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## Pestalotioid species associated with medicinal plants in southwest China and Thailand

Ya-Ru Sun, Ruvishika Jayawardena, Jing-E Sun, and Yong Wang

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### Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

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October 24, 2022

Prof. Yong Wang  
Guizhou University  
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China

Re: Spectrum03987-22 (Pestalotioid species associated with medicinal plants in southwest China and Thailand)

Dear Prof. Yong Wang:

Thank you for submitting your manuscript to Microbiology Spectrum. Your manuscript has been reviewed by two specialists in the field who find your working interesting but also identified questions and concerns. Their comments are given below and are marked in the attached documents. Please also make sure to submit gene sequences to genbank and to deposit new isolates in a culture collection (please provide culture collection numbers).

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Sincerely,

Florian Freimoser

Editor, Microbiology Spectrum

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Reviewer comments:

Reviewer #1 (Comments for the Author):

Dear Authors,

This is an important work for fungi associated with medicinal plants and this study enrich the species diversity for Pestalotioid fungal group. However, there are still some mistakes need to be correct, especially, for the genus Pestalotiopsis, there is a newly published paper which introduced 6 new species, you should check this paper and redo the morphology and phylogeny for your paper. Some other comments and suggestions are marked in the attached file.

Reviewer #2 (Comments for the Author):

Dear Authors,

The manuscript entitled "Pestalotioid species associated with medicinal plants in southwest China and Thailand" describes new Pestalotioid species from various hosts got several serious concerns

1. Authors did not follow the recent taxonomic conflicts on *Neopestalotiopsis* and introduced several new species, I would like to get a clear answer why some studies prefer to introduce new species as *Neopestalotiopsis* sp. while you choose to introduce them as new species
2. As I can see in the material and methods, the authors have not chosen 2022 pulsations to construct a phylogenetic tree, thus I am not sure still your species are new. Therefore, both trees required an update following the 2022 papers
3. Add PHI analysis for all new species, if do not have species in the same clade, you can choose species from nearby clades.
4. Did you isolate pathogens? Or do you only observe fruiting bodies from leaf spots? If isolated, please mention it clearly in the methods
5. In descriptions, I recommend rounding off small values, because 0.5  $\mu\text{m}$  is beyond our eye capacity yet your software for measuring is automated. Also, descriptions are consists of different type font types while considering the avg symbol.
6. Since there are a lot of new species, I do not think we required descriptions of new hosts, you can only include material examined and notes.
7. I have several problems related to PHI analysis; in this case, I would prefer to see the alignment. However, the final alignment of the paper is not available for revision.

All other minor comments are given in the file.

Staff Comments:

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Thank you for submitting your paper to Microbiology Spectrum.

# Pestalotioid species associated with medicinal plants in southwest China and Thailand

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## Abstract

In this paper, a total of 27 pestalotioid isolates associated with different medicinal plants from southwest China and Thailand were studied. Based on the morphological examination and multi-gene analyses of three gene loci (TEF, TUB, and ITS), these 27 isolates represent 17 species distributed in three genera; including six new *Neopestalotiopsis* species, four new *Pestalotiopsis* species, and six new records. Genealogical Concordance Phylogenetic Species Recognition (GCPSR) with a pairwise homoplasy index (PHI) test was also carried out to provide additional evidence for the placements of the ten new species. Further, simplifying the description of pestalotioid species is discussed, and a checklist for pestalotioid species associated with medicinal plants worldwide is provided.

## Importance

Pestalotioid species are an important fungal group, occurring commonly as important plant pathogens, endophytes, and saprophytes. The study of pestalotioid species associated with medicinal plants is significant for agriculture, industry, and pharmaceutical industry but remains poorly studied. In this study, we reported 10 new pestalotioid species and 6 new records based on morphology and molecular analyses. Our study significantly enriches the species richness of pestalotioid and provides a basis for follow-up studies.

**Keywords** – 10 new species – 6 new records – Diversity – Endophytes – Plant pathogens – Phylogeny – Saprophytes – Taxonomy

## Introduction

Medicinal plants play a crucial role in the development of human cultures. They are a rich source of natural products with both biological and chemical properties. They play a health care or treatment role in various ways and have been used since prehistoric times across the world (Rasool-Hassan 2012, Rasool et al. 2020). It is estimated that more than 70% of the world's population relies on medicinal plants



(David et al. 2015). Microfungi can affect the growth and quality of medicinal plants. Some endophytes isolated from medicinal plants have broad developmental prospects (Guo 2016, Jia et al. 2016). Microfungi associated with medicinal plants have always been among the researched hotspots (Weber et al. 2004, Sun et al. 2008, Abtahi and Nourani 2017, Keshri et al. 2021).

Pestalotioid species is a very common group of fungi, which form important associations with different plants as pathogens, endophytes, or saprophytes, and are widely distributed in tropical and temperate regions (Guba 1961, Barr 1975, Nag Raj 1993, Maharachchikumbura et al. 2014, Hyde et al. 2014, Jayawardena et al. 2019, 2021, Norphanphoun et al. 2019, Ul Haq et al. 2021, Yang et al. 2021). Traditionally taxonomy of pestalotioid species mainly depended on their hosts and color intensities of the median conidial cell (Moreau 1949, Steyaert 1949, Guba 1961, Sutton 1980). With the development of DNA-based phylogenetic analyses, the traditional classification system has been proved unreliable. The use of molecular data in resolving pestalotioid species was revisited by Maharachchikumbura et al. (2014) and they separated this group into three genera, viz. *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis*. *Neopestalotiopsis* differs from *Pseudopestalotiopsis* and *Pestalotiopsis* by its versicolourous (two upper median cells darker than the lowest median cell) median cells and indistinct conidiophores, while *Pseudopestalotiopsis* can be easily distinguished from *Pestalotiopsis* by darker colored concolourous (for those possessing equally pigmented median cells) median cells (Maharachchikumbura et al. 2014).

As important plant pathogens, pestalotioid species are almost ubiquitous in agricultural and non-cultivated ecosystems, causing multiple diseases and huge economic losses (Zhang et al. 2012, Maharachchikumbura et al. 2013, Jayawardena et al. 2016, Liu et al. 2017, Yang et al. 2017, Mahapatra et al. 2018, Diogo et al. 2021, Prasannath et al. 2021). For example, grey blight disease of tea plants is caused by *Pseudopestalotiopsis* spp. and *Pestalotiopsis* spp. and accounts for at least 17 % production damage in southern India (Joshi et al. 2009) and 10–20 % yield loss in Japan (Horikawa 1986). *Neopestalotiopsis clavispora* caused the leaf blight of *Elettaria cardamomu* in India (Biju et al. 2018), and leaf spot of *Taxus chinensis* in China (Wang et al. 2019). Diogo et al. (2021) reported that pestalotioid fungi caused stem girdling and dieback in young eucalyptus plants in Portugal. Li et al. (2021) identified five new pestalotioid species associated with symptomatic leaves of *Camellia oleifera* in China. Thus, it is necessary to study the pathogenic pestalotioid species related to medicinal plants, which could provide the research foundation for the prevention and treatment of diseases and reduce economic losses.

The study of endophytic fungi in medicinal plants is of great significance for elucidating their distribution, growth and developmental characteristics and resource regeneration (Weber et al. 2004, Huang et al. 2008, Sun et al. 2008, Schafhauser et al. 2019, Keshri et al. 2021). Many pestalotioid fungi have been found as endophytes from different medicinal plants with richer secondary metabolites (Huang et al. 2008, Jia et al. 2016, Xu et al. 2010, 2014, Reddy et al. 2016, Ma et al. 2019). For example,

the endophytic fungus, *Pestalotiopsis versicolor*, was isolated from the healthy leaves of *Taxus cuspidata* and it is an excellent candidate for an alternate source of Taxol supply (Kumaran et al. 2010). Therefore, the study of endophytic pestalotioid species related to medicinal plants could be of very importance to pharmaceuticals and therapeutic medicine.

This study aims to identify the pestalotioid fungi associated with medicinal plants in southwest China and Thailand based on morphology, phylogenetic analyses, and GCPSR. This paper describes, illustrates, and compares ten new species and six new records with allied species. In addition, we provided a checklist for pestalotioid species associated with medicinal plants worldwide.

## Materials and methods

### Collection, examination and isolation

The fresh samples of different medicinal plants were collected in southwest China and Thailand from 2019 to 2022. Samples were brought to the laboratory in Ziplock plastic bags or paper envelopes for examination. The fruiting bodies on natural substrates were observed and photographed using a stereo-microscope (SteREO Discovery, V12, Carl Zeiss Microscopy GmbH, Germany; VHX-7000, Keyence, Japan). Morphological characters were observed using a Nikon ECLIPSE Ni compound microscope (Nikon, Japan) and photographed with a Nikon DS-Ri2 digital camera (Nikon, Japan); Carl Zeiss compound microscope (Carl Zeiss AG, Germany) and photographed with an Axiocam 208 color digital camera (Carl Zeiss AG, Germany). The photo plates were made by the Adobe Photoshop CS6 Extended v. 13.0 software. Measurements were obtained with the Tarosoft (R) Image Frame Work software.

For pathogens and saprophytes, single spore isolations were used to obtain pure cultures, following the methods described by Senanayake et al. (2020). Germinated conidia were transferred to fresh potato dextrose agar (PDA) plates and incubated at 25°C for four weeks. For endophytes, materials were washed under running tap water, immersed in 70% ethanol for 1 min, followed by soaking in 4% NaOCl for 1 min, rinsed three times in sterile distilled water, and dried on a sterile filter paper. For the control, the final sterile water rinsed was plated and observed during the post-incubation period. The absence of any fungal (microbial) growth indicated that the leaf surface was sterile (Kjer et al. 2010). The sterilized materials were cut into 2-5 mm<sup>2</sup> segments and placed on PDA containing 50 µg/ml penicillin and 50 µg/ml streptomycin (Otero et al. 2002). The plates were observed daily and the mycelial growth on the edge of fungal colonies were transferred to fresh PDA dishes to obtain pure cultures. The pure cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand and the Culture Collection of the Department of Plant Pathology, Agriculture College, Guizhou University (GUCC), Guiyang, China. Specimens were deposited in the herbarium of Mae Fah Luang University (MFLU) Chiang Rai, Thailand, the herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP), Guiyang, China, and

the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS), Kunming, China. Facesoffungi (FoF) and Index Fungorum numbers were acquired as described in Jayasiri et al. (2015) and in Index Fungorum (2022).

### **DNA extraction, PCR amplification and sequencing**

BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416, BIOMIGA, San Diego, California, USA) was used to extract DNA from fresh fungal mycelia, which were grown on PDA medium for 4 weeks at 25 °C. Three genes were selected in this study: the internal transcribed spacers (ITS), the translation elongation factor 1 (TEF), and the partial  $\beta$ -tubulin region (TUB). Polymerase chain reaction (PCR) was carried out in 20  $\mu$ L of reaction volume which contained 10  $\mu$ L 2  $\times$  PCR Master Mix, 7  $\mu$ L of ddH<sub>2</sub>O, 1  $\mu$ L of each primer, and 1  $\mu$ L of template DNA. The PCR thermal cycle program and primers are given (Table 1). Purification and sequencing of PCR products were carried out at SinoGenoMax (Beijing) Co., China.

**Table 1.** Primers and PCR procedures used in this study.

Locus	Primers		PCR procedures	Reference
	Name	Sequence (5'-3')		
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	94 °C 3 min; 94 °C 30 s; 52 °C 30 s; 72 °C 1 min; Repeat 2–4	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	for 35 cycles; 72 °C 8 min; 4 °C on hold	
TEF	EF1-728F	CAT CGA GAA GTT CGA GAA GG	94 °C 5 min; 94 °C 30 sec; 52 °C 30 sec; 72 °C 1 min;	Carbone & Kohn (1999), O'Donnell et al. (1998)
	EF2	GGA RGT ACC AGT SAT CAT GTT	Repeat 2–4 for 40 cycles; 72 °C 8 min; 4 °C on hold	
TUB	T1	AACATGCGTGAGATTGTAAGT	95 °C 3 min; 94 °C 30 sec; 55 °C 50 sec; 72 °C 1 min;	Glass & Donaldson (1995), O'Donnell & Cigel'nik (1997)
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	Repeat 2–4 for 40 cycles; 72 °C 8 min; 4 °C on hold	

**Table 2.** The best-fit evolutionary models used in our phylogenetic analyses

Data set	Method	Model		
		TEF	TUB	ITS
<i>Neopestalotiopsis</i>	ML	HKY+F+G4	TNe+I+G4	TIM2+F+I+G4
	BYPP	GTR+I+G	GTR+I+G	GTR+I+G TPM3u+F+I+G
<i>Pestalotiopsis</i>	ML	TN+F+I+G4	K2P+I+G4	4
	BYPP	GTR+I+G	GTR+I+G	GTR+I+G
<i>Pseudopestalotiopsis</i>	ML	TIM+F+I+G4	GTR+F+I+G4	TIM2+I+G4
	BYPP	GTR+I+G	GTR+I+G	GTR+I+G

## Phylogenetic analyses

Phylogenetic analysis and its preparatory work were carried out according to the method described in Dissanayake et al. (2020). The sequences were compared against the NCBI GenBank nucleotide database using BLASTn to evaluate the closest phylogenetic matches. All sequences used in this study were downloaded from GenBank referring to previous studies (Maharachchikumbura et al. 2016b, Liu et al. 2017, Nozawa et al. 2017, Norphanphoun et al. 2019, Prasannath et al. 2021) (Table 3). The single gene sequences were checked with BioEdit v. 7.0.9.0 (Hall 1999). Alignments for each locus were generated using MAFFT v.7 (<https://mafft.cbrc.jp/alignment/server/>) and manually improved using AliView (Larsson 2014). The final single-gene alignments were combined by SequenceMatrix 1.7.8 (Vaidya et al. 2011).

The maximum likelihood (ML) analyses were carried out using IQ-TREE (Nguyen et al. 2015, Trifinopoulos et al. 2016) on the IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at>, 1 May 2022) under partitioned models. The best-fit substitution models (Table 2) were determined by jModelTest (Chernomor et al. 2016). Ultrafast bootstrap analyses were implemented with 1,000 replicates (Minh et al. 2013, Hoang et al. 2018).

The Bayesian posterior probability (BYPP) analyses were performed in CIPRES (Miller et al. 2010) with MrBayes on XSEDE 3.2.7a (Ronquist et al. 2012). MrModeltest 2.2 (Nylander 2004) was used to evaluate the best nucleotide substitution models (Table 2) for each data. The Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP) (Rannala and Yang 1996). Six simultaneous Markov chains were run for 10 million generations and trees were sampled every 1,000<sup>th</sup> generation. The first 20% of trees, representing the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree.

The final phylogenetic trees were viewed and changed format with FigTree v1.4.0 (Rambaut and Drummond 2008) and modified in Microsoft Office PowerPoint 2010. The new sequences used in this study were deposited in GenBank.

## Genealogical Concordance Phylogenetic Species Recognition Analysis

The Genealogical Concordance Phylogenetic Species Recognition (GCPSR) was used to analyze the new species, their species boundaries, and their most closely related taxa as described by Quaedvlieg et al. (2014). The pairwise homoplasy index (PHI) test (Bruen et al. 2006) was performed in SplitsTree4 (Huson 1998, Huson and Bryant 2006). If the PHI is above 0.05 threshold ( $\Phi_w > 0.05$ ), it indicates there is no significant recombination present in the dataset. The relationships between closely related species were visualized in splits graphs with both, the Log-Det transformation and splits decomposition options.

**Table 3.** Taxa of the three genera; *Neopestalotiopsis*, *Pestalotiopsis*, and *Pseudopestalotiopsis* used in the phylogenetic analysis with the corresponding GenBank accession numbers.

Taxa	Strain_number	TEF	TUB	ITS
<i>Neopestalotiopsis acrostichi</i>	MFLUCC 17-1754	MK764316	MK764338	MK764272
<i>N. alpapicalis</i>	MFLUCC 17-2544	MK463547	MK463545	MK357772
<i>N. amomumica</i>	HKAS 124563			
<i>N. amomumica</i>	HKAS 124564			
<i>N. aotearoa</i>	CBS 367.54	KM199526	KM199454	KM199369
<i>N. asiatica</i>	MFLUCC 12-0286	JX399049	JX399018	JX398983
<i>N. australis</i>	CBS 114159	KM199537	KM199432	KM199348
<i>N. brachiata</i>	MFLUCC 17-1555	MK764318	MK764340	MK764274
<i>N. brasiliensis</i>	COAD 2166	MG692402	MG692400	MG686469
<i>N. camelliae-oleiferae</i>	CSUFTCC81	OK507955	OK562360	OK493585
<i>N. camelliae-oleiferae</i>	CSUFTCC82	OK507956	OK562361	OK493586
<i>N. cavernicola</i>	KUMCC 20-0269	MW550735	MW557596	MW545802
<i>N. cavernicola</i>	KUMCC 20-0332	MW590327	MW590328	MW581238
<i>N. Chiangmaiensis</i>	MFLUCC 18-0113	MH388404	MH412725	N/A
<i>N. chinensis</i>	HKAS 124565			
<i>N. chinensis</i>	HKAS 124565			
<i>N. chinensis</i>	GUCC 808			
<i>N. chrysea</i>	MFLUCC 12-0261	JX399051	JX399020	JX398985
<i>N. clavispora</i>	MFLUCC 12-0281	JX399045	JX399014	JX398979
<i>N. cocoas</i>	MFLUCC 15-0152	KX789689	N/A	NR_156312
<i>N. coffea arabicae</i>	HGUP4015	KF412644	KF412641	KF412647
<i>N. cubana</i>	CBS 600.96	KM199521	KM199438	KM199347
<i>N. dendrobii</i>	MFLUCC 14-0106	MK975829	MK975835	MK993571
<i>N. dendrobii</i>	MFLUCC 14-0132	MK975830	N/A	MK993572
<i>N. drethii</i>	BRIP 72264a	MZ344172	MZ312680	MZ303787
<i>N. drethii</i>	BRIP 72263a	MZ344171	MZ312679	MZ303786
<i>N. ellipsozona</i>	MFLUCC 12-0283	JX399047	JX399016	JX398980
<i>N. egyptiaca</i>	CBS 140162	KP943748	KP943746	KP943747
<i>N. eucalypticola</i>	CBS 264.37	KM199551	KM199431	KM199376
<i>N. eucalyptorum</i>	PE194	MW805398	MW802831	MW794098
<i>N. eucalyptorum</i>	CBS 147684	MW805397	MW802841	MW794108
<i>N. foedans</i>	CGMCC 2-0123	JX399053	JX399022	JX398987
<i>N. formicarum</i>	CBS 362.72	KM199517	KM199455	KM199358
<i>N. formicarum</i>	CBS 115.83	KM199519	KM199444	KM199344
<i>N. formicarum</i>	GUCC 809			
<i>N. guajavae</i>	FMB0026	MH460868	MH460871	MF783085
<i>N. guajavicola</i>	FMB0129	MH460870	MH460873	MH209245
<i>N. haikouensis</i>	SAUCC212272	OK104877	OK104870	OK087294
<i>N. haikouensis</i>	SAUCC212272	OK104878	OK104871	OK087295
<i>N. hadrolaeliae</i>	VIC 47180	MK465122	MK465120	MK454709
<i>N. hispanica</i>	CBS 147686	MW805399	MW802840	MW794107
<i>N. honoluluana</i>	CBS 114495	KM199548	KM199457	NR_145245
<i>N. hydeana</i>	MFLUCC 20-0132	MW251129	MW251119	MW266069

<i>N. hyperica</i>	<b>HKAS 124561</b>			
<i>N. hyperica</i>	<b>GUCC 811</b>			
<i>N. iberica</i>	<b>CBS 147688</b>	<b>MW805402</b>	<b>MW802844</b>	<b>MW794111</b>
<i>N. iraniensis</i>	<b>CBS 137768</b>	<b>KM074051</b>	<b>KM074057</b>	<b>KM074048</b>
<i>N. javaensis</i>	<b>CBS 257 31</b>	<b>KM199543</b>	<b>KM199437</b>	<b>NR_145241</b>
<i>N. keteleeria</i>	<b>MFLUCC 13-0915</b>	<b>KJ503822</b>	<b>KJ503821</b>	<b>KJ503820</b>
<i>N. longiappendiculata</i>	<b>MEAN 1315</b>	<b>MW805404</b>	<b>MW802845</b>	<b>MW794112</b>
<i>N. lusitanica</i>	<b>MEAN 1317</b>	<b>MW805406</b>	<b>MW802843</b>	<b>MW794110</b>
<i>N. lusitanica</i>	<b>MEAN 1320</b>	<b>MW805409</b>	<b>MW802830</b>	<b>MW794097</b>
<i>N. macadamiae</i>	<b>BRIP 63737c</b>	<b>KX186627</b>	<b>KX186654</b>	<b>NR_161002</b>
<i>N. maddoxii</i>	<b>BRIP 72266a</b>	<b>MZ344167</b>	<b>MZ312675</b>	<b>MZ303782</b>
<i>N. magna</i>	<b>MFLUCC 12-0652</b>	<b>KF582791</b>	<b>KF582793</b>	<b>KF582795</b>
<i>N. mesopotamica</i>	<b>CBS 336.86</b>	<b>KM199555</b>	<b>KM199441</b>	<b>KM199362</b>
<i>N. musae</i>	<b>MFLUCC 15-0776</b>	<b>KX789685</b>	<b>KX789686</b>	<b>NR_156311</b>
<i>N. natalensis</i>	<b>CBS 138.41</b>	<b>KM199552</b>	<b>KM199466</b>	<b>NR_156288</b>
<i>N. nebuloides</i>	<b>BRIP 66617</b>	<b>MK977633</b>	<b>MK977632</b>	<b>MK966338</b>
<i>N. olumideae</i>	<b>BRIP 72273a</b>	<b>MZ344175</b>	<b>MZ312683</b>	<b>MZ303790</b>
<i>N. pandanicola</i>	<b>KUMCC 17-0175</b>	<b>MH388389</b>	<b>MH412720</b>	<b>N/A</b>
<i>N. pernambucana</i>	<b>GS 2014-RV01</b>	<b>KU306739</b>	<b>N/A</b>	<b>KJ792466</b>
<i>N. petila</i>	<b>MFLUCC 17-1738</b>	<b>MK764319</b>	<b>MK764341</b>	<b>MK764275</b>
<i>N. phangngaensis</i>	<b>MFLUCC 18-0119</b>	<b>MH388390</b>	<b>MH412721</b>	<b>MH388354</b>
<i>N. photiniana</i>	<b>GUCC 810</b>			
<i>N. perukae</i>	<b>FMB0127</b>	<b>MH523647</b>	<b>MH460876</b>	<b>MH209077</b>
<i>N. piceana</i>	<b>CBS 394.48</b>	<b>KM199527</b>	<b>KM199453</b>	<b>KM199368</b>
<i>N. protearum</i>	<b>CBS 114178</b>	<b>KM199542</b>	<b>KM199463</b>	<b>JN712498</b>
<i>N. psidii</i>	<b>FMB0028</b>	<b>MH460874</b>	<b>MH477870</b>	<b>MF783082</b>
<i>N. rhapsidis</i>	<b>GUCC21501</b>	<b>MW980442</b>	<b>MW980441</b>	<b>MW931620</b>
<i>N. rhapsidis</i>	<b>HKAS 124559</b>			
<i>N. rhizophorae</i>	<b>MFLUCC 17-1550</b>	<b>MK764321</b>	<b>MK764343</b>	<b>MK764277</b>
<i>N. rhododendri</i>	<b>GUCC 2150</b>	<b>MW980444</b>	<b>MW980443</b>	<b>MW979577</b>
<i>N. rhododendri</i>	<b>MFLUCC 22-</b>			
<i>N. rosae</i>	<b>CBS 101057</b>	<b>KM199523</b>	<b>KM199429</b>	<b>KM199359</b>
<i>N. rosicola</i>	<b>CFCC 51992</b>	<b>KY885243</b>	<b>KY885245</b>	<b>KY885239</b>
<i>N. samarangensis</i>	<b>MFLUCC 12-0233</b>	<b>JQ968611</b>	<b>JQ968610</b>	<b>JQ968609</b>
<i>N. saprophytica</i>	<b>MFLUCC 12-0282</b>	<b>KM199538</b>	<b>KM199433</b>	<b>KM199345</b>
<i>N. sichuanensis</i>	<b>CFCC 54338</b>	<b>MW199750</b>	<b>MW218524</b>	<b>MW166231</b>
<i>N. sichuanensis</i>	<b>SM15-1C</b>	<b>MW199751</b>	<b>MW218525</b>	<b>MW166232</b>
<i>N. sonneratae</i>	<b>MFLUCC 17-1745</b>	<b>MK764323</b>	<b>MK764345</b>	<b>MK764279</b>
<i>N. steyaertii</i>	<b>IMI 192475</b>	<b>KF582792</b>	<b>KF582794</b>	<b>KF582796</b>
<i>N. subtropicalis</i>	<b>GUCC 805</b>			
<i>N. subtropicalis</i>	<b>MFLUCC 22-</b>			
<i>N. suphanburica</i>	<b>MFLUCC 22-</b>			
<i>N. surinamensis</i>	<b>CBS 450 74</b>	<b>KM199518</b>	<b>KM199465</b>	<b>KM199351</b>
<i>N. thailandica</i>	<b>MFLUCC 17-1730</b>	<b>MK764325</b>	<b>MK764347</b>	<b>MK764281</b>



<i>N. umbrinospora</i>	MFLUCC 12-0285	JX399050	JX399019	JX398984
<i>N. vheena</i>	BRIP 72293a	MZ344177	MZ312685	MZ303792
<i>N. vitis</i>	MFLUCC 15-1265	KU140676	KU140685	KU140694
<i>N. zakeelii</i>	BRIP 72282a	MZ344174	MZ312682	MZ303789
<i>N. zimbabwana</i>	CBS 111495	KM199545	KM199456	MH554855
<i>Neopestalotiopsis</i> sp1	CFCC 54337	MW199752	MW218526	MW166233
<i>Neopestalotiopsis</i> sp1	ZX12-1	MW199753	N/A	MW166234
<i>Neopestalotiopsis</i> sp2	CFCC 54340	MW199754	MW218528	MW166235
<i>Neopestalotiopsis</i> sp2	ZX22B	MW199755	MW218529	MW166236
<i>Pestalotiopsis adusta</i>	ICMP 6088	JX399070	JX399037	JX399006
<i>P. adusta</i>	MFLUCC 10-0146	JX399071	JX399038	JX399007
<i>P. aggestorum</i>	LC6301	KX895234	KX895348	KX895015
<i>P. anacardiacearum</i>	IFRDCC 2397	KC247156	KC247155	KC247154
<i>P. arceuthobii</i>	CBS 434.65	KM199516	KM199427	KM199341
<i>P. arengae</i>	CBS 331.92	KM199515	KM199426	KM199340
<i>P. australasiae</i>	CBS 114126	KM199499	KM199409	KM199297
<i>P. australasiae</i>	CBS 11141	KM199501	KM199410	KM199298
<i>P. australis</i>	CBS 114193	KM199475	KM199383	KM199332
<i>P. biciliata</i>	CBS 124463	KM199505	KM199399	KM199308
<i>P. biciliata</i>	CAA1011	MW959090	MW934601	MW969738
<i>P. brachiata</i>	LC2988	KX895150	KX895265	KX894933
<i>P. brachiata</i>	LC8189	KY464153	KY464163	KY464143
<i>P. brassicae</i>	CBS 170.26	KM199558	N/A	KM199379
<i>P. camelliae</i>	MFLUCC 12-0277	JX399074	JX399041	JX399010
<i>P. camelliae-oleiferae</i>	CSUFTCC08	OK507963	OK562368	OK493593
<i>P. camelliae-oleiferae</i>	CSUFTCC09	OK507964	OK562369	OK493594
<i>P. chamaeropis</i>	CBS 186.71	KM199473	KM199391	KM199326
<i>P. chamaeropis</i>	GUCC 800			
<i>P. chiangmaiensis</i>	MFLUCC 22-			
<i>P. chiangmaiensis</i>	MFLUCC 22-			
<i>P. chinensis</i>	MFLUCC 12-0273	N/A	N/A	NR_111786
<i>P. clavata</i>	MFLUCC 12-0268	JX399056	JX399025	JX398990
<i>P. colombiensis</i>	CBS 118553	KM199488	KM199421	KM199307
<i>P. digitalis</i>	ICMP 5434	N/A	KP781883	KP781879
<i>P. diploclisiae</i>	CBS 115587	KM199486	KM199419	KM199320
<i>P. dilucida</i>	LC3232	KX895178	KX895293	KX894961
<i>P. diversiseta</i>	MFLUCC 12-0287	JX399073	JX399040	NR_120187
<i>P. dracaenae</i>	HGUP4037	MT598644	MT598645	
<i>P. dracaenicola</i>	MFLUCC 18-0913	MN962732	MN962733	MN962731
<i>P. dracontomelon</i>	MFUCC 10-0149	KP781880	N/A	KP781877
<i>P. dracontomelon</i>	MFLUCC 22-			
<i>P. endophytica</i>	MFLUCC 18-0932	MW417119	N/A	NR_172439
<i>P. ericacearum</i>	IFRDCC 2439	KC537814	KC537821	KC537807
<i>P. etonensis</i>	BRIP 66615	MK977635	MK977634	MK966339



<i>P. formosana</i>	NTUCC 17-009	MH809389	MH809385	MH809381
<i>P. furcata</i>	MFLUCC 12-0054	JQ683740	JQ683708	JQ683724
<i>P. gaultheria</i>	IFRD 411.014	KC537812	KC537819	KC537805
<i>P. gibbosa</i>	NOF 3175	LC311591	LC311590	LC311589
<i>P. grevilleae</i>	CBS 114127	KM199504	KM199407	KM199300
<i>P. hawaiiensis</i>	CBS 114491	KM199514	KM199428	KM199339
<i>P. hydei</i>	MFLUCC 20-0135	MW251113	MW251112	NR_172003
<i>P. hydei</i>	<b>GUCC 816</b>			
<i>P. hollandica</i>	CBS 265.33	KM199481	KM199388	KM199328
<i>P. hollandica</i>	MEAN 1091	MT374691	MT374703	MT374678
<i>P. humus</i>	CBS 336.97	KM199484	KM199420	KM199317
<i>P. hunanensis</i>	CSUFTCC15	OK507969	OK562374	OK493599
<i>P. hunanensis</i>	CSUFTCC18	OK507970	OK562375	OK493600
<i>P. iberica</i>	CAA1006	MW759039	MW759036	MW732249
<i>P. inflexa</i>	MFLUCC 12-0270	JX399072	JX399039	JX399008
<i>P. intermedia</i>	MFLUCC 12-0259	JX399059	JX399028	JX398993
<i>P. italiana</i>	MFLUCC 12-0657	KP781881	KP781882	KP781878
<i>P. jesteri</i>	CBS 109350	KM199554	KM199468	KM199380
<i>P. jiangxiensis</i>	LC4399	KX895227	KX895341	KX895009
<i>P. jinchanghensis</i>	LC6636	KX895247	KX895361	KX895028
<i>P. kandelicola</i>	NCYU 19-0355	MT563101	MT563099	MT560722
<i>P. kaki</i>	KNU-PT-1804	LC553555	LC552954	LC552953
<i>P. kenyana</i>	CBS 442.67	KM199502	KM199395	KM199302
<i>P. kenyana</i>	CBS 911.96	KM199503	KM199396	KM199303
<i>P. knightiae</i>	CBS 114138	KM199497	KM199408	KM199310
<i>P. knightiae</i>	CBS 111963	KM199495	KM199406	KM199311
<i>P. licualacola</i>	HGUP4057	KC481684	KC481683	KC492509
<i>P. linearis</i>	MFLUCC 12-0271	JX399058	JX399027	JX398992
<i>P. longiappendiculata</i>	LC3013	KX895156	KX895271	KX894939
<i>P. lushanensis</i>	LC4344	KX895223	KX895337	KX895005
<i>P. lushanensis</i>	LC8182	KY464146	KY464156	KY464136
<i>P. macadamiae</i>	BRIP 63738B	KX186621	KX186680	KX186588
<i>P. malayana</i>	CBS 102220	KM199482	KM199411	KM199306
<i>P. monochaeta</i>	CBS 144.97	KM199479	KM199386	KM199327
<i>P. monochaeta</i>	CBS 440.83	KM199480	KM199387	KM199329
<i>P. montellica</i>	MFLUCC 12-0279	JX399076	JX399043	JX399012
<i>P. nanjingensis</i>	CSUFTCC16	OK507972	OK562377	OK493602
<i>P. nanjingensis</i>	CSUFTCC20	OK507973	OK562378	OK493603
<i>P. nanningensis</i>	CSUFTCC10	OK507966	OK562371	OK493596
<i>P. nanningensis</i>	CSUFTCC11	OK507967	OK562372	OK493597
<i>P. neolitseae</i>	NTUCC 17-011	MH809391	MH809387	MH809383
<i>P. novae-hollandiae</i>	CBS 130973	KM199511	KM199425	KM199337
<i>P. oryzae</i>	CBS 353.69	KM199496	KM199398	KM199299
<i>P. oryzae</i>	CL107	MN022941	MN015425	MK156295

<i>P. papuana</i>	CBS 331.96	KM199491	KM199413	KM199321
<i>P. papuana</i>	MFLU 19-2764	MW192204	MW296942	MW114337
<i>P. parva</i>	CBS 265.37	KM199508	KM199404	KM199312
<i>P. pallidotheae</i>	MAFF 240993	LC311585	LC311584	NR_111022
<i>P. peristrophidis</i>	<b>GUCC 803</b>			
<i>P. peristrophidis</i>	<b>GUCC 804</b>			
<i>P. peristrophidis</i>	<b>GUCC 802</b>			
<i>P. peristrophidis</i>	<b>GUCC 802</b>			
<i>P. photinicola</i>	GZCC 16-0028	KY047662	KY047663	KY092404
<i>P. pinisp</i>	CBS 146841	MT374694	MT374706	MT374681
<i>P. portugalia</i>	CBS 393.48	KM199510	KM199422	KM199335
<i>P. rhizophorae</i>	MFLUCC 17-0416	MK764327	MK764349	MK764283
<i>P. rhizophorae</i>	MFLUCC 17-0417	MK764328	MK764350	MK764284
<i>P. rhododendri</i>	IFRDCC 2399	KC537811	KC537818	NR_120265
<i>P. rhodomyrtus</i>	HGUP4230	KF412645	KF412642	KF412648
<i>P. rhodomyrtus</i>	MG7	MZ126725	MZ126718	MZ089458
<i>P. rosea</i>	MFLUCC 12-0258	JX399069	JX399036	JX399005
<i>P. scoparia</i>	CBS 176.25	KM199478	KM199393	KM199330
<i>P. sequoiae</i>	MFLUCC 13-0399	N/A	N/A	NR_153271
<i>P. shandogensis</i>	JZB340038	MN62674	MN626729	MN625275
<i>P. shorea</i>	MFLUCC 12-0314	KJ503817	KJ503814	KJ503811
<i>P. smilaxe</i>	<b>MFLUCC 22-</b>			
<i>P. smilaxe</i>	<b>MFLUCC 22-</b>			
<i>P. spathulata</i>	CBS 356.86	KM199513	KM199423	KM199338
<i>P. spathuliappendiculata</i>	CBS 144035	MH554607	MH554845	MH554172
<i>P. telopeae</i>	CBS 114161	KM199500	KM199403	KM199296
<i>P. telopeae</i>	CBS 114137	KM199559	KM199469	KM199301
<i>P. thailandica</i>	MFLUCC 17-1616	MK764329	MK764351	MK764285
<i>P. thailandica</i>	MFLUCC 17-1617	MK764329	MK764351	MK764285
<i>P. trachycarpicola</i>	IFRDCC 2240	JQ845946	JQ845945	NR_120109
<i>P. unicolor</i>	MFLUCC 12-0276	N/A	JX399030	JX398999
<i>P. verruculosa</i>	MFLUCC 12-0274	JX399061	N/A	JX398996
<i>P. yanglingensis</i>	LC4553	KX895231	KX895345	KX895012
<i>Pestalotiopsis</i> sp	LC3637	KX895210	KX895324	KX894993
<i>Pseudopestalotiopsis</i>				
<i>ampullacea</i>	LC6618	KX895244	KX895358	KX895025
<i>Ps. annellata</i>	NTUCC 17-030	MT321988	MT321889	MT322087
<i>Ps. avicenniae</i>	MFLUCC 17-0434	MK764331	MK764353	MK764287
<i>Ps. camelliae-sinesis</i>	LC3490	KX895202	KX895316	KX894985
<i>Ps. chinensis</i>	LC3011	KX895154	KX895269	KX894937
<i>Ps. curvatispora</i>	MFLUCC 17-1722	MK764332	MK764354	MK764288
<i>Ps. cocos</i>	CBS 272.29	KM199553	KM199467	KM199378
<i>Ps. dawaina</i>	MM14 F0015	LC324752	LC324751	LC324750

<i>Ps. gilvanii</i>	<b>INPA 2913</b>	<b>MN385957</b>	<b>MN385954</b>	<b>MN385951</b>
<i>Ps. gilvanii</i>	INPA 2914	MN385958	MN385955	MN385952
<i>Ps. ignota</i>	<b>NN 42909</b>	<b>KU500016</b>	N/A	<b>KU500020</b>
<i>Ps. indica</i>	<b>CBS 459.78</b>	<b>KM199560</b>	<b>KM199470</b>	<b>KM199381</b>
<i>Ps. ixorae</i>	<b>NTUCC 17-001.1</b>	<b>MG816336</b>	<b>MG816326</b>	<b>MG816316</b>
<i>Ps. kawthaungina</i>	<b>MM14 F0083</b>	<b>LC324755</b>	<b>LC324754</b>	<b>LC324753</b>
<i>Ps. kubahensis</i>	<b>UMAS-KUB-P20</b>	N/A	N/A	<b>KT006749</b>
<i>Ps. myanmarina</i>	<b>NBRC 112264</b>	<b>LC114065</b>	<b>LC114045</b>	<b>LC114025</b>
<i>Ps. rhizophorae</i>	<b>MFLUCC 17-1560</b>	<b>MK764335</b>	<b>MK764357</b>	<b>MK764291</b>
<i>Ps. simitheae</i>	<b>MFLUCC 12-0121</b>	<b>KJ503818</b>	<b>KJ503815</b>	<b>KJ503812</b>
<i>Ps. solicola</i>	<b>CBS 386.97</b>	<b>MH554474</b>	<b>MH554715</b>	<b>NR_161086</b>
<i>Ps. taiwanensis</i>	<b>NTUCC 17-002.1</b>	<b>MG816339</b>	<b>MG816329</b>	<b>MG816319</b>
<i>Ps. thailandica</i>	<b>MFLUCC 17-1724</b>	<b>MK764336</b>	<b>MK764358</b>	<b>MK764292</b>
<i>Ps. theae</i>	<b>MFLUCC 12-0055</b>	<b>JQ683743</b>	<b>JQ683711</b>	<b>JQ683727</b>
<i><b>Ps. theae</b></i>	<b>MFLUCC 22-</b>			
<i>Ps. vietnamensis</i>	<b>NBRC 112252</b>	<b>LC114074</b>	<b>LC114054</b>	<b>LC114034</b>

Ex-type strains are in bold. The newly generated strains are indicated in red. N/A: Not available.

## Results

### Part1: *Neopestalotiopsis*

#### Phylogenetic analyses

The combined datasets consist of 98 *Neopestalotiopsis* strains along with the outgroup *Pestalotiopsis diversiseta* (MFLUCC 12-0287) and *P. spathulata* (CBS 356.86), which were analyzed to infer the interspecific relationships within *Neopestalotiopsis*. The aligned sequence matrix comprised TEF (1–500), TUB (501–1,015), and ITS (1,016–1,453), sequence data for a total of 1,305 characters, including coded alignment gaps. Similar tree topologies were obtained by ML and BYPP methods, and the most likely tree ( $-\ln = 7569.2805$ ) is presented in Figure 1. The phylogenetic tree which analyzed the 14 *Neopestalotiopsis* isolates from medicinal plants indicated six novel species and two new records.

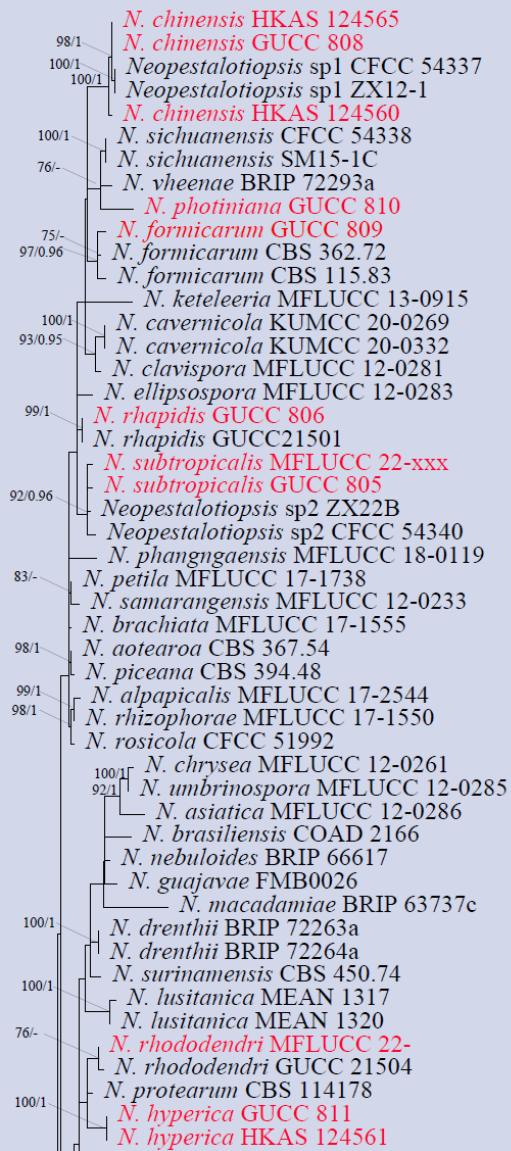
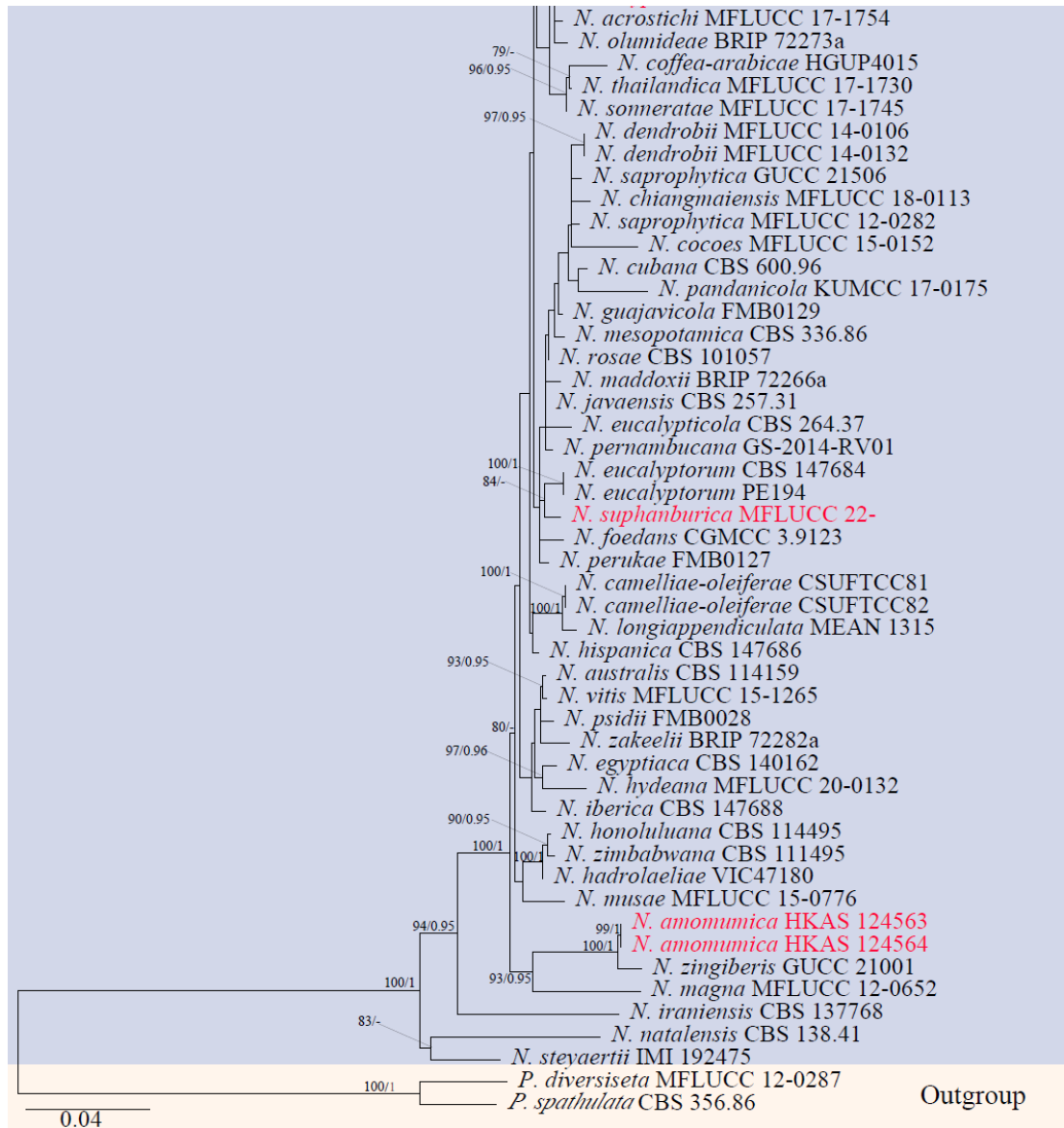


Figure 1. Cont.

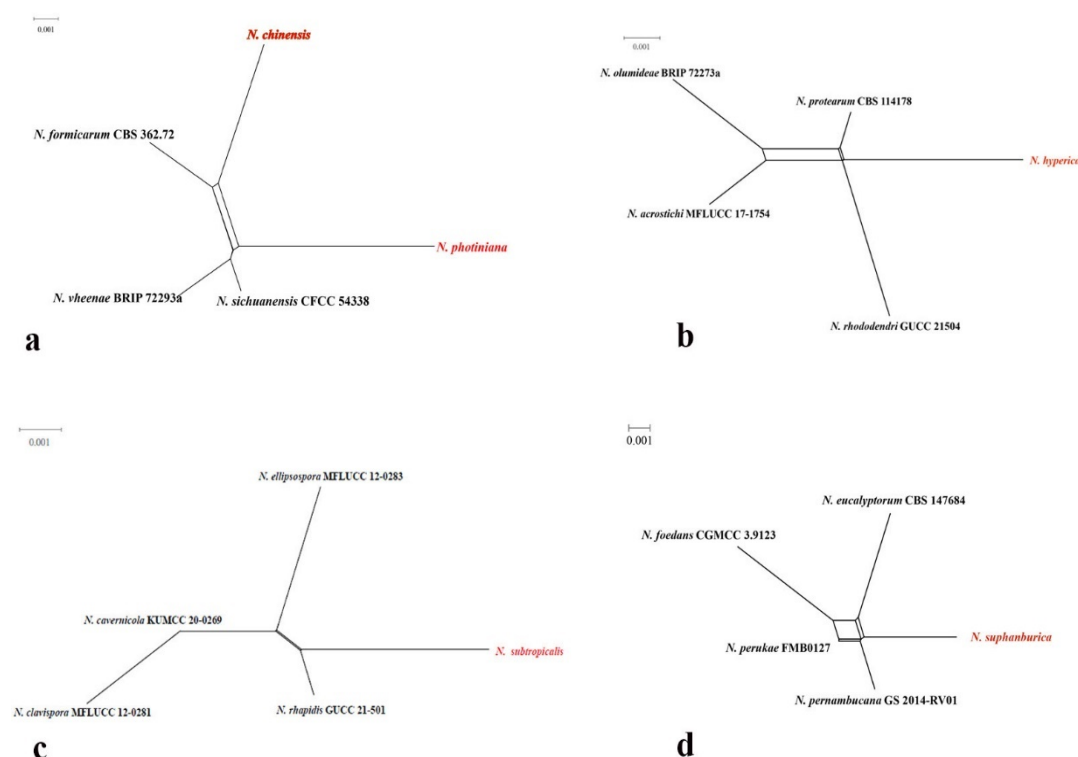


**Figure 1** – Maximum likelihood (RAxML) tree for *Neopestalotiopsis*, based on the analysis of a combined dataset of TEF, TUB and ITS sequence data. The tree is rooted with *Pestalotiopsis diversiseta* (MFLUCC 12-0287) and *P. spathulata* (CBS 356.86). Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. The new isolates are in red.

### Genealogical Concordance Phylogenetic Species Recognition Analysis

The PHI test revealed that there is no significant recombination ( $\Phi_w = 1.0$ ), between *N. photiniana* and its closely related taxa *N. chinensis* (HKAS 124560), *N. formicarum* (CBS 362.72), *N. sichuanensis* (CFCC 54338) and *N. vheenae* (BRIP 72293a) (Fig. 2a). *Neopestalotiopsis hyperica* based on the PHI test resulted that there is no significant recombination ( $\Phi_w = 1.0$ ), between *N. hyperica* and its closely related taxa *N. acrostichi* (MFLUCC 17-1754), *N. olumideae* (BRIP 72273a), *N. protearum* (CBS 114178) and *N. rhododendri* (GUCC 2150) (Fig. 2b). Similar result

also occurs in *N. subtropicalis* ( $\Phi_w = 1.0$ ) (Fig. 2c) and *N. suphanburica* ( $\Phi_w = 1.0$ ) (Fig. 2d), indicating there is no significant recombination between them and their closely related taxa.



**Figure 2** – Split graphs showing the results of PHI test of new *Neopestalotiopsis* species with their most closely related species using Log-Det transformation and splits decomposition options. The new taxon in each graph is shown in red font.

## Taxonomy

*Neopestalotiopsis amomumica* Y.R. Sun & Yong Wang bis, sp. nov. Fig. 3

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology: Refers to the name of the host plant from which the fungus was isolated.

Holotype – HKAS 124563

*Associated* with leaf blight of *Amomum villosum*. *Symptoms* irregular shape, pale to brown, slightly sunken spots appear on the leaves of *Amomum villosum*, which later expand outwards. **Sexual morph**: Not observed. **Asexual morph**: *Conidiomata* solitary, subglobose to globose, unilocular, brown, semi-immersed on leaves. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* 1.2–2.0  $\mu\text{m}$  wide, subcylindrical, ampulliform, hyaline. *Conidia* 18.5–30.0  $\times$  4.5–7.5  $\mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 25 \pm 2.5 \times 6.0 \pm 0.68 \mu\text{m}$  (n = 40), L/W ratio = 4.2, fusiform, straight to slightly curved, 4-septate; basal cell obconic with truncate base, hyaline, smooth-walled, 3.2–6.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.7 \pm 0.75 \mu\text{m}$ ); three median cells 12.0–19.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 15.7 \pm 1.6 \mu\text{m}$ ), pale brown to brown, concolourous, wall rugose, septa darker than the rest of the cell; second cell from base pale brown to

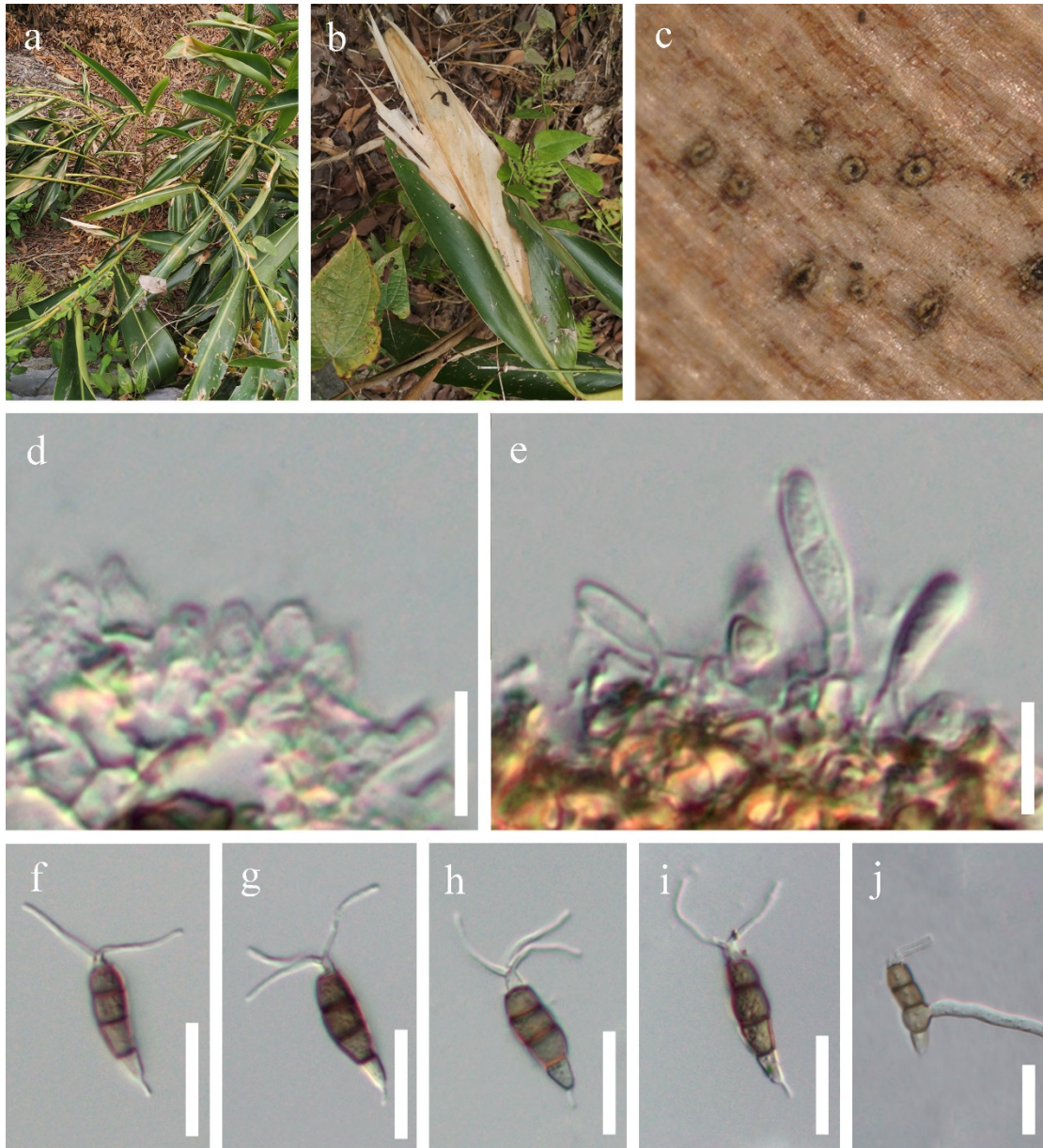
brown, 3.5–8.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.6 \pm 0.93 \mu\text{m}$ ); third cell pale brown to brown, 3.5–6.8  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.0 \pm 0.84 \mu\text{m}$ ); fourth cell pale brown to brown, 3.5–6.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.0 \pm 0.7 \mu\text{m}$ ); apical cell 1.9–4.6  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.5 \pm 0.8 \mu\text{m}$ ), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 7.0–17.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 12.5 \pm 2.8 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, 2.5–5.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.8 \pm 0.7 \mu\text{m}$ ).

Culture characteristics – Conidia germinated on PDA within 12 hours from single-spore isolation. Colony diameter reached 8 cm after two weeks at 25 °C on PDA media, circular, surface rough, flat, white from above and below.

Material examined – China, Guizhou Province, Qiannan Buyei and Miao Autonomous Prefecture, Luodian District, leaf blight of *Amomum villosum* Lour. (Zingiberaceae), 3 September 2021, Y.R. Sun, L8 (HKAS 124563, holotype); ex-type-living cultures, GUCC 814, *ibid.*, on leaf blight of *Amomum villosum*, 3 September 2021, Y.R. Sun, L8-1 (HKAS 124564); living cultures, GUCC 815.

Notes – *Neopestalotiopsis amomumica* was isolated from the diseased leaves of *Amomum villosum* in China. Two strains GUCC 814 and GCUU 815 clustered together with good support (ML-BS = 99%, BYPP = 1) and formed a sister clade to *N. zingiberis* (GUCC 21001) which was also isolated from a Zingiberaceae plant (He et al. 2022). The former differs in producing thinner conidia with basal appendages (4.5–7.5  $\mu\text{m}$  in *N. amomumica* vs. 6–9.5  $\mu\text{m}$  in HN89-1, without basal appendage). In addition, there are 4 base pairs differences between GUCC 814 and GUCC 21001 in the ITS gene and 10 base pairs differences in the TEF gene. *Neopestalotiopsis amomumica* also differs by smaller conidia (18.5–30.0  $\times$  4.5–7.5  $\mu\text{m}$  vs. 42–46  $\times$  9.5–12  $\mu\text{m}$ ) from *N. magna*. Thus, we introduce *N. amomumica* as a new species. Due to the lack of enough closely related species, the GCSPP was not used to evaluate its placement.





**Figure 3** – *Neopestalotiopsis amomumica* (MFLU 22-xx, holotype). a Host. b Leaf blight on *Acrostichum aureum*. c Close up view of conidiomata. d conidiogenous cells. e Immature conidia attached to conidiogenous cells. f–i Conidia. j Germinated conidium. Scale bars: d, e = 10  $\mu$ m, f–j = 20  $\mu$ m.

*Neopestalotiopsis chinensis* Y.R. Sun & Yong Wang bis, sp. nov.

Fig. 4

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology: The specific epithet is referring to China, the country from where the taxa were isolated.

Holotype – HKAS 124560

Associated with leaf spot of *Cyrtomium fortune*, *Lithocarpus* sp. and *Smilax scobinicaulis*. Symptoms irregular shape, pale brown, small spots gradually enlarged, changing to brown circular ring spots with a dark brown border. **Sexual morph**: Not observed. **Asexual morph**: *Conidiomata* solitary, subglobose to globose, unilocular,

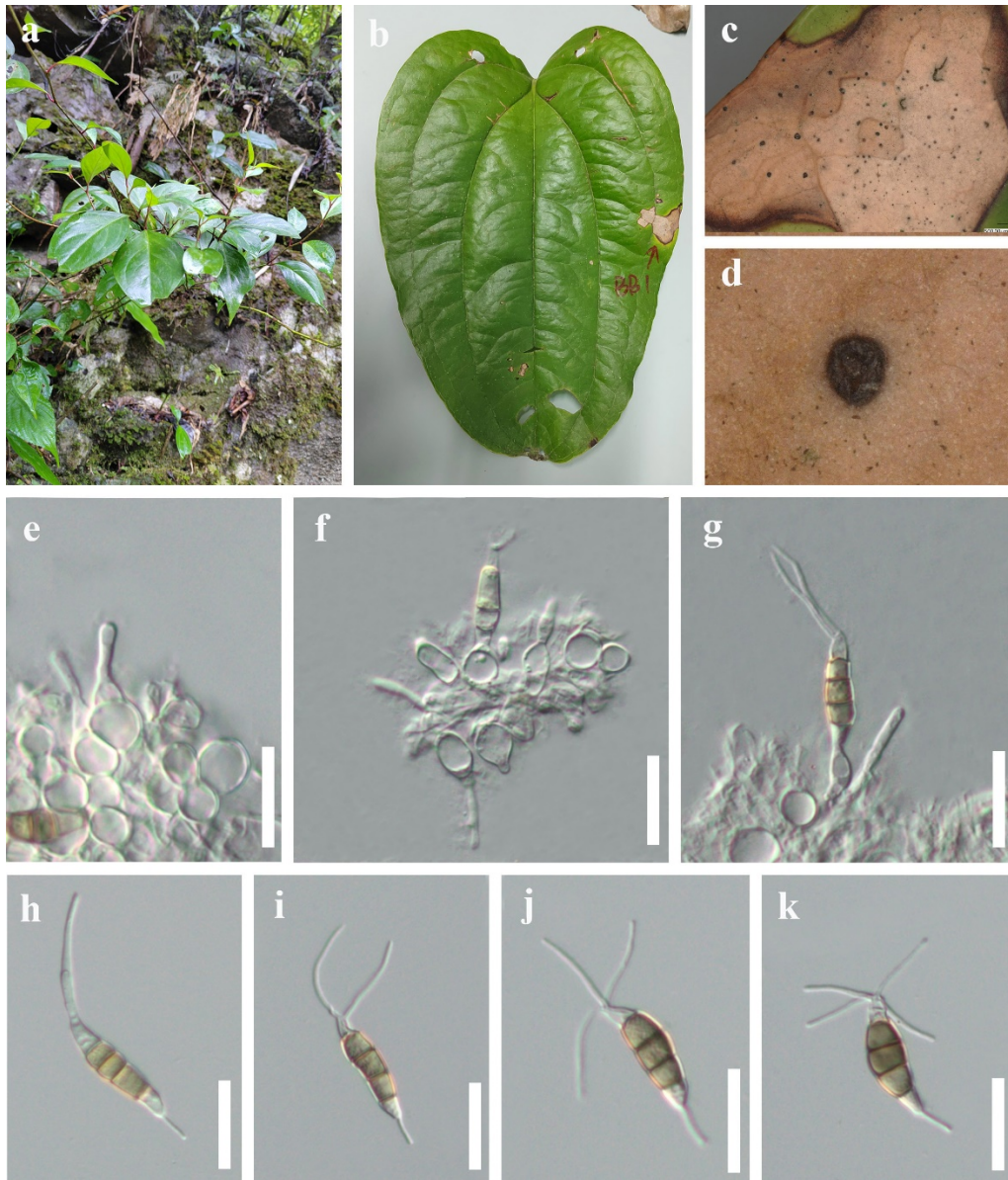


dark brown, semi-immersed on leaves. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* subcylindrical or ampulliform, hyaline. *Conidia* 21.0–31.0 × 4.0–7.0 μm,  $\bar{x} \pm SD = 26.5 \pm 2.3 \times 6.0 \pm 0.7 \mu\text{m}$  (n = 30), L/W ratio = 4.4, fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline, 3.0–6.8 μm long ( $\bar{x} \pm SD = 5.2 \pm 0.85 \mu\text{m}$ ); three median cells doliiform to cylindrical, 11.5–18.0 μm long ( $\bar{x} \pm SD = 15.5 \pm 1.4 \mu\text{m}$ ), yellow to brown, concolourous, septa darker than the rest of the cell; second cell from base yellow to brown, 3.5–6.5 μm long ( $\bar{x} \pm SD = 5.0 \pm 0.6 \mu\text{m}$ ); third cell yellow to brown, 3.5–6.5 μm long ( $\bar{x} \pm SD = 5.0 \pm 0.64 \mu\text{m}$ ); fourth cell yellow to brown, 4.0–6.5 μm long ( $\bar{x} \pm SD = 5.0 \pm 0.9 \mu\text{m}$ ); apical cell 2.5–6.0 μm long ( $\bar{x} \pm SD = 4.0 \pm 0.7 \mu\text{m}$ ), hyaline, conic to acute; with 1–4 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 13.0–26.0 μm long ( $\bar{x} \pm SD = 19.5 \pm 3.2 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, 2.5–7.0 μm long ( $\bar{x} \pm SD = 4.5 \pm 1.1 \mu\text{m}$ ).

Culture characteristics – *Conidia* germinated on PDA within 12 hours at 25 °C from single-spore isolation. Apical cells produced germ tubes. Colony diameter reached 80 mm after two weeks at 25 °C on PDA media, circular, surface rough, flat, white from above, yellow from below.

Material examined – China, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo District, leaf spot of *Smilax china* L.(Liliaceae), 12 March 2022, Y.R. Sun, bb1 (HKAS 124560, holotype); ex-type-living cultures, GUCC 807; China, Guizhou Province, Tongren City, Jiangkou District, Yamugou Parkland, leaf spot of *Lithocarpus* sp.(Fagaceae), 20 May 2022, Y.R. Sun, JK15-2 (HKAS 124565, Paratype); living cultures, GUCC 808; China, Guizhou Province, Guiyang City, Baiyun District, Changpoling National Forest Park, leaf spot of *Dryopteris crassirhizoma* Nakai (Dryopteridaceae), 20 August 2021, Y.R. Sun, CL1-2 (HGUP 22-xxx, dried culture); living cultures, GUCC 813.

Notes – Three strains of *Neopestalotiopsis chinensis* (GUCC 807, GUCC 813 and GUCC 808) have identical ITS, TEF, and TUB sequences to isolates CFCC-54337 and ZX12-1, which were previously provided by Jiang et al. (2021). However, they did not introduce it as a new species due to the lack of neighboring species to compare the morphology. In this study, GUCC 807, GUCC 808 and GUCC 813 have the same morphology. However, they have longer conidia than CFCC-54337 and ZX12-1 (21.0–31.0 × 4.0–7.0 vs. 19.9–23 × 5.8–7.6). In phylogenetic analyses, *N. chinensis* is close to *N. formicarum*, *N. photiniana*, *N. sichuanensi* and *N. vheena*. The PHI test on *N. chinensis* indicated that there is no significant recombination ( $\Phi_w = 1.0$ ) between *N. chinensis* and its closely related taxa. Thus, we introduce *N. chinensis* as a new species and assign GUCC 807 as the holotype, due to CFCC-54337 and ZX12-1 are invalid. *Neopestalotiopsis chinensis* appears to be a common phytopathogen as it has been found in leaf spots on different plants.



**Figure 4** – *Neopestalotiopsis chinensis* (HUGP 22-xx, holotype). a Host. b Leaf spot on *Smilax scobinicaulis*. c, d Close up view of conidiomata. e–g Conidia attached to conidiogenous cells. h–k Conidia. Scale bars: e–k = 20  $\mu$ m.

*Neopestalotiopsis hyperica* Y.R. Sun & Yong Wang bis, sp. nov.

Fig. 5

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology: The specific epithet is referring to *Hypericum*, the host plant from which the fungus was isolated.

Holotype – HKAS 124561

Associated with leaf spots of *Hypericum monogynum*. Symptoms irregular shape, pale to brown, slightly sunken spots appear on the leaves of *Hypericum* sp., which later expand outwards. **Sexual morph**: Not observed. **Asexual morph**: Conidiomata solitary, unilocular, dark. Conidiophores often reduced to conidiogenous cells. Conidiogenous cells indistinct. Conidia 17.0–22.0(–24.2)×5.0–8.2  $\mu$ m  $\bar{x} \pm SD = 19.0 \pm 1.3 \times 6.8 \pm 0.9 \mu$ m (n = 30), L/W ratio = 2.8, fusoid, sub-cylindrical, straight to

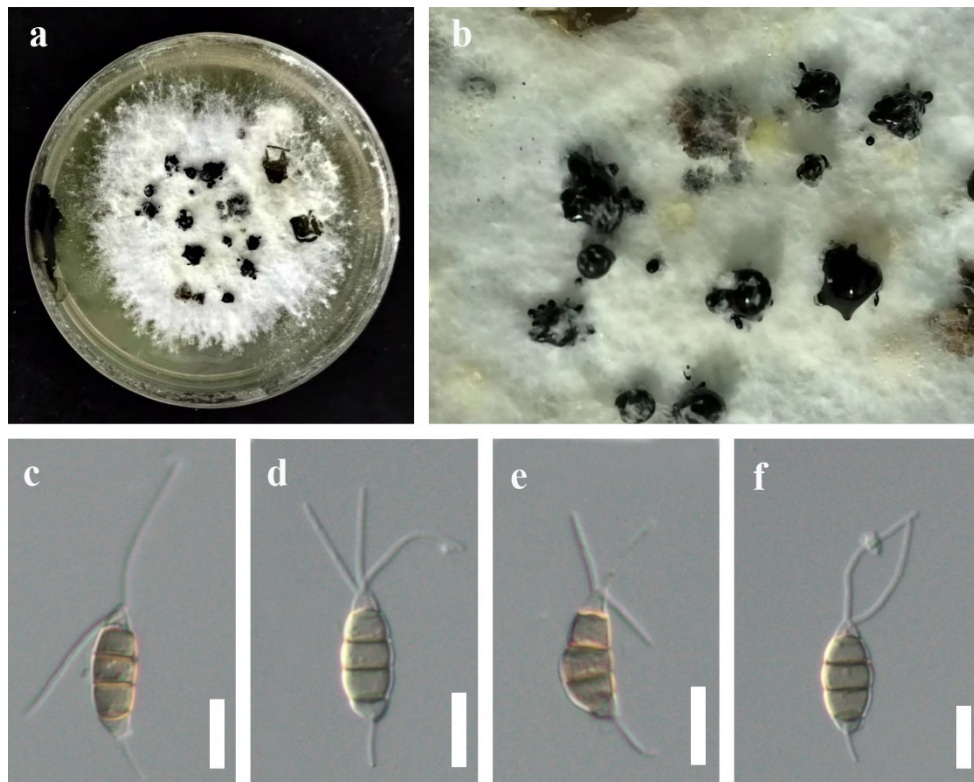
slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline to sub-hyaline, 2.0–4.0 (–4.6)  $\mu\text{m}$  long; three median cells 10.5–14.5(–16.5)  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 12.5 \pm 1.0 \mu\text{m}$ ), wall rugose, concolorous; second cell from base pale brown to brown, 3.0–4.7  $\mu\text{m}$  long; third cell pale brown to brown, 3.0–5.2(–6.1)  $\mu\text{m}$  long; fourth cell pale brown to brown, 2.5–5.5  $\mu\text{m}$  long; apical cell 1.5–3.5  $\mu\text{m}$  long, hyaline, rugose and thin-walled; with 2–3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 11.5–22.5  $\mu\text{m}$  long; single basal appendage 3.7–6.8  $\mu\text{m}$  long, unbranched, tubular, centric.

Culture characteristics – Colonies on PDA reaching up to 10 cm after 2 weeks, dense mycelium on the surface, white from above and below. Fruiting bodies were observed after 14 days.

Material examined – China, Guizhou Province, Guiyang City, Baiyun District, Changpoling National Forest Park, leaf spot of *Hypericum monogynum* L.(Clusiaceae), 20 August 2021, Y.R. Sun, CL5-1 (HKAS 124561, holotype); ex-type-living cultures, GUCC 812; *ibid.*, on leaf spots of *Hypericum monogynum*, 20 August 2021, Y.R. Sun, CL5-1-1 (HGUP 22-xxx), living culture GUCC 811.

Notes – *Neopestalotiopsis hyperica* is related to *N. rhododendri* and *N. protearum* in the phylogenetic analyses (Fig 1), but they can be distinct from concolorous conidia and the size of their median cells (10.5–14.5  $\mu\text{m}$  in *N. hyperica* vs. 13.5–19.5  $\mu\text{m}$  in *N. rhododendri* vs. 16–17  $\mu\text{m}$  in *N. protearum* (Maharachchikumbura et al. 2014, Yang et al. 2021). In addition, there are 13 base pairs differences between *N. hyperica* and *N. rhododendri* in the TEF region. Moreover, the PHI test on *N. hyperica* indicated that there is no significant recombination ( $\Phi_w = 1.0$ ) between *N. hyperica* and its closely related taxa (Fig 2b). Thus, we introduce *N. hyperica* as a new species.





**Figure 5** – *Neopestalotiopsis hyperica* (MFLU 22-xx, holotype). a Culture. b Close up view of conidiomata. c–f Conidia. Scale bars: c–f = 10  $\mu$ m.

*Neopestalotiopsis photiniana* Y.R. Sun & Yong Wang bis, sp. nov.

Fig. 6

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology: Referring to the host plant from which the fungus was isolated.

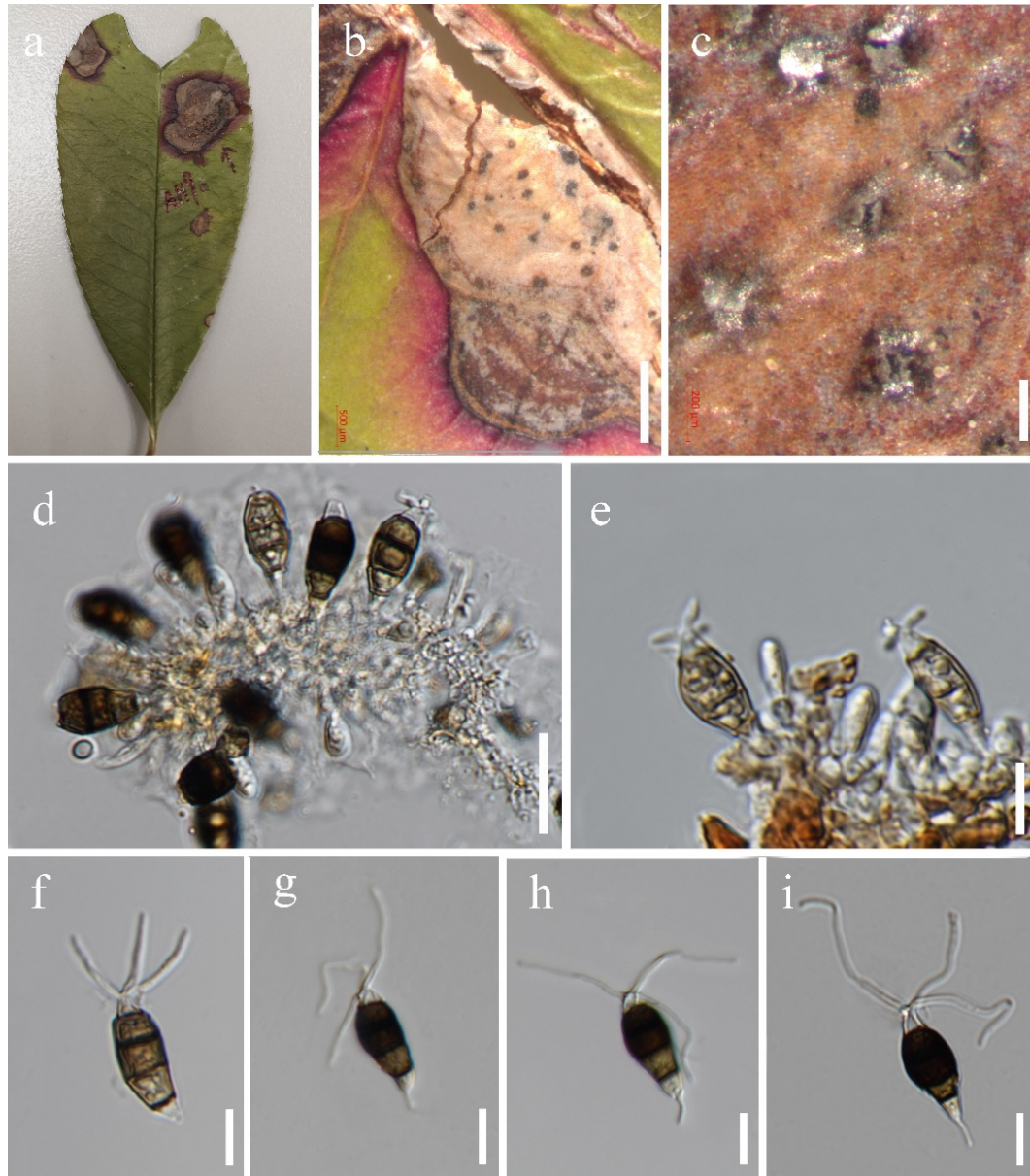
Holotype – MFLU 22-0XXX

*Associated* with leaf spots of *Photinia serrulata*. *Symptoms* irregular shape, pale to brown, slightly sunken spots appear on the leaves of *Photinia serrulata*, which later expand outwards. Small spots gradually enlarged, changing to brown circular ring spots with a dark brown border. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, subglobose to globose, unilocular, dark brown, semi-immersed on leaves. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* sub-cylindrical, ampulliform, hyaline. *Conidia* 20.0–28.5  $\times$  5.5–11.5  $\mu$ m,  $\bar{x} \pm SD = 23.0 \pm 1.9 \times 8.5 \pm 0.89 \mu$ m (n = 40), L/W ratio = 2.7, broadly fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline to pale brown, 1.5–5.5  $\mu$ m long ( $\bar{x} \pm SD = 3.5 \pm 0.89 \mu$ m); three median cells 13.0–19.5  $\mu$ m long ( $\bar{x} \pm SD = 15.5 \pm 1.5 \mu$ m), brown to dark, wall rugose, versicolourous; second cell from base pale brown to brown, 3.5–6.5  $\mu$ m long ( $\bar{x} \pm SD = 4.8 \pm 0.6 \mu$ m); the third and fourth cells dark brown to **dark** are not easily distinguished, septate indistinct, 9.5–13.0  $\mu$ m long ( $\bar{x} \pm SD = 11.5 \pm 1.0 \mu$ m); apical cell 2.0–4.0  $\mu$ m long ( $\bar{x} \pm SD = 3.0 \pm 0.5 \mu$ m), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 17.0–32.5  $\mu$ m long ( $\bar{x} \pm SD = 24.5 \pm 4.2 \mu$ m); single basal appendage, unbranched, tubular, centric, 1.5–5.5  $\mu$ m long ( $\bar{x} \pm SD = 3.0 \pm 1.2 \mu$ m).

Culture characteristics – Conidia germinated on PDA within 12 hours at 25 °C from single-spore isolation. Apical cells produced germ tubes. Colony diameter reached 80 mm after three weeks at 25 °C on PDA media, circular, surface rough, flat, white from above and below.

Material examined – China, Guizhou Province, Guiyang City, Nanming District, xiaochuhe road, Guiyang Ahahu National Wetland Park, leaf spots of *Photinia serrulata* Lindl.(Rosaceae), 21 September 2019, Y.R. Sun, AH9 (MFLU 22-xxx, holotype); ex-type-living culture GUCC 810.

Notes – *Neopestalotiopsis photiniana* is phylogenetically related to *N. sichuanensis* and *N. vheena* (Fig. 1). *Neopestalotiopsis photiniana* differs by its thinner conidia (L/W ratio = 2.7 vs. L/W ratio = 4.1) from *N. sichuanensis* (Jiang et al. 2021). *Neopestalotiopsis photiniana* is morphologically indistinguishable from *N. vheena* (Prasannath et al. 2021). However, they have 13 base pairs (without gap, 473 bp) in the TEF region. The result of the PHI test showed there is no obvious recombination ( $\Phi_w = 1.0$ ) between *N. photiniana* and its closely related taxa (Fig 2a). Therefore, *N. photiniana* is introduced as a new species.



**Figure 6** – *Neopestalotiopsis photiniana* (MFLU 22-xx, holotype). a Host. b Leaf spot on *Photinia serrulata*. c Close up view of conidiomata. d, e conidia attached to conidiogenous cells. f–i Conidia. Scale bars: d = 1000  $\mu\text{m}$ , c = 200  $\mu\text{m}$ , d = 20  $\mu\text{m}$ , f–i = 10  $\mu\text{m}$ .

*Neopestalotiopsis subtropicalis* Y.R. Sun & Yong Wang bis, sp. nov.

Fig. 7

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology: Referring to the subtropical regions in which the collections were encountered.

Holotype – MFLU 22-0XXX

Saprobic on *Ceiba pentandra* leaves and endophytic from *Pinellia ternata*.

**Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, unilocular, dark, immersed on stems. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* indistinct. *Conidia* 19–24.5  $\times$  6.0–8.0  $\mu\text{m}$   $\bar{x} \pm \text{SD} =$

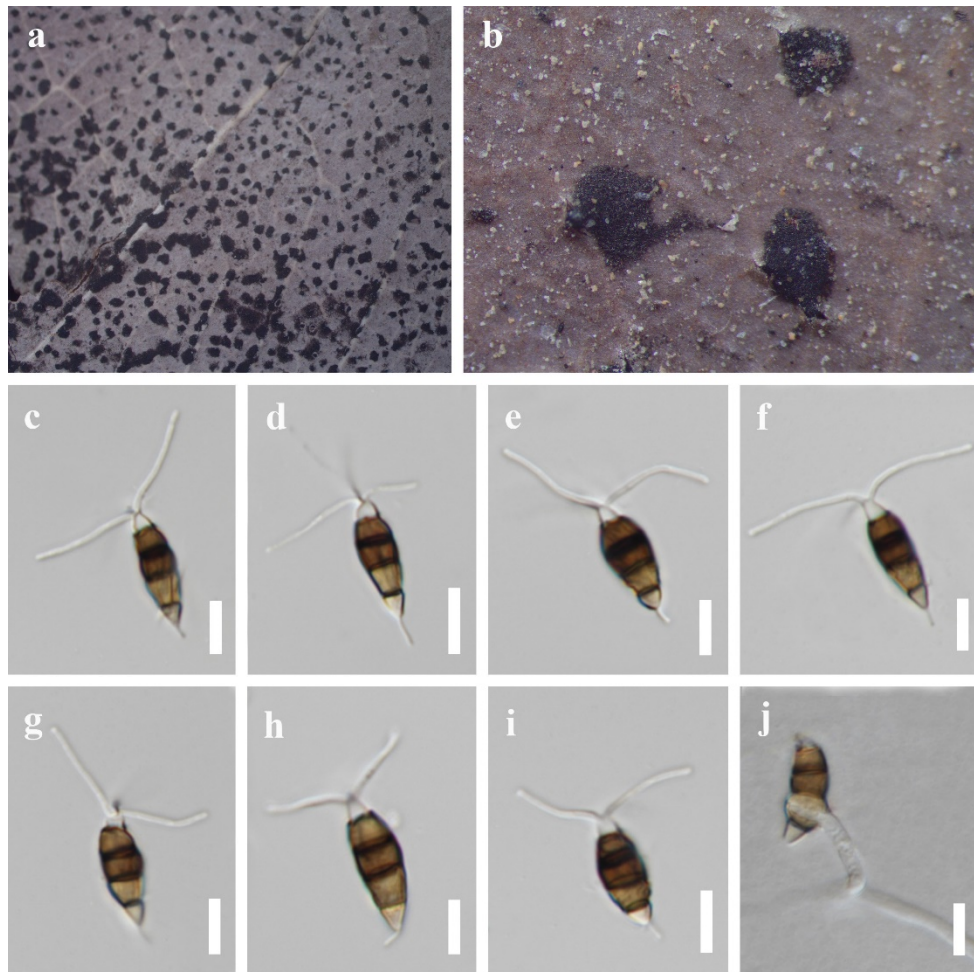
21.5±1.2 × 7.0 ± 0.6 μm (n = 30), L/W ratio = 3.1, fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline to subhyaline, 3–5.5 μm long; three median cells 13.0–15.2 μm long ( $\bar{x} \pm SD = 14.0 \pm 0.6 \mu\text{m}$ ), wall rugose, versicolourous, septa darker than the rest of the cell; second cell from base pale brown to brown, 3.5–5.0 μm long; third cell brown, 3.5–5.5 μm long; fourth cell brown, 3.0–5.5 μm long; apical cell 2.5–4.0 μm long, hyaline, rugose and thin-walled; with 2 (seldom 3) tubular apical appendages, arising from the apical crest, unbranched, filiform, 11.5–20.0 μm long; single basal appendage 2.0–5.0 μm long, unbranched, tubular, centric.

Culture characteristics – Colonies on PDA reaching up to 8 cm in two weeks, dense aerial mycelium on the surface with undulate edge, white. Fruiting bodies were observed after 14 days.

Material examined – Thailand, Chiang Rai Province, dead leaves of *Ceiba pentandra* (L.) Gaertn. (Bombacaceae), 16 Jan 2020, Y.R. Sun, CR20 (MFLU 22-xxx, holotype); ex-type living cultures, MFLUCC 22-xxx. China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy leaves of *Pinellia ternata* (Thunb.) Breit. (Araceae), 1 May 2022, Y.R. Sun, E2 (HGUP 22-xxxx, paratype); living culture GUCC 805.

Notes – Our isolates GUCC 22-xxxx and MFLUCC 22-xxx clustered together with *Neopestalotiopsis* sp2 (CFCC 54340 and ZX22B) and these four isolates formed a distinct clade in the phylogenetic tree (Fig. 1). Four isolates have similar characteristics. The PHI test on *N. subtropicalis* indicated that there is no significant recombination ( $\Phi_w = 1.0$ ), between *N. subtropicalis* and its closely related taxa (Fig 2c). Hence, we identify *N. subtropicalis* as a new species. Interestingly, GUCC 22-xxxx, MFLUCC 22-xxx, ZX22B and CFCC 54340 have different habitats. GUCC 22-xxxx was endophytic in healthy leaves of *Pinellia ternata*, MFLUCC 22-xxx was saprobic on decaying leaves of *Ceiba pentandra*, and CFCC 54340, ZX22B were isolated from leaf spots of *Castanea mollissima*. This seems to indicate that the same species has different lifestyles in different hosts or regions.





**Figure 7** – *Neopestalotiopsis subtropicalis* (MFLU 22-xx, holotype). a, b Conidiomata on the host. c–i Conidia. j Germinated conidium. Scale bars: c–j = 10  $\mu$ m.

*Neopestalotiopsis suphanburica* Y.R. Sun & Yong Wang bis, sp. nov. Fig. 8

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology – refers to the province where the fungus was collected, Suphan Buri Province.

Holotype – MFLU 22-xxx

*Saprobic* on stems of an unidentified plant. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, subglobose to globose, unilocular, brown to dark, immersed on stems. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* sub-cylindrical, ampulliform, hyaline. *Conidia* 18.5–29.0  $\times$  3.7–6.8  $\mu$ m,  $\bar{x} \pm SD = 24.5 \pm 2.8 \times 5.0 \pm 0.6 \mu$ m (n = 40), L/W ratio = 4.9, fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline, smooth-walled, 2.8–6.7  $\mu$ m long ( $\bar{x} \pm SD = 4.5 \pm 1.0 \mu$ m); three median cells 12.0–19.0  $\mu$ m long ( $\bar{x} \pm SD = 15.7 \pm 2.0 \mu$ m), pale brown to brown, wall rugose, concolor, septa darker than the rest of the cell, versicolourous; second cell from base pale brown to brown, 3.0–7.3  $\mu$ m long ( $\bar{x} \pm SD = 5.5 \pm 0.9 \mu$ m); third cell pale brown to brown, 3.3–6.0  $\mu$ m long ( $\bar{x} \pm SD = 4.6 \pm 0.7 \mu$ m); fourth cell pale brown to brown,

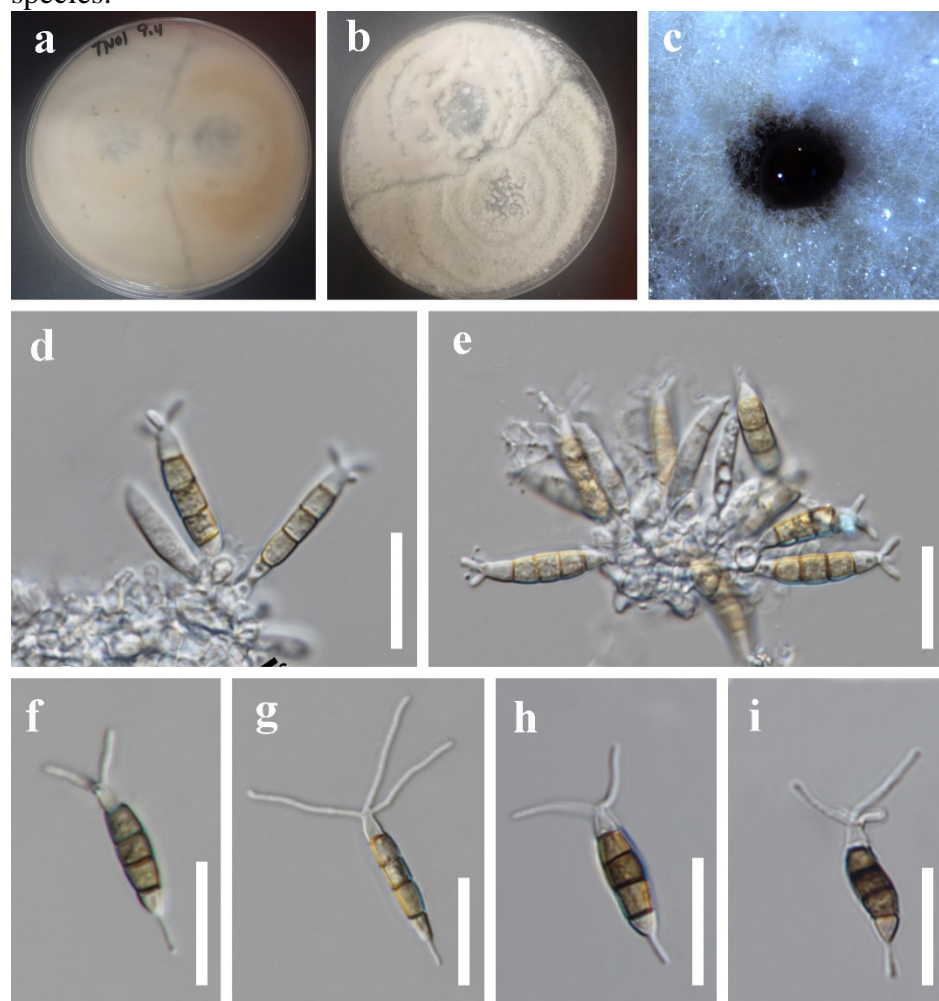


3.5–6.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.0 \pm 0.6 \mu\text{m}$ ); apical cell 3.0–6.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.2 \pm 0.8 \mu\text{m}$ ), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 9.0–21.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 13.5 \pm 3.5 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, 2.3–11.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.5 \pm 1.9 \mu\text{m}$ ).

Culture characteristics – Colony diameter reached 8 cm after two weeks at 25 °C on PDA media, circular, surface rough, flat, white from above, white to pale gray from below.

Material examined – Thailand, Suphan Buri Province, dead stem of an unidentified plant, 5 September 2020, S Wang, TN01 (MFLU 22-xxx, holotype); ex-type-living cultures, MFLUCC 22-xxx.

Notes – *Neopestalotiopsis suphanburica* is phylogenetically sister to *N. eucalyptorum* which was isolated from leaves and stems of *Eucalyptus globulus* (Fig. 1). In morphology, *N. suphanburica* differs from *N. eucalyptorum* in having thinner conidia (3.7–6.8  $\mu\text{m}$  vs. 7.6–8.1  $\mu\text{m}$ ). In addition, there are 10 base pair differences (without gap, 445 bp) in the TEF region. The PHI test on *N. suphanburica* also indicated there is no significant recombination ( $\Phi_w = 1.0$ ) between *N. suphanburica* and its closely related taxa (Fig 2d). We thus introduce *N. suphanburica* as a new species.



**Figure 8** – *Neopestalotiopsis suphanburica* (MFLU 22-xx, holotype). a, b Cultures. c

Colony on culture. d, e Conidia attached to conidiogenous cells, f–i Conidia. Scale bars: d–i = 10 µm.

*Neopestalotiopsis rhapsidis* Qi Yang & Yong Wang bis, Biodiversity Data Journal 9(no. e70446): 9 (2021) Fig. 9

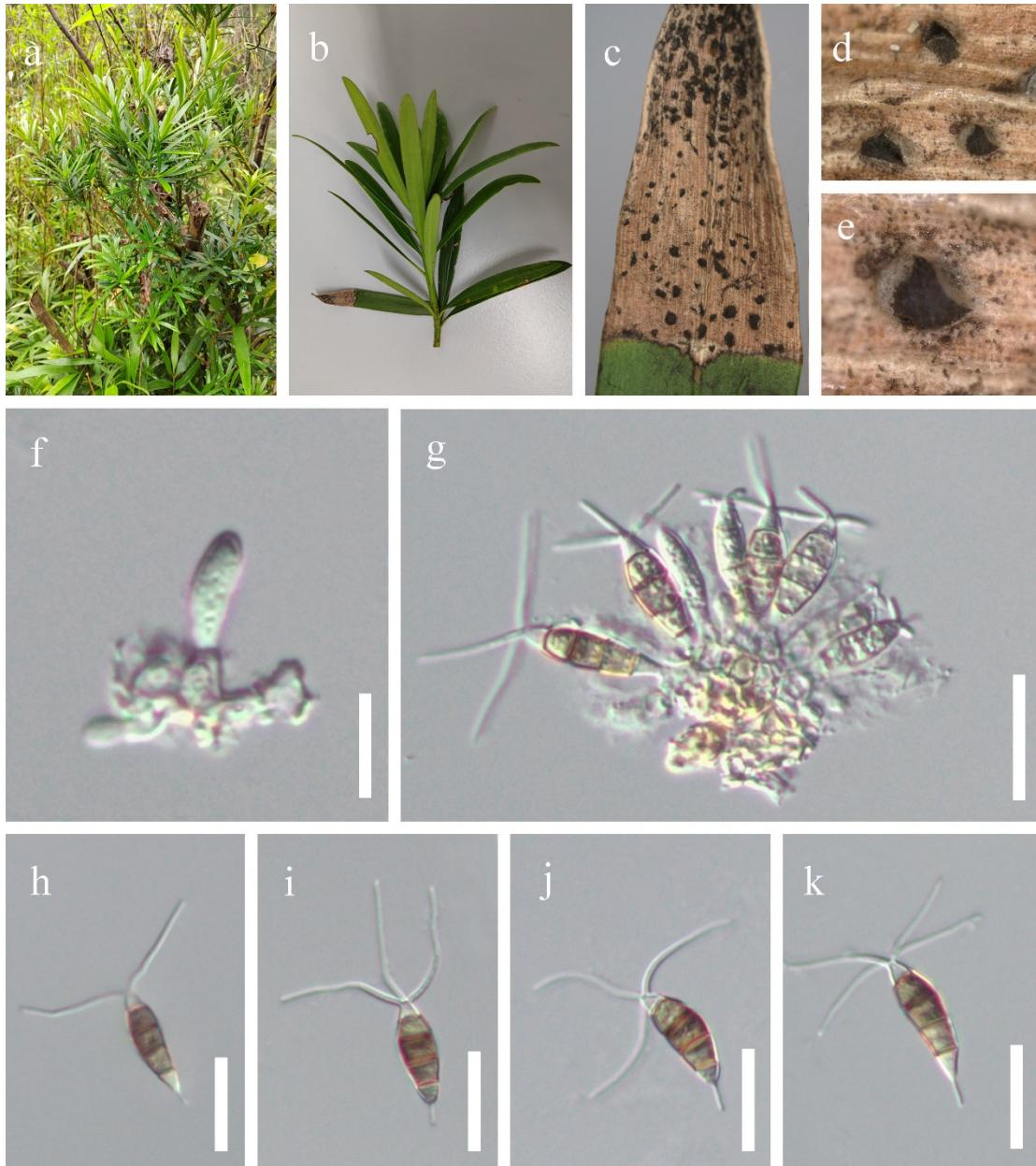
Index Fungorum number: IF 840065; Facesoffungi number: FoF XX

*Associated* with leaf spot on leaves tip of *Podocarpus macrophyllus*. *Symptoms* irregular shape, brown, dry, slightly sunken on leaves tip. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, subglobose to globose, unilocular, dark brown to dark, semi-immersed on leaves. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* subcylindrical or ampulliform, short, hyaline. *Conidia* 17.5–25.0 × 5.0–8.0 µm,  $\bar{x} \pm SD = 22.0 \pm 1.4 \times 6.5 \pm 0.7$  µm (n = 40), L/W ratio = 3.4, fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline, 3.0–6.2 µm long ( $\bar{x} \pm SD = 4.3 \pm 0.65$  µm); three median cells doliiiform to cylindrical, 11.5–17.0 µm long ( $\bar{x} \pm SD = 14.5 \pm 1.0$  µm), pale to brown, versicolourous, septa darker than the rest of the cell; second cell from base pale to brown, 3.0–6.0 µm long ( $\bar{x} \pm SD = 4.5 \pm 0.65$  µm); third cell pale to brown, 3.3–6.5 µm long ( $\bar{x} \pm SD = 5.0 \pm 0.6$  µm); fourth cell pale to brown, 2.5–5.5 µm long ( $\bar{x} \pm SD = 4.2 \pm 0.7$  µm); apical cell 2.0–5.0 µm long ( $\bar{x} \pm SD = 3.0 \pm 0.6$  µm), hyaline, conic to acute; with 2–4 (mostly 3) tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 12.0–28.0 µm long ( $\bar{x} \pm SD = 20.5 \pm 3.4$  µm); single basal appendage, unbranched, tubular, centric, 2.5–7.0 µm long ( $\bar{x} \pm SD = 4.8 \pm 1.2$  µm).

*Culture characteristics* – Conidia germinated on PDA within 12 hours at 25 °C from single-spore isolation. Apical cells produced germ tubes. Colony diameter reached 80 mm after 7 days at 25 °C on PDA media, circular, surface rough, flat, white from above, yellow from below.

*Material examined* – China, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo District, leaf spots of *Podocarpus macrophyllus* (Thunb.) D. Don (Podocarpaceae), 12 March 2022, Y.R. Sun, ML3 (HKAS 124559); living cultures, GUCC 806.

*Notes* – *Neopestalotiopsis rhapsidis* was introduced by Yang et al. (2021) from leaf spot of *Rhapis excelsa* (Arecaceae) in China. Our isolate GUCC 806 clustered together with *N. rhapsidis* (GUCC 21501) in the phylogenetic tree. These two species have overlapping conidial measurements (17.5–25.0 × 5.0–8.0 µm for GUCC 806 vs. (22–)25.5 × 4(–6) µm for GUCC 21501) (Yang et al. 2021). Both isolates were associated with leaf spots in China. Therefore, we identify GUCC 806 and GUCC 21501 to be conspecific species, and GUCC 806 represents a new host record.



**Figure 9** – *Neopestalotiopsis rhapsidis* (MFLU 22-0XXX, new host record). a, b Host. c Leaf blight on *Podocarpus macrophyllus*. d, e Close up view of conidiomata. f, g Conidia attached to conidiogenous cells. h–k Conidia. Scale bars: f = 10  $\mu$ m, g–k = 20  $\mu$ m.

*Neopestalotiopsis rhododendri* Qi Yang & Yong Wang bis, Biodiversity Data Journal 9(no. e70446): 9 (2021) Fig. 10

Index Fungorum number: IF 840066; Facesoffungi number: FoF **XX**

*Associated* with leaf spots of *Dracaena fragrans*. *Symptoms* subcircular to circular, yellow to brown, slightly sunken spots appear on the leaves of *Dracaena* sp, which later expand outwards. Small auburn spots appeared initially and then gradually enlarged. **Sexual morph**: Not observed. **Asexual morph**: *Conidiomata* on PDA pycnidial, subglobose to cylindrical, solitary or aggregated, dark, semi-immersed or

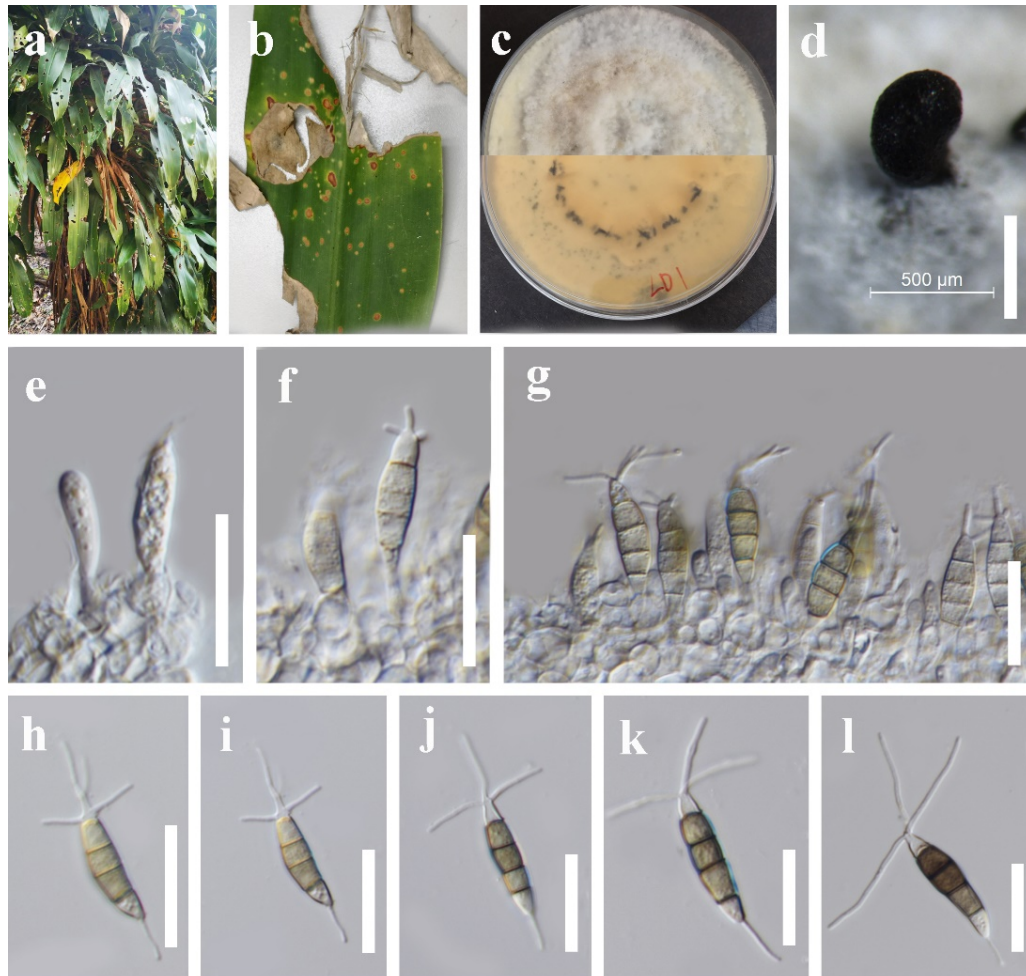


partly erumpent; exuding black conidial masses. *Conidiophores* indistinct. *Conidiogenous cells* cylindrical to sub-cylindrical or ampulliform to lageniform, hyaline. *Conidia* pale brown to dark brown, fusiform, straight to slightly curved, 4-septate,  $19.0\text{--}28.0 \times 5\text{--}7 \mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 24.0 \pm 2.0 \times 6.0 \pm 0.5 \mu\text{m}$  ( $n = 40$ ), L/W ratio = 4; basal cell obconic with a truncate base, hyaline or pale brown,  $3.0\text{--}6 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.3 \pm 0.6 \mu\text{m}$ ,  $n = 40$ ); three median cells  $12.0\text{--}18.0\text{--}(18.5) \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 12.8 \pm 1.5 \mu\text{m}$ ,  $n = 40$ ), pale brown to dark brown, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa, versicolourous; second cell from base pale brown,  $3.5\text{--}6.5 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.8 \pm 0.7 \mu\text{m}$ ,  $n = 40$ ); third cell brown,  $3.0\text{--}6.0 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.5 \pm 0.7 \mu\text{m}$ ,  $n = 40$ ); fourth cell brown,  $3.5\text{--}6 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.5 \pm 0.66 \mu\text{m}$ ,  $n = 40$ ); apical cell  $2.0\text{--}5.5 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.8 \pm 0.7 \mu\text{m}$ ,  $n = 40$ ), hyaline, conic to acute; with 3–5 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched,  $10.0\text{--}26.0 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 15.5 \pm 4.8 \mu\text{m}$ ); 1–3 basal appendage, unbranched, tubular, centric,  $3.5\text{--}12.0 \mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 7.5 \pm 2.6 \mu\text{m}$ ).

Culture characteristics – Colony diameter reached 8 cm after two weeks at  $25 \text{ }^\circ\text{C}$  on PDA medium, circular, surface rough, flat, white from above, yellow from below.

Material examined – Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf spots of *Dracaena fragrans* (L.) Ker Gawl. (Liliaceae), 15 September 2020, S Wang, LD1 (MFLU 22-xxx, holotype); ex-type-living cultures, MFLUCC 22-xxx.

Notes – *Neopestalotiopsis rhododendri* was introduced by Yang et al. (2021) from the diseased leaf of *Rhododendron simsii* (Ericaceae) in China. Based on our phylogenetic analysis of combined TEF, TUB and ITS sequence data, our isolate MFLUCC 22-xxx clustered with the type species, *N. rhododendri* (GUCC 21504) with maximum support (ML-BS = 100%, BYPP = 1). Our collection also shares similar morphological features with the holotype of *N. rhododendri* (GUCC 21504). Both isolates were associated with leaf spots. Therefore, we identify our collection as *N. rhododendri*, which represents a new host and geographical record.



**Figure 10** – *Neopestalotiopsis rhododendri* (MFLU 22-xx, new record). a Host. b Leaf spot of *Dracaena fragrans*. c Cultures. d Colonies on PDA. e–g Conidiogenous cells and developing conidia. h–l Conidia. Scale bars: d = 500 µm, e–l = 20 µm.

## Part 2: *Pestalotiopsis*

### Phylogenetic analyses

The phylogenetic tree (*Pestalotiopsis*) comprised 112 ingroups and two outgroups, *Neopestalotiopsis protearum* (CBS 114178) and *N. cubana* (CBS 600.96). A total of 1,389 characters including gaps (498 for TEF, and 412 for TUB, 479 for ITS) were included in the phylogenetic analysis. Similar tree topologies were obtained by ML and BYPP methods, and the most likely tree ( $-\ln = 66,531.894$ ) is presented (Figure 11). The phylogenetic tree analyzed 12 *Pestalotiopsis* taxa isolated from medicinal plants and indicated four novel species and three new records of *Pestalotiopsis*.

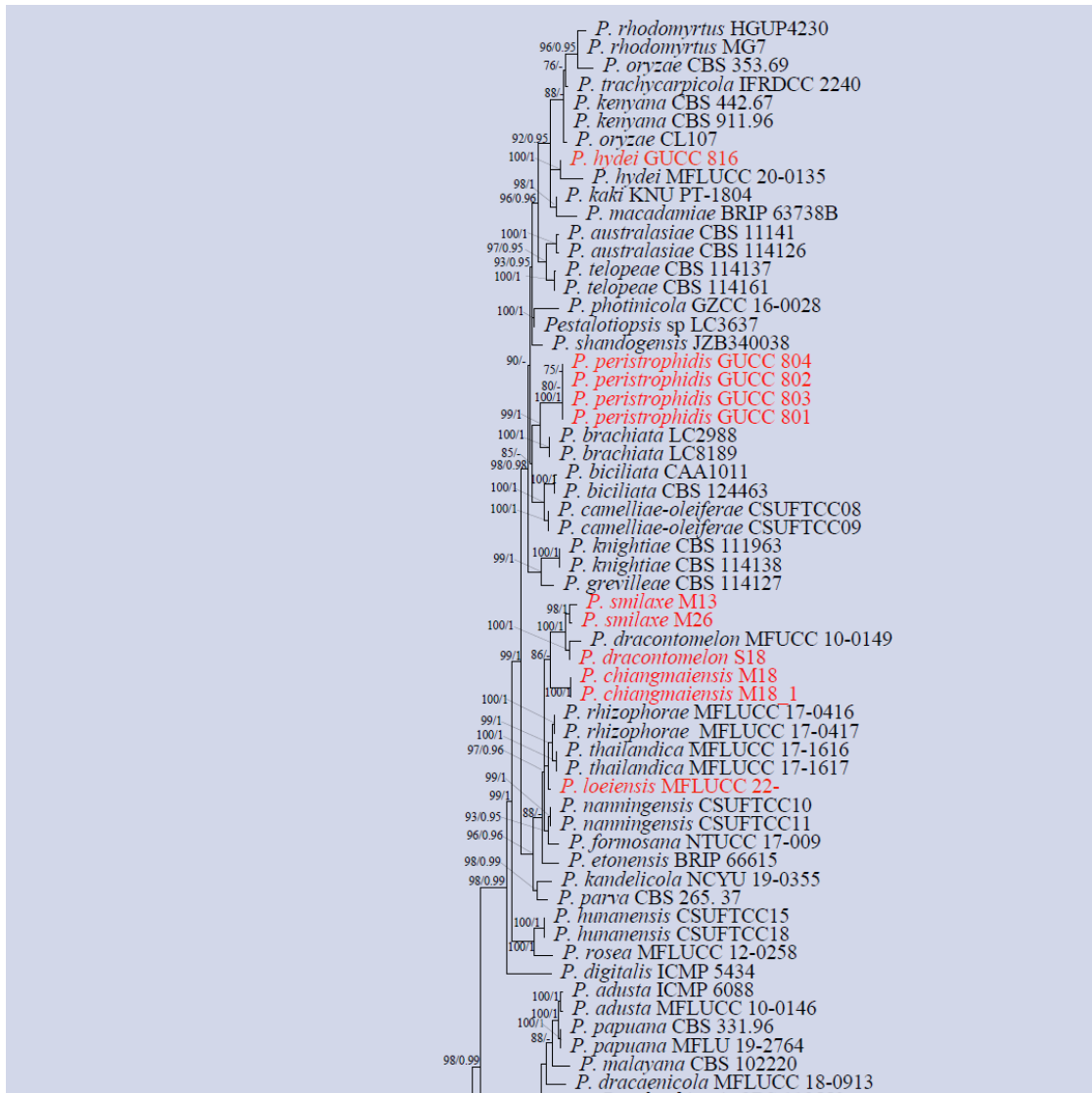
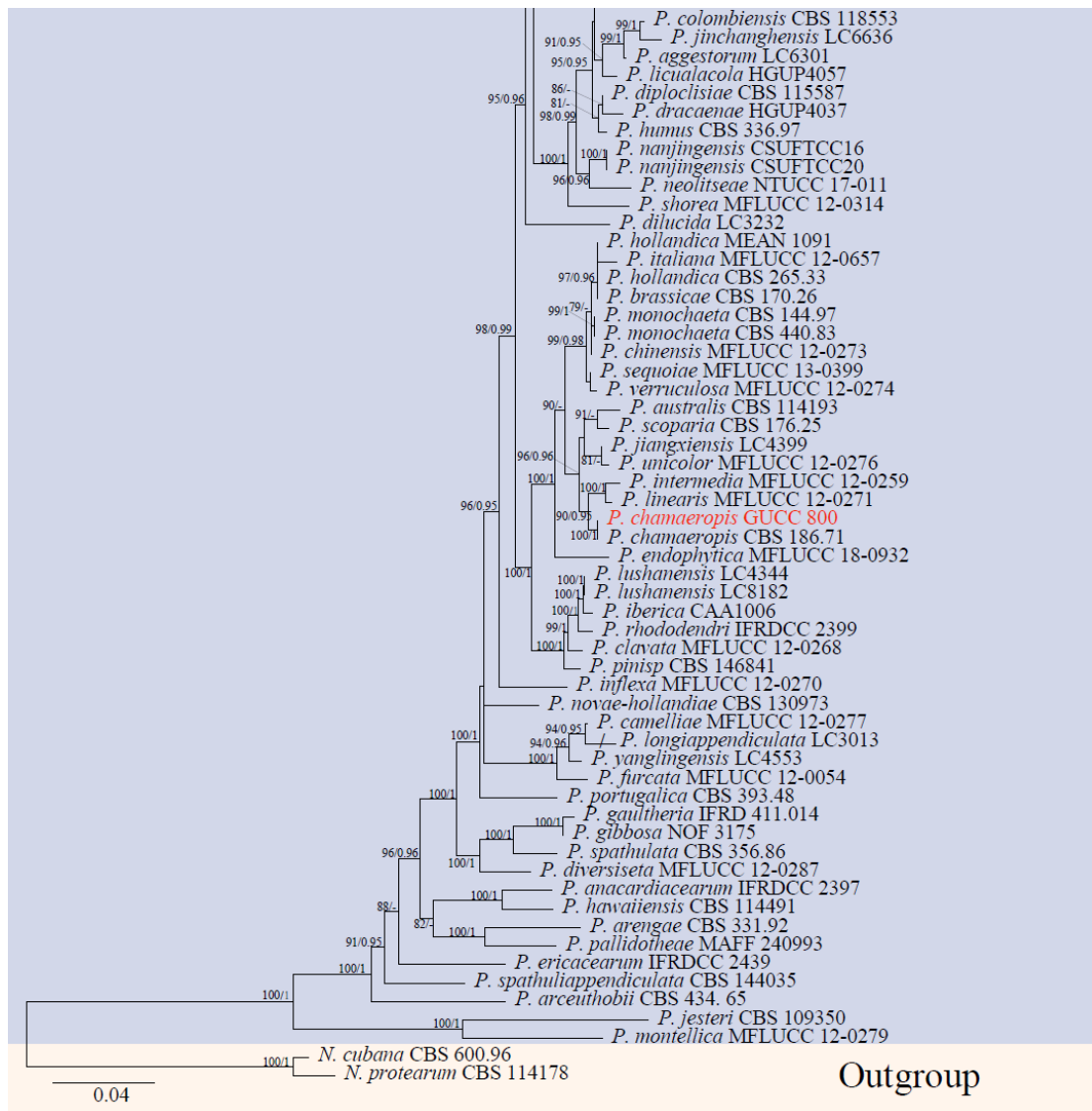


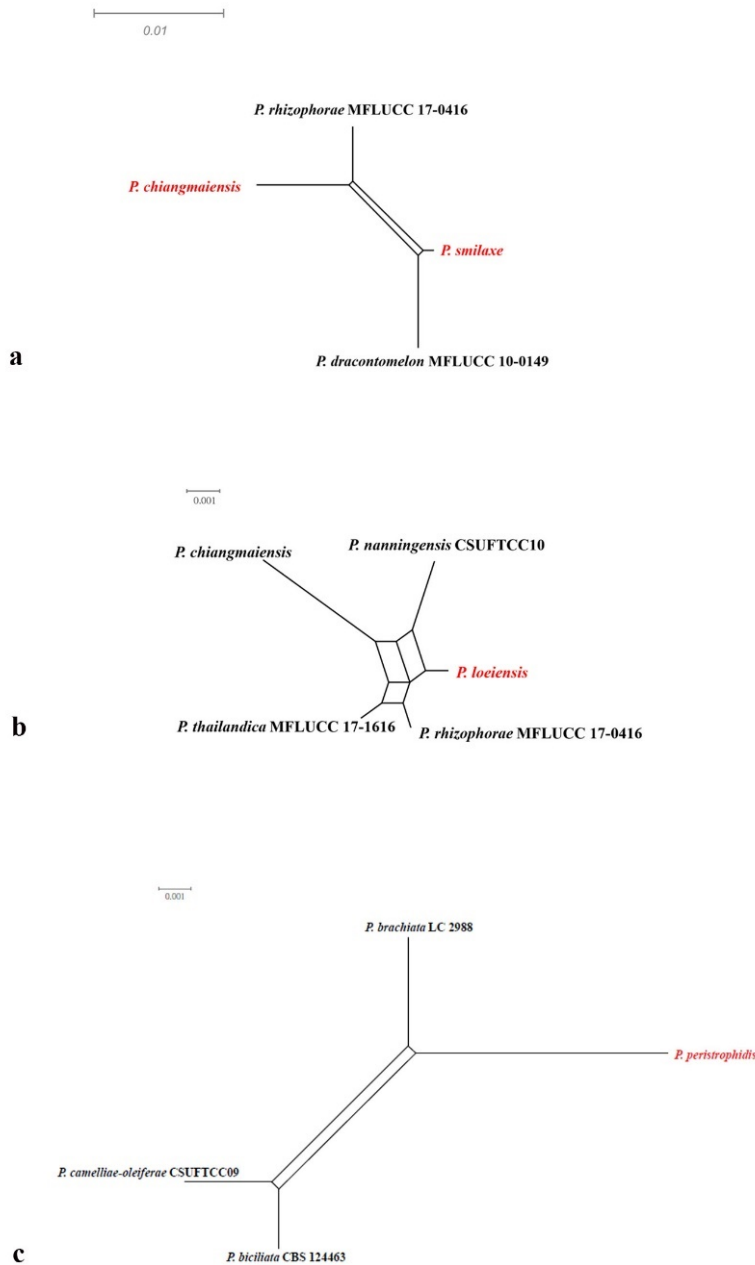
Figure 11. Cont.



**Figure 11** – Maximum likelihood (RAxML) tree for *Pestalotiopsis*, based on analysis of a combined dataset of TEF, TUB and ITS sequence data. The tree is rooted with *Neopestalotiopsis protearum* (CBS 114178) and *N. cubana* (CBS 600.96). Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. The new isolates are in red.

### Genealogical Concordance Phylogenetic Species Recognition Analysis

The PHI test revealed that there is no significant recombination ( $\Phi_w = 0.2563$ ), between *P. chiangmaiensis* and its closely related taxa *P. smilaxe* (MFLUCC 22-xxx), *P. dracontomelon* (MFLUCC 10-0149) and *P. rhizophorae* (MFLUCC 17-0416) (Fig 12a). The *P. loeiensis* based PHI test confirmed that there is no significant recombination ( $\Phi_w = 0.1318$ ), between *P. loeiensis* and its closely related taxa *P. chiangmaiensis* (MFLUCC 22-xxx), *P. nanningensis* (CSUFTCC10), *P. rhizophorae* (MFLUCC 17-0416) and *P. thailandica* (MFLUCC 17-1616) (Fig 12b). The pairwise homoplasy index (PHI) test revealed that there is no significant recombination ( $\Phi_w = 5.827$ ), between *P. peristrophidis* and its closely related taxa *P. biciliate* (CBS 124463), *P. brachiata* (LC2988), and *P. camelliae-oleiferae* (CSUFTCC09) (Fig 12c).



**Figure 12** – Split graphs showing the results of PHI test of new *Pestalotiopsis* species with their most closely related species, using Log-Det transformation and split decomposition options. The new taxon in each graph is shown in red font.

### Taxonomy

*Pestalotiopsis chiangmaiensis* Y.R. Sun & Yong Wang bis, sp. nov. Fig. 13

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology – Refers to the location where the fungus was encountered.

Holotype – MFLU 22-0XXX

*Associated* with leaf strips of *Phyllostachys edulis*. **Sexual morph:** Not observed.

**Asexual morph:** *Conidiomata* on PDA pycnidial, subglobose to globose, solitary or aggregated, dark, semi-immersed or partly erumpent; exuding black conidial masses.

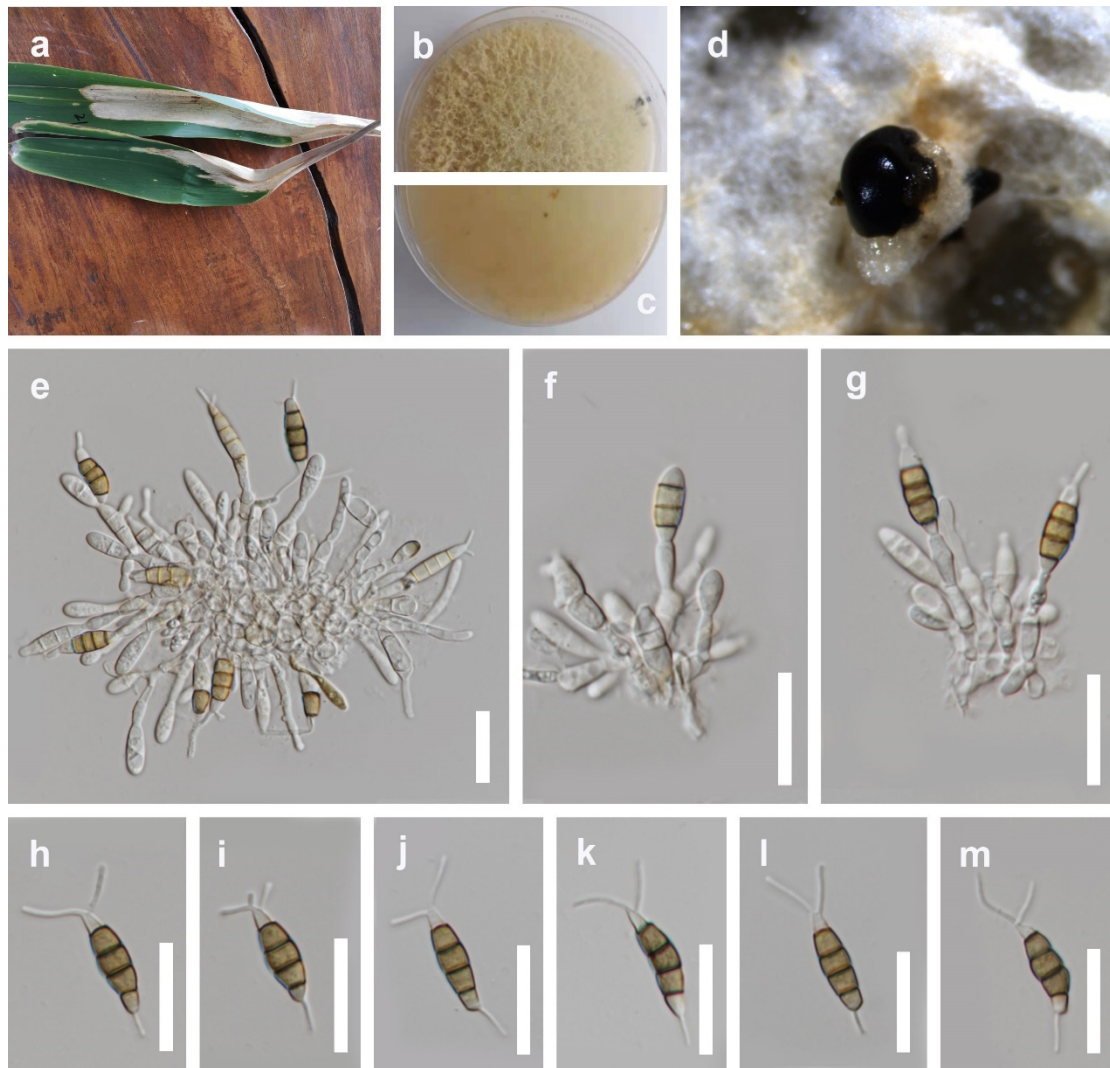


*Conidiophores* hyaline, smooth, simple, reduced to conidiogenous cells. *Conidiogenous cells* 5.0–11.0 × 1.5–3.0 µm, cylindrical to sub-cylindrical or ampulliform to lageniform, hyaline, smooth. *Conidia* pale brown, fusiform, straight to slightly curved, (3)4-septate, 15.5–26.0 × 4.0–6.5 µm,  $\bar{x} \pm SD = 21.0 \pm 2.2 \times 5 \pm 0.5$  µm (n = 40), L/W ratio = 4.2; basal cell obconic with a truncate base, hyaline or sometimes pale brown, smooth-walled, (2.5–)3.5–6 µm long ( $\bar{x} \pm SD = 4.5 \pm 0.7$  µm); three median cells 10.0–16.0 µm long ( $\bar{x} \pm SD = 14.0 \pm 1.4$  µm), pale brown, concolourous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base pale brown, 3.0–6.0 µm long ( $\bar{x} \pm SD = 4.3 \pm 0.6$  µm); third cell brown, 2.5–6.5 µm long ( $\bar{x} \pm SD = 4.0 \pm 0.7$  µm); fourth cell brown, 2.5–5.5 µm long ( $\bar{x} \pm SD = 4 \pm 0.7$  µm); apical cell 2.5–5.0 µm long ( $\bar{x} \pm SD = 3.6 \pm 0.5$  µm), hyaline, conic to acute; with 2(–3) tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 8.5–13.0 µm long ( $\bar{x} \pm SD = 10.0 \pm 1.7$  µm); single basal appendage, unbranched, tubular, centric, 2.5–6.5 µm long ( $\bar{x} \pm SD = 5.0 \pm 1.7$  µm).

Culture characteristics – Colonies on PDA reaching 5–6 cm diam after 7 d at 25 °C, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform (curle) margin, fluffy, white from above and below; fruiting bodies black.

Material examined – Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf strip of *Phyllostachys edulis* (Carriere) J. Houzeau (Poaceae), 15 July 2020, Y.R. Sun, M18 (MFLU 22-xxx, holotype); ex-type-living cultures, MFLUCC 22-xxx; *ibid.*, on leaf strip of *Phyllostachys edulis*, 15 July 2020, Y.R. Sun, M18-1 (MFLU 22-xxx), living cultures, MFLUCC 22-xxx.

Notes – *Pestalotiopsis chiangmaiensis* formed a distinct lineage and was sister to *P. smilaxe* (GUCC 22-xxx) and *P. dracontomelon* (MFLUCC 10-0149) in the phylogenetic tree (Fig. 11). It differs by visible, longer conidiophores from *P. smilaxe* and shorter apical appendages (8.5–13 µm vs. 10.0–22 µm) from *P. dracontomelon*. In addition, there are 14 base pair differences (without gap, 474bp) in the TEF region between MFLUCC 22-xxx and MFLUCC 22-M13, and 15 base pair differences (without gap, 464bp) between MFLUCC 22-xxx and MFLUCC 10-0149. The PHI test on *P. chiangmaiensis* also showed that there is no significant recombination ( $\Phi_w = 0.2563$ ), between *P. chiangmaiensis* and its closely related taxa (Fig 12a). Therefore, we introduce *P. chiangmaiensis* as a new species.



**Figure 13** – *Pestalotiopsis chiangmaiensis* (MFLU 22-0XXX, holotype). a Host. b, c Cultures. d Colonies on PDA. e–g Conidiogenous cells and developing conidia. h–m Conidia. Scale bars: e–m = 20  $\mu$ m.

*Pestalotiopsis loeiensis* Y.R. Sun & Yong Wang bis, sp. nov. Fig. 14

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology – Refers to the collected site, Loei province.

Holotype – MFLU 22-0XXX

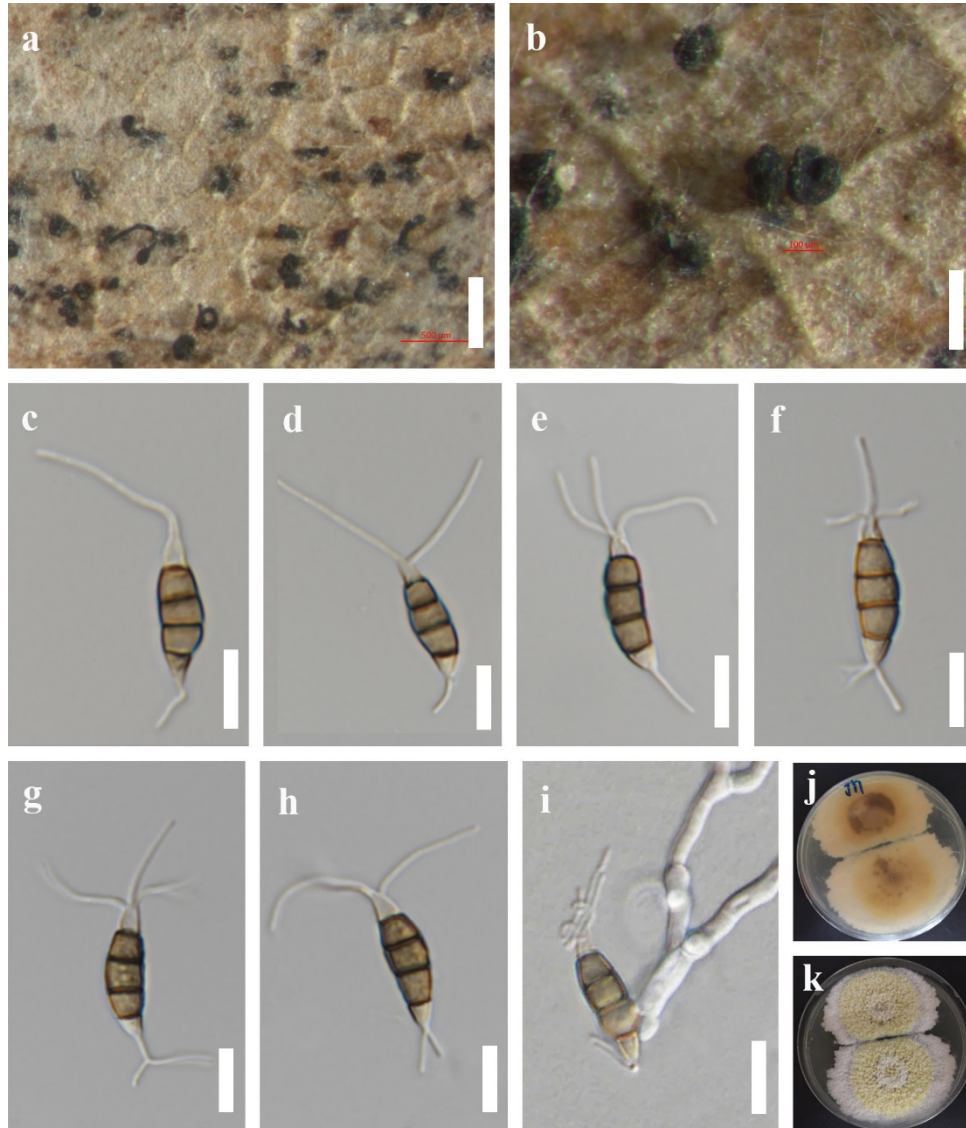
*Saprobic* on dead leaves. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, black, semi-immersed on leaves. *Conidiophores* indistinct and *conidiogenous cells* indistinct. *Conidia* 17.0–22.0  $\times$  4.0–6.0  $\mu$ m,  $\bar{x} \pm SD = 19.0 \pm 1.2 \times 5.2 \pm 0.5 \mu$ m (n = 40), L/W ratio = 3.7, fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, rugose walled, 2.8–5.5  $\mu$ m long ( $\bar{x} \pm SD = 3.6 \pm 0.6 \mu$ m), with 1–3 basal appendages, unbranched, tubular, centric, 3.0–13.0  $\mu$ m long ( $\bar{x} \pm SD = 8.7 \pm 2.8 \mu$ m); three median cells 10.0–14.0  $\mu$ m long ( $\bar{x} \pm SD = 12.0 \pm 0.8 \mu$ m), doliiform to cylindrical, brown, concolourous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base brown, 3.0–5.5  $\mu$ m long ( $\bar{x} \pm SD = 4.1$

$\pm 0.5 \mu\text{m}$ ); third cell brown, 3.0–5.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.9 \pm 0.4 \mu\text{m}$ ); fourth cell brown, 2.0–5.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.0 \pm 0.6 \mu\text{m}$ ); apical cell 2.8–4.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.8 \pm 0.4 \mu\text{m}$ ), 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, unbranched, 13–24  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 18.0 \pm 2.7 \mu\text{m}$ ).

Culture characteristics – Colonies on PDA reaching 8 cm diam after two weeks at 25 °C, colonies filamentous to circular, medium dense, mycelium on surface flat or raised, with filiform margin, fluffy, yellow circle in the middle surrounded by white mycelium from above, light yellow to pale brown from the reverse.

Material examined – Thailand, Loei Province, dead leaves of an identified plant, 27 February 2020, J.Y. Zhang, JY1 (MFLU 22-xxx, holotype); ex-type-living cultures, MFLUCC 22-xxx.

Notes –*Pestalotiopsis Loeiensis* (MFLUCC 22-xxx) is phylogenetically related to *P. rhizophorae* and *P. thailandica*, which were isolated from leaf spots of mangroves (Fig. 11). *P. loeiensis* has a similar conidial size to *P. rhizophorae* and *P. thailandica*. However, *P. loeiensis* is distinguishable by its more than one basal appendage. The result of the PHI test ( $\Phi_w = 0.2881$ ) also showed there is no significant recombination between *P. loeiensis* and its closely related taxa (Fig 12b). Therefore, we introduce *P. loeiensis* as a new species.



**Figure 14** – *Pestalotiopsis loeiensis* (MFLU 22-xx, holotype). a, b Conidiomata on the host. c–h Conidia. i Germinated conidium. j, k Colonies on PDA. Scale bars: a = 500  $\mu$ m, b = 200  $\mu$ m, c–i = 10  $\mu$ m.

*Pestalotiopsis peristrophidis* Y.R. Sun & Yong Wang bis, sp. nov.

Fig. 15

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology – Name reflects the host's name, *Peristrophe japonica*

Holotype – HUGP 801

*Endophytic* from *Peristrophe japonica*. Colonies on PDA white with grey mycelia in the center, felted, black conidiomata, lobed edge; reverse white with pale to brown concentric circles. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* on PDA pycnidial, solitary or aggregated, subglobose to globose, dark, semi-immersed or erumpent; exuding black conidial masses. *Conidiophores* hyaline, smooth, simple, reduced to conidiogenous cells. *Conidiogenous cells* 1.2–3.0  $\mu$ m wide, cylindrical to sub-cylindrical or ampulliform to lageniform, hyaline, smooth. *Conidia* pale to brown, fusiform, straight to slightly curved, 4-septate, 20.0–29.0  $\times$

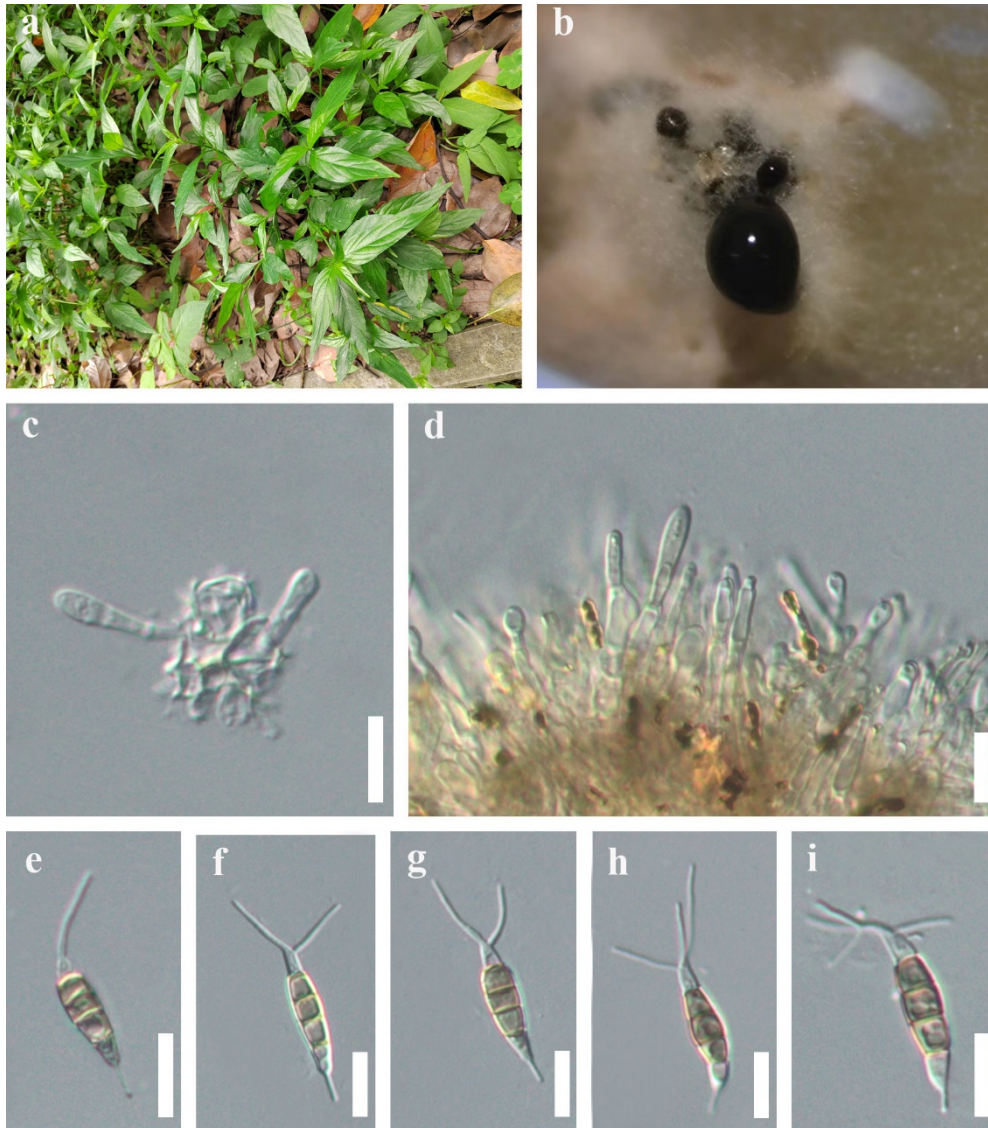


4.5–6.2  $\mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 24.5 \pm 2.0 \times 5.2 \pm 0.4 \mu\text{m}$  ( $n = 40$ ), L/W ratio = 4.7; basal cell obconic with a truncate base, hyaline, smooth walled, 4.5–7.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.5 \pm 0.6 \mu\text{m}$ ); three median cells 12.0–17.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 14.0 \pm 1.1 \mu\text{m}$ ), pale to brown, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base pale to brown, 3.5–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.0 \pm 0.4 \mu\text{m}$ ); third cell pale to brown, 3.5–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.5 \pm 0.4 \mu\text{m}$ ); fourth cell pale to brown, 3.5–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.5 \pm 0.5 \mu\text{m}$ ); apical cell 2.7–5.7  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.0 \pm 0.6 \mu\text{m}$ ), hyaline, conic to acute; with 1–4 (mostly 2) tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 7.5–17.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 12.2 \pm 2.5 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, 2.5–5.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.8 \pm 0.5 \mu\text{m}$ ).

Material examined – China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy leaves of *Peristrophe japonica* (Thunb.) Bremek. (Acanthaceae), 1 May 2022, Y.R. Sun, E53 (HUGP 801, died culture, holotype); ex-type living culture GUCC 803; China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy stems of *Peristrophe japonica*, 1 May 2022, Y.R. Sun, E56 (HUGP 802, dried culture, paratype; living culture GUCC 801).

Notes – An endophyte, *P. peristrophidis* was isolated from the medicinal plant, *Peristrophe japonica* in China. Our four strains GUCC 801, GUCC 802, GUCC 803 and GUCC 804 clustered together and formed a distinct inner clade, which is sister to *P. brachiata* (LC 2988 and LC 8189) with strong support (ML-BS = 99%, BYBB = 1.00) in the phylogenetic tree (Fig 12). In morphology, *P. peristrophidis* differs from *P. brachiata* by the numbers and size of the basal appendages (1 basal appendage, unbranched, 2.5–5.0  $\mu\text{m}$  long in *P. peristrophidis* vs. 1–4 basal appendages, branched or unbranched, 5.5–9.5  $\mu\text{m}$  long in *P. brachiata*) (Liu et al. 2017). The PHI test on *P. peristrophidis* revealed there is no significant recombination ( $\Phi_w = 0.05827$ ) between *P. peristrophidis* and its closely related taxa (Fig 12c). Therefore, we introduce *P. peristrophidis* as a new species.





**Figure 15** – *Pestalotiopsis peristrophidis* (HUGP 22-xx, Holotype). a Host. b Close up view of conidiomata, c, d Conidia attached to conidiogenous cells, e–i Conidia. Scale bars: c–i = 10  $\mu$ m.

*Pestalotiopsis smilaxe* Y.R. Sun & Yong Wang bis, sp. nov.

Fig. 16

Index Fungorum number: IFXX; Facesoffungi number: FoF XX

Etymology – Refers to the host plant from which the fungus was first isolated.

Holotype – MFLU 22-0XXX

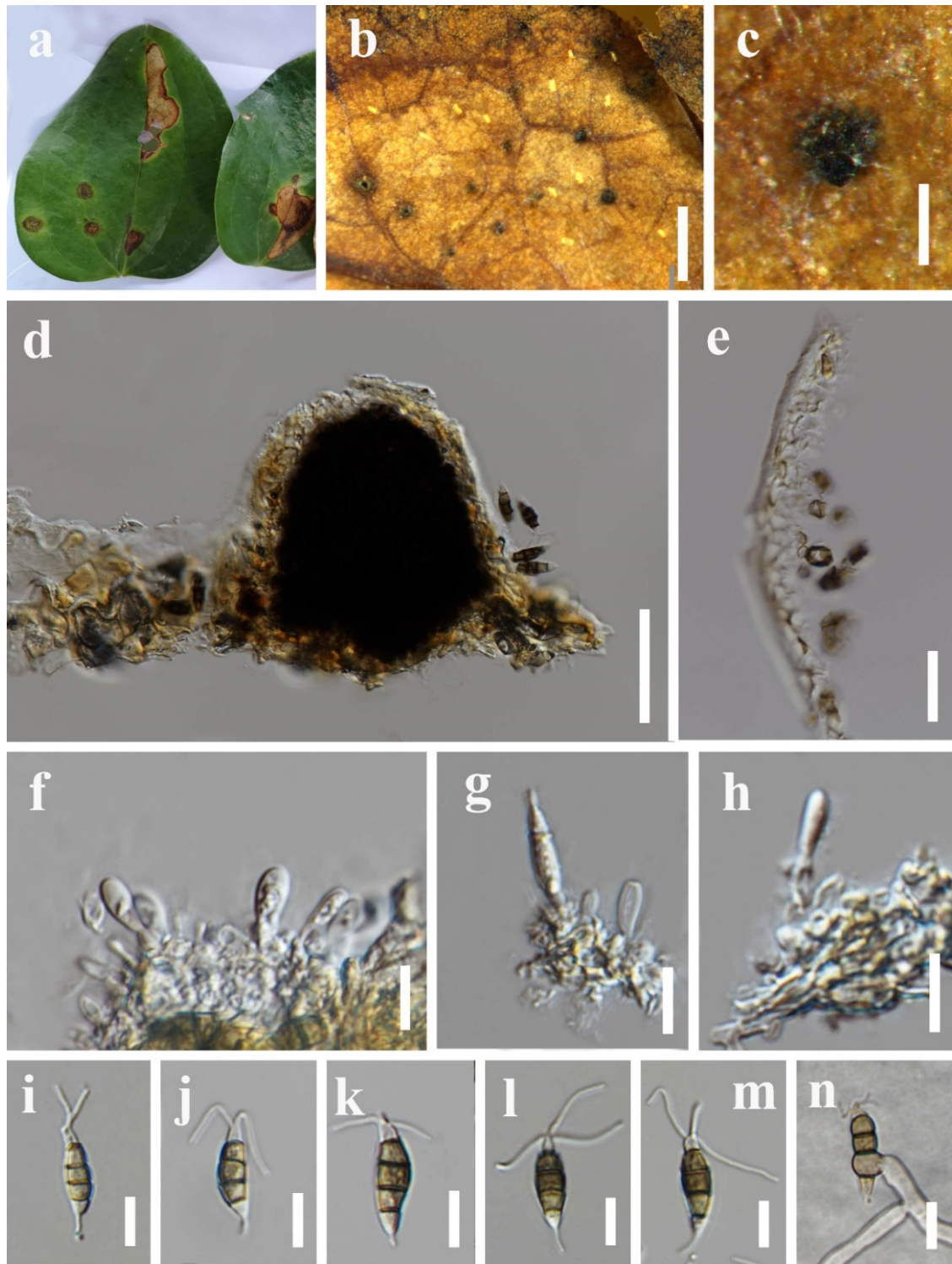
Associated with leaf spots of *Smilax china* and *Dioscorea sp.*. Symptoms subcircular to irregular shape, brown, slightly sunken spots appear on the leaves of *Smilax china*, which later expand outwards. Small auburn spots appeared initially and then gradually enlarged. **Sexual morph**: Not observed. **Asexual morph**: *Conidiomata* solitary, subglobose, unilocular, black, semi-immersed on leaves. *Conidiomatal wall* 7.0–10.0  $\mu$ m wide, thin walled, pale brown. *Conidiophores* indistinct. *Conidiogenous cells* subcylindrical to ampulliform, hyaline, smooth, 1.4–2.6  $\mu$ m wide. *Conidia* 18.0–21.5  $\times$  4.5–6.5  $\mu$ m,  $\bar{x} \pm SD = 20 \pm 0.94 \times 5.3 \pm 0.6 \mu$ m (n = 40), L/W ratio =

3.8, fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, smooth walled, 2.8–5.3  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.0 \pm 0.6 \mu\text{m}$ ); three median cells 9.5–14.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 12.0 \pm 0.9 \mu\text{m}$ ), pale brown to brown, concolourous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base pale brown to brown, 2.5–4.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.8 \pm 0.5 \mu\text{m}$ ); third cell brown, 2.5–5.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4 \pm 0.5 \mu\text{m}$ ); fourth cell brown, 3.0–5.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4 \pm 0.5 \mu\text{m}$ ); apical cell 2.5–4.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.3 \pm 0.4 \mu\text{m}$ ), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 6–14  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 10.0 \pm 2.0 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, (1.5–)2–5(–6)  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.0 \pm 0.7 \mu\text{m}$ ).

Culture characteristics – Colonies on PDA reaching 10 cm diam after two weeks at 25 °C, colonies filamentous to circular, medium dense, aerial mycelium on surface flat or raised, with filiform margin, fluffy, white from above and reverse.

Material examined – Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf spots of *Smilax china* L. (Liliaceae), 15 July 2020, Y.R. Sun, M13 (MFLU 22-xxx, holotype); ex-type-living cultures, MFLUCC 22-xxx. Thailand, Chiang Mai Province, Mae Taeng District, Mushroom Research Center, leaf spots of *Dioscorea sp.* (Dioscoreaceae), 16 July 2020, Y.R. Sun, M26 (MFLU 22-xxx, paratype), living cultures, MFLUCC 22-xxx.

Notes – Two collections MFLU 22-xxx and MFLU 22-xxx share similar morphology. These two isolates clustered together and formed a sister clade to *P. dracontomelon* (MFLUCC 10-0149) in the phylogenetic tree. There are only 1 base pair difference in TEF and TUB genes and 3 base pair differences in the ITS gene between these two isolates. For the differences between *P. smilaxe* and its related species see the notes of *P. chiangmaiensis* (this study). Therefore, these two isolates are identified as conspecific, representing a new species.



**Figure 16** – *Pestalotiopsis smilaxe* (MFLU 22-0XXX, holotype). a Host. b, c Close up view of conidiomata. d Section through conidioma. e Section through pycnidial wall. f–h Immature conidia attached to conidiogenous cells. i–m Conidia. n Germinated conidium. Scale bars: b = 1000  $\mu$ m, c = 200  $\mu$ m, d = 50  $\mu$ m, e = 20  $\mu$ m, f–n = 10  $\mu$ m.

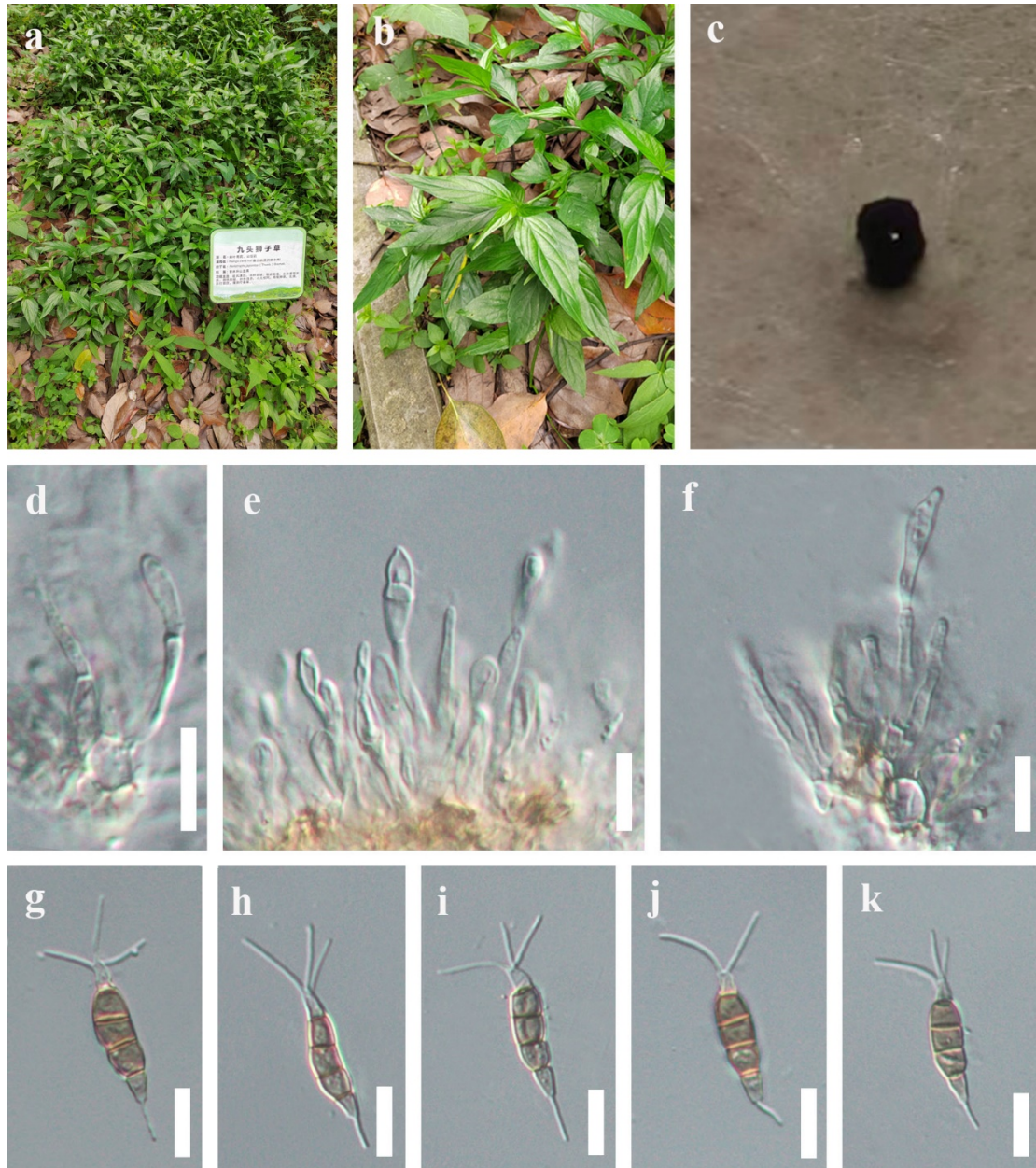


Index Fungorum number: IF809735; Facesoffungi number: FoFxx

*Endophytic* from *Peristrophe japonica*. Colonies on PDA white with grey mycelia in the center, felty, black conidiomata, lobed edge. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* on PDA pycnidial, solitary or aggregated, subglobose to globose, dark, semi-immersed or erumpent; exuding black conidial masses. *Conidiophores* hyaline, smooth, simple, reduced to conidiogenous cells. *Conidiogenous cells* 1.2–2.0  $\mu\text{m}$  wide, cylindrical to sub-cylindrical, hyaline, smooth. *Conidia* brown, fusiform, straight to slightly curved, 4-septate, 20.0–27.5  $\times$  3.7–5.8  $\mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 24.5 \pm 1.3 \times 5.0 \pm 0.4 \mu\text{m}$  (n = 40), L/W ratio = 4.9; basal cell obconic with a truncate base, hyaline to pale brown, smooth walled, 3.2–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.8 \pm 0.5 \mu\text{m}$ ); three median cells 12.5–17.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 14.5 \pm 0.9 \mu\text{m}$ ), brown, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base brown, 4.0–5.8  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.0 \pm 0.4 \mu\text{m}$ ); third cell brown, 3.5–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.7 \pm 0.4 \mu\text{m}$ ); fourth cell brown, 4.0–5.8  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.7 \pm 0.4 \mu\text{m}$ ); apical cell 3.0–5.4  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.1 \pm 0.5 \mu\text{m}$ ), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 8.5–18.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 13.8 \pm 2.1 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, 4.0–9.7  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.6 \pm 0.7 \mu\text{m}$ ).

Material examined – China, Guizhou Province, Guiyang City, Nanming District, Guiyang Medicinal Botanical Garden, on healthy leaves of *Peristrophe japonica* (Thunb.) Bremek. (Acanthaceae), 1 May 2022, Y.R. Sun, E33 (HUGP 803, dried culture; living culture GUCC 800).

Notes – *Pestalotiopsis chamaeropsis* was originally reported on leaves of *Chamaerops humilis* in Italy by Maharachchikumbura et al. (2014). Subsequently, many studies have proven that *P. chamaeropsis* is a serious phytopathogen, which can cause diseases of *Camellia sinensis*, *Camellia oleifera* and *Eurya nitida* (in China), *Erica arborea* (in Tunisia), *Japanese andromeda* (in Japan) and *Prostanthera rotundifolia* (in Australia) (Moslemi and Taylor 2015, Jiang et al. 2017, Ariyawansa and Hyde 2018, Hlaiem et al. 2018, Nozawa et al. 2019, Wang et al. 2019, Chen et al. 2021, Qiu et al. 2022, Santos et al. 2022). Park et al. (2017) reported *P. chamaeropsis* as an endophyte, from the leaves of woody plants in Korea. In this study, our strain GUCC 800 is phylogenetically clustered with *P. chamaeropsis* CBS 186.71 with maximum support (ML-BS = 100%, BYPP = 1), and it has overlapping characteristics with *P. chamaeropsis* (CBS 186.71). Thus, we identify GUCC 800 as *P. chamaeropsis*, representing a new host record.



**Figure 17** – *Pestalotiopsis chamaerapis* (HUGP 803, new record). a, b Host. c Close up view of conidiomata, d–f Conidia attached to conidiogenous cells, g–k Conidia. Scale bars: d–k = 10  $\mu$ m.

*Pestalotiopsis dracontomelon* Maharachch & K.D. Hyde, Fungal Diversity. 15: (2015)  
Fig.18

Index Fungorum number: IF550943; Facesoffungi number: FoF00457

Associated with dead leaves of *Podocarpus* sp.. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, subglobose, unilocular, black, semi-immersed on leaves. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* sub-cylindrical to cylindrical, hyaline, rugose walled, 1.5–3.0  $\mu$ m wide. *Conidia* 19.0–26.0  $\times$  5.5–8.0  $\mu$ m,  $\bar{x}$   $\pm$ SD = 23.0  $\pm$ 1.7  $\times$  7.0  $\pm$ 0.6  $\mu$ m (n = 30), L/W ratio = 3.3, fusiform, ellipsoid, straight to slightly curved, 4-septate; basal cell

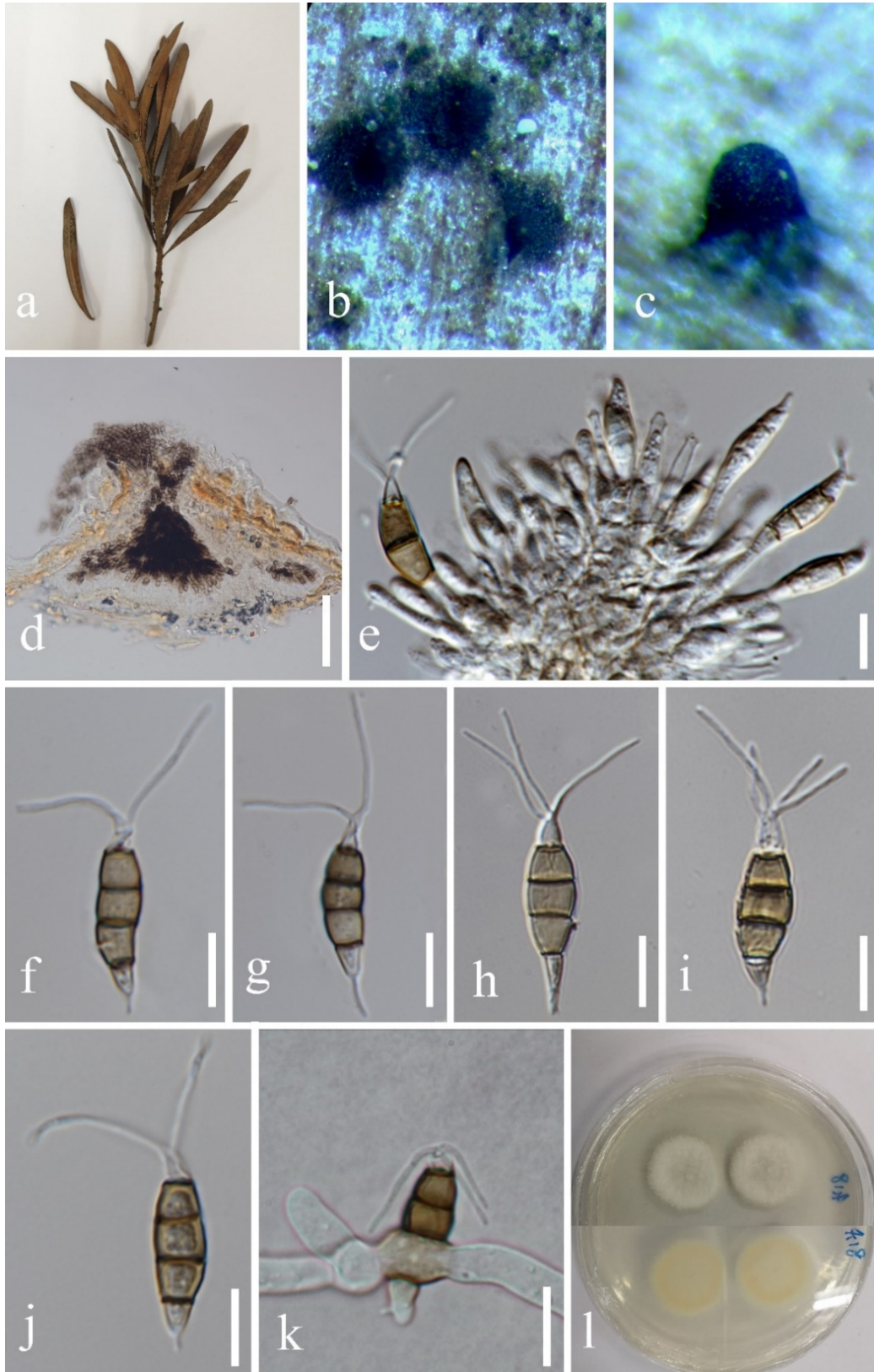


obconic with a truncate base, hyaline or sometimes pale brown, rugose walled, 3.3–5.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.0 \pm 0.5 \mu\text{m}$ ); three median cells 11.0–16.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 14.5 \pm 1.6 \mu\text{m}$ ), doliiform to cylindrical, pale brown to brown, concolourous, wall rugose, septa darker than the rest of the cell, somewhat constricted at the septa; second cell from base pale brown to brown, 4.0–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 5.0 \pm 0.6 \mu\text{m}$ ); third cell pale brown to brown, 3.0–5.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.5 \pm 0.6 \mu\text{m}$ ); fourth cell pale brown to brown, 3.5–5.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.5 \pm 0.5 \mu\text{m}$ ); apical cell 3.0–5.5  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 4.3 \pm 0.6 \mu\text{m}$ ), hyaline, conic to acute; with 2–4 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 10.0–22  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 13.5 \pm 4 \mu\text{m}$ ); single basal appendage, unbranched, tubular, centric, 2.0–6.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 3.8 \pm 1.3 \mu\text{m}$ ).

Culture characteristics – Colonies on PDA reaching 2.5 cm diam after 7 days at 25 °C, colonies filamentous to circular, medium dense, aerial mycelium on the surface flat or raised, fluffy, white from above and below.

Material examined – Thailand, Chiang Rai Province, Mae Fah Luang University, leaf spots of *Podocarpus sp.* (Podocarpaceae), 15 January 2019, Y.R. Sun, S18 (MFLU 22-xxx); living cultures, MFLUCC 22-xxx.

Notes – *Pestalotiopsis dracontomelon* was isolated from diseased leaves of *Dracontomelon mangifera* (Anacardiaceae) in Thailand (Liu et al. 2015). Our isolate MFLUCC 22-xxx was grouped with *P. dracontomelon* (MFLUCC 10-0149) in the phylogenetic tree. Morphologically, they have overlapping conidial measurements (19–26  $\times$  5.5–8  $\mu\text{m}$  for MFLUCC 22-xxx vs. 18–23  $\times$  5.5–7.5  $\mu\text{m}$  for MFLUCC 10-0149). Therefore, we identify MFLUCC 22-xxx as the new host record of *P. dracontomelon*.



**Figure 18** – *Pestalotiopsis dracontomelon* (MFLU 22-xx, new host record). a Host. b, c Close up view of conidiomata, d Section through conidioma, e Conidia attached to conidiogenous cells, f–j Conidia, k Germinated conidium. l Colony. Scale bars: b = 1000  $\mu$ m, d = 50  $\mu$ m, e, k = 20  $\mu$ m, f–j = 10  $\mu$ m.

*Pestalotiopsis hydei* Huanraluek & Jayaward, Phytotaxa. 479(1): 35 (2021) Fig. 19

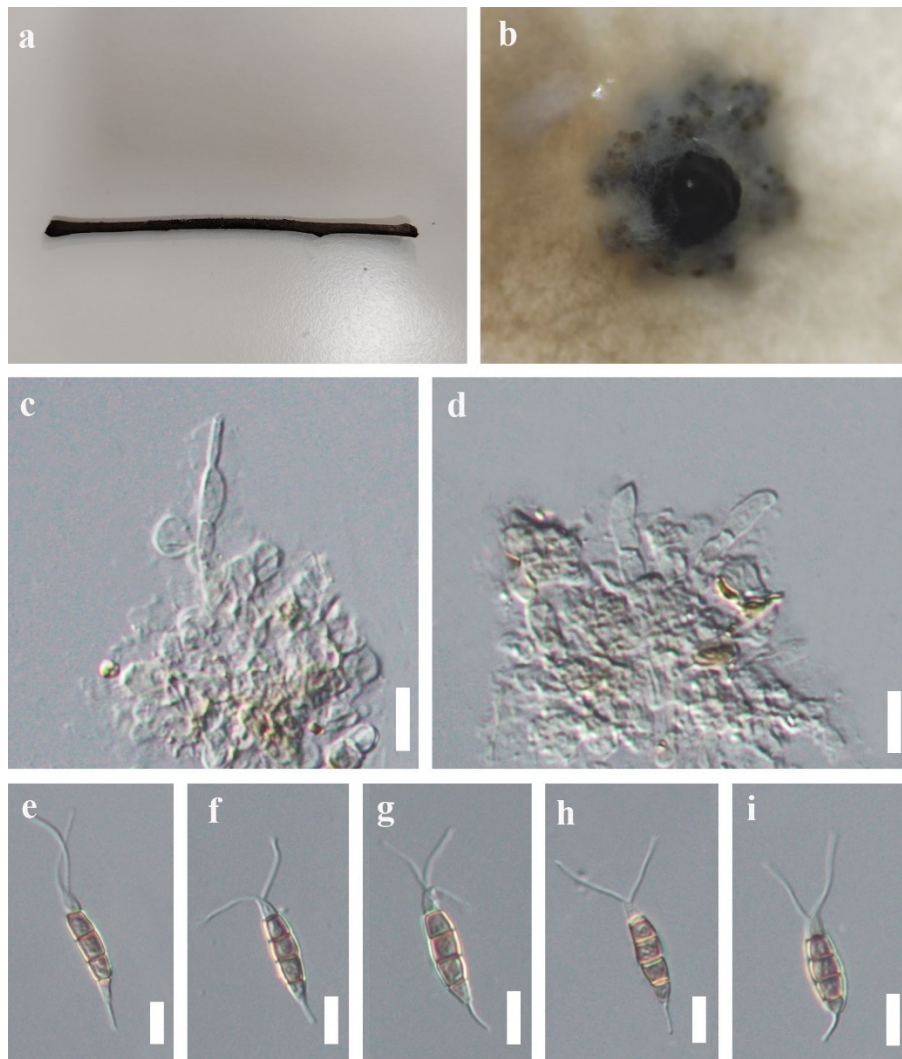
Index Fungorum number: IF558100; Facesoffungi number: FoF 09460

*Saprobic* on dead twigs. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, unilocular, dark, immersed on stems. *Conidiophores* hyaline, smooth, simple, reduced to conidiogenous cells. *Conidiogenous cells* cylindrical to subcylindrical, hyaline, 1.8–2.5  $\mu\text{m}$  wide. *Conidia* 19.5–26.0  $\times$  4.5–5.8  $\mu\text{m}$   $\bar{x} \pm \text{SD} = 22.5 \pm 1.1 \times 5.0 \pm 0.3 \mu\text{m}$  (n = 40), L/W ratio = 4.5, fusiform, straight to slightly curved, 4-septate; basal cell conic to obconic with a truncate base, hyaline to subhyaline, 2.8–6.0  $\mu\text{m}$  long; three median cells 11.5–16.0  $\mu\text{m}$  long ( $\bar{x} \pm \text{SD} = 13.5 \pm 0.7 \mu\text{m}$ ), wall rugose, pale to brown, concolourous, septa darker than the rest of the cell; second cell from base pale to brown, 3.2–5.5  $\mu\text{m}$  long; third cell pale to brown, 3.0–5.0  $\mu\text{m}$  long; fourth cell pale to brown, 4.0–5.5  $\mu\text{m}$  long; apical cell 3.0–5.0  $\mu\text{m}$  long, hyaline, rugose and thin-walled; with 1–3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 7.0–17.5  $\mu\text{m}$  long; single basal appendage 3.0–8.5  $\mu\text{m}$  long, unbranched, tubular, centric.

Culture characteristics – Colonies on PDA reaching 8 cm diam after 2 weeks at 25 °C, colonies filamentous to circular, medium dense, aerial mycelium on the surface flat or raised, fluffy, white from above and reverse.

Material examined – China, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo District, on dead twigs, 12 March 2022, J.E. Sun, L19-1 (HGUP 804); living cultures, GUCC 819.

Notes – *Pestalotiopsis hydei* was isolated from the leaf spots of *Litsea petiolate* in Thailand (Huanaluek et al. 2021). Our collection HGUP 804 clustered together with *P. hydei* (MFLUCC 20-0135) in the phylogenetic tree. HGUP 804 also has a similar conidial measurement to MFLUCC 20-0135 (19.5–26.0  $\times$  4.5–5.8  $\mu\text{m}$  in HGUP 804, 18–35  $\times$  3–6  $\mu\text{m}$  in MFLUCC 20-0135). Besides, there are only 3 base pair differences in the ITS and TEF gene. Therefore, we identify GUCC 819 as a new geographical record of *P. hydei*.



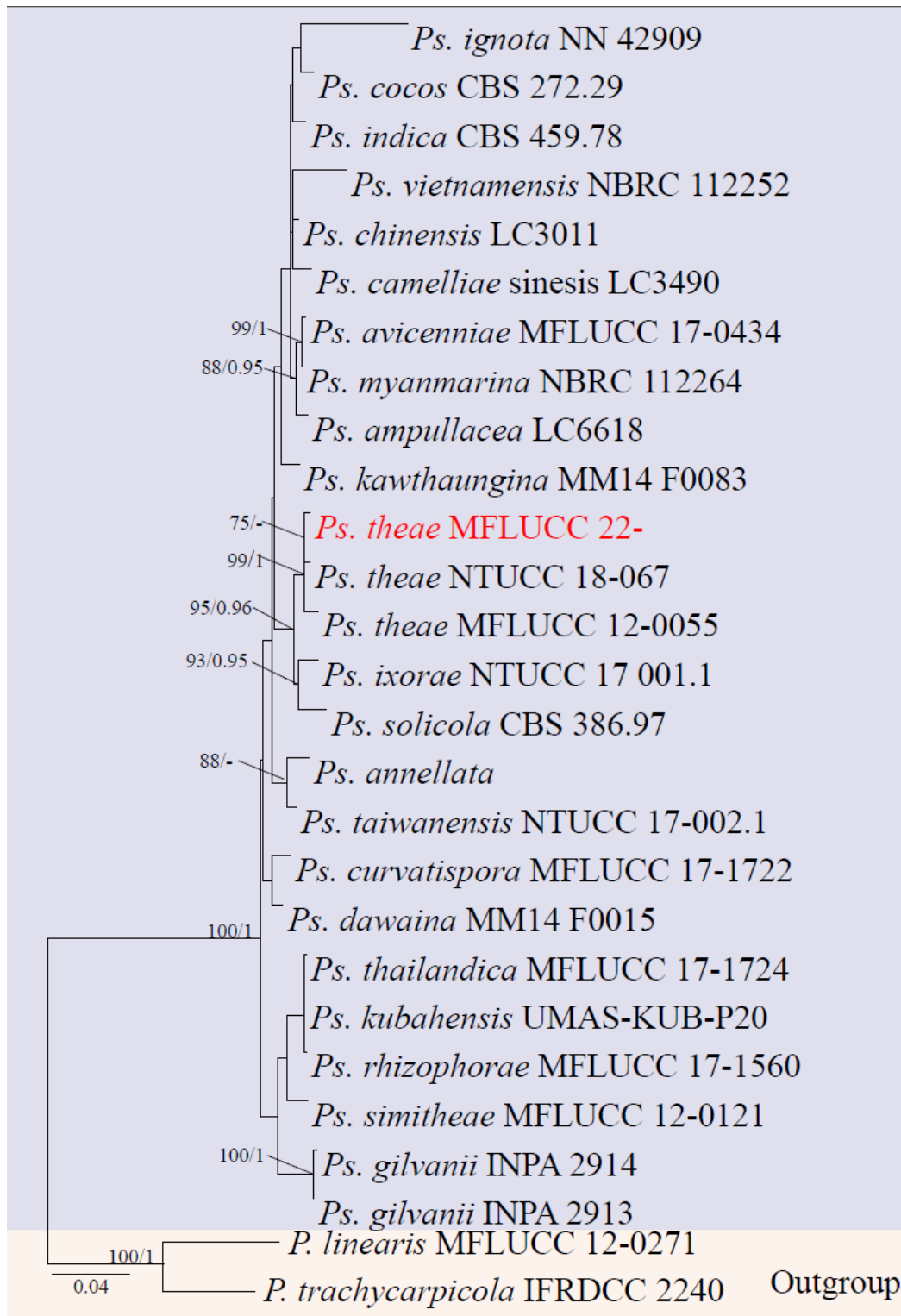
**Figure 19** – *Pestalotiopsis hydei* (HGUP 804, new record). a Host. b Close up view of conidiomata, c, d Conidiogenous cells, e–i Conidia. Scale bars: c–i = 10  $\mu$ m.

### **Part3: *Pseudopestalotiopsis***

#### **Phylogenetic analyses**

The sequence datasets for TEF, TUB and ITS, were analyzed in combination to infer the interspecific relationships within *Pseudopestalotiopsis*. The aligned sequence matrix consisted of 27 sequences, including two outgroups *Pestalotiopsis trachycarpicola* (IFRDCC 2240) and *P. linearis* (MFLUCC 12-0271). Similar tree topologies were obtained by ML and BI methods, and the most likely tree ( $-\ln = 66,531.894$ ) is presented (Figure 20). Our collection is clustered with the type species of *Pseudopestalotiopsis*, *P. theae*, in the phylogenetic tree (Fig 20).





**Fig. 20** Maximum likelihood (rAxML) tree for *Pseudopezalotiopsis*, based on analysis of a combined dataset of TEF, TUB and ITS sequence data. The tree is rooted with *P. trachycarpicola* (IFRDCC 2240) and *P. linearis* (MFLUCC 12-0271). Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. The new isolates are in red.

### Taxonomy



*Pseudopestalotiopsis theae* (Sawada) Maharachch., K.D. Hyde & Crous, Studies in Mycology. 79: 183 (2014) Fig. 21

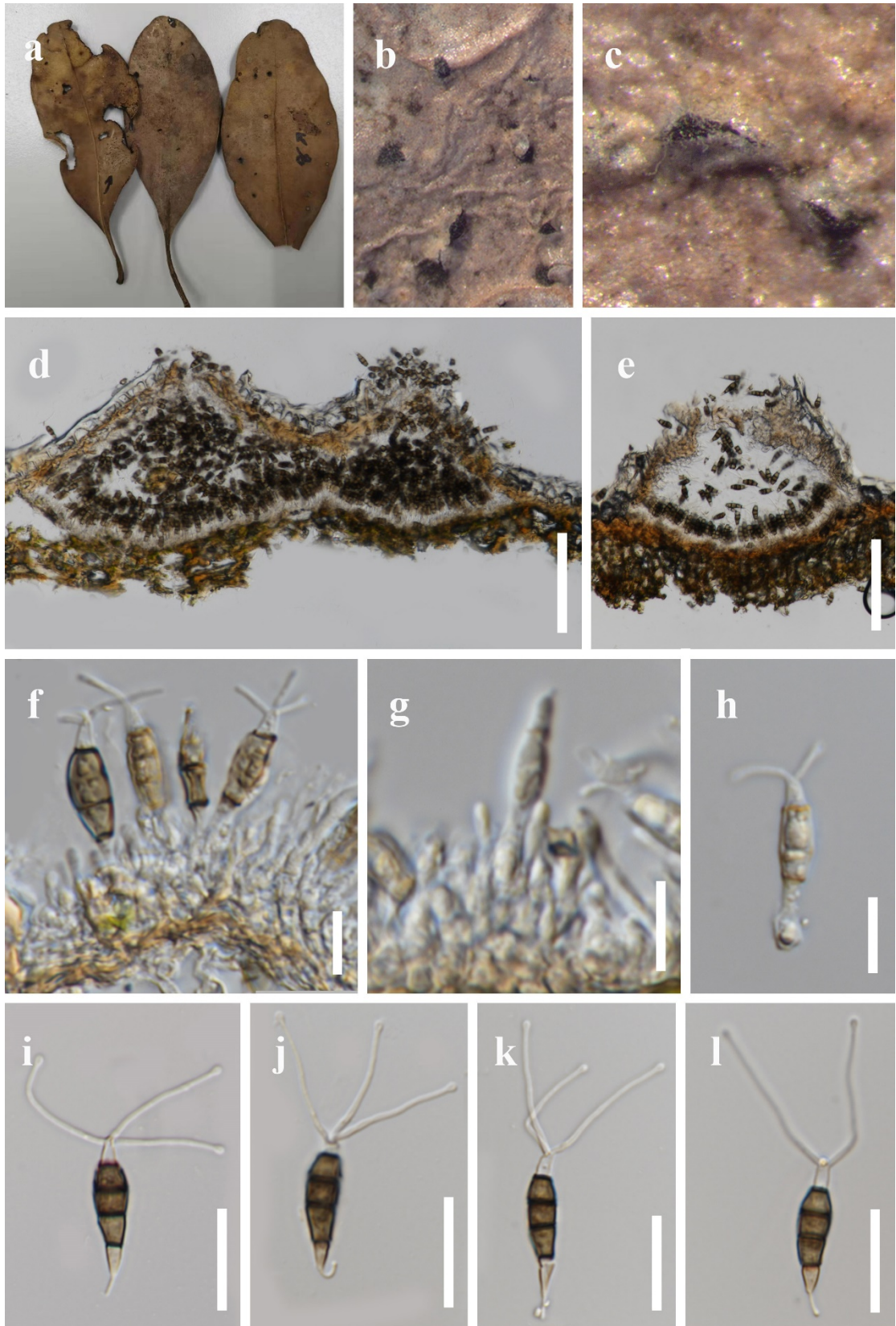
Index Fungorum number: IF 631991; Facesoffungi number: FoF XX

Associated with dead leaves of *Ceriops tagal*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* solitary, subglobose, unilocular or bilocular, brown, semi-immersed on leaves. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* sub-cylindrical to cylindrical, hyaline, rugose walled. *Conidia* 22.0–31.0 × 5.0–7.0 μm,  $\bar{x} \pm SD = 26.0 \pm 1.9 \times 6 \pm 0.5$  μm (n = 40), L/W ratio = 4.3, fusiform, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, rugose walled, 3.0–7.0 μm long ( $\bar{x} \pm SD = 5.2 \pm 0.7$  μm); three median cells 14.0–20.0 μm long ( $\bar{x} \pm SD = 15.8 \pm 1.3$  μm), doliiform to cylindrical, pale brown to brown, concolourous, wall rugose, septa darker than the rest of the cell; second cell from base pale brown to brown, 4.0–7.5 μm long ( $\bar{x} \pm SD = 5.5 \pm 0.7$  μm); third cell pale brown to brown, 3.5–6.5 μm long ( $\bar{x} \pm SD = 5.0 \pm 0.7$  μm); fourth cell pale brown to brown, 2.5–7.0 μm long ( $\bar{x} \pm SD = 5.0 \pm 0.9$  μm); apical cell 2.5–5.5 μm long ( $\bar{x} \pm SD = 4.0 \pm 0.7$  μm), hyaline, conic to acute; with 2–3 tubular appendages on apical cell, inserted at different loci in a crest at the apex of the apical cell, unbranched, 18.0–29.0 μm long ( $\bar{x} \pm SD = 24.5 \pm 2.7$  μm); single basal appendage, unbranched, tubular, centric, 3.0–6.0 μm long ( $\bar{x} \pm SD = 4.5 \pm 0.6$  μm).

Culture characteristics – Colonies on PDA reaching 10 cm diam after two weeks at 25 °C, colonies filamentous to circular, medium dense, aerial mycelium on the surface flat or raised, fluffy, white from above and below.

Material examined – Thailand, Suphan Buri Province, dead leaf of *Ceriops tagal* (perr.) C. B. Rob. (Rhizophoraceae), 5 September 2020, S Wang, TN07 (MFLU 22-xxx); living cultures, MFLUCC 22-xxx.

Notes – Our isolate MFLUCC 22-xxx is phylogenetically grouped with the type species of *Pseudopestalotiopsis*, *Ps. theae*. Morphologically, our new collection MFLU 22-xx resembles *Ps. theae* (MFLUCC 12-0055) in color and size of the conidiogenous cells, conidia and appendages. Therefore, we report this isolate as a new host record of *Ps. theae* from the *Ceriops tagal*.



**Figure 21** – *Pseudopestalotiopsis theae* (MFLU 22-0xx). a Host. b, c Close up view of conidiomata. d, e Section through conidioma. e Section through pycnidial wall. f, h Conidia attached to conidiogenous cells. h–l Conidia. Scale bars: d, e = 100  $\mu$ m, f–h = 10  $\mu$ m, i–l = 20  $\mu$ m.

## Discussion

During the research of microfungi on medicinal plants in southwest China and Thailand, 27 pestalotioid strains representing 17 species were isolated from 16 medicinal plants. Six new *Neopestalotiopsis* species, namely, *N. amomumica*, *N. ceibania*, *N. chinensis*, *N. photiniana*, *N. suphanburica* and *N. hyperica*, four new *Pestalotiopsis* species, namely, *P. Chiangmaiensis*, *P. loeiensis*, *P. smilaxe*, and *P. peristrophidis*, and five new records are introduced with descriptions and illustrations. Among them, ten species are related to leaf diseases of medicinal plants, three species are saprobes, two species are endophytes, and one species has various lifestyles. A worldwide checklist of pestalotioid species associated with medicinal plants is provided (Table 4). Among them, most are related to diseases of medicinal plants, and 51 (a total of 87) were found in China and Thailand. By analysis, the majority of pestalotioid species are associated with dicotyledonous medicinal plants (Fig 22).

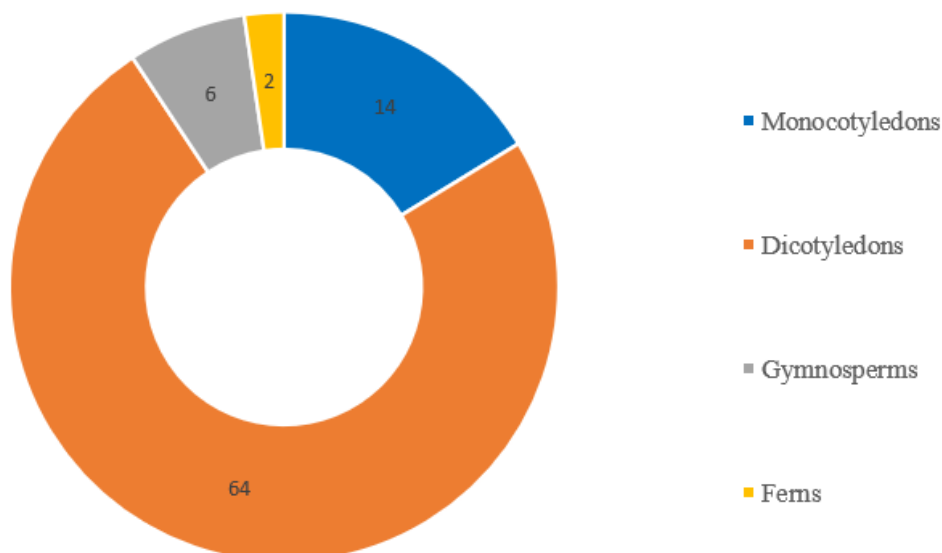
In this study, *N. subtropicalis* was isolated from healthy leaves of *Pinellia ternata* and dead leaves of *Ceiba pentandra*, and it was previously isolated from the leaf spots of *Castanea mollissima* (Jiang et al. 2021). *Pestalotiopsis chamaeropsis* was found as an endophyte from an important medicinal plant here. However, it was reported as a serious phytopathogen in different countries (Moslemi and Taylor 2015, Jiang et al. 2017, Ariyawansa and Hyde 2018, Nozawa et al. 2019, Wang et al. 2019, Chen et al. 2021, Qiu et al. 2022, Santos et al. 2022). *Pestalotiopsis photiniae* was reported as an endophytic fungus on the branches of *Roystonea regia*, and it also has been isolated from the disease leaves of blueberry (Chen et al. 2011, Ding et al. 2012). These seem to indicate that one fungus can be endophytic, pathogenic, or saprophytic in different (or the same) plants or organs. Understanding the external factors that influence the fungal lifestyle, could have major implications for agriculture, industrial development and human health.

It is worth noting that three *Neopestalotiopsis* species, *N. amomumica*, *N. chinensis* and *N. hyperica* introduced in this paper do not match the characteristics of versicolourous median cells described in *Neopestalotiopsis* (Maharachchikumbura et al. 2014). In addition, *N. chinensis* also has obvious conidiophores which do not match the characteristics of indistinct conidiophores described in *Neopestalotiopsis*. This phenomenon was also mentioned by Liu et al. (2017). The reason for this phenomenon probably could be the type of medium or the time of cultivation. However, there is not enough data to explain this phenomenon.

*Neopestalotiopsis* and *Pseudopestalotiopsis* were separated from *Pestalotiopsis* by Maharachchikumbura et al. (2014) based on phylogenetic analyses and conidial color. After that, the number of species in *Neopestalotiopsis* increased from 24 to 70 and the number of species in *Pseudopestalotiopsis* increased from three to 23 (<http://www.indexfungorum.org/>, 28 May 2022). In recent years, there have been more reports on diseases caused by *Neopestalotiopsis*, for example; *N. vitis* caused grapevine leaf spots in China, *N. rosicola* caused stem canker of *Rosa chinensis* in China, *N. clavispora* caused leaf spots and fruit rot of strawberry in India, *N. maddoxii* caused flower diseases of *Macadamia integrifolia* in Australia, *N. eucalyptorum* was associated with disease of *Eucalyptus* plant in Portugal

(Jayawardena et al. 2016, Jiang et al. 2018, Mahapatra et al. 2018, Diogo et al. 2021, Prasannath et al. 2021). However, only six *Pseudopestalotiopsis* species were reported as plant pathogens (Maharachchikumbura et al. 2016a, Nozawa et al. 2017, Nozawa et al. 2018, Norphanphoun et al. 2019, Gualberto et al. 2021). In this study, there are nine *Neopestalotiopsis* species and only one *Pseudopestalotiopsis* species was encountered. This seems to indicate that *Neopestalotiopsis* has richer species diversity, and the *Neopestalotiopsis* species are more likely to infect the plant and cause disease than the *Pseudopestalotiopsis*. By comparing the differences between the two genera through whole-genome sequence analysis, and finding related disease-causing genes probably explain this phenomenon.

The interspecific morphological differences of pestalotioid species have been unclear. In their attempt to find a reliable criterion for interspecific differences, taxonomists have complicated the description of pestalotioid species (Nag Raj 1993, Wei and Xu 2004, Maharachchikumbura et al. 2014, Huanaluek et al. 2021). In the past, the conidia were divided into apical cells, basal cells and the three median cells, describing them. The three median cells were divided into the second cells from the base, the third cells, and the fourth cells. And every cell is measured. Obviously, the length of the three median cells is the sum length of the second, the third, and the fourth cells. Therefore, the descriptions are repeated. In addition, the characteristics of each cell were not treated as a criterion for interspecies differences (Nag Raj 1993, Maharachchikumbura et al. 2012, Jayawardena et al. 2016, Liu et al. 2017). Therefore, we suggest removing the measurement description for each cell.



**Figure 22** – Distribution of pestalotioid species in different medicinal plants

**Table 4** Checklist of pestalotioid species associated with medicinal plants.

Species	Life mode	Disease (if any)	Host	Location	Reference
<i>Neopestalotiopsis acrostichi</i>	P	Leaf spot	<i>Acrostichum aureum</i> (F)	Thailand	Norphanphoun et al. (2019)
<i>Neopestalotiopsis alpapicalis</i>	E		<i>Rhizophora mucronate</i> (D)	Thailand	Kumar et al. (2019)
<i>Neopestalotiopsis amomumica</i>	P	Leaf spot	<i>Amomum villosum</i> (M)	China	This study
<i>Neopestalotiopsis brachiata</i>	P	Leaf spot	<i>Rhizophora apiculata</i> (D)	Thailand	Norphanphoun et al. (2019)
<i>Neopestalotiopsis chinensis</i>	P	Leaf spot	<i>Smilax china</i> , <i>Cyrtomium fortune</i> (M/F)	China	This study Cao et al. (2022), Li et al. (2022)
<i>Neopestalotiopsis clavispora</i>	P	Leaf spot, branch blight	<i>Dendrobium officinale</i> , <i>Taxus×media</i> (M/G)	China	(2022)
<i>Neopestalotiopsis cubana</i>	P	Leaf blight	<i>Ixora chinensis</i> (D)	Malaysia	Khoo et al. (2022)
<i>Neopestalotiopsis dendrobii</i>	E		<i>Dendrobium cariniferum</i> (M)	Thailand	Ma et al. (2019) Maharachchikumbura et al. (2014)
<i>Neopestalotiopsis ellipsospor</i>	Unknown		<i>Ardisia crenata</i> (D)	Hong Kong, China	(2014) Maharachchikumbura et al. (2014)
<i>Neopestalotiopsis eucalypticola</i>	Unknown		<i>Eucalyptus globulus</i> (D)	Unknown	(2014)
<i>Neopestalotiopsis eucalyptorum</i>	P	Leaf necrosis, stem basal cankers	<i>Eucalyptus globulus</i> (D)	Fundão/Guarda/Portugal	Diogo et al. (2021)
<i>Neopestalotiopsis haikouensis</i>	P	Leaf spot	<i>Ilex chinensis</i> (D)	China	Zhang et al. (2022)
<i>Neopestalotiopsis hispanica</i>	P	Leaves and stem necrosis	<i>Eucalyptus globulus</i> (D)	Fundão/Guarda/Spain	Diogo et al. (2021)
<i>Neopestalotiopsis hydeana</i>	P	Leaf spot	<i>Alpinia malaccensis</i> (M)	Thailand	Huanaluek et al. (2021)
<i>Neopestalotiopsis hyperica</i>	P	Leaf spot	<i>Hypericum monogynum</i> (D)	China	This study
<i>Neopestalotiopsis iberica</i>	P	Leaves and stem necrosis	<i>Eucalyptus globulus</i> (D) <i>Eucalyptus globulus</i> /	Pegões/Portugal/Spain	Diogo et al. (2021)
<i>Neopestalotiopsis longiappendiculata</i>	P	Leaves and stem necrosis	<i>E. nitens</i> (D)	Furadouro/Portugal	Diogo et al. (2021)



<i>Neopestalotiopsis lusitanica</i>	P	Leaves and stem necrosis	<i>Eucalyptus globulus</i> (D)	Pegões/Portugal	Diogo et al. (2022)
<i>Neopestalotiopsis pernambucana</i>	Unknown		<i>Vismia guianensis</i> (D)	Brazil	Silvério et al. (2016)
<i>Neopestalotiopsis petila</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	Norphanphoun et al. (2019)
<i>Neopestalotiopsis photiniana</i>	P	Leaf spot	<i>Photinia serrulate</i> (D)	China	This study
<i>Neopestalotiopsis rhapsidis</i>	P	Leaf spot	<i>Podocarpus macrophyllus</i> (G)	China	This study
<i>Neopestalotiopsis rhizophorae</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	Norphanphoun et al. (2019)
<i>Neopestalotiopsis rhododendri</i>	P	Leaf spot	<i>Dracaena fragrans</i> (M)	Thailand	This study Maharachchikumbura et al.
<i>Neopestalotiopsis rosae</i>	Unknown		<i>Paeonia suffruticosa</i> (D)	U.S.A	(2014)
<i>Neopestalotiopsis rosicola</i>	P*	Stem canker	<i>Rosa chinensis</i> (D)	China	Jiang et al. (2018) Maharachchikumbura et al.
<i>Neopestalotiopsis saprophytica</i>	S		<i>Litsea rotundifolia</i> (D)	Hong Kong, China	(2014)
<i>Neopestalotiopsis subtropicalis</i>	E, S		<i>Ceiba pentandra</i> , <i>Pinellia ternate</i> (D/M)	China	This study
<i>Neopestalotiopsis surinamensis</i>	E		<i>Scurrula atropurpurea</i> (D)	Indonesia	Elfita et al. (2020 a,b)
<i>Neopestalotiopsis thailandica</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D) <i>Clerodendrum canescens/Sinopodophyllum</i>	Thailand	Norphanphoun et al. (2019) Xu et al. (2016), Xiao et al.
<i>Pestalotiopsis adusta</i>	E, P*	Leaf spot	<i>hexandrum/Rubus idaeus</i> (D)	China	(2018), Yan et al. (2019)
<i>Pestalotiopsis affinis</i>	P	Leaf spot	<i>Taxus chinensis</i> (G)	China	Chen et al. (2002)
<i>Pestalotiopsis alpiniae</i>	P	Leaf spot	<i>Alpinia galanga</i> (M)	China	Chen et al. (2002)
<i>Pestalotiopsis antenniformis</i>	P	Stem canker	<i>Rubus cissoides</i> , <i>Rubus australis</i> (D)	New Zealand	Index fungorum (2022)
<i>Pestalotiopsis antiaris</i>	P	Leaf spot	<i>Antiaris toxicaria</i> (M)	China	Chen et al. (2002)
<i>Pestalotiopsis apiculata</i>	Unknown		<i>Cunninghamia lanceolata</i> (G)	China	Index fungorum (2022)
<i>Pestalotiopsis arborei</i>	Unknown		<i>Rhododendron arboretum</i> (D)	India	Index fungorum (2022)
<i>Pestalotiopsis bicilia</i>	S		<i>Viburnum opulus</i> (D)	Canada	Index fungorum (2022)

<i>Pestalotiopsis biciliata</i>	P*	Stem canker	<i>Pistacia lentiscus</i> (D)	Tunisia	Hlaiem et al. (2022)
<i>Pestalotiopsis bicolor</i>	S		<i>Smilax</i> sp. (M)	U.S.A	Index fungorum (2022)
<i>Pestalotiopsis brideliae</i>	Unknown		<i>Bridelia monoica</i> (D)	China	Index fungorum (2022)
<i>Pestalotiopsis bruguierae</i>	Unknown		<i>Bruguiera gymnorhiza</i> (D)	India	Index fungorum (2022)
			<i>Bulbophyllum</i>		
<i>Pestalotiopsis bulbophylli</i>	S		<i>thouars</i> (M)	China	Wang et al. (2017)
<i>Pestalotiopsis canarii</i>	Unknown		<i>Canarium album</i> (D)	China	Index fungorum (2022)
<i>Pestalotiopsis caroliniana</i>	Unknown		<i>Euonymus japonicus</i> (D)	U.S.A	Index fungorum (2022)
<i>Pestalotiopsis chamaeropsis</i>	E, P*	Leaf spot	<i>Eurya nitida</i> , <i>Peristrophe japonica</i> (D)	China	Qiu et al. (2022), this study
<i>Pestalotiopsis Chiangmaiensis</i>	P	Leaf strip	Bamboo (M)	Thailand	This study
<i>Pestalotiopsis cruenta</i>	Unknown		<i>Polygonum lasianthum</i> (D)	Japan	Index fungorum (2022)
<i>Pestalotiopsis digitalis</i>	p	Leaf spot	<i>Digitalis purpurea</i> (D)	New Zealand	Liu et al. (2015)
<i>Pestalotiopsis dilleniae</i>	p	Leaf spot	<i>Dillenia turbinata</i> (M)	China	Chen et al. (2002)
					Maharachchikumbura et al. (2014)
<i>Pestalotiopsis diploclisiae</i>	Unknown		<i>Diploclisia glaucescens</i> (D)	Hong Kong, China	(2014)
<i>Pestalotiopsis dracaenae</i>	S		<i>Dracaena fragrans</i> (M)	China	Ariyawansa et al. (2015)
<i>Pestalotiopsis ellipsozpora</i>	p*	Stem canker	<i>Acanthopanax divaricatus</i> (D)	Korea	Yun et al. (2015)
<i>Pestalotiopsis gibbosa</i>	S		<i>Gaultheria shallon</i> (D)	U.S.A	Watanabe et al. (2018)
<i>Pestalotiopsis heucherae</i>			<i>Heuchera parviflora</i> (D)	U.S.A	Index fungorum (2022)
<i>Pestalotiopsis hughesii</i>	Unknown		<i>Cyperus articulatus</i> (M)	Ghana	Index fungorum (2022)
<i>Pestalotiopsis japonica</i>	Unknown		<i>Cedrela sinensis</i> (D)	Japan	Index fungorum (2022)
<i>Pestalotiopsis jinchanghensis</i>	E		<i>Vaccinium dunalianum</i> (D)	China	Fan et al. (2020)
<i>Pestalotiopsis kenya</i>	P*	Leaf spot	<i>Zanthoxylum schinifolium</i> (D)	China	Liu et al. (2021)
<i>Pestalotiopsis kunmingensis</i>	E		<i>Podocarpus macrophyllus</i> (G)	China	Wei and Xu (2004)
<i>Pestalotiopsis kwangsiensis</i>	P	Leaf spot	<i>Sinopimelodendron kwangsiensis</i> (D)	China	Chen et al. (2002)
<i>Pestalotiopsis lawsoniae</i>	Unknown		<i>Lawsonia alba</i> (D)	India	Index fungorum (2022)

<i>Pestalotiopsis lespedezae</i>	Unknown		<i>Lepedeza bicolor</i> (D)	Japan	Index fungorum (2022) Maharachchikumbura et al. (2012)
<i>Pestalotiopsis linearis</i>	E		<i>Trachelospermum</i> sp. (D)	China	(2012)
<i>Pestalotiopsis lushanensis</i>	P*	Brown leaf spot, leaf blight	<i>Sarcandra glabra</i> , <i>Podocarpus macrophyllus</i> (G)	China	Zhang et al. (2021)
<i>Pestalotiopsis microspora</i>	S		<i>Hedera helix</i> (D)	Argentina	Index fungorum (2022)
<i>Pestalotiopsis moluccensis</i>	Unknown		<i>Xylocarpus moluccensis</i> (D)	India	Index fungorum (2022)
<i>Pestalotiopsis neolitsea</i>	P*	Leaf spot	<i>Neolitsea villosa</i> (D)	Taiwan, China	Ariyawansa and Hyde (2018)
<i>Pestalotiopsis oenotherae</i>	Unknown		<i>Oenothera laciniata</i> (D)	U.S.A	Venkatasubbaiah et al. (1991)
<i>Pestalotiopsis pandani</i>	Unknown		<i>Pandanus odoratissimus</i> (M)	Taiwan, China	Index fungorum (2022)
<i>Pestalotiopsis paraguariensis</i>	Unknown		<i>Ilex paraguariensis</i> (D)	Brazil	Index fungorum (2022)
<i>Pestalotiopsis peristropheidis</i>	E		<i>Peristrophe japonica</i> (D)	China	This study
<i>Pestalotiopsis pestalozzioides</i>	Unknown		<i>Clematis ligusticifolia</i> (D)	New Mexico	Index fungorum (2022)
<i>Pestalotiopsis pipericola</i>	Unknown		<i>Piper nigrum</i> (D)	India	Index fungorum (2022)
<i>Pestalotiopsis quadriciliata</i>	Unknown		<i>Vitis vulpina</i> (D)	Canada	Index fungorum (2022)
<i>Pestalotiopsis rhodomyrtus</i>	Unknown		<i>Rhodomyrtus tomentosa</i> (D)	China	Song et al. (2013)
<i>Pestalotiopsis smilaxe</i>	P	Leaf spot	<i>Smilax china</i> , <i>Dioscorea</i> sp. (M)	Thailand	This study
<i>Pestalotiopsis sinensis</i>	Unknown		<i>Ginkgo biloba</i> (G)	China	Index fungorum (2022)
<i>Pestalotiopsis tecomicola</i>	Unknown		<i>Tecoma radicans</i> (D)	U.S.A	Index fungorum (2022)
<i>Pestalotiopsis thailandica</i>	P	Leaf spot	<i>Rhizophora apiculata</i> (D)	Thailand	Norphanphoun et al. (2019)
<i>Pseudopestalotiopsis ampullace</i>	E		<i>Magnolia candolli</i> (D)	China	de Silva et al. (2021)
<i>Pseudopestalotiopsis curvatispora</i>	P	Leaf spot	<i>Rhizophora mucronata</i> (D)	Thailand	Norphanphoun et al. (2019)
<i>Pseudopestalotiopsis gilvanii</i>	P*	Leaf spot	<i>Paullinia cupana</i> (D)	Brazil	Gualberto et al. (2021) Maharachchikumbura et al. (2014)
<i>Pseudopestalotiopsis indica</i>	Unknown		<i>Hibiscus rosa-sinensis</i> (D)	India	(2014) Song et al. (2014), de Silva et al. (2021)
<i>Pseudopestalotiopsis simitheae</i>	S, E		<i>Pandanus odoratissimus</i> / <i>Magnolia candolli</i> (M/D)	Thailand/China	al. (2021)

<i>Pseudopestalotiopsis thailandica</i>	P	Leaf spot	<i>Rhizophora mucronate</i> (D)	Thailand	Norphanphoun et al. (2019)
<i>Pseudoestalotiopsis theae</i>	S		<i>Ceriops tagal</i> (D)	Thailand	This study

<sup>a</sup>The checklist includes species names, life modes, disease names (if any), hosts, locations and references. The current name is used according to Index Fungorum (2022).

<sup>b</sup>The mode of life is given as (E) endophyte, (P) pathogen and (S) saprobe. For the species, those with confirmed pathogenicity data are marked with an asterisk (\*).

<sup>c</sup>The taxonomic status of the host is given as (D) Dicotyledons, (F) Ferns, (G) Gymnosperms and (M) Monocotyledons.

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Dear Editor,

We appreciate you and the reviewers for your precious time in reviewing our paper and providing valuable comments. We have carefully considered the comments and tried our best to address every one of them. We hope the manuscript after careful revisions meet your high standards.

Here is a point-by-point response to the reviewers' comments and concerns. All modifications in the "Marked-Up Manuscript" file have been highlighted.

### Reviewer 1

1. “these 27 isolates represent 17 species distributed in three genera; including six new *Neopestalotiopsis* species, four new *Pestalotiopsis* species, and six new records.”  $6+4+6=16$ , but it mentioned that there are 17 species, what about the other one

R: Thank you so much. We corrected this sentence to “these 26 isolates represent 17 species distributed in three genera: including seven new specie and eight new records.” By looking up google, we found that "including" means containing as part of the whole being considered. So contents after “including” are not all species.

2. “Genealogical Concordance Phylogenetic Species Recognition (GCPSR)”. Should be “Genealogical concordance phylogenetic species recognition (GCPSR)”

R: Thank you so much. We corrected these.

3. “occurring commonly as important plant pathogens, endophytes, and saprophytes.” delete "important"

R: Thank you so much. We deleted this word on page 1, line 22.

4. “(Guba 1961, Barr 1975, Nag Raj 1993, Maharachchikumbura et al. 2014, Hyde et al. 2014, Jayawardena et al. 2019, 2021, Norphanphoun et al. 2019, Ul Haq et al. 2021, Yang et al. 2021).” cite the major references is enough

R: Thanks for your suggestion. I deleted some literature.

5. “analyses” should be “analysis”

R: Thank you so much. We corrected it on page 2, line 13.

6. “*viz. Neopestalotiopsis, Pestalotiopsis, and Pseudopestalotiopsis*” i would suggest the authors provide more information for these three genera.

R: Thank you so much. We added some information as follows: The use of molecular data in resolving pestalotioid species was revisited by Maharachchikumbura et al. (2014) and they separated this group into three genera, *viz. Neopestalotiopsis, Pestalotiopsis, and Pseudopestalotiopsis*. Phylogenetically, these three genera formed distinct clades. Morphologically, *Neopestalotiopsis* differs from *Pseudopestalotiopsis* and *Pestalotiopsis* by its versicolourous (two upper median cells darker than the lowest median cell) median cells and indistinct conidiophores, while *Pseudopestalotiopsis* can be easily distinguished from *Pestalotiopsis* by darker colored concolourous (for those possessing equally pigmented median cells) median cells (Maharachchikumbura et al. 2014). Also, quantitatively, *Neopestalotiopsis, Pestalotiopsis* seem to infect plants more readily than *Pseudopestalotiopsis*.

7. “(Huang et al. 2008, Jia et al. 2016, Xu et al. 2010, 2014, Reddy et al. 2016, Ma et



al. 2019).” i guess the references are list year by year, if so, "Jia et al. 2016" should list after Xu et al. 2010, 2014. check all of MS carefully please.

R: We are sorry for our carelessness. We agreed with you and corrected it on page 3, line 3.

8. “The fresh samples of different medicinal plants were collected in southwest China and Thailand from 2019 to 2022.” leaves? twigs? or any other parts of the plant, please provide detailed information

R: Thank you so much. We provided more information as follows: The fresh healthy leaves, diseased leaves and twigs of different medicinal plants were collected from terrestrial habitats in southwest China and Thailand from 2019 to 2022.

9. “followed by soaking in 4% NaOCl for 1 min” 1 min is enough? normally it should be 3 mins.

R: Thank you so much. We isolated endophytes from young fresh leaves. At first, we soaked the leaves in 4% NaOCl for 3 minutes, but no mycelium grew, and later we shortened the time to 2 minutes, which also failed. Then we adjusted the time to 1 minute, there were mycelia growth, and there was no contamination in the control experiment. So, we think, for fresh young leaves of plants, one minute is enough.

10. “the Culture Collection of the Department of Plant Pathology, Agriculture College, Guizhou University (GUCC)” GZCC may not a international even national culture collection, please deposit the pure cultures to an national culture collection. for your cultures which collected from China, deposit them to CGMCC.

R: Thank you so much. We deposited our cultures to CGMCC and KUNCC and updated the KUNCC codes in the MS. But we haven't got the CGMCC codes due to COVID-19.

11. “the herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP), Guiyang, China” same as above, store the specimens to HKAS, otherwise the new taxa may invalid

R: Thank you so much. We already deposited our specimens into HKAS and updated the specimen codes in the MS.

12. “The maximum likelihood (ML) analyses were carried out using IQ-TREE” “analyses were” should be “analysis was”

R: Thank you so much. But in this study, we carried out the ML and BYPP tree for three genera and we prefer using “analyses were”.

13. do not forget to provide the accession number

R: Thank you so much. We provided the accession numbers in the MS now.

14. this is Herbarium number, not strain number, is it mean that you directly extract DNA from fruiting body of the fungi? if so, please update the method parts. if not, please provide strain number for all of newly obtained.

R: Thanks for your suggestions. Yes, we directly extract DNA from the fruiting body of some fungi, and we updated the method on page 4, line 10. For others, we provided strain numbers in the MS.

15. “SAUCC212272” add space, check all

R: Thank you so much. We checked the original literature (doi: 10.3897/mycokeys.88.82229), no spaces needed.

16. "GUCC 21504" i guess 21-504??

R: Thank you so much. We checked the original literature (doi: 10.3897/BDJ.9.e70446) and determined that it was "GUCC 21504".

17. "HKAS1124560" add space, check all

R: Thank you so much. We checked my MS carefully and corrected them.

18. "IFXX;" "update all of IF and FOF numbers please"

R: Thank you so much. We registered the names of the new species at Fungorum names and also applied for the FOF numbers. We provided codes in the MS now.

19. "Conidia 18.5–30.0 × 4.5–7.5µm, x± SD = 25 ± 2.5 × 6.0 ± 0.68 µm (n = 40)," "Conidia" should italic. in case of we use the µm as unit, so please use integer for the measurement value: at least the nearest half, need to do decimals to round up and round down numbers

R: Thank you for pointing this out. We carefully checked the manuscript and corrected all measurement values, and words that needed to be italicized.

20. "Qiannan Buyei and Miao Autonomous Prefecture" "Buyei" should be "Bouyei"

R: Thank you for pointing this out. We corrected these names throughout the MS.

21. "ex-type-living" delete "living"

R: Thank you so much. We deleted them following your suggestion.

22. "GUCC 21001" 21-001?

R: Thank you so much. We checked the original literature (doi: 10.3897/BDJ.10.e90709) and determined that it was "GUCC 21001".

23. "dark brown to dark" ?? i guess "black"

R: Thank you so much. We agree with you and correct it to black.

24. "ex-type culture" make all of this in same format, please check it is singular or plural carefully for the whole paper as well.

R: Thank you so much. We carefully checked the manuscript and made them in the same format.

25. "Part 2 Pestalotiopsis" For all of new Pestalotiopsis species introduced in this paper, the authors need to check the newly paper on Frontiers in Microbiology: Gu R, Bao D-F, Shen H-W, Su X-J, Li Y-X and Luo Z-L (2022) Endophytic Pestalotiopsis species associated with Rhododendron in Cangshan Mountain, Yunnan Province, China. Front. Microbiol. 13:1016782.

In case of this paper have been accepted by the Journal and it is process to paper proof state now, so please make sure the species in your paper are not repeat with the published species.

R: Thanks for your suggestion. We added these species to our phylogenetic tree and we confirmed that our species were not conspecific with these published species.

26. "Index fungorum (2022)" the authors need to cite the original references, not the IF dataset, it is incorrect check and update all, please.

R: Thanks for your suggestion. We agreed with you and updated all missing references in the MS.

## Reviewer 2

1. Authors did not follow the recent taxonomic conflicts on *Neopestalotiopsis* and introduced several new species, I would like the get a clear answer why some

studies prefer to introduce new species as *Neopestalotiopsis* sp. while you choose to introduce them as new species.

R: Thanks for your comments. As far as we know, since the establishment of *Neopestalotiopsis*, a total of 49 new species have been introduced by mycologists based on morphological and phylogenetic analysis (Maharachchikumbura et al. 2014, Jayawardena et al. 2016, Jiang et al. 2018, Freitas et al. 2019, Kumar et al. 2019, Norphanphoun et al. 2019, Diogo et al. 2021, Prasannath et al. 2021, Fiorenza et al. 2022). Although some scholars believe that these two methods are not enough to introduce new species, we can see from their analysis that the taxonomic status of their strains is not stable enough or lacks sister branches (Belisário et al. 2020, Gerardo-Lugo et al. 2020, Jiang et al. 2021). Therefore, we suggest that new species can be introduced if the following conditions are met: a) there are obvious differences in morphological characteristics, b) there are obvious branch lengths in the phylogenetic analysis, c) strains can form stable clades with known species. In our study, there are 15 isolates representing 9 different clades, and only 4 new species are introduced. For the strains that do not meet the above conditions, we still keep *Neopestalotiopsis* sp.

2. As I can see in the material and methods, the authors have not chosen 2022 pulsations to construct a phylogenetic tree, thus I am not sure still your species are new. Therefore, both trees required an update following the 2022 papers.

R: Thanks for your reminder. We added sequences from the most recently published species and reanalyzed the phylogenetic tree. The updated tree in the MS.

3. Add PHI analysis for all new species, if do not have species in the same clade, you can choose species from nearby clades.

R: Thanks for your suggestion. We updated PHI analysis for every new species in the MS.

4. Did you isolate pathogens? Or do you only observe fruiting bodies from leaf spots? If isolated, please mention it clearly in the methods.

R: Thank you so much. We only isolated fungi from fruiting bodies on leaf spots.

5. In descriptions, I recommend rounding off small values, because 0.5  $\mu\text{m}$  is beyond our eye capacity yet your software for measuring is automated. Also, descriptions are consists of different type font types while considering the avg symbol.

R: Thanks for your suggestion. We carefully checked the manuscript and corrected all measurement values and font types.

6. Since there are a lot of new species, I do not think we required descriptions of new hosts, you can only include material examined and notes.

R: Thanks for your suggestion. We agreed with you and deleted descriptions and photo-plates for new records.

7. I have several problems related to PHI analysis; in this case, I would prefer to see the alignment. However, the final alignment of the paper is not available for revision.

R: Thank you so much. We provided the final alignment in supplementary file1, namely, *N*-Alignment and *P*-Alignment. However, since the journal only accepts Excel and PDF formats as supplementary materials, we cannot provide FASTA

format.

8. are you sure PHI analysis is a GCPSR? PHI is an independent approach to understand the recombination, yet the GCPSR has its own meaning

R: Thanks for your reminder. PHI analysis is not a GCPSR. GCPSR used to identify independent evolutionary lineages. PHI is to check for recombination. We corrected it.

9. i do not agree with the morphology is unreliable. authors need to read and choose the facts carefully. this happened because everyone is totally relying on phylogenetic species

R: Thank you so much. We agree with you. We are not saying that morphology is unreliable, but that there are doubts about only relying on the host and conidial color to identify species. we have changed “With the development of DNA-based phylogenetic analyses, the traditional classification system has been proved unreliable.” into “With the development of DNA-based phylogenetic analysis, the traditional classification system based on host and conidial color is controversial.”

10. “For pathogens and saprophytes, single spore isolations were used to obtain pure cultures, following the methods described by Senanayake et al. (2020).” this sentence need to re write, 1st pathogens will be isolated and then do single spore to get pure cultures? did all pathogens isolates sporulated? to do the single spore isolation as saprobe samples

R: Thank you so much. Usually, the diseased specimens we collect have fruiting bodies. We isolated these fungi using single spore isolation rather than tissue isolation. Some of them sporulated.

11. “TEF” this should have 1 alpha. “TUB” it would be nice to abbreviate in to lower case italic

R: Thank you very much. We changed every “TEF” to “*tef1- $\alpha$* ” and “TUB” to “*tub2*” in the MS.

12. “(Maharachchikumbura et al. 2016b, Liu et al. 2017, Nozawa et al. 2017, Norphanphoun et al. 2019, Prasannath et al. 2021)” seems you are missing the most recently published papers. De Silva et al 2021; Yang et al 2021 please follow the other most recently published papers

R: Thank you so much. We added recently published papers on page 6, line 8 as follows: (De Silva et al 2021, Yang et al 2021, Chaiwan et al. 2022, Crous et al. 2022, Fiorenza et al.2022, Gu et al. 2022).

13. “ $\bar{x}$ ” check these throughout the paper and correct accordingly, do not use as a picture

R: Thank you so much. We checked these throughout the MS and corrected them.

14. “there are 4 base pairs differences between GUCC 814 and GUCC 21001 in the ITS gene and 10 base pairs differences in the TEF gene.” what about tub?

R: Thank you so much. There are two base pairs differences in the *tub2* gene.

15. “Due to the lack of enough closely related species, the GCSPR was not used to evaluate its placement” this can not be accepted, you can use several different taxa which are not in the same clade yet nearby to make sure. if they are different, indeed will give a different values

R: Thanks for your suggestion. We redid this analysis and put the final result in the MS (Fig 2).

10. “However, they did not introduce it as a new species due to the lack of neighboring species to compare the morphology.” are you sure this is the reason?

follow this paper to understand what is exactly going on with this genus

Gerardo-Lugo, S.S.; Tovar-Pedraza, J.M.; Maharachchikumbura, S.S.N.; Apodaca-Sánchez, M.A.; Correia, K.C.; Saucedo-Acosta, C.P.; Camacho-Tapia, M.; Hyde, K.D.; Marraiki, N.; Elgorban, A.M.; et al. Characterization of *Neopestalotiopsis* species associated with mango grey leaf spot disease in Sinaloa, Mexico. *Pathogens* 2020

R: Thank you so much. We checked Jiang et al. (2021) and found that they introduced their taxa as *Neopestalotiopsis* sp.1. rather than new species due to a lack of distinguished characters from close clades. We read Gerardo-Lugo et al. (2020) following your suggestion and agreed with you and keep these taxa as *Neopestalotiopsis* sp.1.

16. “*Neopestalotiopsis chinensis* appears to be a common phytopathogen as it has been found in leaf spots on different plants.” can you confirm or you suggest it could be

R: Thank you so much. We corrected this sentence to “we speculate *Neopestalotiopsis* sp.1 could be a common phytopathogen as it has been found in leaf spots on different plants.”

17. “*Conidia* 17.0–22.0(–24.2) × 5.0–8.2” throughout the MS, round off your values.

R: Thank you so much. We checked throughout the paper and corrected these values.

18. “Part 2: *Pestalotiopsis*” add genetic authority

R: Thank you so much. We added genetic authority for these three genera on page 12, line 4, page 26, line 28 and page 36, line 13 following your suggestions.

Thank you so much!

Best wishes,

Ya-Ru Sun

Ruvishika S. Jayawardena

Jing-E Sun

Yong Wang



November 23, 2022

Prof. Yong Wang  
Guizhou University  
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China

Re: Spectrum03987-22R1 (Pestalotioid species associated with medicinal plants in southwest China and Thailand)

Dear Prof. Yong Wang:

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Supplemental Material: Accept