



Two new endophytic species of *Phyllosticta* (Phyllostictaceae, Botryosphaerales) from Southern China

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Abstract

Phyllosticta is an important genus known to cause various leaf spots and fruit diseases worldwide on a large range of hosts. Two new endophytic species of *Phyllosticta* (*P. dendrobii* and *P. illicii*) are described and illustrated from *Dendrobium nobile* and *Illicium verum* in China. Phylogenetic analysis based on combined ITS, LSU, *tef1-a*, ACT and GPDH loci supported their separation from other species of *Phyllosticta*. Morphologically, *P. dendrobii* is most comparable with *P. aplectri*, while the large-sized pycnidia of *P. dendrobii* differentiate it from *P. aplectri*. Members of *Phyllosticta* are first reported from *Dendrobium* and *Illicium*.

Key words – Asia – Botryosphaerales – leaf spots – Multilocus phylogeny

Introduction

Phyllosticta Pers. was introduced by Persoon (1818) and typified by *P. convallariae* Pers. Many species of *Phyllosticta* cause leaf and fruit spots on various host plants, such as *P. citricarpa* (McAlpine) Aa, which causes citrus black spot (Baayen et al. 2002, Glienke et al. 2011), *P. ampellicida* (Engelm.) Aa species complex that causes black rot disease on grapevines (Wicht et al. 2012), and the *P. musarum* (Cooke) Aa species complex that causes banana freckle disease (Pu et al. 2008, Wong et al. 2012). Some species of *Phyllosticta* have also been isolated as endophytes from a wide range of hosts, and other species are regarded as saprobes, e.g. *P. capitalensis* Henn., *P. carpogena* (Shear) Aa and *P. ericae* Allesch. (van der Aa 1973, Baayen et al. 2002, van der Aa & Vanev 2002, Glienke et al. 2011, Wikee et al. 2011, 2013).

The generic concept of *Phyllosticta* has undergone substantial changes since its establishment in 1818. Allescher (1898) considered *Phyllosticta* as *Phoma*-like, but as foliar pathogens, and *Phoma* Sacc. on other plant parts, and Grove (1935) regarded *Phyllosticta* as a parasite and *Phoma* as saprobe or wound parasite. Host association has been used in separating “*Phyllosticta*” species (Seaver 1922, Grove 1935), which has been widely followed in the 20th century, and led to the introduction of numerous species names. Presently there are approximately 3,213 epithets known for *Phyllosticta* (<http://www.indexfungorum.org>, accessed August 2017), but many of these reflect old concepts of the genus, and have been accommodated elsewhere (van der Aa & Vanev 2002). *Phyllosticta s. str.* was first monographed by van der Aa (1973), who described and illustrated 46 species, and listed the sexual morphs for 12 species, and the spermatial morphs for 17 based mostly on material collected in Europe and North America. More recently, van der Aa & Vanev (2002)

revised all species described in *Phyllosticta*, and accepted 190 epithets. A multi-locus phylogeny of 129 isolates of *Phyllosticta*, representing about 170 species names indicated that *Phyllosticta* is sister to the Botryosphaeriaceae (Botryosphaeriales, Dothideomycetes) and that many species are synonyms of the cosmopolitan *P. capitalensis* (Wikee et al. 2013). The family *Phyllostictaceae* was first proposed by Fries (1849) and resurrected by Wikee et al. (2013) to accommodate *Phyllosticta*. The sexual stage of *Phyllosticta* is *Guignardia* Viala & Ravaz, and the earlier and widely used name *Phyllosticta* was chosen over *Guignardia* (Glienke et al. 2011, Sultan et al. 2011, Wikee et al. 2011, 2013, Wong et al. 2012).

Dendrobium nobile Lindley is a member of the family Orchidaceae, which is a fundamental herb used in traditional Chinese medicine. *Illicium verum* Hook. f. is an aromatic evergreen tree of the family Illiciaceae, the fruit of which (Chinese star anise) has long been used in traditional Chinese medicine and the food industry with the actions of dispelling cold, regulating the flow of *Qi* and relieving pain. Both *Dendrobium nobile* and *Illicium verum* are native to China. During a survey of endophytic fungi associated with *D. nobile* and *I. verum* in China, two *Phyllosticta* species were isolated. Based on morphological and molecular phylogenetic inferences, we introduce them as *P. dendrobii* and *P. illicii* respectively.

Material & Methods

Fungal isolation

Wood segments of 5 mm × 5 mm × 2 mm were cut from the healthy tissue, and washed in tap water and wiped with 70 % ethanol. They were suspended in 70 % ethanol for 15 min, and washed in distilled water (three times) before placing on MEA. All plates were incubated at 28°C. The growing tips of hyphae of *Phyllosticta* colonies that developed were cut out and transferred to fresh MEA plates.

Morphological study of fungi

Growth rates and culture characteristics of the isolates were determined at 28 °C on MEA. Fungal structures (conidia, conidiogenous cells etc.) were mounted in water for observation with a microscope (Nikon Eclipse E600). Nomenclatural novelties and descriptions were deposited in MycoBank (www.mycobank.org, Crous et al. 2004) and Faces Of Fungi (<http://www.facesoffungi.org/>, Jayasiri et al. 2015). Type specimens were deposited in Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), with extype living cultures deposited in China General Microbiological Culture Collection Center (CGMCC). At least 30 pycnidia, conidiogenous cells or conidia were measured to calculate the mean dimensions and standard deviations (SD) given in the formal descriptions.

DNA extraction, PCR amplification, and sequencing

Isolates for DNA extraction were grown on MEA, and total genomic DNA extracted from mycelia with CTAB plant genome DNA fast extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China). Internal transcribed spacer (ITS), Actin (ACT), translation elongation factor 1-alpha (*tef1-α*), 28S large subunit ribosomal RNA gene (LSU), and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes were amplified with primer pairs ITS1/ITS4 (White et al. 1990), ACT512F/ACT783R (Carbone & Kohn 1999), EF1-728F/EF1-786R (Carbone & Kohn 1999), LROR/LR5 (Moncalvo et al. 1995) and GDF1/Gpd2-LM (Myllys et al. 2002, Guerber et al. 2003) respectively.

Sequence alignment and phylogenetic analysis

Sequence data of combined ITS, ACT, *tef1-α*, LSU and GPDH loci were used to infer the phylogenetic relationships of *P. dendrobium*, *P. illicium* and other reported *Phyllosticta* species for which confirmed sequence data were available from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). A multiple alignment was done in MEGA v. 6 (Tamura et

al. 2013) and analyses were performed in PAUP v. 4.0b10 (Swofford 2002) and MrBayes v. 3.1.2. (Ronquist & Huelsenbeck 2003). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. Maximum Likelihood bootstrap values (ML-BS) obtained from 1000 replicates were performed using raxml GUI 1.31 (Michalak 2012) with the GTRGAMMA and GTRCAT models to assess the reliability of the nodes. Maximum Parsimony (MP) analysis was conducted using heuristic searches as implemented in PAUP, with the default options method. Analyses were done under different parameters of maximum parsimony criteria as outlined in Zhang et al. (2008). Clade stability was assessed in a bootstrap analysis with 1000 replicates, random sequence additions with maxtrees set to 1000 and other default parameters as implemented in PAUP. For the Bayesian analysis, the best-fit model of nucleotide evolution (GTR+I+G) was selected by Akaike information criterion (AIC; Posada & Buckley 2004) in MrModeltest 2.3. The metropolis-coupled Markov Chain Monte Carlo (MCMCMC) approach was used to calculate posterior probabilities (Huelsenbeck & Ronquist 2005). A preliminary Bayesian inference (BI) analysis using MrBayes revealed that the Markov Chain Monte Carlo steady state was reached after less than 10,000 generations (the average standard deviation of split frequencies was constantly below 0.01). A conservative burn-in of 100 dendrograms was chosen and a full analysis of 5,000,000 generations was carried out with sampling every 100 generations. Trees were viewed in TREEVIEW (Page 1996). The nucleotide sequences reported in this paper were deposited in GenBank, and alignments in TreeBASE (S21576).

Results

The combined ITS, ACT, *tef1-a*, LSU and GPDH dataset comprised 114 strains. The dataset consisted of 2521 characters after alignment, and all sites were included in the MP analysis. Of the included bases, 768 sites (30.5%) were parsimony-informative. A heuristic search with random addition of taxa (1000 replicates) and treating gaps as missing characters generated 720 equally parsimonious trees. All trees were similar in topology and not significantly different (figures not shown). One of the most parsimonious trees (TL = 3898, CI = 0.408, RI = 0.780, RC = 0.319) is shown in Fig. 1.

Taxonomy

Phyllosticta dendrobii S. Lin, Y. Zhang ter, sp. nov.

Fig. 2

Mycobank: MB821784; Facesoffungi number: FoF03422

Etymology – Named after the host genus from which it was collected, *Dendrobium nobile*.

Conidiomata pycnidial, mostly aggregated in clusters, black, erumpent, globose to clavate or elongated with necks up to 700 µm long, exuding colourless to opaque conidial masses. *Pycnidia* up to 280 µm diam. *Pycnidial wall* of several layers of *textura angularis*, up to 40 µm thick, inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* reduced to conidiogenous cells, *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, 6–10 × 3–4 µm, proliferating several times percurrently near apex. *Conidia* (5–)8–9(–10) × 5(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ovoid to irregularly ellipsoid, enclosed in a thin mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (4–)6–9(–10) × 2(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip. After 2 months, mucoid sheath and apical mucoid appendage disappear. *Ascomata* similar to conidiomata in general anatomy. *Asci* bitunicate, hyaline, clavate to broadly fusoid-ellipsoid, 54–95 × 8–13 µm, with visible apical chamber, 2 µm diam. *Ascospores* bi- to multi-seriate, hyaline, smooth, granular to guttulate, aseptate, straight and slightly curved, widest in the middle, limoniform with obtuse ends, (12–)14–16(–18) × 3–6 µm, with distinct hyaline gelatinous caps at both ends, mostly less than 10 µm, seldom over 15 µm long.

Table 1. *Phyllosticta* isolates investigated in this study.

Species	Culture	Host	Country	GenBank Number				
				ITS	LSU	<i>tef1-a</i>	ACT	GPDH
<i>Botryosphaeria obtusa</i>	CMW 8232	<i>Conifers</i>	South Africa	AY972105	N/A	DQ280419	AY972111	N/A
<i>Guignardia gaultheriae</i>	CBS 447.70	<i>Gaultheria humifusa</i>	USA	JN692543	KF206298	JN692531	KF289248	JN692508
<i>G. mangiferae</i>	IMI 260.576	<i>Mangifera indica</i>	India	JF261459	KF206222	JF261501	JF343641	JF343748
	CPC 20260	<i>Arecaceae</i>	Thailand	KF206193	KF206243	KF289187	KF289294	KF289114
<i>G. musicola</i>	CBS123405	<i>Musa acuminata</i>	Thailand	FJ538334	N/A	FJ538392	FJ538450	N/A
<i>G. rhodora</i>	CBS 901.69	<i>Rhododendron</i>	Netherlands	KF206174	KF206292	KF289230	KF289256	KF289166
<i>Phyllosticta abieticola</i>	CBS 112067	<i>Abies concolor</i>	Canada	KF170306	EU754193	N/A	KF289238	N/A
<i>P. alliacea</i>	MUCC0014	<i>Allium fistulosum</i>	Japan	AB454263	N/A	N/A	N/A	N/A
<i>P. aloecicola</i>	CPC 21020	<i>Aloe ferox</i>	South Africa	KF154280	KF206214	KF289193	KF289311	KF289124
	CPC 21021	<i>Aloe ferox</i>	South Africa	KF154281	KF206213	KF289194	KF289312	KF289125
<i>P. ampellicida</i>	ATCC200578	<i>Vitis riparia</i>	USA	KC193586	N/A	N/A	KC193581	KC193584
<i>P. ardisiicola</i>	NBRC102261	<i>Ardisia crenata</i>	Japan	AB454274	N/A	N/A	N/A	N/A
<i>P. aspidisticola</i>	NBRC102244	<i>Aspidistra elatior</i>	Japan	AB454260	N/A	N/A	N/A	N/A
<i>P. beaumarisii</i>	CBS 535.87	<i>Muehlenbekia adpressa</i>	Australia	AY042927	KF306229	KF289170	KF306232	KF289074
<i>P. bifrenariae</i>	CBS 128855	<i>Bifrenaria harrisoniae</i>	Brazil	JF343565	KF206209	JF343586	JF343649	JF343744
	CPC 17467	<i>Bifrenaria harrisoniae</i>	Brazil	KF170299	KF206260	KF289207	KF289283	KF289138
<i>P. brazilliana</i>	CBS 126270	<i>Mangifera indica</i>	Brazil	JF343572	KF206217	JF343593	JF343656	JF343758
	LGMF 333	<i>Mangifera indica</i>	Brazil	JF343574	KF206216	JF343595	JF343658	JF343760
<i>P. capitalensis</i>	CPC 18848	<i>Stanhopea graveolens</i>	Brazil	JF261465	KF206255	JF261507	KF289289	JF343776
	CPC 20267	<i>Baccaurea ramiflora</i>	Brazil	KF206195	KF206237	KF289173	KF306233	KF289078
<i>P. carochlae</i>	CGMCC 3.17317	<i>Caryota ochlandra</i>	China	KJ847422	N/A	KJ847444	KJ847430	KJ847438

	CGMCC 3.17318	<i>Caryota ochlandra</i>	China	KJ847423	N/A	KJ847445	KJ847431	KJ847439
<i>P. cavendishii</i>	BRIP554196	<i>Musa</i> cv. Formosana	Taiwan	JQ743562	N/A	KF009743	KF014080	N/A
	BRIP58008	<i>Banana</i>	Australia	KC988365	N/A	KF009742	KF014071	N/A
<i>P. citriasiana</i>	CBS 120486	<i>Citrus maxima</i>	Thailand	FJ538360	KF206314	FJ538418	FJ538476	JF343686
	CBS 123371	<i>Citrus maxima</i>	Vietnam	FJ538356	KF206309	FJ538414	FJ538472	JF343690
<i>P. citribraziliensis</i>	CBS 100098	<i>Citrus limon</i>	Brazil	FJ538352	KF206221	FJ538410	FJ538468	JF343691
	CPC 17464	<i>Citrus sp.</i>	Brazil	KF170300	KF206263	KF289224	KF289280	KF289159
<i>P. citricarpa</i>	CBS 127454	<i>Citrus limon</i>	Australia	JF343583	KF206306	JF343604	JF343667	JF343771
	CBS 127455	<i>Citrus sinensis</i>	Australia	JF343584	KF206305	JF343605	JF343668	JF343772
<i>P. citrichinaensis</i>	ZJUCC 200956	<i>Citrus reticulata</i>	China	JN791620	N/A	JN791459	JN791533	N/A
	ZJUCC 200964	<i>Citrus maxima</i>	China	JN791611	N/A	JN791461	JN791535	N/A
<i>P. citrimaxima</i>	CPC 20276	<i>Citrus maxima</i>	Thailand	KF170304	KF206229	KF289222	KF289300	KF289157
<i>P. concentrica</i>	CBS 937.70	<i>Hedera helix</i>	Italy	FJ538350	KF206291	FJ538408	KF289257	JF411745
	CBS 134749	<i>Hedera sp.</i>	Spain	KF170310	KF206256	KF289228	KF289288	KF289163
<i>P. cordylinophila</i>	CPC 20261	<i>Cordyline fruticosa</i>	Thailand	KF170287	KF206242	KF289172	KF289295	KF289076
	CPC 20277	<i>Cordyline fruticosa</i>	Thailand	KF170288	KF206228	KF289171	KF289301	KF289075
<i>P. cornicola</i>	CBS 111639	<i>Cornus florida</i>	USA	KF170307	N/A	N/A	KF289234	N/A
<i>P. cussonia</i>	CPC 14873	<i>Cussonia sp.</i>	South Africa	JF343578	KF206279	JF343599	JF343662	JF343764
	CPC 14875	<i>Cussonia sp.</i>	South Africa	JF343579	KF206278	JF343600	JF343663	JF343765
<i>P. dendrobii</i>	CGMCC 3.18665	<i>Dendrobium nobile</i>	China	MF180192	MF180209	N/A	MF180218	MF180227
	CGMCC 3.18666	<i>Dendrobium nobile</i>	China	MF180193	MF180210	MF180201	MF180219	MF180228
	CGMCC 3.18667	<i>Dendrobium nobile</i>	China	MF180194	MF180211	MF180202	MF180220	MF180229
<i>P. elongata</i>	CBS 126.22	<i>Oxycoccus macrocarpos</i>	USA	FJ538353	N/A	FJ538411	FJ538469	KF289164
<i>P. ericarum</i>	CBS 132534	<i>Erica gracilis</i>	South Africa	KF206170	KF206253	KF289227	KF289291	KF289162
<i>P. eugeniae</i>	CBS 445.82	<i>Eugenia</i>	Indonesia	AY042926	KF206288	KF289208	KF289246	KF289139

<i>P. fallopiae</i>	MUCC0113	<i>aromatica</i> <i>Fallopia</i>	Japan	AB454307	N/A	N/A	N/A	N/A
<i>P. gaultheriae</i>	CBS 447.70	<i>japonica</i> <i>Gaultheria</i>	USA	JN692543	KF206298	JN692531	KF289248	JN692508
<i>P. foliorum</i>	CBS 174.77	<i>humifusa</i> <i>Cryptomeria</i>	USA	KF170308	KF206290	KF289200	KF289245	KF289131
<i>P. hamamelidis</i>	CBS 447.68	<i>japonica</i> <i>Taxus baccata</i>	Netherlands	KF170309	KF206287	KF289201	KF289247	KF289132
	MUCC 149	<i>Hamamelis</i>	Japan	KF170289	N/A	N/A	KF289309	N/A
<i>P. hostae</i>	CGMCC	<i>japonica</i> <i>Hosta</i>	China	JN692535	N/A	JN692524	JN692512	JN692504
	3.14355	<i>plantaginea</i> <i>Hosta</i>	China	JN692536	N/A	JN692525	JN692513	JN692505
<i>P. hubeiensis</i>	CGMCC	<i>plantaginea</i> <i>Viburnum</i>	China	JX025037	N/A	JX025042	JX025032	JX025027
	3.14986	<i>odoratissimim</i> <i>Viburnum</i>	China	JX025038	N/A	JX025043	JX025033	JX025028
	CGMCC	<i>odoratissimim</i> <i>Hymenocallis</i>	Australia	JQ044423	JQ044443	KF289211	KF289242	KF289142
<i>P. hymenocallidicola</i>	CBS 131309	<i>littoralis</i> <i>Hymenocallis</i>	Australia	KF170303	KF206254	KF289212	KF289290	KF289143
<i>P. hypoglossi</i>	CBS 101.72	<i>littoralis</i> <i>Ruscus aculeatus</i>	Italy	FJ538365	KF206326	FJ538423	FJ538481	JF343694
	CBS 434.92	<i>Ruscus aculeatus</i>	Italy	FJ538367	KF206299	FJ538425	FJ538483	JF343695
<i>P. ilicis-aquifolii</i>	CGMCC	<i>Ilex aquifolium</i>	China	JN692538	N/A	JN692526	JN692514	N/A
	3.14358	<i>Ilex aquifolium</i>	China	JN692539	N/A	JN692527	JN692515	N/A
<i>P. illicii</i>	CGMCC	<i>Illicium verum</i>	China	MF180195	MF180212	MF180203	MF180221	N/A
	3.18670	<i>Illicium verum</i>	China	MF180196	MF180213	MF180204	MF180222	N/A
	CGMCC	<i>Illicium verum</i>	China	MF180197	MF180214	MF180205	MF180223	N/A
	3.18672							
	CGMCC							
	3.18673							

	CGMCC 3.18674	<i>Illicium verum</i>	China	MF180198	MF180215	MF180206	MF180224	N/A
	CGMCC 3.18675	<i>Illicium verum</i>	China	MF180199	MF180216	MF180207	MF180225	N/A
	CGMCC 3.18676	<i>Illicium verum</i>	China	MF180200	MF180217	MF180208	MF180226	N/A
	CGMCC 3.18668	<i>Illicium verum</i>	China	MF198233	MF198238	N/A	MF198241	N/A
	CGMCC 3.18669	<i>Illicium verum</i>	China	MF198234	MF198239	MF198236	MF198242	N/A
	CGMCC 3.18671	<i>Illicium verum</i>	China	MF198235	MF198240	MF198237	MF198243	N/A
<i>P. kerriae</i>	MAFF240047	<i>Kerria japonica</i>	Japan	AB454266	N/A	N/A	N/A	N/A
<i>P. leucothoicola</i>	CBS 136073	<i>Leucothoe catesbaei</i>	Japan	AB454370	AB454370	N/A	KF289310	N/A
<i>P. ligustricola</i>	MUCC0024	<i>Ligustrum obtusifolium</i>	Japan	AB454269	N/A	N/A	AB704212	N/A
<i>P. maculata</i>	CPC18347	<i>Musa</i> cv. Goly- goly pot-pot	Australia	JQ743570	N/A	KF009700	KF014016	N/A
	BRIP46622	<i>Musa</i> cv. Goly- goly pot-pot	Australia	JQ743567	N/A	KF009692	KF014013	N/A
<i>P. mangifera-indica</i>	CPC 20274	<i>Mangifera indica</i>	Thailand	KF170305	KF206240	KF289190	KF289296	KF289121
<i>P. minima</i>	CBS 585.84	<i>Acer rubrum</i>	USA	KF206176	KF206286	KF289204	KF289249	KF289135
<i>P. musaechinensis</i>	GZAAS6.1247	<i>Musa</i> sp.	China	KF955294	N/A	KM816639	KM816627	KM816633
	GZAAS6.1384	<i>Musa</i> sp.	China	KF955295	N/A	KM816640	KM816628	KM816634
<i>P. musarum</i>	BRIP57803	<i>Musa</i> sp.	Malaysia	JX997138	N/A	KF009737	KF014055	N/A
	BRIP58028	<i>Musa</i> sp.	Australia	KC988377	N/A	KF009738	KF014054	N/A
<i>P. neopyrolae</i>	CPC 21879	<i>Pyrola asarifolia</i>	Japan	AB454318	AB454241	N/A	AB704233	N/A
<i>P. owaniana</i>	CBS 776.97	<i>Brabejum stellatifolium</i>	South Africa	FJ538368	KF206293	FJ538426	KF289254	JF343767
	CPC 14901	<i>Brabejum stellatifolium</i>	South Africa	JF261462	KF206303	JF261504	KF289243	JF343766
<i>P. pachysandricola</i>	MUCC 124	<i>Pachysandra terminalis</i>	Japan	AB454317	AB454317	N/A	AB704232	N/A

<i>P. parthenocissi</i>	CBS111645	<i>Parthenocissus quinquefolia</i>	USA	EU683672	N/A	JN692530	JN692518	N/A
<i>P. partricuspidatae</i>	NBRC9466	<i>Parthenocissus tricuspidata</i>	Japan	KJ847424	N/A	KJ847446	KJ847432	KJ847440
	NBRC9757	<i>Parthenocissus tricuspidata</i>	Japan	KJ847425	N/A	KJ847447	KJ847433	KJ847441
<i>P. paxistimae</i>	CBS 112527	<i>Paxistima mysinites</i>	USA	KF206172	KF206320	KF289209	KF289239	KF289140
<i>P. philoprina</i>	CBS 587.69	<i>Ilex aquifolium</i>	Spain	KF154278	KF206297	KF289206	KF289250	KF289137
	CBS 616.72	<i>Ilex aquifolium</i>	Germany	KF154279	KF206296	KF289205	KF289251	KF289136
<i>P. podocarpicola</i>	CBS 728.79	<i>Podocarpus maki</i>	USA	KF206173	KF206295	KF289203	KF289252	KF289134
<i>P. podocarpi</i>	CBS 111646	<i>Podocarpus falcatus</i>	South Africa	AF312013	KF206323	KC357671	KC357670	KF289169
	CBS 111647	<i>Podocarpus lanceolata</i>	South Africa	KF154276	KF206322	KF289232	KF289235	KF289168
<i>P. pseudotsugae</i>	CBS 111649	<i>Pseudotsuga menziesii</i>	USA	KF154277	KF206321	KF289231	KF289236	KF289167
<i>P. raphiolepidis</i>	MUCC 432	<i>Raphiolepis indica</i>	Japan	DQ632660	N/A	N/A	AB704242	N/A
<i>P. rubra</i>	CBS 111635	<i>Acer rubrum</i>	USA	KF206171	EU754194	KF289198	KF289233	KF289129
<i>P. schimae</i>	CGMCC 3.14354	<i>Schima superba</i>	China	JN692534	N/A	JN692522	JN692510	JN692506
<i>P. schimicola</i>	CGMCC 3.17319	<i>Schima superba</i>	China	KJ847426	N/A	KJ847448	KJ847434	KJ854895
	CGMCC 3.17320	<i>Schima superba</i>	China	KJ847427	N/A	KJ847449	KJ847435	KJ854896
<i>P. speewahensis</i>	BRIP 58044	<i>Orchids</i>	Australia	KF017269	N/A	KF017268	N/A	N/A
<i>P. sphaeropsoides</i>	CBS 756.70	<i>Aesculus hippocastanum</i>	Germany	AY042934	KF206294	KF289202	KF289253	KF289133
<i>P. spinarum</i>	CBS 292.90	<i>Chamaecyparis pisifera</i>	France	JF343585	KF206301	JF343606	JF343669	JF343773
<i>P. styracicola</i>	CGMCC 3.14985	<i>Styrax gradiflorus</i>	China	JX052040	N/A	JX025045	JX025036	KF289141
	CGMCC	<i>Styrax</i>	China	JX052041	N/A	JX025046	KF289255	KF289165

<i>P. telopeae</i>	3.14989 CBS 777.97	<i>gradiflorus</i> <i>Telopea</i>	Tasmania	KF206205	N/A	KF289210	KF289255	KF289141
<i>P. vaccinii</i>	ATCC 46255	<i>speciosissima</i> <i>Vaccinium</i>	USA	KC193585	N/A	KC193582	KC193580	KC193583
<i>P. vacciniicola</i>	CPC18590	<i>macrocarpon</i> <i>Vaccinium</i>	USA	KF170312	N/A	KF289229	KF289287	KF289165
<i>P. vitis-rotundifoliae</i>	CGMCC 3.17321	<i>macrocarpum</i> <i>Vitis rotundifolia</i>	USA	KJ847429	N/A	KJ847451	KJ847437	KJ847443
	CGMCC 3.17322	<i>Vitis rotundifolia</i>	USA	KJ847428	N/A	KJ847450	KJ847436	KJ847442
<i>P. yuccae</i>	CBS 112065	<i>Yucca</i> <i>elephantipes</i>	USA	KF206175	N/A	N/A	KF289237	N/A
<i>Phyllosticta</i> sp.	CPC 11336	<i>Eucalyptus</i> <i>globulus</i>	Spain	KF206177	KF206284	KF289199	KF289258	KF289130
	MUCC 147	<i>Rhododendron</i> <i>keiskei</i>	Japan	AB454319	AB454319	N/A	AB704234	N/A
	CPC 17454 CPC 17455	<i>Mangifera indica</i> <i>Mangifera indica</i>	Brazil Brazil	KF206206 KF206207	KF206265 KF206264	KF289192 KF289191	KF289278 KF289279	KF289123 KF289122

a. New species described and strains sequenced in this study are shown in bold.

b. **ATCC**: American Type Culture Collection, Virginia, USA; **BRIP**: Plant Pathology Herbarium, Biosecurity Queensland, Dutton Park, Queensland, Australia; **CBS**: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; **CGMCC**: China, General Microbiological Culture Collection, Beijing, China; **CPC**: Culture collection of P.W. Crous, housed at CBS; **GZAAS** : Guizhou Academy of Agricultural Sciences Collection , China; **IMI**: International Mycological Institute, CABI-Bioscience, Egham, Bakenham Lane, U.K.; **LGMF**: Culture collection of Laboratory of Genetics of Microorganisms, Federal University of Parana, Curitiba, Brazil; **MAFF**: the Microbiological Genbank, National Institute of Agrobiological Sciences, Japan; **MUCC**: Culture Collection, Laboratory of Plant Pathology, Mie University; **NBRC**: Biological Resource Center, the National Institute of Technology, and Evaluation, Japan; **ZJUCC**: Zhejiang University Culture Collection, China.

c. Type and ex-type cultures are in bold.

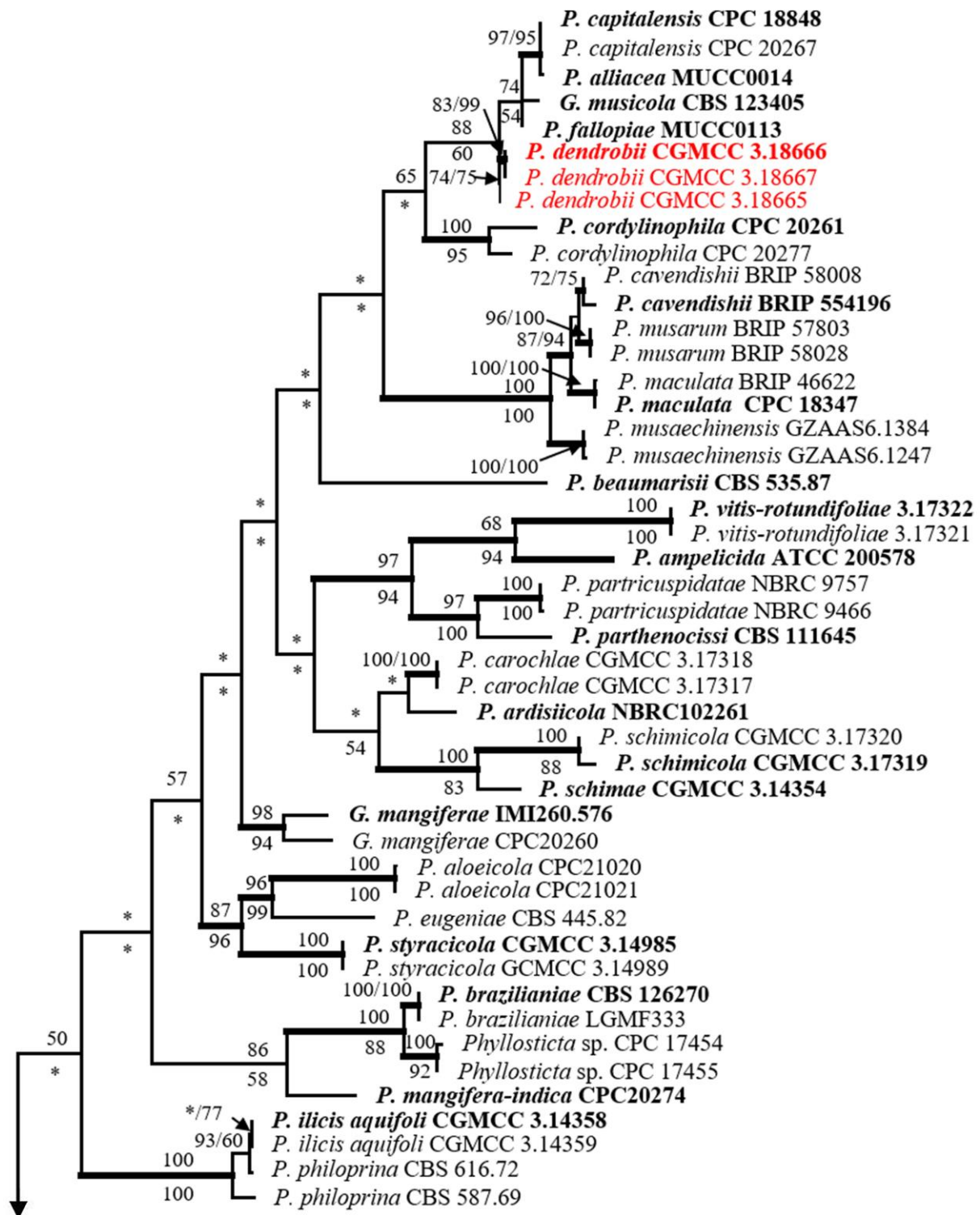
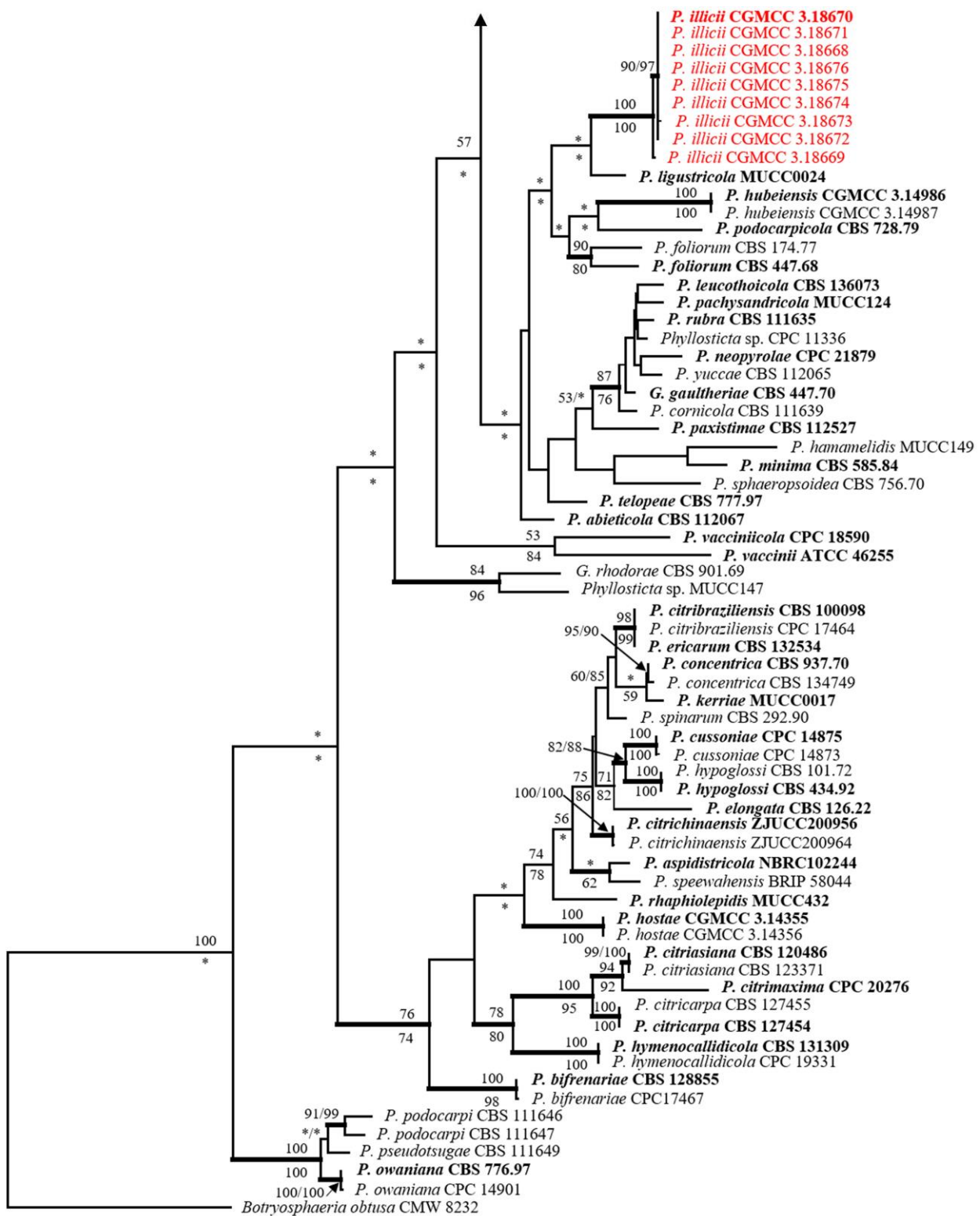


Figure 1 – Maximum parsimony tree generated from sequence analysis of the combined ITS, LSU, *tefl-a*, ACT and GPDH dataset. Bootstrap support values for maximum parsimony (MP) equal to or greater than 50% are shown above the nodes. Bootstrap support values for maximum likelihood (ML) equal to or greater than 50% are shown under the nodes. Bayesian bootstrap (BP) posterior probability scores above 0.90 are indicated as bold branches. Bootstrap support values lower than 50% for MP and ML are indicated with an asterisk. The species characterized in this study are in red, and the ex-type strains are in boldface.



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Figure 1 – (continued).

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, diameter up to 31 mm after 1 week and 68 mm after 2 weeks at 28°C. On MEA surface olivaceous-grey, reverse iron-grey.

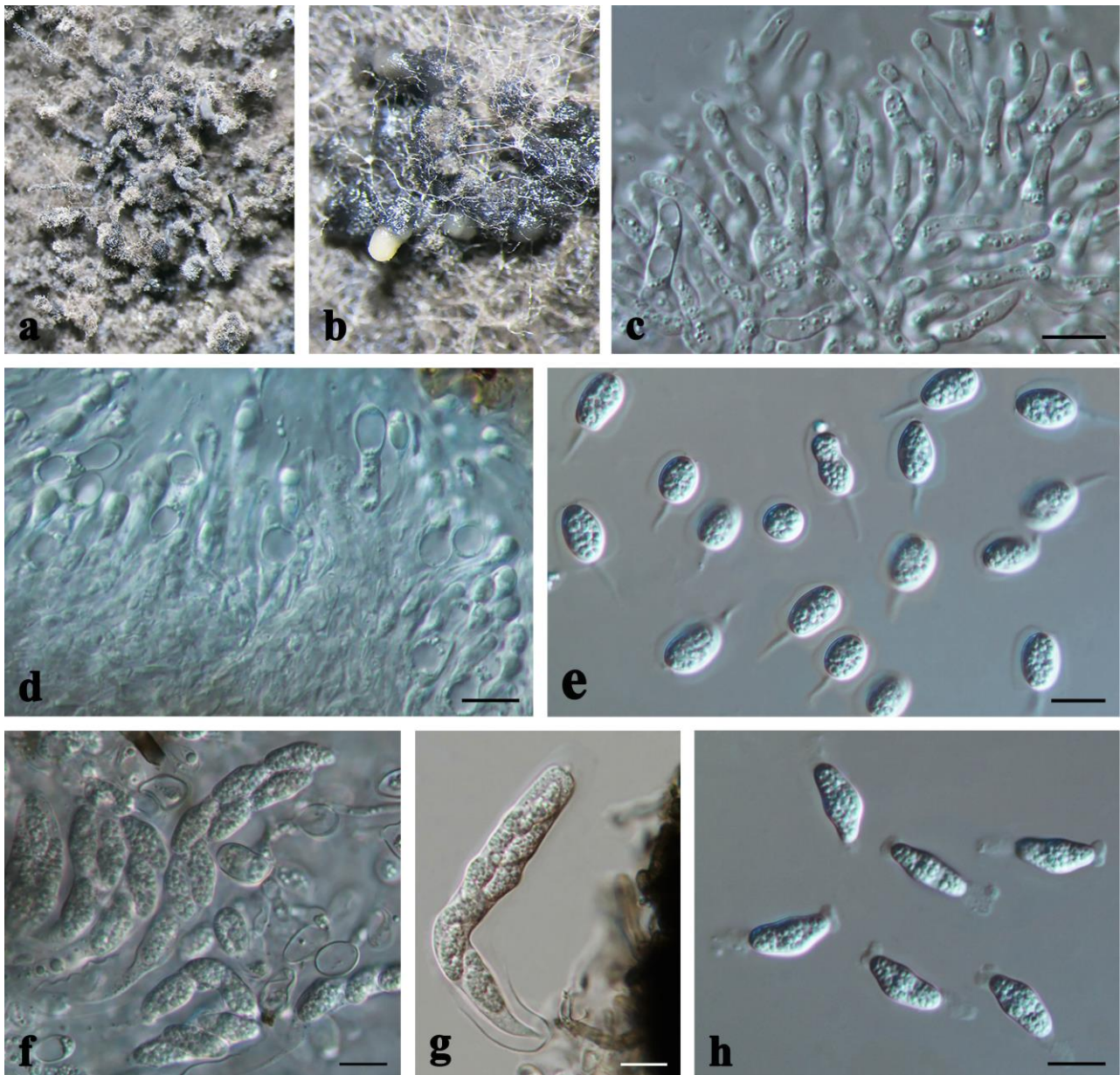


Figure 2 – *Phyllosticta dendrobii* (HMAS255228, holotype). a, b Conidiomata on MEA. c initial conidiogenous cells. d conidia developing on conidiogenous cells. e conidia. f, g ascomata. h ascospores. – Scale bars: c–h = 10 µm.

Specimen examined – CHINA, Jiangxi province, associated from *Dendrobium nobile*, 9 October, 2014, leg. X. Sun (HMAS255228, **holotype**); CGMCC 3.18666 (*ex-type*), CGMCC 3.18665; CGMCC 3.18667.

Notes – The subclade consists of *P. alliacea* Motohashi, Jun. Nishikawa & C. Nakash., *P. capitalensis*, *P. fallopiae* Motohashi, I. Araki & C. Nakash., *Guignardia musicola* Wulandari, L. Cai & K.D. Hyde and *P. dendrobii* received high bootstrap support with *P. dendrobii* basal to all other species (Fig 1). The conidia of *Phyllosticta* are mostly 10–25 µm long (Wikee et al. 2011), while the small conidia of *P. dendrobii* can be easily distinguishable from most reported species of *Phyllosticta*. Morphologically, *P. dendrobii* is comparable with *P. aplectri*, which was reported from *Aplectrum hyemale* (Orchidaceae). Although the conidial dimensions of *P. dendrobii* and *P. aplectri* are similar (5–8 × 4–6 µm vs. (5–)8–9(–10) × 5(–7) µm), the pycnidia of *P. dendrobii* are larger those of *P. aplectri* (up to 280 µm vs. 65–90 µm). *Phyllosticta dendrobii* is the first *Phyllosticta* species reported from *Dendrobium*.

Phyllosticta illicii S. Lin, Y. Zhang ter, sp. nov.

Fig. 3

Mycobank: 821785; Facesoffungi number: FoF03423

Etymology – Named after the host genus from which it was collected, *Illicium verum*.

Conidiomata pycnidial, mostly aggregated in clusters, black, erumpent, globose to clavate or elongated with necks up to 500 μm long, exuding colourless to opaque conidial masses. *Pycnidia* up to 200 μm diam. *Pycnidial wall* of several layers of *textura angularis*, up to 40 μm thick, inner wall of hyaline *textura angularis*. *Ostiole* central, up to 25 μm diam. *Conidiophores* reduced to conidiogenous cells, *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, 7–13 \times 3–5 μm ; proliferating several times percurrently near apex. *Conidia* (12–)13–15(–17) \times 7(–9) μm , solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ovoid to irregularly ellipsoid, enclosed in a thin mucoid sheath, 1 μm thick, and bearing a hyaline apical mucoid appendage, (6–)18–35(–36) \times 1 (–2) μm , flexible, unbranched, tapering towards an acutely rounded tip. After 2 months, mucoid sheath and apical mucoid appendage disappear. *Spermatia* occurring in the same conidioma with conidia, hyaline, smooth, guttulate to granular, bacilliform, 7–13 \times 2–3 μm .

Culture characteristics – Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, diameter up to 33 mm after 1 week and 76 mm after 2 weeks at 28°C. On MEA surface olivaceous-grey, reverse iron-grey.

Specimen examined – CHINA, Guangxi Province, associated from *Illicium verum*, 9 October 2014, leg. X. Sun (HMAS 255230, **holotype**), CGMCC 3.18670 (ex-type), CGMCC3.18668, CGMCC3.18669, CGMCC3.18671, CGMCC3.18672, CGMCC3.18673, CGMCC3.18674, CGMCC3.18675, CGMCC3.18676.

Notes – Phylogenetically, the sub clade comprised of *P. illicii* is well supported, and sibling to other species of *Phyllosticta* (Fig 1). Morphologically, the long apical mucoid appendage is distinguishable from most other species of *Phyllosticta* (mostly shorter than 15 μm , van der Aa 1973, van der Aa & Vanev 2002). *Phyllosticta illicii* is the first *Phyllosticta* species reported from *Illicium*.

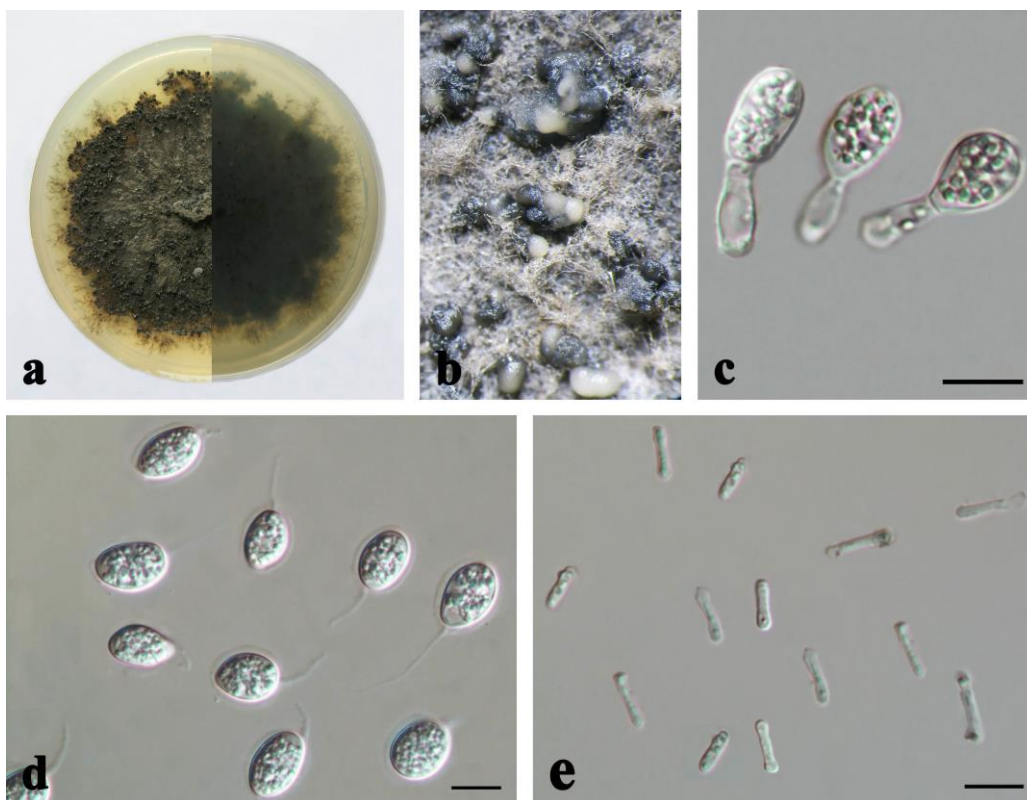


Figure 3 – *Phyllosticta illicii* (HMAS 255230, holotype). a, b Conidiomata on MEA. c Conidia developing on conidiogenous cells. d Conidia. e Spermatia. – Scale bars: c–e = 10 μm .

Discussion

The morphological characteristics of *P. dendrobii* and *P. illicii*, such as aseptate, hyaline conidia that are usually covered by a mucoid layer and bearing a single apical appendage of fit well within *Phyllosticta* well (van der Aa 1973, van der Aa & Vanev 2002). The long apical appendage of *P. illicii* and small conidia of *P. dendrobii* differ from most reported species of *Phyllosticta*.

Both *P. dendrobii* and *P. illicii* were isolated as endophytic fungi from healthy tissues of *Dendrobium nobile* and *Illicium verum* respectively in this study. Several other species of *Phyllosticta* have been reported as endophytes, such as *P. capitalensis*, which is a cosmopolitan endophytic species reported on more than 20 hosts in eight countries (Okane et al. 2001, Baayen et al. 2002, Okane et al. 2003, Glienke et al. 2011). *Phyllosticta bifrenariae* and *P. brazilianiae* were reported as endophytes on *Bifrenaria* (*Orchidaceae*) and *Mangifera indica* (*Anacardiaceae*) respectively in Brazil (Glienke et al. 2011). *Phyllosticta ilicina* and *P. spinarum* were isolated from healthy leaves of *Quercus ilex* (*Fagaceae*) and *Platyclusus orientalis* (*Cupressaceae*) respectively (Collado et al. 1996, Wijeratne et al. 2008). The relationships between hosts and endophytes, however, can be mutualistic, symbiotic, antagonistic or neutral, and may turn pathogenetic during host senescence (Hyde & Soyong 2008, Rodriguez & Redman 2008, Rodriguez et al. 2009, Aly et al. 2011).

Orchid seems a common host for *Phyllosticta*, and several species have been reported from plants of *Orchidaceae*. *Phyllosticta capitalensis* and *P. bifrenariae* were reported on *Stanhopea* and *Bifrenaria* (*Orchidaceae*) respectively in Brazil, and *P. speewahensis* from *Vanda* (*Waxy Blue*, *Orchidaceae*) in Australia (Hennings 1908, Glienke et al. 2011, Shivas et al. 2013). Host association has been used in species identification of *Phyllosticta*, and this has been proved to be unacceptable (van der Aa 2002). For instance, there are six species of *Phyllosticta* associated with *Citrus* spp., namely *P. capitalensis*, *P. citricarpa*, *P. citriasiana* Wulandari, Crous & Gruyter, *P. citrimaxima* S. Wikee, Crous, K.D. Hyde & McKenzie, *P. citribraziliensis* O.L. Pereira, Glienke & Crous and *P. citrichinaensis* X.H. Wang, Chen, Huang, Zhang, K.D. Hyde & H.Y. Li. Of these *P. capitalensis* can be associated with various host plants (Wikee et al. 2013). The host specificity of *Phyllosticta* is not clear yet, while it may depend on the particular species or their life style. Some species that cause plant diseases show host specificity, while the endophytic ones tend to be generalists (Wikee et al. 2013).

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