living plants and humans and have also been found as saprobes in terrestrial habitats or aquatic hyphomycetes worldwide (Hermansen et al. 1999, Stewart et al. 1999, Wijayawardene et al. 2018). This genus is poorly studied and only sequences of *M. acerina* are available in GenBank. There are 12 epithets of *Mycocentrospora* listed in Index Fungorum (2019), but four species were accepted by Wijayawardene et al. (2018). More sampling of taxa in this genus is needed for a better understanding.

Previous phylogenetic studies showed that *Phaeomycocentrospora* belongs in Didymellaceae (Trakunyingcharoen et al. 2014). However, phylogenetic analyses in the present study show that *Phaeomycocentrospora* (Figs 2, 3; clade F) forms a well-resolved clade in Dothidotthiaceae concurring with Marin-Felix et al. (2017). Even though the morphological characters of *Phaeomycocentrospora* are different from other genera in Dothidotthiaceae in having mononematous conidiophores, the conidiophores are sometimes reduced to conidiogenous cells, with filiform to cylindrical conidia (Crous et al. 2013). There is a putative species, *P. cantuariensis* (E.S. Salmon & Wormald) Crous, H.D. Shin & U. Braun, available in

Index Fungorum (2019). Thus, it is necessary to study more taxa coupled with analyses of informative genes for a better understanding of their relationships in the family Dothidotthiaceae.

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