Studies in Fungi 6(1): 92–115 (2021) www.studiesinfungi.org ISSN 2465-4973
Article

Doi 10.5943/sif/6/1/5

New host and geographic records of five pleosporalean hyphomycetes associated with *Musa* spp. (Banana)

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Samarakoon BC, Phookamsak R, Karunarathna SC, Jeewon R, Chomnunti P, Xu JC, Li YJ 2021 – New host and geographic records of five pleosporalean hyphomycetes associated with *Musa* spp. (Banana). Studies in Fungi 6(1), 92–115, Doi 10.5943/sif/6/1/5

Abstract

A study was undertaken to collect and identify saprobic fungi associated with *Musa* spp. (banana) from Taiwan, China, and Thailand. Samples were collected during the dry season and their morpho–molecular relationships were investigated. Five brown pleosporalean hyphomycetous taxa in Periconiaceae and Torulaceae viz. *Periconia cortaderiae*, *P. delonicis*, *Torula chromolaenae*, *T. fici*, and *T. masonii* were identified for the first time from *Musa* spp. (Musaceae). Phylogenetic analyses of a combined SSU, LSU, ITS, RPB2 and TEF DNA sequence dataset further justified the taxonomic placements of these five taxa in the above mentioned families. *Periconia delonicis* is reported for the first time on a monocotyledonous host and *T. masonii* is the first geographical record from Taiwan.

Key words – Dothideomycetes – *Periconia* – Pleosporales – taxonomy – *Torula*

Introduction

Studies of fungi on *Musa* spp. have been conducted since the 19th century, especially in tropics and subtropics (Sawada 1959, Ellis 1971, 1976, Matsushima 1971, Ebbels & Allen 1979, Shaw 1984, Sivanesan 1984, Arnold 1986, Aptroot 1995, Heredia–Abarca et al. 1997, Photita et al. 2001a, b, 2002, 2003b, Somrithipol 2007). However, these studies were restricted to some fungal groups (e.g. hyphomycetous and endophytic fungi) and were based mostly on morphological characteristics. Hence, less novel species on *Musa* spp. were consequently introduced based on morphological characteristics coupled with phylogenetic analyses (Gao et al. 2014, Hernández–Restrepo et al. 2015, Liu et al. 2015, Crous et al. 2016, 2017, Samarakoon et al. 2020a, b).

Submitted 9 January 2021, Accepted 3 February 2021, Published 24 February 2021
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Many genera of fungi have been reported as endophytes, saprobes or pathogens on *Musa* spp. Worldwide. The most common species are from *Acremonium*, *Alternaria*, *Arthrobotryum*, *Bipolaris*, *Brevistachys*, *Chalara*, *Cladosporium*, *Coniothyrium*, *Corynespora*, *Cylindrocarpon*, *Dactylella*, *Diplodia*, *Dothiorella*, *Epicoccum*, *Helminthosporium*, *Lasiodiplodia*, *Leptosphaeria*, *Myrothecium*, *Nectria*, *Parapallidocercospora*, *Phaeodothis*, *Phaeosphaeria*, *Pseudoramularia*, *Pyricularia*, *Pyriculariopsis*, *Radaisiella*, *Scolecobasidium*, *Stagonospora*, *Torulopsis*, *Trichoderma*, *Trichothecium*, *Uwebraunia*, *Virgariella*, *Volutella*, *Wojnowicia*, *Zygophiala* and *Zasmidium* (Shaw 1984, Arnold 1986, Aptroot 1995, Heredia–Abarca et al. 1997, Photita et al. 2001a, b, 2002, Cao et al. 2002, Hao et al. 2013, Gao et al. 2014, Quaedvlieg et al. 2014, Videira et al. 2017). However, few species among the latter genera have been identified based on DNA sequence data and the phylogenetic relationships of many taxa are not well investigated (Hao et al. 2013, Wikee et al. 2013, Gao et al. 2014, Liu et al. 2015, Giraldo et al. 2017).

Documentation of saprobic fungi on *Musa* spp. across the Asian region is also scanty (Matsushima 1975, Photita et al. 2001a, 2002, 2003b, Somrithipol 2007, Samarakoon et al. 2020a, b). Based on morphology, Photita et al. (2001a) reported 46 saprobes on *Musa acuminata* from Hong Kong and they belong to 40 genera in Ascomycota (*Anthostomella*, *Bipolaris*, *Chloridium*, *Colletotrichium*, *Corynesporopsis*, *Curvularia*, *Dactylaria*, *Dactylella*, *Deightoniella*, *Diaporthe*, *Dictyosporium*, *Didymosphaeria*, *Diplodia*, *Durispora*, *Glomerella*, *Hansfordia*, *Hyponectria*, *Leptosphaeria*, *Massarina*, *Memnoniella*, *Mycosphaerella*, *Nectria*, *Nigrospora*, *Ophioceras*, *Periconia*, *Periconiella*, *Phaeosphaeria*, *Phialocephala*, *Phoma*, *Phomatospora*, *Phomopsis*, *Pyriculariopsis*, *Stachybotrys*, *Stachylidium*, *Stemphylium*, *Tetraploa*, *Torula*, *Veronaea*, *Verticillium* and *Zygosporium*). In addition, *Cylindrocarpon* (Matsushima 1975), *Dictyoarthrinium* (Somrithipol 2007, Samarakoon et al. 2020b), *Dictyosporium*, *Pseudopithomyces* (Photita et al. 2002), *Spegazzinia* (Samarakoon et al. 2020a) and *Stachybotrys* (Photita et al. 2003a) were also documented as fungal saprobes on *Musa* spp.

Periconia (Periconiaceae) was introduced by Tode (1791) and typified by *P. lichenoides* Tode. Both sexual and hyphomycetous asexual morphs are recorded in *Periconia* (Tanaka et al. 2015, Liu et al. 2017). The sexual morph has immersed or erumpent ascomata with an ostiolar neck. The eight ascospores are biseriate, hyaline and broadly fusiform with a gelatinous sheath (Tanaka et al. 2015). The asexual morph of *Periconia* has pale to dark brown branched or unbranched conidiophores (referred as stipe) (Tanaka et al. 2015). The conidiogenous cells are usually monoblastic or polyblastic which form at the terminal ends or intercalary parts of the conidiophore. In addition, the conidia of *Periconia* are usually catenate or solitary, pale to dark brown, spherical and aseptate (Ellis 1971, 1976, Seifert & Gams 2011). *Periconia* has been reported as plant pathogens, endophytes and as common saprobes in terrestrial and aquatic environments worldwide (Ellis 1971, 1976, Markovskaja & Kačergius 2014, Liu et al. 2017). Currently 187 epithets are listed in *Periconia* (Index Fungorum 2021). In addition, there are about 2575 DNA sequence data available for *Periconia* in GenBank (NCBI; https://www.ncbi.nlm.nih.gov/nuccore/?term=Periconia, accessed on January 10, 2021), which only restricted to 36 species. This suggests more than 150 species still need correct taxonomic placements based on molecular data, especially *P. lichenoides*, the generic type of *Periconia*.

Torula (Torulaceae) was introduced by Persoon (1975) and is typified by *T. herbarum* (Pers.) Link. The neotype of *T. herbarum* was designated by Crous et al. (2015). The genus is only known by its asexual morph and is characterized by doliiform to ellipsoid or clavate, cupulate conidiogenous cells which the walls are thick and heavily melanized at the base while thin at the apex. The conidia are subcylindrical, phragmosporous, brown, smooth to verrucose, and appear as branched or unbranched chains (Ellis 1971, 1976, Crous et al. 2015, Crane & Miller 2016, Bhat 2017, Li et al. 2017, 2020). Previously, conidiogenesis was a key morphological feature used to delineate species in *Torula* (Mason 1941, Hughes 1953, Crane & Schoknecht 1977, Li et al. 2020). Crous et al. (2015), Su et al. (2016, 2018) and Li et al. (2017, 2020) investigated the phylogenetic relationships of *Torula* using DNA sequence data (ITS, LSU, SSU, TEF, and RPB2). Currently, 17 species epithets have molecular data in GenBank (Hongsanan et al. 2020, Hyde et al. 2020a, Li et

al. 2020), while more than 520 species are listed in Index Fungorum (2021). *Torula* is common on terrestrial and aquatic habitats mainly as a saprobe across tropical and temperate regions (Crous et al. 2015, Su et al. 2018, Li et al. 2020).

Most taxonomic studies have focused mostly on fungal pathogens on *Musa* spp. due to their diseases and agricultural losses (Giatgong 1980, Carlier et al. 2002, Zakaria et al. 2009, Wulandari et al. 2010, Churchill 2011, Su et al. 2011, Guarnaccia et al. 2017, Dita et al. 2018, Marin–Felix et al. 2019, Maryani et al. 2019). Therefore, the hidden saprobic and endophytic fungal diversity on *Musa* spp. are yet to be investigated as only 8% of fungi have possibly been described from the estimated fungal number of 2.2–3.8 million (Hyde et al. 2020b). In addition, a growing number of saprobic fungi recovered from *Musa* spp. need modern taxonomic treatments (Marin–Felix et al. 2019, Maryani et al. 2019, Samarakoon et al. 2020a, b).

We investigated the hidden saprobic fungi associated with different tissue types of *Musa* spp. (i.e. dead or fallen down leaves, leaf sheaths, pseudostems and decaying fruits) from different geographical localities across Asia. Fungi were identified based on morpho–molecular data and the taxonomic relationships were evaluated. In this study, we report *Periconia cortaderiae* (Thailand), *P. delonicis* (Thailand), *Torula chromolaenae* (Thailand), *T. fici* (Yunnan, China) and *T. masonii* (Taiwan, China) for the first time on *Musa* spp. with detailed morphological illustrations and DNA sequence data.

Materials and methods

Fungal collections, isolation and morphological characterization

Dead leaves of *Musa* spp. were collected from China and Thailand. Specimens were transferred to the laboratory in cardboard boxes. Samples were examined with a Motic SMZ 168 Series microscope. Powder like masses of conidia of hyphomycetous taxa on *Musa* samples were mounted in water for microscopic studies and photomicrography. The hyphomycetous taxa were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 550D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures processed with Adobe Photoshop CS6 Extended version 12.0 software (Adobe, USA).

Single spore isolation was carried out following the method described in Senanayake et al. (2020). Germinated spores were individually transferred to potato dextrose agar (PDA) plates and grown at 25°C in normal light. Colony characteristics were observed and measured after 3 weeks. The specimens are deposited at the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures are deposited at the Culture Collection of Mae Fah Luang University (MFLUCC). Faces of fungi numbers are registered as in Jayasiri et al. (2015).

DNA extraction and PCR amplification

Fungal isolates derived from single spore cultures were grown on potato dextrose agar (PDA) for 4 weeks at 25°C and the axenic mycelia (50–100 mg) of each isolate were scrapped off for DNA extraction purposes. Mycelia were ground to a fine powder with liquid nitrogen and fungal DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit–BSC14S1 (BioFlux®, P.R. China) according to the instructions of the manufacturer. Five gene regions were used for the polymerase chain reaction (PCR) amplification, including partial 18S small subunit rDNA (SSU), partial 28S large subunit rDNA (LSU), internal transcribed spacer (ITS), RNA polymerase II second largest subunit (RPB2) and partial translation elongation factor 1–alpha gene (TEF) using the primers NS1/NS4 (White et al. 1990), LR0R/LR5 (Vilgalys & Hester 1990), ITS5/ITS4 (White et al. 1990), fRPB2–5f/fRPB2–7cR (Liu et al. 1999) and EF1–983F/EF1–2218R (Rehner 2001), respectively.

The final volume of the PCR reaction was 25 µl, consisting of 2 µl of DNA template, 1 µl of each forward and reverse primer, 12.5 µl of 2×Easy Taq PCR SuperMix (mixture of EasyTaqTM DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Ltd., Beijing, P.R.

China) and 8.5 µl of the sterilized double–distilled water (ddH_2O). The thermal cycle programs were set up following the procedures described by Samarakoon et al. (2019, 2020b) for the respective genes. The amplified PCR fragments were sent to a commercial sequencing provider (TsingKe Biological Technology (Beijing) Co., Ltd, China) for PCR purification and sequencing. The Sanger DNA sequences obtained from this study were deposited in GenBank (Tables 1, 2).

Sequence alignment

Obtained sequences were subjected to BLASTn search tool in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for finding the closely related taxa. BLASTn search results and initial morphological studies have supported that our isolates belong to Periconiaceae and Torulaceae. Other sequences used in the analyses were obtained from GenBank (Tables 1, 2) based on recently published data (Jayasiri et al. 2019, Hongsanan et al. 2020, Hyde et al. 2020a, Li et al. 2020). The single gene alignments were automatically analysed by MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/large.html; Katoh et al. 2019) using the default settings and refined where necessary, using BioEdit v. 7.0.5.2 (Hall 1999). The single gene matrixes were prior analyzed by maximum likelihood (ML) criterion for checking the congruence of the tree topologies and if the tree topologies were congruent, the concatenated sequence dataset were performed for further analyses.

Table 1 Taxa used in the phylogenetic analyses of Periconiaceae with the corresponding GenBank accession numbers. Type strains are superscripted with "T" and newly generated strains are indicated in black bold.

Taxa	Culture collection/ Voucher no.	GenBank accession numbers			
		LSU	SSU	ITS	TEF
Flavomyces fulophazii	CBS 135761^T	KP184040	KP184082	KP184001	NA
F. fulophazii	MF09	MN515261	NA	MN537663	MN535259
Helminthosporium dalbergiae	H 4628	AB807521	AB797231	LC014555	AB808497
Massarina cisti	CBS 266.62^T	AB807539	AB797249	NA	AB808514
Noosia banksiae	CPC:17282	JF951167	NA	JF951147	NA
N. banksiae	CBS 129526	MH878062	NA	NA	NA
Periconia aquatica	MFLUCC 16–0912^T	KY794705	NA	KY794701	KY814760
P. byssoides	MFLUCC 17–2292	MK347968	MK347858	MK347751	MK360069
P. byssoides	MFLUCC 18–1548	MK348013	MK347902	MK347794	MK360070
P. caespitosa	LAMIC_110_16	MH051907	NA	MH051906	NA
P. cortaderiae	MFLUCC 15–0457^T	NG_068238	NG_068373	NR_165853	KY310703
P. cortaderiae	MFLUCC 15–0451	KX954403	KX986346	KX965734	KY429208
P. cortaderiae	**MFLUCC 20–0236**	**MW406971**	**MW406969**	**MW406973**	**MW422156**
P. cyperacearum	CPC:32138^T	NG_064549	NA	NR_160357	MH327882
P. delonicis	MFLUCC 17–2584^T	MK347941	MK347832	NA	MK360071
P. delonicis	**MFLUCC 20–0235**	**MW406970**	**MW406968**	**MW406972**	**MW422155**
P. digitata	CBS 510.77	AB807561	AB797271	NA	AB808537
P. epilithographicola	CBS 144017^T	NA	NA	NR_157477	NA
P. epilithographicola	PL5–1B	NA	NA	MF422162	NA
P. homothallica	HHUF 29105	NG_059397	NG_064851	NR_153446	AB808541
P. homothallica	KT 916	AB807565	AB797275	AB809645	NA
P. igniaria	CBS 379.86	AB807566	AB797276	LC014585	AB808542
P. igniaria	CBS 845.96	AB807567	AB797277	LC014586	AB808543
P. macrospinosa	CBS 135663	KP184038	KP184080	KP183999	NA
P. neobrittanica	CPC 37903^T	NG_068342	NA	NR_166344	NA
P. palmicola	MFLUCC 14–0400^T	NG_068917	MN648319	NA	MN821070
P. pseudobyssoides	DLUCC 0850	MG333494	NA	MG333491	MG438280
P. pseudobyssoides	H 4151	AB807568	AB797278	LC014587	AB808544
P. pseudobyssoides	H 4790	AB807560	AB797270	LC014588	AB808536
P. pseudodigitata	KT 644	AB807562	AB797272	LC014589	AB808538
P. pseudodigitata	KT 1195A	AB807563	AB797273	LC014590	AB808539

Table 1 Continued.

Taxa	Culture collection/ Voucher no.	GenBank accession numbers			
		LSU	SSU	ITS	TEF
P. pseudodigitata	KT 1395	AB807564	AB797274	LC014591	AB808540
P. salina	MFLU 19–1235^{T}	MN017846	MN017912	MN047086	NA
P. submersa	MFLUCC 16–1098^{T}	KY794706	NA	KY794702	KY814761
P. thailandica	MFLUCC 17–0065^{T}	KY753888	KY753889	KY753887	NA

Table 2 Taxa used in the phylogenetic analyses of Torulaceae with the corresponding GenBank accession numbers. Type strains are superscripted with "T" and newly generated strains are indicated in black bold.

Taxa	Culture collection/ Voucher no.	ITS	LSU	SSU	RPB2	TEF
Dendryphion aquaticum	MFLUCC 15–0257^{T}	KU500566	KU500573	KU500580	NA	NA
D. comosum	CBS 208.69^{T}	MH859293	MH871026	NA	NA	NA
D. europaeum	CPC 23231	KJ869145	KJ869202	NA	NA	NA
D. hydei	KUMCC 18–0009^{T}	MN061343	MH253927	MH253929	NA	NA
Neotorula aquatica	MFLUCC 15–0342^{T}	KU500569	KU500576	KU500583	NA	NA
N. submersa	HKAS 92660	NR_154247	KX789217	NA	NA	NA
Rostriconidium aquaticum	KUMCC 15–0297	MG208165	MG208144	NA	MG207975	MG207995
R. aquaticum	MFLUCC 161113^{T}	MG208164	MG208143	NA	MG207974	MG207994
R. pandanicola	KUMCC 17–0176^{T}	MH275084	MH260318	MH260358	MH412759	MH412781
Roussoella nitidula	MFLUCC 11–0182^{T}	KJ474835	KJ474843	NA	KJ474859	KJ474852
R. scabrispora	MFLUCC 11–0624^{T}	KJ474836	KJ474844	NA	KJ474860	KJ474853
Roussoellopsis tosaensis	KT 1659	NA	AB524625	AB524484	AB539104	AB539117
Rutola graminis	CPC 33267	MN313814	MN317295	NA	NA	NA
R. graminis	CPC 33695	MN313815	MN317296	NA	NA	NA
R. graminis	CPC 33715^{T}	MN313816	MN317297	NA	NA	NA
Sporidesmioides thailandica	MFLUCC 13–0840	MN061347	NG_059703	NG_061242	KX437761	KX437766
S. thailandica	KUMCC 16–0012^{T}	MN061348	KX437758	KX437760	KX437762	KX437767
Thyridaria broussonetiae	TB1	KX650569	NA	KX650515	KX650586	KX650539
Thyridariella mahakoshae	NFCCI 4215	MG020435	MG020438	MG020441	MG020446	MG023140
Th. mangrovei	PUFD 17–98^{T}	MG020434	MG020437	MG020440	MG020445	MG020443
Torula acaciae	CPC 29737^{T}	NR_155944	NG_059764	NA	KY173594	NA
T. aquatica	DLUCC 0550	MG208166	MG208145	NA	MG207976	MG207996
T. aquatica	MFLUCC 16–1115^{T}	MG208167	MG208146	NA	MG207977	NA
T. breviconidiophora	KUMCC 18–0130^{T}	MK071670	MK071672	MK071697	NA	MK077673
T. camporesii	KUMCC 19–0112^{T}	MN507400	MN507402	MN507401	MN507404	MN507403
T. chiangmaiensis	KUMCC 16–0039^{T}	MN061342	KY197856	KY197863	NA	KY197876
T. chromolaenae	**MFLUCC 20–0237**	**MW412524**	**MW412518**	**MW412515**	**MW422161**	**MW422158**
T. fici	CBS 595.96^{T}	KF443408	KF443385	KF443387	KF443395	KF443402
T. fici	KUMCC 15–0428	MG208172	MG208151	NA	MG207981	MG207999
T. fici	KUMCC 16–0038	MN061341	KY197859	KY197866	KY197872	KY197879
T. fici	**MFLUCC 20–0238**	**MW412525**	**MW412519**	**MW412516**	**NA**	**MW422159**

Table 2 Continued.

Taxa	Culture collection/ Voucher no.	ITS	LSU	SSU	RPB2	TEF
T. gaodangensis	MFLUCC 17–0234^{T}	MF034135	NG_059827	NG_063641	NA	NA
T. goaensis	MTCC 12620^{T}	NR_159045	NG_060016	NA	NA	NA
T. herbarum	CPC 24414	KR873260	KR873288	NA	NA	NA
T. hollandica	CBS 220.69	NR_132893	NG_064274	KF443389	KF443393	KF443401
T. hydei	KUMCC 16–0037^{T}	MN061346	MH253926	MH253928	NA	MH253930
T. mackenziei	MFLUCC 13–0839^{T}	MN061344	KY197861	KY197868	KY197874	KY197881
T. masonii	CBS 245.57^{T}	NR_145193	NG_058185	NA	NA	NA
T. masonii	DLUCC 0588	MG208173	MG208152	NA	MG207982	MG208000
T. masonii	**MFLUCC 20–0239**	**MW412523**	**MW412517**	**MW412514**	**MW422160**	**MW422157**
T. pluriseptata	MFLUCC 14–0437^{T}	MN061338	KY197855	KY197862	KY197869	KY197875
T. polyseptata	KUMCC 18–0131^{T}	MK071671	MK071673	MK071698	NA	MK077674

Abbreviations of culture collections: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands. CPC: Working collection of Pedro Crous housed at CBS. DLUCC: Dali University Culture Collection, China. H: University of Helsinki, Helsinki, Finland. HKAS: Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica, China. KT: K. Tanaka. LAMIC: Laboratorio Asociaciones suelo, planta microorganismos, Pontificia Universidad Javeriana, Bogotá, D.C., Colombia. MTCC: Microbial Type Culture Collection, CSIR–Institute of Microbial Technology, Sector 39–A, Chandigarh – 160036, India. KUMCC: Kunming Institute of Botany Culture Collection, China. MFLU: Mae Fah Luang University, Chiang Rai, Thailand. MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. NFCCI: National Fungal Culture Collection of India. NA: DNA sequence data are not available in GenBank.

Phylogenetic analyses

Phylogenetic analyses were preformed based on maximum likelihood (ML) and Bayesian inference (BI) criteria. The phylogenetic trees showing relationships of taxa in Periconiaceae and Torulaceae were generated separately. Data matrixes used in these analyses were followed as; Periconiaceae (Analysis 1): the combined SSU, LSU, ITS and TEF data matrix comprised 35 sequences of representative taxa in Periconiaceae. *Helminthosporium dalbergiae* (MAFF 243853) and *Massarina cisti* (CBS 266.62) were selected as outgroup taxa. Torulaceae (Analysis 2): the combined SSU, LSU, ITS, TEF and RPB2 matrix comprised 40 sequences of selected genera in Torulaceae. Taxa in Roussoellaceae were selected as the outgroup taxa viz. *Roussoella nitidula* (MFLUCC 11–0182), *R. scabrispora* (MFLUCC 11–0624) and *Roussoellopsis tosaensis* (KT 1659).

Maximum likelihood (ML) trees were generated using the RAxML–HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution and 1,000 replicates of rapid bootstrap. Bayesian inference (BI) analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: four simultaneous Markov chains were run for 2,000,000 generations (Analysis 1, 2) and trees were sampled every 100th generation and 20,000 trees were obtained. The first 4,000 trees, representing the burn–in phase of the analyses were discarded. The remaining 16,000 trees were used for calculating PP in the 50% majority rule consensus tree.

Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2011) and reorganized in Microsoft PowerPoint (2007, USA) and converted to jpeg file in Adobe Photoshop CS6 Extended version 12.0 software (Adobe, USA). The final trees and data matrixes were submitted in TreeBASE (https://www.treebase.org/), submission ID: 27468 for Periconiaceae and 27469 for Torulaceae.

Results

Phylogenetic analyses

Analysis 1: Periconiaceae

The best scoring RAxML tree resulted from the combined gene analysis of SSU, LSU, ITS and TEF sequence data is shown in Fig. 1 with a final ML optimization likelihood value of –9645.86. The matrix had 561 distinct alignment patterns, with 26.15% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.233866, C = 0.256179, G = 0.274088, T = 0.235866; substitution rates AC = 2.126081, AG = 2.703293, AT = 1.718216, CG = 1.412442, CT = 10.292987, GT = 1.000000; proportion of invariable sites I =0.624461; gamma distribution shape parameter α = 0.615919. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with a final average standard deviation of split frequencies was 0.008. All trees (ML & BI) were similar in topology and did not differ significantly with respects to generic placements and this is in agreement with previous studies based on multi–gene phylogeny (Jayasiri et al. 2019, Hongsanan et al. 2020, Hyde et al. 2020a). Our MFLUCC 20–0235 strain is sister to *Periconia delonicis* (MFLUCC 17−2584) and grouped with *P. palmicola* (MFLUCC 14−0400) and *P. verrucosa* (MFLUCC 17−2158) with a strong statistical support (ML = 98%, BYPP =1.00; Fig. 1). The MFLUCC 20–0236 strain grouped with *P. cortaderiae* (MFLUCC 15−0457, MFLUCC 15−0451) with high support (ML = 100%, BYPP = 1.00), and has close phylogenetic affinity to *Noosia banksiae* (CPC: 17282, CBS 129526) and *Periconia homothallica* (KT 916, HHUF 29105). Therefore, we identify MFLUCC 20–0235 as *P. delonicis* and MFLUCC 20–0236 as *P. cortaderiae*, respectively.

Analysis 2: Torulaceae

The best scoring RAxML tree (Fig. 2) has a final ML optimization likelihood value of –19754.05. The matrix had 1189 distinct alignment patterns, with 31.37% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.241559, C = 0.263242, G = 0.273563, T = 0.221637; substitution rates AC = 1.375308, AG = 2.974871, AT = 1.345285, CG = 0.922655, CT = 6.841524, GT = 1.000000; proportion of invariable sites I = 0.486989; gamma distribution shape parameter α = 0.482115. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with a final average standard deviation of split frequencies was 0.005. All the phylogenetic trees (ML & BI) were similar in topology and no considerable difference at the generic level and similar with the phylogenetic analyses of Li et al. (2017, 2020), Su et al. (2018) and Hyde et al. (2020a). Our isolate (MFLUCC 20–0237) formed a clade with *Torula chromolaenae* (KUMCC 16–0036) with high support (ML = 100%, BYPP = 1.00), and clustered with *T. breviconidiophora* (KUMCC 18–0130) and *T. mackenziei* (MFLUCC 13−0839). The MFLUCC 20–0238 strain clustered with *T. fici* (CBS 595.96, KUMCC 15–0428, KUMCC 16–0038) with moderate support in ML analysis (ML = 80%) and not significant support in BI analysis and has a close relationship with *T. hydei* (KUMCC 16–0037; ML 98%, BYPP = 1.00). The MFLUCC 20–0239 strain clustered with *T. masonii* (CBS 245.57, DLUCC 0588, KUMCC 16–0033) with high support (ML = 100%, BYPP = 1.00), and also close to *T. acaciae* (CPC 29737). We therefore identify MFLUCC 20–0237 as *T. chromolaenae*, MFLUCC 20–0238 as *T. fici* and MFLUCC 20–0239 as *T. masonii*, respectively.

Taxonomy

Periconia delonicis Jayasiri, E.B.G. Jones & K.D. Hyde, in Jayasiri et al., Mycosphere 10(1): 95 (2019) Fig. 3

Index Fungorum number: IF555562; Facesoffungi number: FoF 05268

Saprobic on a dead leaf of *Musa* sp. Sexual morph: Undetermined. Asexual morph: hyphomycetous. *Conidiophores* 320−400 × 8−12 μm ($\bar{x}$ = 365 × 9.5 μm, n = 10) macronematous,

rarely micronematous. Macronematous conidiophores and conidia resembled a stipe and a globular head. *Stipe of the Conidiophores* unbranched, straight or flexuous from the middle, septate, pale to dark brown, often appearing black, smooth, globular heads shining by reflected light. *Conidiogenous cells* 4−7.5 × 3.4−7.4 µm ($\bar{x}$ = 5.5 × 5.3 µm, n = 15) monoblastic or polyblastic, discrete on the stipe, determinate, ellipsoidal, pale brown to brown. *Conidia* 4−8 × 4−6 µm ($\bar{x}$ = 5.6 × 5.4 µm, n =15), catenate, in chains, arising at one or more points on the curved surface of the conidiogenous cell, simple, usually spherical or subspherical, pale to dark brown, smooth to minutely veruculose, aseptate.

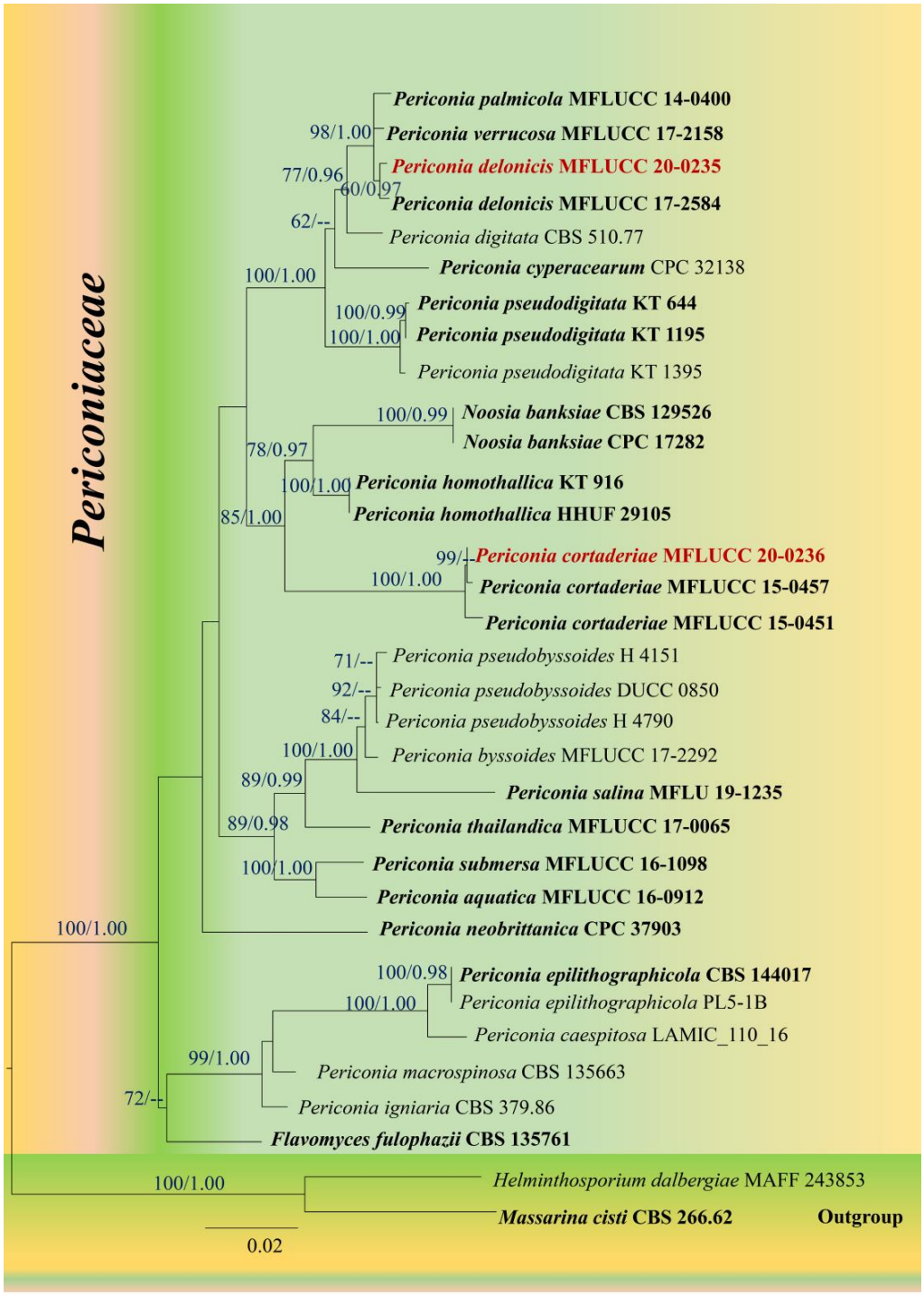

Fig. 1 − Maximum likelihood tree revealed by RAxML from an analysis of a concatenated SSU, LSU, ITS and TEF sequence dataset of the species in Periconiaceae, showing the phylogenetic position of *Periconia delonicis* (MFLUCC 20–0235) and *P. cortaderiae* (MFLUCC 20–0236). ML

bootstrap supports (≥ 60%) and Bayesian posterior probabilities (≥ 0.95 BYPP) are given above the branches as ML/BYPP. The tree is rooted with *Helminthosporium dalbergiae* (MAFF 243853) and *Massarina cisti* (CBS 266.62). Strains generated in this study are indicated in red bold. Ex–type strains are indicated in black bold. The scale bar 0.02 represents the expected number of nucleotide substitutions per site.

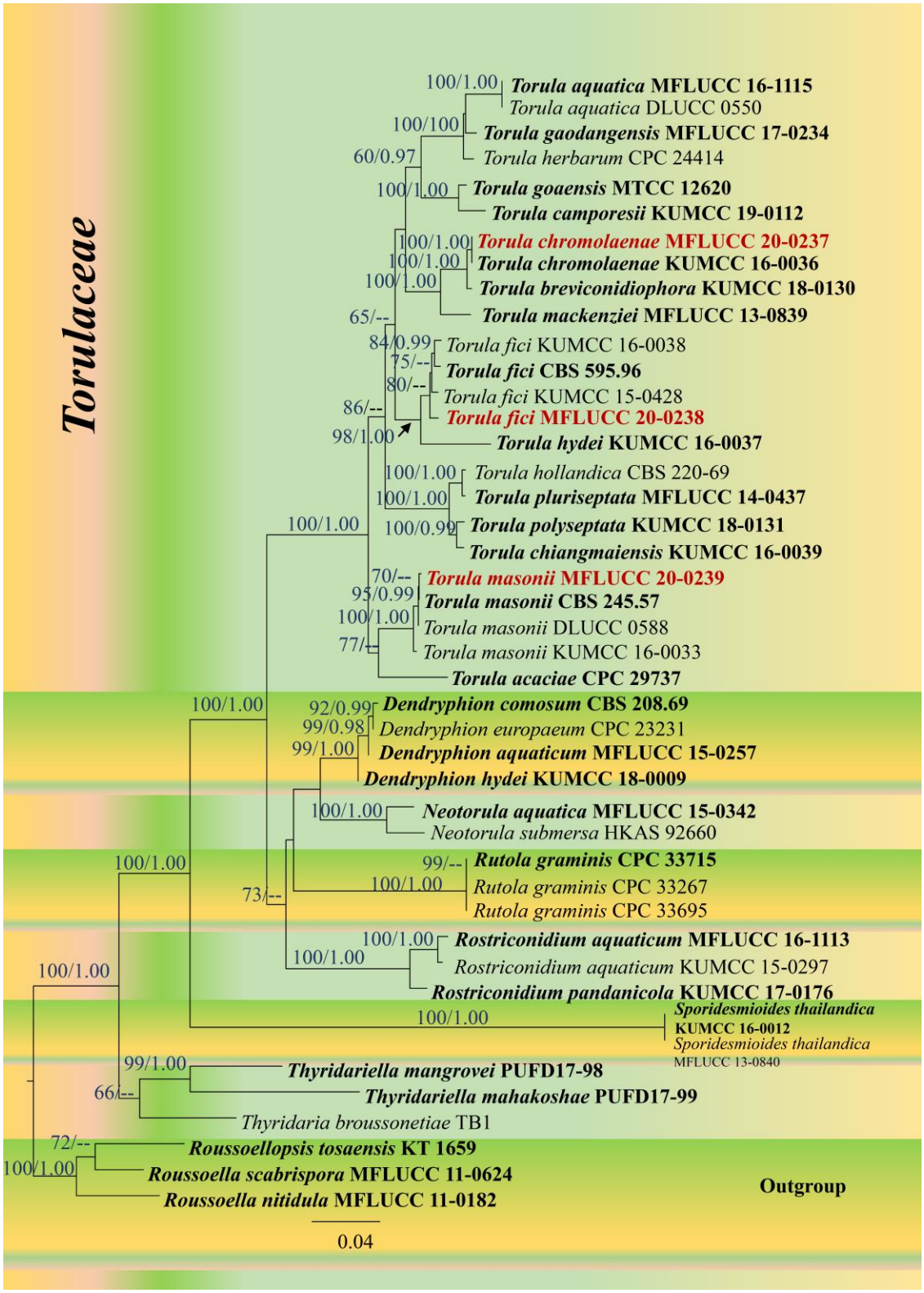

Fig. 2 − Maximum likelihood tree (RAxML) revealed by an analysis of a concatenated SSU, LSU, ITS, TEF and RPB2 sequence dataset of the species in Torulaceae, showing the phylogenetic

position of *Torula chromolaenae* (MFLUCC 20–0237), *T. fici* (MFLUCC 20–0238) and *T. masonii* (MFLUCC 20–0239). ML bootstrap supports (≥ 60%) and Bayesian posterior probabilities (≥ 0.95 BYPP) are given above the branches as ML/BYPP. The tree is rooted with *Roussoella nitidula* (MFLUCC 11–0182), *R. scabrispora* (MFLUCC 11–0624) and *Roussoellopsis tosaensis* (KT 1659). Strains generated in this study are indicated in red bold. Ex–type strains are indicated in black bold. The scale bar 0.04 represents the expected number of nucleotide substitutions per site.

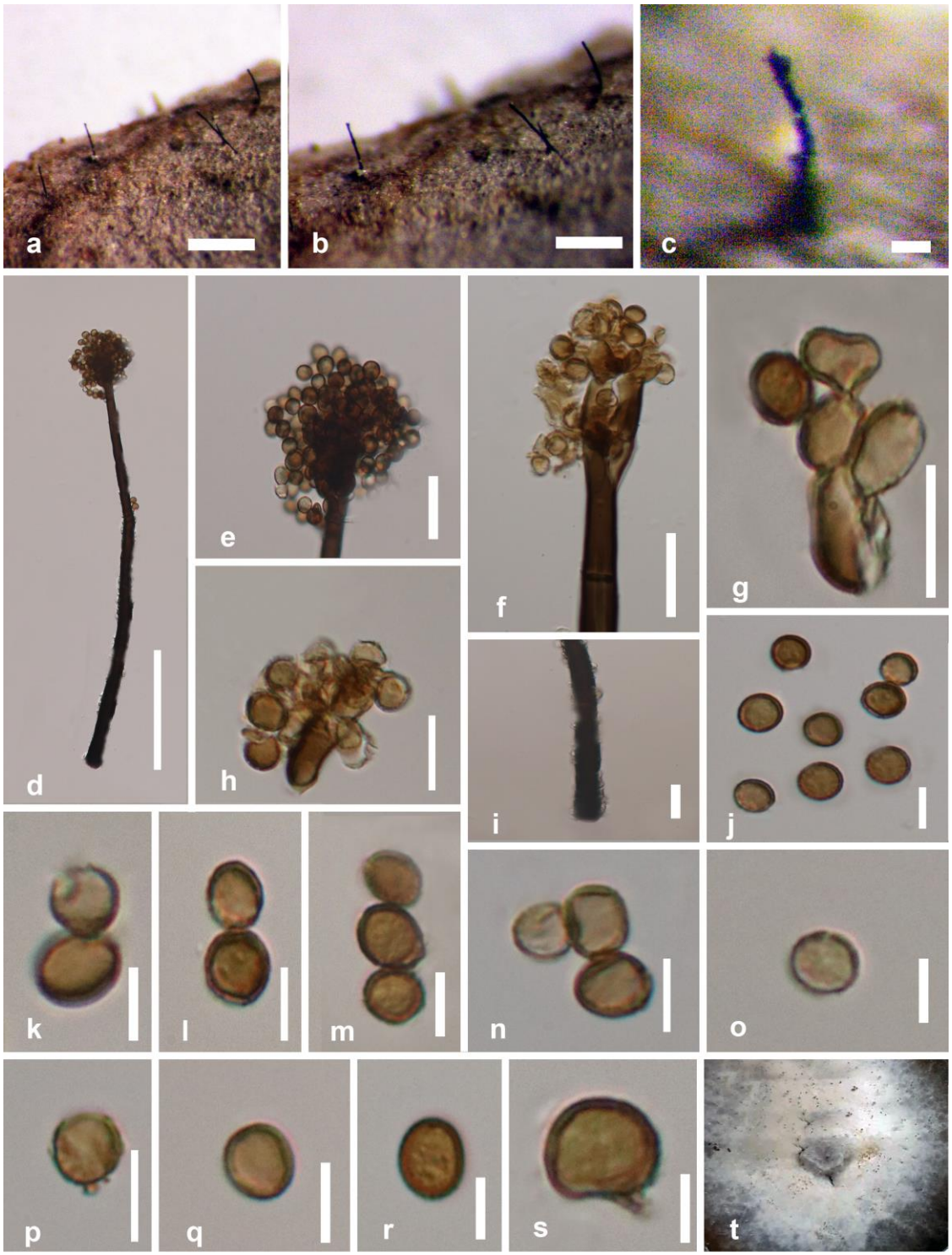

Fig. 3 − *Periconia delonicis* (MFLU 20−0696). a−c Conidiophores bearing conidia on host. d−f Conidia and conidiophores. g, h Conidiogenous cells bearing conidia. i Base of the conidiophore. j−s Mature and immature conidia. t Colonies on PDA after 14 days. Scale bars: a, b = 500 µm, c, d = 100 µm, e, f, i = 20 µm, g, h = 10 µm, j−s = 5 µm.

Culture characteristics – Conidia germinated on PDA within 48 hrs, reaching 40 mm diam. in 2 weeks at 25°C. Colonies on PDA with sparse, pinkish white mycelia on the surface, circular and flattened. Surface is smooth, with small, brown granular–like, powdery masses at maturity. The reverse of the colony is dark brown and yellow in the center with a white margin. Conidia and conidiophores are not observed in mature colonies.

Material examined – THAILAND, Chiang Mai Province, Mae Taeng District, on a dead leaf of *Musa* sp. (Musaceae), 22 September 2018, B.C. Samarakoon, BNS012 (MFLU 20–0696), living culture MFLUCC 20–0235.

Known hosts & distribution – on pods of *Delonix regia* (Fabaceae, Dicotyledon) from Thailand (Jayasiri et al. 2019); on dead leaf of *Musa* sp. (Musaceae, Monocotyledon) from Thailand (this study).

Notes – Based on BLASTn search results of SSU, LSU, ITS, TEF sequence data, our strain (MFLUCC 20−0235) showed high identity to the taxa in GenBank as follows; SSU = 99.75% similarity to *Periconia palmicola* (MFLUCC 14−0400), LSU = 99.88% similarity to *P. delonicis* (MFLUCC 17−2584), ITS = 99.20% similarity to *P. palmicola* (MFLUCC 14−0400). Multi–loci phylogenetic analysis (Fig. 1) showed that the new strain MFLUCC 20–0235 forms a clade together with *P. palmicola* (MFLUCC 14−0400) and *P. verrucosa* (MFLUCC 17−2158) with high statistical support (ML = 98%, BYPP = 1.00).

Our strain (MFLUCC 20–0235) shares similar morphology to the type of *Periconia delonicis* in having macronematous, unbranched conidiophores, with monoblastic, terminal conidiogenous cells and brown, globose to subglobose, aseptate, veruculose conidia (Jayasiri et al. 2019). Our strain (MFLUCC 20−0235) also shares similar size range of conidiophores (320−400 × 8−12 μm vs. 360−420 × 8−12 μm) and conidia (4−8 × 4−6 μm vs. 5.5−7 μm diam.). Based on recommendations by Jeewon & Hyde (2016), we also compare the nucleotide bases of TEF region for our new strain and the type strain of *P. delonicis*. MFLUCC 20−0235 differs from *P. delonicis* (MFLUCC 17−2584) in 5/746 bp (0.67%).

In our phylogenetic analyses, MFLUCC 20−0235 and ex–type stain of *Periconia delonicis* (MFLUCC 17−2584) showed a close phylogenetic affinity to *P. palmicola* (MFLUCC 14−0400) and *P. verrucosa* (MFLUCC 17−2158). The conidiophores, conidiogenous cells and the conidia of MFLUCC 20−0235 shares similar morphology with the holotype illustration of *P. palmicola* in Hyde et al. (2020a) (i.e. dark brown to black conidiophores, hyaline conidiogenous cells, subglobose to globose conidia). However, *P. palmicola* differs from MFLUCC 20−0235 in having comparatively short conidiophores (151–188 × 5.6–8 μm vs. 320−400 × 8−12 μm) which were branched at apex. The conidiophores of MFLUCC 20−0235 are unbranched and comparatively long with respect to *P. palmicola*. A nucleotide base pair comparison of TEF region showed that MFLUCC 20−0235 differs from *P. palmicola* (MFLUCC 14−0400) in 4/746 (0.53%). MFLUCC 20−0235 also has similar morphology to *P. verrucosa* (MFLUCC 17−2158) but differs in having longer conidiophores (320−400 × 8−12 μm vs. 170–296 × 10–12 μm) and smaller conidia (4−8 × 4−6 μm. vs. 7–15 μm diam.) (Phukhamsakda et al. 2020). A nucleotide base comparison of ITS and TEF regions showed that MFLUCC 20−0235 differs from *P. verrucosa* (MFLUCC 17−2158) in 7/501 bp (1.39%) of ITS and 4/746 bp (0.53%) of TEF. Based on morphological comparison with the types of *P. delonicis*, *P. palmicola* and *P. verrucosa*, a nucleotide base comparison of ITS and TEF regions and phylogenetic evidence, we thus identify our new collection as *P. delonicis*. In this study, we report *P. delonicis* on *Musa* sp. (Musaceae, monocotyledon) for the first time.

Periconia cortaderiae Thambugala & K.D. Hyde, in Thambugala et al., Mycosphere 8(4): 734 (2017) Fig. 4

Index Fungorum number: IF553165; Facesoffungi number: FoF 03226

Saprobic on a dead leaf of *Musa* sp. Sexual morph: Undetermined. Asexual morph: hyphomycetous. *Colonies* on host effuse, powdery, gregarious, with a black, conidial masses on the host. *Mycelium* composed of cottony, branched, hyphae forming dark clusters with conidia scattered on the host surface. *Conidiophores* 40–120 × 1−6 μm ($\bar{x}$ = 58.5 × 3.5 μm, n = 15),

macronematous, mononematous, single or rarely 2–3 together, erect, thick–walled, brown to dark brown, septate, rough, 4–5 µm wide at base, and 1–2 µm wide at apex; base shoe–shaped, distinct. *Conidiogenous cells* 2.5–4.5 µm × 2.5–3.5 µm ($\bar{x}$ = 3.5 × 2.8 µm, n = 10), annellidic, monoblastic, discrete on stipe, percurrent proliferations present as scars at the apex of conidiophore. *Conidia* 4–7.6 × 4–6.5 µm ($\bar{x}$ = 6.5 × 5.9 µm, n = 40), catenate, globose, one–celled, hyaline to pale brown when immature, becoming brown to dark brown, smooth or minutely verruculose.

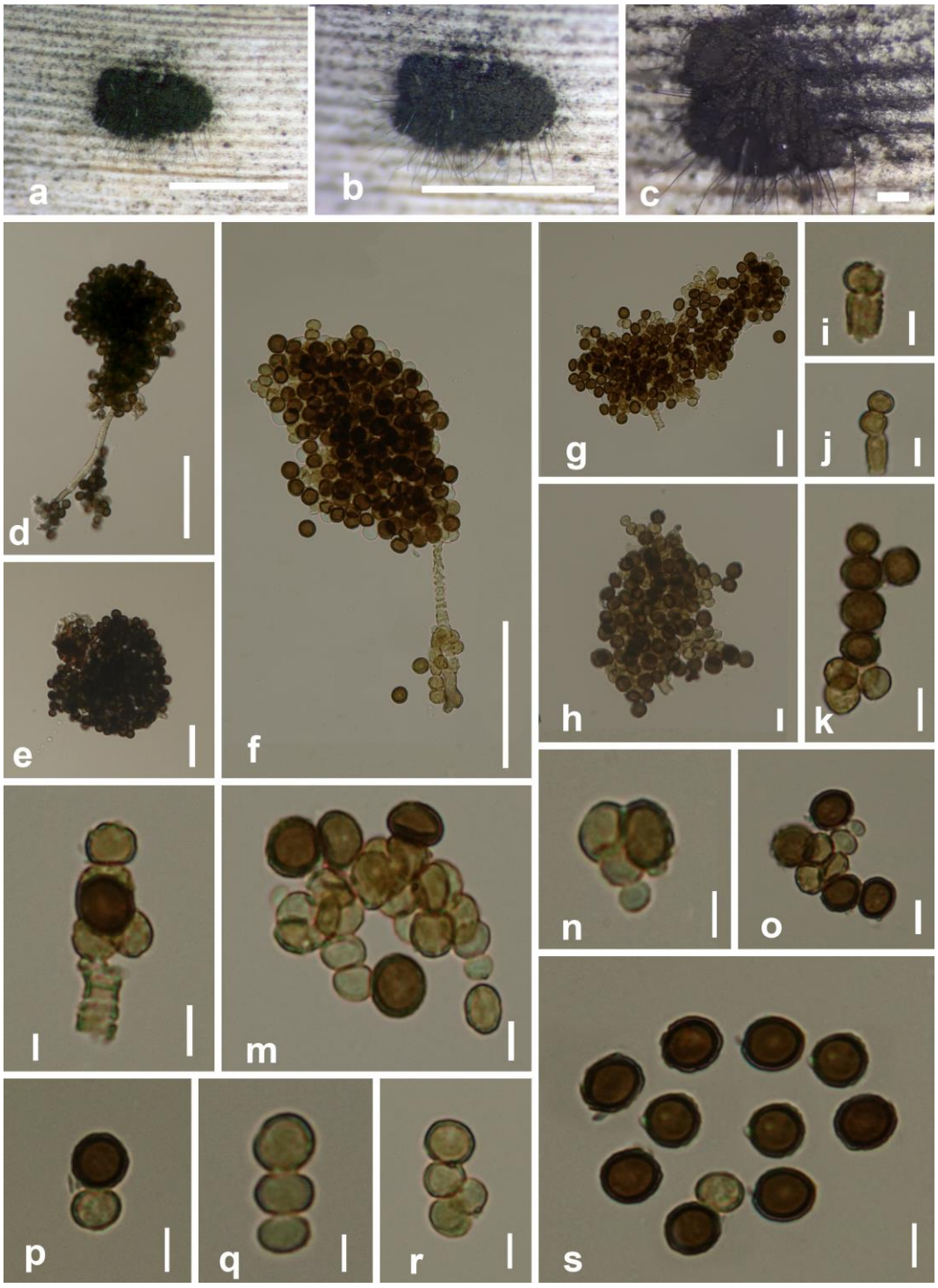

Fig. 4 − *Periconia cortaderiae* (MFLU 20−0697). a−c Colonies on host substrate. d, f Conidiophores bearing conidia. e, g, h Conidial masses. i, j, m−o Conidiogenesis from terminal mother cells. l Monoblastic, annellidic conidiogenous cells bearing conidial chains. k, p, q, r Conidial chains. s Conidia. Scale bars: a = 3 mm, b = 3.5 mm, c = 50 µm, d, f = 50 µm, e, g = 20 µm, h−s = 5 µm.

Culture characteristics – Conidia germinating on PDA within 48 hrs. Colonies growing on MEA, reaching a diameter of 20 mm after 14 days at 25°C, flat when immature, raised at maturity with unevenly distributed radial furrows or linear marks, surface is smooth at immature stage and notably rough at maturity with crenulate to crenate margin. Colony is initially white, moderately dense and completely black at maturity with the sporulation, reverse white to black.

Material examined – THAILAND, Nan Province, Nai Wiang, on a dead leaf of *Musa* sp. (Musaceae), 1 March 2019, B.C. Samarakoon, BNS100 (MFLU 20−0697), living culture MFLUCC 20−0236.

Known hosts & distribution – on *Cortaderia* sp. (Poaceae, Monocotyledon) from Thailand (Thambugala et al. 2017); on *Caragana arborescens* (Fabaceae, Dicotyledon) from Yunnan, China (Phookamsak et al. 2019); on *Musa* sp. (Musaceae, Monocotyledon) from Thailand (this study).

Notes – Based on BLASTn search results of SSU, LSU, ITS sequence data, our strain (MFLUCC 20−0236) showed high identity to the taxa in GenBank as follows; SSU = 99.76%, LSU = 99.88%, ITS = 99.80% similarities to *Periconia cortaderiae* (MFLUCC 15−0457). The morphology of our strain is similar to the holotype of *P. cortaderiae* (Thambugala et al. 2017), except the length of the conidiophores. Our strain has short conidiophores with respect to the holotype (40–120 × 1−6 μm vs. 400–800 × 4–9.4 μm). A nucleotide base comparison of ITS and TEF regions showed that MFLUCC 20−0236 differs from *P. cortaderiae* (MFLUCC 15−0457) in 1/493 bp (0.2%) of ITS and 1/829 bp (0.12%) of TEF. In addition, our MFLUCC 20−0236 strain differs from *P. cortaderiae* (MFLUCC 15−0451) in 2/493 bp (0.4%) of ITS and 2/872 bp (0.2%) of TEF. Therefore, we identify our new collection as *P. cortaderiae*, and this is reported from *Musa* sp. (monocotyledon) from Thailand for the first time.

Torula chromolaenae Jun F. Li, Phook., Mapook & K.D. Hyde, in Li et al., Mycol. Progr. 16(4): 454 (2017) Fig. 5

Index Fungorum number: IF819536; Facesoffungi number: FoF 02713

Saprobic on a dead leaf vein of *Musa* sp. (Musaceae). Sexual morph: Undetermined. Asexual morph: hyphomycetous. *Colonies* effuse, black, powdery, thread–like on host. *Mycelium* slightly immersed, septate, unbranched, smooth, pale brown hyphae. *Conidiophores* 2–5 × 2–4 μm ($\bar{x}$ = 3.8 × 3.3 μm, n = 10), micronematous or semi–macronematous, unbranched, straight or flexuous, subhyaline or pale brown, smooth or minutely verruculose. *Conidiogenous cells* 2–6 × 2–4.5 μm ($\bar{x}$ = 4.5 × 3.7 μm, n =10), polyblastic or sometimes monoblastic, integrated, terminal, discrete, determinate, usually spherical, smooth, distal fertile part thin–walled, subhyaline to pale brown, proximal sterile part dark brown, thick–walled, produced conidia in multiple planes. *Conidia* 10–15 × 5–7 μm ($\bar{x}$ = 11.4 × 6.2 μm, n = 40) dry, in simple or branched chains arising from the surface of the upper half of the characteristic conidiogenous cells, cylindrical with rounded ends, ellipsoidal or subspherical, brown or dark brown, minutely verruculose, 1–3 transverse septa, usually strongly constricted at the septa; conidial chains arranged in multiple planes.

Culture characteristics – Conidia germinating on PDA within 18 hrs and germ tubes produced from the tip cell. Colonies growing on PDA, reaching 50 mm diam. in 14 days at 25°C. Mycelium partly immersed to superficial, slightly effuse, and hairy, with dentate margin, pale pink at periphery golden brown in the middle with whitish hairy mycelial clumps. Sporulation was not observed in mature cultures.

Material examined – THAILAND, Chiang Mai Province, Mae Taeng District, on a dead leaf vein of *Musa* sp. (Musaceae), 15 February 2019, B.C. Samarakoon, BNS083 (MFLU 20−0698), living culture MFLUCC 20−0237.

Known hosts & distribution – on *Chromolaena odorata* (Asteraceae, Dicotyledon) from Thailand (Li et al. 2017, Mapook et al. 2020); on *Clematis fulvicoma*, (Ranunculaceae, Dicotyledon) from Thailand (Phukhamsakda et al. 2020); on *Pandanus tectorius* (Pandanaceae, Monocotyledon) from Yunnan, China (Tibpromma et al. 2018); on herbaceous litter (Dicotyledon) from Yunnan, China (Hyde et al. 2020a); *Musa* sp. (Monocotyledon) from Thailand (this study).

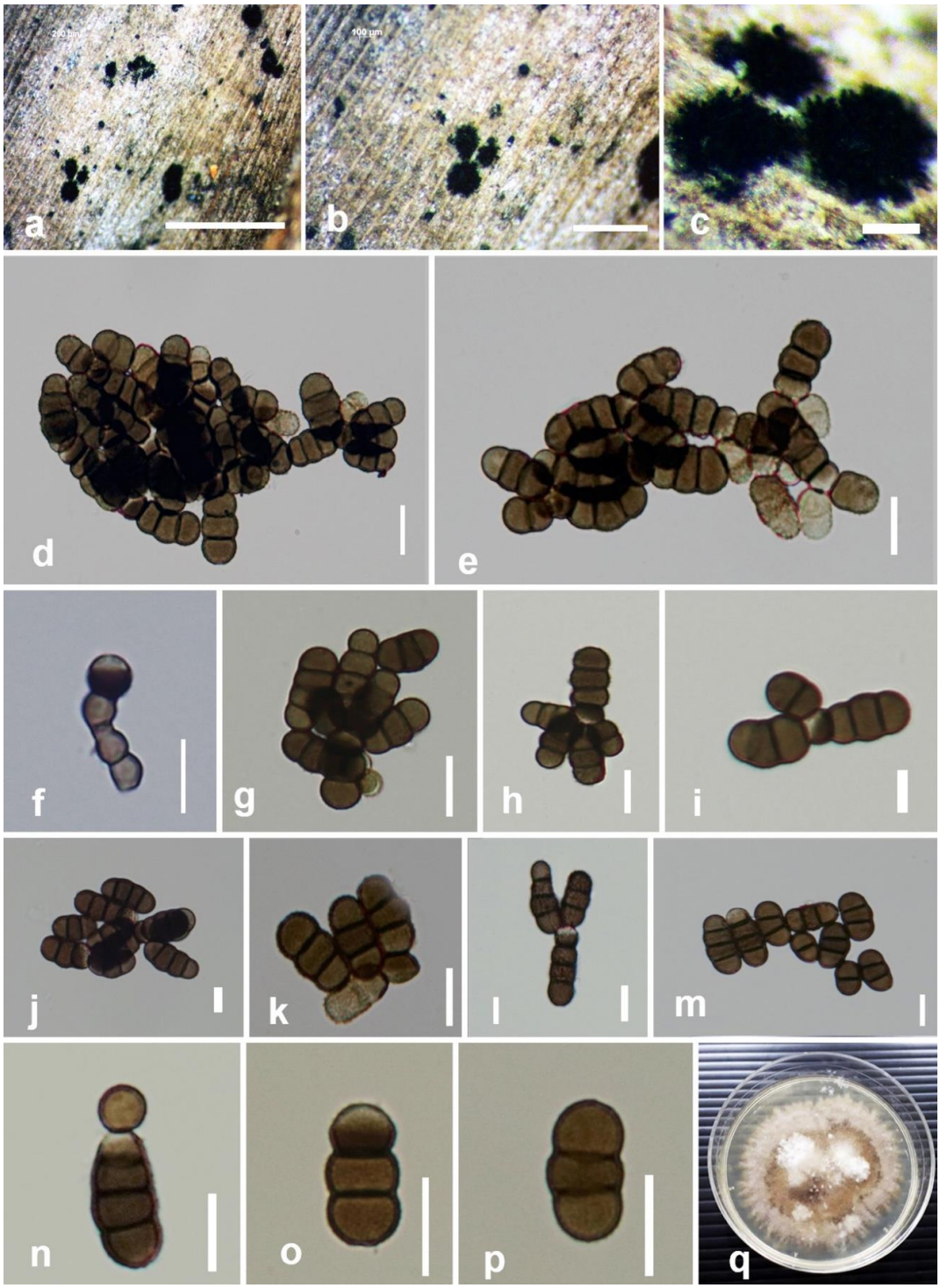

Fig. 5 − *Torula chromolaenae* (MFLU 20–0698). a−c Colonies on leaf vein of *Musa* sp. d, e Conidia arranged in multi planes. f Conidiophore and conidiogenous cell. g−p Conidia and the formation of immature conidia. q Colonies on PDA after 21 days. Scale bars a = 1000 µm, b = 500 µm, c = 50 µm, d−h, k, o, p = 10 µm, l−n, i, j = 5 µm.

Notes – The BLASTn search results of SSU, LSU, ITS and TEF sequence data, indicated that our strain (MFLUCC 20−0237), showed a high identity to *Torula chromolaenae* (KUMCC 16–0036) as follows; SSU = 100.00%, LSU = 100.00%, ITS = 100.00%, TEF = 99.5% similarities. Morphological comparison with the type specimen of *T. chromolaenae* showed that our new collection (MFLU 20−0698/ MFLUCC 20−0237) is typical to *T. chromolaenae* in having brown or dark brown, minutely verruculose, 1–3–septate conidia. However, our collection (MFLU 20−0698) has slightly smaller conidiophores (2–5 × 2–4 µm vs. 5–6.3 × 3.5–4.6 µm) and conidia (10–15 × 5–

7 μm vs. 2.1–16.5 × (3.6–) 4.1–5 μm) with respect to Li et al. (2017). A nucleotide base comparison of ITS, TEF and RPB2 regions also showed that MFLUCC 20–0237 is conspecific with *T. chromolaenae* (KUMCC 16–0036) (0/502 bp of ITS, 0/787 bp of TEF, and 0/817 bp of RPB2). We thus identify our new collection (MFLU 20–0698) as *T. chromolaenae*. Previously, *T. chromolaenae* was reported as a saprobe on dicotyledonous and monocotyledonous hosts from China and Thailand, indicating that the species is not specific on hosts and normally found in tropical region.

Torula fici Crous [as 'ficus'], in Crous et al., IMA Fungus 6(1): 192 (2015) Fig. 6

Index Fungorum number: IF816154; Facesoffungi number: FoF 02712

Saprobic on dead leaf vein of *Musa* sp. (Musaceae). Sexual morph: Undetermined. Asexual morph: hyphomycetous. *Colonies* superficial, black, appearing as powdery masses on the host. *Mycelium* slightly immersed, septate, smooth, pale brown. *Conidiophores* 1–6 × 1.5–3 μm ($\bar{x}$ = 4.8 × 1.8 μm, n = 10), micronematous or semi–macronematous, unbranched, straight or slightly flexuous, subhyaline or pale grayish brown, verruculose. *Conidiogenous cells* 3.5–4 × 2.5–3.5 μm ($\bar{x}$ = 3.2 × 2.7 μm, n = 10) polyblastic or sometimes monoblastic, terminal, discrete, determinate, brown to dark brown, usually spherical, thin–walled, veruculose, having rough distal fertile part and a notably thick–walled, proximal sterile part. Base of sterile part of the conidiogenous cells that arise from mycelium is notably flat. *Conidia* (4–) 10–25(–75) × 3.5–4.5 μm ($\bar{x}$ = 24.8 × 3.8 μm, n = 40) dry, in simple or branched chains arising from the surface of the upper half of the characteristic conidiogenous cells, cylindrical with rounded ends, ellipsoidal or subspherical, brown or dark brown, notably verruculose, 1–3 transverse septa, slightly constricted at the septa, immature conidia hyaline to subhyaline, comparatively thin–walled, mature conidia notably thick–walled.

Culture characteristics – Conidia germinating on PDA within 20 hrs and germ tubes produced from the tip cell. Colonies growing on PDA, reaching 50 mm diam. in 14 days at 25°C, mycelium partly immersed to superficial, slightly effuse, hairy, with dentate to irregular margin, pinkish white.

Material examined – CHINA, Yunnan, Xishuangbanna, on a dead leaf vein of *Musa* sp. (Musaceae), 20 December 2018, D.N Wanasinghe, BNSWN5 (MFLU 20–0699), living culture MFLUCC 20–0238.

Known hosts & distribution – on *Chromolaena odorata* (Asteraceae, Dicotyledon) from Thailand (Li et al. 2017, Mapook et al. 2020); on *Ficus religiosa* (Moraceae, Dicotyledon) from Cuba (Crous et al. 2015); on *Garcinia* sp. (Clusiaceae, Dicotyledon) from Thailand (Jayasiri et al. 2019); on *Magnolia grandiflora* (Magnoliaceae, Dicotyledon) from Yunnan, China (Jayasiri et al. 2019); on *Olea europaea* (Oleaceae, Dicotyledon) from South Africa (Spies et al. 2020); on *Pandanus* sp. (Pandanaceae, Monocotyledon) from Thailand (Tibpromma et al. 2018); on submerged decaying wood in Yunnan, China (Su et al. 2018); on *Musa* sp. (Musaceae, Monocotyledon) from Yunnan, China (this study).

Notes – The BLASTn search results of SSU, LSU, ITS and TEF sequence data, indicated that our strain (MFLUCC 20–0238), has a high identity to *Torula fici* (KUMCC 16–0038) as follows; SSU = 99.88%, LSU = 100.00%, ITS = 98.99%, TEF = 98.53% similarities. Our new collection (MFLU 20–0699) is morphologically similar to *T. fici* in having short conidiophores, or reduced to conidiogenous cells, mono– to polyblastic, brown, verruculose conidiogenous cells and brown or dark brown, 1–3–septate conidia (Crous et al. 2015). However, our collection has slightly smaller conidiogenous cells (3.5–4 × 2.5–3.5 μm vs. (5–) 6(–8) × 5(–7) μm) and overlapped size of conidia (4–16 × 3.5–4.5 μm vs. (12–) 13–14(–15) (–19) × 5(–6) μm; Crous et al. 2015). A nucleotide base comparison of ITS and TEF regions also showed that MFLUCC 20–0238 differs from *T. fici* (CBS 595.96, type strain) in 7/467 bp (1.49%) of ITS and 16/776 bp (2.06%) of TEF. In addition, our strain MFLUCC 20–0238 differs from the strains KUMCC 15–0428 and KUMCC 16–0038 in 3/429 bp (0.69%) and 5/494 bp (1.01%) of ITS, and 15/808 bp (1.8%) and 11/813 bp (1.35%) of TEF, respectively. Nevertheless, our new strain (MFLUCC 20–0238) shows a high variation of TEF sequence with the type strain of *T. fici* (CBS 595.96; 2.06%) and strain KUMCC 15–0428

(1.8%). Based on current phylogenetic results (Fig. 2), we thus identify our new collection (MFLU 20–0699/ MFLUCC 20–0238) as *T. fici*. The conspecific of these strains is needed to be clarified in further studies. In this study, we report *T. fici* on *Musa* sp. (Monocotyledon) from Yunnan, China for the first time.

Fig. 6 − *Torula fici* (MFLU 20−0699). a−c Colonies on dead leaf of *Musa* sp. d−n Conidiogenesis. o, p Conidial masses. q Conidia (in chain). r−u Disposed conidia. v Colonies on PDA after 28 days. Scale bars: a = 500 µm, b = 100 µm, c = 20 µm, d−u = 5 µm.

Torula masonii Crous, in Crous et al., IMA Fungus 6(1): 195 (2015) Fig. 7

Index Fungorum number: IF812806; Facesoffungi number: FoF 02711

Saprobic on a dead leaf of *Musa* sp. (Musaceae). Sexual morph: Undetermined. Asexual morph: hyphomycetous. *Colonies* superficial, appearing as black, powdery masses on the host. *Mycelium* slightly immersed, septate, smooth, grayish brown. *Conidiophores* 3–6.5 × 4–5 μm ($\bar{x}$ = 5.4 × 4.3 μm, n = 10), micronematous or semi–macronematous, unbranched, straight or slightly flexuous, pale grayish brown to brown, subcylindrical, septate, or reduced to conidiogenous cells. *Conidiogenous cells* 5.5–6.5 × 4.5–6 μm ($\bar{x}$ = 6.3 × 5.3 μm, n = 10) polyblastic or sometimes monoblastic, terminal, discrete, determinate, grayish brown to dark brown, darker than the conidiophores or conidia, spherical, cup–shaped, or inverted cup–shaped, thin–walled, smooth, pale brown of distal fertile part and subhyaline or pale grayish brown of proximal sterile part. *Conidia* 8–25 × 4–6.5 μm ($\bar{x}$ = 18.3 × 5.6 μm, n = 40), dry, in simple or branched chains arising from the surface of the upper half of the characteristic conidiogenous cells, cylindrical with rounded ends, ellipsoidal or subspherical in each cell, grayish brown to dark brown, (2–) 3–5(–12) transverse septa, notably constricted at the septa, verruculose, arranged in different planes with respect to the main axis.

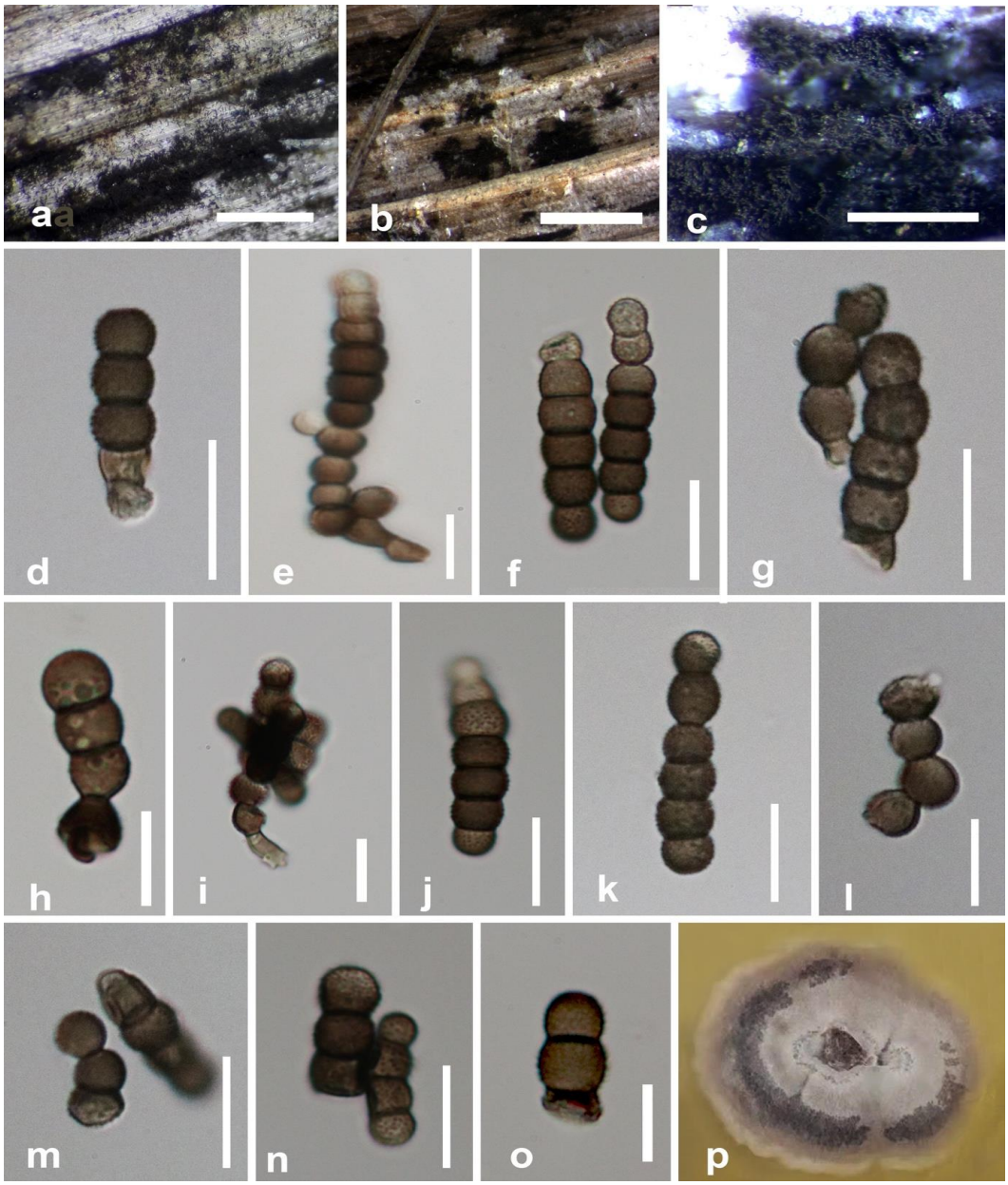

Fig. 7 –*Torula masonii* (MFLU 20–0700). a Colonies on dead leaf of *Musa* sp. b, c Masses of conidia on host surface. d–h, j–o Conidia. i Conidiophore, conidiogenous cell and budding of

conidia. p Colonies on PDA after 28 days. Scale bars: a = 50 μm, b = 500 μm, c = 1000 μm, d, f–o = 10 μm, e = 5 μm.

Culture characteristics – Conidia germinating on PDA within 14 hrs and germ tubes produced from the apex. Colonies growing on PDA, reaching 50 mm diam. in 15 days at 25°C, mycelium partly immersed to superficial, slightly effuse and hairy. Colonies medium dense, circular, flattened, slightly raise, surface smooth, with edge entire to sinuate margin, hairy at the center and margin, with dense, floccose to cottony at the middle, three distinct colour zones present in the culture. Periphery pinkish white, middle gray and grayish white next.

Material examined – CHINA, Taiwan region, Alishan, on a dead leaf of *Musa* sp. (Musaceae), 20 September 2018, D.S. Tennakoon, BNS023 (MFLU 20–0700), living culture MFLUCC 20–0239.

Known host & distribution – on *Brassica* sp. (Brassicaceae, Dicotyledon) from United Kingdom (Crous et al. 2015); *Iris germanica* (Iridaceae, Monocotyledon) from Italy (Li et al. 2017); on submerged decaying wood (Dicotyledon) from Yunnan, China (Su et al. 2018); on *Musa* sp. (Musaceae, Monocotyledon) from Taiwan (this study).

Notes – The BLASTn search results of SSU, LSU, ITS, TEF and RPB2 sequence data, showed that our strain (MFLUCC 20−0239), is similar to taxa in GenBank as follows; SSU = 99.87% similarity to *Torula chromolaenae* (MFLUCC 17−1514), LSU = 100.00% similarity to *T. masonii* (CBS 245.57), ITS = 99.88% similarity to *T. masonii* (KUMCC 16–0033), TEF = 100% and RPB2 = 99.75% similarities to *T. masonii* (DLUCC 0588). Comparing with the type specimen, our new isolate (MFLU 20–0700) is morphologically similar to *T. masonii*; however, the type of *T. masonii* has slightly larger size of conidiophores and conidia. The conidia of the type of *T. masonii* are predominantly 6–septate, but also vary in conidial septation from 2– to 12–septate (Crous et al. 2015), whereas the conidia of our new isolate are predominantly 3–5–sepate, occasionally 2– to 12–septate. A nucleotide base pair comparison of ITS showed that our strain (MFLUCC 20−0239) differs from other *T. masonii* strains (CBS 245.57, DLUCC 0588 and KUMCC 16–0033) in 8/509 (1.57%), 2/441 (0.45%) and 1/508 (0.19%). Our strain MFLUCC 20−0239 also differs from the strains DLUCC 0588 and KUMCC 16–0033 in 2/798 bp (0.25%) and 2/830 (0.34%) of RPB2 but has no different nucleotide base in TEF gene region. We identify our new isolate as *T. masonii*, based on phylogenetic evidence, although there is more than 1.5% variation in the ITS DNA regions as compared to the type strain (CBS 245.57). We document *T. masonii* on *Musa* sp. for the first time. This is the first geographical record of *T. masonii* from Taiwan.

Discussion

Many *Torula* and *Periconia* species have been reported on *Musa* spp. worldwide. *Torula herbarum* has been reported on *Musa* spp. from Papua New Guinea, Somalia, Taiwan, Thailand, and Zambia (Castellani & Ciferri 1937, Riley 1956, Matsushima 1971, 1980, Photita et al. 2001a, 2003b). *Periconia byssoides* (Venezuela, Cuba, and Somalia) (Matsushima 1971, Urtiaga 1986, Delgado–Rodriguez & Mena–Portales 2004), *P. digitata* (Thailand, Malaysia) (Williams & Liu 1976, Photita et al. 2001b), *P. lateralis* (Thailand) (Photita et al. 2001b) and *P. minutissima* (Ghana) (Hughes 1953) have also been reported on *Musa* spp. The identification of the latter taxa on *Musa* spp. was solely based on morphology and molecular data were not integrated. Therefore, more taxon sampling of saprobic and endophytic fungi on *Musa* spp. should be carried out by integrating morpho–molecular data in taxonomy.

Some *Periconia* species have been reported as plant pathogens (i.e., *P. cicirnata*, *P. digitata* and *P. macrospinosa*) on leaves, roots and stems of economically important crops such as maize, sorghum and pointed gourd (Stojkov et al. 1996, Sarkar et al. 2019). In addition, *P. keratitis* has been reported as a human pathogen from India (Gunasekaran et al. 2020). *Periconia* produce some economically important bioactive compounds with antimicrobial activities (Kim et al. 2004, Bhilabutra et al. 2007, Hongsanan et al. 2020). It is interesting to note that *P. delonicis* and *P. cortaderiae* may also produce bioactive compounds which were discovered from taxa in the same

genus. In addition, some species of *Torula* also produce chemically active compounds (i.e., Dichlorinated Aromatic Lactones and erythritol) which have a wide range of applications in the food industry (Chunyu et al. 2018). Mapook et al. (2020) also reported that *T. chromolaenae*, *T. fici* and *T. polyseptata* showed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Mucor plumbeus* on their preliminary screening of antimicrobial activity of fungi on *Chromolaena odorata*. Therefore, it will be interesting to know whether *T. chromolaenae* and *T. fici* from *Musa* spp. will have the same biological ability.

Recent taxonomic studies integrated DNA sequence data on the introduction of novel taxa in *Periconia* and *Torula* (Crous et al. 2015, Su et al. 2016, 2018, Li et al. 2017, 2020, Liu et al. 2017, Jayasiri et al. 2019, Hyde et al. 2020a, Mapook et al. 2020, Phukhamsakda et al. 2020). Protein–coding genes revealed to be good phylogenetic markers in species delineation of *Periconia* and *Torula* (Su et al. 2016, 2018, Li et al. 2017, 2020, Jayasiri et al. 2019, Hyde et al. 2020a, Mapook et al. 2020, Phukhamsakda et al. 2020). Corresponding protein–coding sequences (RPB2 and TEF) with ribosomal DNA (SSU, LSU and ITS) sequence dataset can provide well–resolved tree topologies for the taxa in Periconiaceae and Torulaceae. However, several taxa of Periconiaceae and Torulaceae lack protein–coding DNA sequences in GenBank. Therefore, many *Periconia* and *Torula* taxa need to be recollected so that valid sequence data are provided to GenBank for better taxonomic resolutions.

Phylogenetic tree of Periconiaceae (Fig. 1) showed that *Periconia delonicis* does not form a well–separated clade with *P. palmicola* (MFLUCC 14−0400) and *P. verrucosa* (MFLUCC 17−2158). The tree topology showed that they are conspecific, and this result is also supported by a nucleotide base comparison of ITS and TEF regions. However, *P. delonicis* is phylogenetically well–resolved and is distinct from *P. verrucosa* in Phukhamsakda et al. (2020), indicating that ITS and TEF regions are not good phylogenetic markers for some species in Periconiaceae. *Periconia delonicis* was not included in phylogenetic analyses of Periconiaceae when Hyde et al. (2020a) introduced *P. palmicola* as a new species. The conspecific of *P. delonicis* and *P. palmicola* is therefore questionable and should be reinvestigated in future studies.

Phylogenetic tree of Torulaceae (Fig. 2) showed that *Torula chromolaenae*, *T. fici* and *T. masonii* form well–resolved subclades within Torulaceae. However, the four strains (including type strain) of *T. fici* and *T. masonii* formed insignificantly separated branch lengths in this analysis and this phylogenetic result is also supported by Hongsanan et al. (2020), Hyde et al. (2020a), Li et al. (2020), Mapook et al. (2020) and Phukhamsakda et al. (2020). This may be the result of a high variation (>1.5%) in the TEF region (see notes under *T. fici* and *T. masonii*). Further studies on the conspecific or complexity of these species are needed for their clarification based on the reliable protein coding genes.

Documentation of fungi from new hosts and geographical locations supports the accurate estimates and taxonomic establishments of fungal diversity and distribution. In addition, new occurrences of fungi from various hosts and habitats further provide insights to determine host jumping patterns, host shift speciation and the adaptations of fungi during their life cycle (Hyde et al. 2020c). Taxonomy and phylogeny of fungal pathogens on *Musa* spp. (i.e., *Colletotrichum*, *Fusarium*, *Mycosphaerella*, *Neocordana* and *Phyllosticta*) have been well–studied worldwide (Giatgong 1980, Wulandari et al. 2010, Churchill 2011, Guarnaccia et al. 2017, Marin–Felix et al. 2019, Maryani et al. 2019). The detailed taxonomic works on endophytic fungi in *Musa* spp. were previously conducted by Brown et al. (1998), Photita et al. (2001b, 2004), Zakaria & Aziz (2018) and Samarakoon et al. (2019). Still the saprobic fungal niches on *Musa* spp. remain unrevealed and many more taxa are yet to be discovered. Hence the morpho–molecular data of this study will be further useful in future taxonomic works of fungi.

Acknowledgements

This study is supported by Key Research Project "Agroforestry Systems for restoration and bio–industry technology development (grant no. 2017YFC0505101)". We also thank Biology Experimental Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese

Academy of Sciences for providing the facilities of molecular laboratory. Binu C. Samarakoon is grateful to Danushka Tennakoon for collecting the specimens from Taiwan, Dr. Dhanushka N. Wanasinghe and Junfu Li for the valuable comments and suggestions on the morphological studies of *Periconia* and *Torula*. Rungtiwa Phookamsak thanks CAS President's International Fellowship Initiative (PIFI) for young staff (grant no. Y9215811Q1), the National Science Foundation of China (NSFC) project code 31850410489 (grant no. Y81I982211) and Chiang Mai University for financial support. Samantha C. Karunarathna thanks CAS President's International Fellowship Initiative (PIFI) young staff under the grant number: 2020FYC0002 and the National Science Foundation of China (NSFC) for funding this work under the project code 31851110759. Jianchu Xu thanks Key Research Program of Frontier Sciences of the Chinese Academy of Sciences (grant no. QYZDY–SSW–SMC014) and the Strategic Priority Research Program of Chinese Academy of Sciences for supporting this research.

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