A phylogenetic re-evaluation of *Phyllosticta* (*Botryosphaeriales*)

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Abstract: *Phyllosticta* is a geographically widespread genus of plant pathogenic fungi with a diverse host range. This study redefines *Phyllosticta*, and shows that it clusters sister to the *Botryosphaeriaceae* (*Botryosphaeriales*, *Dothideomycetes*), for which the older family name *Phyllostictaceae* is resurrected. In moving to a unit nomenclature for fungi, the generic name *Phyllosticta* was chosen over *Guignardia* in previous studies, an approach that we support here. We use a multigene DNA dataset of the ITS, LSU, ACT, TEF and GPDH gene regions to investigate 129 isolates of *Phyllosticta*, representing about 170 species names, many of which are shown to be synonyms of the ubiquitous endophyte *P. capitalensis*. Based on the data generated here, 12 new species are introduced, while epitype and neotype specimens are designated for a further seven species. One species of interest is *P. citrimaxima* associated with tan spot of *Citrus maxima* fruit in Thailand, which adds a fifth species to the citrus black spot complex. Previous morphological studies lumped many taxa under single names that represent complexes. In spite of this *Phyllosticta* is a species-rich genus, and many of these taxa need to be recollected in order to resolve their phylogeny and taxonomy.

Key words: *Botryosphaeriales*, foliar pathogens, *Guignardia*, *Phyllosticta*, *Phyllostictaceae,* Multi-Locus Sequence Typing (MLST), systematics.
Taxonomic novelties: New species – *Phyllosticta abieticola* Wikee & Crous, *P. aloeicola* Wikee & Crous, *P. citrimaxima* Wikee, Crous, K.D. Hyde & McKenzie, *P. leucothoicola* Wikee, Motohashi & Crous, *P. mangifera-indica* Wikee, Crous, K.D. Hyde & McKenzie, *P. neopyrolae* Wikee, Motohashi, Crous, K.D. Hyde & McKenzie, *P. pachysandricola* Wikee, Motohashi & Crous, *P. paxistimae* Wikee & Crous, *P. podocarpicola* Wikee, Crous, K.D. Hyde & McKenzie, *P. rhaphiolepidis* Wikee, C. Nakash. & Crous, *P. rubra* Wikee & Crous, *P. vacciniicola* Wikee, Crous, K.D. Hyde & McKenzie; **New combinations** – *P. foliorum* (Sacc.) Wikee & Crous, *P. philoprina* (Berk. & M.A. Curtis) Wikee & Crous; **Epitypifications (basionyms)** – *P. concentrica* Sacc., *P. cussoniae* Cejp, *P. owaniana* G. Winter; **Neotypifications (basionyms)** – *Phyllosticta cordylinophila* P.A. Young, *Physalospora gregaria* var. *foliorum* Sacc., *Sphaeropsis hypoglossi* Mont., *Sphaeropsis minima* Berk. & M.A. Curtis.

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INTRODUCTION

The genus *Phyllosticta* was introduced by Persoon (1818) with *P. convallariae* (nom. inval., lacking description) designated as the type species (Donk 1968), which is a synonym of *P. cruenta* (van der Aa 1973), which van der Aa & Vanev (2002) cited as type of the genus. Species of *Phyllosticta* are mostly plant pathogens of a broad range of hosts, and responsible for numerous diseases, including leaf and fruit spots. Some of these pathogens cause diseases of significant economic importance, e.g., *P. citricarpa,* the cause of citrus black spot, which is regarded as a quarantine pest in Europe and the USA (Baayen *et al.* 2002, Glienke *et al.* 2011). Other economically important plant pathogenic species include the *P. ampelicida* species complex that causes black rot disease on grapevines (Wicht *et al*. 2012), and the *P. musarum* 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, e.g., *P. capitalensis*. Other species are regarded as saprobes, e.g., *P. carpogena* and *P. ericae* (van der Aa 1973, Baayen *et al*. 2002, van der Aa & Vanev 2002, Glienke *et al*. 2011, Wikee *et al*. 2011). Presently there are approximately 3 340 epithets known for *Phyllosticta* (www.MycoBank.org; accessed August 2013), but many of these reflect old concepts of the genus, and have since been accommodated elsewhere (van der Aa & Vanev 2002). Many species also produce spermatial or sexual states, which in some cases have been named in *Leptodothiorella* and *Guignardia*, respectively (van der Aa 1973).

For many years researchers have confused the generic circumscription of *Phoma* and *Phyllosticta*. Both genera were recognised as pycnidial fungi forming unicellular, hyaline conidia. Allescher (1898) separated the two genera based on the infected part of the plant part, with *Phyllosticta* as foliar pathogens, and *Phoma* on other plant parts. This concept was further refined by Grove (1935) who regarded *Phyllosticta* as a parasite and *Phoma* as saprobe or wound parasite. Seaver (1922) and Grove (1935) separated "*Phyllosticta*" species based on host preference, as was common taxonomic practice in the 20th century. Seaver (1922) described 300 species, and Grove (1935) approximately 150. In both cases the host plant was the main criterion on which species were separated. Indeed, Seaver's classification was largely characterised on spore size on host plants, while Grove arranged species under the alphabetically arranged host genera. Many *Phyllosticta* species were given specific epithets based on the host family, genus or species. For example, *P. iridis* on *Iris versicolor*

(*Iridaceae*), *P. eugeniae* on *Eugenia buxifolia* (*Myrtaceae*), *P. minor* on *Vinca minor* (*Apocynaceae*), etc. (Seaver 1922). For the plant pathogenic *Phyllosticta* species, separation based on host species (or sometimes genus) has proven to be a good method to distinguish species, but this does not hold true for the endophytic or saprobic species.

Viala & Ravaz (1892) introduced *Guignardia* as a replacement name for *Laestadia* Auersw. (1869), which was a later homonym of *Laestadia* Kunth ex Lessing (1832). Viala & Ravaz applied the name only to *Sphaeria bidwellii* (≡ *G. bidwellii*), a species that is different from *L. alnea*, the type species of *Laestadia* Auersw. (Bissett 1986). Petrak (1957) concluded that *G. bidwellii* and related species could be accommodated in *Botryosphaeria,* and Barr (1970, 1972) agreed with Petrak and placed *Guignardia* and *Phyllosticta* in *Botryosphaeria*, and other related species in *Discosphaerina*.

Punithalingam (1974) suggested that the genus *Guignardia* must be confined to only those taxa with *Phyllosticta* morphs as typified by *G. bidwellii* (= *P. ampelicida*, see Zhang *et al*. 2013). He stated that *Botryosphaeria* usually has larger ascomata and ascospores, and also a multilocular stroma, features that distinguish it from *Guignardia*. Van der Aa (1973) also pointed out that these two genera had different growth characteristics in culture. Following molecular studies, Schoch *et al.* (2006) placed *Phyllosticta* in the *Botryosphaeriales.* Since *Botryosphaeria* has been shown to be poly- and paraphyletic, numerous genera have been distinguished in the *Botryosphaeriaceae* (Crous *et al*. 2006, Phillips *et al*. 2008, Liu *et al.* 2012). With the increasing use of molecular data to link asexual and sexual morphs, and the end of dual nomenclature for fungi (Hawksworth *et al.* 2011, Wingfield *et al.* 2012), the oldest, more important and commonly used name, *Phyllosticta*, was chosen over that of *Guignardia* (Glienke *et al*. 2011, Sultan *et al*. 2011, Wikee *et al.* 2011, 2013, Wong *et al*. 2012).

The principal character by which a fungus is recognised as a species of *Phyllosticta* is by the production of pycnidia containing aseptate, hyaline conidia that are usually covered by a mucoid layer and bearing a single apical appendage (van der Aa 1973). However, the mucoid layer and appendage is not necessarily a universal feature, and some species such as *P. colocasiicola*, *P. minima*, and *P. sphaeropsoidea* lack these features. Furthermore, mucoid appendages formed on agar media may disappear with age, or vary in size and shape when the same isolate is compared on different media, e.g., pine needle agar, oatmeal agar, or potato dextrose agar. Presently *Phyllosticta* is circumscribed by pycnidia that are usually globose to subglobose, flattened above, and closely connected with the subepidermal pseudostroma. They are mostly unilocular but may be multilocular. The conidia are commonly hyaline, aseptate, ovoid, obovoid to ellipsoid, or short cylindrical, seldomly pyriform, globose or subglobose, and usually covered by a mucoid layer and bearing a single apical appendage (van der Aa 1973). The sexual morph is characterised by erumpent ascomata that are globose to pyriform in section, often irregularly shaped, unilocular, and with a central ostiole. The peridium is thin, comprising a few layers of angular cells. Asci are 8-spored, bitunicate, clavate to broadly ellipsoid, with a wide, obtusely rounded or slightly square apex, tapering gradually to a small pedicel, and with a well-developed ocular chamber. Ascospores are ellipsoid to limoniform, sometimes slightly elongated, aseptate, hyaline, often multiguttulate or with a large central guttule, and may have mucilaginous polar appendages or a sheath. A spermatial state may form in culture. Spermatia are hyaline, aseptate, cylindrical to dumbbell-shaped with guttules at each end (van der Aa 1973).

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 names described in *Phyllosticta,* and provided a list of 190 accepted epithets, as well as a second list of excluded names that indicated their current disposition if known.

In recent years DNA sequencing of conserved loci has vastly improved our knowledge of fungal phylogeny. Several studies have shown that phylogenetic analysis can resolve the taxonomy and identification of *Phyllosticta* spp. (Baayen *et al.* 2002, Wulandari *et al*. 2009, Glienke *et al*. 2011, Wikee *et al.* 2011). Indeed, new species of *Phyllosticta* are increasingly described based on molecular results (Crous *et al*. 2012, Wang *et al.* 2012, Su & Cai 2012, Wong *et al*. 2012, Zhang *et al*. 2012).

Phyllosticta was placed in the order *Botryosphaeriales* by Schoch *et al*. (2006), who proposed that the *Botryosphaeriaceae* contained both *Botryosphaeria* and *Phyllosticta*, although no support was obtained for this relationship. Crous *et al*. (2006) and Liu *et al*. (2012) also classified *Phyllosticta* in the *Botryosphaeriaceae*. In both studies it was noted that *Phyllosticta* was distinct from other genera in the *Botryosphaeriaceae*, and that these authors eventually expected it to be placed elsewhere. Seaver (1922) used the order *Phyllostictales* and family *Phyllostictaceae* for the genus *Phyllosticta*. The family name *Phyllostictaceae* (as Phyllostictei) was first proposed by Fries (1849) and accepted by Hawksworth & David (1989). This family name is still available for use, and we suggest that *Phyllosticta* again be placed in this family, which is sister to the *Botryosphaeriaceae* (*Botryosphaeriales*).

Although phylogenetic analysis has become a standard approach in fungal identification, phylogenetic studies should combine both molecular and morphological data to help discriminate taxa (Crous & Groenewald 2005, Hyde *et al*. 2010). Suitable type material that can be sequenced is not available for many species of fungi, and thus neo- or epitypification is required in order to create a stable and workable taxonomy. The objectives of this study are: (1) to clarify relationships among species of *Phyllosticta* using multi-gene sequence data [internal transcribed spacer region (ITS), translation elongation factor 1-α gene (TEF1), actin gene (ACT), 28S rRNA gene (LSU) and glyceraldehyde-3-phosphate dehydrogenase gene (GPDH)] combined with morphological characteristics; (2) to provide a phylogenetic backbone for the genus *Phyllosticta*, and (3) to designate neo- or epitype specimens for fungal isolates that correlate well with original type material, thereby fixing the genetic application of these names.

MATERIAL AND METHODS

Isolates

A global collection of 160 strains of *Phyllosticta* associated with both leaf spot diseases and healthy leaves of various host plants were studied (Table 1). All isolates were sequenced and analysed together with sequences downloaded from GenBank. If fruit bodies were present on diseased tissue then a single spore isolation procedure as described by Chomnunti *et al*. (2011) was used to obtain cultures. To obtain isolates of *Phyllosticta* from diseased leaves of host plants when fruit bodies were not present, the leaf surface was cleaned by wiping with 70 % ethanol. Small pieces of

leaf were then cut from the interface between healthy and diseased tissue. These were surface sterilised in 70 % ethanol, washed and plated onto ½ strength potato dextrose agar (½PDA). For isolation of endophytic species, healthy leaves were washed in tap water and wiped with 70 % ethanol. They were then cut into small pieces (about 1 × 1 cm), suspended in 70 % ethanol for 15 min (three times) and washed in distilled water (three times) before placing on ½PDA. All plates were incubated at 27 ºC for 1 wk and observed daily. The growing tips of hyphae of *Phyllosticta* colonies that developed were cut out and transferred to fresh PDA plates. Isolates are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and in the working collection of Pedro Crous housed at the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (CPC). Other fungal isolates of representative *Phyllosticta* spp. were obtained from the CBS and added to this study (Table 1).

Morphology

Growth rates, culture characteristics, and morphology of the isolates were determined at 27 °C. Sporulation was induced by growth on pine needle agar (PNA) (Smith *et al*. 1996) and synthetic nutrient-poor agar (SNA) under near UV-light. Colony colour and growth rate were established on PDA, malt extract agar (MEA) and oatmeal agar (OA) according to Crous *et al.* (2009). Culture characteristics were assessed, and the colour of upper and lower sides of cultures was determined after 14 d in the dark at 27 °C. Colony colour on MEA, OA and PDA was determined with the colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.mycobank.org; Crous *et al*. 2004).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from cultures grown on MEA for 2–3 d using the UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc., California, USA) according to the manufacturer's protocol. Partial regions of five loci were amplified including actin (ACT) using primers ACT-512F and ACT-783R (Carbone & Kohn 1999); the internal transcribed spacer region (ITS) of the nuclear rDNA using primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990), the 28S large subunit nrDNA (LSU) using primers LROR (Moncalvo *et al.* 1995) and LR5 (Vilgalys & Hester 1990); translation elongation factor 1-α using primers EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell *et al.* 1998); and glyceraldehyde-3-phosphate dehydrogenase (GPDH) using primers Gpd1-LM and Gpd2-LM (Myllys *et al*. 2002). For *Phyllosticta citricarpa* isolates, GPDH was amplified using primers Gpd1 (Guerber *et al.* 2003) and GPDHR2 (Glienke *et al.* 2011). The PCR reaction mixtures and cycling conditions were followed as described by Glienke *et al.* (2011).

Amplified fragments were sequenced in both directions using the same primers pairs used for amplification. For this purpose, the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems™, Foster City, CA, USA) containing AmpliTaq DNA Polymerase was used. The amplified products were analysed on an automated 3730xl DNA analyzer (Life Technologies Europe BV, Applied Biosystems™, Bleiswijk, The Netherlands). Sequences generated were assembled and aligned using MEGA v. 5.05 (Tamura *et al*. 2011) and MAFFT v. 6 (http://mafft.cbrc.jp/alignment/server/), respectively. The sequences were manually aligned as necessary.

Molecular phylogeny

Phylogenetic analyses were based on both Maximum Parsimony (MP) and Bayesian inference (BI). The MP analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 1 000 random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on "best trees" only with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony and bootstrap analysis (Hillis & Bull 1993) was based on 1 000 replications.

For BI, the best evolutionary models for each partition were determined using MrModeltest (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.1. (Ronquist & Huelsenbeck 2003) was used to generate phylogenetic trees under optimal criteria per partition. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies came below 0.01 with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the "burn-in" phase and posterior probabilities (PP) determined from the remaining trees.

RESULTS

Phylogenetic relationships were determined for the ITS and ACT gene sequences of 160 *Phyllosticta* strains (including one outgroup). The combined partial dataset of *Phyllosticta* comprised 883 characters (including gaps), of which 341 characters are constant, and 150 characters are variable and parsimony-uninformative. Parsimony analysis generated 1 000 trees, one of which is presented as shown in Fig. 1 (TL = 2099, CI = 0.481, RI = 0.898, RC = 0.432). The phylogenetic tree of the ITS and ACT region resolved 46 clades (see Table 1 for details). The Bayesian consensus tree confirmed the tree topology and bootstrap support of the strict consensus tree obtained with MP.

A second analysis including all isolates for which a complete dataset were available (129 strains including the outgroup) was run based on ITS, LSU, ACT, TEF1 and GPDH (Table 1). The combined partial dataset of *Phyllosticta* comprised 2 577 characters (including gaps), of which 1 547 characters are constant, 296 characters are variable and parsimony-uninformative. Parsimony analysis generated 1 000 trees, of which one is shown in Fig. 2 (TL = 3173, CI = 0.517, RI = 0.906, RC = 0.468). The phylogenetic tree using combined multi-gene data resolved 33 clades (see Table 1 for details). The Bayesian consensus tree confirmed the tree topology and bootstrap support of the strict consensus tree obtained with MP.

Taxonomy

Phyllosticta is distinct from members of the *Botryosphaeriaceae* in cultural characteristics (slow growing, black erumpent colonies vs. grey, fluffy, fast-growing colonies). Morphologically it is also distinct, having conidia encased in a mucoid sheath and often with an apical appendage. The sexual morph has ascomata unilocular, ascospores frequently with mucoid caps, and hamathecial tissue disintegrating at maturity, which collectively differs from those in the *Botryosphaeriaceae*. *Phyllosticta* is also phylogenetically

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

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
Botryosphaeria obtusa	CMW 8232	Conifers	South Africa	AY972105	-	DQ280419	AY972111	-
Guignardia mangiferae	CPC 17469	*Cymbidium* sp.	India	KF206189	-	-	KF289285	-
	IMI 260576	*Mangifera indica*	India	JF261459	KF206222	JF261501	JF343641	JF343748
	CPC 20260	*Arecaceae*	Thailand	KF206193	KF206243	KF289187	KF289294	KF289114
G. rhodorae	CBS 901.69	*Rhododendron* sp.	Netherlands	KF206174	KF206292	KF289230	KF289256	KF289166
Phyllosticta abieticola	**CBS 112067**	*Abies concolor*	Canada	KF170306	EU754193	-	KF289238	-
P. aloeicola	CPC 21020	*Aloe ferox*	South Africa	KF154280	KF206214	KF289193	KF289311	KF289124
	CPC 21021	*Aloe ferox*	South Africa	KF154281	KF206213	KF289194	KF289312	KF289125
	CPC 21022	*Aloe ferox*	South Africa	KF154282	KF206212	KF289195	KF289313	KF289126
	CPC 21023	*Aloe ferox*	South Africa	KF154283	KF206211	KF289196	KF289314	KF289127
	CPC 21024	*Aloe ferox*	South Africa	KF154284	KF206210	KF289197	KF289315	KF289128
P. beaumarisii	**CBS 535.87** = IMI 298910	*Muehlenbekia adpressa*	Australia	AY042927	KF306229	KF289170	KF306232	KF289074
P. bifrenariae	**CBS 128855** = VIC30556	*Bifrenaria harrisoniae*	Brazil	JF343565	KF206209	JF343586	JF343649	JF343744
	CPC 17467	*Bifrenaria harrisoniae*	Brazil	KF170299	KF206260	KF289207	KF289283	KF289138
P. brazillianiae	**CBS 126270** = LGMF330	*Mangifera indica*	Brazil	JF343572	KF206217	JF343593	JF343656	JF343758
	LGMF 333	*Mangifera indica*	Brazil	JF343574	KF206216	JF343595	JF343658	JF343760
	LGMF 334	*Mangifera indica*	Brazil	JF343566	KF206215	JF343587	JF343650	JF343752
P. capitalensis	CBS 173.77	*Citrus aurantiifolia*	New Zealand	KF206179	KF306231	FJ538393	KF289244	KF289100
	CBS 226.77	*Paphiopedilum callosum*	Germany	FJ538336	KF206289	FJ538394	FJ538452	JF343718
	CBS 356.52	*Ilex* sp.	Unknown	FJ538342	KF206300	FJ538400	FJ538458	KF289087
	CBS 100175	*Citrus* sp.	Brazil	FJ538320	KF206327	FJ538378	FJ538436	JF343699
	CBS 101228	*Nephelium lappaceum*	Hawaii	FJ538319	KF206325	FJ538377	FJ538435	KF289086
	CBS 114751	*Vaccinium* sp.	New Zealand	EU167584	EU167584	FJ538407	FJ538465	KF289088
	CBS 115046	*Myracrodruon urundeuva*	Brazil	FJ538322	KF206319	FJ538380	FJ538438	KF289082
	CBS 115047	*Aspidosperma polyneuron*	Brazil	FJ538323	KF206318	FJ538381	FJ538439	KF289077
	CBS 115049	*Bowdichia nitida*	Brazil	FJ538324	KF206317	FJ538382	FJ538440	KF289084
	CBS 117118	*Musa acuminata*	Indonesia	FJ538339	JQ743603	FJ538397	FJ538455	KF289090
	CBS 119720	*Musa acuminata*	Hawaii	KF206178	KF206316	FJ538398	KF289240	KF289098
	CBS 120428	*Sansevieria* sp.	Netherlands	JN692544	KF206315	JN692532	JN692520	JN692509
	CBS 123373	*Musa paradisiaca*	Thailand	FJ538341	JQ743604	FJ538399	FJ538457	JF343703
	CBS 123404	*Musa paradisiaca*	Thailand	FJ538333	JQ743601	FJ538391	FJ538449	KF289095
	CBS 128856	*Stanhopea* sp.	Brazil	JF261465	KF206304	JF261507	JF343647	JF343776
	CPC 11337	*Eucalyptus grandis*	Brazil	KF206180	-	-	KF289259	-
	CPC 11867	*Acacia crassicarpa*	Thailand	KF206181	KF206283	KF289184	KF289260	KF289108
	CPC 12157	*Acacia crassicarpa*	Thailand	KF206182	-	-	KF289261	-
	CPC 13987	*Protea repens*	Portugal	KF206183	KF206281	KF289176	KF289263	KF289083
	CPC 14609	*Zyzygium* sp.	Madagascar	KF206184	KF206280	KF289175	KF289264	KF289081
	CPC 16590	*Citrus limon*	Argentina	KF206185	KF206272	KF289177	KF289271	KF289091
	CPC 16591	*Citrus limon*	Argentina	KF206186	KF206271	KF289179	KF289272	KF289093
	CPC 16592	*Citrus limon*	Argentina	KF206187	KF206270	KF289178	KF289273	KF289092
	CPC 17468	*Cymbidium* sp.	Brazil	KF206188	KF206259	KF289189	KF289284	KF289120
	CPC 17748	*Heliconia* sp.	Thailand	KF206190	KF206258	KF289180	KF289286	KF289096
	CPC 18848	*Stanhopea graveolens*	Brazil	JF261465	KF206255	JF261507	KF289289	JF343776
	CPC 20251	Wild plant	Thailand	KC291333	KF206252	KC342553	KC342530	KF289101
	CPC 20252	*Punica granatum*	Thailand	KC291334	KF206251	KC342554	KC342531	KF289097
	CPC 20253	*Scheffera venulosa*	Thailand	KF206192	KF206250	KF289181	KF289293	KF289102
	CPC 20254	*Saccharum officinarum*	Thailand	KC291335	KF206249	KC342555	KC342532	KF289103
	CPC 20255	*Arecaceae*	Thailand	KC291336	KF206248	KC342556	KC342533	KF289115
	CPC 20256	*Ophiopogon japonicus*	Thailand	KC291337	KF206247	KC342557	KC342534	KF289089
	CPC 20257	*Ficus benjamina*	Thailand	KC291338	KF206246	KC342558	KC342535	KF289099

Table 1. (Continued).

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
	CPC 20258	*Ophiopogon japonicus*	Thailand	KC291339	KF206245	KC342559	KC342536	KF289094
	CPC 20259	*Orchidaceae*	Thailand	KC291340	KF206244	KC342560	KC342537	KF289104
	CPC 20263	*Magnoliaceae*	Thailand	KC291341	KF206241	KC342561	KC342538	KF289085
	CPC 20265	*Euphobiaceae*	Thailand	KF206194	KF206239	KF289182	KF289297	KF289105
	CPC 20266	*Polyscias* sp.	Thailand	KC291342	KF206238	KC342562	KC342539	KF289109
	CPC 20267	*Baccaurea ramiflora*	Thailand	KF206195	KF206237	KF289173	KF306233	KF289078
	CPC 20268	*Hibiscus syriacus*	Thailand	KC291343	KF206236	KC342563	KC342540	KF289117
	CPC 20269	*Ophiopogon japonicus*	Thailand	KC291344	KF206235	KC342564	KC342541	KF289118
	CPC 20270	*Tectona grandis*	Thailand	KC291345	KF206234	KC342565	KC342542	KF289110
	CPC 20271	*Crinum asiaticum*	Thailand	KF206196	KF206233	KF289183	KF289298	KF289106
	CPC 20272	*Orchidaceae*	Thailand	KC291346	KF206232	KC342566	KC342543	KF289079
	CPC 20274	*Mangifera indica*	Thailand	KF206197	KF206231	KF289188	KF289299	KF289119
	CPC 20275	*Polyalthia longifolia*	Thailand	KC291347	KF206230	KC342567	KC342544	KF289107
	CPC 20278	*Euphorbia milii*	Thailand	KC291348	KF206227	KC342568	KC342545	KF289113
	CPC 20423	*Philodendron* sp.	Thailand	KC291349	KF206226	KC342569	KC342546	KF289116
	CPC 20508	*Ixora chinensis*	Thailand	KF206198	KF206225	KF289185	KF289302	KF289111
	CPC 20509	*Cordyline fruticosa*	Thailand	KF206199	KF206224	KF289186	KF289303	KF289112
	CPC 20510	*Pyrrosia adnascens*	Thailand	KF206200	KF206223	KF289174	KF289304	KF289080
	CPC 21035	*Citrus* sp.		KF206201	-	-	KF289305	-
	LGMF 219	*Citrus sinensis*	Brazil	KF206202	KF206220	JF261490	KF289306	JF343737
	LGMF 220	*Citrus sinensis*	Brazil	KF206203	KF206219	JF261488	KF289307	JF343735
	LGMF 222	*Citrus sinensis*	Brazil	KF206204	KF206218	JF261492	KF289308	JF343739
P. citriasiana	**CBS 120486**	*Citrus maxima*	Thailand	FJ538360	KF206314	FJ538418	FJ538476	JF343686
	CBS 120487	*Citrus maxima*	China	FJ538361	KF206313	FJ538419	FJ538477	JF343687
	CBS 120488	*Citrus maxima*	Thailand	JN692545	KF206312	JN692533	JN692521	KF289144
	CBS 123370	*Citrus maxima*	Vietnam	FJ538355	KF206310	FJ538413	FJ538471	JF343689
	CBS 123371	*Citrus maxima*	Vietnam	FJ538356	KF206309	FJ538414	FJ538472	JF343690
	CBS 123372	*Citrus maxima*	Vietnam	FJ538357	KF206308	FJ538415	FJ538473	KF289145
P. citribraziliensis	**CBS 100098**	*Citrus limon*	Brazil	FJ538352	KF206221	FJ538410	FJ538468	JF343691
	CPC 17464	*Citrus* sp.	Brazil	KF170300	KF206263	KF289224	KF289280	KF289159
	CPC 17465	*Citrus* sp.	Brazil	KF170301	KF206262	KF289225	KF289281	KF289160
	CPC 17466	*Citrus* sp.	Brazil	KF170302	KF206261	KF289226	KF289282	KF289161
P. citricarpa	CBS 102374	*Citrus aurantium*	Brazil	FJ538313	KF206324	GU349053	FJ538429	JF343679
	CBS 120489	*Citrus sinensis*	Brazil	FJ538315	KF206311	FJ538373	FJ538431	KF289150
	CBS 127454	*Citrus limon*	Australia	JF343583	KF206306	JF343604	JF343667	JF343771
	CBS 127452	*Citrus reticulata*	Australia	JF343581	KF206307	JF343602	KF289241	JF343769
	CBS 127455	*Citrus sinensis*	Australia	JF343584	KF206305	JF343605	JF343668	JF343772
	CBS 122482	*Citrus sinensis*	Zimbabwe	FJ538317	KF306230	FJ538375	KF289265	KF289146
	CPC 16586	*Citrus limon*	Argentina	KF170293	KF206274	KF289220	KF289269	KF289155
	CPC 16587	*Citrus limon*	Argentina	KF170294	KF206273	KF289219	KF289270	KF289154
	CPC 16603	*Citrus limon*	Uruguay	KF170295	KF206269	KF289213	KF289274	KF289147
	CPC 16604	*Citrus limon*	Uruguay	KF206191	-	-	KF289292	-
	CPC 16605	*Citrus limon*	Uruguay	KF170296	KF206268	KF289214	KF289275	KF289148
	CPC 16606	*Citrus limon*	Uruguay	KF170297	KF206267	KF289215	KF289276	KF289149
	CPC 16609	*Citrus* sp.	Argentina	KF170298	KF206266	KF289217	KF289277	KF289152
	CPC 16149	*Citrus* sp.	Argentina	KF170290	KF206277	KF289216	KF289266	KF289151
	CPC 16151	*Citrus* sp.	South Africa	KF170291	KF206276	KF289221	KF289267	KF289156
	CPC 16152	*Citrus* sp.	South Africa	KF170292	KF206275	KF289218	KF289268	KF289153
P. citrichinaensis	**ZJUCC 200956**	*Citrus reticulata*	China	JN791620	-	JN791459	JN791533	-
	ZJUCC 200964	*Citrus maxima*	China	JN791611	-	JN791461	JN791535	-

Table 1. (Continued).

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
	ZJUCC 2010150	*Citrus maxima*	China	JN791662	-	JN791514	JN791582	-
	ZJUCC 2010152	*Citrus sinensis*	China	JN791664	-	JN791515	JN791589	-
P. citrimaxima	**CPC 20276** = MFLUCC10-0137 = CBS 136059	*Citrus maxima*	Thailand	KF170304	KF206229	KF289222	KF289300	KF289157
P. concentrica	**CBS 937.70**	*Hedera helix*	Italy	FJ538350	KF206291	FJ538408	KF289257	JF411745
	CBS 134749 = CPC 18842	*Hedera* sp.	Spain	KF170310	KF206256	KF289228	KF289288	KF289163
P. cordylinophila	CPC 21880 = MUCCJ 521	*Cordyline fruticosa*	Japan	AB454357	AB454357	-	AB704244	-
	CPC 20261 = MFLUCC10-0166 = WK024	*Cordyline fruticosa*	Thailand	KF170287	KF206242	KF289172	KF289295	KF289076
	CPC 20277 = MFLUCC12-0014 = WK048	*Cordyline fruticosa*	Thailand	KF170288	KF206228	KF289171	KF289301	KF289075
P. cornicola	CBS 111639	*Cornus florida*	USA	KF170307	-	-	KF289234	-
P. cussonia	CPC 13812	*Cussonia* sp.	South Africa	KF170311	KF206282	KF289223	KF289262	KF289158
	CPC 14873	*Cussonia* sp.	South Africa	JF343578	KF206279	JF343599	JF343662	JF343764
	CPC 14875	*Cussonia* sp.	South Africa	JF343579	KF206278	JF343600	JF343663	JF343765
P. elongata	**CBS 126.22**	*Oxycoccus macrocarpos*	USA	FJ538353	AB095508	FJ538411	FJ538469	KF289164
P. ericarum	**CBS 132534** = CPC 19744	*Erica gracilis*	South Africa	KF206170	KF206253	KF289227	KF28291	KF289162
P. eugeniae	CBS 445.82	*Eugenia aromatica*	Indonesia	AY042926	KF206288	KF289208	KF289246	KF289139
P. foliorum	CBS 174.77	*Cryptomeria japonica*	USA	KF170308	KF206290	KF289200	KF289245	KF289131
	CBS 447.68	*Taxus baccata*	Netherlands	KF170309	KF206287	KF289201	KF289247	KF289132
P. gaultheriae	**CBS 447.70**	*Gaultheria humifusa*	USA	JN692543	KF206298	JN692531	KF289248	JN692508
P. hamamelidis	MUCC 149	*Hamamelis japonica*	Japan	KF170289	-	-	KF289309	-
P. hostae	**CGMCC 3.14355**	*Hosta plantaginea*	China	JN692535	-	JN692523	JN692511	JN692503
	CGMCC 3.14356	*Hosta plantaginea*	China	JN692536	-	JN692524	JN692512	JN692504
	CGMCC 3.14357	*Hosta plantaginea*	China	JN692537	-	JN692525	JN692513	JN692505
P. hubeiensis	**CGMCC 3.14986**	*Viburnum odoratissimim*	China	JX025037	-	JX025042	JX025032	JX025027
	CGMCC 3.14987	*Viburnum odoratissimim*	China	JX025038	-	JX025043	JX025033	JX025028
	CGMCC 3.14988	*Viburnum odoratissimim*	China	JX025039	-	JX025044	JX025034	JX025029
P. hymenocallidicola	**CBS 131309**	*Hymenocallis littoralis*	Australia	JQ044423	JQ044443	KF289211	KF289242	KF289142
	CPC 19331	*Hymenocallis littoralis*	Australia	KF170303	KF206254	KF289212	KF289290	KF289143
P. hypoglossi	CBS 101.72	*Ruscus aculeatus*	Italy	FJ538365	KF206326	FJ538423	FJ538481	JF343694
	CBS 167.85	*Ruscus hypoglossum*	Italy	FJ538366	KF206302	FJ538424	FJ538482	JF343696
	CBS 434.92	*Ruscus aculeatus*	Italy	FJ538367	KF206299	FJ538425	FJ538483	JF343695
P. ilicis-aquifolii	**CGMCC 3.14358**	*Ilex aquifolium*	China	JN692538	-	JN692526	JN692514	-
	CGMCC 3.14359	*Ilex aquifolium*	China	JN692539	-	JN692527	JN692515	-
	CGMCC 3.14360	*Ilex aquifolium*	China	JN692540	-	JN692528	JN692516	-
P. leucothoicola	**MUCC 553 = CBS 136073**	*Leucothoe catesbaei*	Japan	AB454370	AB454370	-	KF289310	-
P. mangifera-indica	**CPC 20274** = MFLUCC10-0029	*Mangifera indica*	Thailand	KF170305	KF206240	KF289190	KF289296	KF289121
P. minima	**CBS 585.84** = IFO 32917	*Acer rubrum*	USA	KF206176	KF206286	KF289204	KF289249	KF289135
P. neopyrolae	**CPC 21879** = MUCC 125	*Pyrola asarifolia*	Japan	AB454318	AB454318	-	AB704233	-
P. owaniana	**CBS 776.97** = CPC 1009	*Brabejum stellatifolium*	South Africa	FJ538368	KF206293	FJ538426	KF289254	JF343767
	CPC 14901	*Brabejum stellatifolium*	South Africa	JF261462	KF206303	JF261504	KF289243	JF343766
P. pachysandricola	**MUCC 124** = NBRC 102276	*Pachysandra terminalis*	Japan	AB454317	AB454317	-	AB704232	-
P. paxistimae	**CBS 112527**	*Paxistima mysinites*	USA	KF206172	KF206320	KF289209	KF289239	KF289140
P. philoprina	CBS 587.69	*Ilex aquifolium*	Spain	KF154278	KF206297	KF289206	KF289250	KF289137
	CBS 616.72	*Ilex aquifolium*	Germany	KF154279	KF206296	KF289205	KF289251	KF289136
P. podocarpicola	**CBS 728.79**	*Podocarpus maki*	USA	KF206173	KF206295	KF289203	KF289252	KF289134

Table 1. (Continued).

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	LSU	TEF1	ACT	GPDH
P. podocarpi	CBS 111646	*Podocarpus falcatus*	South Africa	AF312013	KF206323	KC357671	KC357670	KF289169
	CBS 111647	*Podocarpus lanceolata*	South Africa	KF154276	KF206322	KF289232	KF289235	KF268168
P. pseudotsugae	CBS 111649	*Pseudotsuga menziesii*	USA	KF154277	KF206321	KF289231	KF289236	KF289167
P. rhaphiolepidis	**MUCC 432**	*Rhaphiolepis indica*	Japan	DQ632660	-	DQ632724	AB704242	-
P. rubra	**CBS 111635**	*Acer rubrum*	USA	KF206171	EU754194	KF289198	KF289233	KF289129
P. sphaeropsoidea	CBS 756.70 = IFO 32905	*Aesculus hippocastanum*	Germany	AY042934	KF206294	KF289202	KF289253	KF289133
P. spinarum	CBS 292.90	*Chamaecyparis pisifera*	France	JF343585	KF206301	JF343606	JF343669	JF343773
P. styracicola	**CGMCC 3.14985**	*Styrax gradiflorus*	China	JX052040	-	JX025045	JX025035	JX025030
	CGMCC 3.14989	*Styrax gradiflorus*	China	JX052041	-	JX025046	JX025036	JX025031
P. telopeae	**CBS 777.97**	*Telopea speciosissima*	Tasmania	KF206205	KF206285	KF289210	KF289255	KF289141
P. vacciniicola	**CPC 18590**	*Vaccinium macrocarpum*	USA	KF170312	KF206257	KF289229	KF289287	KF289165
P. yuccae	CBS 112065	*Yucca elephantipes*	USA	KF206175	-	-	KF289237	-
Phyllosticta sp.	CPC 11336	*Eucalyptus globulus*	Spain	KF206177	KF206284	KF289199	KF289258	KF289130
	MUCC 147	*Rhododendron keiskei*	Japan	AB454319	AB454319	-	AB704234	-
	CPC 17454	*Mangifera indica*	Brazil	KF206206	KF206265	KF289192	KF289278	KF289123
	CPC 17455	*Mangifera indica*	Brazil	KF206207	KF206264	KF289191	KF289279	KF289122

[1]CPC: Culture collection of P.W. Crous, housed at CBS; IFO: Institute For Fermentation, Osaka, Japan; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; LGMF: Culture collection of Laboratory of Genetics of Microorganisms, Federal University of Parana, Curitiba, Brazil; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; ZJUCC: Zhejiang University Culture Collection, China; MFLUCC: Mae Fah Luang University Culture Collection; CGMCC: China, General Microbiological Culture Collection, Beijing, China; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie prefecture, Japan. Type and ex-type cultures are in bold.
[2]ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: large subunit 28S nrDNA; TEF1: partial translation elongation factor 1-α gene; ACT: partial actin gene; GPDH: partial glyceraldehyde-3-phosphate dehydrogenase gene.

supported as distinct from members of the *Botryosphaeriaceae* (see Slippers *et al*. 2013, this volume), we choose to place it in the *Phyllostictaceae* that was originally erected to accommodate this genus.

Phyllostictaceae Fr. (as "Phyllostictei"), Summa veg. Scand., Section Post. (Stockholm): 420. 1849.

Foliicolous, plant pathogenic, endophytic or saprobic. *Ascomata* pseudothecial, separate to gregarious, globose, brown to black, with a central ostiole. *Asci* bitunicate, fissitunicate, clavate to subcylindrical, 8-spored, fasciculate, stipitate, with an ocular chamber. *Pseudoparaphyses* mostly absent at maturity (see Sultan *et al*. 2013), filamentous, branched, septate when present. *Ascospores* bi- to triseriate, hyaline, aseptate, ellipsoid-fusoid to limoniform, smooth-walled, usually with mucilaginous caps at ends, or surrounded by a mucilaginous sheath. *Asexual morph*: *Conidiomata* pycnidial globose, dark brown, separate to aggregated, with a central ostiole; wall of 3–6 layers of brown *textura angularis*. *Conidiogenous cells* lining the inner wall, hyaline, smooth, subcylindrical to ampulliform or doliiform, proliferating percurrently near apex, frequently covered in mucilaginous sheath. *Conidia* hyaline, smooth, ellipsoid-fusoid to obovoid or ovoid, aseptate, smooth-walled, guttulate or granular, frequently surrounded by a mucilaginous sheath, and bearing a single mucilaginous apical appendage.

Type genus: *Phyllosticta* Pers.

Phyllosticta Pers., Traité sur les Champignons Comestibles (Paris): 55. 147. 1818.

Conidiomata and *spermatogonia* pycnidial, immersed, subepidermal to erumpent, unilocular, rarely multilocular, glabrous, ostiolate, dark brown to black; ostiole circular to oval; pycnidial wall of thick-walled, dark brown *textura angularis*, with inner layers of hyaline to pale brown, thin-walled *textura prismatica* to *angularis*. *Conidiophores* lining the cavity of the conidioma, reduced to conidiogenous cells, invested in mucus. *Conidiogenous cells* discrete, producing macroconidia and spermatia (also produced in separate spermatogonia), ampulliform, lageniform, doliiform to subcylindrical, hyaline, smooth, proliferating several times percurrently near the apex, invested in a mucoid layer. *Spermatogenous cells* ampulliform to lageniform or subcylindrical, hyaline smooth, phialidic. *Conidia* ellipsoid-fusoid to obovoid or ovoid, rarely subcylindrical, aseptate, broadly rounded at the apex, often tapering strongly toward the base, unicellular, hyaline, smooth-walled, guttulate to granular, often enclosed in a persistent mucilaginous sheath, and bearing an unbranched, tapering, straight to curved, mucoid apical appendage. *Spermatia* hyaline, smooth, granular, subcylindrical or dumbbell-shaped, with rounded or blunt ends. *Ascomata* pseudothecial, separate to gregarious, globose to subglobose, brown to black, unilocular with a central ostiole. *Asci* bitunicate, fissitunicate, clavate to subcylindrical, 8-spored, fasciculate, stipitate, with an ocular chamber. *Pseudoparaphyses* mostly absent at maturity, filamentous, branched, septate when present. *Ascospores* bi- to triseriate, hyaline, guttulate to granular, aseptate, ellipsoid, ellipsoid-fusoid to limoniform, smooth-walled, usually with mucilaginous caps at ends, or surrounded by a mucilaginous sheath.

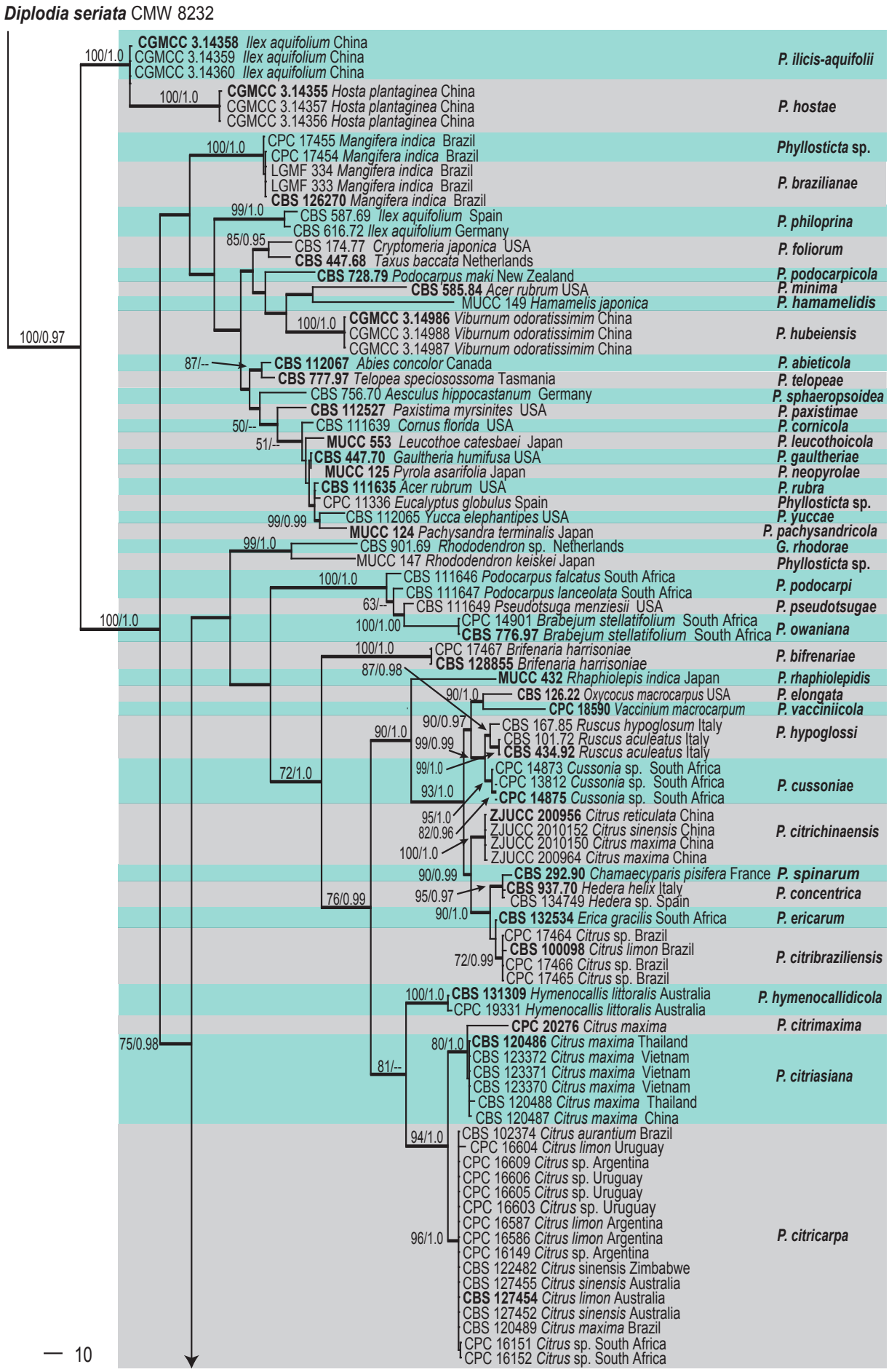

Fig. 1. One of 1 000 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined ACT and ITS sequence alignments. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. Branches present in both the consensus trees of the MP and BI are thickened. Substrate and country of origin, where known, are indicated next to the strain numbers. The tree was rooted to *Diplodia seriata* (CMW 8232)

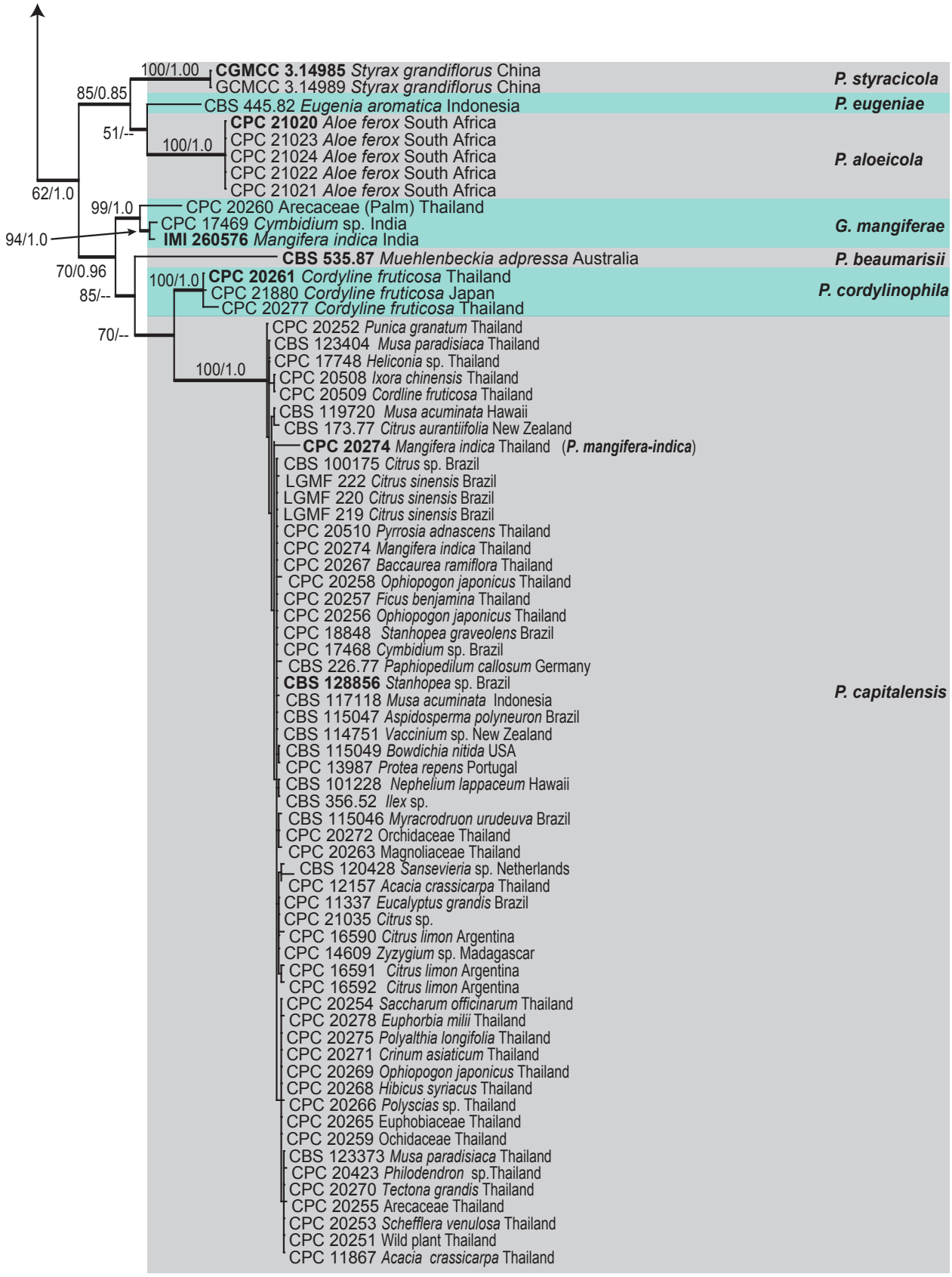

Fig. 1. (Continued).

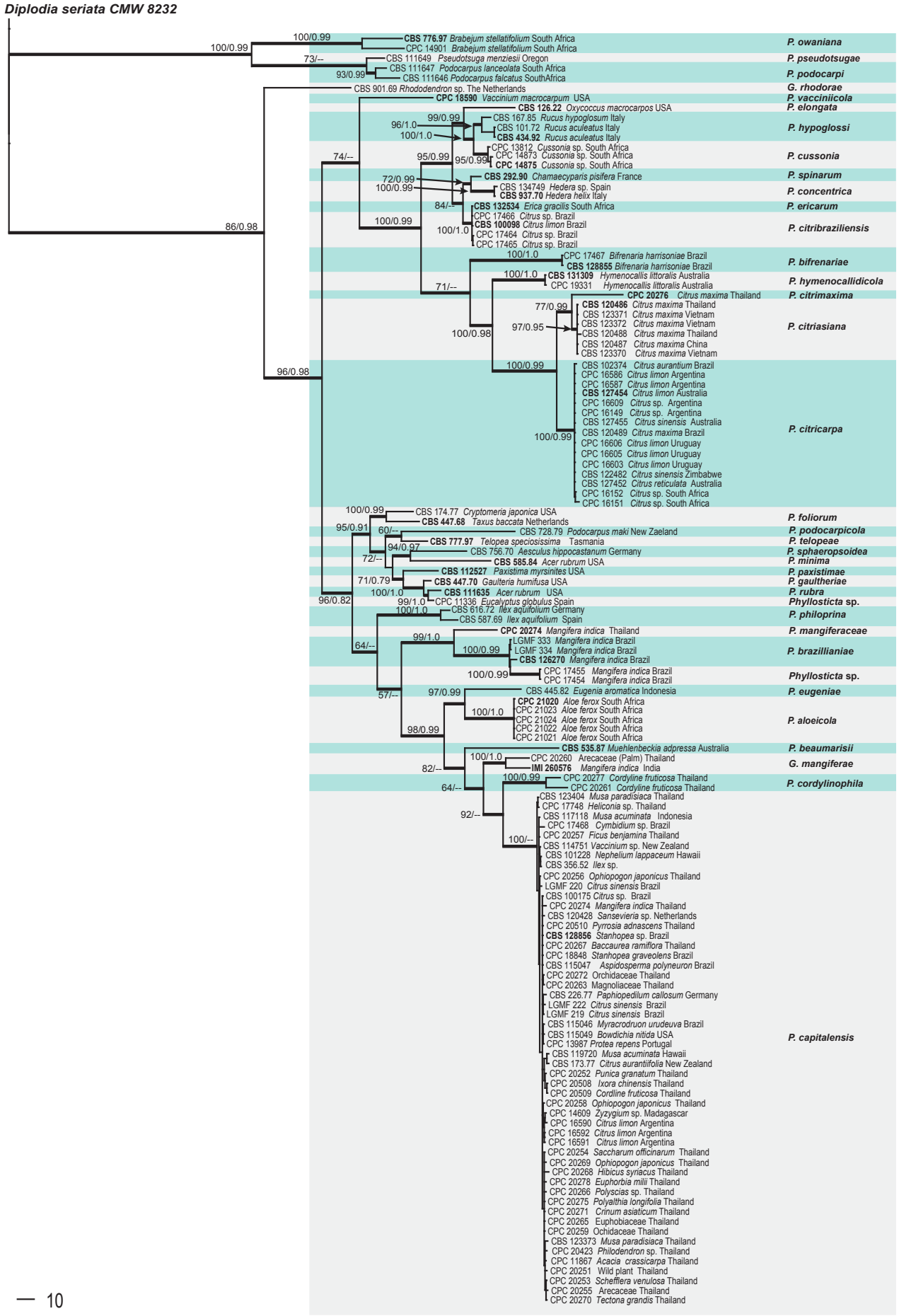

Fig. 2. One of 1 000 equally most parsimonious trees obtained from a heuristic search with 1 000 random taxon additions of the combined ACT, GPDH, ITS, LSU and TEF1 sequence alignments. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. Branches present in both the consensus trees of the MP and BI are thickened. Substrate and country of origin, where known, are indicated next to the strain numbers. The tree was rooted to *Diplodia seriata* (CMW 8232).

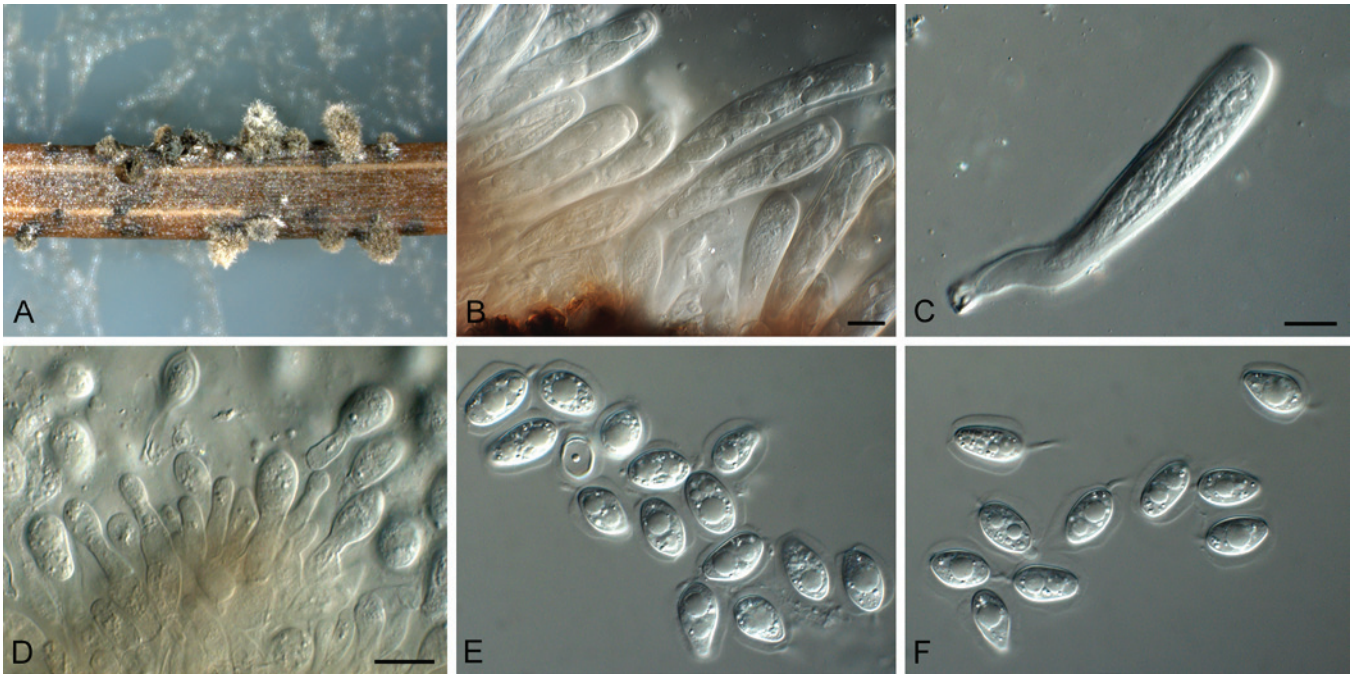

Fig. 3. *Phyllosticta abieticola* (CBS 112067). A. Conidiomata and ascomata forming on PNA. B, C. Asci with ascospores. D. Conidiogenous cells giving rise to conidia. E, F. Conidia with mucoid sheaths and apical appendages. Scale bars = 10 µm.

Type species: *P. convallariae* Pers., nom. inval. (= *P. cruenta* (Fr.) J. Kickx f.)

Phyllosticta abieticola Wikee & Crous, **sp. nov.** MycoBank MB805654. Fig. 3.

Etymology: Named after the host genus from which it was collected, *Abies.*

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 250 µm diam, elongated in culture on PNA; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 10–25 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 7–15 × 3–5 µm; proliferating several times percurrently near apex. *Conidia* (11–)13–16(–18) × (7–)8 µm, solitary, hyaline, aseptate, thin and smooth-walled, granular, or with a single large central guttule, fusoid-ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 3–4 µm thick, and bearing a hyaline, apical mucoid appendage, (15–)20–25(–30) × 1.5(–2) µm, flexible, unbranched, tapering towards an acutely rounded tip. *Ascomata* similar to conidiomata in general anatomy. *Asci* bitunicate, hyaline, clavate to broadly fusoid-ellipsoid, with visible apical chamber, 2 µm diam, 65–120 × 12–15 µm. *Ascospores* bi- to multiseriate, hyaline, smooth, granular to guttulate, aseptate, straight, rarely curved, widest in the middle, limoniform with obtuse ends, (15–)16–18(–20) × (6–)7 µm.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium, covering dish after 1 mo at 25 ºC. On OA surface iron-grey. On PDA and MEA surface grey-olivaceous, reverse iron-grey.

Specimen examined. **Canada**, on living leaf of *Abies concolor*, Jan. 2001, M. Forve (**holotype** CBS H-21389, ex-type culture CBS 112067).

Notes: The present isolate of *P. abieticola* was originally identified as *P. abietis*, which is distinguished by having smaller conidia (7–12 × 6.5–9 µm), and a sheath up to 1.5 µm wide, with apical appendages up to 2.5 µm long when present (Bissett & Palm 1989).

Phyllosticta aloeicola Wikee & Crous, **sp. nov.** MycoBank MB805655. Fig. 4.

Etymology: Named after the host genus from which it was collected, *Aloe*.

Associated with leaf tip blight. *Conidiomata* (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 250 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* subcylindrical to ampulliform, reduced to conidiogenous cells. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 5–13 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (8–)14–18(–27) × (6.5–) 7–8(–9) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid or subcylindrical, tapering towards a narrow truncate base, 3–5 µm diam, enclosed in a thin, persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (7–)15–20(–23) × 2–3(–3.5) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA and PDA iron-grey on surface and reverse.

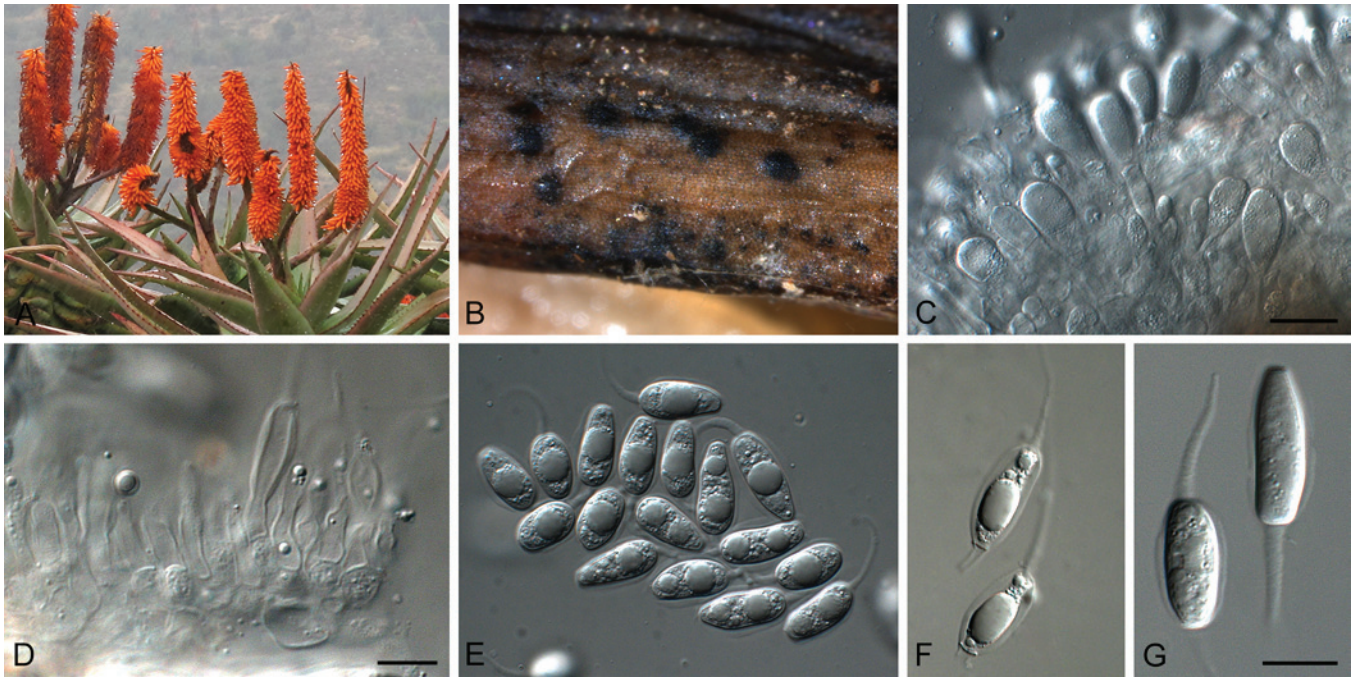

Fig. 4. *Phyllosticta aloeicola* (CPC 20677). A. *Aloe* with dead leaf tips that harbour *P. aloeicola*. B. Immersed conidiomata on leaf tissue. C, D. Conidiogenous cells giving rise to conidia. E–G. Conidia. Scale bars = 10 µm.

Specimen examined: **South Africa**, Free State Province, Bloemfontein Botanical Garden, Bloemfontein, on living leaf of *Aloe ferox*, 7 May 2012, P.W. Crous & W.J. Swart (**holotype** CBS H-21390, culture ex-type CPC 21020 = CBS 136058).

Notes: *Phyllosticta aloeicola* and *P. aloës* were both isolated from *Aloe latifolia* in South Africa. Van der Aa & Vanev (2002) examined the type specimen of *P. aloës* (deposited in B), and concluded that it was either a *Phoma* or *Asteromella* sp.

Phyllosticta citrimaxima Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB803675. Fig. 5.

Etymology: Named after this host on which it occurs, *Citrus maxima*.

Conidiomata pycnidial (on PNA), forming after 4 d of incubation, black, superficial, globose, 150–160 × 120–130 µm; wall 1–3 layers, 20–30 µm thick. *Conidiogenous cells* developing after 5 d, lining wall of pycnidium, phialidic, cylindrical, hyaline, 3–5 × 1–2 µm. *Conidia* ellipsoidal, hyaline, 1-celled, smooth, 5(–8) × (3–)4(–7) µm, surrounded by mucilaginous sheath, 1 µm thick, bearing a single, apical appendage, 2–16 µm long.

Culture characteristics: On OA, colonies flat, with irregular margin, initially hyaline with abundant mycelium, gradually becoming greenish after 2–3 d. On MEA colonies woolly, irregular, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d with white hyphae on the undulate margin, eventually turning black; reverse dark green to black. After 25 d in the dark at 27 ºC the colony covered the whole plate. On PDA, colonies were flat, rather fast growing, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d, with white hyphae at the margin, eventually turning black; reverse black and after 14 d in the dark at 27 ºC colony covered the whole plate.

Specimen examined: **Thailand**, Chiangrai, Weing Khaen, on fruit peel of *Citrus maxima*, Jun. 2011, S. Wikee (**holotype** MFLU 13-0001, ex-type culture CPC 20276 = MFLUCC10-0137 = CBS 136059).

Notes: *Phyllosticta citrimaxima* was isolated from tan spots on the fruit surface of *Citrus maxima*, which is grown as an economically important crop in Thailand and Asia. Recently, *P. citriasiana*, and *P. citrichinaensis* were described from *Citrus maxima* in Vietnam and China (Wulandari *et al.* 2009, Wang *et al*. 2012), and *P. citribraziliensis* from Brazil (Glienke *et al*. 2011). *Phyllosticta citrimaxima* is well supported phylogenetically (Fig. 1). Wang *et al.* (2012) provided a table in which they compared the morphology of five *Phyllosticta* species associated with citrus: *P. citricarpa*, *P. citriasiana*, *P. capitalensis*, *P. citribraziliensis*, and *P. citrichinaensis*. *Phyllosticta citrimaxima* produces smaller conidia (5–8 × 3–7 µm) than *P. citricarpa* (11–12 × 6–8 µm), *P. citriasiana* (12–14 × 6–7 µm), *P. capitalensis* (11–12 × 6–7 µm), *P. citribraziliensis* (10–12 × 6–7 µm) and *P. citrichinaensis* (8–12 × 6–9 µm), and has longer apical appendages (2–16 µm) than any of these four species, except *P. citrichinaensis* (14–26 µm).

Phyllosticta concentrica Sacc., Nuovo Giorn. Bot. Ital. 8: 203. 1876. Fig. 6.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 400 µm diam, elongated in culture on PNA; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 25 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that gives rise to 1–2 conidiogenous cells, 12–20 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 7–10 × 3–6 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–13(–14) × (6–)8(–9) µm, solitary, hyaline, aseptate, thin and smooth-walled,

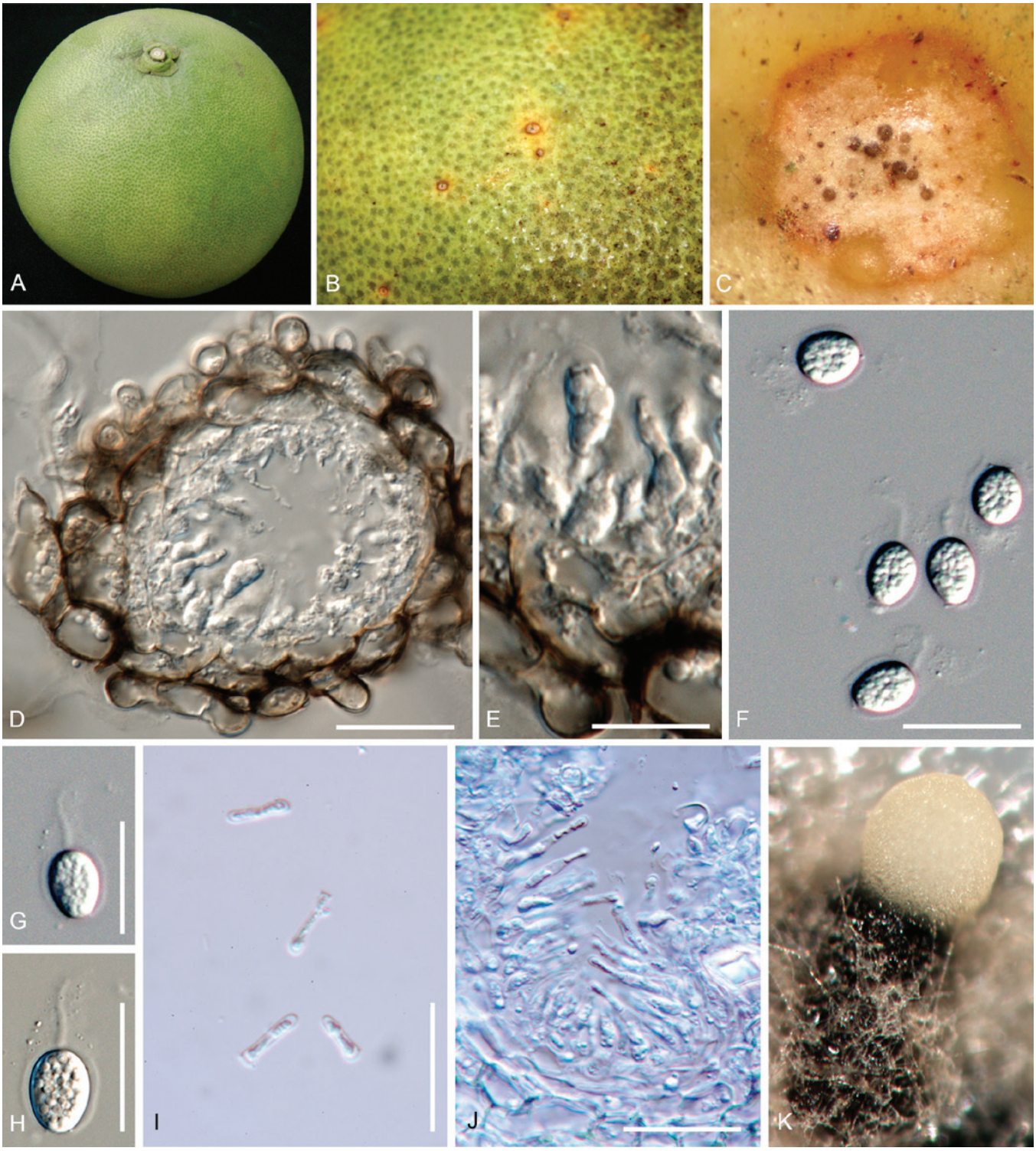

Fig. 5. *Phyllosticta citrimaxima* (CPC 20276). A–C. Symtoms on host. D, E. Vertical section through conidioma showing developing conidia. F–H.Conidia. I, J. Spermatial state, spermogonium. K. Conidia produced on OA. Scale bars: D = 30 µm; E–J = 10 µm.

granular, or with a single large central guttule, ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)8–12(–15) × (1–)1.5 µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium, and feathery, lobate margins, reaching 30 mm after 2 wk at 25 ºC. On PDA surface greenish black, reverse iron-grey; on OA surface iron-grey; on MEA surface olivaceous-grey in centre, pale olivaceous-grey in outer region, olivaceous-grey underneath.

Specimens examined. **Italy**, Padua, on withering leaves of *Hedera helix*, Jul. 1875, **syntype** (L); Sardegna, Cologne near Oleina, leaf litter of *Hedera helix*, 31 Aug. 1970, W. Gams (**epitype designated here** CBS H-16992, culture ex-epitype CBS 937.70; MBT176244). **Spain**, on living leaf of *Hedera* sp., 10 Jul. 2010, U. Damm, culture CPC 18842 = CBS 134749.

Notes: *Phyllosticta concentrica*, and its purported sexual state, *Guignardia philoprina*, represent different taxa, with each name representing a species complex for which numerous old names are available. *Phyllosticta concentrica* was originally introduced by Saccardo for a species occurring on *Hedera helix* in Italy, but which appears to be common in Europe on this host. The present

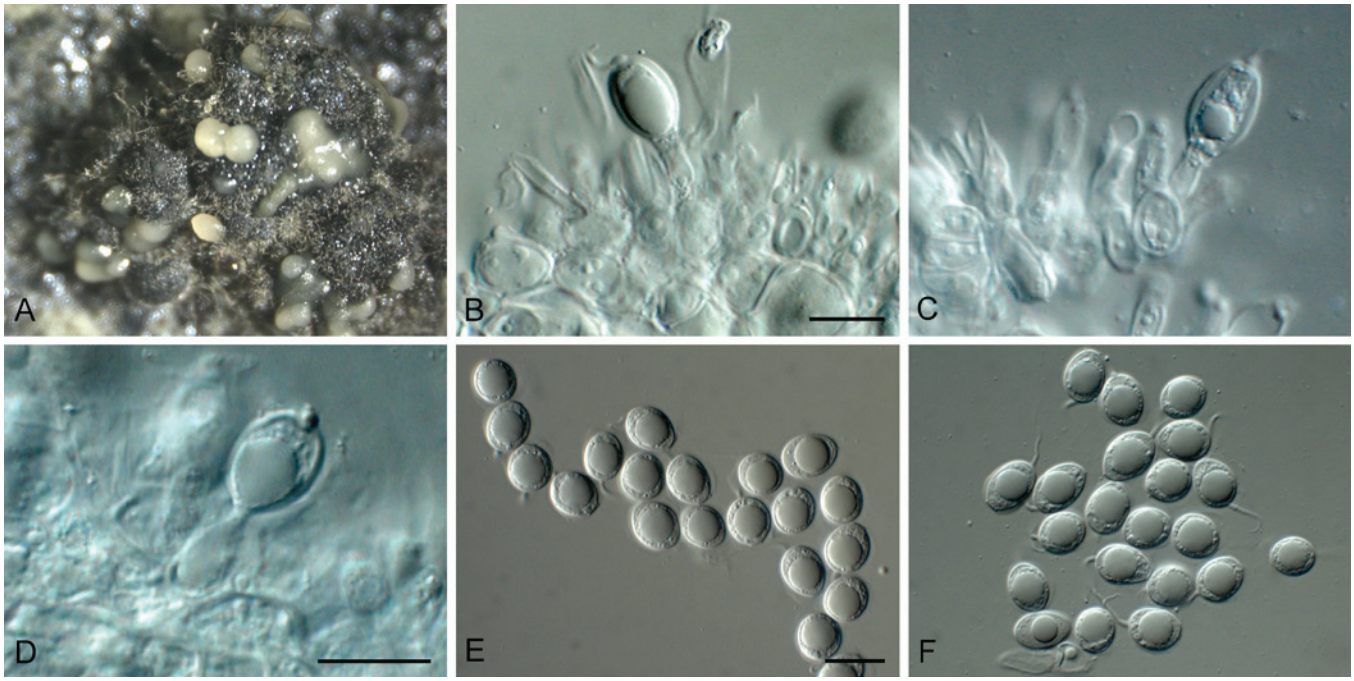

Fig. 6. *Phyllosticta concentrica* (CBS 937.70). A. Conidiomata sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

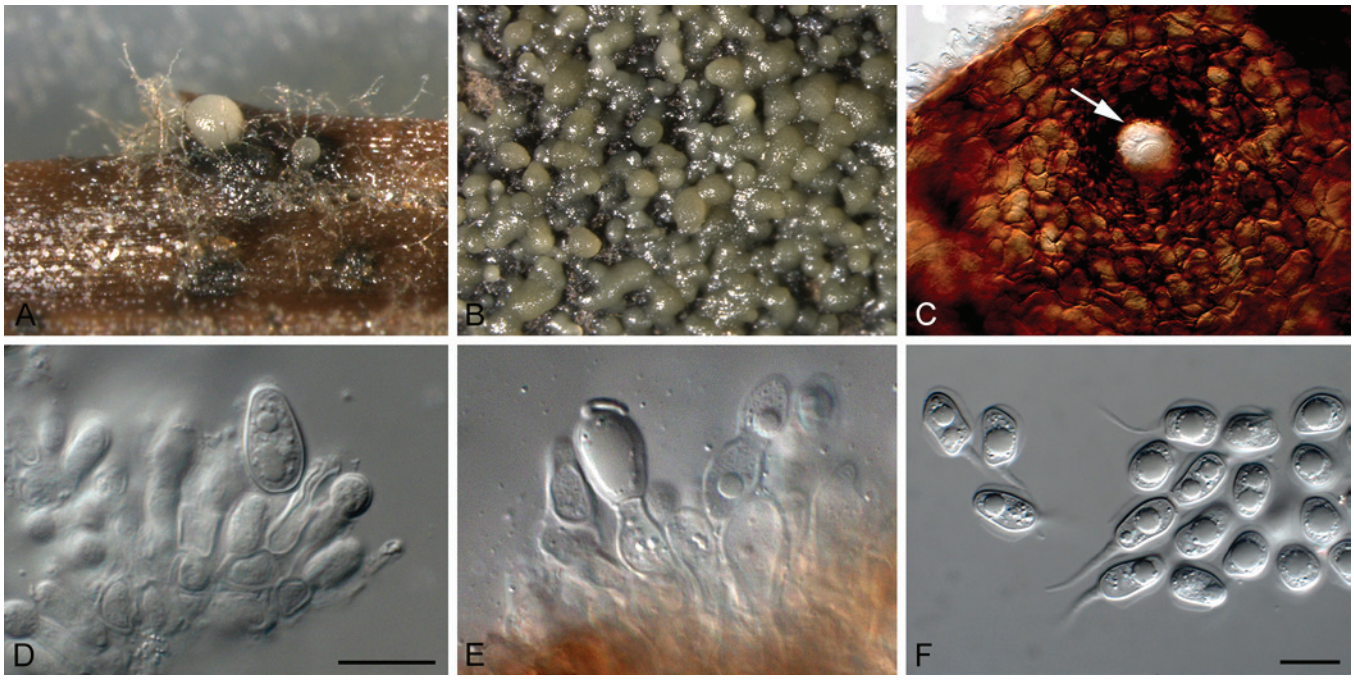

Fig. 7. *Phyllosticta cordylinophila* (CPC 20261). A. Conidiomata sporulating on PNA. B. Conidiomata sporulating on OA. C. Conidioma with ostiole (arrowed). D, E. Conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.

collection closely matches the original description of *P. concentrica* in morphology, for which an epitype is designated.

Phyllosticta cordylinophila P.A. Young, Bulletin of the Bernice P. Bishop Museum, Honolulu, Hawaii 19: 133. 1925. Fig. 7.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless to opaque conidial masses; pycnidia up to 200 µm diam; pycnidial wall of 3–6 layers of *textura angularis*, up to 40 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, at times branched at base, 10–20 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 10–17 × 3–6 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–13(–15) × 7–8(–11) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin, persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (10–)20–35(–40) × 2(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies spreading, erumpent, with sparse aerial mycelium and even, smooth margins. On MEA surface pale olivaceous-grey in centre, dirty white in outer region, reverse iron-

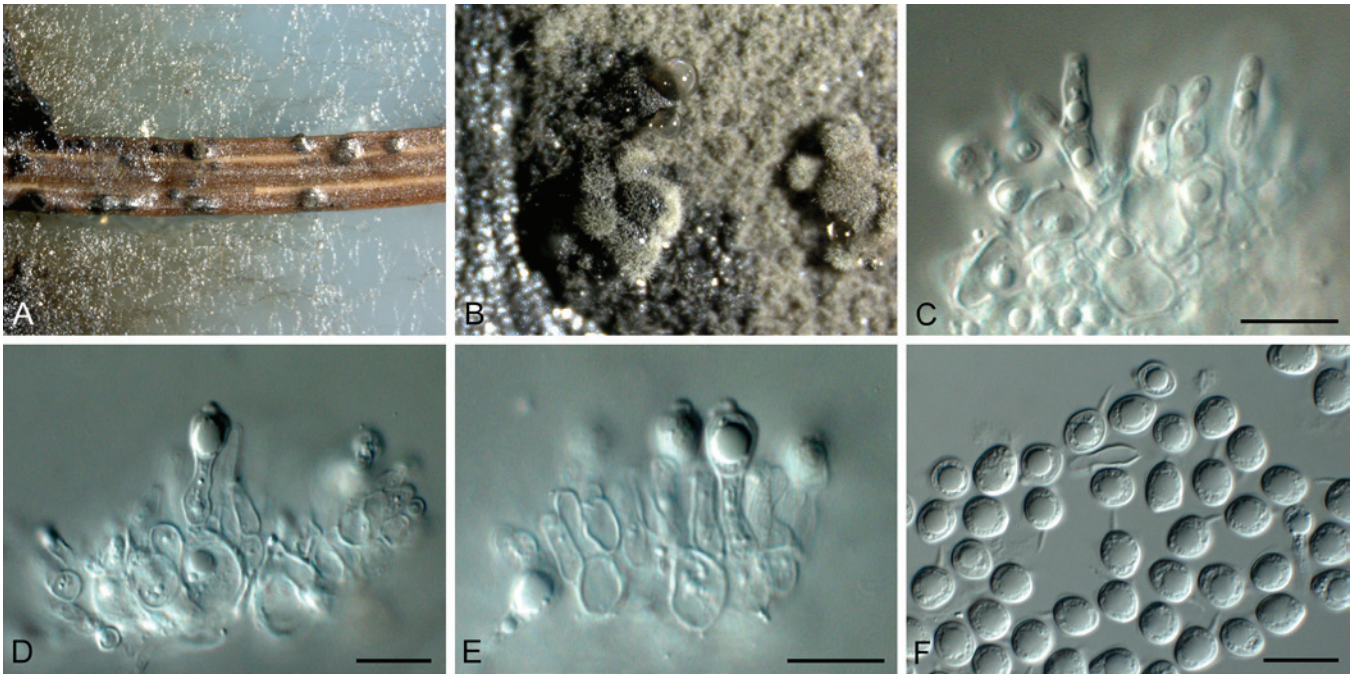

Fig. 8. *Phyllosticta cornicola* (CBS 111639). A. Conidiomata sporulating on PNA. B. Conidiomata forming on OA. C–E. Conidiophores giving rise to conidia. F. Conidia. Scale bars = 10 µm.

grey; on OA olivaceous-grey; on PDA olivaceous-grey on surface and reverse.

Specimens examined: **Thailand**, Chiangrai, Nang lae, Pasang, on leaf spot of *Cordyline fruticosa*, Nov. 2011, S. Wikee (**neotype designated here** CBS H-21391, ex-neotype culture CPC 20261 = WK024 = CBS 136244; MBT176245). **Japan**, Kagoshima, Amami-Ohshima, Amagi, on *C. fruticosa*, 22 Oct. 2003, Y. Ono & T. Kobayashi, culture ex-type MUCCJ 521 = CPC 21880 = CBS 136072.

Notes: Van der Aa (1973) did not locate type material, and the material studied by Petrak & Sydow (1927) was depauperate. As the present collections match the morphology of the original species description [conidia ellipsoid to ovoid, 7–12(–15) × 5–7.5(–8) µm], we herewith designate one specimen as neotype.

Phyllosticta cornicola (DC.) Rabenh., Klotzschii Herb. Viv. Mycol., Edn 2: no. 454. 1857. Fig. 8.
Basionym: *Sphaeria lichenoides* var. *cornicola* DC., in de Candolle & Lamarck, *Fl. franç.*, Edn 3 (Paris) 6: 148. 1815.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 10 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 10–20 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 7–12 × 2.5–4 µm; proliferating several times percurrently near apex. *Conidia* (6–)7–8 × (5.5–)6(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1 µm thick, and bearing a hyaline, apical mucoid appendage, (3–)4–5(–7) × 1(–1.5) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium and feathery, lobate margins, covering dish after 1 mo at 25 ºC. On OA, MEA and PDA surface olivaceous-grey, reverse iron-grey.

Specimen examined. **USA**, on living leaf of *Cornus florida*, Jul. 1999, G. Carroll, CBS H-21392, culture CBS 111639.

Notes: The name *P. cornicola* is based on European collections (*Cornus sanguinea*, Czech Republic), and until fresh European material has been collected, we cannot be sure that the name is authentic for this taxon.

Phyllosticta cussoniae Cejp, Bothalia 10: 341. 1971. Fig. 9.

Leaf spots amphigenous, subcircular, pale to medium brown, 0.5–1 cm diam, frequently surrounded by a red-purple margin. *Conidiomata* (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless to opaque conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, branched at base, 10–25 × 3–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 5–10 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (10–)12–15(–17) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin, persistent mucoid sheath, 2–4 µm thick, and bearing a hyaline, apical mucoid appendage, (8–)10–12(–13) × 2(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip. *Spermatia* occurring in same conidioma with conidia, hyaline, smooth, guttulate to granular, bacilliform, 7–10 × 2–3 µm.

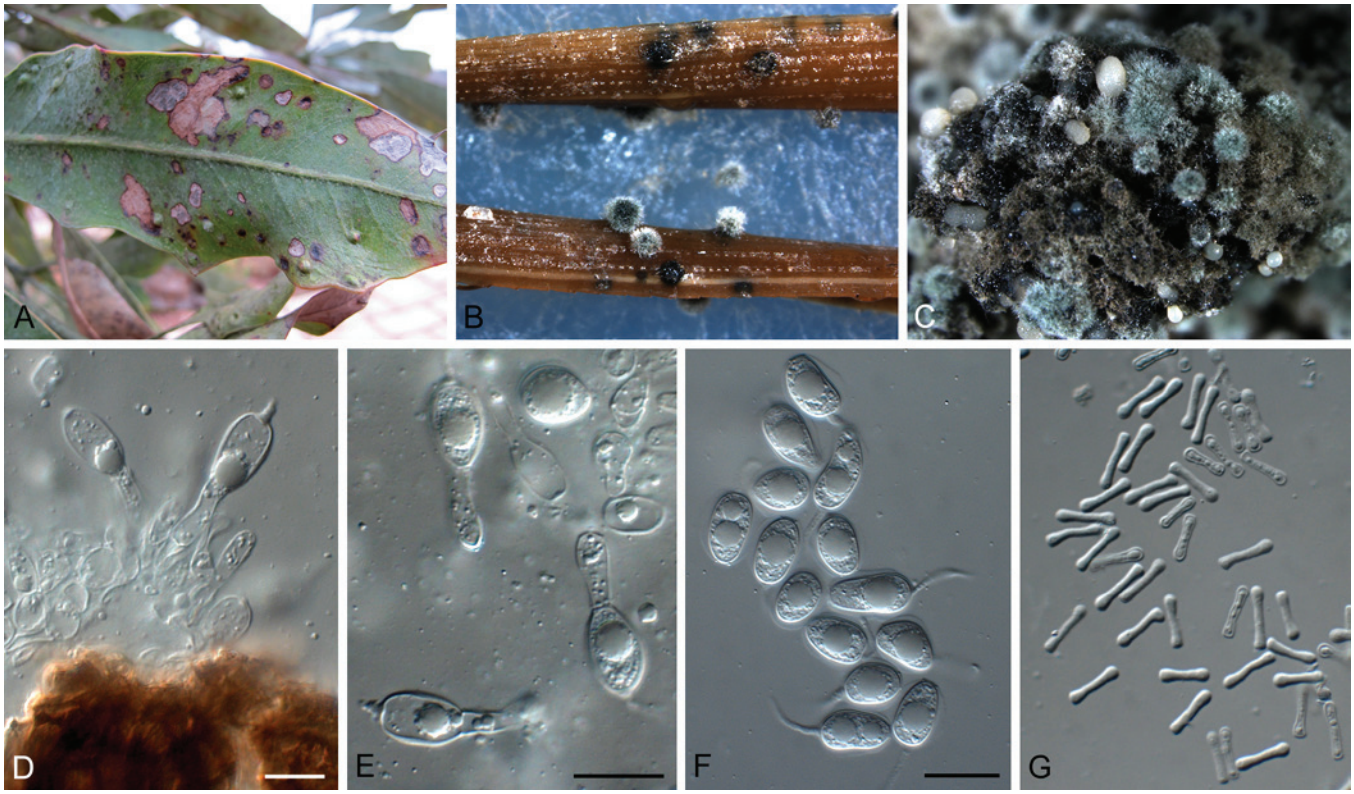

Fig. 9. *Phyllosticta cussoniae* (CPC 14873). A. Symptomatic leaf of *Cussonia* sp. B. Conidiomata forming on PNA. C. Conidiomata sporulating on OA. D, E. Conidiogenous cells giving rise to conidia. F. Conidia. G. Spermatia. Scale bars = 10 µm.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimens examined. **South Africa**, Mpumalanga, Schagen, Nelspruit, on *Cussonia umbellifera*, 25 Dec. 1933, L.C.C. Liebenberg, **holotype** PREM 32871; Eastern Cape, Graaff Reinet, Valley of Desolation, on leaf spot of *Cussonia* sp., 9 Jan. 2008, P.W. Crous (**epitype designated here** CBS H-21393, cultures ex-epitype CPC 14874, 14873 = CBS 136060; MBT176246); Gauteng, Walter Sisulu National Botanical Garden, on leaves of *Cussonia* sp., 2 Mar. 2007, P.W. Crous, cultures CPC 13812–13813.

Notes: *Phyllosticta cussoniae* occurs commonly on various *Cussonia* spp. throughout South Africa, where it causes a prominent leaf spot disease. All isolates collected from the various provinces where this host occurs, appear to have the same species (based on DNA sequence data) associated with the disease.

Phyllosticta foliorum (Sacc.) Wikee & Crous, **comb. nov.** MycoBank MB805656. Fig. 10.

Basionym: *Physalospora gregaria* var. *foliorum* Sacc., Syll. fung. (Abellini) 1: 435. 1882.

≡ *Pyreniella foliorum* (Sacc.) Theiss., Annls mycol. 14(6): 411. 1917 (1916).

≡ *Melanops foliorum* (Sacc.) Petr. (as "foliicola"), Kryptogamenflora Forsch. Bayer. Bot. Ges. Erforsch Leim. Flora 2(2): 165. 1931.

≡ *Botryosphaeria foliorum* (Sacc.) Arx & E. Müll., Beitr. Kryptfl. Schweiz 11(no. 1): 42. 1954.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose or with elongated body, exuding colourless to opaque conidial masses; pycnidia up to 400 µm diam; pycnidial wall of several layers of *textura angularis*; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 40 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, branched at base or not, 10–25 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–20 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (12–)13–14(–15) × (9–)10(–11) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, broadly ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin, persistent mucoid sheath, 2–3 µm thick, and bearing a hyaline, apical mucoid appendage, (10–)12–15(–20) × 1.5(–2) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimens examined. **Italy**, on fallen leaves of *Taxus baccata*, **holotype** of *Physalospora gregaria* var. *foliorum*, Herb. P.A. Saccardo, PAD. **Netherlands**, Baarn, Maarschalksbos, on dead twigs and needles of *Taxus baccata*, Sep. 1969, H.A. van der Aa (**neotype designated here** CBS H-9495, culture ex-neotype CBS 447.68). **USA**, from bonsai tree of *Cryptomeria japonica*, 25 Feb. 1977, G.H. Boerema, specimens CBS H-13111, CBS H-619, culture CBS 174.77.

Notes: *Guignardia philoprina* (from *Ilex*) is a species complex with numerous old names. The oldest name linked to European specimens from *Taxus* appears to be *Physalospora gregaria* var. *foliorum*, which we recombine in *Phyllosticta*. As the holotype specimen in PAD only contains immature ascomata and spermatia, a neotype is herewith designated.

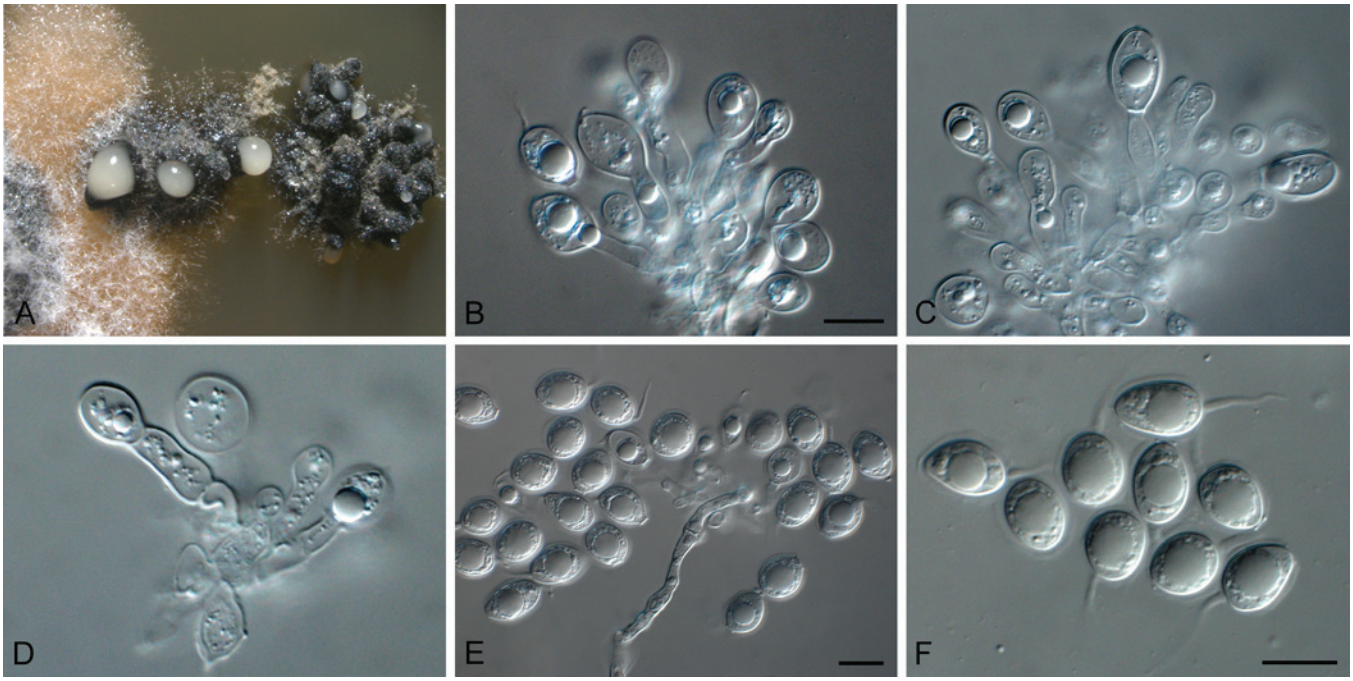

Fig. 10. *Phyllosticta foliorum* (CBS 447.68). A. Colony sporulating on MEA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

Phyllosticta hypoglossi (Mont.) Allesch., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1(6): 163. 1898. Fig. 11.
Basionym: *Sphaeropsis hypoglossi* Mont., Annls Sci. Nat., Bot., sér. 3 12: 307. 1849.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 15–25 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 10–15 × 3–5 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–12(–14) × (9–)10(–11) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid to obovoid or globose, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin, mucoid sheath, 1–3 µm thick, mostly not persistent, and bearing a hyaline, apical mucoid appendage, (8–)10–12(–15) × 1.5(–2) µm, flexible, unbranched, tapering towards an acute tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 25 mm diam on MEA, 30 mm diam on PDA and 35 mm diam on OA after 2 wk at 25 ºC. On OA centre olivaceous-grey, outer zone with diffuse pale yellow pigment in agar. On PDA surface olivaceous-grey, reverse iron-grey. On MEA surface iron-grey in centre, pale grey-olivaceous in outer region, iron-grey in reverse.

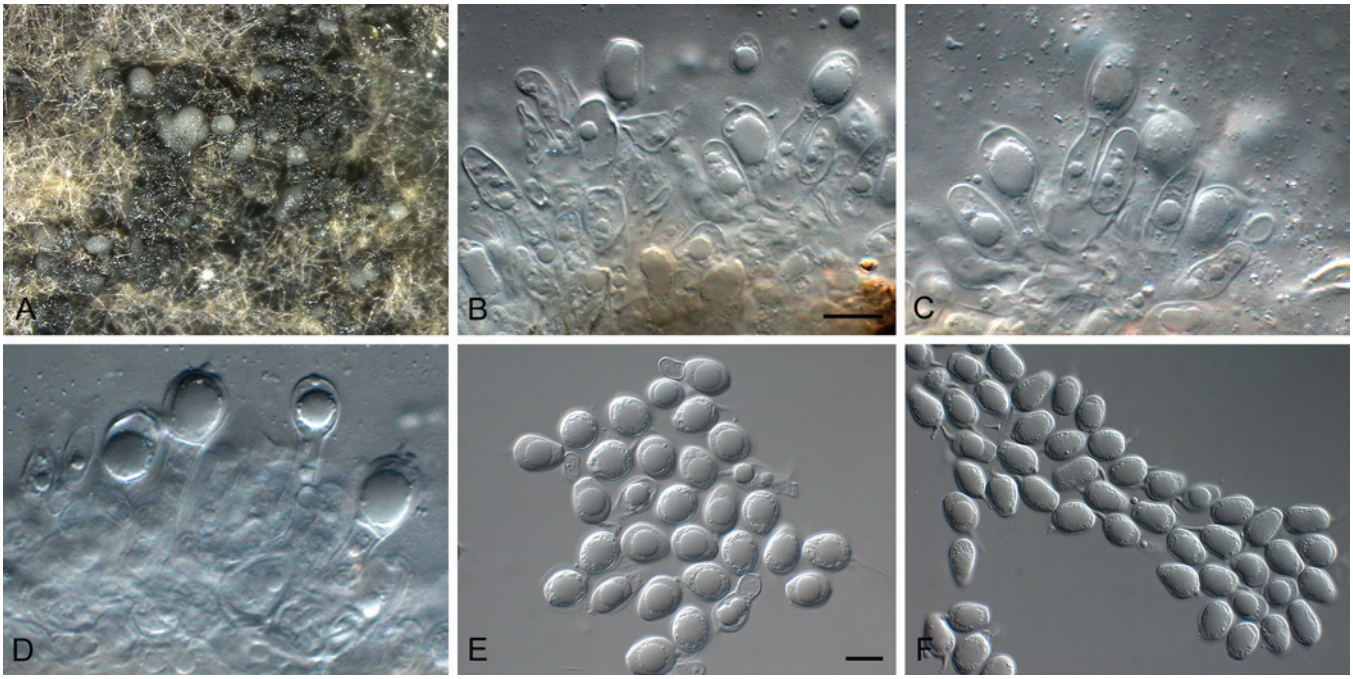

Fig. 11. *Phyllosticta hypoglossi* (CBS 434.92). A. Colony sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

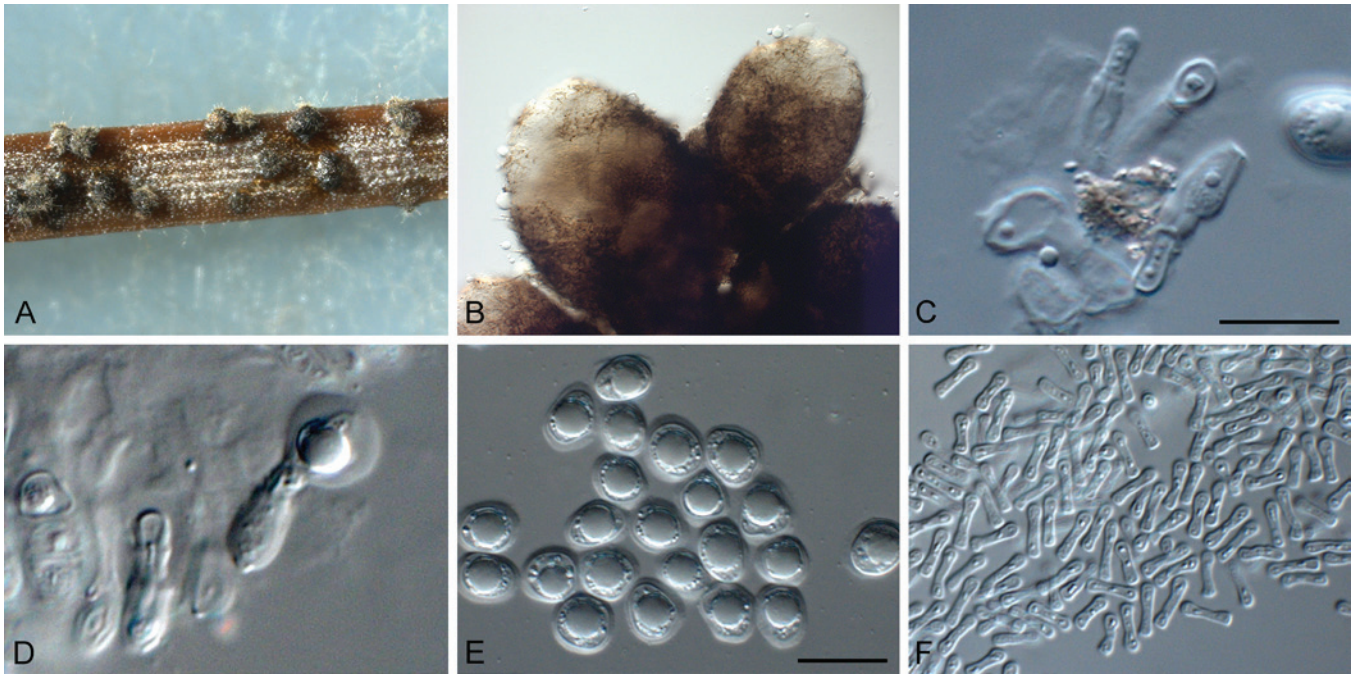

Fig. 12. *Phyllosticta leucothoicola* (MUCC 553). A. Conidiomata forming on PNA. B. Conidiomata. C, D. Conidiogenous cells giving rise to conidia. E. Conidia. F. Spermatia. Scale bars = 10 µm.

Specimens examined: **France**, near Marseille, on cladodes of *Ruscus hypoglossum*, 1845, J.L.M. Castagne, (type not found, presumably missing). **Italy**, Prov. Napoli, Cratere degli Astroni, on dead cladodes of *Ruscus aculeatus*, May 1992, W. Gams (**neotype designated here** CBS H-5331; ex-neotype culture CBS 434.92; MBT176248).

Notes: Judging from the number of specimens and cultures in the CBS collection, *P. hypoglossi* is a common European species on cladodes of *Ruscus hypoglossum.* The morphology of the neotype closely matches that described in the original description.

Phyllosticta leucothoicola Wikee, Motohashi & Crous, **sp. nov.** MycoBank MB805657. Fig. 12.

Etymology: Named after the host genus from which it was collected, *Leucothoe.*

Leaf spots purple-brown, scattered, enlarged and becoming confluent, subcircular to oblong, with brown to dark brown border (Takeuchi & Horie 1998). *Conidiomata* (on PNA) 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 300 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 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, 6–20 × 3–4 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 6–15 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (6–)7–8(–9) × 6(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, ovoid to irregularly ellipsoid, at times enclosed in a thin mucoid sheath, up to 1.5 µm thick; apical mucoid appendage not seen. *Spermatia* developing in same conidioma as conidia, bacilliform, smooth, hyaline, guttulate, 5–7 × 2–3 µm.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimen examined: **Japan**, Tokyo, on living leaf of *Leucothoe catesbaei*, May 1996, J. Takeuchi (holotype CBS H-21394, ex-type culture MUCC 553 = CPC 21881 = CBS 136073).

Notes: *Phyllosticta leucothoës* has been described from *Leucothoe acuminata*, although van der Aa & Vanev (2002) transferred this to *Fusicoccum* based on an examination of type material. *Phyllosticta leucothoicola* represents a distinct taxon on *L. catesbaei*, corroborating the morphological differences noted by Motohashi *et al.* (2009).

Phyllosticta mangifera-indica Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB805657. Fig. 13.

Etymology: Named after the host genus on which it occurs, *Mangifera indica*.

Conidiomata pycnidial (on PNA), initially forming after 4 d of incubation, black, superficial, subglobose or ellipsoidal, 220–300 × 160–180 µm; wall of 1–3 layers of brown *textura angularis*, 20–30 µm thick. *Conidiogenous cells* lining the inner wall, phialidic, cylindrical, hyaline, 3–5 × 3–4 µm. *Conidia* ellipsoidal, hyaline, aseptate, smooth, (6–)9(–13) × (4–)5(–6) µm, surrounded by mucilaginous sheath, 0.5–2 µm thick, bearing single apical appendage, 3–14 µm long.

Culture characteristics: On OA colonies appeared flat, with irregular margins, initially hyaline with abundant mycelium, gradually becoming greenish after 2–3 d. On MEA, colonies woolly, irregular, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d, with white hyphae at the undulate margin, eventually turning black; reverse dark green to black. After 25 d in

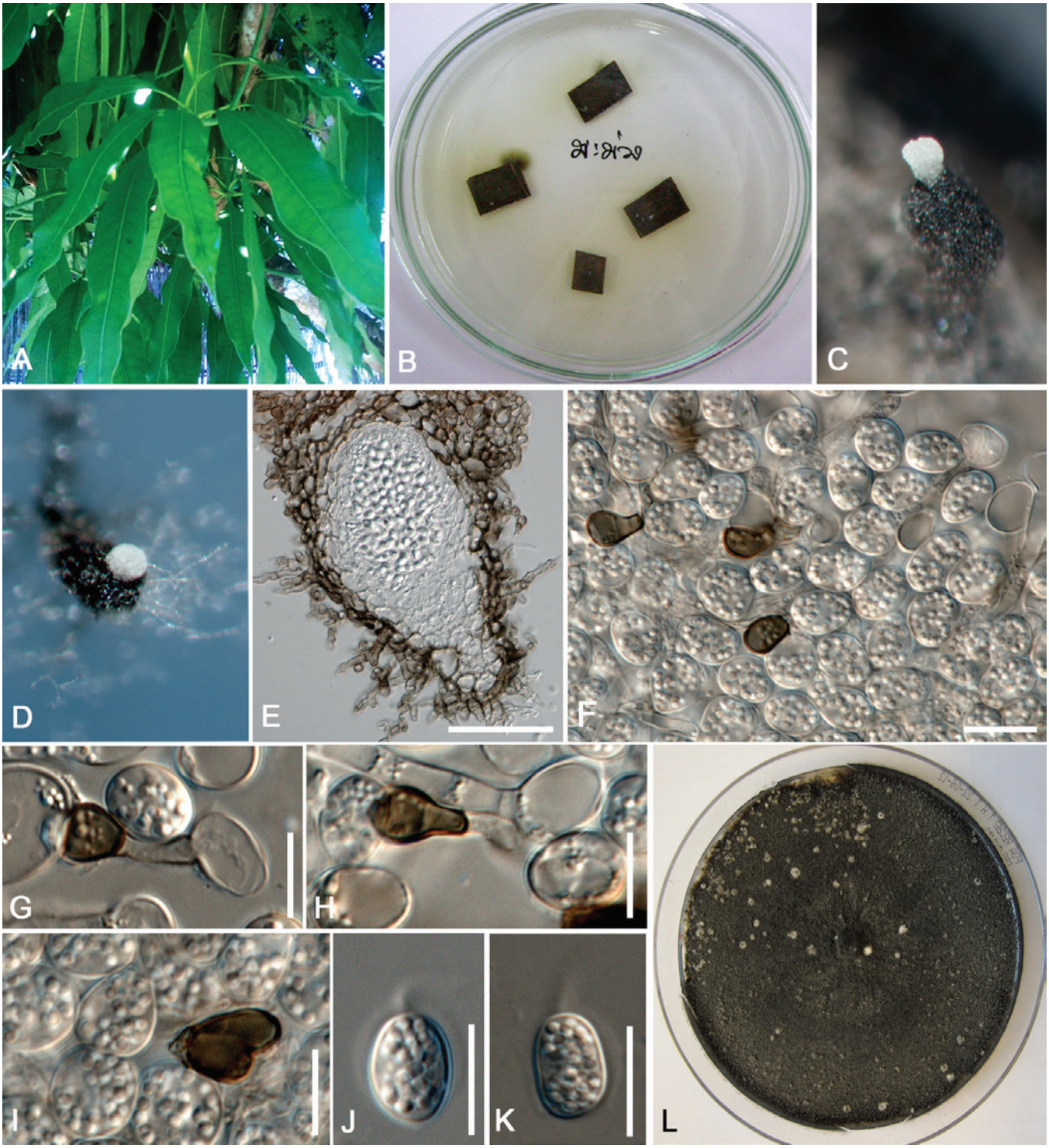

Fig. 13. *Phyllosticta mangifera-indica* (CPC 20274). A. Healthy leaf of *Mangifera indica*. B. Isolation on WA. C. Culture sporulating on OA. D. Culture sporulating on SNA. E. Vertical section through a conidioma showing developing conidia. F–I. Appressoria. J, K. Conidia. L. Culture on MEA. Scale bars: E = 100 µm, F–K = 10 µm.

the dark at 27 ºC colony covering the whole plate. On PDA colonies flat, rather fast growing, initially white with abundant mycelium, gradually becoming greenish to dark green after 2–3 d with white hyphae at the margin, eventually turning black; reverse black and after 14 d in the dark at 27 ºC colony covering the whole plate.

Specimen examined: **Thailand**, Chiangrai, Nanglae, on healthy leaf of *Mangifera indica*, July 2011, S. Wikee (**holotype** MFU13-0108; ex-type culture CPC 20274 = MFLUCC10-0029 = CBS 136061).

Notes: *Phyllosticta mangifera-indica* was isolated as an endophyte from a healthy leaf of *Mangifera indica*. Several species have been reported as pathogens on *M. indica* including *G. mangiferae* and *P. brazilianiae* (Glienke *et al.* 2011). *Phyllosticta mangifera-indica* produced abundant conidia on OA and formed appressoria within 2 d. Morphologically, it is distinct from *P. capitalensis* (conidia 8–11 × 5–6 µm) in having longer conidia (conidia 6–13 × 4–6), and represents a distinct lineage with 99 % bootstrap support with the inclusion of TEF1 and GPDH sequence data. It is phylogenetically distinct from *P. mangiferae*, and most closely related to *P. brazilianiae*, which occurs on the same host.

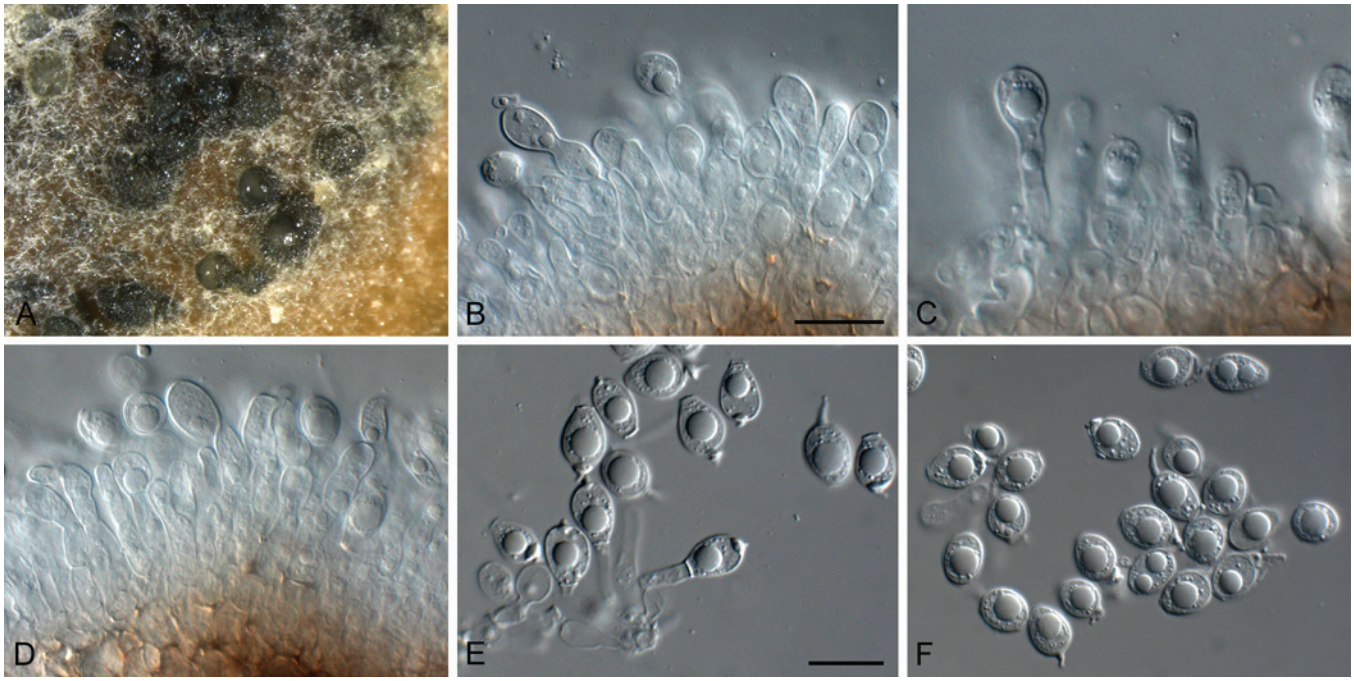

Fig. 14. *Phyllosticta minima* (CBS 585.84). A. Colony sporulating on MEA. B–E. Conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.

Phyllosticta minima (Berk. & M.A. Curtis) Underw. & Earle, Bull. Alabama Agric. Exp. Stn. 80: 168. 1897. Fig. 14.
Basionym: *Sphaeropsis minima* Berk. & M.A. Curtis, N. Amer. Fungi: no. 418. 1874.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 180 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 15–50 × 5–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–20 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (9–)10–11(–12) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid to obovoid or globose, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin mucoid sheath, absent at maturity or 1 µm thick, and bearing a hyaline, apical mucoid appendage, 6–7(–10) × 1.5(–2) µm, flexible, unbranched, tapering towards an acute tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 15 mm diam on MEA, 40 mm diam on PDA and 8 mm diam on OA after 2 wk at 25 ºC. On OA surface olivaceous-grey. On PDA surface and reverse iron-grey. On MEA surface olivaceous-grey with patches of pale luteus.

Specimens examined: **USA**, North Dakota, New England, on *Acer rubrum*, R. Sprague 5314 (holotype not found); Tennessee, Gatlinburg, Great Smoky Mountains National Park, on leaf spot of *Acer rubrum*, June 1984, D.H. Defoe (**neotype designated here** CBS H-17023; ex-neotype culture CBS 585.84 = IFO 32917; MBT176250).

Note: This taxon appears to be common on *Acer* spp. in the USA, where it is associated with leaf spots (Bissett & Darbyshire 1984). The holotype could not be located in NY, LCR, IMI, S, K or BPI, and thus a neotype (from the original host in the USA) is designated.

Phyllosticta neopyrolae Wikee, Motohashi, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB803676. Fig. 15.

Etymology: Named after the host genus on which it occurs, *Pyrola.*

Leaf spots orbicular to ellipsoid, black. *Conidiomata* (on PNA) pycnidial, epiphyllous, sparse, solitary or aggregated, immersed at first, then erumpent breaking through the epidermis, brown to dark brown, subglobose, 60–100 × 60–113 µm; pycnidial wall composed of the depressed or irregular cells in 1–4 layers, brown to dark brown, hyaline or paler toward the inside, with a central ostiole, up to 10 µm diam. *Conidiophores* subcylindrical, reduced to conidiogenous cells, or with 1–2 supporting cells, branched at the base, 15–20 × 2–3 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–15 × 2–3 µm; proliferating several times percurrently near apex. *Conidia* (6–)7(–8) × (5–)6(–7) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid to globose, mucoid sheath and appendage lacking.

Culture characteristics: Colonies erumpent, spreading, with sparse aerial mycelium and feathery margins, covering the dish in 1 mo. On MEA surface olivaceous-grey, reverse iron-grey; on OA iron-grey; on PDA iron-grey on surface and reverse.

Specimen examined. **Japan**, Nagano, Sugadaira, on living leaf of *Pyrola asarifolia* subsp. *incarnata*, 17 June 2006, T. Hosoya (**holotype** TFM : FPH 7887, **isotype** CBS H-21395, ex-type culture MUCC 125 = CPC 21879 = CBS 134750).

Notes: Two species of *Phyllosticta* are known from *Pyrola* spp., namely *P. pyrolae* Ellis & Everh. and *P. pyrolae* (Ehrenb. : Fr) Allesch. Of these, the latter species is an illegitimate homonym, with morphological characteristics (conidia 3–4 µm long) that indicate that it should be excluded from *Phyllosticta* s. str. (van der Aa & Vanev 2002). The other species, *P. pyrolae* Ellis & Everh. (conidia ovoid to globose, 4.5–7.5 × 4–9 µm, with mucoid layer and an apical appendage) resembles *P. neopyrolae*. *Phyllosticta*

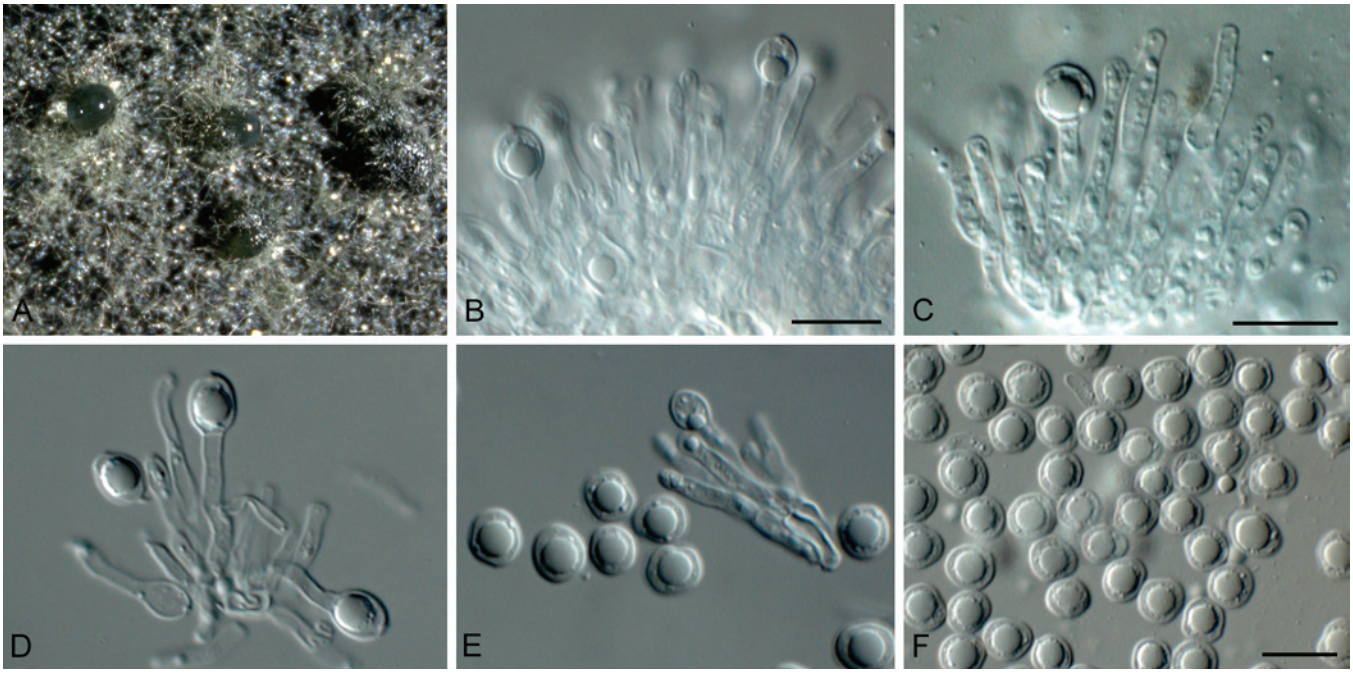

Fig. 15. *Phyllosticta neopyrolae* (CBS 134750). A. Colony sporulating on OA. B–E. Conidiogenous cells giving rise to conidia. F. Conidia. Scale bars = 10 µm.

neopyrolae differs from these two species by having conidia that lack a mucoid sheath and apical appendage.

Phyllosticta owaniana G. Winter, Hedwigia 24: 31. 1885. Fig. 16.

Leaf spots amphigenous, irregular to subcircular, pale to medium brown, turning greyish with age, surrounded by a broad purplish border, and chlorotic margin. *Conidiomata* (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 300 µm diam, frequently with elongated neck on OA and MEA; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 10 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, that can be branched at the base, 10–30 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 10–25 × 3–4.5 µm; proliferating several times percurrently near apex. *Conidia* (10–)11–12(–13) × (7–)8(–9) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a bluntly obtuse or narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)8–12(–15) × (1–)1.5 µm, flexible, unbranched, tapering towards an acutely rounded tip.

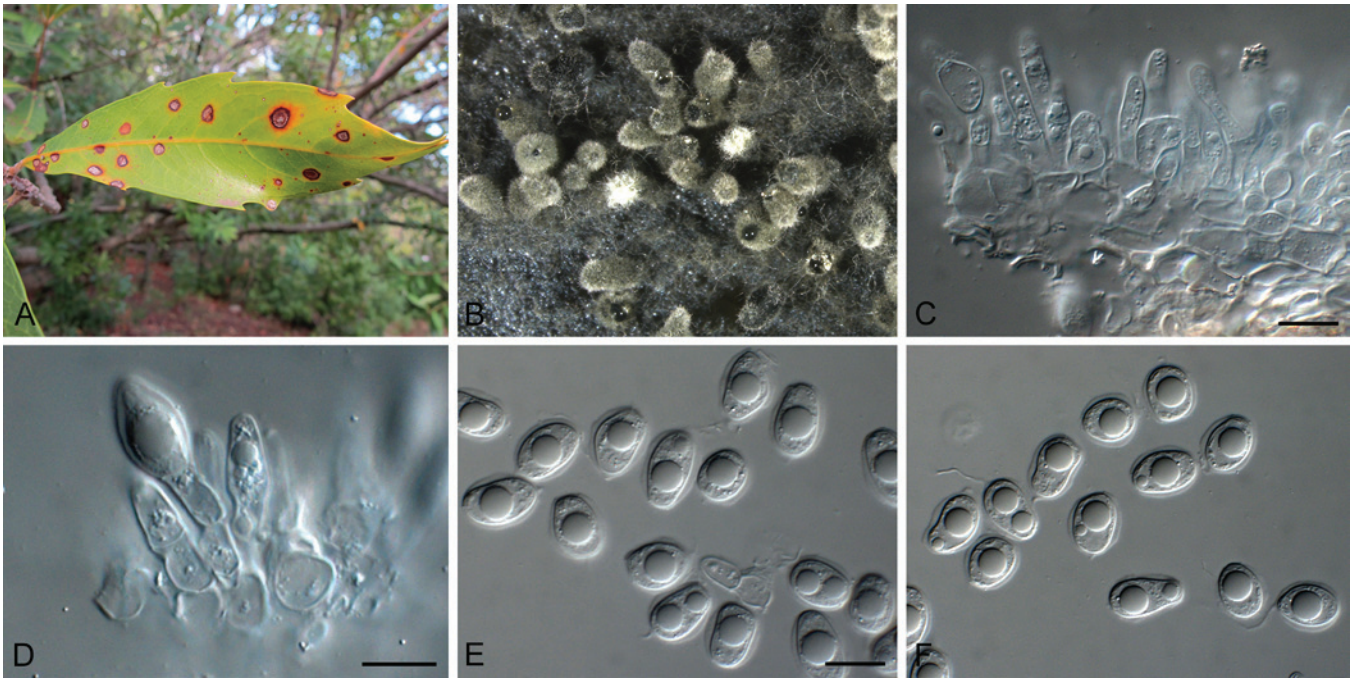

Fig. 16. *Phyllosticta owaniana* (CBS 776.97). A. Symptomatic leaf of *Brabejum stellatifolium*. B. Colony sporulating on OA. C, D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

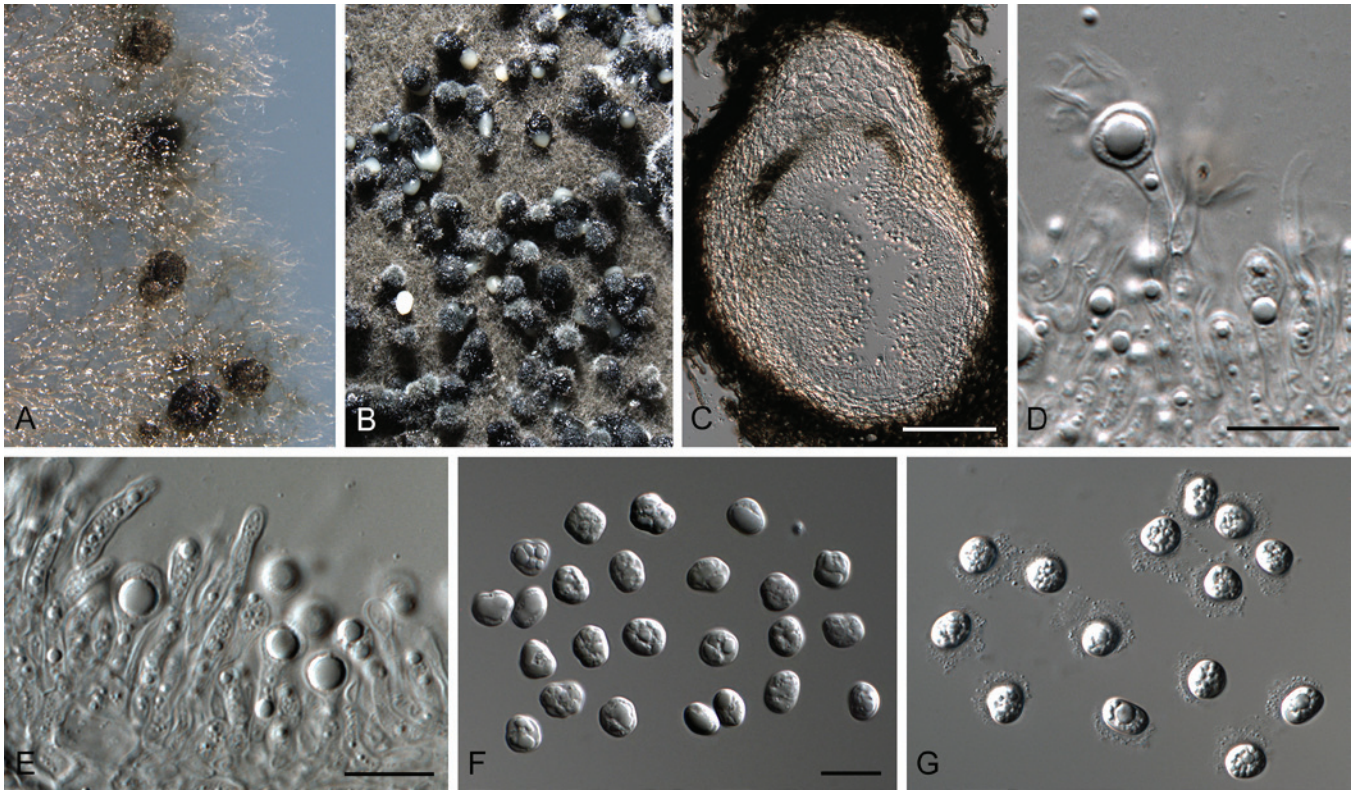

Fig. 17. *Phyllosticta pachysandricola* (NBRC 102276). A. Colony sporulating on SNA. B. Colony sporulating on PDA. C. Vertical section through conidioma. D, E. Conidiogenous cells. F. Conidia mounted in lactic acid. G. Conidia mounted in water. Scale bars: C = 35 µm, all others = 10 µm.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 30 mm diam on MEA, 40 mm diam on PDA and 25 mm diam on OA after 2 wk at 25 ºC. On OA surface iron-grey. On PDA surface and reverse iron-grey. On MEA surface and reverse iron-grey.

Specimens examined: **South Africa**, Western Cape Province, Cape Town, Table Mountain, on leaves of *Brabejum stellatifolium*, 1884, P. McOwan, **holotype** in B; Western Cape Province, Jonkershoek Nature Reserve, on leaf spot of *Brabejum stellatifolium*, 3 Jan. 1995, A. den Breeÿen, (**epitype designated here** CBS H-21396, ex-epitype culture CPC 1009 = CBS 776.97; MBT176251).

Notes: *Phyllosticta owaniana* causes a serious leaf spot disease on *Brabejum stellatifolium*, and is generally found wherever this host occurs in South Africa. All isolates collected thus far (Crous, unpubl. data) are similar based on DNA sequence data, suggesting that it's a common species on this host.

Phyllosticta pachysandricola Wikee, Motohashi & Crous, **sp. nov.** MycoBank MB805658. Fig. 17.

Etymology: Named after the host genus from which it was collected, *Pachysandra.*

Leaf spots circular to ellipsoid, pale brown to brown, often extend with concentric rings, 6–16 mm diam, surrounded by a dark brown border. *Conidiomata* (on PNA) pycnidial, amphiphyllous, sparse, solitary or aggregated, immersed at first, then erumpent breaking through the epidermis, brown to dark brown, subglobular, 90–140 × 25–80 µm diam, with central ostiole; pycnidial wall composed of depressed or irregular cells with 1–4 layers, brown to dark brown, hyaline or paler toward the inside. *Conidiogenous cells* integrated, lining the innermost layer of the pycnidial wall, cylindrical, straight or slightly curved, hyaline, proliferating percurrently at least once, with minute periclinal thickenings, 5–12 × 2–2.5 µm. *Conidia* sporulating holoblastically, solitary, unicellular, spherical, ellipsoid to obovoid, 5.5–8.5 × 4.5–7.5 µm, truncate at the base or rounded at both ends, containing numerous greenish guttulae, surrounded by a mucous sheath, rarely with a short apical appendage.

Specimen examined: **Japan**, Hokkaido, Asahikawa, on *Pachysandra terminalis*, K. Motohashi, C. Nakashima & T. Akashi, 7 June 2006 (**holotype** TFM : FPH7877, **isotype** MUMH 10488, ex-type culture MUCC 124 = NBRC 102276).

Notes: One other species has been recorded from *Pachysandra*, *P. pachysandrae*, which van der Aa & Vanev (2002) excluded from *Phyllosticta s. str*. based on its conidia (unicellular, oblong, 4.5–6 × 1 µm) that indicate placement in *Asteromella*. The Japanese collection on *Pachysandra* is thus described as a new species, *P. pachysandricola*, in accordance to the morphological differences noted by Motohashi *et al*. (2009).

Phyllosticta paxistimae Wikee & Crous, **sp. nov.** MycoBank MB805659. Fig. 18.

Etymology: Named after the host genus from which it was collected, *Paxistima.*

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; pycnidia up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 10 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, 15–30 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline,

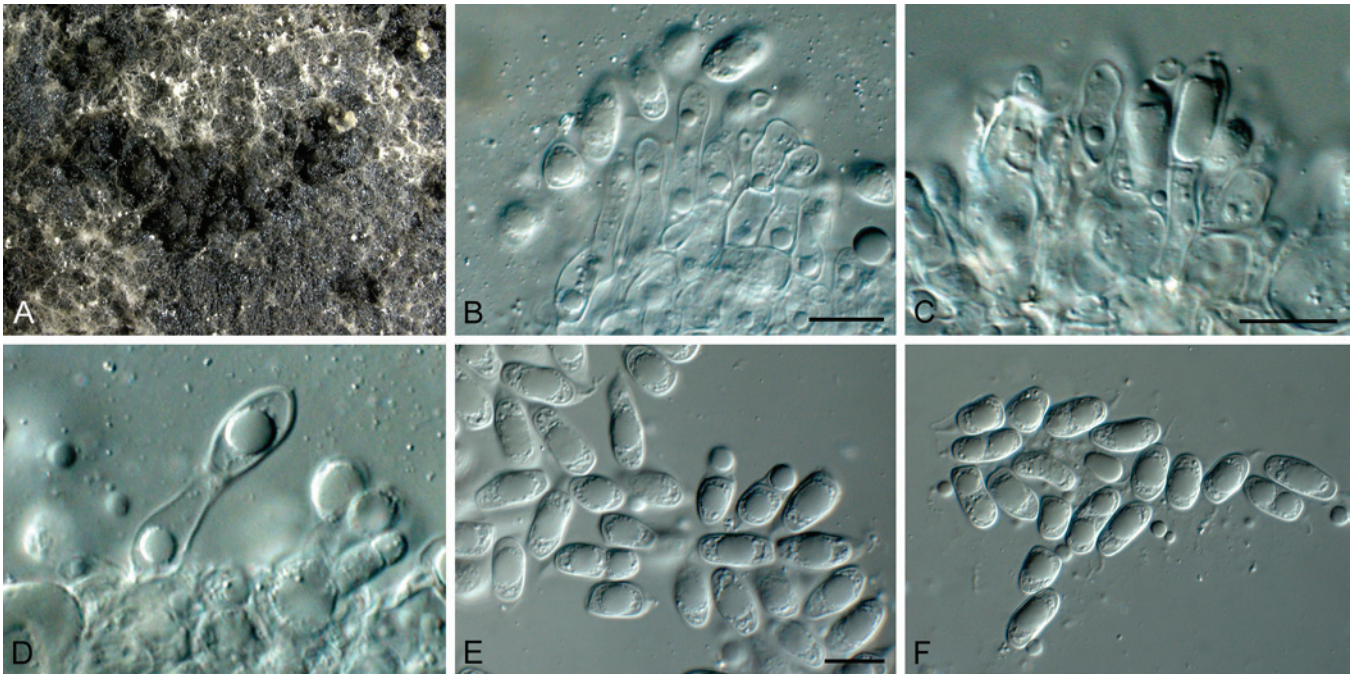

Fig. 18. *Phyllosticta paxistimae* (CBS 112527). A. Colony sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

smooth, coated in a mucoid layer, 10–20 × 4–5 µm; proliferating several times percurrently near apex. *Conidia* (10–)12–14(–16) × 6–7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, broadly ellipsoid, tapering towards a narrow truncate base, 2–3 µm diam, enclosed in a thin persistent mucoid sheath, 1 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)9–11(–13) × 1.5(–2) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium and feathery, lobate margins, reaching 60 mm diam after 1 mo at 25 ºC. On OA surface iron-grey with patches of olivaceous-grey. On PDA surface and reverse iron-grey. On MEA surface dirty white with patches of iron-grey, reverse iron-grey.

Specimen examined. **USA**, Oregon, on living leaf of *Paxistima myrsinites*, 1994, G. Carroll (**holotype** CBS H-21397, ex-type culture CBS 112527).

Notes: We have been unable to trace the holotype of *P. pachystimae* (**USA**, Wyoming, Hoback Canyon, near Granite Creek, on *Paxistima myrsinites*, 1 Aug. 1940, L.E. Wehmeyer No 1198). The conidia of *P. pachystimae* (9–14 × 4–5 µm; Wehmeyer 1946) are much narrower than those of *P. paxistimae* (10–16 × 6–8 µm).

Phyllosticta philoprina (Berk. & M.A. Curtis) Wikee & Crous, **comb. nov.** MycoBank MB805660.

Basionym: *Sphaeria philoprina* Berk. & M.A. Curtis, Grevillea 4 (32): 154. 1876.

≡ *Guignardia philoprina* (Berk. & M.A. Curtis) Aa, Stud. Mycol. 5: 44. 1973.

For additional synonyms see van der Aa (1973).

Specimens examined: **Spain**, on living leaf of *Ilex aquifolium*, July 1970, H.A. van der Aa, specimen CBS H-13113, culture CBS 587.69. **Germany**, on *Ilex aquifolium*, Aug. 1972, R. Schneider, CBS 616.72.

Notes: The oldest name for a *Phyllosticta* sp. occurring on *Ilex* is *Sphaeria philoprina*. However, this name was based on material collected in the USA, and the present isolates were derived from European collections.

Phyllosticta podocarpicola Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB805661. Fig. 19.

Etymology: Named after the host genus from which it was collected, *Podocarpus*.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless to opaque conidial masses; pycnidia up to 200 µm diam; pycnidial wall of 3–6 layers of brown *textura angularis*; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, at times branched at base, 10–25 × 4–6 µm. *Conidiogenous cells* terminal, subcylindrical to doliiform, hyaline, smooth, coated in a mucoid layer, 10–17 × 4–6 µm; proliferating several times percurrently near apex. *Conidia* 12–13(–16) × 8–9(–9.5) µm, solitary, hyaline, aseptate, thin and smooth walled, coarsely guttulate, or with a single large central guttule, broadly ellipsoid, tapering towards a narrow truncate base, 2–5 µm diam, enclosed in a thin, persistent mucoid sheath, 3–4 µm thick, and bearing a hyaline, apical mucoid appendage, (25–)30–45(–55) × 3–4(–5) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies after 3 wk reaching 30 mm diam on MEA, 60 mm on PDA and OA. Colonies flattened, spreading, with sparse aerial mycelium and feathery margins. On MEA surface and reverse olivaceous-grey; on OA olivaceous-grey; on PDA iron-grey on surface and reverse.

Specimen examined: **USA,** Florida, on seed of *Podocarpus maki* (intercepted in New Zealand), Sep. 1979, G. Laundon (**holotype** CBS H-13109; ex-type culture CBS 728.79).

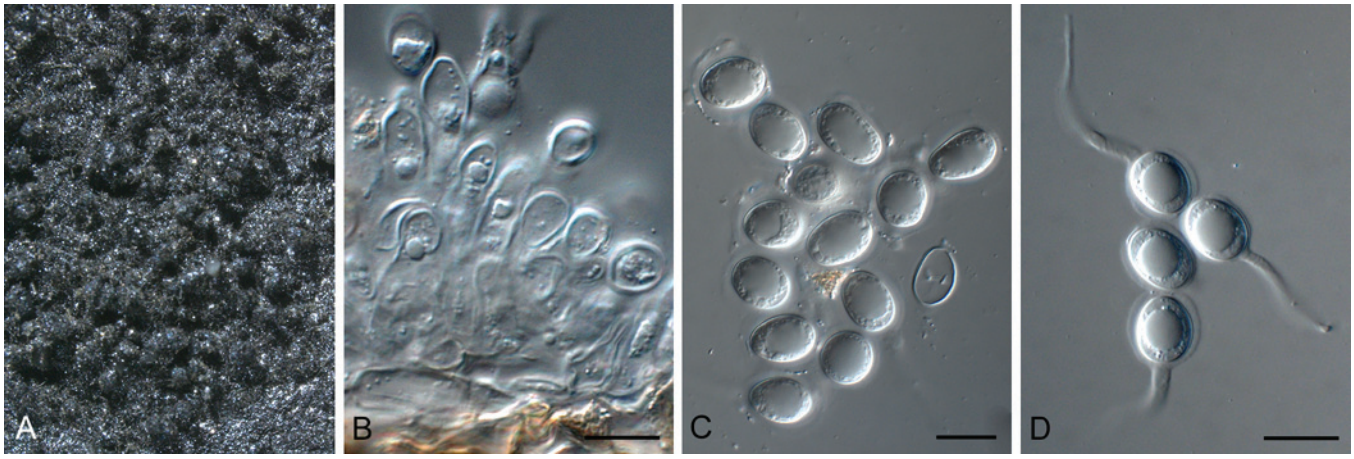

Fig. 19. *Phyllosticta podocarpicola* (CBS 728.79). A. Colony sporulating on OA. B. Conidiogenous cells giving rise to conidia. C, D. Conidia. Scale bars = 10 µm.

Notes: The isolate described here as *Phyllosticta podocarpicola* (CBS 728.79) was originally treated as part of the *G. philoprina* species complex, from which it is phylogenetically distinct (Figs 1, 2). It is also distinct from *Phyllosticta podocarpi*, which was originally described from *Podocarpus elongatus* leaf litter collected in South Africa [conidia (10–)14(–17) × (8–)9(–10) µm, appendages 10–40 × 1.5–2 µm; Crous *et al.* 1996].

Phyllosticta rhaphiolepidis Wikee, C. Nakash. & Crous, **sp. nov.** MycoBank MB805662. Fig. 20.

Etymology: Named after the host genus from which it was collected, *Rhaphiolepis.*

Leaf spots irregular, pale brown. *Conidiomata* (on PDA) pycnidial, amphiphyllous, immersed, subglobose to globose, composed of depressed or irregular cells in 2–3 layers, dark brown to black, hyaline or paler toward the inside, 85–175 × 100–110 µm diam, with central ostiole, 10–13 µm diam. *Conidiogenous cells* integrated, lining the inner layer of pycnidia, hyaline, lageniform, cylindrical or conical, 3–10 × 3–4 µm, proliferating percurrently near apex. *Conidia* unicellular, spherical, ellipsoid to obovoid, truncate at base, later rounded at both ends, surrounded by a mucoid layer, containing numerous minute guttules, 7.5–10 × 4.6–6 µm, with a slender and short apical appendage 4–6 × 1–2 µm.

Specimen examined: **Japan**, Kagoshima, Tokunoshima Is., on living leaf of *Rhaphiolepis indica* var. *umbellata*, T. Kobayashi & Y. Ono, 22 Oct. 2003 (**holotype** CBS H-21408, culture ex-type MUCC 432).

Notes: *Phyllosticta rhaphiolepicola*, which occurs on *Rhaphiolepis japonica* in Germany, has somewhat wider conidia (7–9 × 6–8 µm; van der Aa & Vanev 2002) than the Japanese collection. *Phyllosticta*

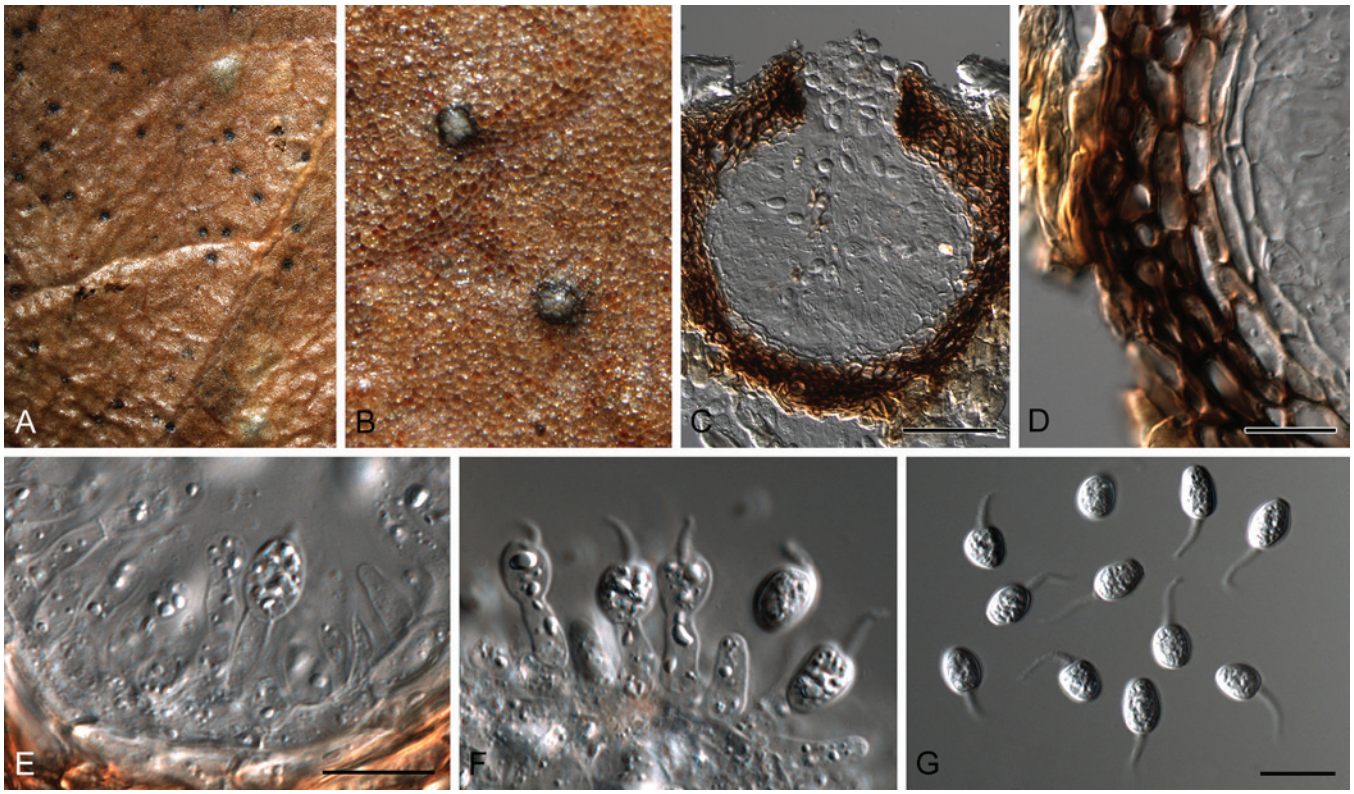

Fig. 20. *Phyllosticta rhaphiolepidis* (MUCC 432). A, B. Close-up of immersed conidiomata on leaf tissue. C. Vertical section through conidioma. D. Conidiomatal wall of *textura angularis*. E, F. Conidiogenous cells. G. Conidia. Scale bars: C = 25 µm, all others = 10 µm.

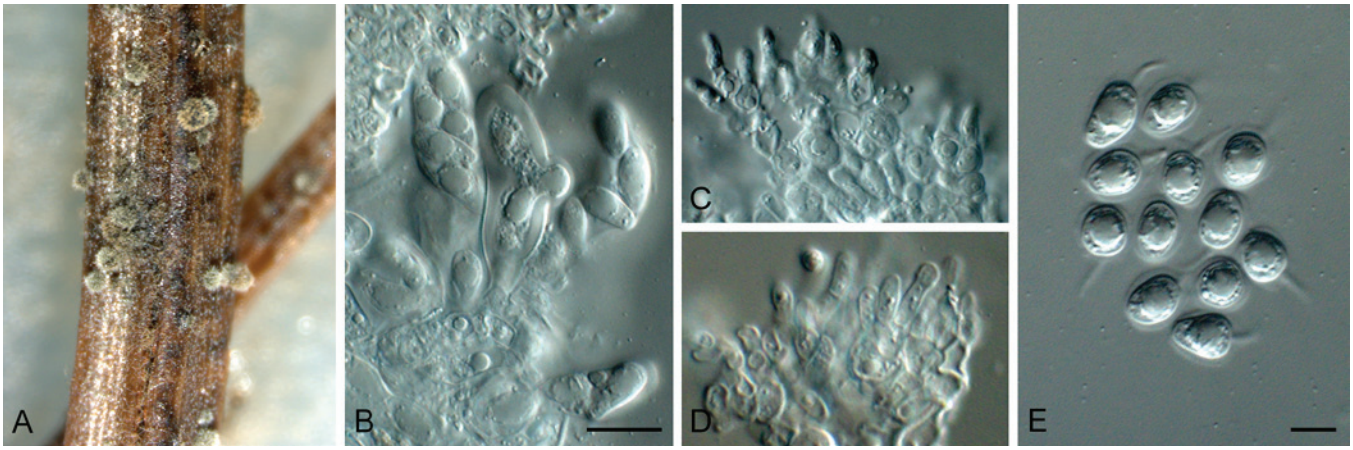

Fig. 21. *Phyllosticta rubra* (CBS 111635). A. Conidiomata forming on PNA. B. Asci with ascospores. C, D. Conidiogenous cells. E. Conidia. Scale bars = 10 µm.

rhaphiolepidis is also phylogenetically distinct from other species of *Phyllosticta* that have been deposited in GenBank (Figs 1, 2).

Phyllosticta rubra Wikee & Crous, **sp. nov.** MycoBank MB805663. Fig. 21.

Etymology. Named after the host species from which it was collected, *Acer rubrum.*

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; *pycnidia* up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 7–10 × 2–3 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 3–8 × 2–3 µm; proliferating several times percurrently near apex. *Conidia* (6–)6.5–7(–8) × (4–)5(–5.5) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 1.5–2 µm diam, enclosed in a thin persistent mucoid sheath, 1–1.5 µm thick, and bearing a hyaline, apical mucoid appendage, (5–)6–7(–9) × (1–)1.5 µm, flexible, unbranched, tapering towards an acutely rounded tip. *Ascomata* similar to conidiomata in general anatomy. *Asci* bitunicate, hyaline, clavate to broadly fusoid-ellipsoid, with visible apical chamber, 1 µm diam, 30–50 × 10–12 µm. *Ascospores* bi- to triseriate, hyaline, smooth, granular to guttulate, aseptate, straight, rarely curved, widest in the upper third, limoniform, (8–)9–10(–12) × (4–)5 µm.

Culture characteristics: Colonies erumpent, spreading with moderate aerial mycelium, covering dish after 1 mo at 25 ºC. On OA surface iron-grey. On PDA and MEA surface olivaceous-grey, to iron-grey, reverse iron-grey.

Specimen examined: **USA**, Missouri, on *Acer rubrum*, July 1999, G. Carroll, (**holotype** CBS H-21398, culture ex-type CBS 111635).

Notes: *Phyllosticta rubra* is part of the *P. minima* species complex. *Phyllosticta rubra* has larger conidia (10 µm long), than two proposed synonyms, namely *P. arida* (on *Acer negundo*, conidia 8–10 × 6–7 µm), and *P. acericola* (on *Acer rubrum*, conidia 5–8 × 3–3.5 µm) (see van der Aa 1973).

Phyllosticta spinarum (Died.) Nag Raj & M. Morelet, Bull. Soc. Sci. nat. Arch. Toulon et du Var 34 (219): 12. 1978. Fig. 22.

Basionym: *Phoma spinarum* Died., Krypt.-Fl. Brandenburg (Leipzig) 9: 148. 1912.

Conidiomata (on PNA) pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; *pycnidia* up to 200 µm diam; pycnidial wall of several layers of *textura angularis*, up to 30 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 15 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 10–15 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 5–12 × 3–5 µm; proliferating several times percurrently near apex. *Conidia* (10–)12–14(–15) × (7–)7.5(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, or with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin persistent mucoid sheath, 1–2 µm thick, and bearing a hyaline, apical mucoid appendage, (7–)8–12(–20) × (2–)2.5(–3) µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 70 mm diam after 1 mo at 25 ºC. On OA surface olivaceous-grey. On PDA surface olivaceous-grey, reverse iron-grey. On MEA surface pale olivaceous-grey in outer region, olivaceous-grey in centre; in reverse iron-grey in centre, smoke-grey in outer region.

Specimens examined: **France**, St. Denis en Val, on living leaf of *Chamaecyparis pisifera*, 1970, M. Morelet (CBS H-17034, culture CBS 292.90). **Germany**, Nieder Lauslitz: Colbus, on *Juniperus* sp., 4 Jul. 1910, Diedicke, **holotype** in B.

Notes: Nag Raj & Morelet (1979) provide a detailed description of the type specimen, which closely corresponds with isolate CBS 292.90 studied here.

Phyllosticta vacciniicola Wikee, Crous, K.D. Hyde & McKenzie, **sp. nov.** MycoBank MB805664. Fig. 23.

Conidiomata pycnidial, solitary, black, erumpent, globose, exuding colourless conidial masses; *pycnidia* up to 200 µm diam;

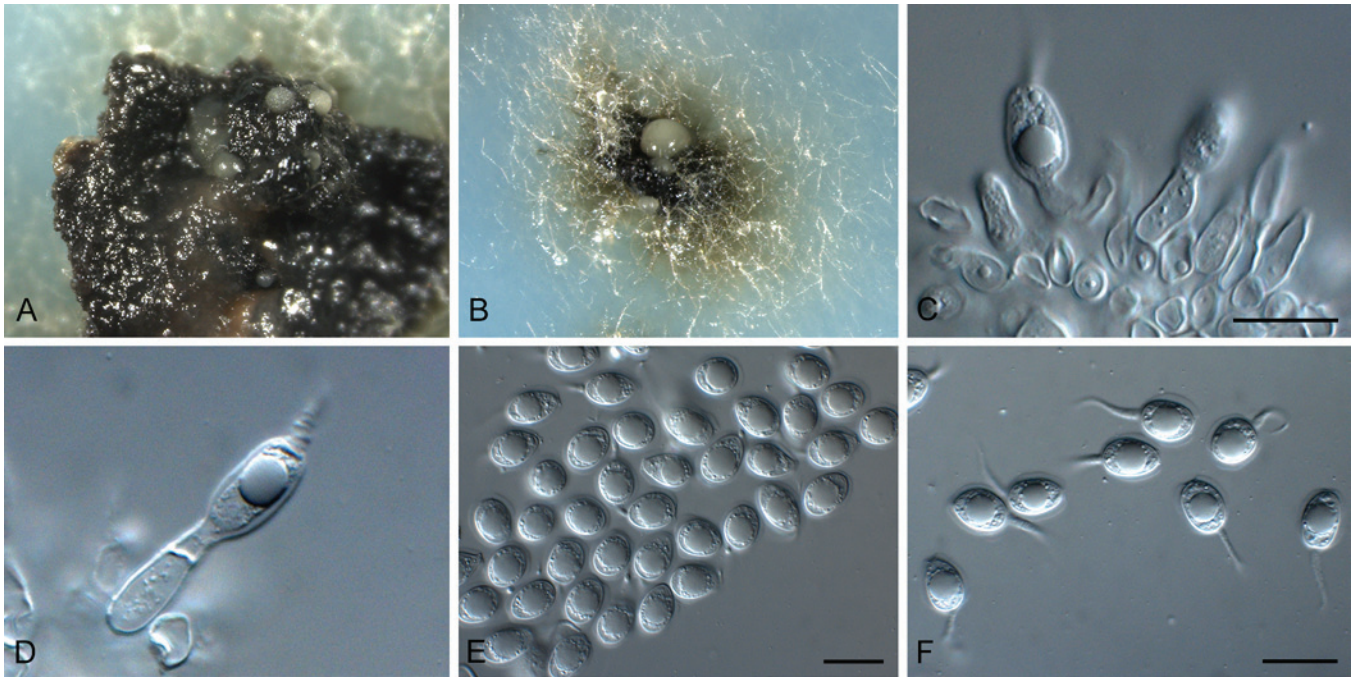

Fig. 22. *Phyllosticta spinarum* (CBS 292.90). A, B. Colony sporulating on SNA. C, D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

pycnidial wall of several layers of *textura angularis*, up to 25 µm thick; inner wall of hyaline *textura angularis*. *Ostiole* central, up to 20 µm diam. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1 supporting cell, that can be branched at the base, 10–20 × 3–5 µm. *Conidiogenous cells* terminal, subcylindrical, hyaline, smooth, coated in a mucoid layer, 8–15 × 3–4 µm; proliferating several times percurrently near apex. *Conidia* (9–)10–12(–13) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin and smooth walled, granular, with a single large central guttule, ellipsoid to obovoid, tapering towards a narrow truncate base, 3–4 µm diam, enclosed in a thin persistent mucoid sheath, 0.5–1 µm thick, and bearing a hyaline, apical mucoid appendage, 7–25 × (1.5–)2 µm, flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics: Colonies flat, spreading with sparse aerial mycelium and feathery, lobate margins, reaching 15 mm diam after 2 wk at 25 ºC. On OA surface iron-grey. On PDA surface and reverse iron-grey. On MEA surface pale olivaceous-grey to olivaceous-grey, reverse olivaceous-grey.

Specimen examined: **USA**, on living leaf of *Vaccinium macrocarpum*, Mariusz Tadych, (**holotype** CBS H-21399, ex-type culture CPC 18590 = CBS 136062).

Notes: A recent study published by Zhang *et al*. (2013) revealed *P. vaccinii* (ex-epitype ATCC 46255) to be distinct from *Guignardia vaccinii* (ex-holotype CBS 126.22), and also revealed that several undescribed *Phyllosticta* spp. are associated with *Vaccinium*, one of which is described here as *P. vacciniicola*. The correct name for

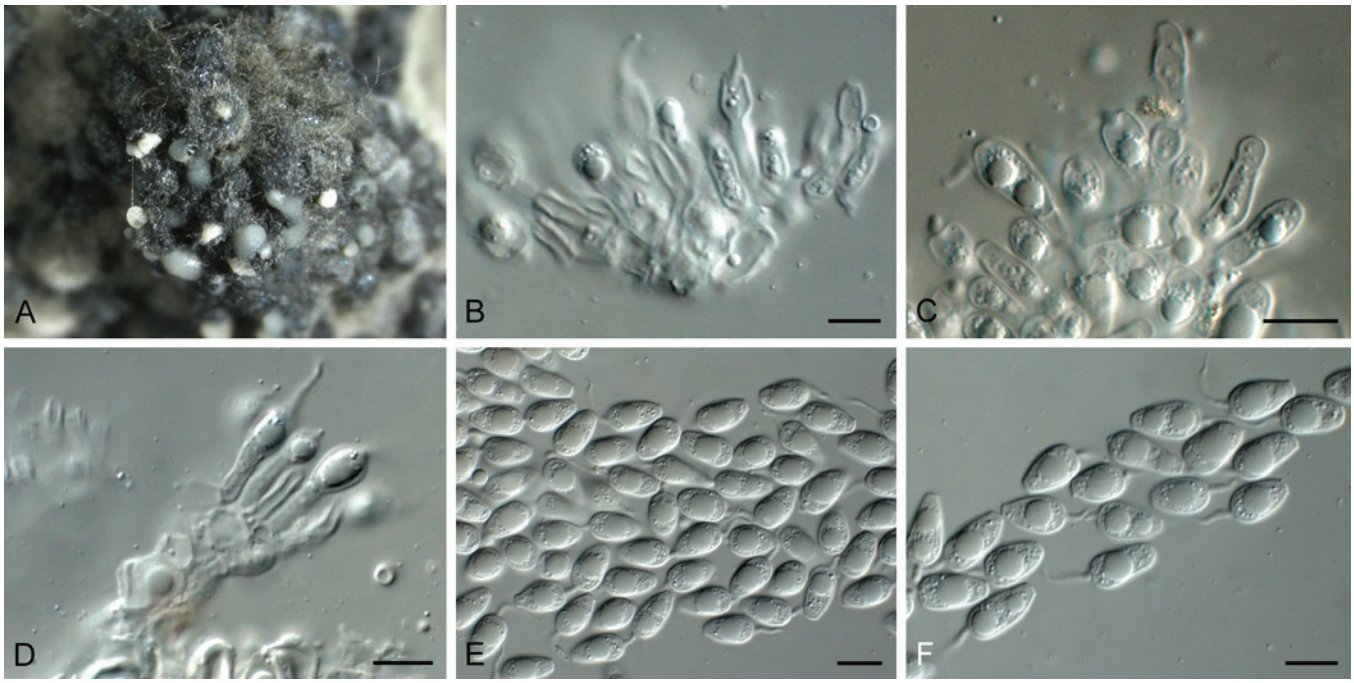

Fig. 23. *Phyllosticta vacciniicola* (CPC 18590). A. Colony sporulating on OA. B–D. Conidiogenous cells giving rise to conidia. E, F. Conidia. Scale bars = 10 µm.

G. vaccinii should thus be that of its asexual morph *P. elongata*, in accordance with Weidemann *et al*. (1982).

Phyllosticta sp.

Specimen examined: **Spain**, on living leaf of *Eucalyptus globulus*, 4 Jan. 2004, M.J. Wingfield, culture CPC 11336.

Notes: Two species of *Phyllosticta* are known from *Eucalyptus*. Van der Aa & Vanev (2002) treated *P. eucalyptorum* (on *E. grandis* from Brazil, conidia (7.5–)11–20 × (5–6(–6.5) µm; Crous *et al*. 1993) as synonymous with *P. eucalyptina* (on *E. globulus*, Tunisia, conidia 18–20 × 5–6 µm). No cultures of *P. eucalyptina* are available, and *P. eucalyptorum* was considered a synonym of *P. capitalensis* (Fig. 1). Although the present collection appears to represent a novel species, it is not treated further as the cultures proved to be sterile.

Phyllosticta sp.

Specimens examined: **Brazil**, São Paulo, Pompeia, on living leaf of *Mangifera indica*, 14 May 2007, C. Glienke & D. Stringari CPC 17454; ibid., CPC 17455.

Notes: Although phylogenetically distinct (Fig. 2), both cultures of this species proved to be sterile, and thus are not treated further.

DISCUSSION

The resurrection of the *Phyllostictaceae*, and its separation from the *Botryosphaeriaceae* is justified based on morphology and DNA phylogeny (Crous *et al.* 2006, Liu *et al.* 2012, Slippers *et al*. 2013, this volume). *Phyllosticta* is a well-established genus, distinct from genera in the *Phoma* complex (Aveskamp *et al*. 2010, de Gruyter *et al*. 2009, 2012, 2013), while the *Botryosphaeria* complex has also been shown to represent numerous genera (Crous *et al*. 2006, Phillips *et al*. 2008, Liu *et al*. 2012), and even families (Slippers *et al*. 2013, this volume).

Traditionally species of *Phyllosticta* have been chiefly identified by their host association. Several recent papers have shown that many traditional morphological species represent complexes of species, e.g. *P. citricarpa* on citrus, *P. musarum* on banana (Glienke *et al.* 2011, Wang *et al*. 2012), and *P. elongata* on *Vaccinium* (Zhang *et al*. 2013).

Freckle disease of banana was usually referred to in literature under its sexual name, *Guignardia musae*, or that of its purported asexual morph, *Phyllosticta musarum*. By employing multigene DNA analysis combined with a morphological comparison, Wong *et al.* (2012) demonstrated that these two names were not conspecific, and that the the common species occurring on banana cultivar Cavendish was in fact a novel taxon, which they described as *P. cavendishii*. The commonly occurring species in Southeast Asia and Oceania on non-Cavendish bananas was in fact another taxon, *P. maculata*. A third species on bananas, *P. musarum* was confirmed from India and Thailand. The most recent studies focusing on the taxonomy of *Phyllosticta* species associated with citrus black spot is that of Glienke *et al*. (2011) and Wang *et al*. (2012). Surprisingly, several species of *Phyllosticta* were shown to cause these symptoms on *Citrus*, although there was a difference in their host range and preference. The citrus black spot pathogen which is presently subjected to phytosanitary legislation in the EU and United States, *P. citricarpa*, was isolated from lemons, mandarins and oranges in China, although Wang *et al*. (2012) did define two subclades, one from mandarins, and another from oranges and lemons. *Phyllosticta citriasiana* was newly described on *Citrus maxima* in Asia by Wulandari *et al.* (2009), while Glienke *et al*. (2011) described *P. citribraziliensis* on *Citrus limon* from Brazil. Wang *et al.* (2012) also described *P. citrichinaensis* on *C. maxima* and *C. reticulata* from China. The present study adds yet a fifth species to this complex, namely *P. citrimaxima*, which is associated with tan spots on the fruit rind of *Citrus maxima* in Thailand. When considering that *P. capitalensis* can still co-occur as an endophyte in fruit or leaf lesions caused by these five species (Wikee *et al*. 2013), it is clear that these taxa are best distinguished by DNA sequence data. This has important biosecurity implications for the *Citrus* industries in many countries.

Guignardia philoprina (asexual morph *P. concentrica*) has been known as the taxon occurring on hosts such as *Rhododendron*, *Hedera*, *Ilex, Magnolia,* and *Taxus* (von Arx & Müller 1954). Not surprisingly, this turned out to represent a species complex, with numerous names available for consideration under the sexual and asexual morph. Although some of these names have been resurrected and applied in the present study, e.g. *P. concentrica* on *Hedera helix*, *P. foliorum* on *Taxus*, and *P. philoprina* on *Ilex*, many taxa still need to be recollected to resolve their phylogeny and correct taxonomy.

One aim of the present paper was to employ multigene DNA sequence analysis to discriminate among all species of *Phyllosticta* that were available to us from the CBS culture collection, supplemented by our own working collections, which resulted in a total of 160 strains. In addition to dealing with old synonymies that represented names that had to be resurrected, a further challenge has been to also merge *Phyllosticta* and *Guignardia* epithets, to obtain the best possible unit nomenclature for these species (Wingfield *et al.* 2012). In the present study we described 12 novel species, and designated a further eight epitype or neotype specimens. From the results obtained here, it is clear that in the case of epitypification, epitypes need to be designated based on the same host, recollected in the same geographic region (see Cannon *et al.* 2012). This is extremely difficult, as American names are commonly used for European of Asian taxa, and also vice versa (see the same situation in *Cercospora* and *Pseudocercospora*; Crous *et al*. 2013, Groenewald *et al*. 2013). In these cases the application of names to collections from other countries that appear morphologically similar, can at best be regarded as tentative, pending further collections.

Results obtained here clearly show that a multi-gene approach works well for distinguishing these taxa. In this study the intron dominated genes (ITS, ACT, TEF), and highly conserved gene coding regions (LSU, GPDH) were used. The result from the five gene analysis compared with the two gene analysis were similar (Figs 1, 2), indicating that the phylogeny of *Phyllosticta* derived from the ITS and ACT gene loci is sufficiently robust to distinguish most taxa, except those closely related to *P. capitalensis*. The biggest challenge ahead is to recollect specimens representative of the more than 3 000 names that exist in this complex.

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