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RESEARCH ARTICLE

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Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot

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Key words

Guignardia endophyllicola
Guignardia mangiferae
Phyllosticta bifrenariae
Phyllosticta brazilianiae
Phyllosticta capitalensis
Phyllosticta citriasiana
Phyllosticta citribraziliensis
Phyllosticta citricarpa
taxonomy

Abstract We investigated the identity and genetic diversity of more than 100 isolates belonging to *Phyllosticta* (teleomorph *Guignardia*), with particular emphasis on *Phyllosticta citricarpa* and *Guignardia mangiferae* s.l. occurring on *Citrus*. *Phyllosticta citricarpa* is the causal agent of Citrus Black Spot and is subject to phytosanitary legislation in the EU. This species is frequently confused with a taxon generally referred to as *G. mangiferae*, the presumed teleomorph of *P. capitalensis*, which is a non-pathogenic endophyte, commonly isolated from citrus leaves and fruits and a wide range of other hosts. DNA sequence analysis of the nrDNA internal transcribed spacer region (ITS1, 5.8S nrDNA, ITS2) and partial translation elongation factor 1-alpha (TEF1), actin and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes resolved nine clades correlating to seven known, and two apparently undescribed species. *Phyllosticta citribraziliensis* is newly described as an endophytic species occurring on *Citrus* in Brazil. An epitype is designated for *P. citricarpa* from material newly collected in Australia, which is distinct from *P. citriasiana*, presently only known on *C. maxima* from Asia. *Phyllosticta bifrenariae* is newly described for a species causing leaf and bulb spots on *Bifrenaria harrisoniae* (*Orchidaceae*) in Brazil. It is morphologically distinct from *P. capitalensis*, which was originally described from *Stanhopea* (*Orchidaceae*) in Brazil; an epitype is designated here. *Guignardia mangiferae*, which was originally described from *Mangifera indica* (*Anacardiaceae*) in India, is distinguished from the non-pathogenic endophyte, *P. brazilianiae* sp. nov., which is common on *M. indica* in Brazil. Furthermore, a combined phylogenetic tree revealed the *P. capitalensis* s.l. clade to be genetically distinct from the reference isolate of *G. mangiferae*. Several names are available for this clade, the oldest being *P. capitalensis*. These results suggest that endophytic, non-pathogenic isolates occurring on a wide host range would be more correctly referred to as *P. capitalensis*. However, more genes need to be analysed to fully resolve the morphological variation still observed within this clade.

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INTRODUCTION

Phyllosticta species have often been reported as endophytes, plant pathogens or saprobes (Baayen et al. 2002, Glienke-Blanco et al. 2002, Okane et al. 2003, Silva et al. 2008, Huang et al. 2009, Wulandari et al. 2009). Many *Phyllosticta* species cause leaf blotch, leaf blight and black spots on fruits of various plants (Glienke-Blanco et al. 2002, Silva & Pereira 2007). Species of *Phyllosticta* s.str. represent anamorphs of *Guignardia* (*Botryosphaeriaceae*) (van der Aa & Vanev 2002, Crous et al. 2006, Schoch et al. 2009). Few studies have to date, however, elucidated the phylogenetic relationships among *Phyllosticta* species and their *Guignardia* teleomorphs. The generic concept of *Phyllosticta* was refined by van der Aa & Vanev (2002) who relocated 2 733 taxa to other coelomycetous genera. However, species concepts within *Phyllosticta* remain problematic.

Phyllosticta capitalensis was originally described on *Stanhopea* (*Orchidaceae*) from Brazil by Hennings (1908). Okane et al. (2001) reported an endophytic *Phyllosticta* in ericaceous plants from Japan, to which they attributed the name *Phyllosticta capitalensis*, describing the teleomorph as a new species, *G. endophyllicola*. Based on DNA sequence data of the ITS gene, Baayen et al. (2002) concluded that there was a common endophytic species associated with a wide host range of plants, which was similar to *G. endophyllicola* in morphology. Although several names were available for this species, they attributed the species to *G. mangiferae* (pathogenic on *Mangifera indica* (*Anacardiaceae*) in India), while the anamorph was referred to as *P. capitalensis*. Although no clear argument was presented for choosing the name *G. mangiferae* for this fungus, the choice of the anamorph name was based on the fact that two isolates from *Orchidaceae* (CBS 398.80, CBS 226.77) clustered in this clade. Uncertainty remains, therefore, as to which name applies to this species.

To determine the identity of the *Phyllosticta* species associated with several hosts including *Citrus*, *Mangifera indica* and the *Orchidaceae*, and to study the phylogenetic relationships among them, fungal isolates were subjected to DNA sequence analysis of the rDNA internal transcribed spacer (ITS1, 5.8S, ITS2) region, and partial translation elongation factor 1-alpha (TEF1), actin (ACT) and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes.

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

Species	Strain no.[1]	Substrate	Country[2]	Collector(s)	GenBank Accession number			
					ITS	TEF1	ACT	GPDH[3]
Guignardia mangiferae	IMI 260576	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	India	M.V. Leksshmi	JF261459	JF261501	JF343641	JF343748
Phyllosticta bifrenariae	VIC30556; CBS 128855	*Bifrenaria harrisoniae* (*Orchidaceae*), living leaves	Brazil: MG	O. Pereira	JF343565	JF343586	JF343649	JF343744
Phyllosticta brazilianiae	LGMF330	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343572	JF343593	JF343656	JF343758
	LGMF333	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343574	JF343595	JF343658	JF343760
	LGMF334	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343566	JF343587	JF343650	JF343752
	LGMF335	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343577	JF343598	JF343661	JF343763
	LGMF338	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343569	JF343590	JF343653	JF343755
	LGMF341	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343575	JF343596	JF343659	JF343761
	LGMF342	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343576	JF343597	JF343660	JF343762
	LGMF343	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343571	JF343592	JF343655	JF343757
	LGMF347	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343567	JF343588	JF343651	JF343753
	LGMF350	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF343573	JF343594	JF343657	JF343759
	LGMF357	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF343570	JF343591	JF343654	JF343756
	LGMF372	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF343568	JF343589	JF343652	JF343754
Phyllosticta capitalensis	16	*Citrus paradisii* (*Rutaceae*), fruit	Florida	–	JF261456	JF261498	JF343638	JF343745
	90	*Smilax kraussiana* (*Smilacaceae*), leaf	South Africa	G.C. Carroll	JF261457	JF261499	JF343639	JF343746
	106	*Encephalartos ferox* (*Zamiaceae*), healthy leaves	South Africa	G.C. Carroll	JF261458	JF261500	JF343640	JF343747
	CBS 100175	*Citrus* sp. (*Rutaceae*), healthy leaves	Brazil: SP	C. Glienke	FJ538320	FJ538378	FJ538436	JF343699
	CBS 100176	*Citrus* sp. (*Rutaceae*), healthy leaves	Brazil: SP	C. Glienke	FJ538321	FJ538379	FJ538437	JF343704
	CBS 100250	*Psidium guajava* (*Myrtaceae*), fruits	Brazil	C. Glienke	FJ538351	FJ538409	FJ538467	JF343710
	CBS 101228	*Nephelium lappaceum* (*Sapindaceae*), discoloured spinters	USA: Hawaii	K.A. Nishijima	FJ538319	FJ538377	FJ538435	JF343697
	CBS 111638	*Capsicum* sp. (*Solanaceae*), fruit	Dominican Republic	G.C. Carroll	FJ538345	FJ538403	FJ538461	JF343709
	CBS 114751	*Vaccinium* sp. (*Ericaceae*), leaf	New Zealand	T. Fluher	FJ538349	FJ538407	FJ538465	JF343722
	CBS 115046	*Myracrodruon urundeuva* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538322	FJ538380	FJ538438	JF343711
	CBS 115047	*Aspidosperma polyneuron* (*Apocynaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538323	FJ538381	FJ538439	JF343705
	CBS 115049	*Bowdichia nitida* (*Fabaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538324	FJ538382	FJ538440	JF343706
	CBS 115051	*Spondias mombin* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538325	FJ538383	FJ538441	JF343715
	CBS 115052	*Spondias mombin* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538326	FJ538384	FJ538442	JF343712
	CBS 115053	*Myracrodruon urundeuva* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538327	FJ538385	FJ538443	JF343717
	CBS 115056	*Anacardium giganteum* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538328	FJ538386	FJ538444	JF343720
	CBS 115057	*Anacardium giganteum* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538329	FJ538387	FJ538445	JF343716
	CBS 115313	*Myracrodruon urundeuva* (*Anacardiaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538330	FJ538388	FJ538446	JF343713
	CBS 115345	*Bowdichia nitida* (*Fabaceae*), leaf or bark	Brazil	K.F. Rodrigues	FJ538331	FJ538389	FJ538447	JF343707
	CBS 117118	*Musa acuminata* (*Musaceae*)	Indonesia	I. Buddenhagen	FJ538339	FJ538397	FJ538455	JF343723
	CBS 119720	*Musa* sp. (*Musaceae*)	USA: Hawaii	I. Buddenhagen	FJ538340	FJ538398	FJ538456	JF343708
	CBS 123373	*Musa paradisiaca* (*Musaceae*)	Thailand	N.F. Wulandari	FJ538341	FJ538399	FJ538457	JF343703
	CBS 123374	*Citrus aurantium* (*Rutaceae*)	Thailand	N.F. Wulandari	FJ538332	FJ538390	FJ538448	JF343702
	CBS 123404	*Musa paradisiaca* (*Musaceae*)	Thailand	N.F. Wulandari	FJ538333	FJ538391	FJ538449	JF343701
	CBS 123405	*Musa acuminata* (*Musaceae*)	Thailand	N.F. Wulandari	FJ538334	FJ538392	FJ538450	JF343726
	CBS 173.77	*Citrus aurantiifolia* (*Rutaceae*)	New Zealand	–	FJ538335	FJ538393	FJ538451	JF343725
	CBS 226.77	*Paphiopedilum callosum* (*Orchidaceae*), leaf spot	Germany	–	FJ538336	FJ538394	FJ538452	JF343718
	CBS 356.52; ATCC 11368	*Ilex* sp. (*Aquifoliaceae*)	–	–	FJ538342	FJ538400	FJ538458	JF343721
	CBS 373.54	*Ilex* sp. (*Aquifoliaceae*)	–	–	FJ538343	FJ538401	FJ538459	JF343698
	CMU131	*Magnolia liliifera* (*Magnoliaceae*), leaf endophyte	Thailand	L.M. Duong	FJ538346	FJ538404	FJ538462	JF343724
	CMU139	*Magnolia liliifera* (*Magnoliaceae*), leaf endophyte	Thailand	L.M. Duong	FJ538347	FJ538405	FJ538463	JF343714
	CMU142	*Magnolia liliifera* (*Magnoliaceae*), leaf endophyte	Thailand	L.M. Duong	FJ538348	FJ538406	FJ538464	JF343719
	CPC 18845	*Stanhopea graveolens* (*Orchidaceae*)	Brazil	O.L. Pereira	JF261463	JF261505	JF343645	JF343774
	CPC 18847	*Stanhopea graveolens* (*Orchidaceae*)	Brazil	O.L. Pereira	JF261464	JF261506	JF343646	JF343775
	CPC 18848; CBS 128856	*Stanhopea graveolens* (*Orchidaceae*)	Brazil	O.L. Pereira	JF261465	JF261507	JF343647	JF343776
	CPC 18849	*Stanhopea graveolens* (*Orchidaceae*)	Brazil	O.L. Pereira	JF261466	JF261508	JF343648	JF343777
	G22	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	A. de Goes	JF261437	JF261479	JF343619	JF343700
	LGMF02	*Citrus latifolia* (*Rutaceae*), healthy leaves	Brazil: SP	A. de Goes	JF261452	JF261494	JF343634	JF343741
	LGMF03	*Citrus latifolia* (*Rutaceae*), healthy leaves	Brazil: SP	A. de Goes	JF261453	JF261495	JF343635	JF343749
	LGMF181	*Citrus reticulata* (*Rutaceae*), black spot on fruit	Brazil: PR	C. Glienke	JF261447	JF261489	JF343629	JF343736
	LGMF217	*Citrus sinensis* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261451	JF261493	JF343633	JF343740

	LGMF219	*Citrus sinensis* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261448	JF261490	JF343630	JF343737
	LGMF220	*Citrus sinensis* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261446	JF261488	JF343628	JF343735
	LGMF222	*Citrus sinensis* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261450	JF261492	JF343632	JF343739
	LGMF231	*Citrus sinensis* (*Rutaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF261441	JF261483	JF343623	JF343730
	LGMF240	*Citrus sinensis* (*Rutaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF261443	JF261485	JF343625	JF343732
	LGMF244	*Citrus limonia* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261442	JF261484	JF343624	JF343731
	LGMF253	*Citrus limonia* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261460	JF261502	JF343642	JF343750
	LGMF259	*Citrus latifolia* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261461	JF261503	JF343643	JF343751
	LGMF317	*Citrus reticulata* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261440	JF261482	JF343622	JF343729
	LGMF318	*Citrus reticulata* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261454	JF261496	JF343636	JF343742
	LGMF319	*Citrus reticulata* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261445	JF261487	JF343627	JF343734
	LGMF326	*Citrus reticulata* (*Rutaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261444	JF261486	JF343626	JF343733
	LGMF332	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: SP	C. Glienke	JF261439	JF261481	JF343621	JF343728
	LGMF358	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261449	JF261491	JF343631	JF343738
	LGMF366	*Mangifera indica* (*Anacardiaceae*), leaf endophyte	Brazil: PR	C. Glienke	JF261438	JF261480	JF343620	JF343727
	VIC30428	*Cymbidium* sp. (*Orchidaceae*), leaf blight	Brazil: MG	M. Silva & O.L. Pereira	JF261455	JF261497	JF343637	JF343743
Phyllosticta citriasiana	CBS 120486; PD 05/01969753	*Citrus maxima* (*Rutaceae*)	Thailand	J. de Gruyter	FJ538360	FJ538418	FJ538476	JF343686
	CBS 120487; PD 05/03081053	*Citrus maxima* (*Rutaceae*)	China	K. Rosendahl-Peters	FJ538361	FJ538419	FJ538477	JF343687
	CBS 123370; PD 08/04453736	*Citrus maxima* (*Rutaceae*)	Vietnam	J. de Gruyter	FJ538355	FJ538413	FJ538471	JF343689
	CBS 123371; PD 08/04454173	*Citrus maxima* (*Rutaceae*)	Vietnam	J. de Gruyter	FJ538356	FJ538414	FJ538472	JF343690
	CBS 123393; PD 08/04453728	*Citrus maxima* (*Rutaceae*)	Vietnam	J. de Gruyter	FJ538358	FJ538416	FJ538474	JF343688
Phyllosticta citribraziliensis	CBS 100098	*Citrus* sp. (*Rutaceae*), healthy leaves	Brazil: PR	C. Glienke	FJ538352	FJ538410	FJ538468	JF343691
	LGMF08	*Citrus* sp. (*Rutaceae*), healthy leaves	Brazil: PR	C. Glienke	JF261435	JF261477	JF343617	JF343692
	LGMF09	*Citrus* sp. (*Rutaceae*), healthy leaves	Brazil: PR	C. Glienke	JF261436	JF261478	JF343618	JF343693
Phyllosticta citricarpa	29	*Citrus sinensis* (*Rutaceae*), black spot on fruit	South Africa	G.C. Carroll	JF261433	JF261475	JF343615	JF343683
	71	*Citrus sinensis* (*Rutaceae*), black spot on fruit	South Africa	G.C. Schutte	JF261432	JF261474	JF343614	JF343682
	CBS 102373	*Citrus aurantium* (*Rutaceae*), black spot on fruit	Brazil	–	FJ538312	FJ538370	FJ538428	JF343678
	CBS 102374	*Citrus aurantium* (*Rutaceae*), black spot on fruit	Brazil	–	FJ538313	FJ538371	FJ538429	JF343679
	CBS 111.20	–	–	–	FJ538314	FJ538372	FJ538430	JF343681
	CBS 120489	*Citrus limon* (*Rutaceae*)	Brazil	J. de Gruyter	FJ538315	FJ538373	FJ538431	JF343685
	CBS 122384	*Citrus limon* (*Rutaceae*)	South Africa	M. Truter	FJ538316	FJ538374	FJ538432	JF343680
	CBS 122482	*Citrus sinensis* (*Rutaceae*), lesions on fruit	Zimbabwe	L. Huisman	FJ538317	FJ538375	FJ538433	JF343677
	CBS 127451; CPC 18173	*Citrus reticulata* (*Rutaceae*)	Australia	S.L. Willingham	JF343580	JF343601	JF343664	JF343768
	CBS 127452; CPC 18174	*Citrus reticulata* (*Rutaceae*)	Australia	S.L. Willingham	JF343581	JF343602	JF343665	JF343769
	CBS 127453; CPC 18175	*Citrus reticulata* (*Rutaceae*)	Australia	S.L. Willingham	JF343582	JF343603	JF343666	JF343770
	CBS 127454; CPC 18176	*Citrus limon* (*Rutaceae*)	Australia	S.L. Willingham	JF343583	JF343604	JF343667	JF343771
	CBS 127455; CPC 18177	*Citrus sinensis* (*Rutaceae*)	Australia	S.L. Willingham	JF343584	JF343605	JF343668	JF343772
	Guig1	*Citrus maxima* (*Rutaceae*), black spot on fruit	Brazil: SP	A. de Goes	JF261429	JF261471	JF343611	JF343674
	LGMF06	*Citrus sinensis* (*Rutaceae*), black spot on fruit	Brazil: SP	A. de Goes	JF261431	JF261473	JF343613	JF343676
	LGMF20	*Citrus sinensis* (*Rutaceae*), black spot on fruit	Brazil: PR	C. Glienke	JF261430	JF261472	JF343612	JF343675
	LGMF25	*Citrus sinensis* (*Rutaceae*), black spot on fruit	Brazil: PR	C. Glienke	JF261428	JF261470	JF343610	JF343673
	LGMF27	*Citrus sinensis* (*Rutaceae*), black spot on fruit	Brazil: PR	C. Glienke	JF261427	JF261469	JF343609	JF343672
	LGMF45	*Citrus reticulata* (*Rutaceae*), black spot on fruit	Brazil: PR	C. Glienke	JF261426	JF261468	JF343608	JF343671
	LGMF63	*Citrus reticulata* (*Rutaceae*), black spot on fruit	Brazil: PR	C. Glienke	JF261425	JF261467	JF343607	JF343670
	LGMF247	*Citrus limonia* (*Rutaceae*), on leaves	Brazil: PR	C. Glienke	JF261434	JF261476	JF343616	JF343684
Phyllosticta cussonia	CPC 14873	*Cussonia* sp.	South Africa	P.W. Crous	JF343578	JF343599	JF343662	JF343764
	CPC 14875	*Cussonia* sp.	South Africa	P.W. Crous	JF343579	JF343600	JF343663	JF343765
Phyllosticta hypoglossi	CBS 101.72; IFO 32916	*Ruscus aculeatus* (*Ruscaceae*), living leaves	Italy	W. Gams	FJ538365	FJ538423	FJ538481	JF343694
	CBS 167.85	*Ruscus hypoglosum* (*Ruscaceae*)	Italy	W. Gams	FJ538366	FJ538424	FJ538482	JF343696
	CBS 434.92	*Ruscus aculeatus* (*Ruscaceae*), dead cladodes	Italy	W. Gams	FJ538367	FJ538425	FJ538483	JF343695
Phyllosticta owaniana	CBS 776.97	*Brabejum stellatifolium* (Proteaceae), leaf spot	South Africa	A. den Breeÿen	FJ538368	FJ538426	FJ538484	JF343767
	CPC 14901	*Brabeijum stellatifolium* (*Proteaceae*), leaf spot	South Africa	P.W. Crous	JF261462	JF261504	JF343644	JF343766
Phyllosticta spinarum	CBS 292.90	*Chamaecyparis pisifera* (*Cupressaceae*)	France	M. Morelet	JF343585	JF343606	JF343669	JF343773
	CBS 937.70	*Hedera helix* (*Araliaceae*), leaf litter	Italy	W. Gams	FJ538350	FJ538408	FJ538466	JF411745

[1] ATCC: American Type Culture Collection, Virginia, USA; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CMU: Microbiology Section, Chiang Mai University (MSCMU), Department of Biology, Faculty of Science, Chang Mai University, Thailand; 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; PD: Plant Protection Service, Wageningen, The Netherlands; VIC: Culture collection of Federal University of Viçosa, Viçosa, Brazil.

[2] Abbreviations used with Brazil: MG: State of Minas Gerais; PR: State of Paraná; SP: State of São Paulo.

[3] ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; TEF1: partial translation elongation factor 1-alpha gene; ACT: partial actin gene; GPDH: partial glyceraldehyde-3-phosphate dehydrogenase gene.

MATERIAL AND METHODS

Isolates

A total of 109 *Phyllosticta* / *Guignardia* isolates were investigated in the present study (Table 1). Single monosporic isolates were obtained from each culture prior to DNA sequence analysis. Isolates were obtained from several sources including the CBS Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands, the working collection of Pedro Crous housed at CBS (CPC), the LabGeM/UFPR collection, Curitiba, Brazil, the Dutch Quarantine Service (PD), and the Department of Primary Industries (BRIP), Brisbane, Australia. Two isolates (VIC30428 and VIC30556) were obtained from UFG collection, Viçosa, Brazil, and two isolates from the UNESP collection, Jaboticabal, Brazil (G22, Guig1). One strain of *G. mangiferae* was obtained from CABI Bioscience, UK (IMI 260576).

DNA isolation, amplification and analyses

Genomic DNA extraction was done using the UltraClean™ Microbial DNA Kit (MO Bio, Carlsbad, CA, USA) according to manufacturer's protocol or according to Glienke-Blanco et al. (2002). The primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5' end of the 28S rRNA gene. The primers EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell et al. 1998) were used to amplify part of the translation elongation factor 1-α gene (TEF1) and the primers ACT-512F and ACT-783R (Carbone & Kohn 1999) were used to amplify part of the actin gene (ACT). Amplification conditions followed Arzanlou et al. (2008). The primers GDF1 (Guerber et al. 2003) and Gpd2-LM (Myllys et al. 2002) or GDR1 (Guerber et al. 2003) were used to amplify part of the glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene of *G. mangiferae* s.l. isolates. Amplification reactions were performed under two different conditions, depending on the laboratory in which those specific reactions were performed. The first condition had a total reaction volume of 15.5 µL, which was composed of 1× PCR Buffer (Applied Biosystems, Foster City, USA), 2 mM $MgCl_2$, 40 µM dNTPs, 0.08 µM of each forward and reverse primer, 0.5 U of *Taq* DNA polymerase (Roche Diagnostics, Indianapolis, USA) and 1–10 ng of genomic DNA. The PCR cycle conditions were 4 min of 94 °C, followed by 13 cycles of 94 °C for 30 s, the annealing temperature was decreased in 0.7 for every subsequent set of cycles, 72 °C for 60 s, followed by 23 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s and a final elongation at 72 °C for 7 min. The second condition had a total reaction volume of 12.5 µL, which was composed of 1× PCR Buffer (Bioline GmbH, Luckenwalde, Germany), 5.6 % DMSO (v/v), 2 mM $MgCl_2$, 20 µM dNTPs, 0.2 µM of each forward and reverse primer, 0.25 U of BioTaq *Taq* DNA polymerase (Bioline GmbH, Luckenwalde, Germany) and 1–10 ng of genomic DNA. The PCR cycle conditions were 5 min of 94 °C, followed by 40 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s and a final elongation step at 72 °C for 7 min. The partial GPDH gene of *G. citricarpa* isolates was amplified with the primers GDF1 (Guerber et al. 2003) and a primer developed in the present study, GPDHR2 (5'-CTCRGMRGCRGCCTT-GATGG-3'). A 1 000 bp fragment was obtained with this primer combination. Amplification reactions were performed in a final reaction volume of 12.5 µL, which was composed of 1× PCR Buffer (Applied Biosystems, Foster City, USA), 2.5 mM $MgCl_2$, 40 µM dNTPs, 0.12 µM of each forward and reverse primer, 0.5 U of *Taq* DNA polymerase (Roche Diagnostics, Indianapolis, USA) and 1–10 ng of genomic DNA. The PCR cycle conditions were 5 min of 95 °C, followed by 35 cycles of 95 °C for 30 s, 50 °C for 45 s, 72 °C for 90 s, and a final elongation at 72 °C for 7 min. Amplicons were sequenced using both PCR primers with a BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and sequences were analyzed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Norwalk, Foster City, CA, USA).

Consensus sequences were manually aligned using MEGA v4 software (Kumar et al. 2008) by inserting gaps. Phylogenetic analyses of the aligned sequence data (no nucleotides were excluded) were performed with PAUP (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford 2003) as described previously (Cheewangkoon et al. 2008). Based on previous phylogenetic studies (e.g. Wulandari et al. 2009), *Phyllosticta owaniana* was used as outgroup in the phylogenetic analyses. Statistical parameters calculated by PAUP included Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). Novel sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Morphology

Isolates were established on 2 % malt extract agar (MEA), 2 % potato-dextrose agar (PDA), pine-needle agar (PNA; tap water agar with autoclaved pine needles; Crous et al. 2006) and oatmeal agar (OA; Crous et al. 2009c), and incubated at 25 °C under near-ultraviolet light to promote sporulation. Fungal structures were mounted on glass slides in clear lactic acid for microscopic examination after 14 d of incubation. Thirty measurements were determined per structure, where possible, from colonies sporulating on PNA. Colony colours (surface and reverse) were determined using the colour charts of Rayner (1970) after 1 mo at 25 °C in the dark. Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

RESULTS

Phylogenetic analysis

The manually adjusted combined (ITS, TEF1, ACT and GPDH) alignment contained 105 isolates (including two outgroup sequences) and, of all 1 580 characters used in the phylogenetic analysis, 442 were parsimony-informative, 61 were variable and parsimony-uninformative, and 1 077 were conserved. Distance analyses using the three substitution models on the sequence data yielded trees with identical topology and similar bootstrap values. Only the first 1 000 equally most parsimonious trees were retained, the first of which is shown in Fig. 1 (TL = 932, CI = 0.790, RI = 0.982, RC = 0.776). These trees only differed with regard to the order of the small terminal branches within the well-supported clades (see the thickened strict consensus branches in Fig. 1).

Ten well-supported clades could be resolved (Fig. 1). The first clade consists of the strain VIC30556, which was isolated from leaf and pseudobulb lesions on *Bifrenaria harrisoniae* (*Orchidaceae*) in Brazil (Silva et al. 2008) and was morphologically identified as *Phyllosticta capitalensis* by the authors. This isolate, described here as *P. bifrenariae* sp. nov., caused dark, large spots on orchid leaves, in contrast to the symptoms associated with endophytic isolates (Silva et al. 2008).

The second clade consists of two isolates of *Phyllosticta cussonia* from South Africa, while the third clade consists of three isolates from *Ruscus hypoglossum* in Italy, representing a species complex presently treated as *P. hypoglossi*. The fourth clade consists of two isolates identified as *P. spinarum* from *Chamaecyparis pisifera* in France and *Hedera helix* in Italy, respectively, and probably also represents a species complex.

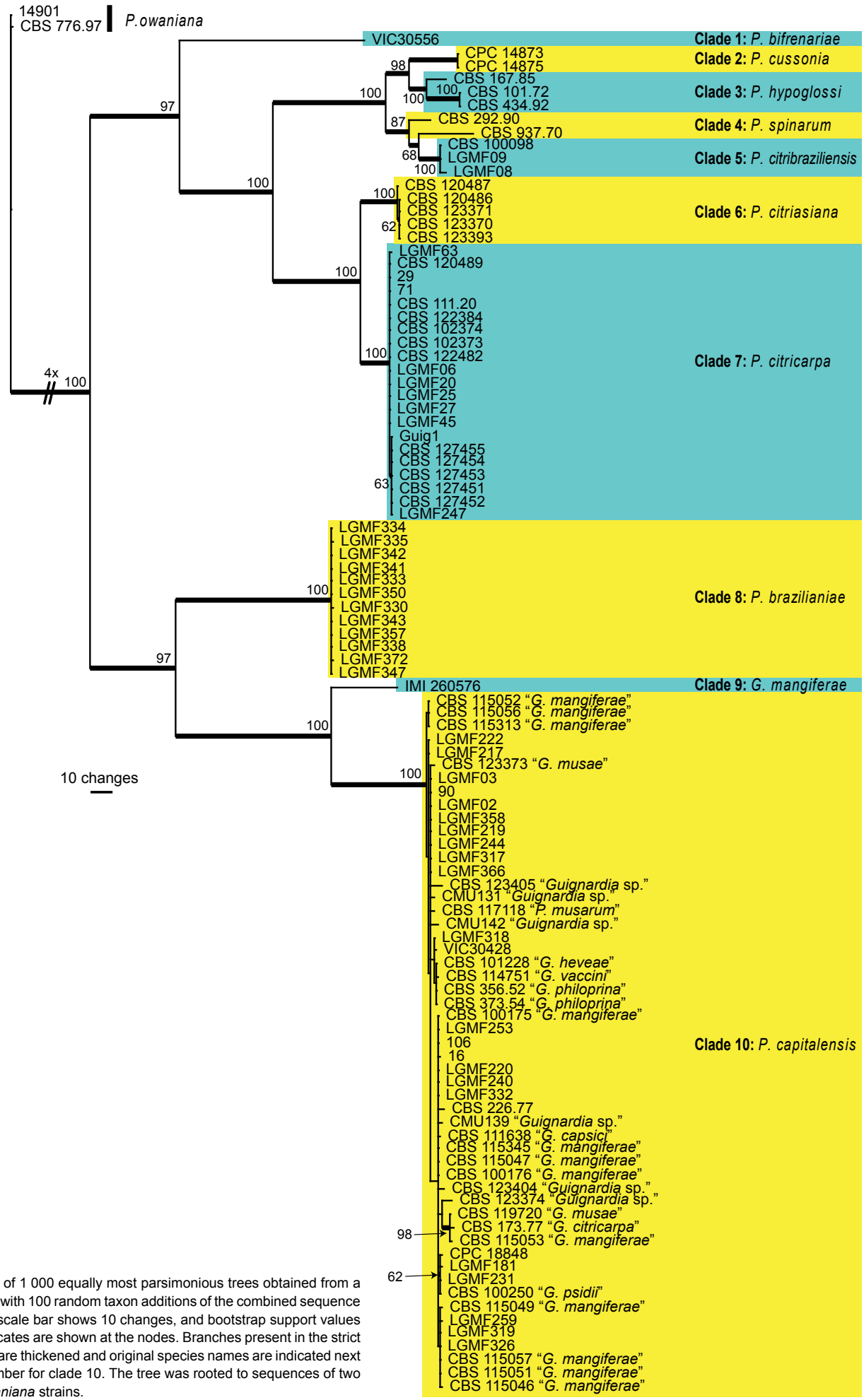


Fig. 1 The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Branches present in the strict consensus tree are thickened and original species names are indicated next to the strain number for clade 10. The tree was rooted to sequences of two *Phyllosticta owaniana* strains.

Three *Citrus* (*Rutaceae*) endophytic isolates from Brazil, described here as *P. citribraziliensis*, make up clade 5.

The sixth clade is represented by isolates of *P. citriasiana* (Wulandari et al. 2009), associated with tan spot on *Citrus maxima* fruits. Clade 7 represents isolates of *P. citricarpa* from Australia, Brazil, South Africa and Zimbabwe. Clade 8 consists of 12 endophytic isolates of *Mangifera indica* (*Anacardiaceae*) from Brazil. These isolates are morphologically distinct, and exhibited insignificant homology to any sequence found in the GenBank nucleotide database, and these are described below as *P. brazilianiae* sp. nov. Clade 9 consists of a single isolate (IMI 260576), which was isolated in India from *Mangifera indica*, and is considered authentic for the name *G. mangiferae*.

Clade 10 represents several different hosts and countries (Fig. 1, Table 1). This clade included isolates from *Rutaceae* (*Citrus* spp.), *Anacardiaceae* (*Mangifera indica*, *Spondias mombin*, *Myracrodruon urundeuva*, *Anacardium giganteum*), *Myrtaceae* (*Psidium guajava*), *Sapindaceae* (*Nephelium lappaceum*), *Solanaceae* (*Capsicum*), *Fabaceae* (*Bowdichia nitida*), *Apocynaceae* (*Aspidosperma polyneuron*), *Musaceae* (*Musa* spp.), *Orchidaceae* (*Cymbidium* sp., *Paphiopedilum callosum*, *Stanhopea graveolens*), *Aquifoliaceae* (*Ilex* sp.), *Magnoliaceae* (*Magnolia liliifera*), *Smilacaceae* (*Smilax kraussiana*) and *Zamiaceae* (*Encephalartos ferox*). This clade contains isolates previously identified as *G. mangiferae*, *G. endophylicolla*, *G. psidii*, *G. capsici*, *G. musae*, *G. vaccini*, *G. philoprina*, *G. musarum*, *Guignardia* sp. and *P. capitalensis*. However, the low sequence homology found between the reference isolate of *G. mangiferae* (clade 9) (IMI 260576) and clade 10 isolates, strongly supports these as two distinct species (Fig. 1).

Morphology

Several new species were identified during this study, which are described below. Furthermore, an epitype could also be designated for *P. citricarpa* based on *Citrus* collections newly obtained from Australia. Similarly, an epitype could be designated for *P. capitalensis*, based on fresh collections obtained on *Stanhopea* from Brazil. Although isolates belonging to clade 10 are all treated as *P. capitalensis*, some morphological variation was observed in conidium morphology (sheath thickness, appendage length and conidium shape), and growth in culture. Most cultures produced conidia with sheaths more than 2 µm thick, as reported by Baayen et al. (2002) for *P. capitalensis*. Several isolates also produced a *Guignardia* state in culture. Additional genes need to be sequenced to determine if the observed variation in clade 10 is intra- or interspecific. Furthermore, in moving to a single nomenclature for species of *Ascomycetes* (Rossman & Samuels 2005, Crous et al. 2006, 2007, 2009a, b, Aveskamp et al. 2010, Lechat et al. 2010, Lombard et al. 2010a–c), the older generic name, *Phyllosticta* (1818), is chosen above the later *Guignardia* (1892), which should be regarded as synonym.

Guignardia mangiferae A.J. Roy, Indian Phytopathol. 20: 348. 1968

Type specimen. India, Shitlakhet in Almora, on leaves of *Mangifera indica*, 9 July 1963, *B.S. Khati*, holotype HFRS 1056 (could not be obtained for examination).

Colonies on OA. *Pycnidia* black, aggregated, erumpent, globose to ampulliform, exuding a colourless, glossy conidial mass; pycnidia up to 300 µm diam, 250 µm tall; pycnidial wall consisting of several layers, up to 40 µm thick, of *textura angularis*. *Ostiole* single, central, up to 30 µm wide, consisting of thickened, brown cells. *Conidiophores* subcylindrical to doliiform, frequently reduced to conidiogenous cells, coated in mucoid layer, 6–15 × 3–6 µm. *Conidiogenous cells* terminal, subcylindrical to doliiform, hyaline, smooth, 6–10 × 3–4 µm; proliferating 2–3 times percurrently near apex. *Conidia* (8–)10–12 × (5–)6–7 µm, solitary, hyaline, aseptate, thin- and smooth-walled, coarsely guttule, ellipsoid to obovoid, tapering toward a narrowly truncate base, enclosed in a mucilaginous sheath, 2–5 µm thick, and bearing a hyaline, mucoid apical appendage, 7–13 × 1–1.5 µm, straight to flexible, unbranched, tapering towards an acute apex. No teleomorph other than ascomatal initials developed in agar (OA, SNA, PDA, MEA, PNA), and the isolate sporulated poorly.

Specimen examined. India, on leaves of *Mangifera indica* (*Anacardiaceae*), 1981, *M.V. Leksshmi*, culture IMI 260576.

Notes — Two other species occurring on *Mangifera indica* in Brazil need to be discussed. *Phyllosticta mangiferae* has fusiform, 11–23 × 6–7 µm conidia, resembling the genus *Fusicoccum* (van der Aa & Vanev 2002). *Phyllosticta anacardiacearum* differs from *G. mangiferae* by having shorter conidiophores, and a narrower sheath, although the conidia are similar in size (van der Aa 1973). No cultures of *P. anacardiacearum* are, however, available for study. Because the name *Phyllosticta mangiferae* is occupied, a new name would have to be proposed for *Guignardia mangiferae* when it eventually is placed in *Phyllosticta*. However, because mango has been poorly studied, we choose to wait until more isolates become available.

Phyllosticta bifrenariae O.L. Pereira, C. Glienke & Crous, *sp. nov*. — MycoBank MB517969; Fig. 2

Phyllostictae capitalensis similis, sed conidiis maioribus, 10–16 × 7–9 µm.

Etymology. Named after the host genus from which it was isolated, *Bifrenaria*.

Colonies on PNA. *Pycnidia* black, solitary, or arranged in clusters of up to 6, ampulliform, base ovoid, up to 250 µm diam, with elongated subcylindrical neck up to 1 100 µm long, and rounded apex, 180 µm diam; pycnidial wall consisting of several layers, up to 40 µm thick; outer region of dark brown *textura angularis* to *globularis*; inner region consisting of 1–2 pale cell layers, that become hyaline toward interior, *textura angularis*. *Ostiole* single, central, up to 40 µm wide. *Conidiophores* reduced to *Conidiogenous cells*, subcylindrical to ampulliform, hyaline, smooth, 7–10 × 4–5 µm; inconspicuously proliferating once or twice percurrently near apex. *Conidia* (10–)11–13(–16) × (7–)8–9 µm, solitary, hyaline, aseptate, thin- and smooth-walled, with large central guttule, ellipsoid to ovoid or obovoid, tapering toward a narrowly truncate base, 3–4 µm wide, enclosed in a thick mucilaginous sheath, 3–6 µm thick, and bearing a hyaline, mucoid apical appendage, 6–20 × 1–1.5 µm, straight to flexible, unbranched, tapering towards an acute tip. *Spermatia* at times forming in conidial conidiomata, hyaline, bacilliform, 5–10 × 1.5–2 µm.

Culture characteristics — Colonies after 14 d at 25 °C in the dark on OA flat, spreading, olivaceous-grey, with moderate aerial mycelium.

Specimen examined. Brazil, Gerdau Açominas RPPN, Serra de Ouro Branco, Ouro Branco, Minas Gerais, on *Bifrenaria harrisoniae* (*Orchidaceae*), 6 Nov. 2007, *O.L. Pereira*, CBS H-20520 holotype, culture ex-type VIC 30556 = CBS 128855.

Notes — Although the isolate now described as *P. bifrenariae* was originally considered to be representative of *P. capitalensis*, it is ecologically distinct in being a pathogen on *Bifrenaria harrisoniae* (*Orchidaceae*) (Silva et al. 2008), and is also phylogenetically distinct (Fig. 1). Morphologically *P. capitalensis* (conidia (10–)11–12(–14) × (5–)6–7 µm) is distinct by having smaller conidia than *P. bifrenariae* (10–16 × 7–9 µm). *Phyllosticta aplectri*, which occurs on *Aplectrum hyemale* (*Orchidaceae*, USA), has smaller conidia, 5–8 × 4–6 µm (van der Aa 1973).

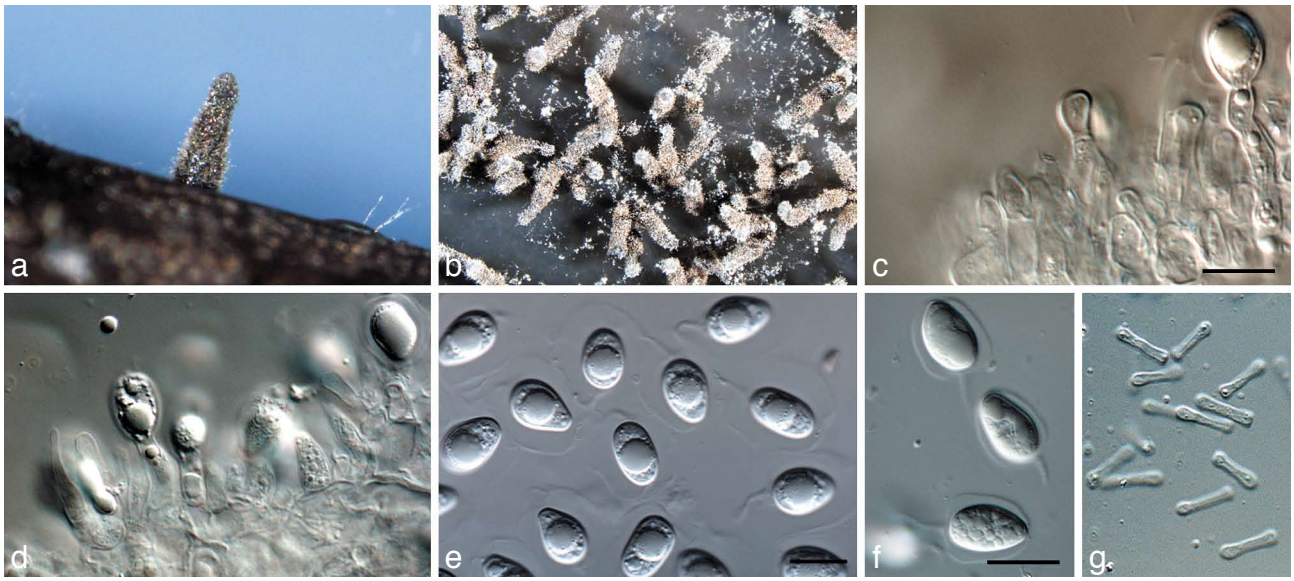


Fig. 2 *Phyllosticta bifrenariae*. a. Pycnidium forming on PNA; b. pycnidia forming on PDA; c, d. conidiophores giving rise to conidia; e, f. conidia; g. spermatia (all: CBS H-20520 holotype). — Scale bars = 10 µm.

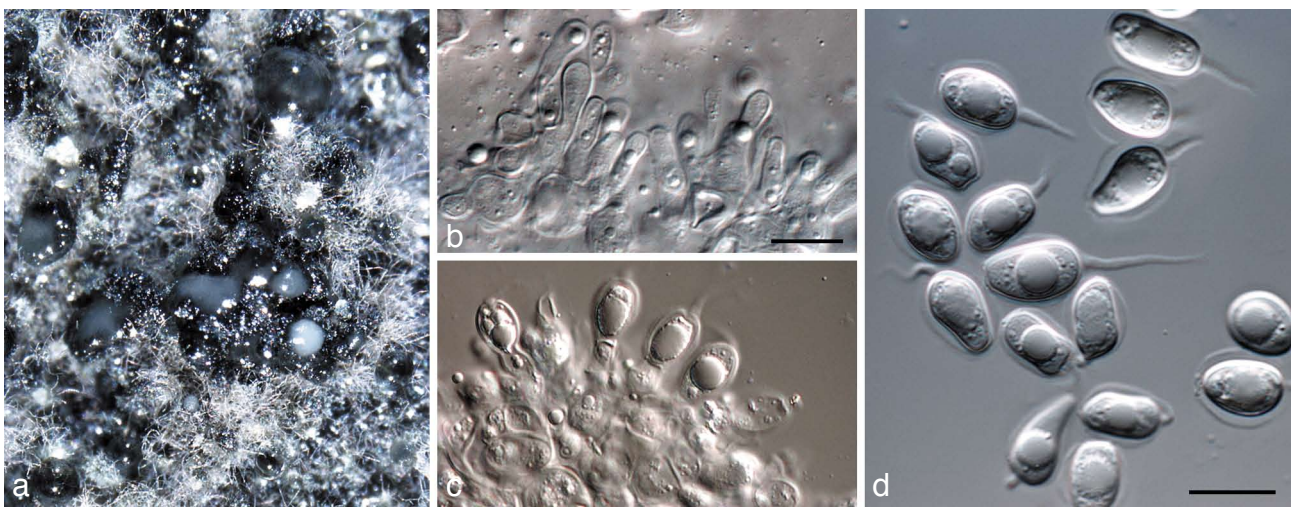


Fig. 3 *Phyllosticta brazilianiae*. a. Pycnidia forming on PDA; b, c. conidiophores giving rise to conidia; d. conidia (all: CBS H-20521 holotype). — Scale bars = 10 µm.

Phyllosticta brazilianiae D. Stringari, C. Glienke & Crous, *sp. nov*. — MycoBank MB517970; Fig. 3

Phyllostictae anacardiacearum similis, sed endophytice, neque vero phytoparasitice crescenti.

Etymology. Named after the country from which it was collected, Brazil.

Colonies on PNA. *Pycnidia* black, aggregated, superficial to erumpent, globose to ampulliform, exuding a colourless, glossy conidial mass; pycnidia up to 300 µm diam; pycnidial wall consisting of several layers, up to 40 µm thick; outer region of dark brown, thickened, *textura angularis* to *globularis*; inner region up to 20 µm wide, consisting of 1–2 pale cell layers of *textura angularis*. *Ostiole* single, central, 5–10 µm wide, consisting of thickened, brown cells. *Conidiophores* subcylindrical to doliiform, reduced to conidiogenous cells, or with one supporting cell, coated in mucoid layer, 10–20 × 4–5 µm. *Conidiogenous cells* terminal, subcylindrical to doliiform, hyaline, smooth, 7–15 × 3–4 µm; proliferating 1–3 times percurrently near apex. *Conidia* (8–)10–11(–12.5) × (5–)6(–7) µm, solitary, hyaline, aseptate, thin- and smooth-walled, coarsely guttulate, ellipsoid to obovoid, tapering toward a narrowly truncate base, enclosed in a thin mucilaginous sheath, 1–2 µm thick, and bearing a hyaline, mucoid apical appendage, (5–)8–10(–15) × 1.5–2 µm, straight to flexible, unbranched, tapering towards an acute apex.

Culture characteristics — Colonies after 14 d at 25 °C in the dark on OA flat, spreading, olivaceous-grey, becoming pale olivaceous-grey towards the margin, with moderate aerial mycelium.

Specimen examined. Brazil, Pompéia, São Paulo, on *Mangifera indica* (*Anacardiaceae*), May 2007, *D. Stringari*, CBS H-20521 holotype, culture ex-type LGMF 330 = CBS 126270.

Notes — Van der Aa (1973) introduced the name *Phyllosticta anacardiacearum* as a nom. nov. for *Phyllostictina mangiferae* occurring on mango in Brazil. The name *Phyllosticta mangiferae* was found to be a species of *Fusicoccum*, while *Phyllosticta mortonii*, occurring on mango in Mexico, was thought to be a species of *Phoma* (van der Aa & Vanev 2002). While no authentic material could be located for *Phyllosticta anacardiacearum*, it was originally described from subcircular to angular leaf spots, reaching 1 cm diam, surrounded by a red-purple margin. The same was also found to be the case when van der Aa (1973) redescribed the fungus from a specimen collected on *Mangifera indica* in Miami. The species described here as *P. brazilianae* is ecologically distinct from *P. anacardiacearum* being an endophyte, and failing to induce leaf spots despite repeated inoculations on mango.

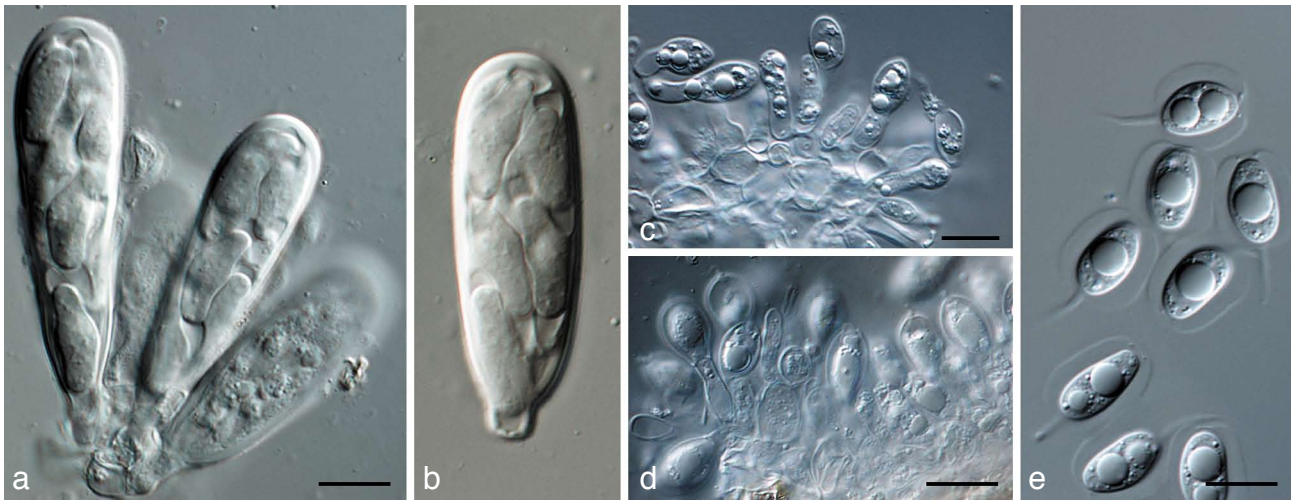


Fig. 4 *Phyllosticta capitalensis*. a, b. Asci with ascospores; c, d. conidiogenous cells giving rise to conidia; e. conidia (all: CBS H-20522 epitype). — Scale bars = 10 µm.

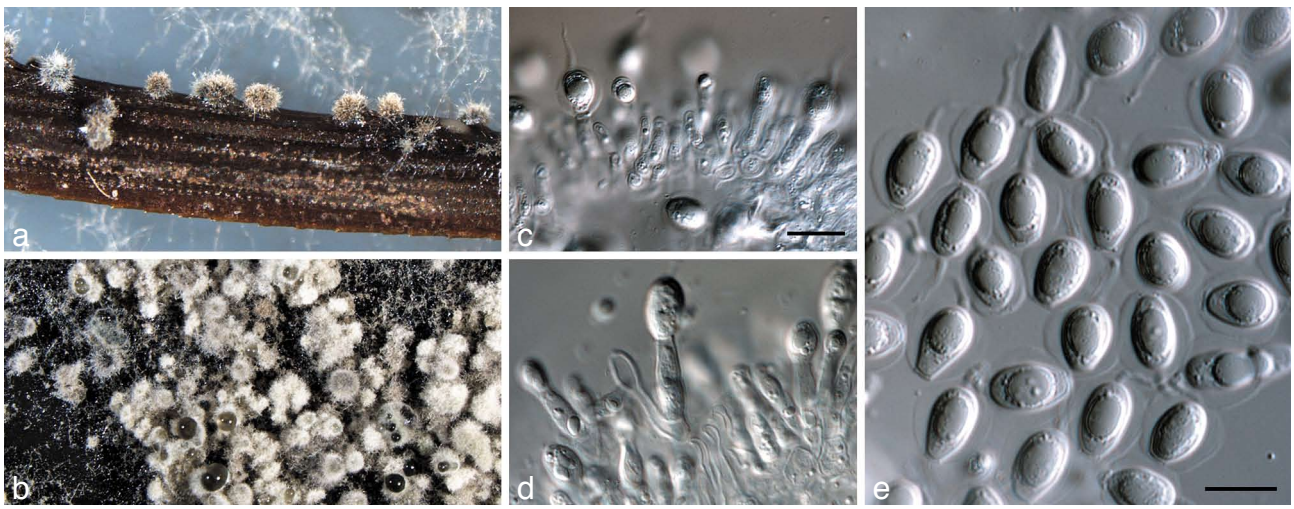


Fig. 5 *Phyllosticta citribraziliensis*. a. Pycnidia forming on PNA; b. pycnidia forming on PDA; c, d. conidiophores giving rise to conidia; e. conidia (all: CBS H-20523 holotype). — Scale bars = 10 µm.

Phyllosticta capitalensis Henn., Hedwigia 48: 13. 1908 — Fig. 4

Colonies on OA. *Ascomata* erumpent, in section globose to pyriform, often irregularly shaped, unilocular, central ostiole forming by dehiscence when mature, up to 250 µm diam. *Peridium* comprising three strata, an outer stratum of thick-walled, small-lumened, brown *textura angularis*, becoming thin-walled with larger lumina in the middle layer, inner layer of thin-walled, hyaline *textura angularis*, altogether 14–45 µm thick. *Asci* attached to the basal peridium, clavate, with a wide, slightly squared apex, tapering gradually to a small pedicel, bitunicate, with a well-developed ocular chamber, 8-spored, 58–80 × 11–15 µm. *Ascospores* limoniform, sometimes slightly elongated, aseptate, hyaline, thick-walled, refractive, with a large central guttule and large mucilaginous polar appendages, overlapping biseriate, 15–17 × 5–6 µm, 3.5 µm wide at each end. *Pycnidia* black, aggregated, erumpent, globose to ampulliform, exuding a colourless, glossy conidial mass; pycnidia up to 300 µm diam, 250 µm tall; pycnidial wall consisting of 6–8 layers, up to 40 µm thick, of *textura angularis*. *Ostiole* single, central, 5–15 µm diam. *Conidiophores* subcylindrical to ampulliform, frequently reduced to conidiogenous cells, or branching from a basal supporting cell, coated in mucoid layer, 7–20 × 3–7 µm. *Conidiogenous cells* terminal, subcylindrical to ampulliform to doliiform, hyaline, smooth, 7–10 × 3–5 µm; proliferating 1–2 times percurrently near apex. *Conidia* (10–)11–12(–14) × (5–)6–7 µm, solitary, hyaline, aseptate, thin- and smooth-walled, coarsely guttule, ellipsoid to obovoid, tapering toward a narrowly truncate base, enclosed in a mucilaginous sheath, 2–4 µm thick, and bearing a hyaline, mucoid apical appendage, 6–8 × 1–1.5 µm, straight to curved, unbranched, tapering towards a bluntly rounded apex.

Specimens examined. Brazil, São Paulo, on leaves of *Stanhopea* sp., Apr. 1903, B, holotype; São Paulo, Lindóia, on leaves of *Stanhopea graveolens*, 17 Oct. 2010, *O.L. Pereira*, epitype designated here CBS H-20522, culture ex-epitype CBS 128856 = CPC 18848, CPC 18849.

Notes — *Phyllosticta capitalensis* is the name proposed for the isolates in clade 10 (formerly incorrectly referred to as *Guignardia mangiferae*; Baayen et al. 2002), representing a taxon that is frequently isolated as endophyte, and has a wide host range and geographic distribution.

Phyllosticta citribraziliensis C. Glienke & Crous, *sp. nov*. — MycoBank MB517971; Fig. 5

Phyllostictae citricarpae similis, sed conidiis maioribus, 10–16 × 5–8 µm.

Etymology. Named after the host (*Citrus*) and country from which it was isolated, Brazil.

Colonies on PNA. *Pycnidia* black, solitary, erumpent, globose, exuding colourless to opague conidial masses; pycnidia up to 250 µm diam; pycnidial wall consisting of several layers,

up to 40 µm thick; outer region of dark brown, thickened, *textura angularis* to *globularis*; inner region up to 25 µm wide, consisting of 1–2 pale cell layers, that become hyaline toward interior, *textura angularis*. *Ostiole* single, central, up to 30 µm wide. *Conidiophores* subcylindrical to ampulliform, reduced to conidiogenous cells, or with 1–2 supporting cells, at times branched at the base, 20–45 × 6–9 µm. *Conidiogenous cells* terminal, subcylindrical to doliiform, hyaline, smooth, coated in a mucoid layer, 7–20 × 3–4 µm; inconspicuously proliferating once or twice percurrently near apex. *Conidia* (8–)10–12(–13) × 6–7(–8) µm, solitary, hyaline, aseptate, thin- and smooth-walled, coarsely guttulate, ellipsoid to obovoid, tapering toward a narrowly truncate base, 2–3 µm wide, enclosed in a thick mucilaginous sheath, 2–4 µm thick, and bearing a hyaline, mucoid apical appendage, 7–15 × 1.5–2 µm, straight to flexible, unbranched, tapering towards an acutely rounded tip.

Culture characteristics — Colonies after 14 d at 25 °C in the dark on OA flat, spreading, olivaceous grey, with moderate aerial mycelium.

Specimen examined. Brazil, Rio Negro, Paraná, on *Citrus limon*, Mar. 1997, *C. Glienke*, CBS H-20523 holotype, culture ex-type CBS 100098.

Notes — Although isolates occurring on *Citrus* have in the past been treated as representative of *P. spinarum* (Stringari et al. 2009), they are phylogenetically distinct (Fig. 1), and can also be distinguished morphologically by having larger conidia (8–)10–12(–13) × 6–7(–8) µm than the type of *P. spinarum* (8–)9.8(–12) × (6–)6.6(–7) µm; Nag Raj & Morelet 1997). Furthermore, *P. citribraziliensis* also has branched conidiophores, a thick mucilaginous sheath surrounding its conidia (2–4 µm), whereas those in *P. spinarum* are reduced to conidiogenous cells, and the sheath is 1–2 µm thick (Nag Raj & Morelet 1997).

Phyllosticta citricarpa (McAlpine) Aa, Stud. Mycol. 5: 40. 1973. — Fig. 6

Basionym. *Phoma citricarpa* McAlpine, Fungus diseases of Citrus trees in Australia, and their treatment: 21. 1899.

Teleomorph. *Guignardia citricarpa* Kiely, Proc. Linn. Soc. New South Wales 73: 259. 1948.

Colonies on OA. *Pycnidia* black, aggregated, superficial to erumpent, globose to ampulliform, exuding a colourless, opaque conidial mass; pycnidia up to 250 µm diam; pycnidial wall consisting of several layers, 20–50 µm thick; outer region of dark brown, thickened, *textura angularis* to *globularis*; inner region consisting of 1–2 pale cell layers of *textura angularis*. *Ostiole* single, central, 10–15 µm wide, consisting of thickened, brown cells. *Conidiophores* subcylindrical to doliiform, reduced to conidiogenous cells, or branched from a supporting cell, coated in mucoid layer, 10–20 × 4–7 µm. *Conidiogenous cells* terminal, subcylindrical to somewhat doliiform, hyaline, smooth, 7–12 × 3–4 µm; proliferating 1–2 times percurrently near apex. *Conidia* (10–)11–12(–14) × (6–)7(–8) µm, solitary, hyaline, aseptate, thin- and smooth-walled, coarsely guttulate, ellipsoid to obovoid, tapering toward a narrowly truncate base, enclosed in a thin mucilaginous sheath, 1(–2) µm thick, and bearing a hyaline, mucoid apical appendage, 5–10(–17) × 1–1.5 µm, straight to flexible, unbranched, tapering towards an acute apex.

Culture characteristics — Colonies after 14 d at 25 °C in the dark on OA flat, spreading, olivaceous-grey, becoming pale olivaceous-grey towards the margin, with sparse to moderate aerial mycelium; surrounded by a diffuse yellow pigment in the agar medium.

Specimens examined. Australia, Sydney, on *Citrus sinensis*, 1898, *D. McAlpine*, VPRI 1536, Lectotype selected here; Queensland, Emerald, ex Citrus black spot on leaf of *Citrus sinensis*, *anon.*, 16 Dec. 2004, BRIP 46098 = CBS 127455; Queensland, Mundubbera, ex Citrus black spot on fruit of *C. reticulata* cv. Imperial, 27 Mar. 2001, *S.L. Willingham*, BRIP 27890 = CBS 127453, BRIP 27889 = CBS 127452, BRIP 27888 = CBS 127451; Gayndah, Queensland, ex Citrus black spot on *C. limon*, 3 Mar. 2009, *A.K. Miles*, CBS H-20524 epitype designated here, culture ex-epitype BRIP 52614 = CBS 127454.

Notes — The most characteristic features of *P. citricarpa* are the narrower sheaths (1(–2) µm thick), compared to that of *P. capitalensis* (2–3 µm thick), and the yellow pigment that diffuses into the agar when isolates of *P. citricarpa* are cultivated on oatmeal agar.

DISCUSSION

The present study aimed to resolve the taxonomy of the *Phyllosticta* species occurring on *Citrus*, either as pathogens, or as harmless endophytes. In the process we also had to resolve the status of the common endophytic taxon with a known wide host range and geographic distribution. Several names have in the past been linked to this taxon, including *Guignardia mangiferae* and *Phyllosticta capitalensis*. By obtaining reference strains considered authentic for these names, we could show that *G. mangiferae* is a distinct taxon from *P. capitalensis*, and that *P. capitalensis* is the name to be used for this cosmopolitan endophyte (clade 10, Fig. 1). In the process we also designated epitypes for *P. capitalensis* and *P. citricarpa*, described a novel species on orchids in Brazil as *P. bifrenariae*, one on *Citrus* as *P. citribraziliensis*, and another on *Mangifera indica* as *P. brazilianiae*.

Several species of *Phyllosticta* are now known to occur on *Citrus*, namely *P. citriasiana*, which is a pathogen of *C. maxima*, causing tan spot in Asia (Wulandari et al. 2009), *P. citricarpa*, which causes Citrus Black Spot in many countries, and is of quarantine concern (Baayen et al. 2002), *P. citribraziliensis*, which is an endophyte on *Citrus* in Brazil, and *P. capitalensis*, which is a wide host range endophyte, that also occurs on *Citrus*.

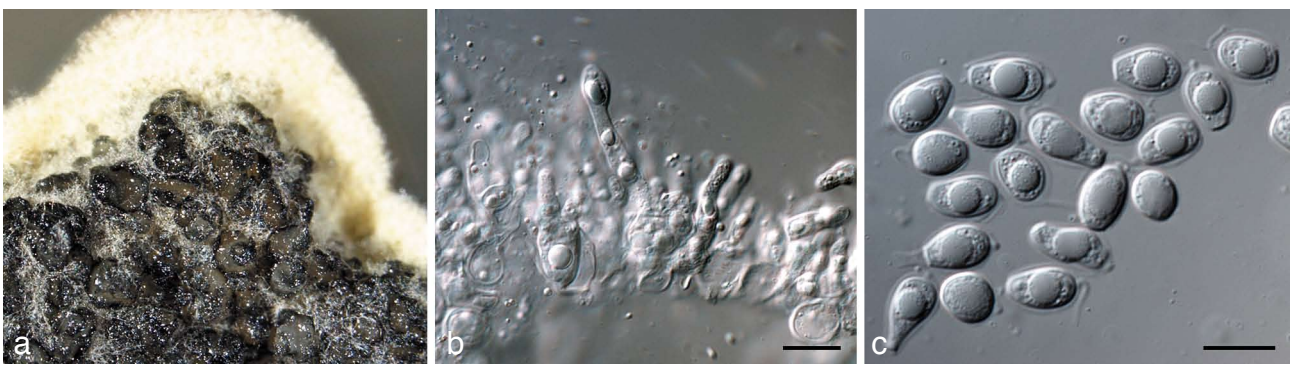


Fig. 6 *Phyllosticta citricarpa*. a. Pycnidia forming on OA, with diffuse yellow pigment visible in agar; b. conidiophores giving rise to conidia; c. conidia (all: CBS H-20524 epitype). — Scale bars = 10 µm.

Although the genus *Phyllosticta* has received much taxonomic attention of late (refs), very few phylogenetic studies have thus far been conducted, and hence the taxonomy of this group is still problematic. Due to the lack of reference strains, and the fact that few gene loci other than ITS have thus far been used for DNA analysis, most of the conclusions reached thus far have been incorrect, meaning that published literature will have to be interpreted with care. Furthermore, in spite of the multigene approach taken in the present study, some morphological variation is still present among isolates treated here as *P. capitalensis* (clade 10), and more gene loci need to be investigated to confirm whether this is indeed a single taxon. Further studies are presently underway to address this issue.

Guignardia mangiferae was first described on *Mangifera indica* in India (Roy 1968), but the type specimen has not been available for study. In spite of the reference isolate (IMI 260576) being genetically distinct from others in the *P. capitalensis* clade (Fig. 1), this isolate proved to only form the anamorph in culture. Furthermore, no cultures are available for the plant pathogenic species, *P. anacardiacearum*, which we regard as distinct from the common endophyte for which the name *P. brazilianiae* has been introduced. This situation on mango is similar to the one on *Citrus*, where the plant pathogenic species are represented by *P. citricarpa* and *P. citriasiana*, and the endophytic strains by *P. citribraziliensis* and *P. capitalensis*. Despite the large production of mango in Brazil, the *Phyllosticta* leaf spot disease has not been found in commercial orchards, and it is possible that the species is either distinct, or vary rare, and not occurring on commercial cultivars. To help clarify the relationship of endophytic *Phyllosticta* spp. and their hosts, pathogenicity tests similar to those performed for endophytes of *Musa acuminata* (Photita et al. 2004), must be conducted on a range of different hosts in future studies.

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