

Phyllosticta taxa from northern Thailand and southern European Russia: a novel species and four new host records

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Abstract

Phyllosticta is a cosmopolitan group of fungi found on various host plants, occurring as pathogens, endophytes and saprobes. Diseases caused by *Phyllosticta* commonly include leaf and fruit spots that affect economically important plants. The genus is characterized mainly by aseptate and hyaline conidia and ascospores. However, its conidia are surrounded by a mucilaginous sheath, with a single mucoid apical appendage while ascospores exhibit a mucoid cap at both ends. Given that many *Phyllosticta* taxa are cryptic and share similar morphological features, it is arduous to depict taxonomically relevant characters solely on the basis of morphological and ecological features. Coupled with morphological description, multi-locus phylogenetic analyses of species comprising complexes are used to broadly describe this genus and understand species boundaries. Despite several published taxonomic revisions and enumerations of *Phyllosticta* species, there is still considerable confusion when identifying these taxa. Herein, we introduce a new species (*P. chiangmaiensis*) and three new host records (*P. capitalensis*) in Thailand, and one new host and country record (*P. citribrasiliensis*) in Russia. We provide an updated phylogenetic tree, including all *Phyllosticta* species with sequence data.

Introduction

Phyllosticta taxa manifest primarily as phytopathogens (Wikee et al. 2013a; Hyde et al. 2014; Jayawardena et al. 2019). They cause diseases such as leaf spots, leaf blights and blotch, tan and black spots of fruits, fruit lesions and freckle disease in numerous plants (Glienke-Blanco et al. 2002; Aa and Vanev 2002; Wulandari et al. 2009; Wong et al. 2012; Wikee et al. 2013a; Zhou et al. 2015; Jayawardena et al. 2019; Anderson et al. 2021). These phytopathogens infect economically important crops and ornamentals globally. For example, *P. vaccinii* causes early rot of cranberries in Wisconsin, United States (McManus 1998). *Phyllosticta citriasiana* is a destructive pathogen that causes necrotic spots of *Citrus maxima* in Asia (Wulandari et al. 2009). *Phyllosticta ophiopogonis* causes leaf spots of *Ophiopogon japonicus*, an important ornamental plant in Thailand (Wikee et al. 2012). *Phyllosticta ampelicida* causes black rot disease of *Vitis vinifera* in North America (Kuo and Hoch 2018). Certain pathogens are considered to cause emerging diseases, impacting largely on the ecosystem and economy (Gomdola et al. 2022). Such an example is *P. citricarpa*, which causes citrus black spots (Kotzé 1981; Baldassari et al. 2008). *Phyllosticta citricarpa* is considered as a quarantine pest in Europe and the USA, thereby jeopardizing international trade (Baayen et al. 2002; Dewdney et al. 2011; Glienke et al. 2011; Gabriela et al. 2014; EPPO 2023). Pathogenic *Phyllosticta* taxa impair host plants by reducing their photosynthetic potential and increasing leaf or fruit fall (Glienke-Blanco et al. 2002; Baldassari et al. 2008).

Phyllosticta species also exist as endophytes (Baayen et al. 2002; Okane et al. 2003; Wulandari et al. 2010; Wikee et al. 2013b; Asiandu et al. 2021), as well as saprobes (van der Aa and Vanev 2002; Glienke et al. 2011). One of the most common endophytes, *P. capitalensis*, has a ubiquitous distribution on a myriad of hosts (Wikee et al. 2013b). *Phyllosticta capitalensis* is also a weak phytopathogen causing leaf spots (Wikee et al. 2013b), suggesting that some species can switch lifestyles depending on the environment and hosts that they colonize. Endophytic *P. capitalensis* also possesses potential antagonistic effects against pathogenic *P. citricarpa* on citrus (Tran et al. 2019). Some *Phyllosticta* species generate appressoria prior to entering their hosts, for example *P. maculata* (Wong et al. 2013). *Phyllosticta* species mainly produce melanized appressoria (Sutton 1980; Chethana et al. 2021a, b).

Phyllosticta (*Phyllostictaceae*, *Botryosphaerales*, *Dothideomycetes*) was established by Persoon (Persoon 1818; Wijayawardene et al. 2022a). The genus has been accommodated in different families, listed chronologically in Table 1. *Phyllosticta convallariae* was designated as the type species (Donk 1968), which was later synonymized to *P. cruenta* (van der Aa 1973; Wikee et al. 2013a). *Phyllosticta* species occur both in the sexual and asexual morphs. *Guignardia*, the sexual morph of *Phyllosticta*, was introduced by Viala and Ravaz (1892). According to current fungal nomenclature rules that employ one name for one fungus (Hawksworth et al. 2011; Wingfield et al. 2011), the use of *Phyllosticta* is recommended, given that it is the earlier and a more commonly used name than *Guignardia* (Glienke et al. 2011; Wikee et al. 2013a; Wijayawardene et al. 2022a).

The sexual morph of *Phyllosticta* species are characterized by erumpent, uniloculate, globose to subglobose ascumata with a central ostiole, displaying pseudoparaphyses at maturity. Asci are usually clavate to broadly ellipsoidal or narrowly ovoid, pedicellate, with an ocular chamber. Ascospores are aseptate, hyaline, ellipsoidal to limoniform, guttulate, and smooth-walled, with a mucoid cap at both ends (van der Aa 1973; Wong et al. 2012; Wikee et al. 2013a). Conidia of *Phyllosticta* are generally aseptate, hyaline, ovoid to ellipsoidal, globose to sub-globose, surrounded by a mucilaginous sheath, bearing an apical appendage (van der Aa 1973; Wikee et al. 2011). However, the sheath and appendages are not present in all species e.g., *P. minima* and *P. sphaerospoidea* lack a sheath and appendage (Wikee et al. 2013a). Conidial appendages that form on agar cultures may be lost upon maturation, or fluctuate in shape and size when grown in different media (Wikee et al. 2013a). Furthermore, spermatia produced in culture are hyaline, aseptate, cylindrical to dumbbell-shaped with guttules at each end (van der Aa 1973). The overlapping morphological features of *Phyllosticta* make it difficult to delineate between species but multi-locus phylogenetic analyses can facilitate species delimitation (Norphanphoun et al. 2020).

Because fungi are integral components of biodiversity, it is important to report novel and existing species from different hosts. Herein, based on the combination of morphological description and multigene phylogenetic analyses, we establish a novel taxon (*P. chiangmaiensis*) from *Musa* sp. and provide three new host records (*P. capitalensis*) from *Phyllanthus emblica*, *Morus alba*, and *Ficus auriculata* in Thailand, and one new host and country record (*P. citribrasiliensis*) from *Laburnum anagyroides* in Russia. We provide an updated phylogenetic tree, including all *Phyllosticta* species with sequence data.

Table 1
Family history of *Phyllosticta*

Events	References
<i>Phyllostictaceae</i> (as <i>Phyllostictaceae</i>) was initially proposed by Fries and accepted by Hawksworth & David.	Fries (1849); Hawksworth (1989)
<i>Phyllosticta</i> was accommodated in <i>Phyllostictaceae</i> (<i>Phyllostictales</i>).	Seaver (1922)
Placement of <i>Phyllosticta</i> in <i>Botryosphaeriaceae</i> Theiss. & Syd. (<i>Botryosphaeriales</i>)	Crous et al. (2006); Schoch et al. (2006); Liu et al. (2012)
<i>Phyllosticta</i> was re-accommodated in <i>Phyllostictaceae</i> (<i>Botryosphaeriales</i>).	Wikee et al. (2013a)

Materials And Methods

Sample collection, isolation and morphology

Dead leaf and fruit specimens, some having leaf and fruit spots, were collected from northern Thailand and brought to the laboratory in paper bags. Other living leaf specimens with leaf spots were collected from southern European Russia and dried in paper bags. Following surface sterilization with 70% ethanol to avoid secondary contaminants, the specimens were incubated in moist plastic boxes at room temperature for 3 days. Following methods outlined by Senanayake et al. (2020), single spore isolation was performed for MFLU22-0175 and MFLU22-0176, but the latter did not germinate despite the use of different media and different incubation temperatures. Tissue isolation was performed for MFLU22-0177, MFLU22-0178 and MFLU22-0179. For tissue isolation, sections including part of the leaf spots and healthy tissue were cut and surface sterilized in 70% ethanol for one minute, followed with 5% sodium hypochlorite for one minute. These leaf sections were rinsed thrice with sterilized water, dried on sterilized tissue paper, and placed on potato dextrose agar (PDA) plates, incubated at 25°C for one to two days. Growing hyphal tips were transferred aseptically to fresh PDA plates containing antibiotics (Amoxicillin, MacroPhar). Resulting pure isolates were incubated for two to three weeks at 25°C.

For MFLU22-0175 and MFLU22-0176, morphological characters were observed from the leaf specimens while living cultures were observed for MFLU22-0177, MFLU22-0178 and MFLU22-0179. Structures that were examined include conidiomata, pycnidial wall and conidia, as well as their attachment. These observations were made using a Motic SMZ 168 Series stereo-microscope. Digital images of micro morphological features were captured with a Cannon 750D camera (Canon, Tokyo, Japan) attached to a Nikon ECLIPSE E600 compound microscope (Nikon, Tokyo, Japan). The photo-plate was prepared using Adobe Photoshop CS6 version (Adobe Systems, USA). The micro morphological features were measured using Tarosoft® Image Frame Work software (version 0.97) by using different calibration settings under different magnification.

Material deposition and reference numbers

Living cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC). Dry leaf specimens and dry cultures, including a holotype of the newly described taxon, were deposited in Mae Fah Luang University herbarium (MFLU). FacesofFungi (<https://www.facesoffungi.org/>), and Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>) numbers are provided (Index Fungorum 2023; Jayasiri et al. 2015). Species descriptions have been updated in the GMS microfungi database (<https://gmsmicrofungi.org/>) (Chaiwan et al. 2021).

DNA extraction, PCR amplification, and sequencing

For MFLU22-0175 and MFLU22-0176, DNA was extracted directly from conidiomata while fresh mycelium scraped from the margin of colonies on PDA were used for DNA extraction of MFLU22-0177, MFLU22-0178 and MFLU22-0179. For direct DNA extraction, approximately 30 conidiomata were picked from the sterilized substrates by using a sterile needle. This was carried out using a Motic SMZ 168 Series stereo-microscope. The conidiomata were collected in a 1.5 ml micro-centrifuge tube. Genomic DNA was extracted using the Forensic DNA Kit (D3396-01, OMEGA bio-tek), following the manufacturer's protocol. The loci ITS, LSU, *ACT*, *TEF-1α*, *GAPDH*, and *RPB2* were amplified using primers listed (Table 2). PCR conditions for each gene region are provided (Fig. 1). The polymerase chain reaction (PCR) mixture (25 µL) comprised 12.5 µL of master mix (PROMEGA GoTaq®, Green), 1.5 µL of genomic DNA, 1 µL of forward and reverse primer each, and 9 µL of double-distilled H₂O. The amplification procedure was performed in an Applied Biosystems C1000 Touch™ Thermal Cycler. The PCR products were verified DNA fluorescent loading dye (FluoroDye, SMOBIO) on 1.7% agarose electrophoresis gels. The latter were purified following the company protocols. DNA was sequenced at Biogenomed Co. Ltd. (Bangkok, Thailand). Forward and reverse DNA sequence data were obtained from the sequencing company and consensus sequences were produced using SeqMan (DNAStar, Inc., Madison, WI, USA).

Newly generated sequences were deposited in NCBI GenBank database (<https://submit.ncbi.nlm.nih.gov/>) and accession numbers for each isolate are provided (Table 3).

Table 2
Primers used

Gene region	Primer pairs	References
Internal transcribed spacer (ITS)	ITS1/ ITS4	White et al. (1990)
Large subunit (LSU)	LR0R/ LR5	White et al. (1990)
Actin (<i>ACT</i>)	Act512F/ Act738R	Carbone and Kohn (1999)
Translation elongation factor 1 α (<i>TEF-1α</i>)	EF1-728F/EF2	O'Donnell et al. (1998); Carbone and Kohn (1999)
Glyceraldehyde-3-phosphate dehydrogenase (<i>GAPDH</i>)	Gpd1-LM/Gpd2-LM	Myllys et al. (2002)
RNA polymerase 2 (<i>RPB2</i>)	RPB2-5F2/RPB2-7CR	Liu et al. (1999); Sung et al. (2007)

Phylogenetic analyses

Newly generated sequences were subjected to BLAST search in NCBI (<https://blast.ncbi.nlm.nih.gov/>) (Nilsson et al. 2014; Dissanayake et al. 2020). Based on latest data, ITS, LSU, *ACT*, *TEF-1 α* , *GAPDH*, and *RPB2* sequences from type and non-type strains were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/>) (Table 3). Sequences of individual gene regions were aligned using the online platform, MAFFT v.7 (<https://mafft.cbrc.jp/alignment/server/>) (Katoh et al. 2019), and trimmed using trimAl to remove uneven ends (Capella-Gutiérrez et al. 2009). Gaps were treated as missing data. Single genes were concatenated using BioEdit v. 7.0.5.2 (Hall 1999). Both single and multi-locus datasets were analyzed. Phylogenetic trees were constructed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) method.

Maximum likelihood analysis was performed in the CIPRES Science Gateway v.3.3 (Miller et al. 2010). RAxML-HPC v.8 on XSEDE with 1,000 bootstrap iterations were performed (Stamatakis 2014). Maximum parsimony analysis was performed by using Phylogenetic Analysis Using Parsimony (PAUP) v.4.0b10 (Swofford 2002) to obtain the most parsimonious tree. Trees were inferred using the heuristic search option with 1,000 random sequence additions. Maxtrees were setup to 5000 with 1000 bootstrap replicates. Descriptive tree statistics for parsimony (tree length [TL], consistency index [CI], retention index [RI], relative consistency index [RC] and homoplasy index [HI]) were calculated for trees generated under different optimality criteria.

Prior to BI analysis, the model of evolution was estimated by using MrModeltest 2.2 under the Akaike information criterion (AIC) implemented in PAUP v.4.0b10. Partitioning of data was carried out for individual gene regions (Table 4) (Nylander 2004). Bayesian inference analysis was executed in the CIPRES Science Gateway v.3.3 by running MrBayes on XSEDE v.3.2.7a (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). Posterior probabilities (PP) were obtained through Markov chain Monte Carlo (MCMC) sampling. Four Markov chains were run simultaneously for 30,000,000 generations, with trees sampled every 100th generation, so that the average standard deviation of split frequencies at the end of the total MCMC generations converged to 0.01 or less. The first 20% of the sampled trees were discarded as 'burn in' and the remaining 80% was used to calculate PP of the majority rule consensus tree.

Phylograms were visualized using FigTree v.1.4.4 (Rambaut and Drummond 2014). Microsoft PowerPoint (2016) was used to edit the resulting phylogenetic trees. Bootstrap support values equal or greater than 70% are given for ML and MP. Posterior probability (PP) values equal or greater than 0.80 are given for BI. In this study, we consider bootstrap values equal or greater than 80% and PP values equal or greater than 0.95 as strong support.

Genealogical Concordance Phylogenetic Species Recognition Analysis (GCPSR)

Genealogical concordance phylogenetic species recognition (GCPSR) model as described by Taylor et al. (2000) was applied to scrutinize any significant recombination event that occurred between phylogenetically related species. The recombination extent of the newly described taxon, *P. chiangmaiensis* (MFLU22-0176) was compared with the type strain of the phylogenetically closely related taxa, *P. maculata* (CPC 18347), *P. musaechinensis* (GZAAS 6.1247) and *P. musarum* (BRIP 55434). This was determined by a pairwise homoplasy index (Φ_w) test (PHI), performed in SplitsTree4 (www.splitstree.org) (Bruen et al. 2006; Huson et al. 2014). The resulting splits graphs were constructed using both LogDet transformation and splits decomposition options (Fig. 2).

Table 3

Isolates and sequences used in this study. The newly generated sequences are indicated in red. Type and ex-type strains are in bold

Species	Strain no.	GenBank accession numbers						Hosts	Country
		ITS	LSU	<i>TEF-1a</i>	<i>ACT</i>	<i>GAPDH</i>	<i>RPB2</i>		
<i>Phyllosticta abieticola</i>	CBS 112067	KF170306	EU754193	-	KF289238	-	-	<i>Abies concolor</i>	Canada
<i>P. acaciigena</i>	CPC 28295	KY173433	KY173523	-	KY173570	-	-	<i>Acacia suaveolens</i>	Australia
<i>P. aloecicola</i>	CPC 21020	KF154280	KF206214	KF289193	KF289311	KF289124	KY855816	<i>Aloe ferox</i>	South Africa
<i>P. aloecicola</i>	CPC 21021	KF154281	KF206213	KF289194	KF289312	KF289125	KY855817	<i>Aloe ferox</i>	South Africa
<i>P. alpina</i>	GZCC 6.1702	MH380033	-	MH380029	MH380027	MH380031	-	<i>Cephalotaxus fortunei</i>	China
<i>P. alpina</i>	GZCC 6.1703	MH380034	-	MH380030	MH380028	MH380032	-	<i>Cephalotaxus fortunei</i>	China
<i>P. ampelicida</i>	ATCC 200578	KC193586	-	-	KC193581	KC193584	-	<i>Vitis riparia</i>	USA
<i>P. ardisiae</i>	MUCC0045	AB454283	-	-	-	-	-	<i>Ardisia japonica</i>	Japan
<i>P. ardisiicola</i>	NBRC 102261	AB454274	-	-	AB704216	-	-	<i>Ardisia crenata</i>	Japan
<i>P. aristolochiicola</i>	BRIP 53316	JX486129	-	-	-	-	-	<i>Aristolochia acuminata</i>	Australia
<i>P. aspidiicola</i>	NBRC 102244	AB454314	-	-	AB704204	-	-	<i>Aspidistra elatior</i>	Japan
<i>P. aucubae-japonicae</i>	MAFF 236703	KR233300	-	KR233310	KR233305	-	-	<i>Aucuba japonica</i>	Japan
<i>P. austroafricana</i>	CBS 144593	MK442613	MK442549	MK442704	MK442640	-	-	Unknown tree	South Africa
<i>P. azevinhi</i>	MUCC0088	AB454302	-	-	AB704226	-	-	<i>Ilex pedunculosa</i>	Japan
<i>P. beaumarisii</i>	CBS 535.87	NR_145235	NG_058040	KF766429	KF306232	KF289074	-	<i>Muehlenbeckia adpressa</i>	Australia
<i>P. bifrenariae</i>	CBS 128855	JF343565	KF206209	JF343586	JF343649	JF343744	KY855818	<i>Bifrenaria harrisoniae</i>	Brazil
<i>P. bifrenariae</i>	CPC 17467	KF170299	KF206260	KF289207	KF289283	KF289138	KY855819	<i>Bifrenaria harrisoniae</i>	Brazil
<i>P. brazilianae</i>	LGMF 330	JF343572	KF206217	JF343593	JF343656	JF343758	-	<i>Mangifera indica</i>	Brazil
<i>P. brazilianae</i>	LGMF 334	JF343566	KF206215	JF343587	JF343650	JF343752	-	<i>Mangifera indica</i>	Brazil
<i>P. capitalensis</i>	CBS 128856	JF261465	KF206304	JF261507	JF343647	JF343776	KY855826	<i>Stanhopea</i> sp.	Brazil
<i>P. capitalensis</i>	MFLU22-0177	OP686473	OP686475	OQ189916	OQ189919	-	OQ189923	<i>Phyllanthus emblica</i>	Thailand
<i>P. capitalensis</i>	MFLU22-0178	-	-	-	-	-	OQ189924	<i>Morus alba</i>	Thailand
<i>P. capitalensis</i>	MFLU22-0179	OP688119	OP688118	-	OQ189920	-	OQ189925	<i>Ficus auriculata</i>	Thailand
<i>P. capitalensis</i>	CBS 226.77	FJ538336	KF206289	FJ538394	FJ538452	JF343718	KY855820	<i>Paphiopedilum callosum</i>	Germany
<i>P. capitalensis</i>	CBS 356.52	FJ538342	KF206300	FJ538400	FJ538458	KF289087	-	<i>Ilex</i> sp.	-
<i>P. capitalensis</i>	CBS 100175	FJ538320	KF206327	FJ538378	FJ538436	JF343699	KY855821	<i>Citrus</i> sp.	Brazil
<i>P. capitalensis</i>	CBS 101228	FJ538319	KF206325	FJ538377	FJ538435	KF289086	KY855822	<i>Nephelium lappaceum</i>	Hawaii
<i>P. capitalensis</i>	CBS 114751	EU167584	-	FJ538407	FJ538465	KF289088	KY855823	<i>Vaccinium</i> sp.	New Zealand
<i>P. capitalensis</i>	CBS 115047	FJ538323	KF206318	FJ538381	FJ538439	KF289077	-	<i>Aspidosperma polyneuron</i>	Brazil
<i>P. capitalensis</i>	CBS 115049	FJ538324	KF206317	FJ538382	FJ538440	KF289084	-	<i>Bowdichia nitida</i>	Brazil

Species	Strain no.	GenBank accession numbers						Hosts	Country
		ITS	LSU	<i>TEF-1α</i>	<i>ACT</i>	<i>GAPDH</i>	<i>RPB2</i>		
<i>P. capitalensis</i>	CBS 117118	FJ538339	JQ743603	FJ538397	FJ538455	KF289090	-	<i>Musa acuminata</i>	Indonesia
<i>P. capitalensis</i>	CBS 123373	FJ538341	JQ743604	FJ538399	FJ538457	JF343703	KY855824	<i>Musa paradisiaca</i>	Thailand
<i>P. capitalensis</i>	CPC 11867	KF206181	KF206283	KF289184	KF289260	KF289108	-	<i>Acacia crassicarpa</i>	Thailand
<i>P. capitalensis</i>	CPC 13987	KF206183	KF206281	KF289176	KF289263	KF289083	-	<i>Protea repens</i>	Portugal
<i>P. capitalensis</i>	CPC 14609	KF206184	KF206280	KF289175	KF289264	KF289081	KY855827	<i>Zyzygium</i> sp.	Republ Madag
<i>P. capitalensis</i>	CPC 16590	KF206185	KF206272	KF289177	KF289271	KF289091	-	<i>Citrus limon</i>	Argentina
<i>P. capitalensis</i>	CPC 16591	KF206186	KF206271	KF289179	KF289272	KF289093	-	<i>Citrus limon</i>	Argentina
<i>P. capitalensis</i>	CPC 17468	KF206188	KF206259	KF289189	KF289284	KF289120	-	<i>Cymbidium</i> sp.	Brazil
<i>P. capitalensis</i>	CPC 20251	KC291333	KF206252	KC342553	KC342530	KF289101	-	Unknown plant	Thailand
<i>P. capitalensis</i>	CPC 20253	KF206192	KF206250	KF289181	KF289293	KF289102	-	<i>Schefflera venulosa</i>	Thailand
<i>P. capitalensis</i>	CPC 20254	KC291335	KF206249	KC342555	KC342532	KF289103	-	<i>Saccharum officinarum</i>	Thailand
<i>P. capitalensis</i>	CPC 20255	KC291336	KF206248	KC342556	KC342533	KF289115	-	<i>Arecaceae</i>	Thailand
<i>P. capitalensis</i>	CPC 20256	KC291337	KF206247	KC342557	KC342534	KF289089	-	<i>Ophiopogon japonicus</i>	Thailand
<i>P. capitalensis</i>	CPC 20257	KC291338	KF206246	KC342558	KC342535	KF289099	-	<i>Ficus benjamina</i>	Thailand
<i>P. capitalensis</i>	CPC 20258	KC291339	KF206245	KC342559	KC342536	KF289094	-	<i>Ophiopogon japonicus</i>	Thailand
<i>P. capitalensis</i>	CPC 20259	KC291340	KF206244	KC342560	KC342537	KF289104	KY855828	<i>Orchidaceae</i>	Thailand
<i>P. capitalensis</i>	CPC 20263	KC291341	KF206241	KC342561	KC342538	KF289085	KY855829	<i>Magnoliaceae</i>	Thailand
<i>P. capitalensis</i>	CPC 20265	KF206194	KF206239	KF289182	KF289297	KF289105	-	<i>Eupobiaceae</i>	Thailand
<i>P. capitalensis</i>	CPC 20266	KC291342	KF206238	KC342562	KC342539	KF289109	-	<i>Polyscias</i> sp.	Thailand
<i>P. capitalensis</i>	CPC 20267	KF206195	KF206237	KF289173	KF306233	KF289078	-	<i>Baccaurea ramiflora</i>	Thailand
<i>P. capitalensis</i>	CPC 20268	KC291343	KF206236	KC342563	KC342540	KF289117	KY855830	<i>Hibiscus syriacus</i>	Thailand
<i>P. capitalensis</i>	CPC 20270	KC291345	KF206234	KC342565	KC342542	KF289110	-	<i>Tectona grandis</i>	Thailand
<i>P. capitalensis</i>	CPC 20271	KF206196	KF206233	KF289183	KF289298	KF289106	-	<i>Crinum asiaticum</i>	Thailand
<i>P. capitalensis</i>	CPC 20272	KC291346	KF206232	KC342566	KC342543	KF289079	-	<i>Orchidaceae</i>	Thailand
<i>P. capitalensis</i>	CPC 20274	KF206197	KF206231	KF289188	KF289299	KF289119	-	<i>Mangifera indica</i>	Thailand
<i>P. capitalensis</i>	CPC 20275	KC291347	KF206230	KC342567	KC342544	KF289107	KY855831	<i>Polyalthia longifolia</i>	Thailand
<i>P. capitalensis</i>	CPC 20278	KC291348	KF206227	KC342568	KC342545	KF289113	KY855832	<i>Euphorbia milii</i>	Thailand
<i>P. capitalensis</i>	CPC 20423	KC291349	KF206226	KC342569	KC342546	KF289116	-	<i>Philodendron</i> sp.	Thailand
<i>P. capitalensis</i>	CPC 20510	KF206200	KF206223	KF289174	KF289304	KF289080	-	<i>Pyrosia adnascens</i>	Thailand
<i>P. capitalensis</i>	LGMF 219	KF206202	KF206220	JF261490	KF289306	JF343737	-	<i>Citrus sinensis</i>	Brazil
<i>P. capitalensis</i>	LGMF 220	KF206203	KF206219	JF261488	KF289307	JF343735	-	<i>Citrus sinensis</i>	Brazil
<i>P. capitalensis</i>	LGMF 222	KF206204	KF206218	JF261492	KF289308	JF343739	-	<i>Citrus sinensis</i>	Brazil
<i>P. carissicola</i>	CPC 25665	KT950849	KT950863	KT950879	KT950872	KT950876	-	<i>Carissa macrocarpa</i>	South Africa

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		ITS	LSU	<i>TEF-1α</i>	<i>ACT</i>	<i>GAPDH</i>	<i>RPB2</i>		
<i>P. carochlae</i>	CGMCC 3.17317	KJ847422	-	KJ847444	KJ847430	KJ847438	-	<i>Caryota ochlandra</i>	China
<i>P. catimbauensis</i>	URM 7672	MF466160	MF466163	MF466155	MF466157	-	-	<i>Mandevilla catimbauensis</i>	Brazil
<i>P. catimbauensis</i>	URM 7674	MF466161	MF466164	MF466153	MF466158	-	-	<i>Mandevilla catimbauensis</i>	Brazil
<i>P. cavendishii</i>	BRIP 55419	JQ743562	-	-	-	-	-	<i>Musa</i> sp.	Taiwan
<i>P. cavendishii</i>	BRIP 57384	KC117644	KU697330	KF009695	KF014059	KU716085	-	<i>Musa</i> sp.	Australia
<i>P. cavendishii</i>	BRIP 57383	KC117643	KU697329	KF009694	KF014058	KU716084	-	<i>Musa</i> sp.	Australia
<i>P. cavendishii</i>	NTP-Dc 40957	KU708538	KU697328	KU716092	KU697321	KU716083	-	<i>Musa</i> sp.	Australia
<i>P. cavendishii</i>	NTP-Dc 40579	KU708537	KU697327	KU716091	KU697320	KU716082	-	<i>Musa</i> sp.	Australia
<i>P. Chiangmaiensis</i>	MFLU22-0176	OP693476	OP693472	OQ189918	OQ189922	-	-	<i>Musa</i> sp.	Thailand
<i>P. citriasiana</i>	CBS 120486	FJ538360	KF206314	FJ538418	FJ538476	JF343686	KY855858	<i>Citrus maxima</i>	Thailand
<i>P. citriasiana</i>	CBS 120487	FJ538361	KF206313	FJ538419	FJ538477	JF343687	KY855859	<i>Citrus maxima</i>	China
<i>P. citribrasilensis</i>	CBS 100098	FJ538352	KF206221	FJ538410	FJ538468	JF343691	KY855861	<i>Citrus limon</i>	Brazil
<i>P. citribrasilensis</i>	MFLU22-0175	-	OP684319	OQ189917	OQ189921	-	-	<i>Laburnum anagyroides</i>	Russia
<i>P. citribrasilensis</i>	CPC 17466	KF170302	KF206261	KF289226	KF289282	KF289161	-	<i>Citrus</i> sp.	Brazil
<i>P. citribrasilensis</i>	CPC 17465	KF170301	KF206262	KF289225	KF289281	KF289160	KY855863	<i>Citrus</i> sp.	Brazil
<i>P. citribrasilensis</i>	CPC17464	KF170300	KF206263	KF289224	KF289280	KF289159	KY855862	<i>Citrus</i> sp.	Brazil
<i>P. citribrasilensis</i>	LGMF09	JF261436	-	JF261478	JF343618	JF343693	-	<i>Citrus</i> sp.	Brazil
<i>P. citribrasilensis</i>	LGMF08	JF261435	-	JF261477	JF343617	JF343692	-	<i>Citrus</i> sp.	Brazil
<i>P. citricarpa</i>	CBS 127454	JF343583	KF206306	JF343604	JF343667	JF343771	KY855866	<i>Citrus limon</i>	Australia
<i>P. citricarpa</i>	CBS 127455	JF343584	KF206305	JF343605	JF343668	JF343772	-	<i>Citrus sinensis</i>	Australia
<i>P. citrichinaensis</i>	ZJUCC 200956	JN791620	-	JN791459	JN791533	-	-	<i>Citrus reticulata</i>	China
<i>P. citrimaxima</i>	MFLUCC 10-0137	KF170304	KF206229	KF289222	KF289300	KF289157	-	<i>Citrus maxima</i>	Thailand
<i>P. concentrica</i>	CPC 18842	KF170310	KF206256	KF289228	KF289288	KF289163	-	<i>Hedera</i> sp.	Italy
<i>P. concentrica</i>	CBS 937.70	-	KF206291	-	KF289257	-	-	<i>Hedera helix</i>	Italy
<i>P. cordylinophila</i>	MFLUCC 10-0166	KF170287	KF206242	KF289172	KF289295	KF289076	KY855887	<i>Cordyline fruticosa</i>	Thailand
<i>P. cordylinophila</i>	MFLUCC 12-0014	KF170288	KF206228	KF289171	KF289301	KF289075	KY855888	<i>Cordyline fruticosa</i>	Thailand
<i>P. comicola</i>	CBS 111639	KF170307	-	-	KF289234	-	-	<i>Cornus florida</i>	USA
<i>P. cruenta</i>	CBS 858.71	MG934458	-	MG934501	MG934465	MG934474	-	<i>Polygonatum odoratum</i>	Czech Republic
<i>P. cruenta</i>	MUCC0206	AB454331	-	-	AB704237	-	-	<i>Polygonatum odoratum</i> var. <i>pluriflorum</i>	Japan
<i>P. cryptomeriae</i>	KACC 48643	MK396559	-	-	-	-	-	<i>Juniperus chinensis</i> var. <i>sargentii</i>	-
<i>P. cryptomeriae</i>	MUCC0028	AB454271	-	-	AB704213	-	-	<i>Cryptomeria japonica</i>	Japan
<i>P. cussonia</i>	CPC 14873	JF343578	KF206279	JF343599	JF343662	JF343764	KY855889	<i>Cussonia</i> sp.	South Korea
<i>P. cussonia</i>	CPC 14875	JF343579	KF206278	JF343600	JF343663	JF343765	KY855890	<i>Cussonia</i> sp.	South Korea
<i>P. doitungensis</i>	MFLUCC 21-0074	OK661034	OK661034	OL345581	-	-	OL345582	<i>Dasymaschalon obtusipetalum</i>	Thailand

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		ITS	LSU	<i>TEF-1α</i>	<i>ACT</i>	<i>GAPDH</i>	<i>RPB2</i>		
<i>P. domestica</i>	MUCC0425	AB454346	-	-	AB704241	-	-	<i>Nandina domestica</i>	Japan
<i>P. elongata</i>	CBS 126.22	FJ538353	-	FJ538411	FJ538469	KF289164	-	<i>Oxycoccus macrocarpos</i>	USA
<i>P. encephalarticola</i>	CPC 35970	MN562101	MN567609	MN556818	MN556783	-	-	<i>Encephalartos</i> sp.	South Africa
<i>P. ericarum</i>	CPC 19744	KF206170	KF206253	KF289227	KF289291	KF289162	-	<i>Erica gracilis</i>	South Africa
<i>P. ericarum</i>	GZAAS 6.1245	KR025419	-	KR025450	KR025460	KR025434	-	<i>Pittosporum tobira</i>	China
<i>P. eugeniae</i>	CBS 445.82	AY042926	KF206288	KF289208	KF289246	KF289139	KY855891	<i>Eugenia aromatica</i>	Indonesia
<i>P. fallopieae</i>	MUCC0113	AB454307	-	-	AB704228	-	-	<i>Fallopia japonica</i>	Japan
<i>P. foliorum</i>	CBS 447.68	KF170309	KF206287	KF289201	KF289247	KF289132	-	<i>Taxus baccata</i>	Netherlands
<i>P. gardeniicola</i>	MUCC0117	AB454310	-	-	AB704230	-	-	<i>Gardenia jasminoides</i>	Japan
<i>P. gardeniicola</i>	MUCC0089	AB454303	-	-	-	-	-	<i>Gardenia jasminoides</i>	Japan
<i>P. gaultheriae</i>	CBS 447.70	JN692543	KF206298	JN692531	KF289248	JN692508	-	<i>Gaultheria humifusa</i>	USA
<i>P. gwangjuensis</i>	CNUFC NJ1-12	OK285195	-	OM038511	OM001471	-	-	<i>Torreya nucifera</i>	South Korea
<i>P. gwangjuensis</i>	CNUFC NJ1-12-1	OK285196	-	OM038512	OM001472	-	-	<i>Torreya nucifera</i>	South Korea
<i>P. hagahagaensis</i>	CBS 144592	MK442614	MK442550	MK442705	MK442641	MK442657	-	<i>Carissa bispinosa</i>	South Africa
<i>P. hakeicola</i>	CBS 143492	MH107907	MH107953	MH108025	MH107984	MH107999	-	<i>Hakea</i> sp.	Australia
<i>P. hamamelidis</i>	MUCC149	KF170289	-	-	KF289309	-	-	<i>Hamamelis japonica</i>	Japan
<i>P. harai</i>	MUCC0038	AB454277	-	-	AB704218	-	-	<i>Aucuba japonica</i>	Japan
<i>P. hostae</i>	CGMCC 3.14355	JN692535	-	JN692523	JN692511	JN692503	-	<i>Hosta plantaginea</i>	China
<i>P. hubeiensis</i>	CGMCC 3.14986	JX025037	-	JX025042	JX025032	JX025027	-	<i>Viburnum odoratissimum</i>	China
<i>P. hubeiensis</i>	CGMCC 3.14987	JX025038	-	JX025043	JX025033	JX025028	-	<i>Viburnum odoratissimum</i>	China
<i>P. hymenocallidicola</i>	CBS 131309	JQ044423	JQ044443	KF289211	KF289242	KF289142	-	<i>Hymenocallis littoralis</i>	Australia
<i>P. hymenocallidicola</i>	CPC 19331	KF170303	KF206254	KF289212	KF289290	KF289143	-	<i>Hymenocallis littoralis</i>	Australia
<i>P. hypoglossi</i>	CBS 434.92	FJ538367	KF206299	FJ538425	FJ538483	JF343695	KY855892	<i>Ruscus aculeatus</i>	Italy
<i>P. hypoglossi</i>	CBS 101.72	FJ538365	KF206326	FJ538423	FJ538481	JF343694	-	<i>Ruscus aculeatus</i>	Italy
<i>P. ilicis-aquifolii</i>	CGMCC 3.14358	JN692538	-	JN692526	JN692514	-	-	<i>Ilex aquifolium</i>	China
<i>P. ilicis-aquifolii</i>	CGMCC 3.14359	JN692539	-	JN692527	JN692515	-	-	<i>Ilex aquifolium</i>	China
<i>P. illicii</i>	24-1-1	MF198235	MF198240	MF198237	MF198243	-	-	<i>Illicium verum</i>	China
<i>P. illicii</i>	16-16-1	MF198234	MF198239	MF198236	MF198242	-	-	<i>Illicium verum</i>	China
<i>P. iridigena</i>	CBS 143410	MG934459	-	MG934502	MG934466	-	-	<i>Iris</i> sp.	South Africa
<i>P. kerriae</i>	MAFF 240047	AB454266	-	-	-	-	-	<i>Kerria japonica</i>	Japan

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<i>P. kobus</i>	MUCC0049	AB454286	-	-	AB704221	-	-	<i>Magnolia kobus</i>	Japan
<i>P. lauridiae</i>	CBS 145559	MK876404	MK876445	MK876498	MK876460	MK876472	MK876489	<i>Lauridia tetragona</i>	South Korea
<i>P. leucothoicola</i>	MUCC553	AB454370	-	-	KF289310	-	-	<i>Leucothoe catesbaei</i>	Japan
<i>P. ligustricola</i>	MUCC0024	AB454269	-	-	AB704212	-	-	<i>Ligustrum obtusifolium</i>	Japan
<i>P. longicauda</i>	BRIP 66984	MH971220	-	-	-	-	-	<i>Eustrephus latifolius</i>	Australia
<i>P. maculata</i>	CPC 18347	JQ743570	JQ743593	KF009700	KF014016	-	-	<i>Musa</i> sp.	Australia
<i>P. maculata</i>	BRIP 46622	JQ743567	-	KF009692	KF014013	-	-	<i>Musa</i> sp.	Australia
<i>P. maculata</i>	NTP-Dc 40103	KU708539	KU697331	KU716093	KU697322	-	-	<i>Musa</i> sp.	Australia
<i>P. mangiferae</i>	IMI 260576	JF261459	KF206222	JF261501	JF343641	JF343748	-	<i>Mangifera indica</i>	India
<i>P. mangifera-indicae</i>	MFLUCC 10-0029	KF170305	KF206240	KF289190	KF289296	KF289121	-	<i>Mangifera indica</i>	Thailand
<i>P. mate</i>	1636497	KP195189	-	-	-	-	-	<i>Ilex paraguariensis</i>	Argentina
<i>P. mate</i>	1636496	KP195188	-	-	-	-	-	<i>Ilex paraguariensis</i>	Argentina
<i>P. mimusopisicola</i>	CBS 138899	KP004447	MH878626	-	-	-	-	<i>Mimusops zeyheri</i>	South Africa
<i>P. minima</i>	CBS 585.84	KF206176	KF206286	KF289204	KF289249	KF289135	-	<i>Acer rubrum</i>	USA
<i>P. miurae</i>	MUCC0065	AB454291	-	-	AB704224	-	-	<i>Lindera praecox</i>	Japan
<i>P. musaechinensis</i>	GZAAS 6.1247	KF955294	-	KM816639	KM816627	KM816633	-	<i>Musa</i> sp.	China
<i>P. musaechinensis</i>	GZAAS 6.1384	KF955295	-	KM816640	KM816628	KM816634	-	<i>Musa</i> sp.	China
<i>P. musarum</i>	BRIP 55434	JQ743584	-	-	-	-	-	<i>Musa</i> sp.	India
<i>P. musarum</i>	BRIP 55435	JQ743583	-	-	-	-	-	<i>Musa</i> sp.	Thailand
<i>P. musarum</i>	GZAAS 6.1228	KF955293	KF955299	KM816638	KM816626	KM816632	-	<i>Musa acuminata</i>	China
<i>P. musarum</i>	BRIP 57360	JX997136	-	KF009740	-	-	-	-	Thailand
<i>P. neopyrolae</i>	CBS 134750	NR_145201	MH877561	-	AB704233	-	-	<i>Pyrola asarifolia</i>	Japan
<i>P. oblongifolae</i>	SAUCC210052	OM248445	OM232088	OM273893	OM273897	OM273901	-	<i>Garcinia oblongifolia</i>	China
<i>P. oblongifolae</i>	SAUCC210055	OM248442	OM232085	OM273890	OM273894	OM273898	-	<i>Garcinia oblongifolia</i>	China
<i>P. oblongifolae</i>	SAUCC210054	OM248443	OM232086	OM273891	OM273895	OM273899	-	<i>Garcinia oblongifolia</i>	China
<i>P. oblongifolae</i>	SAUCC210053	OM248444	OM232087	OM273892	OM273896	OM273900	-	<i>Garcinia oblongifolia</i>	China
<i>P. ophiopogonis</i>	KACC 47754	KP197057	-	-	-	-	-	<i>Ophiopogon japonicus</i>	South Korea
<i>P. ophiopogonis</i>	LrLF11	MG543713	-	-	-	-	-	<i>Lycoris radiata</i>	China
<i>P. owaniana</i>	CBS 776.97	FJ538368	KF206293	FJ538426	KF289254	JF343767	-	<i>Brabejum stellatifolium</i>	South Korea
<i>P. owaniana</i>	CPC 14901	JF261462	KF206303	JF261504	KF289243	JF343766	-	<i>Brabejum stellatifolium</i>	South Korea
<i>P. pachysandricola</i>	MUCC124	AB454317	-	-	AB704232	-	-	<i>Pachysandra terminalis</i>	Japan

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<i>P. paracapitalensis</i>	CPC 26517	KY855622	KY855796	KY855951	KY855677	KY855735	KY855894	<i>Citrus floridana</i>	Italy
<i>P. paracapitalensis</i>	CPC 26518	KY855623	KY855797	KY855952	KY855678	KY855736	KY855895	<i>Citrus floridana</i>	Italy
<i>P. paracitricarpa</i>	CPC 27169	KY855635	KY855809	KY855964	KY855690	KY855748	KY855907	<i>Citrus limon</i>	Greece
<i>P. paracitricarpa</i>	ZJUCC 200933	JN791626	KY855813	JN791468	JN791544	KY855752	KY855911	<i>Citrus sinensis</i>	China
<i>P. parthenocissi</i>	CBS 111645	EU683672	-	JN692530	JN692518	-	-	<i>Parthenocissus quinquefolia</i>	USA
<i>P. partricuspidatae</i>	NBRC 9466	KJ847424	-	KJ847446	KJ847432	KJ847440	-	<i>Parthenocissus tricuspidata</i>	Japan
<i>P. partricuspidatae</i>	NBRC 9757	KJ847425	-	KJ847447	KJ847433	KJ847441	-	<i>Parthenocissus tricuspidata</i>	Japan
<i>P. paxistimae</i>	CBS 112527	KF206172	KF206320	KF289209	KF289239	KF289140	-	<i>Paxistima mysinites</i>	USA
<i>P. persooniae</i>	CBS 143409	MG934460	-	MG934503	MG934467	MG934475	-	-	Austral
<i>P. philoprina</i>	CBS 587.69	KF154278	KF206297	KF289206	KF289250	KF289137	-	<i>Ilex aquifolium</i>	Spain
<i>P. pilospora</i>	MUCC 2915	LC542597	LC543423	LC543445	LC543465	-	-	<i>Chamaecyparis pisifera</i>	Japan
<i>P. pilospora</i>	MUCC 2922	LC542598	LC543424	LC543446	LC543466	-	-	<i>Juniperus chinensis</i>	Japan
<i>P. podocarpus</i>	CBS 111646	AF312013	KF206323	KC357671	KC357670	KF289169	-	<i>Podocarpus falcatus</i>	South
<i>P. podocarpus</i>	CBS 111647	KF154276	KF206322	KF289232	KF289235	KF289168	-	<i>Podocarpus lanceolata</i>	South
<i>P. podocarpicola</i>	CBS 728.79	KF206173	KF206295	KF289203	KF289252	KF289134	-	<i>Podocarpus maki</i>	USA
<i>P. pseudotsugae</i>	CBS 111649	KF154277	KF206321	KF289231	KF289236	KF289167	-	<i>Pseudotsuga menziesii</i>	USA
<i>P. psidii</i>	CBS 100250	FJ538351	-	FJ538409	FJ538467	-	-	<i>Psidium guajava</i>	Brazil
<i>P. pterospermi</i>	SAUCC210104	OM249954	OM249956	OM273902	OM273904	OM273906	-	<i>Pterospermum heterophyllum</i>	China
<i>P. pterospermi</i>	SAUCC210406	OM249955	OM249957	OM273903	OM273905	OM273907	-	<i>Pterospermum heterophyllum</i>	China
<i>P. pyrolae</i>	IFO 32652	AB041242	-	-	-	-	-	<i>Erica carnea</i>	-
<i>P. religiosa</i>	1592	LN865107	-	-	-	-	-	<i>Ficus benjamina</i>	Philipp
<i>P. raphiolepidis</i>	MUCC0432	AB454349	-	-	AB704242	-	-	<i>Raphiolepis indica</i>	Japan
<i>P. rhizophorae</i>	NCYUCC 19-0352	MT360030	MT360039	-	MT363248	MT363250	-	<i>Rhizophora stylosa</i>	Taiwar
<i>P. rhizophorae</i>	NCYUCC 19-0358	MT360031	MT360040	-	MT363249	-	-	<i>Rhizophora stylosa</i>	Taiwar
<i>P. rhodora</i>	CBS 901.69	KF206174	KF206292	KF289230	KF289256	KF289166	-	<i>Rhododendron</i> sp.	Netherl
<i>P. rizhaoensis</i>	CFCC 57579	OP537081	OP542427	OP554271	OP554274	-	-	<i>Ophiopogon japonicus</i>	China
<i>P. rizhaoensis</i>	CFCC 57580	OP537082	OP542428	OP554272	OP554275	-	-	<i>Ophiopogon japonicus</i>	China
<i>P. rizhaoensis</i>	CX2	OP537083	OP542429	OP554273	OP554276	-	-	<i>Ophiopogon japonicus</i>	China
<i>P. rubella</i>	CBS 111635	KF206171	EU754194	KF289198	KF289233	KF289129	-	<i>Acer rubrum</i>	USA
<i>P. schimae</i>	CGMCC 3.14354	JN692534	-	JN692522	JN692510	JN692506	-	<i>Schima superba</i>	China

Species	Strain no.	GenBank accession numbers						Hosts	Country
		ITS	LSU	<i>TEF-1α</i>	<i>ACT</i>	<i>GAPDH</i>	<i>RPB2</i>		
<i>P. schimicola</i>	CGMCC 3.17319	KJ847426	-	KJ847448	KJ847434	KJ854895	-	<i>Schima superba</i>	China
<i>P. schimicola</i>	CGMCC 3.17320	KJ847427	-	KJ847449	KJ847435	KJ854896	-	<i>Schima superba</i>	China
<i>P. speewahensis</i>	BRIP 58044	KF017269	-	KF017268	-	-	-	Orchids	Australia
<i>P. sphaeropsoides</i>	MUCC0112	AB454306	-	-	AB704227	-	-	<i>Aesculus carnea</i>	Japan
<i>P. spinarum</i>	CBS 292.90	JF343585	KF206301	JF343606	JF343669	JF343773	KY855913	<i>Chamaecyparis pisifera</i>	France
<i>P. styracicola</i>	CGMCC3.14985	JX025040	-	JX025045	JX025035	JX025030	-	<i>Styrax grandiflorus</i>	China
<i>P. styracicola</i>	CGMCC3.14989	JX025041	-	JX025046	JX025036	JX025031	-	<i>Styrax grandiflorus</i>	China
<i>P. telopeae</i>	CBS 777.97	KF206205	KF206285	KF289210	KF289255	KF289141	-	<i>Telopea speciosissima</i>	Tasmania
<i>P. vaccinii</i>	ATCC 46255	KC193585	-	KC193582	KC193580	KC193583	-	<i>Vaccinium macrocarpon</i>	-
<i>P. vaccinii</i>	LC 2795	KR233323	-	-	-	-	-	<i>Vitis macrocarpon</i>	USA
<i>P. vacciniicola</i>	CPC 18590	KF170312	KF206257	KF289229	KF289287	KF289165	-	<i>Vaccinium macrocarpum</i>	USA
<i>P. vitis-rotundifoliae</i>	CGMCC 3.17321	KJ847429	-	KJ847451	KJ847437	KJ847443	-	<i>Vitis rotundifolia</i>	USA
<i>P. vitis-rotundifoliae</i>	CGMCC 3.17322	KJ847428	-	KJ847450	KJ847436	KJ847442	-	<i>Vitis rotundifolia</i>	USA
<i>P. westeae</i>	BRIP 7239c	OP599631	-	OP627090	-	-	-	<i>Clerodendrum inerme</i> (= <i>Volkateria inermis</i>)	Australia
<i>P. yuccae</i>	CBS 117136	JN692541	KF766385	JN692529	JN692517	JN692507	-	<i>Yucca elephantipes</i>	New Zealand
<i>P. yuccae</i>	CBS 112065	KF206175	-	-	KF289237	-	-	<i>Yucca elephantipes</i>	USA
<i>Botryosphaeria obtusa</i>	CMW 8232	AY972105	-	DQ280419	AY972111	-	-	Conifers	South Africa
<i>B. obtusa</i>	CMW7775	AY236954	-	AY236903	-	-	-	<i>Ribes</i> sp.	USA
<i>B. stevensii</i>	CBS 112553	AY259093	AY928049	AY573219	-	-	-	<i>Vitis vinifera</i>	Portugal
<i>B. stevensii</i>	CMW7060	AY236955	-	AY236904	AY972112	-	-	<i>Fraxinus excelsior</i>	Netherlands

Table 4
Partition model selected for each locus

Gene region	Model selected under Akaike Information Criterion (AIC)
ITS, LSU, <i>GAPDH</i> , <i>RPB2</i>	GTR + I + G
<i>ACT</i>	GTR + G
<i>TEF-1α</i>	HKY + I + G

Results

Sequence alignment and phylogenetic analyses

The concatenated ITS, LSU, *ACT*, *TEF-1 α* , *GAPDH* and *RPB2* sequence matrix comprised 208 strains that belong to 106 species of *Phyllosticta*, including four outgroups; *Botryosphaeria obtusa* (CMW 8232 and CMW 7775) and *B. stevensii* (CBS 112553 and CMW 7060) (*Botryosphaeriaceae*). The combined alignment contained 3,107 characters (ITS: 1–505, LSU: 506–1,267, *TEF-1 α* : 1,268–1,520, *ACT*:1,521–1,747, *GAPDH*: 1,748–2,366, *RPB2*: 2,367–3,107).

The RAxML analysis yielded the best scoring tree, which was used as the backbone tree (Fig. 3). The results of the ML and MP parameters are provided (Table 5). Single gene trees were also constructed to confirm phylogenetic placement of our isolates. Coupled with combined gene trees, single gene trees support the establishment of our novel taxon, *P. chiangmaiensis* (not illustrated).

Table 5
RAxML and MP analysis parameters

RAxML analysis parameters		
ML optimization likelihood value		-26813.077229
ML Tree length		3.525370
Estimated base frequencies	A	0.213296
	C	0.287794
	G	0.280374
	T	0.218535
Substitution rates	AC	1.080680
	AG	3.097136
	AT	1.233293
	CG	1.131194
	CT	7.420060
	GT	1.000000
Gamma distribution shape parameter α		0.293938
Distinct alignment patterns		1286
Undetermined characters or gaps (%)		40.73
Maximum parsimonious analysis parameters		
MP length: Tree #1		4374
Total number of characters		3107
Constant		1934
Parsimony-informative		974
Parsimony-uninformative		199
Tree #1	CI	0.416
	RI	0.856
	RC	0.356
	HI	0.584

Genealogical Concordance Phylogenetic Species Recognition Analysis (GCPSR)

Using both the LogDet transformation and splits decomposition options, the PHI test resulted in a threshold exceeding 0.05 ($\Phi_w = 0.9207$) for our newly described taxon, *P. chiangmaiensis* (MFLU22-0176), indicating no significant recombination in the dataset (Fig. 2).

Figure 3 Phylogram generated from maximum likelihood analysis (RAxML) based on the combined ITS, LSU, *TEF-1 α* , *ACT*, *GAPDH* and *RPB2* matrices of *Phyllosticta*. Maximum likelihood (ML) and maximum parsimony (MP) with bootstrap support $\geq 70\%$, and the posterior probability (PP) values (≥ 0.8) of Bayesian inference (BI) analyses are given at respective nodes as ML/MP/PP. Hyphen (-) represents support values below 70% (ML and MP) and below 0.80 (PP). The tree is rooted with *Botryosphaeria obtusa* (CMW 8232 and CMW 7775) and *B. stevensii* (CBS 112553 and CMW 7060). Type strains are indicated in bold and our isolates are in red. Different background colours indicate the six *Phyllosticta* species complexes

Taxonomy

Phyllosticta chiangmaiensis D. Gomdola & K.D. Hyde, sp. nov. Figure 4

Index Fungorum number: IF 557882, Facesoffungi number: FoF 12965

Etymology – The specific epithet refers to Chiang Mai province, where the specimen was collected.

Holotype – MFLU22-0176

Saprobic on fallen leaves of *Musa* sp. (*Musaceae*). **Sexual morph:** Not observed. **Asexual morph:** Coelomycetous. *Conidiomata* 50–160 × 50–160 µm (\bar{x} =90 × 95 µm, n = 20), solitary, uniloculate, globose to sub-globose, scattered or gregarious, semi-immersed, conspicuous on host surface, black. *Pycnidial wall* 2.75–14.5 µm wide (\bar{x} =7.7 µm, n = 30), comprising 1–2 layers of thick-walled *textura angularis* cells, outer layer dark brown to black, inner layer pale brown. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 6–14 × 4.5–9 µm (\bar{x} =9.8 × 6.4 µm, n = 25), sub-globose or ellipsoidal or ovoid, guttulate, aseptate, hyaline, smooth. *Conidia* 13–18 × 8.5–12.5 µm (\bar{x} =15.5 × 10.0 µm, n = 60), solitary, ellipsoidal to obovoid, guttulate, verruculose or with a single large central guttule, aseptate, hyaline, smooth-walled, tapering towards a narrowly truncate base and broader apex, surrounded by a mucilaginous *sheath*, thicker on both sides, 1.2–4.7 µm thick (\bar{x} =2.6 µm, n = 60), thinner at the apex and base, 0.2–2.0 µm thick (\bar{x} =1.0 µm, n = 25), with a hyaline, apical mucoid appendage. *Appendages* 4.7–11 × 0.8–1.2 µm (\bar{x} =7.4 × 1.0 µm, n = 10), flexuous, unbranched, straight to curved, tapering towards an acutely rounded tip.

Material examined – Thailand, Chiang Mai Province, forests around the Mushroom Research Center, on fallen dead leaves of *Musa* sp. (*Musaceae*), 1 April 2021, D. Gomdola, (MFLU22-0176, holotype).

Distribution – Thailand.

GenBank accession numbers: ITS = OP693476, LSU = OP693472, *TEF-1α* = OQ189918, *ACT* = OQ189922.

Notes – *Phyllosticta chiangmaiensis* is sister to *P. musaechinensis* (GZAAS 6.1247 and GZAAS 6.1384) with strong support (100%ML, 100%MP, 1.00PP) (Fig. 3). Characters of the conidia match the species concept of *Phyllosticta*. Conidial length of *P. chiangmaiensis* ranges from 13–18 µm and that of *P. musaechinensis* (GZAAS 6.1247) is similar, ranging from 14–18 µm. However, conidiomata diameter and sheath thickness of *P. chiangmaiensis* are larger than that of the phylogenetically closely related taxa (Table 6). Other differences and similarities between *P. chiangmaiensis* and sister taxa are given (Table 6). Even though *P. chiangmaiensis* grouped with other species (*P. musaechinensis*, *P. musarum*, *P. maculata*, *P. cavendishii*) that were also isolated from *Musa* sp., it formed distinct lineages. Excluding gaps, in pairwise nucleotide comparisons of the type species of *P. musaechinensis* and *P. chiangmaiensis* (MFLU22-0176), there are 10 nucleotide base pair (bp) differences across ITS (567 nucleotides), 1 bp difference across *ACT* (195 nucleotides), and 1 bp difference in *TEF-1α* (316 nucleotides). In the ITS region of the type species of *P. musarum*, 14 nucleotide bp differences were observed across 433 nucleotides, excluding gaps. Hence, based on the recommendations provided by Chethana et al. (2021c), Jayawardena et al. (2021), Manawasinghe et al. (2021), Pem et al. (2021), we introduce *P. chiangmaiensis* as a new species.

Table 6
Morphological comparison of *P. chiangmaiensis* and sister taxa

		Species				
Species characters		<i>P. chiangmaiensis</i> MFLU22-0176	<i>P. musaechinensis</i> GZAAS 6.1247	<i>P. musarum</i> BRIP 55434	<i>P. maculata</i> CPC 18347	
Morphological features	Conidiomata	Size (µm)	50–160 diam., 50–160 high	45–145 diam.	69–118 diam., 52–80 high	84–137 diam., 68–132 high
		Shape and colour	Globose to sub-globose, black. Ostiole not observed	Globose or subglobose, black, shiny, with a rounded central ostiole	Pycnidial, ostiolate	Pycnidial, ostiolate
	Conidia	Size (µm)	13–18 × 8.5–12.5	14–18 × 8–12	(12)13–16(20) × (7)9–10(11)	(15)16–19(21) × (9)10–12(13)
		Shape	Ellipsoidal to obovoid, coarsely guttulate, smooth-walled, broad apex and narrowly truncate base	Ellipsoidal or clavate, coarsely guttulate, thin- and smooth-walled	Oblong, obovoid or ellipsoidal, coarsely guttulate, thin- and smooth-walled, broad rounded or obtuse apex, truncate base becoming obtuse with age	Oblong or obovoid to subclavate, coarsely guttulate, smooth-walled, broad rounded or obtuse apex, truncate base becoming obtuse with age
	Mucilaginous sheath	Thickness (µm)	1.2–4.7	0.5–3.5	1–3	2–4(6)
	Appendage	Length (µm)	4.7–11	4–18.5	(12)14–18(20)	(12)15–26(37)
Shape		Straight to curved, tapering towards acutely rounded apex	Straight to curved	Straight to curved, tapering towards acute apex	Straight to curved, tapering towards acute apex	
Reported morph		Asexual	Asexual	Asexual and sexual	Asexual and sexual	
Symptoms		Saprobic	Weak pathogen, causing symptom similar to freckle disease	Leaf spots	Leaf spots	
Hosts		<i>Musa</i> sp.	<i>Musa</i> sp.	<i>Musa paradisiaca</i>	<i>Musa</i> sp.	
Gene region(s)		ITS, LSU, <i>ACT</i> , <i>TEF-1a</i>	ITS, <i>ACT</i> , <i>TEF-1a</i> , <i>GAPDH</i>	ITS	ITS, LSU, <i>ACT</i> , <i>TEF-1a</i>	
References		This study	Wu et al. (2014)	Wong et al. (2012)	Wong et al. (2012)	

Phyllosticta citribraasilensis O.L. Pereira, Glienke & Crous (2011) Fig. 5

Index Fungorum number: IF831482, Facesoffungi number: FoF 12964

Associated with leaf spots of *Laburnum anagyroides* Medik. (*Fabaceae*). **Sexual morph:** Not observed. **Asexual morph:** Coelomycetous. *Conidiomata* 100–160 × 80–110 µm (\bar{x} =111 × 97.5 µm, n = 15), solitary, uniloculate, globose to sub-globose, scattered, semi-immersed, conspicuous on host surface, black. *Pycnidial walls* 13.7–27 µm wide (\bar{x} =18.6 µm, n = 15), comprising several layers of *textura angularis* cells, outer layers dark brown to black, inner layers pale brown to hyaline. *Ostiole* single, central, 12.5–17.5 µm wide (\bar{x} =14.8 µm, n = 5). *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5.6–15 × 1.3–2.6 µm (\bar{x} =10.1 × 2.3 µm, n = 15), enteroblastic, phialidic, integrated, truncate to cylindrical to ampulliform, hyaline, formed from the inner layer of pycnidial wall. *Conidia* 7–9.8 × 4.5–6.6 µm (\bar{x} =8.5 × 5.7 µm, n = 30), solitary, ellipsoidal to obovoid, coarsely guttulate, aseptate, hyaline, smooth-walled, with narrowly truncate base, surrounded by a mucilaginous *sheath*, thicker on both sides, 1.3–2 µm thick (\bar{x} =1.6 µm, n = 20), thinner at apex and base, 0.4–0.9 µm thick (\bar{x} =0.6 µm, n = 20), with a hyaline, apical mucoid appendage. *Appendages* 2.9–27 × 0.7–1.8 µm (\bar{x} =8.3 × 1.1 µm, n = 20), flexuous, unbranched, tapering towards acutely rounded tip.

Material examined – Russia, Krasnodar Region, Sochi, Khostinsky City District, M.V. Frunze Health Care Resort, park, on senescing leaves of *Laburnum anagyroides* (*Fabaceae*), 15 October 2018, Timur S. Bulgakov, T-7583 (MFLU22-0175).

Distribution – Brazil, Russia (Farr and Rossman 2023).

GenBank accession numbers: LSU = OP684319, *TEF-1a* = OQ189917, *ACT* = OQ189921.

Notes – Our strain clusters with the type strain (CBS 100098) as well as other strains of *P. citribraasilensis* (LGMF09, LGMF08, CPC 17466, CPC 17465, CPC 17464) with strong support (88%ML, 83%MP, 1.00PP) (Fig. 3). Excluding gaps, in pairwise nucleotide comparisons of the type strain of *P. citribraasilensis* and our isolate (MFLU22-0175), there is no nucleotide base pair (bp) difference across LSU (761 nucleotides) and *ACT* (181 nucleotides), and 1 bp difference across *TEF-1a* (226 nucleotides). Morphological differences and similarities are also given (Table 7). Based on multigene phylogenetic analyses and morphological characters, we establish our strain as *P. citribraasilensis*, a new host and country record. This is the first time *P. citribraasilensis* is reported from *Laburnum anagyroides* in Russia (Farr and Rossman 2023).

Table 7
Comparison of *P. citribraasilensis* (type strain) and strain obtained in this study

Species characters		MFLU22-0175	CBS 100098	
Morphological features	Conidiomata	Size (µm)	100–160 diam., 80–110 high	Up to 250 diam.
		Shape and color	Solitary, globose to sub-globose, black	Solitary, globose, black, erumpent, exuding colourless to opaque conidial masses
	Central ostiole	Width (µm)	12.5–17.5	Up to 30 µm
	Conidia	Size (µm)	7–9.8 × 4.5–6.6	(8)10–12(13) × 6–7(8)
		Shape	Solitary, ellipsoidal to obovoid, coarsely guttulate, smooth-walled, with narrowly truncate base	Solitary, ellipsoid to obovoid, coarsely guttulate, thin- and smooth-walled, tapering toward a narrowly truncate base
	Mucilaginous sheath	Thickness (µm)	1.3–2	2–4
	Appendage	Length (µm)	2.9–27 × 0.7–1.8	7–15 × 1.5–2
Shape		Straight to curved	Straight to flexuous, unbranched	
Reported morph		Asexual	Asexual	
Hosts		<i>Laburnum anagyroides</i>	<i>Citrus limon</i>	
Gene region(s)		LSU, <i>ACT</i> , <i>TEF-1a</i>	ITS, LSU, <i>ACT</i> , <i>TEF-1a</i> , <i>GAPDH</i> , <i>RPB2</i>	
References		This study	Glienke et al. (2011)	

Phyllosticta capitalensis Henn. (1908) Fig. 6

Index Fungorum number: IF168326, Facesoffungi number: FoF06888

For morphological description, see Glienke et al. (2011) and Wikee et al. (2013b).

Material examined – Thailand, Chiang Rai market, on fruit of *Phyllanthus emblica* (*Phyllanthaceae*), 3 March 2021, N. Huanraluek, FUA 03/3 (MFLU22-0177, dry culture), living culture (MFLUCC 22–0115); Thailand, Chiang Mai, Doi Inthanon National park, on fallen leaves of *Morus alba* (*Moraceae*), 18 October 2021, D. Gomdola, DGO2-L2A (MFLU22-0178, dry culture), living culture (MFLUCC 22–0116); Thailand, Chiang Mai, Doi Inthanon National park, on fallen fruit of *Ficus auriculata* (*Moraceae*), 18 October 2021, D. Gomdola, DGD1-L1-N3B (MFLU22-0179, dry culture), living culture (MFLUCC 22–0117).

GenBank accession numbers: (MFLU22-0177: ITS = OP686473, LSU = OP686475, *ACT* = OQ189919, *TEF-1a* = OQ189916, *RPB2* = OQ189923), (MFLU22-0178: *RPB2* = OQ189924), (MFLU22-0179: ITS = OP688119, LSU = OP688118, *ACT* = OQ189920, *RPB2* = OQ189925)

Notes – In our multi-locus phylogenetic analyses, MFLU22-0177, MFLU22-0178 and MFLU22-0179 are in the *P. capitalensis* species complex (Fig. 3). Our isolates of *P. capitalensis* produced pycnidia on PDA. However, no conidia or other morphological characters were observed. Nonetheless, culture characteristics of our isolates are consistent with those described by Wikee et al. (2013b), also shown herein (Fig. 6).

We report our three strains of *P. capitalensis* as new host records in northern Thailand. This is the first time *P. capitalensis* is isolated from *Phyllanthus emblica*, *Morus alba*, and *Ficus auriculata*. *Phyllosticta capitalensis* has previously been reported on other *Ficus* species such as *F. benjamina* in Thailand and *F. macrophylla* in Iran (Wikee et al. 2013a, b; Sabahi et al. 2022; Farr and Rossman 2023). *Phyllosticta capitalensis* (MFLU22-0177 and MFLU22-0179) are established based on multigene phylogenetic analyses. However, *P. capitalensis* (MFLU22-0178) is reported solely based on the *RPB2* gene region.

Excluding gaps, in pairwise nucleotide comparisons of the type species of *P. capitalensis* and our isolate (MFLU22-0177), there is no nucleotide base pair (bp) difference in ITS (523 nucleotides), *ACT* (201 nucleotides), *TEF-1a* (178 nucleotides) and *RPB2* (749 nucleotides), and 1 nucleotide base pair (bp) difference across LSU (763 nucleotides). There is no nucleotide bp difference across *RPB2* (749 nucleotides) when compared with *P. capitalensis* (MFLU22-0178). There is no nucleotide bp difference across ITS (534 nucleotides), LSU (763 nucleotides) and *ACT* (127 nucleotides), but 2 bp differences across *RPB2* (749 nucleotides) when compared with *P. capitalensis* (MFLU22-0179). Since there are insignificant differences across each gene region, we determine these three strains as *P. capitalensis*.

Morphological And Nucleotide Base Pair Comparison

The following *Phyllosticta* species have unstable placements in the phylogenetic tree (Fig. 3): *P. ericarum* (CPC 19744 and GZAAS 6.1245) located in the *P. concentrica* species complex, and *P. fallopieae* (MUCC0113), *P. miurae* (MUCC0065) and *P. sphaeropsoidea* (MUCC0112) found in the *P. capitalensis* species complex. Table 8 shows the base pair differences of *P. ericarum* compared to those of *P. citribasiliensis* (CBS 100098), and base pair differences of the other species mentioned above compared with those of *P. capitalensis* (CBS 128856). Morphological feature comparison of these taxa is given (Table 9).

No nucleotide base pair differences were found in comparison between the type strains of *P. ericarum* and *P. citribasiliensis* (Table 8). Furthermore, their morphological characters are similar (Table 9). Therefore, we synonymize *P. ericarum* with *P. citribasiliensis*. *Phyllosticta fallopieae* (MUCC0113), *P. miurae* (MUCC0065) and *P. sphaeropsoidea* (MUCC0112) have sequence data only for ITS and *ACT* gene regions. Since each taxon does not have complete sequence data for all gene regions and lacks complete morphological description, further studies involving more DNA sequence data as well as morphological examination are required to resolve and confirm their taxonomic placement.

Table 8
Nucleotide base pair comparison between species that have unstable placements against type strains

Nucleotide base pair differences in each locus		ITS	LSU	<i>ACT</i>	<i>TEF-1a</i>	<i>GAPDH</i>	<i>RPB2</i>
Comparison between:							
P. citribasiliensis CBS 100098 (has all 6 gene regions)	P. ericarum CPC 19744	0 bp (561 nucleotides)	0 (761 nucleotides)	0 (221 nucleotides)	0 (222 nucleotides)	0 (623 nucleotides)	N/A
	P. ericarum GZAAS 6.1245	0 (594 nucleotides)	N/A	2 (236 nucleotides)	1 (282 nucleotides)	0 (650 nucleotides)	N/A
P. capitalensis CBS 128856 (has all 6 gene regions)	P. fallopieae MUCC0113	0 (557 nucleotides)	N/A	0 (225 nucleotides)	N/A	N/A	N/A
	P. miurae MUCC0065	2 (557 nucleotides)	N/A	0 (225 nucleotides)	N/A	N/A	N/A
	P. sphaeropsoidea MUCC0112	0 (557 nucleotides)	N/A	0 (225 nucleotides)	N/A	N/A	N/A
N/A = Gene region not available for those species							

Table 9
Synopsis of similarities and differences in species that have unstable placements

Species characters		Species			
		<i>P. citribasilensis</i> CBS 100098	<i>P. ericarum</i> CPC 19744	<i>P. capitalensis</i> CBS 128856 CPC 20252	
Morphological features	Conidiomata	Size (µm)	Up to 250 diam.	Up to 180 diam.	120–125 × 135–140
		Shape and colour	Solitary, globose, black, erumpent, exuding colourless to opaque conidial masses	Solitary, globose, pycnidial, black, erumpent, exuding colourless to opaque conidial masses	Epiphyllous, globose, brown or black
	Conidia	Size (µm)	(8)10–12(13) × 6–7(8)	(8)9–10(12) × (6)7	8–11 × 5–6
		Shape	Solitary, aseptate, hyaline, ellipsoid to obovoid, coarsely guttulate, thin- and smooth-walled, tapering towards a narrowly truncate base	Solitary, aseptate, hyaline, ellipsoid or obovoid, coarsely guttulate, or with a single large central guttule, thin- and smooth-walled, tapering towards a narrowly truncate base	Ellipsoidal, hyaline, 1-celled, smooth-walled
	Mucilaginous sheath	Thickness (µm)	2–4	3–4	-
	Appendage	Length (µm)	7–15 × 1.5–2	(5)8–10(12) × 1.5(2)	5–8
Shape		Straight to flexuous, unbranched	Flexuous, unbranched, tapering towards acutely rounded tip	-	
Hosts		<i>Citrus limon</i>	<i>Erica gracilis</i>	<i>Punica granatum</i>	
Gene region(s)		ITS, LSU, <i>ACT</i> , <i>TEF-1α</i> , <i>GAPDH</i> , <i>RPB2</i>	ITS, LSU, <i>ACT</i> , <i>TEF-1α</i> , <i>GAPDH</i>	ITS, LSU, <i>ACT</i> , <i>TEF-1α</i> , <i>GAPDH</i> , <i>RPB2</i>	
References		Glienke et al. (2011)	Crous et al. (2012)	Wikee et al. (2013b)	

Discussion

Speculating the number of fungal species is a major challenge for mycologists (Hyde et al. 2020; Wijayawardene et al. 2021a). Based on high throughput sequencing, the most recent estimate of fungal species is 6.28 million (Baldrian et al. 2022). Numerous taxa await discovery, especially in tropical regions (Hawksworth 1991). Considering that Thailand is a tropical country and a fungal biodiversity hotspot, it harbours a huge number of undiscovered taxa (Hyde et al. 2018, 2020). Therefore, more fungal collections and studies should be carried out in tropical regions from a wide number of hosts to report new species and new host records of *Phyllosticta* species.

To date, several *Phyllosticta* species have been reported from different hosts (Tennakoon et al. 2021, 2022). There are 3,212 epithets in Index Fungorum database (Index Fungorum 2023) and 1,495 epithets in Species Fungorum, excluding species that have already been synonymized (Species Fungorum 2023) (accessed 23 February 2023). This huge number might be attributed to the addition of *Phyllosticta* taxa based on host association over the past 200 years. Also, *Phyllosticta* species were previously delineated based on morphology, thereby placing many species having hyaline, unicellular conidia similar to those of *Phoma*, either in the genera *Phoma* or *Phyllosticta* (Wikee et al. 2011). Thenceforth, several of these species have been synonymized (van der Aa and Vanev 2002). To properly estimate the number of *Phyllosticta* species, more research focusing on re-studying type specimens and providing molecular data by re-collecting *Phyllosticta* species that were previously isolated solely on the basis of morphological description is required.

Norphanphoun et al. (2020) introduced six individual *Phyllosticta* species complexes based on five gene loci (ITS, LSU, *ACT*, *GAPDH* and *TEF-1α*), which can be used as a broader delimiter of the genus. In our study, the same complexes have been considered. Our phylogenetic trees are consistent with those of previous studies (Wikee et al. 2013a; Jayawardena et al. 2019; Norphanphoun et al. 2020; Zhang et al. 2022; Wang et al. 2023). However, in our analyses, *P. mimusopisicola* (CBS 138899) and *P. rhodora* (CBS 901.69) clustered in the *P. cruenta* species complex instead of forming a distinct lineage in the *P. rhodora* species complex as shown in Norphanphoun et al. (2020). Therefore, further studies are required to confirm the taxonomic placement of these species complexes.

The host plants from which we isolated the above-mentioned *Phyllosticta* species are greatly considered for their economic and ecological significance. For example, *Musa* spp. are important in the pharmaceutical and food industries (Qamar et al. 2018). Since *Musa* spp. are staple food crops, they are a major source of income in many developing countries in South America, South-East Asia, and Africa (Kaushal et al. 2022). *Laburnum anagyroides* is cultivated as an

ornamental, with decorative golden-yellow flowers (Heywood 1993). *Phyllanthus emblica* is widely used for its nutritional and therapeutic properties including antioxidant, antidiabetic and antimicrobial effects (Krishnaveni and Mirunalini 2010; Ahmad et al. 2021). *Morus alba* is economically important to the silk industry because it is used to nourish larvae of the silk moth (Watanabe 1958). *Ficus* spp. are cultivated to enhance tropical forest restoration. In view of their fruit production, they act as seedling recruitment foci whereby they attract seed dispersers, thus inducing rapid forest restoration (Cottee-Jones et al. 2016). Also, many frugivorous birds feed on *Ficus* spp. (Corlett 2005, 2006; Caughlin et al. 2012; Lok et al. 2013). With respect to the numerous economic benefits and ecological importance of the above-mentioned hosts, it is important to study and report the diverse fungi associated with them.

Phyllosticta species have a wide global distribution. Owing to the fact that fungi exhibit different lifestyles such as pathogenic, endophytic and saprobic, they play essential roles in the ecosystem (De Silva et al. 2017). Given different environmental and biotic factors, many fungi can switch lifestyles based on their nutritional mode, for example from endophytes to pathogens (Promputtha et al. 2007; Rai and Agarkar 2016). In order to understand the biology and ecosystem functioning of a specific fungal group, it is crucial to study the association and relationship between the host plants and the fungal group of interest. In this study, we reported three strains of *P. capitalensis* from three different hosts. *Phyllosticta capitalensis* is the most commonly isolated endophytic species in the genus and is widely distributed (Chethana et al. 2021d; Manawasinghe et al. 2022). Therefore, it is important to study this group of fungi.

From Table 3, we can decipher that *Phyllosticta* is a generalist, rather than host-specific. However, further research is required to confirm this statement because they exhibit different lifestyles. Some species are host genus or family specific when they manifest as pathogens while as endophytes, most species tend to be generalists (Wikee et al. 2011; Bhunjun et al. 2022). One of the important cryptic fungal phytopathogens with species complexes is *Phyllosticta* (Cai et al. 2011). Cryptic species directly impact the number of fungal species (Wijayawardene et al. 2021b, 2022b). Since most *Phyllosticta* species are cryptic and share similar morphological features, it is arduous to pinpoint homologous characters and delineate these taxa. With the use of polyphasic approaches such as DNA-based characters and GCPSR, coupled with morphological description, several cryptic species can be unmasked. However, in view of their overlapping morphological features, phylogenetic analyses using distinct molecular markers are sometimes insufficient to delineate *Phyllosticta* species (Wang et al. 2020). Therefore, along with the above-mentioned approaches, sequence-based species delimitation methods following Maharachchikumbura et al. (2021) are useful for species delineation of *Phyllosticta*.

Declarations

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Author Contributions

D.G. carried out the experiments, analyzed data and wrote the manuscript. R.S.J., E.H.C.M, D.N.W. and K.D.H. reviewed and provided suggestions to improve the manuscript. T.S.B. collected specimens from Russia. N.H. helped to carry out the experiment partially. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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Figures

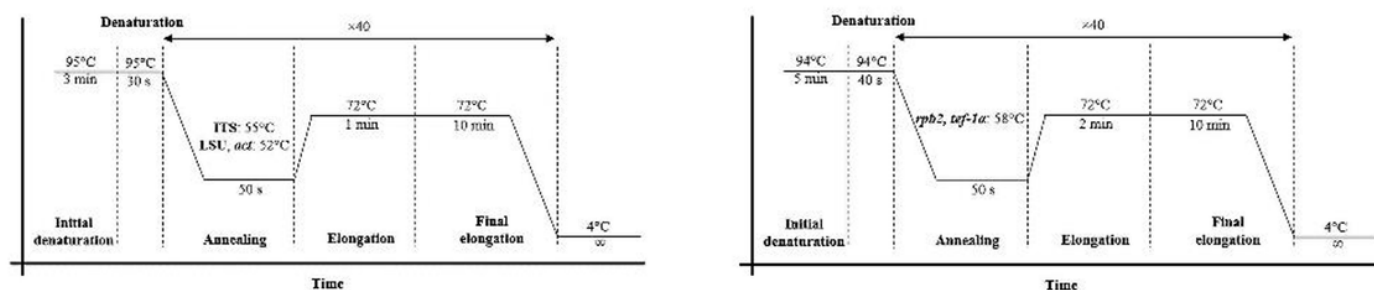


Figure 1

a PCR conditions for ITS, LSU and ACT

b PCR conditions for RPB2 and TEF-1a

P. musaechinensis
GZAAS 6.1247
GZAAS 6.1384

P. chiangmaiensis sp. nov.
MFLU22-0176

P. maculata
CPC 18347
BRIP 46622

$\Phi_w = 0.9207$

0.001

P. musarium
BRIP 55434
GZAAS 6.1228

Figure 2

PHI test result using LogDet transformation and splits decomposition. The new taxon is given in red

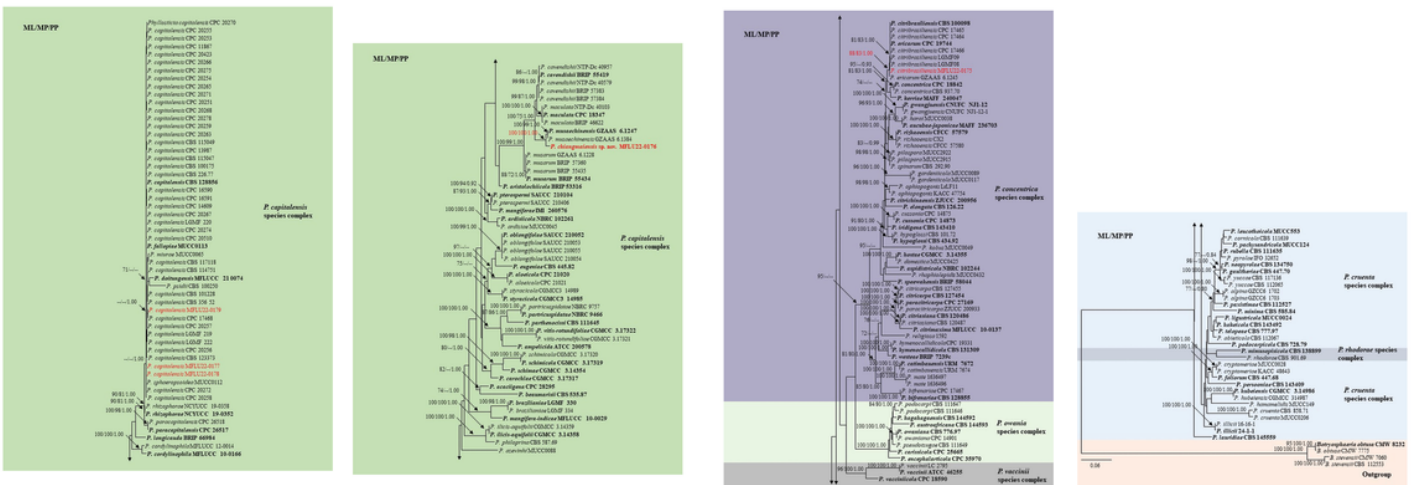


Figure 3

Phylogram generated from maximum likelihood analysis (RAxML) based on the combined ITS, LSU, *TEF-1 α* , *ACT*, *GAPDH* and *RPB2* matrices of *Phyllosticta*. Maximum likelihood (ML) and maximum parsimony (MP) with bootstrap support $\geq 70\%$, and the posterior probability (PP) values (≥ 0.8) of Bayesian inference (BI) analyses are given at respective nodes as ML/MP/PP. Hyphen (-) represents support values below 70% (ML and MP) and below 0.80 (PP). The tree is rooted with *Botryosphaeria obtusa* (CMW 8232 and CMW 7775) and *B. stevensii* (CBS 112553 and CMW 7060). Type strains are indicated in bold and our isolates are in red. Different background colours indicate the six *Phyllosticta* species complexes

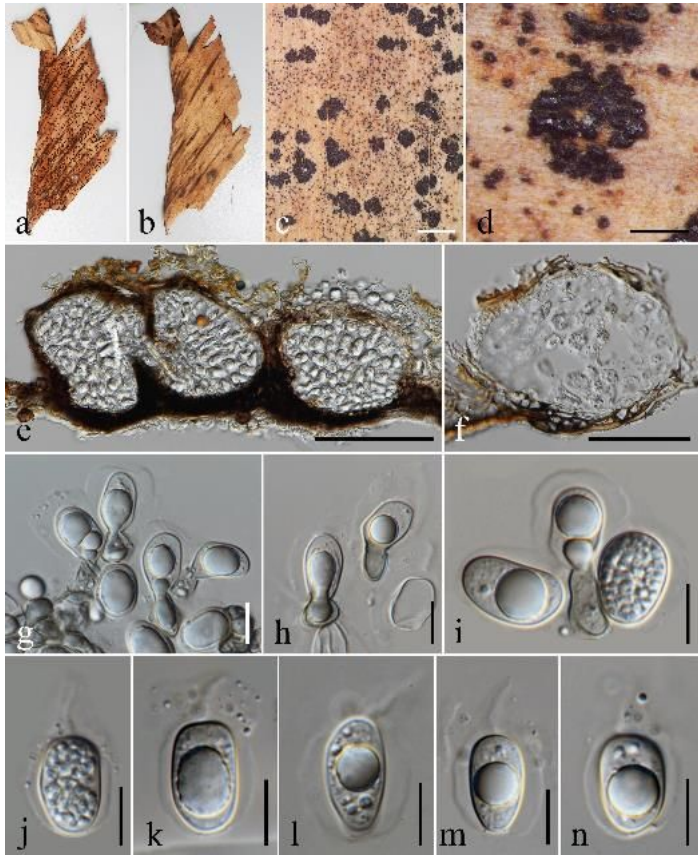


Figure 4
Phyllosticta chiangmaiensis (MFLU22-0176, holotype) **a–b** Appearance of conidiomata on leaves of *Musa* sp. **c–d** Close up of conidiomata on substrate. **e–f** Section through conidiomata showing pycnidial wall. **g–i** Conidiogenous cells and developing conidia. **j–n** Conidia surrounded by mucilaginous sheath, with an apical appendage. Scale bars: **c** = 2 mm, **d** = 500 μ m, **e** = 100 μ m, **f** = 50 μ m, **g–n** = 10 μ m



Figure 5
Phyllosticta citribasilensis (MFLU22-0175) **a** Leaf spot on *Laburnum anagyroides* **b–c** Close up of conidiomata on substrate. **d–e** Section through conidiomata showing pycnidial wall. **f** Ostiole **g–i** Conidiogenous cells and developing conidia. **j–n** Conidia surrounded by mucilaginous sheath, with an apical appendage. Scale bars: **b** = 500 μm **c** = 200 μm , **d–e** = 50 μm , **f, g, j–n** = 10 μm , **h–i** = 5 μm

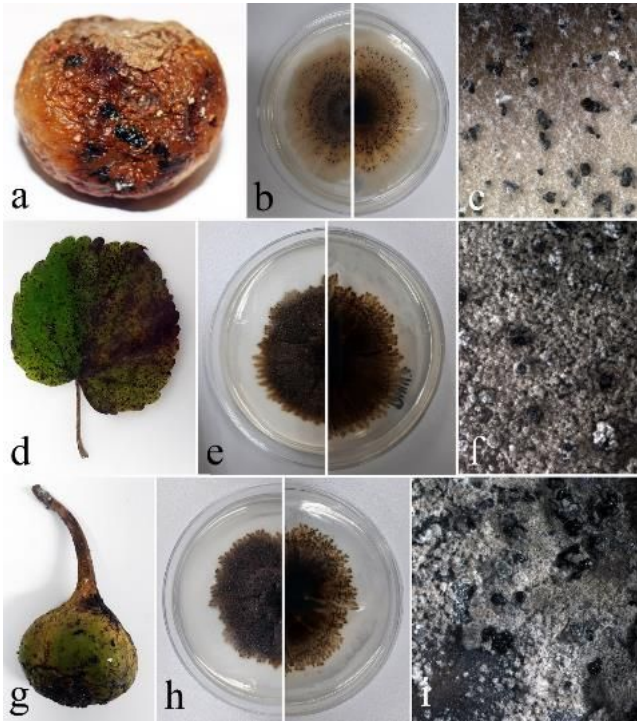


Figure 6
Phyllosticta capitalensis **a** Fruit of *Phyllanthus emblica* **b–c** Colony and pycnidia of MFLU22-0177 **d** Dead leaves of *Morus alba* **e–f** Colony and pycnidia of MFLU22-0178. **g** Fruit of *Ficus auriculata* **h–i** Colony and pycnidia of MFLU22-0179