



Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand

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Abstract

Pestalotioid fungi are associated with a wide variety of plants worldwide and are endophytes, pathogens and saprobes. The present study provides an updated phylogenetic placement of *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis* using fresh collections from mangrove plants in Thailand. Twelve novel species are characterized based on combined sequence data analyses of internal transcribed spacer (ITS), beta tubulin (β -tubulin) and translation elongation factor 1-alpha (EF1 α) coupled with morphological characters. The taxonomy and phylogenetic relationships of pestalotioid-fungi are reappraised with suggestions for future work.

Keywords – 12 new species – Asymptomatic leaves – *Acrostichum aureum* – *Avicenia marina* – Leaf spots – Mangroves – *Neopestalotiopsis* – *Pestalotiopsis* – pestalotiopsis-like – Phylogeny – *Pseudopestalotiopsis* – *Rhizophora* sp. – *Sonneronata alba* – Taxonomy

Introduction

Mangrove forests are one of the most productive ecosystems which play a major role in the ecological communities in coastal tropical and subtropical waters (Hyde & Lee 1995). They serve as hatcheries and nursery habitats for marine organisms and protect coastlines from catastrophic events such as storms and tidal surges (Hyde & Jones 1988, Fisher & Spalding 1993, Hyde & Lee 1995, Hyde et al. 1998). The greatest diversity of mangrove species occurs in Indonesia, Malaysia and Thailand, which also harbour a great diversity of micro-organisms especially fungi (Alias & Jones 2009, Alias et al. 2010). However, the phylogenetic and functional description of microbial diversity in mangrove ecosystems has not been addressed to the same extent as for other environments. Even though mangrove ecosystems are very rich in microbial diversity, less than 5% of species are believed to have been described (Thatoi et al. 2012). Recently developed technologies in molecular biology and genetics offer a great promise to explore the potential of microbial diversity. Hence, the present paper makes an effort to identify the species of fungi in

mangrove ecosystems in Thailand (Guba 1961, Barr 1975, Nag Raj 1993, Norphanphoun et al. 2018, Kumar et al. 2019).

Several studies have focused on the diversity and the ecology of fungi in this special habitat and there are relatively few studies on disease-causing fungi of mangrove (Cribb & Cribb 1955, Kohlmeyer & Kohlmeyer 1979, Hyde & Jones 1988). Most of the mangrove fungi are reported as saprobes and endophytes (e.g. Hu et al. 2007, Jones et al. 2009, Pang et al. 2010, Doilom et al. 2017, Devadatha et al. 2018, Li et al. 2018, Kumar et al. 2019). Zhou et al. (2018) reported that the endophytic fungi *Diaporthe* sp., *Neopestalotiopsis protearum* and *Pestalotiopsis* sp. are predominant in mangrove trees in south China. Endophytic fungal genera such as *Cladosporium*, *Colletotrichum*, *Fusarium*, *Pestalotiopsis*, *Phomopsis*, *Phyllosticta* and *Xylaria* were also reported from Thailand mangroves (Chaeprasert et al. 2010, Doilom et al. 2017). Nevertheless, endophytic fungi are less well-studied and studies mainly identified taxa to the genus level (Chaeprasert et al. 2010, Abraham et al. 2015, Thomas et al. 2016). Pathogenic mangrove fungi have received much less attention (e.g. Hyde & Cannon 1992, Jones & Pang 2012, Jones et al. 2019). In the present study we focused on Pestalotioid taxa on *Acrostichum aureum*, *Avicennia marina*, *Rhizophora* (mainly *R. apiculata* and *R. mucronata*) and *Sonneronata alba*, which were abundant plants in Thailand mangroves. The most notable mangrove plant among Thailand mangroves is *Rhizophora*, a genus of tropical mangrove trees, which are collectively known as true mangroves (Hassan et al. 2018).

Pestalotioid fungi are characterized based on morphological features such as the median cell colour (Maharachchikumbura et al. 2011). They are common phytopathogens causing a variety of diseases or saprobes or endophytes, and are widely distributed in tropical and temperate regions (Guba 1961, Barr 1975, Nag Raj 1993, Maharachchikumbura et al. 2014). However, species identification in this genus remains a major challenge because of overlapping morphologies (Jeewon et al. 2002, 2003, Maharachchikumbura et al. 2012, 2011). Maharachchikumbura et al. (2014) split *Pestalotiopsis* into three genera: *Neopestalotiopsis*, *Pseudopestalotiopsis* and *Pestalotiopsis*, based on conidia pigment colour, conidiophores and molecular phylogeny. *Neopestalotiopsis* can be easily distinguished from *Pseudopestalotiopsis* and *Pestalotiopsis* by its versicolourous median cells (Maharachchikumbura et al. 2014). *Pseudopestalotiopsis* differs from *Pestalotiopsis* by having darker three median cells and knobbed apical appendages (Maharachchikumbura et al. 2014). Many novel species were introduced into this group during recent years (Maharachchikumbura et al. 2016, Liu et al. 2017, Nozawa et al. 2017, Ariyawansa et al. 2018, Watanabe et al. 2018, Tsai et al. 2018, Tibpromma et al. 2018). This study aimed to identify the pestalotioid fungi associated with mangroves in Thailand based on both morphological characters and molecular phylogeny.

Materials & Methods

Sample collection and examination of specimens

Fresh leaf samples (both diseased and healthy) were collected in 2016, from *Acrostichum aureum*, *Avicennia marina*, *Rhizophora apiculata*, *R. mucronata* and *Sonneronata alba* from Phetchaburi, Ranong and Trat provinces, Thailand. Permission for collecting specimens were obtained where necessary. Fresh specimens were taken to the laboratory in paper bags, examined and described following Norphanphoun et al. (2017). Morphological characters of conidiomata were examined using a Motic SMZ 168 dissecting microscope (Motic Incorporation Ltd., Hong Kong). Hand sections were mounted in water and examined for morphological details. Micro-morphology was studied using a Nikon Ni compound microscope (Nikon Instruments Inc., NY, USA) fitted with a Canon EOS 600D digital camera (Canon Inc., Tokyo, Japan). Photo-plates were made using Adobe Photoshop CS6 Extended version 13.0 × 64 (Adobe Systems, CA, USA), while Tarosoft (R) Image Frame Work program v. 0.9.7 (Tarosoft, Thailand) was used for measurements.

Cultures were obtained by the tissue isolation method outlined in Norphanphoun et al. (2018). Single hyphal tips were transferred onto potato dextrose agar (PDA, Hardy Diagnostics, CA, USA)

plates throughout a two-week period. Pure cultures were maintained for further studies on PDA. The specimens/dried cultures and living cultures are deposited in the Herbarium Mae Fah Luang University (MFLU) and culture collection Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand and duplicated in the International Collection of Micro-organisms from Plants (ICMP). Facesoffungi numbers were obtained as in Jayasiri et al. (2015) and the new species were registered in Index Fungorum (Index Fungorum 2019).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh fungal mycelia growing on PDA at room temperature (18–25 °C) for two weeks using a E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA) following the manufacturer's protocols. Polymerase chain reactions (PCR) were carried out using primer pairs of ITS1/ITS4 to amplify the ITS region (White et al. 1990), EF1-728f/EF2 to amplify EF1 α (Carbone & Kohn 1999, O'Donnell et al. 1998) and the partial β -tubulin region was amplified using primers T1/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997).

The amplification reactions were carried out with the following protocol: 50 μ l reaction volume containing 2 μ l of DNA template, 2 μ l of each forward and reverse primers, 25 μ l of 2 \times Bench TopTMTaq Master Mix (mixture of Taq DNA Polymerase (recombinant): 0.05 units/ μ L, MgCl₂: 4 mM, and dNTPs (dATP, dCTP, dGTP, dTTP): 0.4 mM) and 19 μ l of double-distilled water (ddH₂O) (sterilized water). The PCR thermal cycle program for each gene is described in Table 1. Purification and sequencing of PCR products with the same primers mentioned above were carried out at Life Biotechnology Co., Shanghai, China.

Table 1 Polymerase chain reactions (PCR) thermal cycle program for each gene.

Gene	Primers	PCR thermal cycle protocols
ITS	ITS1/ITS4	initially 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30s, annealing at 55 °C for 50s, elongation at 72 °C for 1 min, final extension at 72 °C for 7 min
β -tubulin	T1/Bt2b	initially 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30s, annealing at 55 °C for 50s, elongation at 72 °C for 1 min, final extension at 72 °C for 7 min
EF1 α	EF1-728f/EF2	initially 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30s, annealing at 52 °C for 30s, elongation at 72 °C for 30s, final extension at 72 °C for 7 min

Phylogenetic analysis

The sequences were assembled by Geneious® 11.1.5 (<http://www.geneious.com>). Multiple alignments derived in this study were analysed with similar sequences, acquired from GenBank BLASTn queries and recently published articles (Maharachchikumbura et al. 2016, Liu et al. 2017, Nozawa et al. 2017, Tsai et al. 2018, Watanabe et al. 2018). ITSx 1.1 (a Perl-based software tool) was used to extract ITS1, 5.8S and ITS2 (Bengtsson-Palme et al. 2013). BioEdit 7.2.3 (Hall 1999) was used to extract the partition of β -tubulin, and EF1 α , which follow the partition template based on nucleotide BLAST in GenBank. Combined analyses of ITS1+ITS2, 5.8S, β -tubulin and EF1 α sequence data were performed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis (BI) for each genus separately. The dataset of *Neopestalotiopsis* consisted of 52 taxa (outgroup: *Pestalotiopsis diversiseta* MFLUCC 12-0287); 70 taxa for *Pestalotiopsis* (outgroup: *Neopestalotiopsis saprophytica* MFLUCC 12-0282) and 27 taxa for *Pseudopestalotiopsis* (outgroup: *Neopestalotiopsis natalensis* CBS 138.41). All sequences were aligned for each gene separately using MAFFT v.7.110 online program (<http://mafft.cbrc.jp/alignment/server/>; Katoh & Standley 2013) and Gblocks v. 0.91b was used to exclude ambiguously aligned positions in the ITS, EF1 α and β -tubulin alignments (Castresana 2000, Talavera & Castresana 2007). A partition homogeneity test (PHT) was performed with

PAUP* 4.0b10 to determine whether the individual datasets were congruent and could be combined (Swofford 2002). The combined sequence alignments were obtained from MEGA7 version 7.0.14 (Kumar et al. 2015) and missing data were coded as gap (-) and further manual adjustments were made wherever necessary in BioEdit 7.2.3 (Hall 1999). The combined sequence alignment was converted to NEXUS file for maximum parsimony analysis using ClustalX v. 2 (Larkin et al. 2007). The NEXUS file was prepared for MrModeltest v. 2.2 (Nylander 2004) in PAUP* v.4.0b10 (Swofford 2002).

Maximum parsimony (MP) analysis was performed using PAUP* v. 4.0b10 (Swofford 2002) with 1000 bootstrap replicates using heuristic search with random stepwise addition and tree-bisection reconnection (TBR). Maxtrees were set to 1000 and branches of zero length were collapsed. The following descriptive tree statistics were calculated: parsimony tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI).

For both maximum likelihood and Bayesian analyses, a partitioned analysis was performed with the following six partitions: ITS1+ITS2, 5.8S, β -tubulin (exon), β -tubulin (intron), EF1 α (exon), and EF1 α (intron). Maximum-likelihood (ML) analysis was performed with RAxML (Stamatakis 2006) implemented in the CIPRES Science Gateway web server (RAxML-HPC2on XSEDE; Miller et al. 2010), 1000 rapid bootstrap replicates were run with GTRGAMMA model of nucleotide evolution. Maximum likelihood bootstrap values (MLBS) equal or greater than 50% are presented above each node in the resulting phylogenetic trees.

Bayesian inference (BI) analysis was performed using the Markov Chain Monte Carlo (MCMC) method with MrBayes 3.2.2 (Ronquist et al. 2012). The best-fit nucleotide substitution model for each dataset was separately determined using MrModeltest version 2.2 (Nylander 2004). GTR model was selected a best-fit model for the β -tubulin-exons (*Neopestalotiopsis*), GTR+I model was selected a best-fit model for the β -tubulin-exons (*Pseudopestalotiopsis*), ITS1+ITS2+5.8S (*Neopestalotiopsis*), GTR+I+G model for β -tubulin-exons (*Pestalotiopsis*), HKY model for ITS1+ITS2+5.8S (*Pseudopestalotiopsis*), HKY+G model for EF1 α -introns (*Neopestalotiopsis*), EF1 α -introns (*Pestalotiopsis*), and β -tubulin-introns (*Pseudopestalotiopsis*), HKY+I model for EF1 α -introns (*Pseudopestalotiopsis*), HKY+I+G model for ITS1+ITS2+5.8S (*Pestalotiopsis*) and β -tubulin-introns (*Pestalotiopsis*), K80 model for EF1 α -exons (*Pseudopestalotiopsis*), K80+G model for β -tubulin-introns (*Neopestalotiopsis*), SYM model for EF1 α -exons (*Neopestalotiopsis*), SYM+I model was selected a best-fit model for the EF1 α -exons (*Pestalotiopsis*). The MCMC analyses, with four chains starting from random tree topology, were run between 1,000,000-5,000,000 generations for each combined dataset. Trees were sampled every 100 generations. Tracer v. 1.5.0 was used to check parameters including the effective sampling sizes (ESS) >200, the stable likelihood plateaus and burn-in value (Rambaut et al. 2013). The first 5000 samples were excluded as burn-in.

The phylogram was visualized in FigTree v1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>; Rambaut 2014) and made in Adobe Illustrator CC (Adobe Systems, CA, U.S.A.) and Adobe Photoshop CS6 Extended version 13.1.2 \times 64. Newly generated sequences in this study are deposited in GenBank (Table 2). The finalized alignments and trees were deposited in TreeBASE. The phylogram was visualized in FigTree v1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>; Rambaut 2014) and made in Adobe Illustrator CC (Adobe Systems, CA, U.S.A.) and Adobe Photoshop CS6 Extended version 13.1.2 \times 64. Newly generated sequences in this study are deposited in GenBank (Table 2). The finalized alignments and trees were deposited in TreeBASE, Fig. 1: submission ID: 24243 (Reviewer access URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S24243?x-access-code=9556bf0e6bb1598ab7df74b6c8c86564&format=html>), Fig. 8: submission ID: 24244 (Reviewer access URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S24244?x-access-code=83ce9ad2fa86038ae180067b214435b2&format=html>), Fig. 11: submission ID: 24245 (Reviewer access URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S24245?x-access-code=cd7080d4e86cc3268e7981fcf2b7fc47&format=html>).

Table 2 GenBank accession numbers of the sequences used in phylogenetic analyses.

Taxa	Strain number ^a	Host	Country	ITS	β -tubulin	EF1 α
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754 ^T	<i>Acrostichum aureum</i>	Thailand	MK764272	MK764338	MK764316
	MFLUCC 17-1755	<i>Acrostichum aureum</i>	Thailand	MK764273	MK764339	MK764317
<i>N. brachiata</i>	MFLUCC 17-1555 ^T	<i>Rhizophora apiculata</i>	Thailand	MK764274	MK764340	MK764318
<i>N. petila</i>	MFLUCC 17-1738 ^T	<i>Rhizophora mucronata</i>	Thailand	MK764275	MK764341	MK764319
	MFLUCC 17-1737	<i>Rhizophora mucronata</i>	Thailand	MK764276	MK764342	MK764320
<i>N. rhizophorae</i>	MFLUCC 17-1550 ^T	<i>Rhizophora mucronata</i>	Thailand	MK764277	MK764343	MK764321
	MFLUCC 17-1551	<i>Rhizophora mucronata</i>	Thailand	MK764278	MK764344	MK764322
<i>N. sonneratae</i>	MFLUCC 17-1745 ^T	<i>Sonneronata alba</i>	Thailand	MK764279	MK764345	MK764323
	MFLUCC 17-1744	<i>Sonneronata alba</i>	Thailand	MK764280	MK764346	MK764324
<i>N. thailandica</i>	MFLUCC 17-1730 ^T	<i>Rhizophora mucronata</i>	Thailand	MK764281	MK764347	MK764325
	MFLUCC 17-1731	<i>Rhizophora mucronata</i>	Thailand	MK764282	MK764348	MK764326
<i>N. alpapicalis</i>	MFLUCC 17-2544 ^T	<i>Rhizophora mucronata</i>	Thailand	MK357772	MK463545	MK463547
	MFLUCC 17-2545	symptomatic leaves <i>R. apiculata</i>	Thailand	MK357773	MK463546	MK463548
<i>N. aotearoa</i>	CBS 367.54 ^T	<i>Canva</i>	New Zealand	KM199369	KM199454	KM199526
<i>N. asiatica</i>	MFLUCC 12-0286 ^T	unidentified tree	China	JX398983	JX399018	JX399049
<i>N. australis</i>	CBS 114159 ^T	<i>Telopea</i> sp.	Australia	KM199348	KM199432	KM199537
<i>N. chrysea</i>	MFLUCC 12-0261 ^T	dead leaves	China	JX398985	JX399020	JX399051
<i>N. clavispora</i>	MFLUCC 12-0281 ^T	<i>Magnolia</i> sp.	China	JX398979	JX399014	JX399045
<i>N. cocoes</i>	MFLUCC 15-0152 ^T	<i>Cocos nucifera</i>	Thailand	NR_156312	-	KX789689
<i>N. coffea-arabicae</i>	HGUP4015 ^T	<i>Coffea arabica</i>	China	KF412647	KF412641	KF412644
<i>N. cubana</i>	CBS 600.96 ^T	leaf litter	Cuba	KM199347	KM199438	KM199521
<i>N. ellipsospora</i>	MFLUCC 12-0283 ^T	dead plant material	China	JX398980	JX399016	JX399047
<i>N. egyptiaca</i>	CBS 140162 ^T	<i>Mangifera indica</i>	Egypt	KP943747	KP943746	KP943748
<i>N. eucalypticola</i>	CBS 264.37 ^T	<i>Eucalyptus globulus</i>	-	KM199376	KM199431	KM199551
<i>N. foedans</i>	CGMCC 3.9123 ^T	unidentified mangrove plant	China	JX398987	JX399022	JX399053
<i>N. formicarum</i>	CBS 362.72 ^T	dead ant	Ghana	KM199358	KM199455	KM199517
<i>N. honoluluana</i>	CBS 114495 ^T	<i>Telopea</i> sp.	USA	KM199364	KM199457	KM199548
<i>N. iraniensis</i>	CBS 137768 ^T	<i>Fragaria</i> \times <i>ananassa</i>	Iran	KM074048	KM074057	KM074051
<i>N. javaensis</i>	CBS 257.31 ^T	<i>Cocos nucifera</i>	Indonesia	KM199357	KM199457	KM199548
<i>N. keteleeria</i>	MFLUCC 13-0915 ^T	living leaves of <i>Keteleeria pubescens</i>	China	KJ503820	KJ503821	KJ503822
<i>N. magna</i>	MFLUCC 12-0652 ^T	<i>Pteridium</i> sp.	France	KF582795	KF582793	KF582791
<i>N. mesopotamica</i>	CBS 336.86 ^T	<i>Pinus brutia</i>	Iraq	KM199362	KM199441	KM199555

Table 2 Continued.

Taxa	Strain number ^a	Host	Country	ITS	β -tubulin	EF1 α
<i>N. musae</i>	MFLUCC 15-0776 ^T	<i>Musa</i> sp.	Thailand	NR_156311	KX789686	KX789685
<i>N. natalensis</i>	CBS 138.41 ^T	<i>Acacia mollissima</i>	South Africa	NR_156288	KM199466	KM199552
<i>N. pernambucana</i>	GS-2014 RV01 ^T	<i>Vismia guianensis</i>	Brazil	KJ792466	-	-
<i>N. piceana</i>	CBS 394.48 ^T	<i>Picea</i> sp.	UK	KM199368	KM199453	KM199527
	CBS 254.32	<i>Cocos nucifera</i>	Indonesia	KM199372	KM199452	KM199529
	CBS 225.3	<i>Mangifera indica</i>	-	KM199371	KM199451	KM199535
<i>N. protearum</i>	CBS 114178 ^T	<i>Leucospermum cuneiforme</i> cv. "Sunbird"	Zimbabwe	JN712498	KM199463	KM199542
	CMM1357	-	-	KY549597	KY549632	KY549594
<i>N. rosae</i>	CBS 101057 ^T	<i>Rosa</i> sp.	New Zealand	KM199359	KM199429	KM199523
<i>N. rosicola</i>	CFCC 51992 ^T	<i>Rosa chinensis</i>	China	KY885239	KY885245	KY885243
	CFCC 51993	<i>Rosa chinensis</i>	China	KY885240	KY885246	KY885244
<i>N. samarangensis</i>	MFLUCC 12-0233 ^T	<i>Syzygium samarangense</i>	Thailand	JQ968609	JQ968610	JQ968611
<i>N. saprophytica</i>	MFLUCC 12-0282 ^T	<i>Magnolia</i> sp.	China	KM199345	KM199433	KM199538
<i>N. steyaertii</i>	IMI 192475 ^T	<i>Eucalyptus viminalis</i>	Australia	KF582796	KF582794	KF582792
<i>N. surinamensis</i>	CBS 450.74 ^T	soil under <i>Elaeis guineensis</i>	Suriname	KM199351	KM199465	KM199518
	CBS 111494	<i>Protea eximia</i>	Zimbabwe	-	KM199462	KM199530
<i>N. umbrinospora</i>	MFLUCC 12-0285 ^T	unidentified plant	China	JX398984	JX399019	JX399050
<i>N. vitis</i>	MFLUCC 15-1265 ^T	<i>Vitis vinifera</i> cv. "Summer black"	China	KU140694	KU140685	KU140676
<i>N. zimbabwana</i>	CBS 111495 ^T	<i>Leucospermum cuneiforme</i> cv. "Sunbird"	Zimbabwe	JX556231	KM199456	KM199545
<i>P. rhizophorae</i>	MFLUCC 17-0416^T	<i>Rhizophora apiculata</i>	Thailand	MK764283	MK764349	MK764327
	MFLUCC 17-0417	<i>Rhizophora apiculata</i>	Thailand	MK764284	MK764350	MK764328
<i>P. thailandica</i>	MFLUCC 17-1616^T	<i>Rhizophora apiculata</i>	Thailand	MK764285	MK764351	MK764329
	MFLUCC 17-1617	<i>Rhizophora apiculata</i>	Thailand	MK764286	MK764352	MK764330
<i>P. adusta</i>	ICMP 6088 ^T	on refrigerator door PVC gasket	Fiji	JX399006	JX399037	JX399070
<i>P. aggestorum</i>	LC6301 ^T	<i>Camellia sinensis</i>	China	KX895015	KX895348	KX895234
<i>P. anacardiacearum</i>	IFRDCC 2397 ^T	<i>Mangifera indica</i>	China	KC247154	KC247155	KC247156
<i>P. arceuthobii</i>	CBS 434.65 ^T	<i>Arceuthobium campylopodum</i>	USA	KM199341	KM199427	KM199516
<i>P. arengae</i>	CBS 331.92 ^T	<i>Arenga undulatifolia</i>	Singapore	KM199340	KM199426	KM199515
<i>P. australasiae</i>	CBS 114126 ^T	<i>Knightia</i> sp.	New Zealand	KM199297	KM199409	KM199499
<i>P. australis</i>	CBS 114193 ^T	<i>Grevillea</i> sp.	Australia	KM199332	KM199383	KM199475

Table 2 Continued.

Taxa	Strain number^a	Host	Country	ITS	β-tubulin	EF1α
<i>P. biciliata</i>	CBS 124463 ^T	<i>Platanus × hispanica</i>	Slovakia	KM199308	KM199399	KM199505
<i>P. brachiata</i>	LC2988 ^T	<i>Camellia</i> sp.	China	KX894933	KX895265	KX895150
<i>P. brassicae</i>	CBS 170.26 ^T	<i>Brassica napus</i>	New Zealand	KM199379	-	KM199558
<i>P. camelliae</i>	MFLUCC 12-0277 ^T	<i>Camellia japonica</i>	China	JX399010	JX399041	JX399074
<i>P. chamaeropsis</i>	CBS 186.71 ^T	<i>Chamaerops humilis</i>	Italy	KM199326	KM199391	KM199473
<i>P. clavata</i>	MFLUCC 12-0268 ^T	<i>Buxus</i> sp.	China	JX398990	JX399025	JX399056
<i>P. colombiensis</i>	CBS 118553 ^T	<i>Eucalyptus eurograndis</i>	Colombia	KM199307	KM199421	KM199488
<i>P. digitalis</i>	ICMP 5434 ^T	<i>Digitalis purpurea</i>	New Zealand	KP781879	KP781883	-
<i>P. diploclisiae</i>	CBS 115587 ^T	<i>Diploclisia glaucescens</i>	Hong Kong	KM199320	KM199419	KM199486
<i>P. distincta</i>	LC3232 ^T	<i>Camellia sinensis</i>	China	KX894961	KX895293	KX895178
<i>P. diversiseta</i>	MFLUCC 12-0287 ^T	dead plant material	China	NR_120187	JX399040	JX399073
<i>P. dracontomelon</i>	MFUCC 10-0149 ^T	<i>Dracontomelon dao</i>	Thailand	KP781877	-	KP781880
<i>P. ericacearum</i>	IFRDCC 2439 ^T	<i>Rhododendron delavayi</i>	China	KC537807	KC537821	KC537814
<i>P. formosana</i>	NTUCC 17-009 ^T	on dead grass	Taiwan	MH809381	MH809385	MH809389
	NTUCC 17-010	on dead grass	Taiwan	MH809382	MH809386	MH809390
<i>P. furcata</i>	MFLUCC 12-0054 ^T	<i>Camellia sinensis</i>	Thailand	JQ683724	JQ683708	JQ683740
<i>P. gaultheria</i>	IFRD 411-014 ^T	<i>Gaultheria forrestii</i>	China	KC537805	KC537819	KC537812
<i>P. gibbosa</i>	NOF 3175 ^T	<i>Gaultheria shallon</i>	Canada	LC311589	LC311590	LC311591
<i>P. grevilleae</i>	CBS 114127 ^T	<i>Grevillea</i> sp.	Australia	KM199300	KM199407	KM199504
<i>P. hawaiiensis</i>	CBS 114491 ^T	<i>Leucospermum</i> sp. (coral)	USA	KM199339	KM199428	KM199514
<i>P. hollandica</i>	CBS 265.33 ^T	<i>Sciadopitys verticillata</i>	The Netherlands	KM199328	KM199388	KM199481
<i>P. humus</i>	CBS 336.97 ^T	soil	Papua New Guinea	KM199317	KM199420	KM199484
<i>P. inflexa</i>	MFLUCC 12-0270 ^T	dead plant material	China	JX399008	JX399039	JX399072
<i>P. intermedia</i>	MFLUCC 12-0259 ^T	dead plant material	China	JX398993	JX399028	JX399059
<i>P. italiana</i>	MFLUCC 12-0657 ^T	<i>Cupressus glabra</i>	Italy	KP781878	KP781882	KP781881
<i>P. jester</i>	CBS 109350 ^T	<i>Fagraea bodenii</i>	Papua New Guinea	KM199380	KM199468	KM199554
<i>P. jiangxiensis</i>	LC4399 ^T	<i>Camellia</i> sp.	China	KX895009	KX895341	KX895227
<i>P. jinchanghensis</i>	LC6636 ^T	<i>Camellia sinensis</i>	China	KX895028	KX895361	KX895247
<i>P. kenyana</i>	CBS 442.67 ^T	<i>Coffea</i> sp.	Kenya	KM199302	KM199395	KM199502
<i>P. knightiae</i>	CBS 114138 ^T	<i>Knightsia</i> sp.	New Zealand	KM199310	KM199408	KM199497

Table 2 Continued.

Taxa	Strain number a	Host	Country	ITS	β-tubulin	EF1α
<i>P. licualacola</i>	HGUP4057 T	<i>Licuala grandis</i>	China	KC492509	KC481683	KC481684
<i>P. linearis</i>	MFLUCC 12-0271 T	dead plant material	China	JX398992	JX399027	JX399058
<i>P. longiappendiculata</i>	LC3013 T	<i>Camellia sinensis</i>	China	KX894939	KX895271	KX895156
<i>P. lushanensis</i>	LC4344 T	<i>Camellia</i> sp.	China	KX895005	KX895337	KX895223
<i>P. macadamiae</i>	BRIP 63738B T	<i>Macadamia</i> sp.	Australia	KX186588	KX186680	KX186621
<i>P. malayana</i>	CBS 102220 T	<i>Macaranga triloba</i>	Malaysia	KM199306	KM199411	KM199482
<i>P. monochaeta</i>	CBS 144.97 T	<i>Quercus robur</i>	Netherlands	KM199327	KM199386	KM199479
<i>P. montellica</i>	MFLUCC 12-0279 T	dead plant material	China	JX399012	JX399043	JX399076
<i>P. neglecta</i>	TAP1100 T	<i>Quercus myrsinaefolia</i>	Japan	AB482220	LC311599	LC311600
<i>P. neolitsea</i>	NTUCC 17-011 T	on leaf of <i>Neolitsea villosa</i>	Taiwan	MH809383	MH809387	MH809391
<i>P. novae-hollandiae</i>	CBS 130973 T	<i>Banksia grandis</i>	Australia	KM199337	KM199425	KM199511
<i>P. oryzae</i>	CBS 353.69 T	<i>Oryza sativa</i>	Denmark	KM199299	KM199398	KM199496
<i>P. papuana</i>	CBS 331.96 T	coastal soil	Papua New Guinea	KM199321	KM199413	KM199491
<i>P. parva</i>	CBS 265.37 T	<i>Delonix regia</i>	-	KM199312	KM199404	KM199508
	CBS 278.35	<i>Leucothoe fontanesiana</i>	-	KM199313	KM199405	KM199509
<i>P. pallidotheae</i>	MAFF 240993 T	<i>Pieris japonica</i>	Japan	NR111022	LC311584	LC311585
<i>P. portugalica</i>	CBS 393.48 T	-	Portugal	KM199335	KM199422	KM199510
<i>P. rhododendri</i>	IFRDCC 2399 T	<i>Rhododendron sinogrande</i>	China	KC537804	KC537818	KC537811
<i>P. rhodomyrtus</i>	HGUP4230 T	<i>Rhodomyrtus tomentosa</i>	China	KF412648	KC537818	KF412645
<i>P. rosea</i>	MFLUCC 12-0258 T	dead plant material	China	JX399005	JX399036	JX399069
<i>P. scoparia</i>	CBS 176.25 T	<i>Chamaecyparis</i> sp.	-	KM199330	KM199393	KM199478
<i>P. shorea</i>	MFLUCC 12-0314 T	dead seed wing of <i>Shorea obtusa</i>	Thailand	KJ503811	KJ503814	KJ503817
<i>Pestalotiopsis</i> sp.	LC3637 T	<i>Camellia</i> sp.	China	KX894993	KX895324	KX895210
<i>P. spathulata</i>	CBS 356.86 T	<i>Gevuina avellana</i>	Chile	KM199338	KM199423	KM199513
<i>P. telopeae</i>	CBS 114161 T	<i>Telopea</i> sp.	Australia	KM199296	KM199403	KM199500
<i>P. trachicarpicola</i>	IFRDCC 2240 T	<i>Trachycarpus fortunei</i>	China	NR_120109	-	-
<i>P. unicolor</i>	MFLUCC 12-0276 T	dead plant material	China	JX398999	JX399030	-
<i>P. verruculosa</i>	MFLUCC 12-0274 T	dead plant material	China	JX398996	-	JX399061
<i>P. yanglingensis</i>	LC4553 T	<i>Camellia sinensis</i>	China	KX895012	KX895345	KX895231
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434 T	<i>Avicennia marina</i>	Thailand	MK764287	MK764353	MK764331

Table 2 Continued.

Taxa	Strain number ^a	Host	Country	ITS	β-tubulin	EF1α
<i>Ps. curvatispora</i>	MFLUCC 17-1722 ^T	<i>Rhizophora mucronata</i>	Thailand	MK764288	MK764354	MK764332
<i>Ps. curvatispora</i>	MFLUCC 17-1723	<i>Rhizophora mucronata</i>	Thailand	MK764289	MK764355	MK764333
<i>Ps. curvatispora</i>	MFLUCC 17-1747	<i>Rhizophora mucronata</i>	Thailand	MK764290	MK764356	MK764334
<i>Ps. rhizophorae</i>	MFLUCC 17-1560 ^T	<i>Rhizophora apiculata</i>	Thailand	MK764291	MK764357	MK764335
<i>Ps. thailandica</i>	MFLUCC 17-1724 ^T	<i>Rhizophora mucronata</i>	Thailand	MK764292	MK764358	MK764336
<i>Ps. thailandica</i>	MFLUCC 17-1725	<i>Rhizophora mucronata</i>	Thailand	MK764293	MK764359	MK764337
<i>Ps. ampullaceae</i>	LC6618 ^T	<i>Camellia sinensis</i>	China	KX895025	KX895358	KX895244
<i>Ps. camelliae-sinensis</i>	LC3011 ^T	<i>Camellia sinensis</i>	China	KX894985	KX895316	KX895202
<i>Ps. chinensis</i>	LC3011 ^T	<i>Camellia sinensis</i>	China	KX894937	KX895269	KX895154
<i>Ps. cocos</i>	CBS 272.29 ^T	<i>Cocos nucifera</i>	Java, Indonesia	KM199378	KM199467	KM199553
<i>Ps. dawaina</i>	MM14-F0015 ^T	unknown	Dawei, Myanmar	LC324750	LC324751	LC324752
<i>Ps. ignota</i>	NN 42909 ^T	unknown	-	KU500020	-	KU500016
<i>Ps. indica</i>	CBS 459.78 ^T	<i>Hibiscus rosa-sinensis</i>	-	KM199381	KM199470	KM199560
<i>Ps. ixorae</i>	NTUCC 17-001.1 ^T	<i>Ixora</i> sp.	-	MG816316	MG816326	MG816336
<i>Ps. jiangxiensis</i>	LC4479 ^T	<i>Eurya</i> sp.	China	KX895034	KX895343	KX895229
<i>Ps. kawthaungina</i>	MM14-F0083 ^T	unknown	Kawthaung, Myanmar	LC324753	LC324754	LC324755
<i>Ps. kubahensis</i>	UMAS KUB-P20 ^T	<i>Macaranga</i> sp.	Sarawak, Malaysia	KT006749	-	-
<i>Ps. myanmarina</i>	NBRC 112264 ^T	<i>Averrhoa carambola</i>	Dawei, Myanmar	LC114025	LC114045	LC114065
<i>Ps. simitheae</i>	MFLUCC 12-0121 ^T	<i>Pandanus odoratissimus</i>	Thailand	KJ503812	KJ503815	KJ503818
	MFLUCC 12-0125	living leaves of <i>Pandanus odoratissimus</i>	Thailand	KJ503813	KJ503816	KJ503819
<i>Ps. taiwanensis</i>	NTUCC 17-002.1 ^T	<i>Ixora</i> sp.	Taiwan	MG816319	MG816329	MG816339
	NTUCC 17-002.2	<i>Ixora</i> sp.	Taiwan	MG816320	MG816330	MG816340
	NTUCC 17-002.3	<i>Ixora</i> sp.	Taiwan	MG816321	MG816331	MG816341
	NTUCC 17-002.4	<i>Ixora</i> sp.	Taiwan	MG816322	MG816332	MG816342
<i>Ps. theae</i>	MFLUCC 12-0055 ^T	<i>Camellia sinensis</i>	Thailand	JQ683727	JQ683711	JQ683743
<i>Ps. vietnamensis</i>	NBRC 112252 ^T	<i>Fragaria</i> sp.	Hue, Vietnam	LC114034	LC114054	LC114074

^aBRIP Queensland Plant Pathology Herbarium; CBS CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC China Forestry Culture Collection Center; CGMCC China General Microbiological Culture Collection Center; HGUP the Plant Pathology Herbarium of Guizhou University; IFRDCC the International Fungal Research & Development Centre, Kunming, Yunnan (culture collection); ICMP International Collection of Microorganisms from Plants; IMI International Mycological Institute; MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NBRC the Biological Resource Center, Japan; NTUCC the Department of Plant Pathology and Microbiology, National Taiwan University Culture Collection. ^T Ex-type strains. Strains in this study are in bold.

Results

Phylogenetic analysis of combined ITS, β -tubulin and EF1 α sequences

The results from the partition homogeneity tests (PHT) for all three phylogenetic trees were not significant (level $\leq 95\%$), indicating that the individual datasets were congruent and could be combined. The combined alignment of the three phylogeny analyses trees included 22 strains, of which 11 strains belong to *Neopestalotiopsis*, four strains belong to *Pestalotiopsis* and seven strains belong to *Pseudopestalotiopsis*.

The alignment of *Neopestalotiopsis* (Fig. 1) comprised 52 taxa, with the outgroup taxon, *Pestalotiopsis diversiseta* (MFLUCC 12-0287). The total length of the dataset was 1358 characters including alignment gaps, 1–324, 325–482, 483–700, 701–886, 887–1054 and 1055–1358 corresponding to ITS1+ITS2, 5.8S, β -tubulin (exon), β -tubulin (intron), EF1 α (exon) and EF1 α (intron), respectively. The combined dataset contained 1001 constant, 175 parsimony uninformative and 182 parsimony informative characters. The combined dataset was analyzed using MP, ML and BI. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics generated from MP analysis based on the combined dataset of ITS1+ITS2, 5.8S, β -tubulin (exon), β -tubulin (intron), EF1 α (exon), and EF1 α (intron) were TL = 643, CI = 0.673, RI = 0.670, RC = 0.451, HI = 0.327. The best scoring likelihood tree selected with a final value for the combined dataset = -5197.543923.

Neopestalotiopsis acrostichi Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 2

Index Fungorum number: IF556434; Facesoffungi number: FoF 05780

Etymology – refers to the host from which the fungus was isolated, *Acrostichum aureum* L.

Holotype – MFLU 19-0774

Associated with leaf spots of *Acrostichum aureum* L. *Symptoms* sub circular to irregular shape, pale brown, appear on adaxial surface leaves of *A. aureum*, which later expand outwards (Fig. 2c). Small brown spots appeared initially and then gradually enlarged, changing to pale brown circular spots with a black border. They were usually 4–5 circular spots occurred on a single affected leaf (Fig. 2b). Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth, thin-walled, proliferating once percurrently, collarete present and not flared, 10–25 \times 2–5 μ m. *Conidia* (22–)23–26(–27) \times (5–)5.5–6.5(–7) μ m, (mean \pm SD = 24.3 \pm 1.3 \times 6 \pm 0.6 μ m), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (4.5–)5–6(–7) μ m long (mean \pm SD = 5.5 \pm 0.8 μ m); three median cells (14–)15–16(–18) μ m long (mean \pm SD = 15.9 \pm 1 μ m), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–6.5) μ m long (mean \pm SD = 5.6 \pm 0.6 μ m); third cell brown, 4.5–6 μ m long (mean \pm SD = 4.9 \pm 0.5 μ m); fourth cell brown, (4–)4.5–5(–6.5) μ m long (mean \pm SD = 5.4 \pm 0.6 μ m); apical cell (3–)4.5–5(–6) μ m long (mean \pm SD = 4.9 \pm 0.8 μ m), hyaline, conic to acute, with 3–5 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (16–)19–28.5(–33.5) μ m long (mean \pm SD = 24.5 \pm 4.7 μ m); single basal appendage, tubular, unbranched, centric, (4.5–)5–7(–12) μ m long, (mean \pm SD = 7.1 \pm 2 μ m).

Culture characteristics – Colonies on PDA reaching 6–8 cm diam after 7 d at room temperature (± 25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Chanthaburi Province, leaf spots of *Acrostichum aureum* L., 25 April 2017, Norphanphoun Chada JT12-1 (MFLU 19-0774 dried culture, holotype; PDD,

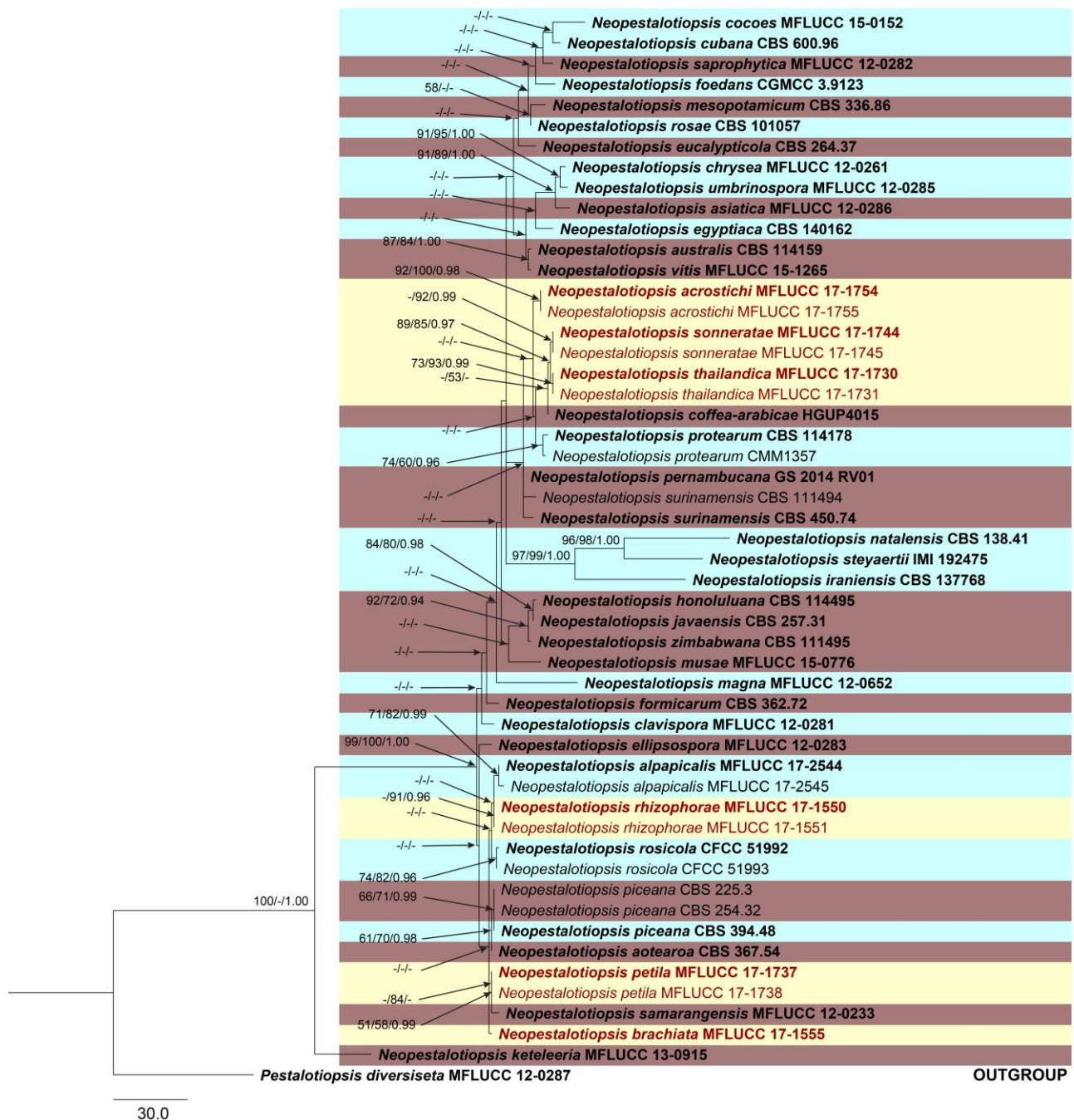


Figure 1 – One of the 1000 most parsimonious trees obtained from a heuristic search of combined ITS, β -tubulin and EF1 α sequence data for the genus *Neopestalotiopsis*. The tree is rooted to *Pestalotiopsis diversiseta* (MFLUCC 12-0287). Maximum parsimony and maximum likelihood bootstrap values $\leq 50\%$, Bayesian posterior probabilities ≤ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. The species obtained in this study are in red. Ex-type taxa from other studies are in black bold.

isotype); ex-type-living cultures, MFLUCC 17-1754, TNCC. THAILAND, Chanthaburi Province, leaf spots of *Acrostichum aureum* L., 25 April 2017, Norphanphoun Chada JT12-2 (MFLU 19-0775 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1755.

Notes – *Neopestalotiopsis acrostichi* was isolated from a leaf spot of *Acrostichum aureum*. The new species resembles several other *Neopestalotiopsis* species. The combined phylogenetic tree indicated that *N. acrostichi* is sister to *N. protearum* (CBS 114178) and *N. surinamensis* (CBS 450.74) (Fig. 1), but they differ in morphology. *Neopestalotiopsis acrostichi* have larger conidia than *N. protearum* (*N. protearum*: (14–)16–17(–18) \times (6.5–)8–9(–10)). *Neopestalotiopsis acrostichi* is very much similar to *N. surinamensis* (Crous et al. 2011, Maharachchikumbura et al.

2014). However, *N. acrostichi* differs from this species by having smaller conidia (*N. surinamensis*: (23–)24–28(–29) × (7–)7.5–9(–9.5)) with four apical tubular appendages (*N. surinamensis*: (15–)18–27(–28)) (Table 3, Maharachchikumbura et al. 2014). This is the first report of *Neopestalotiopsis* on *A. aureum*.

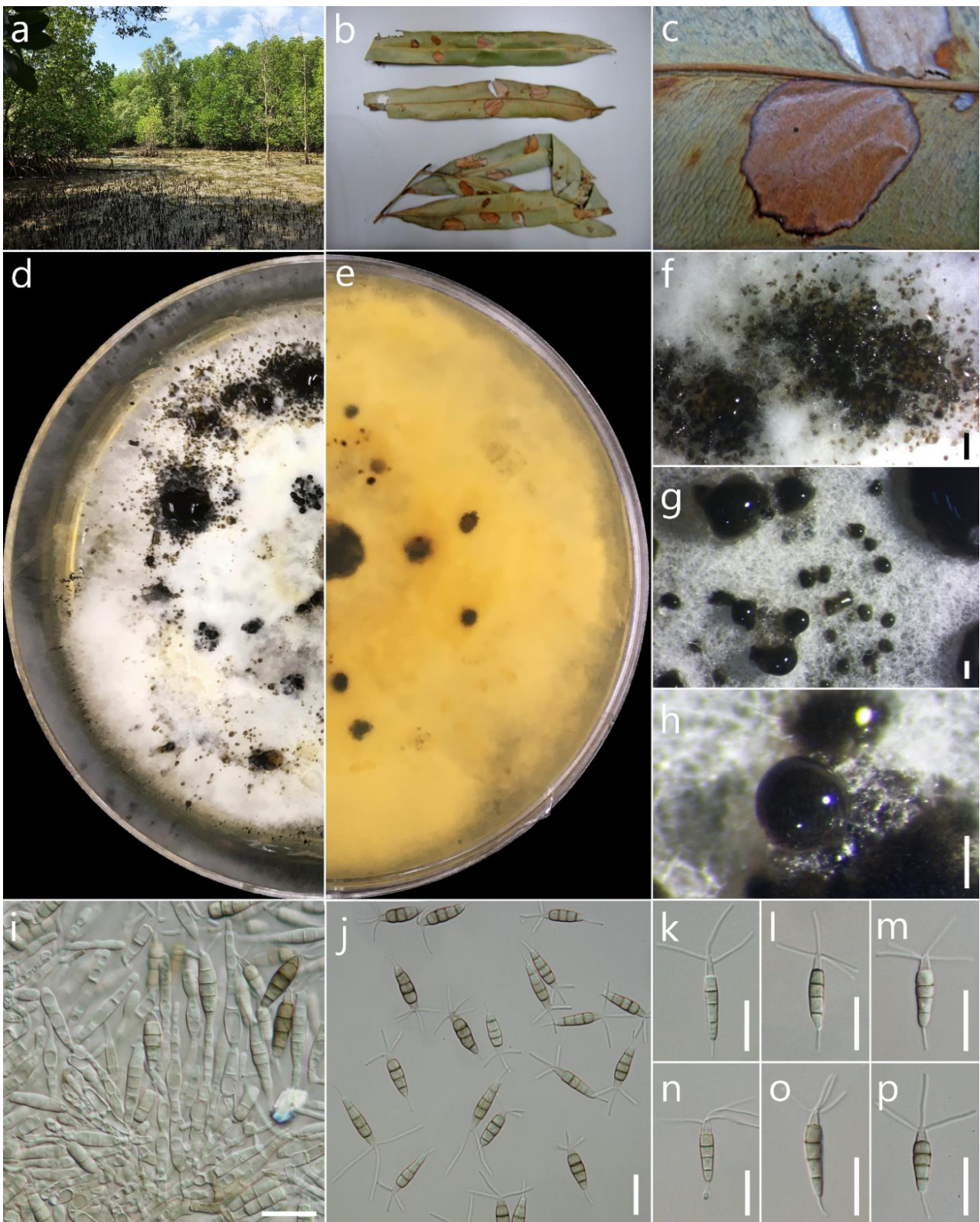


Figure 2 – *Neopestalotiopsis acrostichi* (MFLU 19-0774, holotype). a Habitat. b, c Leaf spots on *Acrostichum aureum*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f–h = 200 μ m, i–p = 20 μ m.

Neopestalotiopsis brachiata Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 3

Index Fungorum number: IF556435; Facesoffungi number: FoF 05771

Etymology – the specific epithet “brachiata” refers to the character of apical appendages (Latin ‘brachiata’ means ‘branches’).

Holotype – MFLU 19-0776

Associated with leaf spots of *Rhizophora apiculata* Blume. *Symptoms* circular or sub circular shape, grayish brown, slightly sunken spots appear on adaxial surface leaves of *R. apiculata*, which later expand outwards (Fig. 3c). Small auburn spots appeared initially and then gradually enlarged, changing to grayish brown circular ring spots with a dark brown border. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spreaded evenly on the leaves (Fig. 3b). Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to ampulliform to lageniform, hyaline, smooth- and thin-walled, simple, proliferating 1–2 times percurrently, collarete present and not flared, 5–10 × 5–8 μm. *Conidia* (18–)18.5–25(–26) × (5–)5.5–6(–6.5) μm (mean ± SD = 21 ± 2.2 × 5.5 ± 0.6 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)3.5–4(–5) μm long (mean ± SD = 3.8 ± 0.7 μm); three median cells (10–)10.5–15(–16) μm long (mean ± SD = 12.7 ± 1.7 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (3–)4–5(–6) μm long (mean ± SD = 4.4 ± 0.8 μm); third cell brown, (3.5–)4–5(–6) μm long (mean ± SD = 4.2 ± 0.7 μm); fourth cell brown, (3–)4–5(–5.5) μm long (mean ± SD = 4.1 ± 0.5 μm); apical cell (4–)4.5–5(–6) μm long (mean ± SD = 4.5 ± 0.7 μm), hyaline, conic to acute; with 1–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, branched, flexuous, (8.5–)9.5–33(–34) μm long (mean ± SD = 20 ± 7.6 μm); single basal appendage, tubular, unbranched, centric, (3.5–)4–9(–10) μm long (mean ± SD = 6.5 ± 1.6 μm).

Culture characteristics – Colonies on PDA reaching 6–7 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora apiculata*, 6 December 2016, Norphanphoun Chada NG33 (MFLU 19-0776 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1555, TNCC.

Notes – *Neopestalotiopsis brachiata* is similar to *N. rosicola* (strain CFCC 51992) in conidial size (*N. brachiata* (18–)18.5–25(–26) × (5–)5.5–6(–6.5) μm vs. *N. rosicola*: (19–)20–25.5(–26) × (5–)5.5–8(–8.5) μm) but phylogenetically distinct (Jiang et al. 2018). In the multigene phylogenetic analysis presented here, the new species formed a sister clade to *N. aotearoa* (strain CBS 367.54), *N. piceanae* (strain CBS 394.48) and *N. petila* (in this study), which appear to be phylogenetically distinct (Fig. 1). The conidia of *N. brachiata* are different from *N. aotearoa* ((19.5–)21–28(–29) × (6–)6.5–8.5(–9) μm), *N. piceanae* ((19–)19.5–25(–26) × (7–)7.5–9(–9.5) μm) and *N. petila* ((20–)21–26.5(–27.5) × (5.5–)6–7(–8) μm) and also differs by having branched, flexuous apical tubular appendages (Maharachchikumbura et al. 2014).

Neopestalotiopsis petila Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 4

Index Fungorum number: IF556436; Facesoffungi number: FoF 05772

Etymology – the latin “petilus” meaning slender/slim, which refers to the shape of conidia.

Holotype – MFLU 19-0777

Associated with leaf spots of *Rhizophora mucronata* Lam. *Symptoms* subcircular to the irregular shape, pale brown, slightly sunken spots appear on adaxial surface leaves of *R. mucronata*, which later expand outwards (Fig. 4c). Small auburn spots appeared initially and then gradually enlarged, changing to pale-auburn circular ring spots with a dark auburn border.

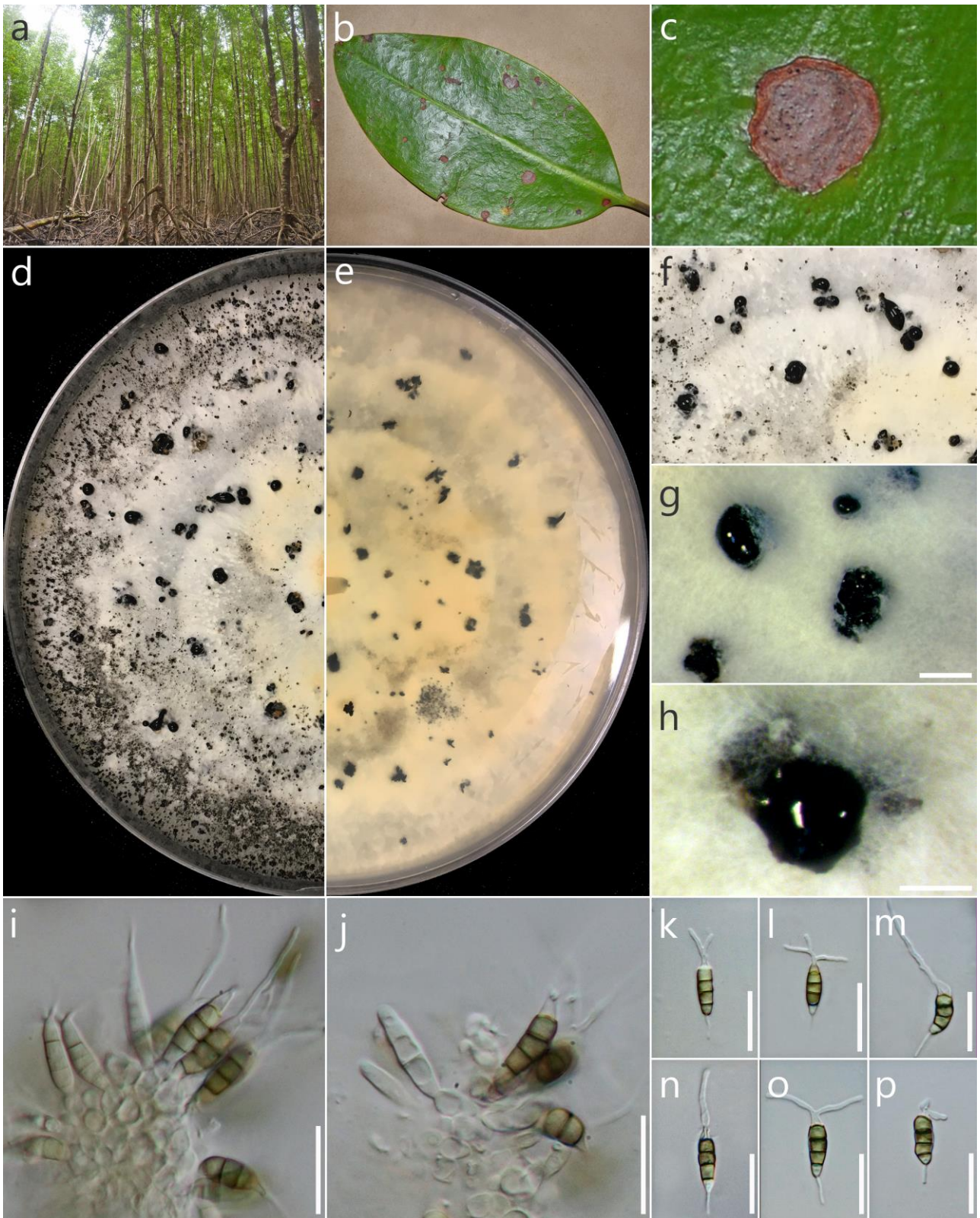


Figure 3 – *Neopestalotiopsis brachiata* (MFLU 19-0776, holotype). a Habitat. b, c Leaf spots on *Rhizophora apiculata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 1000 μ m, h = 500 μ m, i–p = 20 μ m.

They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 4b). Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass.

Conidiophores distinct. *Conidiogenous cells* discrete to integrated, hyaline, smooth- and thin-walled, proliferating one time percurrently, collarete present and not flared. *Conidia* (20–)21–26.5(–27.5) × (5.5–)6–7(–8) μm (mean ± SD = 24.5 ± 2.0 × 6.7 ± 0.7 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)4–5.5(–6) μm long (mean ± SD = 4.52 ± 0.8 μm); three median cells (12.5–)13.5–15(–17) μm long (mean ± SD = 15.3 ± 1.0 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) μm long (mean ± SD = 5.2 ± 0.5 μm); third cell brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.8 ± 0.5 μm); fourth cell brown, (4.5–)5–5.5(–6) μm long (mean ± SD = 5.3 ± 0.5 μm); apical cell (3–)4–5(–7) μm long (mean ± SD = 4.6 ± 1.0 μm), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (21–)22–29(–33) μm long (mean ± SD = 25.5 ± 3.1 μm); single basal appendage, tubular, unbranched, centric, (2–)3–8(–9) μm long (mean ± SD = 6 ± 2.1 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Kor Chang, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC05-1 (MFLU 19-0777 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1737, TNCC. THAILAND, Kor Chang, Trat Province, leaf spots of *R. mucronata*, 27 April 2017, Norphanphoun Chada KC05-2 (MFLU 19-0778 dried culture, paratype); living cultures, MFLUCC 17-1738.

Notes – Based on multigene analyses, *Neopestalotiopsis petila* is closely related to *N. aotearoa* (strain CBS 367.54), *N. piceana* (strain CBS 394.48), *N. brachiata* (in this study) and *N. samarangensis* (strain MFLUCC 12-0233) (Fig. 1). However, *N. petila* differs from *N. aotearoa* in having long apical appendages (*N. petila*: (21–)22–29(–33) vs. *N. aotearoa*: (3–)5–12(–13)) and differs from *N. piceana* by having 2–3 apical appendages (*N. piceana* containing only 3 appendages) and short basal appendage (*N. petila*: (2–)3–8(–9) vs. *N. piceana*: 6–23) and larger conidia than *N. samarangensis* (*N. petila*: (20–)21–26.5(–27.5) × (5.6–)6–7(–7.8) vs. *N. samarangensis*: 18–21 × 6.5–7.5) (Maharachchikumbura et al. 2014, 2013). The morphological differences between *N. petila* and *N. brachiata* have been mentioned above as notes of *N. brachiata*. Thus, *N. petila* is considered a novel species.

Neopestalotiopsis rhizophorae Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 5

Index Fungorum number: IF556437; Facesoffungi number: FoF 05773

Etymology – refers to the host from which the fungus was collected, *Rhizophora mucronata* Lam.

Holotype – MFLU 19-0779

Associated with leaf spots of *Rhizophora mucronata* Lam. Symptoms irregular to subcircular shape, brown, slightly sunken spots appear on adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves (Fig. 5c). Small auburn spots appeared initially and then gradually enlarged, changing to tawny circular ring spots with a dark mahogany border and jagged edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 5b). Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* distinct. *Conidiogenous cells* discrete or integrated, ampulliform, clavate or subcylindrical, hyaline, smooth, 8–20 × 4–8 μm. *Conidia* (20–)20.5–27(–27.5) × (6–)6.5–7.5(–8) μm (mean ± SD = 24.5 ± 0.3 × 7.2 ± 0.6 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2–)3–4(–5) μm long (mean ± SD = 3.7 ± 0.6 μm); three median cells (14–)15.5–17(–19.5) μm long (mean ± SD = 16.7 ± 1.4 μm), brown, septa and periclinal walls darker than rest

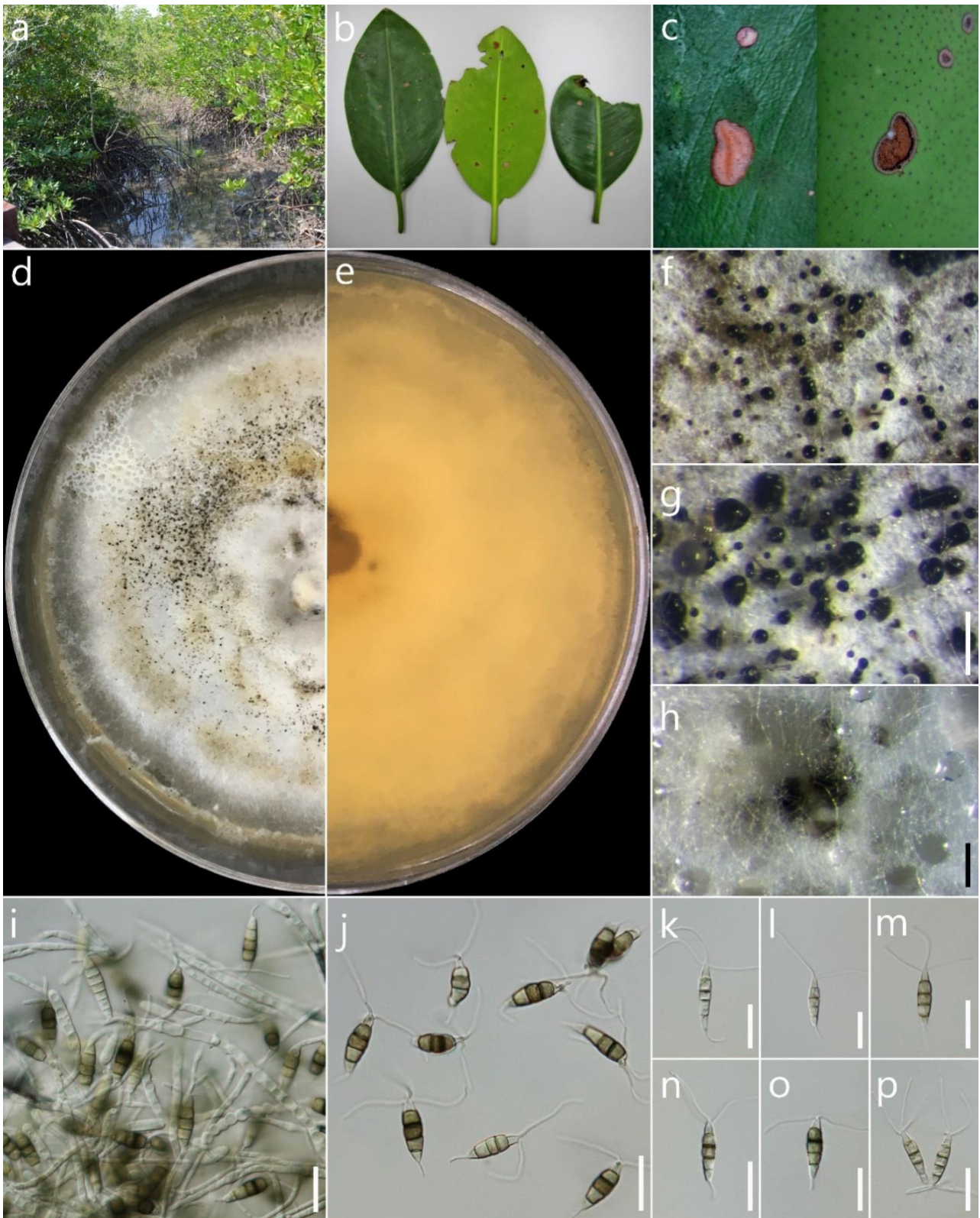


Figure 4 – *Neopestalotiopsis petila* (MFLU 19-0777, holotype). a Habitat. b, c Leaf spots on *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g–h = 200 μ m, i–p = 20 μ m.

of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) μ m long (mean \pm SD = 5.9 \pm 0.7 μ m); third cell brown, (4–)5–7(–10) μ m long (mean \pm SD = 5.2 \pm 0.6 μ m); fourth cell brown, (4–)5–5.5(–6.5) μ m long (mean \pm SD = 5.5 \pm 0.6 μ m); apical cell (3–)3.5–4(–5.5) μ m long (mean \pm SD = 4.1 \pm 0.7 μ m), hyaline, conic to acute; with 2–3 tubular appendages on

apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (6–)12.5–22(–24) μm long (mean \pm SD = 17.5 \pm 4.4 μm); single basal appendage, tubular, unbranched, centric, with or without knob (2.5–)3–9.5(–10) μm long (mean \pm SD = 5.2 \pm 1.8 μm).

Culture characteristics – Colonies on PDA reaching 7–8 cm diam after 7 d at room temperature (± 25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora mucronata*, 6 December 2016, Norphanphoun Chada NG16a (MFLU 19-0779 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1550, TNCC. THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora mucronata*, 6 December 2016, Norphanphoun Chada NG16b (MFLU 19-0780 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1551.

Notes – The new species *Neopestalotiopsis rhizophorae* (MFLUCC 17-1550, MFLUCC 17-1551) is isolated from *R. mucronata*, from Ranong province in Thailand. *Neopestalotiopsis rhizophorae* is most similar to *N. petila* and *N. thailandica* (this study) in its conidial size (*N. petila*: (20–)21–26.5(–27.5) \times (5.5–)6–7(–8) μm , *N. thailandica*: (20–)21–25(–25.5) \times (5.5–)6–7(–7.5) μm) (Table 3). However, based on combined gene phylogenetic analysis, *N. rhizophorae* is separated from *N. petila* and *N. thailandica*, and nested between these two species; *N. alpapicalis* (MFLUCC 17-2544, MFLUCC 17-2545), the collections from *R. mucronata* in Phuket, Thailand and *N. rosicola* (strain CFCC 51992, CFCC 51993), the collections from *Rosa chinensis* in China (Fig. 1). *Neopestalotiopsis rhizophorae* can be distinguished from *N. alpapicalis* by larger conidia and longer appendages (*N. alpapicalis*: conidial size 14–22.5 \times 5–7 μm , apical appendages 5.5–15 μm , basal appendages 3–6.5 μm , Table 3) with polymorphic nucleotide differences of β -tubulin (5-bp) and EF1 α (3-bp) sequence data. It differs from *N. rosicola* by ITS (4-bp) and EF1 α (4-bp) sequence data.

Neopestalotiopsis sonneratae Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 6

Index Fungorum number: IF556438; Facesoffungi number: FoF 05774

Etymology – refers to the host from which the fungus was isolated, *Sonneronata alba* L.

Holotype – MFLU 19-0781

Associated with leaf spots on *Sonneronata alba* L. *Symptoms* small circular shape, auburn, slightly spots appear on adaxial surface leaves of *S. alba*, which later expand outwards on the surface of the leaves (Fig. 6c). Small auburn spots appeared initially and then gradually enlarged, changing to dark brown circular spots and border with blurred edge. They were usually > 5 circulars, which occurred on a single affected leaf (Fig. 6b). Asexual morph: *Conidiomata* pycnidial, 200–400 μm diam, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 3–8 \times 2–6 μm , proliferating one time percurrently, collarete present and not flared. *Conidia* (21.5–)24–26(–28) \times 7–7.5(–8), (mean \pm SD = 24 \pm 1.6 \times 7.51 \pm 0.4 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2–)3–3.5(–4) μm long (mean \pm SD = 3 \pm 0.6); three median cells (14.5–)15–16.5(–17.5) μm long (mean \pm SD = 15.8 \pm 0.9), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) μm long (mean \pm SD = 5.6 \pm 0.8 μm); third cell brown, (4–)5–5.5(–6) μm long (mean \pm SD = 5.2 \pm 0.7 μm); fourth cell brown, (4–)5–6(–7) μm long (mean \pm SD = 5.1 \pm 0.7 μm); apical cell (3.5–)4–4.5(–5) μm long (mean \pm SD = 4 \pm 0.6 μm), hyaline, conic to acute; with 1–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (5.5–)7–8(–14) μm long (mean \pm SD = 8.5 \pm 2); single basal appendage, tubular, unbranched, centric, (2.5–)3–4(–5) μm long (mean \pm SD = 3.4 \pm 0.9) long.

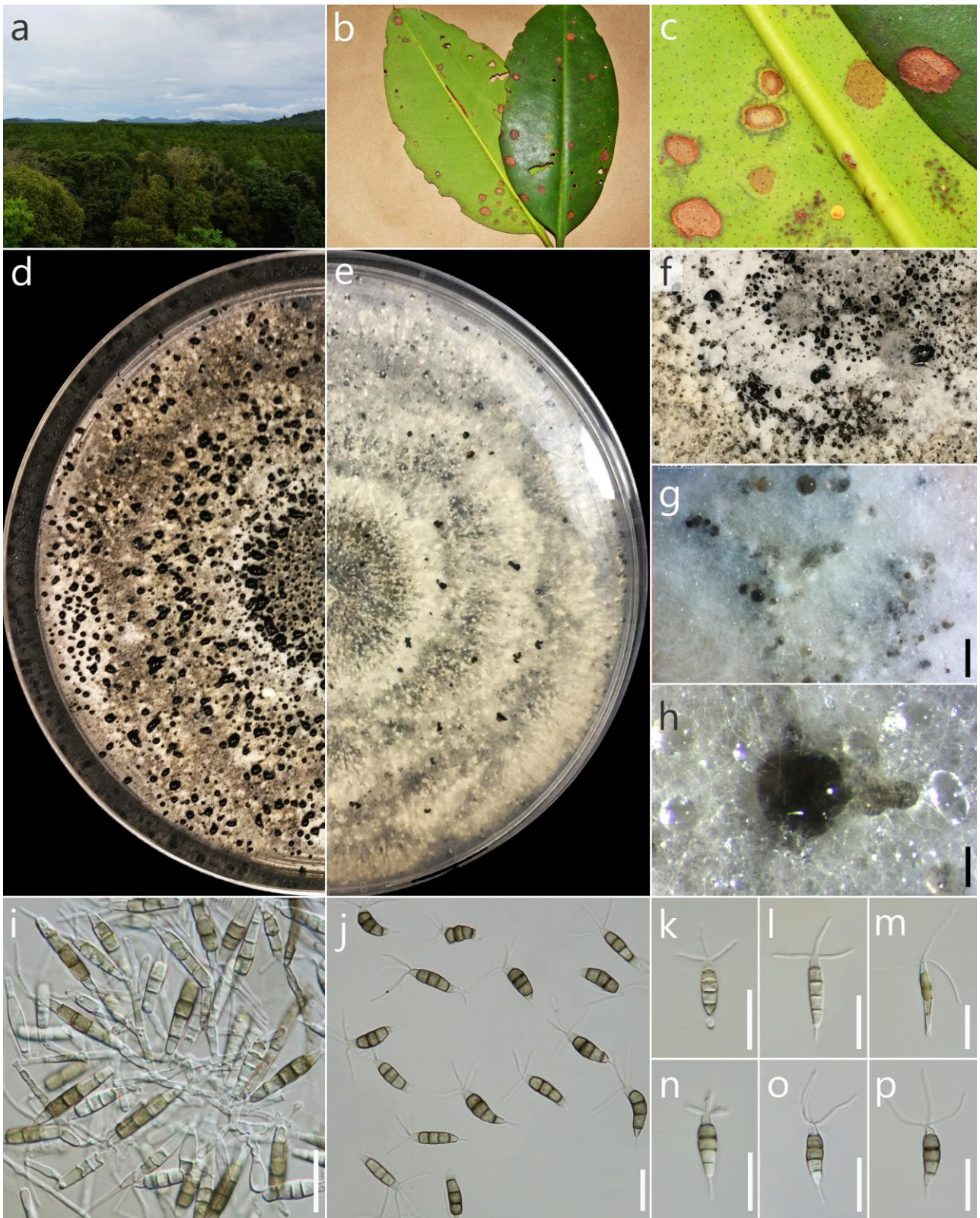


Figure 5 – *Neopestalotiopsis rhizophorae* (MFLU 19-0779, holotype). a Habitat. b, c Leaf spots on *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 1000 μ m, h = 100 μ m, i = 50 μ m, j–p = 20 μ m.

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (± 25 $^{\circ}$ C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial

mycelium on surface flat or raised, with filiform margin (curled margin), moderate-to-strongly fluffy, fluffy to floccose, white from above and reverse; fruiting bodies black.

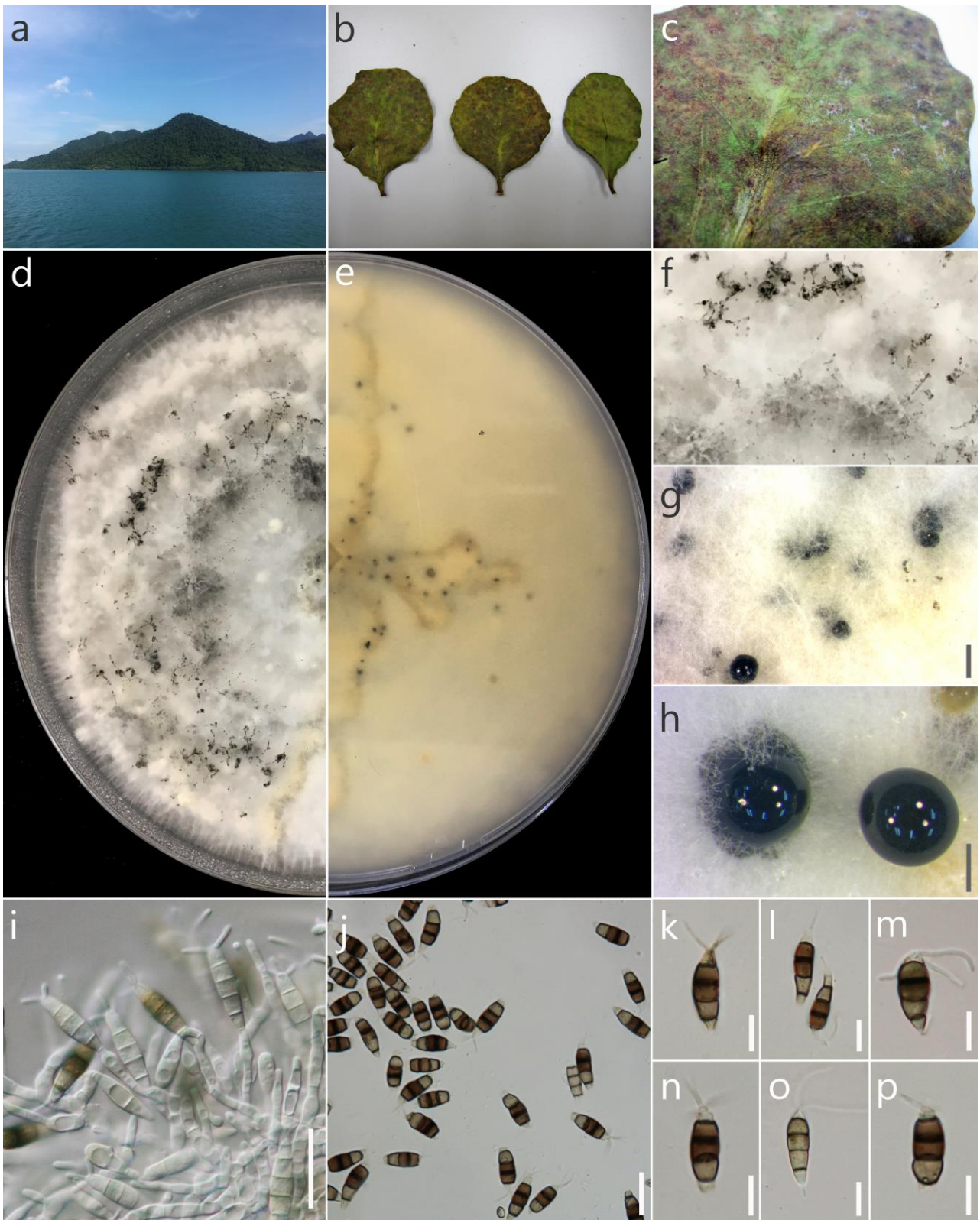


Figure 6 – *Neopestalotiopsis sonneratae* (MFLU 19-0781, holotype). a Habitat. b, c Leaf spots on *Sonneronata alba*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 1000 μ m, h = 500 μ m, i, j = 20 μ m, k–p = 10 μ m.

Material examined – THAILAND, Kor Chang, Trat Province, leaf spots of *Sonneronata alba*, 27 April 2017, Norphanphoun Chada KC01-1 (MFLU 19-0781 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1744, TNCC. THAILAND, Kor Chang, Trat Province, leaf spots of *Sonneronata alba*, 27 April 2017, Norphanphoun Chada KC01-2 (MFLU 19-0782 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1745.

Notes – *Neopestalotiopsis sonneratae* was isolated from a leaf spot of *Sonneronata alba* in Thailand. In the phylogenetic analyses of combined genes, *N. sonneratae* forms a sister group to *N. coffea-arabicae* (strain HGUP4015) and *N. thailandica* (in this study) (Fig. 1). However, there are significant differences in morphological characteristics; *N. coffea-arabicae* and *N. thailandica* conidia are smaller (*N. coffea-arabicae*: 16–20 × 5–7 µm, *N. thailandica*: (20–)21–25(–25.5) × (5.7–)6–7(–7.3) µm) with both species containing longer apical appendages (*N. coffea-arabicae*: 11–16 µm, *N. thailandica*: (30–)32.5–38(–40) µm) and *N. thailandica* having two apical appendages (Song et al. 2013).

Neopestalotiopsis thailandica Norphanphoun & K.D. Hyde, sp. nov.

Fig. 7

Index Fungorum number: IF556439; Facesoffungi number: FoF 05775

Etymology – refers to the country where the fungus was collected, Thailand.

Holotype – MFLU 19-0783

Associated with leaf spots of *Rhizophora mucronata* Lam. *Symptoms* small irregular to subcircular shape, brown, slightly sunken spots appear on adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves (Fig. 7c). Small auburn spots appeared initially and then gradually enlarged, changing to tawny circular ring spots with a dark mahogany border and jagged edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 7b). Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* distinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* (20–)21–25(–25.5) × 6–7(–7.5) µm (mean ± SD = 22.6 ± 1.3 × 6.6 ± 0.5 µm), fusiform to clavate, straight to slightly curved, 4(–7)-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2.5–)3–4(–4.5) µm long (mean ± SD = 3.7 ± 0.5 µm); threemedian cells (12–)12.5–15(–16) µm long (mean ± SD = 14.2 ± 0.9 µm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)4.5–5(–5.5) µm long (mean ± SD = 4.6 ± 0.3 µm); third cell brown, (3.5–)11–26(–27.5) µm long (mean ± SD = 4.5 ± 0.4 µm); fourth cell brown, (4–)5–5.5(–6) µm long (mean ± SD = 5.1 ± 0.6 µm); apical cell (3.5–)4–5.5(–6) µm long (mean ± SD = 4.7 ± 0.6 µm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (30–)32.5–38(–40) µm long (mean ± SD = 34.5 ± 3.7 µm); single basal appendage, tubular, unbranched, centric, (3–)6–9(–10) µm long (mean ± SD = 7.6 ± 2.2 µm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Kor Chang, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC11-1 (MFLU 19-0783 dried culture, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1730, TNCC. THAILAND, Kor Chang, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC11-2 (MFLU 19-0784 dried culture, paratype); ex-type-living cultures, MFLUCC 17-1731.

Notes – The collections (MFLUCC 17-1730, MFLUCC 17-1731) were observed and introduced as *N. thailandica*. The new species is introduced with the type from *Rhizophora mucronata* from Trat Province in Thailand. *Neopestalotiopsis thailandica* is most similar to

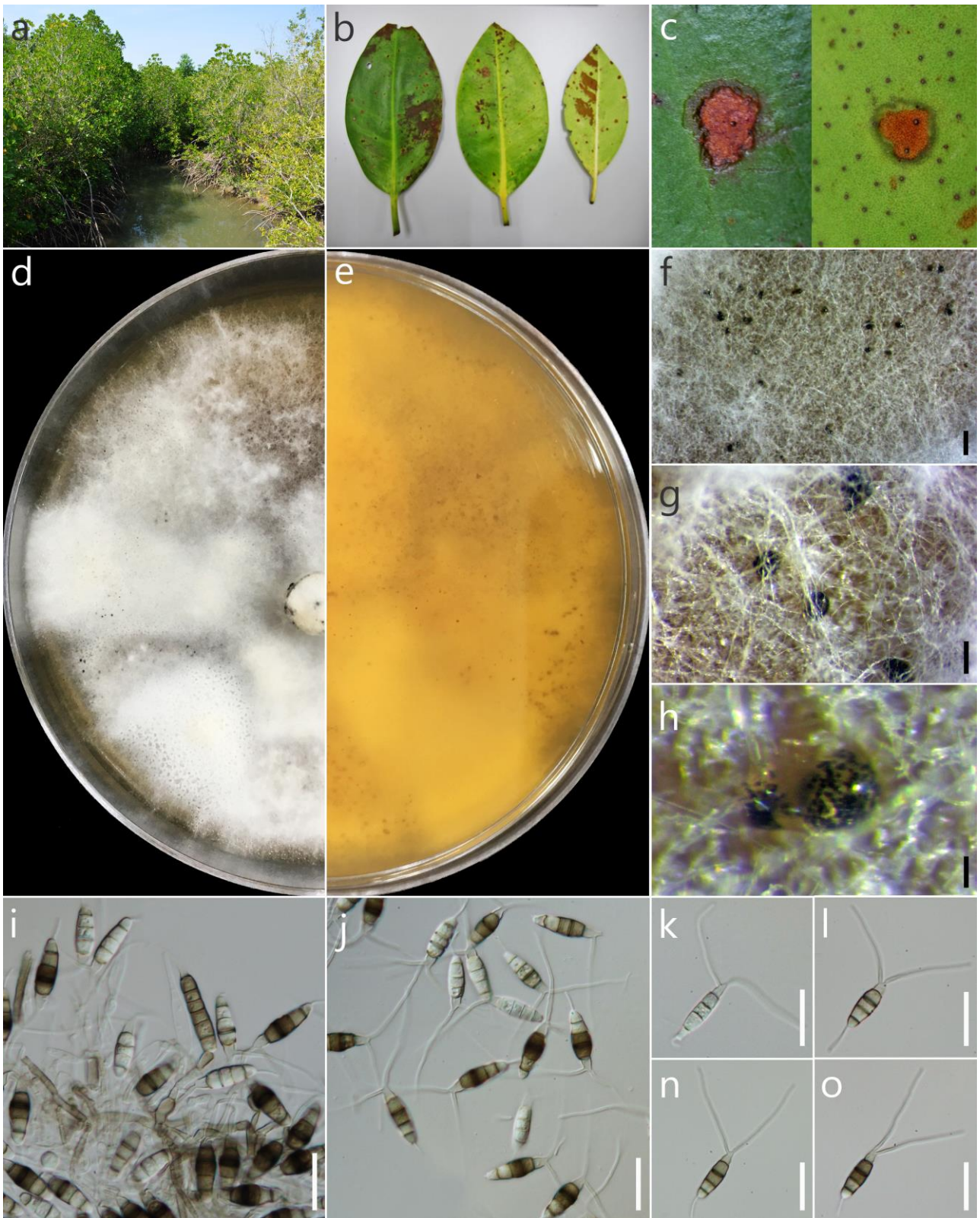


Figure 7 – *Neopestalotiopsis thailandica* (MFLU 19-0783, holotype). a Habitat. b, c Leaf spots on *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–o Conidia. Scale bars: f = 500 μ m, g = 200 μ m, h = 50 μ m, i–o = 20 μ m.

N. petila and *N. rhizophorae* (both species are introduced in this study) with its conidial size. The morphological differences between *N. petila* and *N. rhizophorae* have been mentioned above as notes of *N. rhizophorae* (Table 3). However, based on the combined gene phylogenetic analysis, *N.*

thailandica is separated from other species in the genus and is sister to *N. coffea-arabicae* (HGUP4015, Song et al. 2013), which was isolated from leaves of *Coffea arabica* L. and *N. sonneratae* (in this study) from leaf spots of *Sonneronata alba* (Fig. 1). However, their morphology is different. *Neopestalotiopsis thailandica* differs from *N. coffea-arabicae* by having larger conidia (*N. thailandica*: (20–)21–25(–25.5) × 6–7(–7.5) vs. *N. coffea-arabicae*: 16–20 × 5–7)), and longer apical appendages (*N. thailandica*: (30–)32.5–38(–40) vs. *N. coffea-arabicae*: 11–16) (Table 3). The morphological differences between *N. thailandica* and *N. sonneratae* are mentioned above under the notes of *N. sonneratae*. Thus, based on morphology and phylogeny it is considered that *N. thailandica* is a novel species.

The second phylogenetic tree (*Pestalotiopsis*) comprised 70 taxa with the outgroup, *Neopestalotiopsis saprophytica* (MFLUCC 12-0282). The total length of the dataset was 1440 characters including alignment gaps, 1–380, 381–538, 539–779, 780–979, 980–1147, and 1148–1440 corresponding to ITS1+ ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron), respectively. The combined dataset contained 887 constant, 174 parsimony uninformative and 379 parsimony informative characters. The combined dataset was analyzed using MP, ML and BI. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics generated from MP analysis based on the combined dataset of ITS1+ITS2, 5.8S, β-tubulin (exon), β-tubulin (intron), EF1α (exon), and EF1α (intron) were TL = 1664, CI = 0.510, RI = 0.685, RC = 0.349, HI = 0.490. The best scoring likelihood tree selected with a final value for the combined dataset = -10362.397645.

Pestalotiopsis rhizophorae Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 9

Index Fungorum number: IF556440; Facesoffungi number: FoF 05781

Etymology – refers to the host from which the fungus was isolated, *Rhizophora apiculata* Blume.

Holotype – MFLU 19-0785

Associated with leaf spots of Rhizophora apiculata Blume. *Symptoms* small irregular spots shape, rufous, slightly sunken spots adaxial surface leaves of *R. apiculata*, which later expand outwards on the surface of the leaves (Fig. 9c). Small rufous spots appeared initially and then gradually enlarged, changing to tawny circular ring spots with a dark brown border and smooth edge. They were usually few circulars, which occurred on a single affected leaf (Fig. 9b). Asexual morph: *Conidiomata* 2 0–7 0 μm diam, pycnidial, globose, brown, semi-immersed on PDA, rreleasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 1 0–2 0 × 1–2 μm, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* (17–)17.5–23(–23.5) × (5.5–)6–6.5(–7) μm (mean ± SD = 20 ± 1.6 × 6.3 ± 0.5 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (2–)3–3.5(–5) μm long (mean ± SD = 3.7 ± 0.7 μm); three median cells (11–)11.5–14(–14.5) μm long (mean ± SD = 13.2 ± 1.2 μm), brown, septa and periclinal walls darker than rest of the cell, concolorous, wall rugose; second cell from base pale brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.6 ± 0.6 μm); third cell brown, (3–)4–4.5(–5) μm long (mean ± SD = 4.1 ± 0.6 μm); fourth cell brown, (3.5–)4–5(–5.5) μm long (mean ± SD = 4.6 ± 0.5 μm); apical cell (1.8–)2–3(–4.5) μm long (mean ± SD = 3.1 ± 0.5 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (7.5–)8–13(–14.5) μm long (mean ± SD = 11.4 ± 2.4 μm); single basal appendage, tubular, unbranched, centric, 1.5–4.5(–5) μm long (mean ± SD = 2.5 ± 1.1 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

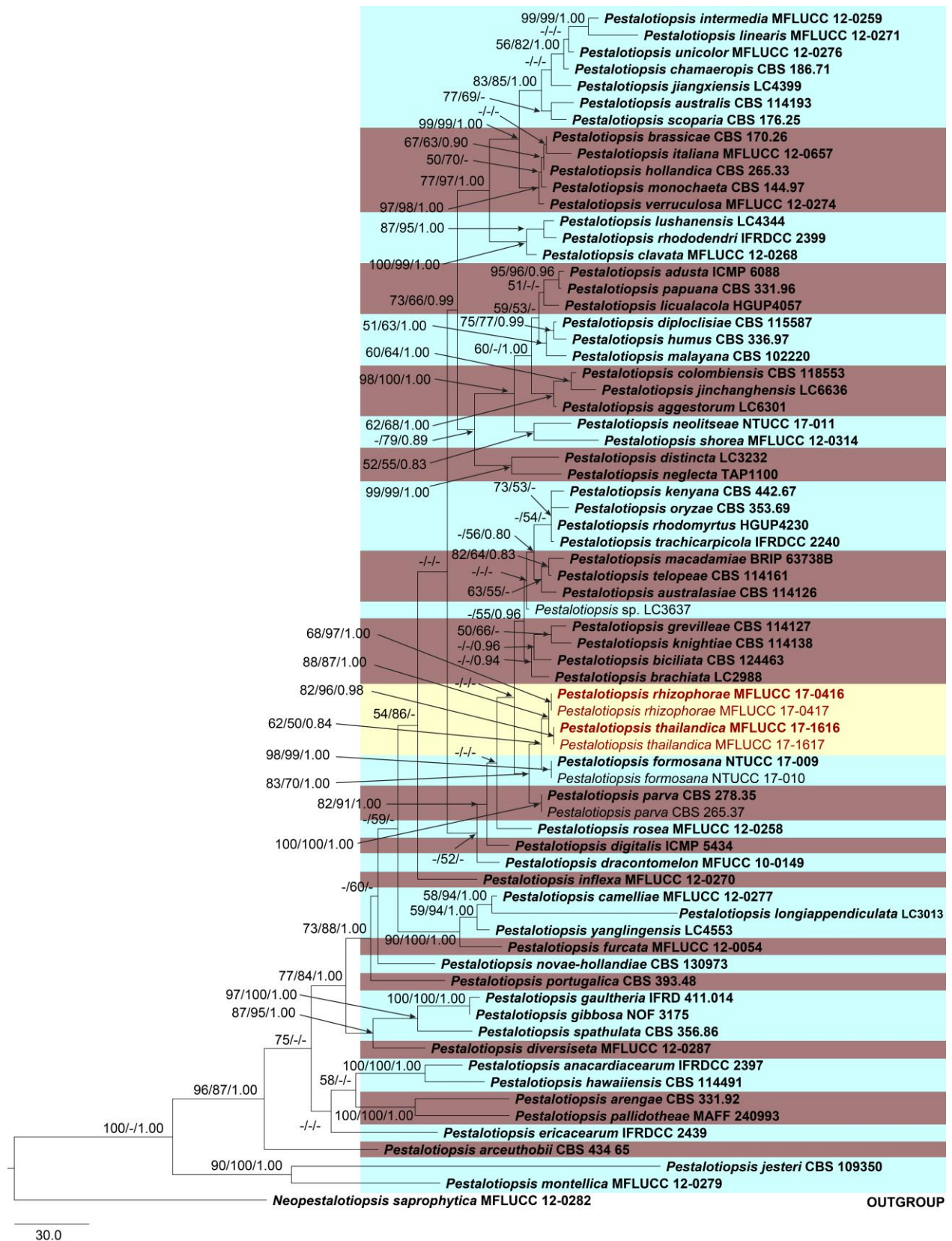


Figure 8 – One of the 1000 most parsimonious trees obtained from a heuristic search of combined ITS, β -tubulin and EF1 α sequence data for the genus *Pestalotiopsis*. The tree is rooted to *Neopestalotiopsis saprophytica* (MFLUCC 12-0282). Maximum parsimony and maximum likelihood bootstrap values $\leq 50\%$. Bayesian posterior probabilities ≤ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. The species obtained in this study are in red font. Ex-type taxa from other studies are in black bold.

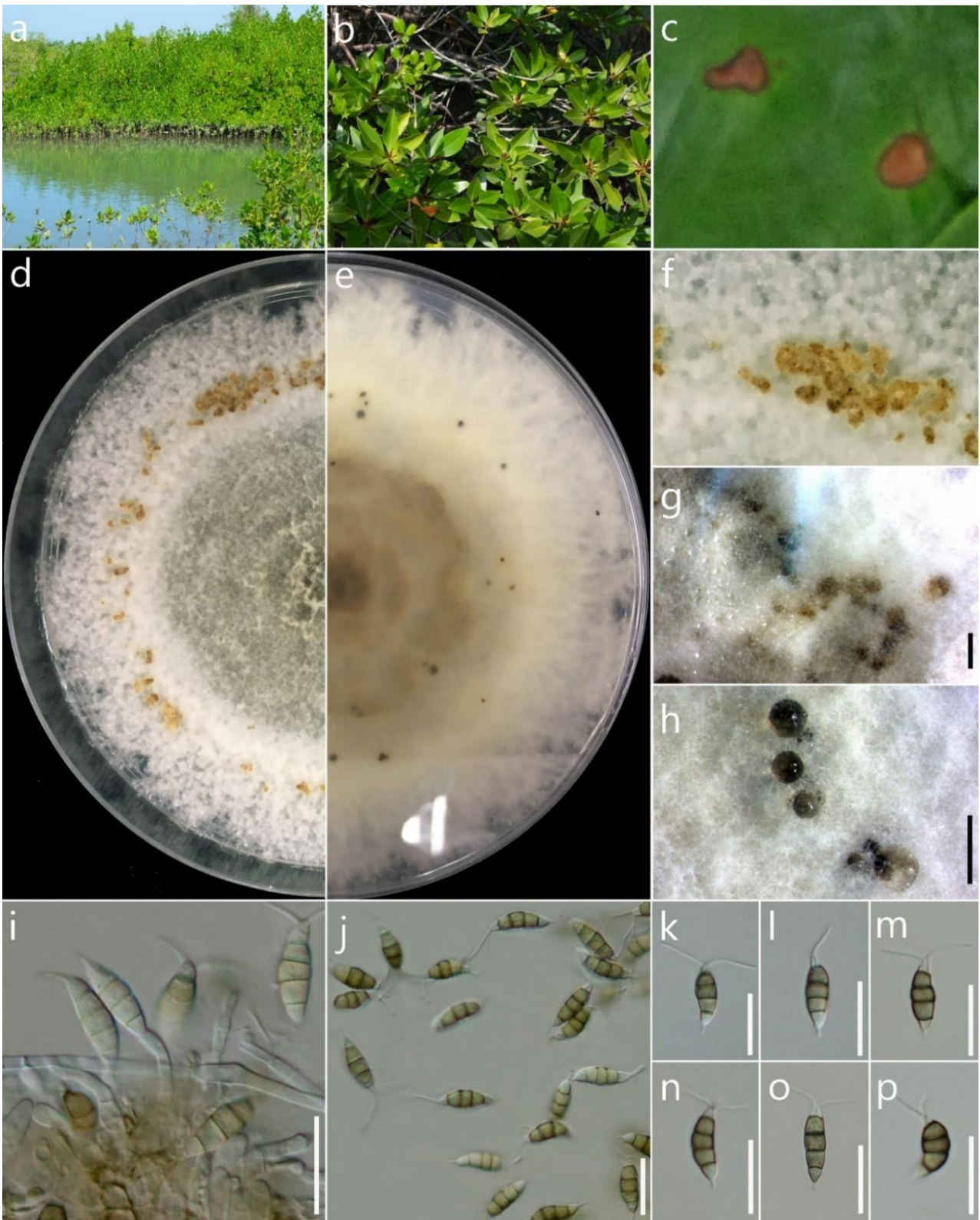


Figure 9 – *Pestalotiopsis rhizophorae* (MFLU 19-0785, holotype). a Habitat b, c Leaf spots of *Rhizophora apiculata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f–h = 100 µm, i–p = 20 µm.

Material examined – THAILAND, The Sirindhorn International Environmental Park, Cha-am District, Phetchaburi Province, leaf spots on *Rhizophora apiculata*, 30 November 2016, Norphanphoun Chada NNS28a (MFLU 19-0785, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-0416, TNCC. THAILAND, The Sirindhorn International Environmental Park, Cha-

am District, Phetchaburi Province, leaf spots on *Rhizophora apiculata*, 30 November 2016, Norphanphoun Chada NNS28b (MFLU 19-0786, paratype); living cultures, MFLUCC 17-0417.

Notes – *Pestalotiopsis rhizophorae* formed a distinct clade in the multi-locus tree and is sister to *P. formosana* (strain NTUCC 17-009), *P. parva* (strain CBS 265.37) and *P. thailandica* (in this study) (Fig. 2). *Pestalotiopsis rhizophorae* differs from *P. thailandica* and *P. formosana* by shorter conidia (*P. rhizophorae*: (17–)17.5–23(–23.5) μm vs. *P. thailandica*: (17–)17.5–28(–29) μm , *P. formosana*: (15–)18–22(–26) \times (5–)6–7 μm); shorter apical appendages (*P. rhizophorae*: (7.5–)8–13(–14.5) μm vs. *P. thailandica*: (5.5–)11–34(–38) μm) and basal appendages (*P. rhizophorae*: (1.3–)1.5–4.5(–5) μm vs. *P. thailandica*: (2–)2.5–9.5(–10) μm , *P. formosana*: (2–)3–5(–6) μm) (Table 4, Ariyawansa and Hyde 2018, Maharachchikumbura et al. 2014). *Pestalotiopsis rhizophorae* is similar to *P. parva* in conidial size (*P. parva*: (16–)16.5–20(–21) \times 5–7(–7.5) μm) but differs by having 1–2 apical appendages (Table 4, Ariyawansa and Hyde 2018, Maharachchikumbura et al. 2014). The two species (*P. rhizophorae*, *P. thailandica*) are also different in four base pairs in ITS, one base pair in β -tubulin and EF1 α . Therefore, the collection in the present study is designated as a new species.

Pestalotiopsis thailandica Norphanphoun, Doilom & K.D. Hyde, sp. nov.

Fig. 10

Index Fungorum number: IF556441; Facesoffungi number: FoF 05782

Etymology – refers to the country where the fungus was collected, Thailand.

Holotype – MFLU 19-0787

Isolated from asymptomatic leaf of *Rhizophora apiculata* Blume. Asexual morph: *Conidiomata* pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* (17–)17.5–28(–29) \times (4.9–)5.5–6.5(–7.1) μm (mean \pm SD = 23.3 \pm 3.0 \times 5.8 \pm 0.5 μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (1.8–)2–4(–6) μm long (mean \pm SD = 3.9 \pm 1.3 μm); three median cells (12–)12.5–16(–18) μm long (mean \pm SD = 15.3 \pm 1.5 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)4.5–6(–7) μm long (mean \pm SD = 5.6 \pm 0.8 μm); third cell brown, (3.5–)4–4.5(–5.5) μm long (mean \pm SD = 4.6 \pm 0.5 μm); fourth cell brown, (3.5–)4–5(–6.5) μm long (mean \pm SD = 5.1 \pm 0.7 μm); apical cell (2–)3.5–4(–6) μm long (mean \pm SD = 4.2 \pm 1.0 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at same loci at the apex of the apical cell, unbranched, flexuous, (5.5–)11–34(–38) μm long (mean \pm SD = 22.2 \pm 8.5 μm); single basal appendage, tubular, unbranched, centric, (2–)2.5–9.5(–10) μm long (mean \pm SD = 4 \pm 2.0 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (± 25 $^{\circ}\text{C}$), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, The Sirindhorn International Environmental Park, Cha-am, Cha-am District, Phetchaburi Province, asymptomatic leaf of *Rhizophora apiculata*, 30 November 2016, Mingkwan Doilom NNSE03AL (MFLU 19-0787, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1616, TNCC. THAILAND, The Sirindhorn International Environmental Park, Cha-am, Cha-am District, Phetchaburi Province, asymptomatic leaf of *Rhizophora apiculata*, 30 November 2016, Mingkwan Doilom NNSE03DL (MFLU 19-0788, paratype); living cultures, MFLUCC 17-1617.

Notes – *Pestalotiopsis thailandica* isolated as an endophyte from a living leaf of *Rhizophora apiculata* is introduced here as a new species. In the phylogenetic analyses based on combined genes, the species appeared as a distinct species represented by two strains and is sister to *P. rhizophorae* (in this study) (Fig. 2). Although these two species are found on the same host and

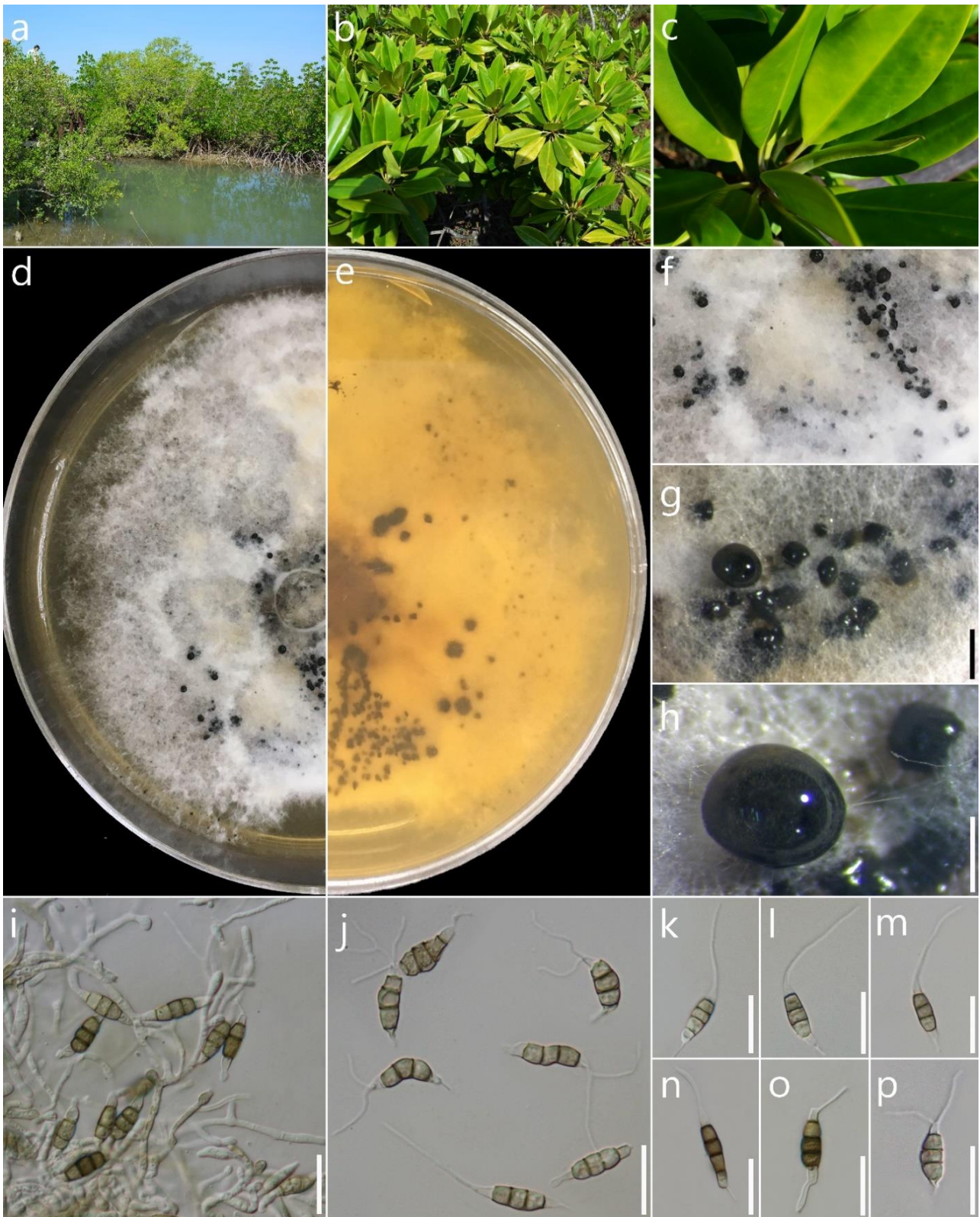


Figure 10 – *Pestalotiopsis thailandica* (MFLU 19-0787, holotype). a Habitat. b, c Leaf of *Rhizophora apiculata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 1000 μm , h = 100 μm , i–p = 20 μm .

location, *P. thailandica* differs by having larger conidia (*P. rhizophorae*: (17–)17.5–23(–23.5) \times (5.5–)6–6.5(–7) μm) and longer apical appendages (*P. rhizophorae*: (7.5–)8–13(–14.5) μm) than *P. rhizophorae* (Table 4) (see notes under *P. rhizophorae*). In the phylogenetic analyses, *P.*

rhizophorae is also related to *P. formosana* (NTUCC 17-009) and *P. parva* (CBS 265. 37). However, *Pestalotiopsis formosana* and *P. parva* are different from *P. thailandica* by having smaller conidia (*P. formosana*: (15–)18–22(–26) μm , *P. parva*: (16–)16.5–20(–21) μm) and shorter apical appendages (*P. formosana*: (8–)11–16(–20) μm , *P. parva*: (6–)6.5–12(–13) μm) with 2–3 tubular apical appendages (Table 4, Ariyawansa & Hyde 2018, Maharachchikumbura et al. 2014).

The third phylogenetic tree comprised 27 taxa, with *Neopestalotiopsis natalensis* (CBS 138.41) as the outgroup taxon. The total length of the dataset was 1404 characters including alignment gaps, 1–335, 336–493, 494–742, 743–925, 926–1093 and 1094–1404 corresponding to ITS1+ ITS2, 5.8S, β -tubulin (exon), β -tubulin (intron), EF1 α (exon), and EF1 α (intron), respectively). The combined dataset contained 1122 constant, 193 parsimony uninformative and 89 parsimony informative characters. The combined dataset was analyzed using MP, ML and BI. The trees generated under different optimality criteria were essentially similar in topology and did not differ significantly (data not shown). The descriptive statistics generated from MP analysis based on the combined dataset of ITS1+ITS2, 5.8S, β -tubulin (exon), β -tubulin (intron), EF1 α (exon), and EF1 α (intron) were TL = 386, CI = 0.832, RI = 0.823, RC = 0.685, HI = 0.168. The best scoring likelihood tree selected with a final value for the combined dataset = -3905.071762.

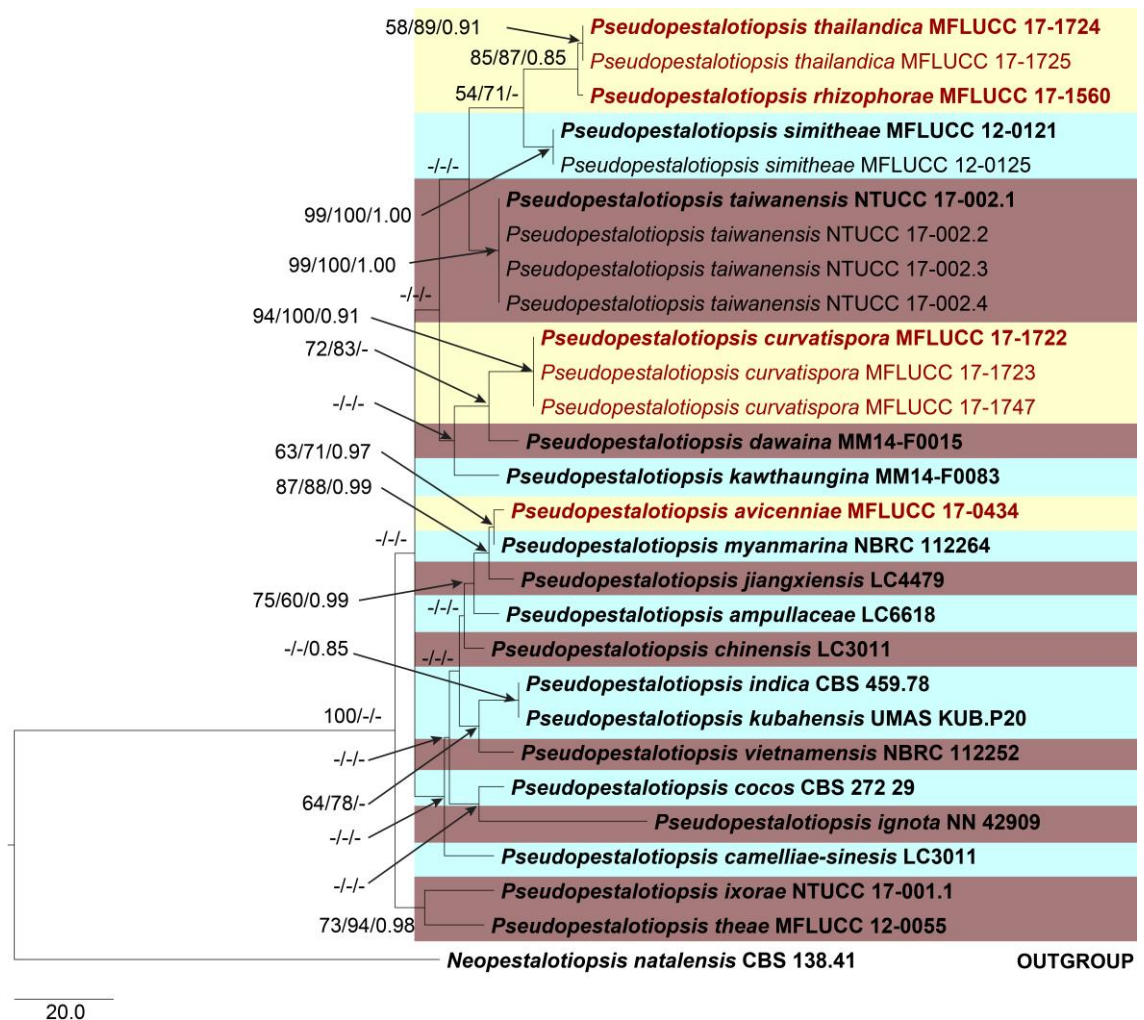


Figure 11 – One of the 93 most parsimonious trees obtained from a heuristic search of combined ITS, β -tubulin and EF1 α sequence data of the genus *Pseudopestalotiopsis*. The tree is rooted with *Neopestalotiopsis natalensis* (CBS 138.41). Maximum parsimony and maximum likelihood bootstrap values $\leq 50\%$, Bayesian posterior probabilities ≤ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. The species obtained in this study are in red font. Ex-type taxa from other studies are in black bold.

Pseudopestalotiopsis avicenniae Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 12

Index Fungorum number: IF556442; Facesoffungi number: FoF 05776

Etymology – refers to the host from which the fungus was isolated, *Avicennia marina* (Forssk.) Vierh.

Holotype – MFLU 19-0789

Associated with leaf spots of *Avicennia marina* (Forssk.) Vierh. *Symptoms* circular to irregular, grayish-brown, slightly sunken spots adaxial surface leaves of *A. marina*, which later expand outwards on the surface of the leaves (Fig. 12c). Small black spots appeared initially and then gradually enlarged, changing to beige circular ring spots with a black border and smooth edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 12b). Asexual morph: *Conidiomata* 300–1000 µm diam, pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, 3–6 × 2–56 µm, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* (22–)22.5–26.5(–27) × (5–)5.5–6(–6.5) µm (mean ± SD = 23.9 ± 1.4 × 6 ± 0.4 µm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3–)4–4.5(–6) µm long (mean ± SD = 4 ± 0.7 µm); three median cells (15–)15.5–17(–18) µm long (mean ± SD = 16.3 ± 0.8 µm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–6.5) µm long (mean ± SD = 5.5 ± 0.5 µm); third cell brown, (4.5–)5–5.5(–6) µm long (mean ± SD = 4.9 ± 0.4 µm); fourth cell brown, (5–)5.5–6(–7) µm long (mean ± SD = 5.9 ± 0.5 µm); apical cell (2–)2.5–4(–5) µm long (mean ± SD = 3.5 ± 1.0 µm), hyaline, conic to acute; with 1–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (14–)15.5–28.5(–35.5) µm long (mean ± SD = 21.3 ± 5.9 µm); single basal appendage, tubular, unbranched, centric, (2–)3–4(–4.5) µm long (mean ± SD = 3.1 ± 0.7 µm).

Culture characteristics – Colonies on PDA reaching 5–7 cm diam after 7 d at room temperature (±25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, The Sirindhorn International Environmental Park, Cha-am, Cha-am District, Phetchaburi Province, leaf spots of *Avicennia marina*, 30 November 2016, Norphanphoun Chada NNS05-1 (MFLU 19-0789, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-0434, TNCC.

Notes – The combined phylogenetic tree showed that *Ps. avicenniae* is sister to *Ps. jiangxiensis* (strain LC4479) and *Ps. elaeidis* (= *Ps. myanmarina*, strain NBRC 112264) (Fig. 1, Liu et al. 2017, Nozawa et al. 2017). *Pseudopestalotiopsis avicenniae* is morphologically similar to *Ps. jiangxiensis*, but phylogenetically clearly distinct as an independent lineage (Fig. 3). *Pseudopestalotiopsis avicenniae* is phylogenetically closer to *Ps. elaeidis* (strain NBRC 112264), which was collected from *Averrhoa carambola* in Myanmar, but can be distinguished by its larger conidia (*Ps. elaeidis*: 31–38.5 × 6.5–9 µm) and 2–3 apical appendages (Table 5, Nozawa et al. 2017).

Pseudopestalotiopsis curvatispora Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov.

Fig. 13

Index Fungorum number: IF556443; Facesoffungi number: FoF 05777

Etymology – in reference to the Latin word “curvatus” in reference to the shape of the conidia.

Holotype – MFLU 19-0790

Associated with leaf spots of *Rhizophora mucronata* Blume. *Symptoms* small subcircular, grayish brown, slightly sunken spots adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves (Fig. 13c). Small brown spots appeared initially and then

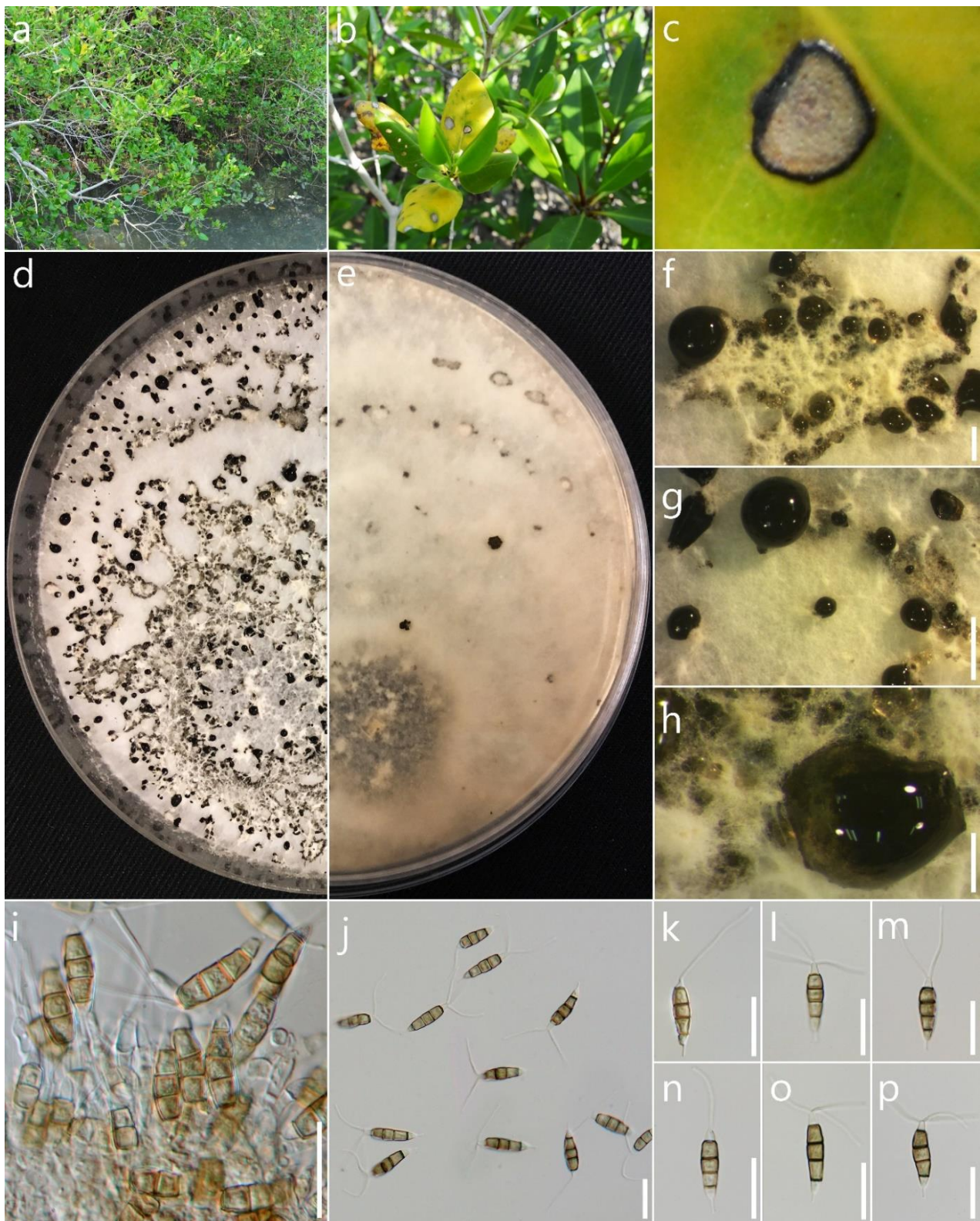


Figure 12 – *Pseudoestalotiopsis avicenniae* (MFLU 19-0789, holotype). a Habitat. b, c Leaf spots on *Avicennia marina*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f = 1000 μ m, g = 200 μ m, h = 500 μ m, i–p = 20 μ m.

gradually enlarged, changing to beige circular ring spots with a dark brown border and smooth edge. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 13b). Asexual morph: *Conidiomata* 200–400 μ m diam,

pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, $5.5\text{--}11 \times 3\text{--}5.5 \mu\text{m}$, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* $(18.5\text{--})22\text{--}25(\text{--}26.5) \times (6\text{--})6.5\text{--}7 \mu\text{m}$ (mean \pm SD = $23.5 \pm 2 \times 6.6 \pm 0.4 \mu\text{m}$), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, $(3\text{--})3.5\text{--}4(\text{--}5) \mu\text{m}$ long (mean \pm SD = $4.3 \pm 0.7 \mu\text{m}$); three median cells $(11\text{--})13\text{--}14(\text{--}15) \mu\text{m}$ long (mean \pm SD = $13.8 \pm 1.1 \mu\text{m}$), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, $4\text{--}5 \mu\text{m}$ long (mean \pm SD = $4.5 \pm 0.5 \mu\text{m}$); third cell brown, $(3.5\text{--})4\text{--}5(\text{--}5.5) \mu\text{m}$ long (mean \pm SD = $4.4 \pm 0.6 \mu\text{m}$); fourth cell brown, $(3.5\text{--})4\text{--}5(\text{--}5.5) \mu\text{m}$ long (mean \pm SD = $4.6 \pm 0.6 \mu\text{m}$); apical cell $(3.5\text{--})4\text{--}6(\text{--}7) \mu\text{m}$ long (mean \pm SD = $5.1 \pm 1.1 \mu\text{m}$), hyaline, conic to acute; with 1–2 tubular appendages on an apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched or branched irregularly along their length resulting in 1–2 branches, flexuous, $(10\text{--})20\text{--}29(\text{--}35) \mu\text{m}$ long (mean \pm SD = $22.7 \pm 8.1 \mu\text{m}$); single basal appendage, tubular, unbranched, centric, $(5.5\text{--})9\text{--}12(\text{--}13.5) \mu\text{m}$ long (mean \pm SD = $9.2 \pm 2 \mu\text{m}$).

Culture characteristics – Colonies on PDA reaching 3–4 cm diam after 7 d at room temperature ($\pm 25^\circ\text{C}$), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC12-1, (MFLU 19-0791, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1722, TNCC. THAILAND, Trat Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC12-2, (MFL 19-0792, paratype); living cultures, MFLUCC 17-1723. THAILAND, Trat Province, leaf spots of *Rhizophora apiculata*, 27 April 2017, Norphanphoun Chada KC18-2 (MFLU 19-0790, paratype); ex-type-living cultures, MFLUCC 17-1747.

Notes – The new species *Pseudopezalotiopsis curvatispora* is introduced, which was isolated from a leaf spot on *Rhizophora mucronata*, with the morphology of curved conidia and flexuous branched apical appendages. Based on combined gene phylogenetic analyses, it showed that *Ps. curvatispora* is nested in between *Ps. simitheae* (MFLUCC 12-0121) and *Ps. thailandica* (in this study), which are morphologically different (Table 5). *Pseudopezalotiopsis simitheae* (MFLUCC 12-0121), distinct from other species by forming a well-separated clade (Fig. 3). This is also supported by morphological differences (larger conidia $22\text{--}30 \mu\text{m}$) and 2–4 tubular, shorter apical and basal appendages (apical appendages: $14.5\text{--}26.5 \mu\text{m}$, basal appendages: $4\text{--}6.5 \mu\text{m}$, Song et al. 2014). *Pseudopezalotiopsis thailandica* is different from *Ps. simitheae* by having larger conidia ($(24\text{--})24.5\text{--}30(\text{--}30.5) \mu\text{m}$), longer apical appendages ($(26.5\text{--})28\text{--}36(\text{--}39.5) \mu\text{m}$) and shorter basal appendages ($(3.5\text{--})4.5\text{--}5(\text{--}6.5) \mu\text{m}$) (Table 5, Song et al. 2014).

Pseudopezalotiopsis rhizophorae Norphanphoun, T.C. Wen & K.D. Hyde, sp. nov. Fig. 14

Index Fungorum number: IF556444; Facesoffungi number: FoF 05778

Etymology – refers to the host from which the fungus was isolated, *Rhizophora apiculata* Blume.

Holotype – MFLU 19-0793

Associated with leaf spots of *Rhizophora apiculata* Blume. Symptoms irregular shape, pale brown, slightly sunken spots adaxial surface leaves of *R. apiculata*, which later expand outwards on the surface of the leaves (Fig. 14c). Small brown spots appeared initially and then gradually enlarged, changing to pale brown irregular spots with auburn border. They were usually >5 circular spots occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves with defected leaves (Fig. 14b). Asexual morph: *Conidiomata* $100\text{--}200 \mu\text{m}$ diam, pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and

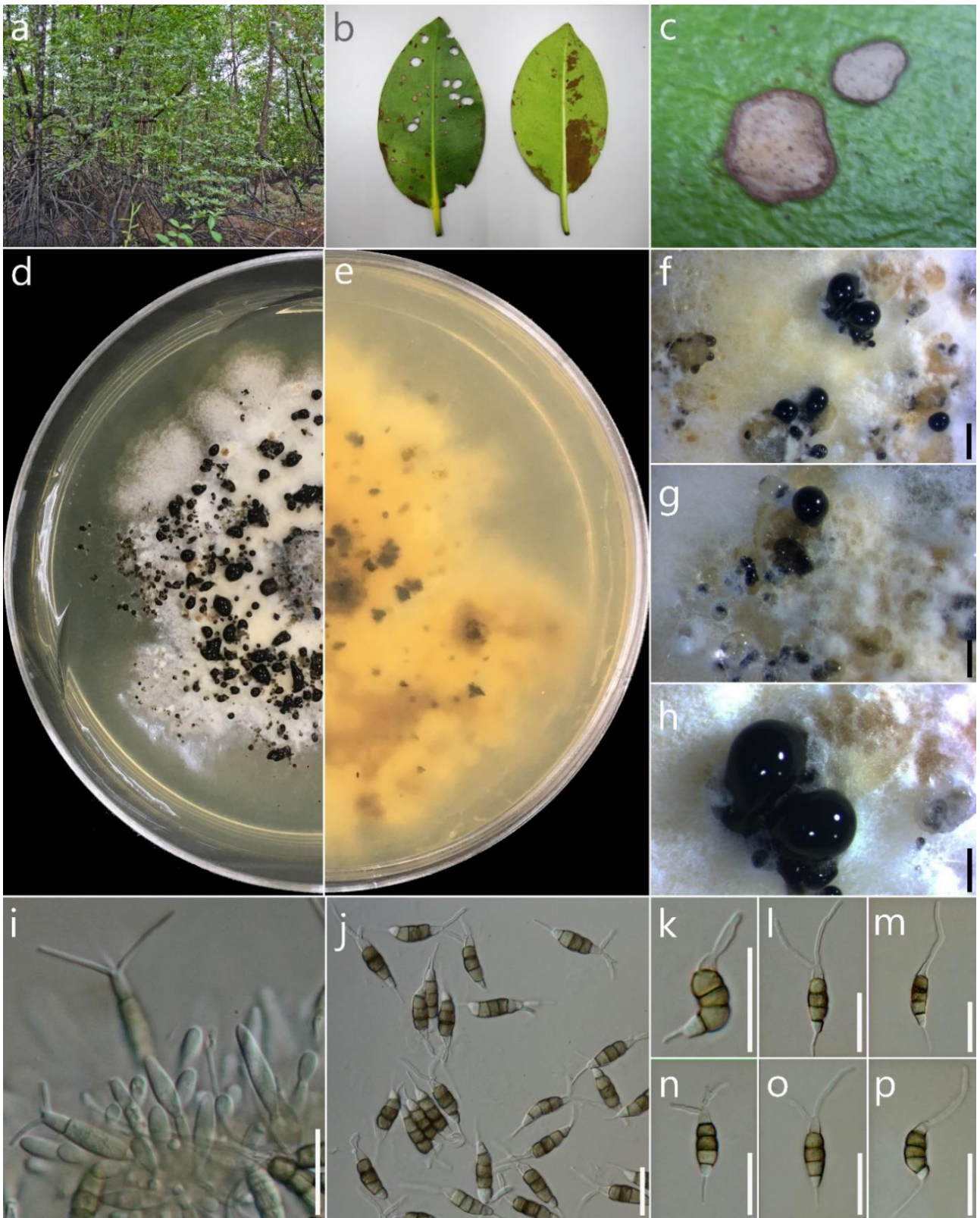


Figure 13 – *Pseudopestalotiopsis curvatispora* (MFLU 19-0791, holotype). a Habitat. b, c Leaf spots of *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f = 1000 μ m, g, h = 500 μ m, i–p = 20 μ m.

thin-walled, $5\text{--}8 \times 5\text{--}6 \mu\text{m}$, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* $(23\text{--})24\text{--}29.5\text{--}(30) \times 5\text{--}5.5\text{--}(6) \mu\text{m}$ (mean \pm SD = $26 \pm 2.5 \times 5.4 \pm 0.4 \mu\text{m}$), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or

sometimes pale brown, thin- and smooth-walled, (3–)4–5(–6) μm long (mean \pm SD = 4.5 ± 0.9 μm); three median cells (14.5–)15–19(–19.5) μm long (mean \pm SD = 16.4 ± 2 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)5–5.5(–7) μm long (mean \pm SD = 5.8 ± 1 μm); third cell brown, 4–5(–5.5) μm long (mean \pm SD = 4.7 ± 0.4 μm); fourth cell brown, (4.5–)5.5–6(–7.5) μm long (mean \pm SD = 5.9 ± 1.0 μm); apical cell (3.5–)4–5(–7) μm long (mean \pm SD = 5.2 ± 0.8 μm), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (16.5–)18–25(–26) μm long (mean \pm SD = 22 ± 3.1 μm); single basal appendage, tubular, unbranched, centric, (3–)3.5–6(–7) μm long (mean \pm SD = 5 ± 1.2 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (± 25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

Material examined – THAILAND, Ngao, Ranong Province, Ngao Mangrove Forest Research Centre, leaf spots of *Rhizophora apiculata*, 6 December 2016, Norphanphoun Chada NG38a (MFLU 19-0793, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1560, TNCC.

Notes – *Pseudopestalotiopsis rhizophorae* formed an independent branch in the phylogeny presented here (Fig. 3) and is closely related to *Ps. dawaina* (MM14-F0015) and *Ps. kubahensis* (UMAS KUB-P20). *Pseudopestalotiopsis rhizophorae* differs from *Ps. dawaina* and *Ps. kubahensis* in length of appendage in both ends: shorter apical appendage (*Ps. dawaina*: 20.5–33.5 μm , *Ps. kubahensis*: 16–29.5 μm) and longer basal appendage (*Ps. dawaina*: 2.5–6.5 μm , *Ps. kubahensis*: 3–6 μm) (Table 5). Thus, *Ps. rhizophorae* is introduced as a new species.

Pseudopestalotiopsis thailandica Norphanphoun & K.D. Hyde, sp. nov.

Fig. 15

Index Fungorum number: IF556445; Facesoffungi number: FoF 05779

Etymology – refers to the country where the fungus was collected, Thailand.

Holotype – MFLU 19-0794

Associated with leaf spots of *Rhizophora mucronata* Blume. *Symptoms* subcircular to the irregular shape, pale brown, slightly sunken spots adaxial surface leaves of *R. mucronata*, which later expand outwards on the surface of the leaves (Fig. 15c). Small auburn spots appeared initially and then gradually enlarged, changing to pale-auburn circular ring spots with a dark auburn border. They were usually >5 circulars, which occurred on a single affected leaf. In severe cases, lesions spread evenly on the leaves (Fig. 15b). Asexual morph: *Conidiomata* 250–500 μm diam, pycnidial, globose, brown, semi-immersed on PDA, releasing conidia in a black, slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth- and thin-walled, proliferating 1–2 times percurrently, collarete present and not flared. *Conidia* (24–)24.5–30(–30.5) \times (5–)5.5–6(–6.7) μm (mean \pm SD = $26.6 \pm 2.2 \times 5.9 \pm 0.3$ μm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, (3.5–)4–5(–6.6) μm long (mean \pm SD = 4.4 ± 1 μm); three median cells (13.5–)16–18(–19) μm long (mean \pm SD = 17.2 ± 1.5 μm), brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (5–)5.5–6(–7.5) μm long (mean \pm SD = 5.8 ± 0.9 μm); third cell brown, (5–)5.5–6(–6.2) μm long (mean \pm SD = 5.6 ± 0.5 μm); fourth cell brown, (5.5–)6–6.5(–7) μm long (mean \pm SD = 6.2 ± 0.6 μm); apical cell (3.5–)4.5–5(–7) μm long (mean \pm SD = 4.5 ± 1.1 μm), hyaline, conic to acute; with 1–2 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (26.5–)28–36(–39.5) μm long (mean \pm SD = 31.3 ± 3.9 μm); single basal appendage, tubular, unbranched, centric, (3.5–)4.5–5(–6.5) μm long (mean \pm SD = 4.8 ± 1 μm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at room temperature (± 25 °C), under light 12 hr/dark 12 hr, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin (curled margin), fluffy, white from above and reverse; fruiting bodies black.

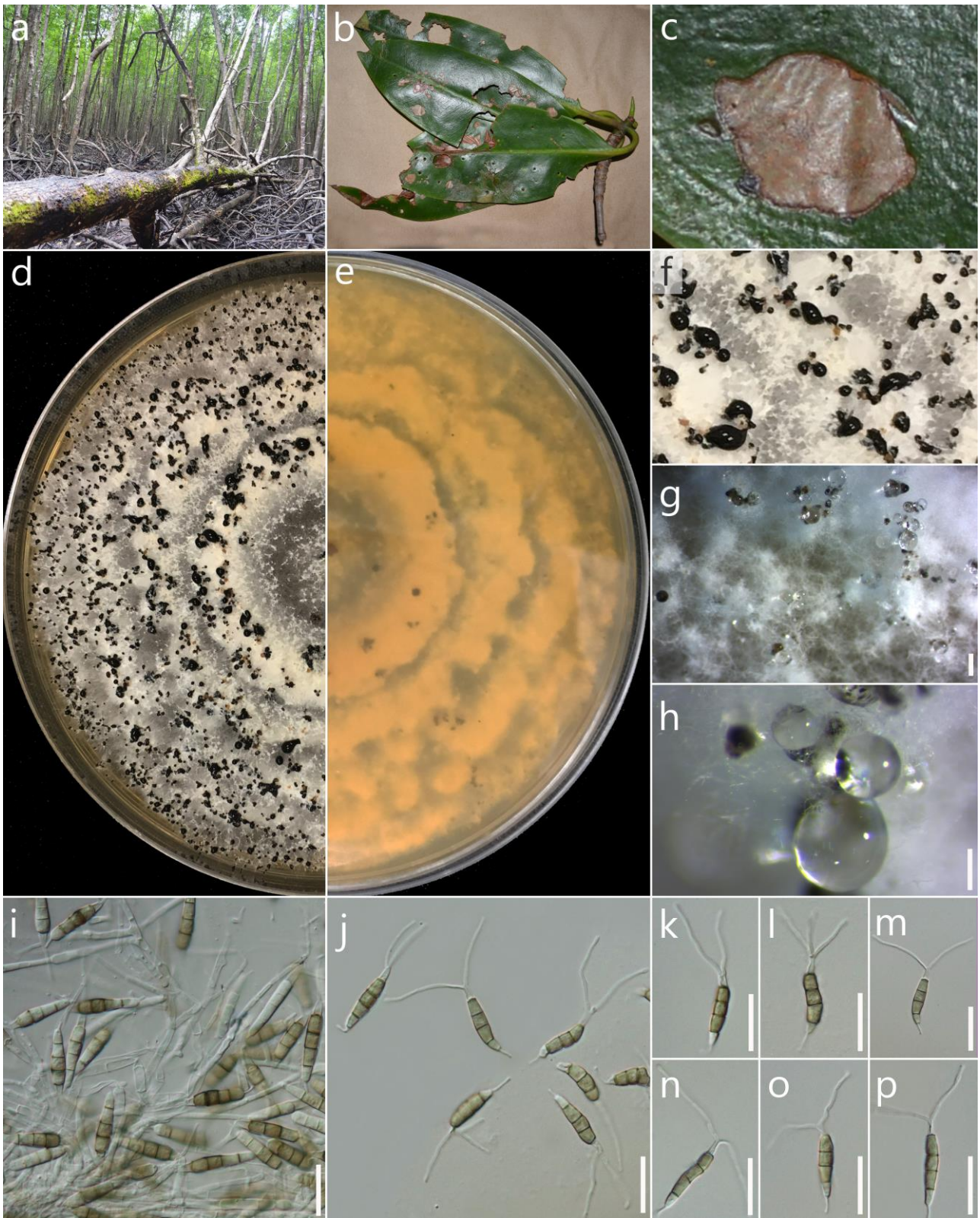


Figure 14 – *Pseudopestalotiopsis rhizophorae* (MFLU 19-0793, holotype). a Habitat. b, c Leaf spots of *Rhizophora apiculata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: g = 500 μ m, h = 200 μ m, i–p = 20 μ m.

Material examined – THAILAND, Chanthaburi Province, leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC21-1 (MFLU 19-0794, holotype; PDD, isotype); ex-type-living cultures, MFLUCC 17-1724, TNCC. THAILAND, Chanthaburi Province,

leaf spots of *Rhizophora mucronata*, 27 April 2017, Norphanphoun Chada KC21-12 (MFLU 19-0795, paratype); ex-type-living cultures, MFLUCC 17-1725.

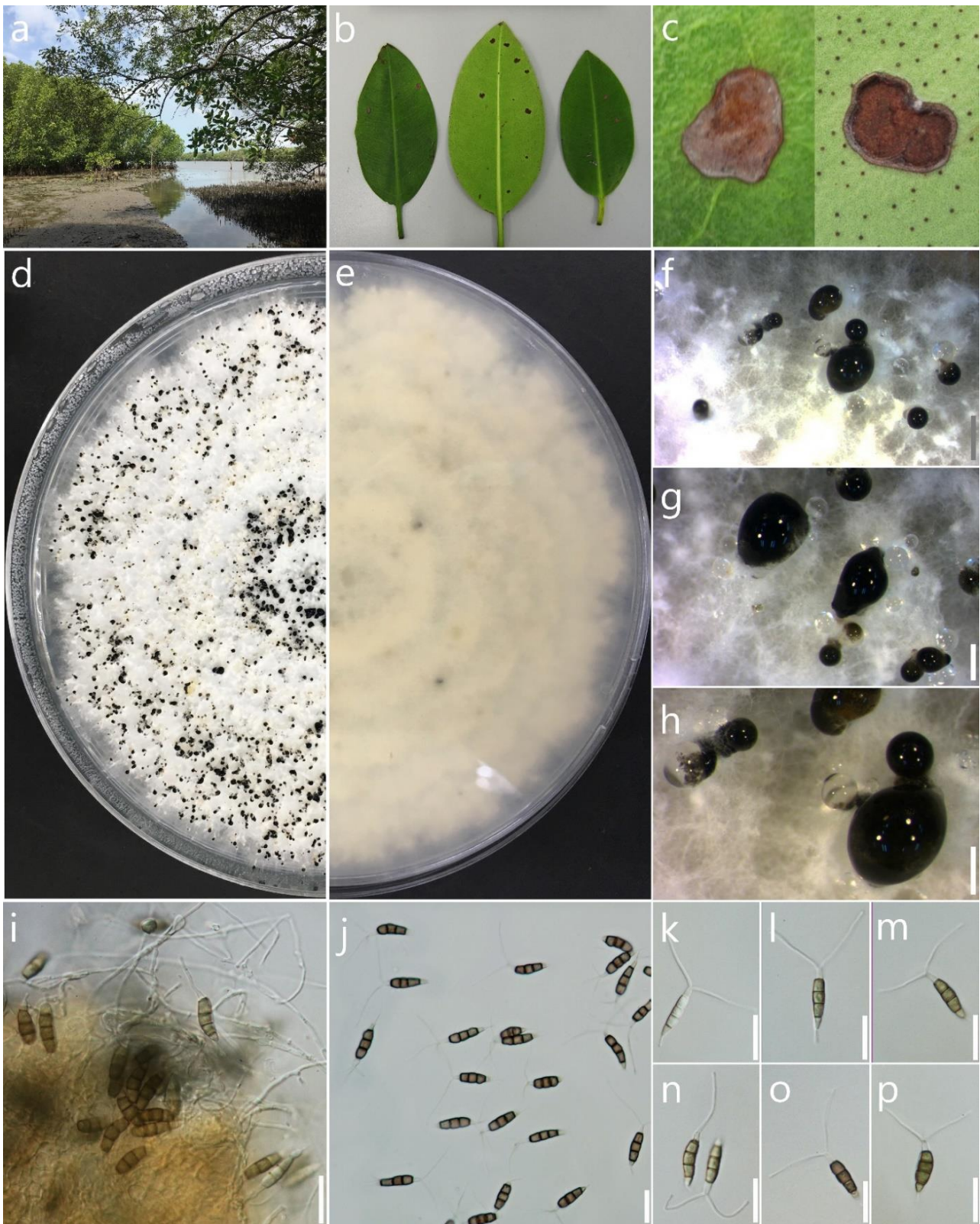


Figure 15 – *Pseudopestalotiopsis thailandica* (MFLU 19-0794, holotype). a Habitat. b, c Leaf spots on *Rhizophora mucronata*. d, e Culture on PDA (d-above, e-reverse). f–h Colony sporulating on PDA. i Conidiogenous cells giving rise to conidia. j–p Conidia. Scale bars: f = 1000 μ m, g, h = 500 μ m, i–p = 20 μ m.

Notes – The new species, *Pseudopestalotiopsis thailandica* was isolated from a leaf spot from *Rhizophora mucronata* from Chanthaburi Province, Thailand. Based on the combined gene phylogenetic analysis, it showed that *Ps. thailandica* is sister to *Ps. curvatispora* (in this study), and morphology differences as mentioned in the notes of *Ps. curvatispora*. Thus, it is considered that *Ps. thailandica* is a novel species.

Discussion

Mangrove forests occurring at the interface of terrestrial and marine ecosystems harbour a rich biological diversity of plants, animals and microorganisms (Beger et al. 2010). However, the phylogenetic and functional description of microbial diversity in mangrove ecosystems has not been addressed to the same extent as for other environments. A previous study on mangrove fungi reported some pathogenic fungi causing leaf spots on *Rhizophora*, e.g. leaf spots on *Rhizophora mangle* in Puerto Rico caused by *Anthostomella* (Stevens 1920), leaf spots on *R. mangle* in Florida caused by *Cercospora* (McMillan 1964), leaf spots on *Rhizophora* sp. caused by *Pestalotiopsis* sp., *Colletotrichum* sp. and *Polystigma sonneratae* (Hyde & Cannon 1992, Xu et al. 2009). Heart and butt rot disease on *Rhizophora* sp. caused by *Heterobasidion annosum*, *Ganoderma*, *Phellinus* sp., and *Inonotus* sp. (Ryvarden 2000, Fox 2001, Sakayarojet et al. 2012) and canker and dieback on *Rhizophora* sp. caused by *Phytophthora cinnamomi* (Vollbrecht et al. 1995, Barnard 2000, Kinge & Mih 2011) have also been reported. A leaf spot of *Avicennia marina* caused by *Pseudocercospora avicenniae* (Shivas et al. 2009) was recorded in Queensland, Australia. Thatoi et al. (2013) reported more than 200 species of endophytic fungi from mangroves in India. Norphanphoun et al. (2018) reported three novel species of *Cytospora* (*C. lumnitzericola*, *C. thailandica* and *C. xylocarpi*) from *Lumnitzera racemosa*, *Xylocarpus granatum* and *X. moluccensis* from Phetchaburi and Ranong provinces in Thailand, and Kumar et al. (2019) reported an endophytic species, *Neopestalotiopsis alpapicalis* on *R. apiculata* and *R. mucronata* from Krabi Province, Thailand.

In this study, we examined 22 strains isolated from leaf collections from Phetchaburi, Ranong and Trat Provinces in Thailand. As a result of these sampling endeavors, we described 12 novel species, including six novel species of *Neopestalotiopsis* (*N. acrostichi*, *N. brachiata*, *N. petila*, *N. rhizophorae*, *N. sonneratae*, *N. thailandica*), two novel species of *Pestalotiopsis* (*P. rhizophorae*, *P. thailandica*) and four novel species of *Pseudopestalotiopsis* (*Ps. avicenniae*, *Ps. curvatispora*, *Ps. rhizophorae* and *Ps. thailandica*), based on morphological and molecular phylogenetic evidence, which clearly differentiated these new species from known species. As many pestalotioid species have overlapping morphological traits, sequence data is essential to resolve these three genera and introduce new species. Combined gene sequences of ITS, β -tubulin and EF1 α can provide a better resolution for *Pestalotiopsis* and *Pseudopestalotiopsis*. However, more genes are needed to provide better resolution and support in *Neopestalotiopsis*. Furthermore, this is the first report of *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis* fungi associated on mangrove plants in Thailand (*Acrostichum aureum*, *Avicennia marina*, *Rhizophora mucronata*, *R. apiculata* and *Sonneronata alba*), which indicates that there may be a high undescribed diversity of fungi in this ecosystem as has been shown elsewhere in terrestrial habitats in northern Thailand (Hyde et al. 2018). We found 12 new species from mangrove ecosystems, however further study is needed to establish whether these species are unique to such hosts and habitats.

Table 3 Comparison of conidia of *Neopestalotiopsis* species related to this study.

Species	Strain	Conidial size (μm)	Apical appendages		Basal appendage Length (μm)
			Number	Length (μm)	
<i>N. acrostichi</i>	MFLUCC 17-1754/ MFLUCC 17-1755	(22-)23-26(-27.2) \times (5-)5.5-6.5(-7.1)	3-4 (4)	(16-)19-65(-69.5)	(7-)12.5-36(-37)
<i>N. alpapicalis</i> ^g	MFLUCC 17-2544	14-22.3 \times 5-6.8	1-4(1, 2)	5.6-15	3.1-6.4
<i>N. aotearoa</i> ^d	CBS 367.54	(19.5-)21-28(-29) \times (6-)6.5-8.5(-9)	2-3	(3-)5-12(-13)	1.5-4
<i>N. coffea-arabicae</i> ^f	HGUP4015	16-20 \times 5-7	2-4	11-16	3-5
<i>N. musae</i> ^b	MFLUCC 15-0776	18.6-25.9 4.1-5	2-3	16.3-25	4.6-10.3
<i>N. piceana</i> ^d	CBS 394.48	(19-)19.5-25(-26) \times (7-)7.5-9(-9.5)	3	(19-)21-31(-33)	6-23
<i>N. protearum</i> ^a	CBS 114178	(14-)16-17(-18) \times (6.5-)8-9(-10)	2-4	(10-)15-17(-22)	(2-)3-3.5(-5)
<i>N. brachiata</i>	MFLUCC 17-1555	(18-)18.5-25(-26) \times (4.7-)5.5-6(-6.3)	1-3	(8.5-)9.5-33(-34)	(3.5-)4-9(-10)
<i>N. rhizophorae</i>	MFLUCC 17-1550/ MFLUCC 17-1551	(20-)20.5-27(-27.5) \times (6-)6.5-7.5(-8.2)	1-4 (3)	(6-)12.5-22(-24)	(2.5-)4-9.5(-10)
<i>N. rosicola</i> ^c	CFCC 51992	(18.9-)20.2-25.5(-26.2) \times (5-)5.5-8(-8.5)	2-4 (2, 3)	(16.5-)17-22.8(-25.9)	2-9.5
<i>N. samarangensis</i> ^e	MFLUCC 12-0233	18-21 \times 6.5-7.5	3	12-18	3.5-5.2
<i>N. sonneratae</i>	MFLUCC 17-1744/ MFLUCC 17-1745	(21.6-)24-26(-28.2) \times (6.8-)7-7.5(-8.1)	1-3	(5.3-)7-8(-13.8)	(2.5-)3-4(-4.7)
<i>N. surinamensis</i> ^d	CBS 450.74	(23-) 24-28(-29) \times (7-)7.5-9(-9.5)	2-3	(15-)18-27(-28)	Up to 5-7
<i>N. thailandica</i>	MFLUCC 17-1730/ MFLUCC 17-1731	(20-)21-25(-25.5) \times (5.7-)6-7(-7.3)	1-2	(30-)32.5-38(-40)	(3-)6-9(-10)
<i>N. petila</i>	MFLUCC 17-1737/ MFLUCC 17-1738	(20-)21-26.5(-27.5) \times (5.6-)6-7(-7.8)	2-3	(21-)22-29(-33)	(2-)3-8(-9)

Strains in this study are in bold.

^aCrous et al. (2011); ^bHyde et al. (2016); ^cJiang et al. (2018); ^dMaharachchikumbura et al. (2014); ^eMaharachchikumbura et al. (2013); ^fSong et al. (2013); ^gKumar et al. (2019).

Table 4 Comparison of conidia of *Pestalotiopsis* species related to this study.

Species	Strain	Conidial size (μm)	Apical appendages (μm)		Basal appendage (μm)
			Number	Length	
<i>P. formosana</i> ^a	NTUCC 17-009	(15-)18-22(-26) \times (5-)6-7	2-3	(8-)11-16(-20)	(2-)3-5(-6)
<i>P. parva</i> ^b	CBS 265.37	(16-)16.5-20(-21) \times 5-7(-7.5)	2-3	(6-)6.5-12(-13)	2-4
<i>P. rhizophorae</i>	MFLUCC 17-0416/ MFLUCC 17-0417	(17-)17.5-23(-23.5) \times (5.5-)6-6.5(-7)	1-2	(7.5-)8-13(-14.5)	(1.3-)1.5-4.5(-5)
<i>P. thailandica</i>	MFLUCC 17-1616/ MFLUCC 17-1617	(17-)17.5-28(-29) \times (4.9-)5.5-6.5(-7.1)	1-2	(5.5-)11-34(-38)	(2-)2.5-9.5(-10)

Strains in this study are in bold.

^aAriyawansa & Hyde (2018); ^bMaharachchikumbura et al. (2014).

Table 5 Comparison of conidia of *Pseudopestalotiopsis* species related to this study.

Species	Strain	Conidial size (µm)	Apical appendages (µm)		Basal appendage (µm)
			Number	Length	
<i>Ps. ampullacea</i> ^b	LC6618	21–31.5 × 6.5–9	2–3	17–25	3.5–7
<i>Ps. avicenniae</i>	MFLUCC 17-0434	(22–)22.5–26.5(–27) × (5–)5.5–6(–6.4)	1–3	(14–)15.5–28.5(–35.5)	(2–)3–4(–4.5)
<i>Ps. chinensis</i> ^b	LC3011	25.5–35.5 × 6–9	2–3	24–41	5–12
<i>Ps. rhizophorae</i>	MFLUCC 17-1560	(18.5–)22–25(–26.5) × (6.2–)6.5–7(–7.2)	1–2	(10.2–)20–29(–35)	(5.5–)9–12(–13.5)
<i>Ps. dawaina</i> ^d	MM14-F0015	22–31 × 8–9.5	3	20.5–33.5	2.5–6.5
<i>Ps. elaeidis</i> (= <i>Ps. myanmarina</i>) ^c	NBRC 112264	31–38.5 × 6.5–9	2–3	22.5–38.5	unbranched
<i>Ps. jiangxiensis</i> ^b	LC4479	22–29 × 6–9	2–4(3)	16.5–32	6.5–19.5
<i>Ps. kawthaungina</i> ^d	MM14-F0083	29.5–34.5 × 7–9	3	28–41	4.5–9
<i>Ps. kubahensis</i> ^a	UMAS KUB-P20	(26–)27–30(–33) × 5.6–7.3	2–4(3)	15.9–29.4	3.1–6.0
<i>Ps. curvatispora</i>	MFLUCC 17-1722/ MFLUCC 17-1723	(18.6–)19–26(–26.4) × (5.5–)6–7(–7.4)	2–3	(5.5–)6–24(–26.6)	(5.8–)5–11(–12.2)
<i>Ps. simitheae</i> ^e	MFLUCC 12-0121	22–30 × 5–6.5	2–4	14.5–26.5	4–6.5
<i>Ps. taiwanensis</i> ^f	NTUCC 17-002.1	21–26 × 6–7	2–5	16–25	3–7
<i>Ps. thailandica</i>	MFLUCC 17-1724/ MFLUCC 17-1725	(24–)24.5–30(–30.5) × (5–)5.5–6(–6.7)	1–3	(26.5–)28–36(–39.5)	(3.5–)4.5–5(–6.6)

Strain in this study are in bold.

^aLateef et al. (2015); ^bLiu et al. (2017); ^cNozawa et al. (2017); ^dNozawa et al. (2018); ^eSong et al. 2014; ^fTsai et al. (2018).

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Supplementary Table 1 Nucleotides differences in the ITS, β -tubulin and EF1 α sequences of *Neopestalotiopsis* discussed in the paper.

Taxon	Strain	ITS										β -tubulin			
		68	85	100	370	372	373	374	375	449	487	163	169	201	205
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	T	A	T	T	C	A	T	T	T	T	C	T	T	A
<i>N. alpapicalis</i>	MFLUCC 17-2544	-	A	T	T	-	-	A	T	T	T	-	-	-	-
<i>N. aotearoa</i>	CBS 367.54	T	G	T	T	-	-	A	T	T	T	C	T	C	T
<i>N. brachiata</i>	MFLUCC 17-1555	T	A	T	T	-	-	A	T	T	T	T	T	C	T
<i>N. coffea-arabicae</i>	HGUP4015	T	A	T	T	-	-	A	T	T	T	-	-	-	-
<i>N. ellipospora</i>	MFLUCC 12-0283	T	A	T	C	-	-	G	G	T	T	-	-	-	-
<i>N. petila</i>	MFLUCC 17-1737	T	A	T	T	-	-	A	T	T	T	T	T	C	T
<i>N. piceana</i>	CBS 394.48	T	G	T	T	-	-	A	T	T	T	T	T	C	T
<i>N. protearum</i>	CBS 114178	T	A	T	C	-	-	G	G	T	C	C	T	T	A
<i>N. rhizophorae</i>	MFLUCC 17-1550	T	A	T	T	-	-	A	T	T	T	T	T	C	T
<i>N. rosicola</i>	CFCC 51992	T	A	T	T	-	-	T	A	C	T	T	T	C	T
<i>N. samarangensis</i>	MFLUCC 12-0233	T	A	T	T	-	-	A	T	T	T	-	-	-	-
<i>N. sonneratae</i>	MFLUCC 17-1744	T	A	T	T	-	-	A	T	T	T	C	T	T	A
<i>N. surinamensis</i>	CBS 450.74	T	A	T	T	-	-	T	T	T	T	C	A	T	A
<i>N. thailandica</i>	MFLUCC 17-1730	T	A	C	T	-	-	A	T	T	T	C	T	T	A
Taxon	Strain	β -tubulin													
		218	341	355	364	369	370	373	374	375	376	378	379	382	383
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	G	C	C	C	G	G	T	G	T	T	C	C	T	C
<i>N. alpapicalis</i>	MFLUCC 17-2544	-	-	C	T	G	G	T	G	T	A	T	C	T	C
<i>N. aotearoa</i>	CBS 367.54	T	C	T	C	G	G	T	G	T	A	T	C	T	C
<i>N. brachiata</i>	MFLUCC 17-1555	T	C	T	C	G	G	T	G	T	A	T	C	T	C
<i>N. coffea-arabicae</i>	HGUP4015	-	-	-	-	A	A	C	A	C	A	C	G	A	T
<i>N. ellipospora</i>	MFLUCC 12-0283	-	-	C	T	G	G	T	G	T	A	T	C	T	C
<i>N. petila</i>	MFLUCC 17-1737	T	C	T	C	G	G	T	G	T	A	T	C	T	C
<i>N. piceana</i>	CBS 394.48	T	C	T	C	G	G	T	G	T	A	T	C	T	C
<i>N. protearum</i>	CBS 114178	G	C	C	C	G	G	T	G	T	T	C	C	T	C
<i>N. rhizophorae</i>	MFLUCC 17-1550	T	C	T	C	G	G	T	G	T	A	T	C	T	C
<i>N. rosicola</i>	CFCC 51992	T	C	T	C	G	G	T	G	T	A	T	C	T	C
<i>N. samarangensis</i>	MFLUCC 12-0233	-	-	C	T	G	G	T	G	T	A	T	C	T	C
<i>N. sonneratae</i>	MFLUCC 17-1744	T	T	C	C	G	G	T	G	T	T	C	C	T	C
<i>N. surinamensis</i>	CBS 450.74	T	C	C	C	G	G	T	G	T	T	C	C	T	C
<i>N. thailandica</i>	MFLUCC 17-1730	T	T	C	C	G	G	T	G	T	T	C	C	T	C

Supplementary Table 1 Continued.

Taxon	Strain	β -tubulin													
		384	385	386	393	402	428	437	438	441	446	459	469	471	472
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	T	G	C	A	C	C	A	C	-	A	G	G	T	A
<i>N. alpapicalis</i>	MFLUCC 17-2544	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. aotearoa</i>	CBS 367.54	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. brachiata</i>	MFLUCC 17-1555	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. coffea-arabicae</i>	HGUP4015	C	T	G	-	A	C	A	C	T	A	G	A	G	A
<i>N. ellipospora</i>	MFLUCC 12-0283	T	G	C	A	A	T	T	C	-	T	C	G	T	G
<i>N. petila</i>	MFLUCC 17-1737	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. piceana</i>	CBS 394.48	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. protearum</i>	CBS 114178	T	G	C	A	C	C	A	A	-	A	G	G	T	A
<i>N. rhizophorae</i>	MFLUCC 17-1550	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. rosicola</i>	CFCC 51992	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. samarangensis</i>	MFLUCC 12-0233	T	G	C	A	A	C	T	C	-	T	C	G	T	G
<i>N. sonneratae</i>	MFLUCC 17-1744	T	G	C	A	A	C	A	C	T	A	G	A	T	A
<i>N. surinamensis</i>	CBS 450.74	T	G	C	A	C	C	A	C	-	A	G	G	T	A
<i>N. thailandica</i>	MFLUCC 17-1730	T	G	C	A	A	C	A	C	T	A	G	A	G	A

Taxon	Strain	β -tubulin					EF1 α								
		543	544	571	674	683	70	81	84	85	86	87	88	89	90
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	A	C	T	T	C	T	C	C	A	T	-	-	-	-
<i>N. alpapicalis</i>	MFLUCC 17-2544	T	T	C	T	C	-	-	C	A	T	-	-	-	-
<i>N. aotearoa</i>	CBS 367.54	T	T	C	T	C	T	C	C	A	T	-	-	-	-
<i>N. brachiata</i>	MFLUCC 17-1555	T	T	C	T	C	T	C	C	A	T	-	-	-	-
<i>N. coffea-arabicae</i>	HGUP4015	A	C	C	T	T	T	C	C	A	T	-	-	-	-
<i>N. ellipospora</i>	MFLUCC 12-0283	T	T	C	T	C	T	A	C	A	T	-	-	-	-
<i>N. petila</i>	MFLUCC 17-1737	T	T	C	C	C	T	C	C	A	T	-	-	-	-
<i>N. piceana</i>	CBS 394.48	T	T	C	T	C	T	C	C	A	T	-	-	-	-
<i>N. protearum</i>	CBS 114178	A	C	T	T	C	T	C	-	-	-	-	-	-	-
<i>N. rhizophorae</i>	MFLUCC 17-1550	T	T	C	T	C	T	C	C	A	T	-	-	-	-
<i>N. rosicola</i>	CFCC 51992	T	T	C	T	C	T	C	C	A	T	-	-	-	-
<i>N. samarangensis</i>	MFLUCC 12-0233	T	T	C	C	C	T	C	C	A	T	-	-	-	-
<i>N. sonneratae</i>	MFLUCC 17-1744	A	C	C	T	T	T	C	C	A	T	C	A	T	C
<i>N. surinamensis</i>	CBS 450.74	A	C	T	T	C	C	C	C	A	T	-	-	-	-
<i>N. thailandica</i>	MFLUCC 17-1730	A	C	C	T	T	T	C	C	A	T	C	A	T	C

Supplementary Table 1 Continued.

Taxon	Strain	EF1 α													
		91	92	93	97	104	105	112	113	114	118	120	124	133	167
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	-	-	-	C	C	A	C	A	T	A	T	T	T	T
<i>N. alpapicalis</i>	MFLUCC 17-2544	-	-	-	C	C	A	-	-	-	A	T	C	T	T
<i>N. aotearoa</i>	CBS 367.54	-	-	-	C	C	A	C	A	T	A	T	C	T	T
<i>N. brachiata</i>	MFLUCC 17-1555	-	-	-	C	C	A	C	A	T	A	A	C	T	T
<i>N. coffea-arabicae</i>	HGUP4015	-	-	-	C	C	A	C	A	T	A	T	C	T	T
<i>N. ellipospora</i>	MFLUCC 12-0283	-	-	-	C	C	A	C	A	T	A	T	C	T	T
<i>N. petila</i>	MFLUCC 17-1737	-	-	-	C	C	A	C	A	T	A	T	C	T	T
<i>N. piceana</i>	CBS 394.48	-	-	-	C	C	A	C	A	T	A	T	C	T	G
<i>N. protearum</i>	CBS 114178	-	-	-	C	C	A	C	A	T	A	T	C	T	T
<i>N. rhizophorae</i>	MFLUCC 17-1550	-	-	-	C	C	A	-	-	-	A	T	C	T	T
<i>N. rosicola</i>	CFCC 51992	-	-	-	C	C	A	C	A	T	A	T	C	T	T
<i>N. samarangensis</i>	MFLUCC 12-0233	-	-	-	C	G	A	C	A	T	A	T	C	T	T
<i>N. sonneratae</i>	MFLUCC 17-1744	C	C	C	C	C	A	C	A	T	A	T	C	T	T
<i>N. surinamensis</i>	CBS 450.74	-	-	-	T	C	T	C	G	T	G	T	C	C	T
<i>N. thailandica</i>	MFLUCC 17-1730	C	C	C	C	C	A	C	A	T	A	T	C	T	T

Taxon	Strain	EF1 α													
		169	186	210	246	287	290	407	456	462	480	485			
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	-	G	G	G	A	T	T	C	A	A	A			
<i>N. alpapicalis</i>	MFLUCC 17-2544	-	G	G	G	A	T	C	T	A	A	G			
<i>N. aotearoa</i>	CBS 367.54	-	G	G	G	A	T	C	C	A	T	A			
<i>N. brachiata</i>	MFLUCC 17-1555	-	G	G	G	A	T	C	C	A	T	A			
<i>N. coffea-arabicae</i>	HGUP4015	-	G	G	G	A	T	C	C	A	T	A			
<i>N. ellipospora</i>	MFLUCC 12-0283	-	A	A	A	A	T	C	C	A	T	A			
<i>N. petila</i>	MFLUCC 17-1737	-	G	G	G	A	T	C	C	A	T	A			
<i>N. piceana</i>	CBS 394.48	-	G	G	G	A	T	C	C	A	T	A			
<i>N. protearum</i>	CBS 114178	T	G	G	G	A	T	C	C	A	T	A			
<i>N. rhizophorae</i>	MFLUCC 17-1550	-	G	G	G	A	T	C	C	A	A	G			
<i>N. rosicola</i>	CFCC 51992	-	G	G	G	A	T	C	C	A	A	A			
<i>N. samarangensis</i>	MFLUCC 12-0233	-	G	G	G	A	T	C	C	T	A	A			
<i>N. sonneratae</i>	MFLUCC 17-1744	C	G	G	G	A	C	C	C	T	A	A			
<i>N. surinamensis</i>	CBS 450.74	-	G	G	T	G	T	C	C	T	A	A			
<i>N. thailandica</i>	MFLUCC 17-1730	C	G	G	G	A	C	C	C	T	A	A			

Supplementary Table 2 Nucleotides differences in the ITS, β -tubulin and EF1 α sequences of *Pestalotiopsis* discussed in the paper.

Taxon	Strain	ITS											β -tubulin		
		14	23	162	164	433	491	539	547	551	558	559	14	36	38
<i>Pestalotiopsis rhizophorae</i>	MFLUCC 17-0416	A	T	C	A	A	A	T	T	T	T	G	A	T	C
<i>P. thailandica</i>	MFLUCC 17-1616	A	T	C	G	G	-	C	T	T	T	G	T	T	C
<i>P. formosana</i>	NTUCC 17-009	-	-	T	G	A	-	C	T	-	G	T	-	-	-
<i>P. parva</i>	CBS 278.35	T	A	T	G	A	-	C	C	-	T	G	A	G	G
Taxon	Strain	β -tubulin													
		39	43	130	131	132	153	174	260	360	368	372	403	428	430
<i>Pestalotiopsis rhizophorae</i>	MFLUCC 17-0416	C	C	A	A	G	C	C	C	C	T	A	G	A	T
<i>P. thailandica</i>	MFLUCC 17-1616	C	C	A	A	G	C	C	C	C	T	A	G	A	T
<i>P. formosana</i>	NTUCC 17-009	-	-	-	-	-	-	-	-	-	-	C	G	A	T
<i>P. parva</i>	CBS 278.35	A	C	-	-	-	G	T	A	T	C	A	A	T	C
Taxon	Strain	EF1 α													
		762	33	34	36	56	57	58	71	81	123	126	226	261	434
<i>Pestalotiopsis rhizophorae</i>	MFLUCC 17-0416	A	T	C	C	C	C	A	C	C	A	C	T	C	G
<i>P. thailandica</i>	MFLUCC 17-1616	G	T	C	C	C	C	A	C	C	A	C	T	C	G
<i>P. formosana</i>	NTUCC 17-009	A	T	C	C	-	-	-	G	T	G	A	C	C	A
<i>P. parva</i>	CBS 278.35	A	A	T	T	T	C	A	G	C	A	C	T	T	A

Supplementary Table 3 Nucleotides differences in the ITS, β -tubulin and EF1 α sequences of *Pseudopestalotiopsis* discussed in the paper.

Taxon	Strain	ITS													
		30	39	75	119	147	409	410	482	527	545	546	549	553	554
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	A	T	A	G	A	-	-	T	T	G	A	A	G	C
<i>Ps. curvatispora</i>	MFLUCC 17-1722	A	T	A	G	G	-	-	C	T	G	A	A	G	C
<i>Ps. dawaina</i>	MM14 F0015	T	A	A	T	G	-	-	C	T	G	A	A	G	C
<i>Ps. jiangxiensis</i>	LC4479	-	-	-	G	A	-	-	T	T	G	A	A	G	C
<i>Ps. kawthaungina</i>	MM14 F0083	T	A	A	G	A	-	-	T	T	G	A	A	G	C
<i>Ps. myanmarina</i>	NBRC 112264	T	A	A	G	A	-	-	T	T	G	A	A	G	C
<i>Ps. rhizophorae</i>	MFLUCC 17-560	A	T	A	G	G	A	A	C	T	G	A	A	G	C
<i>Ps. simitheae</i>	MFLUCC 12-0121	T	A	A	G	A	-	-	T	-	-	-	G	A	T
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	T	A	A	G	A	-	-	T	-	-	-	-	-	-
<i>Ps. thailandica</i>	MFLUCC 17-1724	A	T	A	G	G	A	A	C	T	G	A	A	G	C

Supplementary Table 3 Continued.

Taxon	Strain	ITS	β-tubulin												
		556	22	107	125	134	148	149	150	151	153	158	183	192	194
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	G	G	T	A	C	T	-	-	-	T	C	A	A	A
<i>Ps. curvatispora</i>	MFLUCC 17-1722	G	G	G	C	C	G	C	A	G	C	C	G	G	A
<i>Ps. dawaina</i>	MM14 F0015	G	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. jiangxiensis</i>	LC4479	G	G	T	A	C	T	-	-	-	T	C	A	A	A
<i>Ps. kawthaungina</i>	MM14 F0083	G	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. myanmarina</i>	NBRC 112264	G	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. rhizophorae</i>	MFLUCC 17-560	G	A	T	C	T	G	C	A	G	T	A	A	G	G
<i>Ps. simitheae</i>	MFLUCC 12-0121	C	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. thailandica</i>	MFLUCC 17-1724	G	A	T	C	T	G	-	-	-	T	A	A	G	G

Taxon	Strain	β-tubulin													
		198	206	223	225	250	302	330	355	361	362	363	364	365	372
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	A	G	A	G	C	G	A	C	G	T	A	T	G	A
<i>Ps. curvatispora</i>	MFLUCC 17-1722	T	A	G	A	T	G	G	C	G	T	A	T	G	A
<i>Ps. dawaina</i>	MM14 F0015	-	-	-	-	-	-	-	-	G	T	A	T	G	A
<i>Ps. jiangxiensis</i>	LC4479	A	G	A	G	C	G	A	C	G	T	A	T	G	A
<i>Ps. kawthaungina</i>	MM14 F0083	-	-	-	-	-	-	-	-	G	T	A	T	G	A
<i>Ps. myanmarina</i>	NBRC 112264	-	-	-	-	-	-	-	-	-	-	-	-	-	A
<i>Ps. rhizophorae</i>	MFLUCC 17-560	T	A	A	A	T	A	A	C	G	T	A	T	G	A
<i>Ps. simitheae</i>	MFLUCC 12-0121	-	-	-	-	-	-	-	-	A	G	T	C	A	-
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	-	-	-	-	-	-	-	T	G	T	A	T	G	A
<i>Ps. thailandica</i>	MFLUCC 17-1724	T	A	A	A	T	G	A	C	G	T	A	T	G	A

Taxon	Strain	β-tubulin													
		376	413	434	435	445	453	459	505	538	539	540	558	559	560
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	A	T	T	G	C	-	G	T	-	-	-	C	A	T
<i>Ps. curvatispora</i>	MFLUCC 17-1722	A	T	T	A	A	G	G	T	C	C	C	T	C	C
<i>Ps. dawaina</i>	MM14 F0015	A	T	T	A	A	G	G	T	C	C	C	T	C	C
<i>Ps. jiangxiensis</i>	LC4479	A	T	T	G	C	-	G	T	-	-	-	C	A	C
<i>Ps. kawthaungina</i>	MM14 F0083	A	T	T	A	A	G	G	T	C	C	C	T	C	C
<i>Ps. myanmarina</i>	NBRC 112264	A	T	T	G	C	-	G	T	-	-	-	C	A	T
<i>Ps. rhizophorae</i>	MFLUCC 17-560	C	C	G	A	A	G	G	C	T	T	C	C	A	C
<i>Ps. simitheae</i>	MFLUCC 12-0121	-	C	G	A	A	G	G	T	T	T	C	C	A	C
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	C	C	G	A	A	G	G	T	T	T	C	C	A	C
<i>Ps. thailandica</i>	MFLUCC 17-1724	C	C	G	A	A	G	A	C	T	T	C	C	A	C

Supplementary Table 3 Continued.

Taxon	Strain	β -tubulin										EF1 α			
		571	572	573	581	625	628	691	715	739	772	88	89	93	95
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	T	T	G	G	T	C	T	C	T	C	A	C	T	T
<i>Ps. curvatispora</i>	MFLUCC 17-1722	T	C	A	T	T	C	C	T	C	T	A	T	T	C
<i>Ps. dawaina</i>	MM14 F0015	T	C	A	T	T	C	C	T	C	T	A	C	T	T
<i>Ps. jiangxiensis</i>	LC4479	T	T	G	G	T	C	T	C	T	C	A	C	T	T
<i>Ps. kawthaungina</i>	MM14 F0083	T	C	A	T	T	C	C	T	C	T	A	C	T	T
<i>Ps. myanmarina</i>	NBRC 112264	T	T	G	G	T	C	T	C	T	C	A	C	T	T
<i>Ps. rhizophorae</i>	MFLUCC 17-560	T	C	A	T	C	G	C	T	C	T	C	C	T	T
<i>Ps. simitheae</i>	MFLUCC 12-0121	C	C	A	T	T	C	C	T	C	T	G	C	A	T
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	C	C	A	T	T	C	C	T	C	T	A	C	T	T
<i>Ps. thailandica</i>	MFLUCC 17-1724	T	C	A	T	C	C	C	T	C	T	C	C	T	T
Taxon	Strain	EF1 α													
		99	100	102	105	114	115	123	125	127	128	134	137	140	150
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	T	C	A	G	-	-	A	C	T	C	C	G	C	C
<i>Ps. curvatispora</i>	MFLUCC 17-1722	T	G	T	A	A	A	A	T	C	C	A	A	T	C
<i>Ps. dawaina</i>	MM14 F0015	C	-	-	A	-	-	T	C	C	C	C	A	T	A
<i>Ps. jiangxiensis</i>	LC4479	T	C	A	G	-	-	A	C	T	C	C	G	C	C
<i>Ps. kawthaungina</i>	MM14 F0083	T	C	A	G	-	-	A	C	T	C	C	G	C	C
<i>Ps. myanmarina</i>	NBRC 112264	T	C	A	G	-	-	A	C	T	C	C	G	C	C
<i>Ps. rhizophorae</i>	MFLUCC 17-560	T	C	T	A	-	-	G	C	C	C	C	A	T	C
<i>Ps. simitheae</i>	MFLUCC 12-0121	T	C	T	A	-	-	G	C	C	C	C	A	T	C
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	T	C	T	A	A	-	A	C	T	T	C	G	T	C
<i>Ps. thailandica</i>	MFLUCC 17-1724	T	C	T	A	-	-	G	C	C	C	C	A	T	C
Taxon	Strain	EF1 α													
		151	152	157	167	172	176	186	252	267	268	269	270	271	272
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	C	A	A	C	A	G	T	A	C	A	G	C	A	A
<i>Ps. curvatispora</i>	MFLUCC 17-1722	-	-	C	T	A	C	C	A	C	A	G	C	A	A
<i>Ps. dawaina</i>	MM14 F0015	G	A	A	T	A	C	C	A	C	A	G	C	A	A
<i>Ps. jiangxiensis</i>	LC4479	C	A	A	T	A	C	T	T	C	A	G	C	A	A
<i>Ps. kawthaungina</i>	MM14 F0083	C	N	A	T	A	C	T	A	C	A	G	C	A	A
<i>Ps. myanmarina</i>	NBRC 112264	C	A	A	T	A	C	T	A	C	A	G	C	A	A
<i>Ps. rhizophorae</i>	MFLUCC 17-560	G	A	A	T	C	C	C	A	-	-	-	-	-	-
<i>Ps. simitheae</i>	MFLUCC 12-0121	G	A	A	T	C	C	C	A	-	-	-	-	-	-
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	A	A	A	T	A	C	T	A	C	A	G	C	A	A
<i>Ps. thailandica</i>	MFLUCC 17-1724	G	A	A	T	C	C	C	A	-	-	-	-	-	-

Supplementary Table 3 Continued.

Taxon	Strain	EF1 α													
		273	274	275	276	277	278	279	280	281	282	283	286	290	304
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	C	-	-	-	C	A	T	G	C	A	C	C	T	A
<i>Ps. curvatispora</i>	MFLUCC 17-1722	C	-	-	-	C	A	T	G	C	A	C	T	T	A
<i>Ps. dawaina</i>	MM14 F0015	C	T	A	C	C	A	T	G	C	A	C	C	T	A
<i>Ps. jiangxiensis</i>	LC4479	C	-	-	-	C	A	T	G	C	A	C	C	T	A
<i>Ps. kawthaungina</i>	MM14 F0083	C	-	-	-	C	A	T	G	C	A	C	C	T	A
<i>Ps. myanmarina</i>	NBRC 112264	C	-	-	-	C	A	T	G	C	A	C	C	T	A
<i>Ps. rhizophorae</i>	MFLUCC 17-560	-	-	-	-	-	-	-	-	-	-	-	C	C	A
<i>Ps. simitheae</i>	MFLUCC 12-0121	-	-	-	-	-	-	-	-	-	-	-	C	T	C
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	C	-	-	-	C	A	T	G	C	A	C	C	T	A
<i>Ps. thailandica</i>	MFLUCC 17-1724	-	-	-	-	-	-	-	-	-	-	-	C	C	A
Taxon	Strain	EF1 α													
		306	308	312	318	321	343	475	476	478	479	480	484	487	488
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	T	A	C	C	C	C	G	T	C	C	T	C	G	C
<i>Ps. curvatispora</i>	MFLUCC 17-1722	C	A	C	C	C	C	T	T	C	C	T	A	A	C
<i>Ps. dawaina</i>	MM14 F0015	C	A	C	C	C	C	T	T	C	T	T	C	A	C
<i>Ps. jiangxiensis</i>	LC4479	T	A	C	C	C	C	G	T	C	C	T	C	G	C
<i>Ps. kawthaungina</i>	MM14 F0083	C	A	C	C	C	C	T	T	A	C	T	C	G	T
<i>Ps. myanmarina</i>	NBRC 112264	T	A	C	C	C	C	G	T	C	C	T	C	G	C
<i>Ps. rhizophorae</i>	MFLUCC 17-560	C	G	C	T	A	T	T	C	C	T	C	C	G	C
<i>Ps. simitheae</i>	MFLUCC 12-0121	C	A	C	C	C	C	T	C	C	T	T	C	G	C
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	C	A	T	C	C	C	T	T	C	C	C	C	A	C
<i>Ps. thailandica</i>	MFLUCC 17-1724	C	G	C	T	A	T	T	C	C	T	C	C	G	C
Taxon	Strain	EF1 α													
		491	492	493	496	500	501	504	510	512	521	524			
<i>Pseudopestalotiopsis avucenniae</i>	MFLUCC 17-0434	A	G	C	C	G	A	T	T	-	T	C			
<i>Ps. curvatispora</i>	MFLUCC 17-1722	A	G	C	C	G	C	T	C	-	T	T			
<i>Ps. dawaina</i>	MM14 F0015	A	G	T	C	G	A	T	T	T	T	T			
<i>Ps. jiangxiensis</i>	LC4479	A	G	C	C	G	A	T	T	-	T	C			
<i>Ps. kawthaungina</i>	MM14 F0083	A	A	C	C	G	A	G	T	-	T	C			
<i>Ps. myanmarina</i>	NBRC 112264	A	G	C	C	G	A	T	T	-	T	C			
<i>Ps. rhizophorae</i>	MFLUCC 17-560	T	C	C	C	G	A	T	T	-	C	C			
<i>Ps. simitheae</i>	MFLUCC 12-0121	A	C	G	C	A	A	T	T	-	C	C			
<i>Ps. taiwanensis</i>	NTUCC 17-002.1	A	G	C	T	G	A	T	T	-	T	G			
<i>Ps. thailandica</i>	MFLUCC 17-1724	T	C	C	C	G	A	T	T	-	C	C			