Phyllosticta—an overview of current status of species recognition

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Abstract *Phyllosticta* is an important coelomycetous plant pathogenic genus known to cause leaf spots and various fruit diseases worldwide on a large range of hosts. Species recognition in *Phyllosticta* has historically been based on morphology, culture characters and host association. Although there have been several taxonomic revisions and enumerations of species, there is still considerable confusion when identifying taxa. Recent studies based on molecular data have resolved some cryptic species and

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e-mail: kdhyde3@gmail.com some novel taxa have been discovered. However, compared to the wide species diversity and taxonomic records, there is a lack of molecular studies to resolve current names in the genus. A phylogenetic tree is here generated by combined gene analysis (ITS, partial actin and partial elongation factor $l\alpha$) using a selected set of taxa including type-derived sequences available in GenBank. Life modes, modal lifecycle and applications of the genus in biocontrol and metabolite production are also discussed. We present a selected set of taxa as an example of resolved and newly described species in the genus and these are annotated with host range, distribution, disease symptoms and notes of additional information with comments where future work is needed.

Keywords Biocontrol \cdot Endophyte \cdot *Guignardia* \cdot Leaf spot \cdot Morphology \cdot Molecular phylogeny \cdot Secondary metabolites

Introduction

The genus *Phyllosticta* Pers. ex Desm. is a taxonomically confused group of microfungi comprising mostly important phytopathogens with a wide host range (van der Aa 1973; van der Aa and Vanev 2002). Although the generic concept of *Phyllosticta* has been refined and species names were enumerated in a monographic treatment by van der Aa and Vanev (2002), species recognition still remains problematic (Hyde et al. 2010a, b; Glienke et al. 2011). Several species of *Phyllosticta* have also been reported as endophytes and saprobes (van der Aa and Vanev 2002; Baayen et al. 2002; Okane et al. 2003; Wulandari et al. 2009, 2010; Glienke et al. 2011). Species of *Phyllosticta* (teleomorph *Guignardia* Viala & Ravaz) cause leaf spot symptoms and fruit diseases on a range of hosts including some economically important

crops and ornamentals such as citrus, banana, apple, grapes, cranberry, orchids, Ficus sp., Buxus sp. and maple (Uchida and Aragaki 1980; Paul and Blackburn 1986; Baayen et al. 2002; McManus 1998; Olatinwo et al. 2003; Paul et al. 2005; Liu et al. 2009). Phyllosticta species are also potential biocontrol agents (Yan et al. 2011), and have been reported to produce novel bioactive metabolites such as phyllostine and phyllostoxin (Evidente et al. 2008a). Molecular data has improved the knowledge of species relationships and taxonomic classifications in the recent decade with reference to different complex groups of plant pathogenic fungi (Crous and Groenewald 2005; Shenoy et al. 2007; Rossman and Palm-Hernández 2008; Cai et al. 2011; Udayanga et al. 2011). Similarly, Phyllosticta (and its sexual Guignardia state) is an important genus requiring modern revisionary treatment employing morphological characters and a molecular phylogenetic approach, as the understanding of species is less advanced, but molecular data are expected to reveal cryptic novel species (Crous and Groenewald 2005; Hyde et al. 2010a, b). The objectives of this review are to (1) review the taxonomic and nomenclatural history of *Phyllosticta* (2) review studies of species recognition by morphological and molecular phylogenetic criteria (3) discuss the life styles and biology of species of the genus (4) summarize the applications of some species in metabolite production and biocontrol, and (5) provide notes on selected resolved and recently described species.

History

The genus Phyllosticta Pers. was established by Persoon (1818) when he introduced the generic name Phyllosticta for Sphaeria lichenoides DC. Over the past 200 years numerous species have been added to the genus, often based on host association so that more than 3,100 names have been associated with Phyllosticta at various times (http://www.indexfungorum.org/names/Names.asp: available 21 Sep, 2011). Desmazieres (1847) validated Phyllosticta Pers., and Donk (1968) designated Phyllosticta convallariae Pers. as the type species. Many of the 3,100 names do not refer to what is now considered to be Phyllosticta sensu stricto. Initially, many fungi with unicellular conidia, similar to those of Phoma were named as either Phoma or Phyllosticta, depending on the location of conidiomata on the host. Those fruiting on leaves were described as *Phyllosticta* while those occurring on other parts of the plant were placed in Phoma. van der Aa (1973) provided a key for 46 species of *Phyllosticta* he accepted, and this has been widely followed. Furthermore, in their monographic study, van der Aa and Vanev (2002) accepted 190 species in this genus, while Kirk et al. (2008) estimated that there are only 92 Phyllosticta species. Since the revision by van der Aa and Vanev (2002), a further nine new species have been described (Box 1).

| Box | 1 | History | v of the | study | of Ph | ıvli | losticta |
|-----|---|---------|----------|-------|-------|------|----------|
| | | | | | | | |

| Year | Event | References |
|-----------|---|--------------------------------|
| 1818 | <i>Phyllosticta</i> was introduced the generic name for <i>Sphaeria lichenoides</i> DC | Persoon (1818) |
| 1847 | Phyllosticta Pers. was validated | Desmazieres (1847) |
| 1849 | The genus <i>Phyllosticta</i> Pers. ex Desm typified with <i>Phyllosticta cruenta</i> (Kunze ex Fr.) Kickx (1849). | Kickx (1849) |
| 1968 | <i>Phyllosticta convallariae</i> Pers. designated as the type species | Donk (1968) |
| 1927 | A compilation of <i>Phyllosticta</i> names published | Petrak and Sydow, (1927) |
| 1973 | <i>Phyllosticta</i> outline with 46 accepted species | Van der Aa (1973) |
| 2002 | A further revision of the species described in <i>Phyllosticta</i> with notes on each of the 191 accepted species | Van der Aa and Vanev (2002) |
| 2003-2011 | P. ardisiicola Motohashi, I. Araki & C. Nakash. P. aspidistricola Motohashi, I. Araki & C. Nakash. | Motohashi et al. (2008) |
| | P. kerriae Motohashi, I. Araki & C. Nakash. | |
| | <i>P. fallopiae</i> Motohashi, I. Araki & C. Nakash. | |
| | P. citriasiana Wulandari, Crous & Gruyter. | |
| | Phyllosticta (Guignardia) musicola N.F. Wulandari, L. | Wulandari et al. (2009) |
| | Cai & K. D. Hyde. <i>P. bifrenariae</i> O.L. Pereira, C. Glienke & Crous. | Wulandari et al. (2010) |
| | P. brazilianiae D. Stringari, C. Glienke & Crous. P. citribraziliensis C. Glienke & Crous. | Glienke et al. (2011) |
| | Phyllosticta citrichinaensis H.X. Wang, K.D. Hyde & H.Y. Li | Wang et al. 2011 |

The teleomorph state of *Phyllosticta* is *Guignardia* Viala & Ravaz, represented by the generic type, *Guignardia bidwellii* (Ellis) Viala & Ravaz. There are 335 epithets associated with this genus (http://www.indexfungorum.org/names/Names.asp) and in a similar fashion to *Phyllosticta*, most species where described based on host association. The genus has not been well-studied, and there is no comprehensive monograph, although individual species or groups of species have been treated (Hyde 1995; Motohashi et al. 2009; Wulandari et al. 2011). Most species of *Phyllosticta* and *Guignardia* have been described indepen-

dently from each other, and only a few *Phyllosticta* species have been linked to their *Guignardia* teleomorphs. Consequently, their classification is confusing and the relation between host range and disease are often poorly understood (van der Aa 2002)

Using Phyllosticta versus Guignardia

The name Phyllosticta (asexual state) and Guignardia (sexual state) have been used separately following the dual classification system used by mycologists over several decades (Hawksworth 2004; McNeil et al. 2006; Shenoy et al. 2007, 2010). For instance Phyllosticta musarum (Cooke) Aa and Guignardia musae Racib. are the same biological species but have different names, P. musarum being the asexual state and G. musae being the sexual state (van der Aa 1973; Wulandari et al. 2010). However with the use of molecular data it is now possible to link asexual and sexual states (Berbee and Taylor 2001) and the use of the dual nomenclature system of classification in fungi has become redundant (Hawksworth 2011). Therefore a single name should be adopted and there are various views to which names should be followed, i.e. the oldest, the sexual state name, the most conserved name, and view maintaining both names (Berbee and Taylor 2001; Seifert and Rossman 2010; Hyde et al. 2011). Our view is that we should generally adopt the oldest name for each genus, which will soon be enforced in the International Code of Nomenclature for algae, fungi and plants, but also taking into account which name is the most important and commonly used. Phyllosticta Pers. (1818) is a much older name than Guignardia Viala & Ravaz (1892) and generally Phyllosticta species are known to cause important diseases (e.g. leaf spot, citrus black spot, black rot of horse chestnut). There are also many more species of Phyllosticta than Guignardia. There are exceptions, for example Guignardia candeloflamma K.D. Hyde is only known in it teleomorph state (Wulandari et al. 2010a), while banana freckle is caused by both states (Wulandari et al. 2010b). Because Phyllosticta is the oldest name and generally more important as the causal agent of disease we chose to adopt this name and treat all Guignardia species as synonyms of Phyllosticta, in the sense of Glienke et al.

(2011). Because of this decision we use the name *Phyllosticta* throughout this review unless we specifically refer to a *Guignardia* species. *Leptodothiorella*, which previously represented the spermatial state of some *Phyllosticta* species (e.g. *Leptodothiorella aesculicola* (Sacc.) Sivan.), are also treated as synonyms of *Phyllosticta* (van der Aa 1973).

Morphological characteristics to differentiate species

Phyllosticta pycnidia are usually globose, subglobose or tympaniform, flattened above, and closely connected with the subepidermal pseudostroma (Fig. 3a, b). They are mostly unilocular but occasionally may be multilocular (van der Aa 1973). The conidia are commonly hyaline, one-celled, ovoid, obovate or ellipsoid, or short cylindrical, seldom pyriform, globose or subglobose, 10-25 µm long, and usually covered by a slime layer and bearing a single apical appendage (Fig. 3c) (van der Aa 1973). Cultural characteristics when grown on specific media may also be used as differentiating characters. In the case of P. citricarpa colonies can be characterised after 14 days at 25°C in the dark on OA as 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 (Wulandari et al. 2009).

The sexual state *Guignardia* can be characterized by erumpent ascomata, which are globose to pyriform in section, often irregularly shaped, unilocular, and with a central ostiole forming by dehiscence when mature. The peridium is thin, comprising a few layers of angular cells. Asci are 8-spored, bitunicate, clavate to broadly ellipsoid, with a wide, slightly square apex, tapering gradually to a small pedicel, and with a welldeveloped ocular chamber. Ascospores are ellipsoid to limoniform, sometimes slightly elongated, aseptate, hyaline, often guttulate or with a large central guttule, and some have mucilaginous polar appendages (van der Aa 1973; Wulandari et al. 2011, Fig. 3d–f).

A spermatial state is often present in the life cycle of *Guignardia* species, and readily forms in culture. Spermatia are cylindrical to dumbbell-shaped with guttules at each end (Fig. 3g). In the past several spermatial states were



Fig. 1 Phyllosticta sp. on living leaf of jackfruit. a Leaf spots. b-c Lesion on adaxial surface. d Banana freckle disease



spores infect leaves, canes and berries if

conditions are favourable

Fig. 2 Schematic representation of the life cycle of *Phyllosticta* and its teleomorph redrawn from: http://www.oardc.ohio-state.edu/fruitpathology/ organic/grape/All-Grapes.html

officially named. For instance, the spermatial state of *Melanops concinna* Syd. (= *Guignardia concinna* (Syd.) Aa; van der Aa 1973) was described as *Leptodothiorella concinna* Sydow (1926).

Molecular studies advance the understanding of *Phyllosticta*

The rapid development of molecular phylogenetic tools have improved our understanding of several other coelomycetous genera such as *Colletotrichum* (Cai et al. 2009; Crouch et al. 2009; b; Hyde et al. 2009), *Phomopsis* (Santos and Phillips 2009; Udayanga et al. 2011), *Phoma* (Aveskamp et al. 2008, 2010; de Gruyter et al. 2010), *Fusicoccum* (Crous et al. 2006), *Diplodia* (Phillips et al. 2008) and *Pestalotiopsis* (Liu et al. 2010; Maharachchikumbura et al. 2011), to name but a few. There have also been several molecular phylogenetic studies concerning *Phyllosticta* species that have helped to facilitate the identification of species and resolution of species complexes (Baayen et al. 2002; Okane et al. 2003; Motohashi et al. 2009; Wulandari et al. 2009; Glienke et al. 2011).

ITS rDNA sequence-based studies in Phyllosticta

ITS rDNA sequences are often used to infer phylogenetic relationships in many groups of fungi including *Phyllos-ticta* (Okane et al. 2003; Motohashi et al. 2009; Wulandari et al. 2009). Motohashi et al. (2009) evaluated the phylogenetic relationships among Japanese species of *Phyllosticta sensu stricto* and its teleomorph *Guignardia* using 18S rDNA sequence data. They observed that *Phyllosticta sensu stricto* is a monophyletic clade. In the same study, ITS and 28S rDNA sequences were used in a



Fig. 3 Comparison of *Phyllosticta* and *Guignardia* states. **a** Vertical section through pycnidium. **b** Pycnidial wall with conidiogenous cells. **c** Conidia. **d** Section of ascoma. **e** Bitunicate and fissitunicate ascus. **f**

phylogenetic analysis of *Phyllosticta* strains from various host plants. Results from this study revealed isolates to cluster in two subgroups based on molecular data as, (1) cultures from a wide range of host plants mainly derived as endophytes from symptomless plants (*P. capitalensis* complex, see below) and (2) relatively host-specific

Ascospores. g *Leptodothiorella* spermatial state. Scale Bars: a, b, d, e = 50 μ m, c, f, g =10 μ m

strains (often isolated as foliar pathogens from diverse plants).

ITS-RFLP and ITS sequence analysis were used to examine genetic variation of foliar endophytic *Phyllosticta* strains from different tropical trees (Pandey et al. 2003). Although ITS-RFLP failed to infer genetic diversity among

isolates used, the ITS phylogram supported the identity of P. capitalensis as a common foliar endophyte and pathogen with wide range of hosts. In a similar study, the diversity of strains of Guignardia (or Phyllosticta) was evaluated using rDNA ITS sequence data (Okane et al. 2003). Guignardia endophyllicola (anamorph Phyllosticta capitalensis) was shown to have an extensive host range. The taxon was identified in 53 isolates from the same number of different plants belonging to 43 genera. Phylogeny based on rDNA ITS sequence analyses derived from 18 tropical endophytic strains from different plants confirmed conspecificity of the Brazilian isolates with Phyllosticta captalensis (as G. mangiferae) (Rodrigues and Sieber. 2004). In the ITS sequence comparison, some Guignardia and Phyllosticta strains from unrelated hosts were more closely related than other isolates derived from closely related plants. However, the diversity across the wide range of hosts should be evaluated by incorporating more genes in analyses and isolating strains from a wide range of hosts in future studies.

The population structure and phylogenetic relationships of *Guignardia citricarpa* (citrus black spot) were investigated by Baayen et al. (2002) using ITS, AFLP and morphological comparison. The observations supported the historic distinction between slowly growing pathogenic isolates and fast growing non-pathogenic isolates, which proved to belong to *P. capitalensis* (as *G. mangiferae*), the ubiquitous endophyte reported in various studies (Okane et al. 2003; Rodriguez et al. 2009; Glienke et al. 2011). Numerous synonyms for *P. capitalensis* have been used in earlier studies, reviewed in this section. We have used the name as it appeared in the original publication, although the need for careful refinement using the currently accepted name is recommended.

Studies employing multi-locus analyses in Phyllosticta

Identification of *Phyllosticta* species is problematic as few characters are available to differentiate species. Although ITS sequence data have been widely used for species discrimination, multi-locus phylogenies might resolve cryptic species (Wulandari et al. 2009). A combined phylogenetic analysis based on the rDNA ITS, translation elongation factor 1 (TEF1), and actin (ACT) genes resolved three species, namely P. mangiferae, P. citricarpa and a new species, P. citriasiana (Wulandari et al. 2009), the latter causing tan spot of Citrus maxima in Asia. Glienke et al. (2011) investigated the genetic diversity of endophytic and pathogenic Phyllosticta species, with particular emphasis on Phyllosticta citricarpa and Guignardia mangiferae occurring on Citrus. Combined DNA sequence analysis based on rDNA ITS, translation elongation factor 1 (TEF1), actin (ACT) and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes resolved nine well-supported clades related to seven known species and two apparently undescribed species. They have designated epitypes for *P. citricarpa* collected from Australia and *Phyllosticta capitalensis* collected from Brazil (Glienke et al. 2011). Furthermore, *P. brazilinae*, *P. bifinariae* and *P. citribraziliensis* were described as novel species based on morphology and a multilocus phylogeny. The combined gene analysis further revealed that the allocation of various synonyms for the endophytic, non-pathogenic isolates occurring on wide range of hosts would be more correctly referred to as *P. capitalensis*. Further work is needed, however, to resolve whether this taxon is a complex of cryptic species.

Multi-locus phylogeny inferred from available sequences in GenBank

A selected set of ITS rDNA, ACT, and TEF1 sequences, including the available ex-type, and ex-epitype materials were downloaded from GenBank (Table 1). The sequences were aligned using Bioedit, alignment was optimized manually and the genes are combined to perform phylogenetic analysis. Parsimony analysis was carried out by PAUP v. 4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded from all analyses and the gaps were treated as missing data. Trees were figured in Treeview (Page 1996). One of the most parsimonious trees generated from combined gene analysis for 35 strains is provided (Fig. 4).

The phylogenetic tree based on GenBank sequences including sequences originating from seven ex-type cultures, and other sequences are identified as appeared in recent publications. We have used 35 isolates in the multilocus phylogenetic tree, as there is a lack of sequence data of all three genes for all known ex-type cultures, some of which therefore had to be excluded from the analyzed dataset (see Table 1). However we recommend the improvement of the multi-locus phylogenetic analysis by using more phylogenetically informative genes, and more ex-type isolates in future work. Incorporation of more ex-type sequences will accelerate the accurate identification of other species from various geographical locations and a wide range of hosts.

Ecology of Phyllosticta species

Phyllosticta species are important plant pathogens and, although taxa are also commonly identified as endophytes (Baayen et al. 2002; Rodrigues and Sieber 2004), a few species have also been reported as saprobes. In some cases a species may occupy more than one life mode. For example, *P. capitalensis* was originally described on *Stanhopea* (*Orchidaceae*) from Brazil as a fungal pathogen. Recently, Silva et al. (2008) reported that *P. capitalensis*

| Guignardia bidwellii Guignardia citricarpa Guignardia citricarpa | Strain" | Substrate | Country | Collector(s) | ITS | ACT | TEF1 |
|--|----------------------|---------------------------------------|--------------|-----------------|----------|----------|-----------|
| Guignardia citricarpa Guignardia citricarpa | CBS 111645 | Parthenocissus quinquefolia | USA | G. Carroll | EU683672 | I | EU683653- |
| Guignardia citricarpa | CBS 102345 | Citrus aurantium, lesion on peel | Brazil | I | FJ538311 | FJ538427 | FJ538369 |
| | CBS 122482 | Citrus sinensis | Zimbabwe | L. Huisman | FJ538317 | FJ538433 | FJ538375 |
| Guignardia citricarpa (| CBS 122384 | Citrus limon | South Africa | M. Truter | FJ538316 | FJ538432 | FJ538374 |
| Guignardia citricarpa (ex-epitype) (| CBS 127454 | Citrus limon | Australia | S.L. Willingham | JF343583 | JF343667 | JF343604 |
| Guignardia mangiferae (| CBS 115046 | Myradcrodruon urundeuva, leaf or bark | Brazil | K.F. Rodriques | FJ538322 | FJ538438 | FJ538380 |
| Guignardia mangiferae (| CBS 115047 | Aspidosperma polyneuron, leaf or bark | Brazil | K.F. Rodriques | FJ538323 | FJ538439 | FJ538381 |
| Guignardia mangiferae (| CBS 114751 | Spondias mombin, leaf or bark | Brazil | K.F. Rodriques | FJ538349 | FJ538465 | FJ538407 |
| Guignardia mangiferae (| CBS 115049 | Bowdichia nitida, leaf or bark | Brazil | K.F. Rodriques | FJ538324 | FJ538440 | FJ538382 |
| Guignardia mangiferae | IMI 260576 | Mangifera indica, leaf endophyte | India | M.V. Leksshmi | JF261459 | JF343641 | JF261501 |
| Guignardia psidii (| CBS 100250 | Psidium guajava, fruit | Brazil | C. Glienke | FJ538351 | FJ538467 | FJ538409 |
| Guignardia vaccinii (| CBS 126.22 | Oxycocus macrocarpus | U.S.A | I | FJ538353 | FJ538469 | FJ538411 |
| Phyllosticta bifrenariae(ex-type) | VIC30556; CBS 128855 | Bifrenaria harrisoniae, living leaf | Brazil | O. Pereira | JF343565 | JF343649 | JF343586 |
| Phyllosticta brazilianiae | LGMF 333 | Mangifera indica, leaf endophyte | Brazil | C. Glienke | JF343574 | JF343658 | JF343595 |
| Phyllosticta brazilianiae | LGMF 334 | Mangifera indica, leaf endophyte | Brazil | C. Glienke | JF343566 | JF343650 | JF343587 |
| Phyllosticta brazilianiae(ex-type) (| CBS 126270 | Mangifera indica, leaf endophyte | Brazil | C. Glienke | JF343572 | JF343656 | JF343593 |
| Phyllosticta capitalensis (| CBS 100175 | Citrus sp., healthy leaf | Brazil | C. Glienke | FJ538320 | FJ538436 | FJ538378 |
| Phyllosticta capitalensis (| CBS 123373 | Musa paradisiaca | Thailand | N. F. Wulandari | FJ538341 | FJ538457 | FJ538399 |
| Phyllosticta capitalensis (ex-epitype) (| CBS 128856 | Stanhopea graveolens | Brazil | O.L. Pereira | JF261465 | JF343647 | JF261507 |
| Phyllosticta citriasiana (| CBS 120488 | Citrus maxima | Thailand | J. de Gruyter | FJ538354 | FJ538470 | FJ538412 |
| Phyllosticta citriasiana (| CBS 123393 | Citrus maxima | Vietnam | J. de Gruyter | FJ538358 | FJ538474 | FJ538416 |
| Phyllosticta citriasiana (| CBS 123372 | Citrus maxima | Vietnam | J. de Gruyter | FJ538357 | FJ538473 | FJ538415 |
| Phyllosticta citriasiana (ex-type) (| CBS 120486 | Citrus maxima | Thailand | J. de Gruyter | FJ538360 | FJ538476 | FJ538418 |
| Phyllosticta citribraziliensis (ex-type) (| CBS 100098 | Citrus sp., healthy leaves | Brazil | C. Glienke | FJ538352 | FJ538468 | FJ538410 |
| Phyllosticta citribraziliensis | LGMF09 | Citrus sp., healthy leaves | Brazil | C. Glienke | JF261436 | JF343618 | JF261478 |
| Phyllosticta citricarpa (| CBS 122348 | Citrus sinensis, lesions on fruit | Zimbabwe | L. Huisman | FJ538315 | FJ538431 | FJ538373 |
| Phyllosticta citricarpa (| CBS 127455 | Citrus sinensis | Australia | S.L. Willingham | JF343584 | JF343668 | JF343605 |
| Phyllosticta citricarpa (ex-epitype) (| CBS 127454 | Citrus limon | Australia | S.L. Willingham | JF343583 | JF343667 | JF343604 |
| Phyllosticta cussonia (| CPC 14873 | Cussonia sp. | South Africa | P.W. Crous | JF343579 | JF343663 | JF343600 |
| Phyllosticta cussonia (| CPC 14875 | Cussonia sp | South Africa | P.W. Crous | JF343578 | JF343662 | JF343599 |
| Phyllosticta hypoglossi (| CBS 101.72 | Ruscus aculeatus, living leaf | Italy | W. Gams | FJ538365 | FJ538481 | FJ538423 |
| Phyllosticta hypoglossi (| CBS 434.92 | Ruscus aculeatus | Italy | W. Gams | FJ538367 | FJ538483 | FJ538425 |
| Phyllosticta hypoglossi (| CBS 167.85 | Ruscus hypoglossum | Italy | W. Gams | FJ538366 | FJ538482 | FJ538424 |
| Phyllosticta owaniana (| CBS 776.97 | Brabejum stellatifolium | South Africa | A. den Breeÿen | FJ538368 | FJ538484 | FJ538426 |
| Phyllosticta spinarum (| CBS 292.90 | Chamaecyparis pisifera | France | M. Morelet | JF343585 | JF343669 | JF343606 |
| Phyllosticta spinarum (| CBS 937.70 | Hedera helix, leaf litter | Italy | W. Gams | FJ538350 | FJ538466 | FJ538408 |

Egham, Bakeham Lane, UK; LGMF: Culture collection of Laboratory of Genetics of Mycroorganisms, Federal University of parana, Curitiba, Brazil; VIC: Culture Collection of Federal University of Vicosa, Nicosa, Brazil; VIC: Culture Collection of Federal University of Vicosa, Brazil: VIC: Culture Collection of Federal

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◄ Fig. 4 Phylogram generated from the parsimony analysis based on combined rDNA ITS, ACT, TEF1 sequence data for *Phyllosticta* spp. downloaded from GenBank. Strictly consensus branches are thickened and bootstrap support values >70% are shown below or above the branch. Cultures derived from type specimens are in bold. The tree is rooted with *Phyllosticta owaniana*

caused disease of leaves and pseudobulbs of *Bifrenaria harrisoniae* (*Orchidaceae*) in Brazil. *Phyllosticta capitalensis* has also been reported as an endophyte on ericaceous plants in Japan by Okane et al. (2001), and non-pathogenic strains have been isolated from *Citrus* sp. (Table 2) (Baayen et al. 2002; Glienke et al. 2011).

Host specificity of *Phyllosticta* species and disease symptoms

Phyllosticta species may cause leaf spots on many plant species and it is not clear if they are generalists or hostspecific; this may depend on the particular species or their life style. It is known that several species that cause diseases are host, genus or family specific, while endophytes may be generalists. For example, P. sphaeropsoidea Ellis & Everh. causes leaf blotch disease specific to horse chestnut in Europe and North America (Hudson 1987). P. citricarpa causes leaf spot disease of Citrus species, while P. citriasiana can infect fruits of Citrus maxima (pomeloes), and causes tan spot and has only been isolated from pomeloes, but has never been found from lemons, mandarins and oranges, and Phyllosticta musarum is specific on Musa spp. (Wulandari et al. 2009, 2010, 2011). P. capitalensis is an endophyte of a wide range of hosts (Okane et al. 2003; Baayen et al. 2002).

Knowledge of disease symptoms on hosts are important for field identification by taxonomists as well as plant pathologist interested in disease occurrence, management and distribution. Generally, *Phyllosticta* species cause necrotic lesions on leaves, which are characteristically small, often 1–2 mm in diameter, circular, brown in the middle and dark brown or sometimes reddish at the margin (Fig. 1a). One to more than 10 pycnidia are often found in one lesion (Fig. 1b, c). Pycnidia on leaves are usually black, globose or subglobose, and semi-immersed (Fig. 1c). After infection by Phyllosticta the leaf may become dry in the centre of the lesion, causing the infected tissue to drop out, forming a hole, and hence this is known as target spot or shot hole spot. Leaf spots often occur in living leaves in the late dry and wet seasons or in winter in temperate countries. There are four types of leaf spot symptoms-hard spot, false melanose, freckle spot and virulent spot (Kotzé 2000). In July 1984, Phyllosticta species were the cause of problems on Muehlenbeckia adpressa in Victoria, Australia. Virtually all mature leaves of plants contained distinctive necrotic spots for an area of 10 sq. m. Spots were roughly circular to elliptical in shape and were tan with a maroon margin (Paul and Blackburn 1986). Freckle disease occurs on several species and varieties of banana (Fig. 1d). Characteristic spots (pycnidia and ascomata) form on fruit, giving the lesion a sandpaper texture. Leaves of banana will turn yellow when infected with this fungus (Wulandari et al. 2010).

Phyllosticta species as endophytes

Endophytes are fungi that asymptomatically colonize plant tissues during some phase of their life cycle (Petrini 1991; Hyde and Soytong 2008; Saikkonen 2007), but may turn pathogenic during host senescence (Rodriguez and Redman 2008; Rodriguez et al. 2009). The relationship may be symbiotic, antagonistic, neutral or mutualistic (Hyde and Soytong 2008; Aly et al. 2011). Endophytes are horizontally transmitted, and transfer to their host plants via airborne spores. However, some endophytes may also be vertically transmitted to the next host plant generations via seeds (Hartley and Gange 2009). Although the first discovery of endophytes dates already back to 1904, they did not receive considerable attention until the recent recognition of their pharmaceutical and ecological significance (Gunatilaka 2006). Recent development of screening technologies revealed the great potential of endophytes as a major source of biologically active compounds (Strobel and

Table 2 Phyllosticta species recorded as endophytes in selected studies

| Species | Host | Country | Plant organ | Reference |
|------------------|---------------------------------------|-------------|----------------------|---|
| P. bifrenariae | Orchidaceae | Brazil | Leaf and bulb | Glienke et al. (2011) |
| P. brazilianiae | Anacardiaceae | Brazil | Leaf and fruit | Glienke et al. (2011) |
| P. capitalensis | Various hosts, woody plant | New Zealand | Leave and fruit | Baayen et al. (2002), Glienke et al. (2011) |
| P. ilicina | Quercus ilex (Fagaceae) | Switzerland | Leaves or needles | Collado et al. (1996) |
| P. spinarum | Platycladus orientalis (Cupressaceae) | USA | Leaves | Wijeratne et al. (2008) |
| Phyllosticta sp. | Ginkgo biloba (Ginkgoaceae) | Japan | Leaf, petiole, twigs | Thongsandee et al. (2011) |
| Phyllosticta sp. | Abies grandis (Pinaceae) | USA | Leaves or needles | Carroll and Carroll (1978) |

Daisy 2003; Huang et al. 2009; Xu et al. 2010; Tan and Zou 2001). Investigations related to endophytic microorganisms in plants and especially tropical hosts have increased, due to the significance of using endophytes in biological control and the discovery of biologically active compounds (Wijeratne et al. 2008; Le Calvé et al. 2011).

Although Phyllosticta species have been reported as endophytes there are relatively few reports of Phyllosticta species being recorded as endophytes in recent studies. In two volumes of the journal Fungal Diversity (Volume 41, 2010, Volume 47, 2011) there were 13 manuscripts devoted to biodiversity of fungal endophytes and only one (Lin et al. 2010) reported an endophytic Guignardia species. Phyllosticta capitalensis however has commonly been recorded as an endophyte in several studies (Baayen et al. 2002; Glienke et al. 2011, Okane et al. 2001; Okane et al. 2003) and was reported as an endophyte on more than 20 hosts in eight countries (Wulandari et al. 2010; Glienke et al. 2011). Therefore the species is thought to be one of the most common endophytic species of Phyllosticta (Glienke et al. 2011). There are few records of other Phyllosticta species recorded as endophytes and they are usually listed as unidentified *Phyllosticta* sp. (Pandey et al. 2003). Some Phyllosticta species reported as endophytes are listed in Table 2.

Phyllosticta species as saprobes

Most fungi have the ability to grow as saprobes, and degrade organic material from dead plant material as a food source. Plant pathogenic fungi can often survive as saprobes between growing seasons (Trigiano et al. 2004). For example, *Phyllosticta carpogena* and *P. ericae* occurred as saprobes on *Rubus* sp. (*Rosaceae*) and *Erica carnea* (*Ericaceae*), respectively (van der Aa and Vanev 2002) (Table 3).

Life cycle

Concepts concerning life cycles of plant pathogens may have significant practical consequences for plant pathologists and taxonomists. Herein, we provide a schematic diagram of the life cycle of a typical species of *Phyllosticta* (*Phyllosticta ampelicida*, Fig. 2). After infection by the *Phyllosticta* or *Guignardia* propagules, pycnidia and/or ascomata develop under the leaf tissue, and produce leaf spots on the host. During the wet season conidia and ascospores, and sometimes the spermatial stage are present. Subsequently spores are released and ejected from the pycnidia and ascomata. The spores are carried by rain and wind to other leaves and young fruits. Germ tubes develop from spores and grow into leaves and develop within the plant tissue. The disease spreads by transmission by warm wind and rain during the wet season.

Secondary metabolites from species of Phyllosticta

Fungi are well-known as a good source of important metabolites, some of which are useful in the pharmocological industry and agriculture (Pearce 1997, Smith and Casey 2008, Aly et al. 2010; Xu et al. 2010; Udayanga et al. 2011). Both novel and previously known metabolites have been isolated from species of *Phyllosticta* (Tables 4, 5). Metabolites produced by *Phyllosticta* species include phyllostin and phyllostoxin. Phyllostictines A-D that were isolated from P. cirsii (Evidente et al. 2008b). Phytotoxins, including phyllosinol, brefeldin, and PM-toxin (Sakamura et al. 1969; Sakai et al. 1970) were extracted from Phyllosticta maydis (Comstock et al. 1973) and Phyllosticta medicaginis (Entwistle et al. 1974), respectively. Phyllostictines A-D have been tested with on five cancer cell lines which displayed growth-inhibitory activity (Le Calvé et al. 2011). In addition, five new metabolites were isolated from P. spinarum, reported by Wijeratne et al. (2008) namely (+)-(5S,10S)-4'hydroxymethylcyclozonarone, 3ketotauranin, 3-hydroxytauranin, 12-hydroxytauranin, phyllospinarone.

Taxol was initially known as a phytochemical derived from the bark of *Taxus brevifolia* (Western Yew) and is an expensive and important diterpenoid anti-cancer intensive treatment drug used against breast, ovarian and lung cancers (Wani et al. 1971). It has been reported that the molecule has anti-tumour activity in several experimental

 Table 3 Phyllosticta species recorded as saprobes in selected studies

| Species | Host | Country | Plant organ | References |
|----------------------------------|---------------------|----------|------------------|--|
| P. acetosellae A.L. Sm. & Ramsb. | Rumicis acetosellae | England | Leaves | Smith and Ramsbottom (1913) |
| P. capitalensis | Magnolia liliifera | Thailand | Senescent leaves | Okane et al. (2003) |
| P. cocoicola | Palm | Europe | Leaves | Punithalingam (1974), Taylor and Hyde (2003) |
| P. pyrolae Ellis & Everh. | Pyrola rotundiforia | America | Leaves | Ellis and Everhart (1889) |

| Table 4 Metabolites produced by <i>Phyllosticta</i> spec |
|---|
|---|

| Compound | Properties | Name of taxa as in publication | References |
|--|----------------------------------|--------------------------------|--|
| Befeldin | Bioactive metabolite | P. medicaginis | Entwistle et al. (1974) |
| Phyllosinal | Bioactive metabolite | Phyllosticta. sp., P. maydis | Sakamura et al.(1969), Sakai et al. (1970) |
| Phyllostictine | Mycoherbicide | P. cirsii | Evidente et al. (2008a) |
| Phyllostin | Anti-microbial, anti-cancer | P. cirsii | Evidente et al. (2008a), Le Calvé et al. (2011) |
| Phyllostoxin | Mycoherbicide | P. cirsii | Evidente et al. (2008a) |
| PM-toxin | Mycoherbicide | P. maydis | Comstock et al. (1973) |
| Tauranine | Anti-cancer activity | P. spinarum | Wijeratne et al. (2008) |
| Taxol | Anti-cancer activity | P. tabernaemontanae | Kumaran et al. (2009a) |
| (+)-(5S,10S)-4' hydroxymethylcyclozonarone | Inhibition of cell proliferation | P. spinarum | Wijeratne et al. (2008) |
| 3-ketotauranin | Inhibition of cell proliferation | P. spinarum | Wijeratne et al. (2008) |
| 3-hydroxytauranin | Inhibition of cell proliferation | P. spinarum | Wijeratne et al. (2008) |
| 12-hydroxytauranin | Inhibition of cell proliferation | P. spinarum | Wijeratne et al. (2008) |
| Phyllospinarone | Inhibition of cell proliferation | P. spinarum | Wijeratne et al. (2008) |

trials. Taxol is produced by various fungal strains of *Pestalotiopsis* (Strobel et al. 1996, 1997), *Phomopsis* (Kumaran and Hur 2009) and *Phyllosticta* (Kumaran et al. 2008a, b, 2009a) in culture media under various conditions. Taxol has been reported from *P. citricarpa*, from *Citrus medica* and *P. dioscoreae* from *Hibiscus rosa-sinensis* (Kumaran et al. 2008a, 2009b). Species of *Phyllosticta* are therefore potential sources for discovery of pharmaceutical, medical and agricultural novel compounds.

Phyllosticta in biocontrol

Biocontrol is "the control of unwanted organisms such as weeds by the use of other organisms, as through the use of organisms that are natural predators, parasites, or pathogens (http://www.answers.com/topic/biological-pest-control#ixz z1YSqa0e00)" Fungi are commonly used as biological control agents (Charudattan and Dinoor 2000, Mortensen 1998; Trujillo 2005; Rosskopf et al. 2000). *Phyllosticta* species may have potential for use as biocontrol agents although there are presently few examples (Table 5). A strain of *Phyllosticta* (Ph511) was shown to produce compounds

that had high affect on motility of the second stage juveniles of *Meloidogyne incognita* and has potential in parasitic nematode control (Yan et al. 2011). *P. cirsii*, a pathogen isolated from diseased leaves of *Cirsium arvense* has been evaluated as a potential biocontrol agent of this noxious perennial weed, also produces different phytotoxic metabolites with potential herbicidal activity when grown in liquid cultures (Evidente et al. 2008a). The metabolites reported are Phyllostictines A–D which are potential mycoherbicides (Berestetskiy et al. 2008; Evidente et al. 2008a,b).

Need for epitypification of Phyllosticta species

An epitype is a fresh specimen (usually with accompanying culture) selected to serve as a representative type when such authentic material has been recollected, and confirmed to represent the same species as the original type material (Phillips et al. 2006; Hyde et al. 2010a, b; Abd-Elsalam et al. 2010). This practice enables mycologists to link older names to DNA data derived from fresh collections. The sole purpose of epitypification, is to move mycology into the culture and DNA era. When an epitype is designated the original material

Table 5 Phyllosticta species used in biocontrol

| Species | Host | Compound name | Used against | References |
|-------------------------|------------------|--|--|-------------------------|
| Phyllosticta cirsii | Cirsium arvense | Phyllostictine A-D | Cirsium arvense (weed) | Evidente et al. (2008b) |
| <i>Phyllosticta</i> sp. | Cirsium arvense | Phyllostin (8-hydroxy-3-methyl- 2-oxo-2,3,4a, 5,8,8a-hexahydro- benzo[1,4]dioxine-6-carboxylic acid methyl ester) | Cirsium arvense | Tuzi et al. (2010) |
| Phyllosticta sp. | Curcumis sativus | Ph511 | Meloidogyne incognita root knot nematode | Yan et al. (2011) |

that the epitype supports must be explicitly cited. Several authors have discussed the urgent need for epitypication in plant pathogenic genera (Verkley et al. 2004; Crous 2005; Crous et al. 2007, Shenoy et al. 2007; Hyde et al. 2010a, b; Cai et al. 2011). Since few species of Phyllosticta have been epitypied or have ex-type cultures available it is imperative that pathogenic species are recollected and epitypified as has been done for other plant pathogenic genera.

Notes on selected species of Phyllosticta

Molecular data has to date proven to be inadequate in resolving many species in the genus *Phyllosticta* (Wulandari et al. 2009). Either there are very few species of *Phyllosticta* with some having a very wide host range, or the genes that we are presently using do not resolve species complexes. Differentiation of the 192 species accepted by van der Aa (1973) and van der Aa and Vanev (2002) was based on morphological data with often minor differences, and molecular evidence is not available to support this differentiation. Most species also lack living cultures and their uniqueness cannot be confirmed. For this reason it is not possible to list which species can be stated as currently in use in *Phyllosticta*, as has been done for *Cochliobolus* (Manamgoda et al. 2011), *Colletotrichum* (Hyde et al. 2009), *Fusarium* (Summerell et al. 2010; Summerell and Leslie 2011) and *Phomopsis* (Udayanga et al. 2011).

Below we discuss alphabetically selected *Phyllosticta* names which includes the generic type, an earlier name for the generic type, plant pathogens and endophytes resolved using molecular data, all taxa introduced since van der Aa and Vanev (2002) and some other taxa that we believe warrant discussion and is partly based on the most recent literature. This cannot be considered as a thorough account of the generic species, but provides a starting point towards establishing the number of acceptable species in future revisionary treatments of *Phyllosticta* as phylogenetic data accumulates and helps to resolve the species.

The account of selected species names provides authorities and publication details as appeared in *Index Fungorum* (http://www.indexfungorum.org/names/Names.asp). Synonyms are not given as these can be searched for in *Index Fungorum*. The teleomorph is given where known. This is not a rigorous list as it is impossible to verify at this stage whether collections of each taxon on a host are correctly identified. We have annotated the notes with host range, symptoms and known distribution, and additional notes on pathological, taxonomic and phylogenetic research. Also the additional notes emphasize the need for molecular data in future studies. We recommend that other resolved species are added to this selected list based on future studies of *Phyllosticta* employing molecular and morphological data as has been done in *Colletotrichum* (Phoulivong 2011).

When referring to *Phyllosticta* species one also has to consider the teleomorph Guignardia. However, although there is molecular data for this teleomorphic genus, it is mostly for the endophyte incorrectly identified as G. mangiferae (e.g. P. capitalensis). We therefore do not discuss Guignardia species below. However, Guignardia psidii Ullasa & Rawal, was shown to be distinct in the molecular analysis of Wang et al. (2011). This strain (CBS 100250) was isolated from a fruit of Psidium guajava collected from Sao Paulo, Brazil. The Phyllosticta state is unknown. Several new species of Guignardia have also been described in recent years (e.g. G. musicola N.F. Wulandari, L. Cai & K.D Hyde, G. bispora N.F. Wulandari & K.D. Hyde, Wulandari et al. 2010, 2011) and these also need recollecting and sequencing to establish their relationships with species of *Phyllosticta* and whether they can be considered as distinct species.

Phyllosticta ampelicida (Engelm.) Aa, Stud. Mycol. 5: 28 (1973)

Teleomorph: *Guignardia bidwellii* (Ellis) Viala & Ravaz, Bull. Soc. mycol. Fr. 8:63 (1892)

Hosts: Vitis spp., Ampelopsis spp., Cissus spp., Parthenocissus spp. (Vitaceae).

Disease symptoms: Black rot

Distribution: Asia, Canada, South America, UK and USA.

Notes: This *Phyllosticta* species is linked to the generic type of *Guignardia* and has a *Leptodothiorella* spermatial state. The black rot fungus can infect all parts of the vine, although the most significant losses are caused by berry infection (Reddick 1911, Miller 1968; Kuo and Hoch 1996; Wilcox 2003; Ellis et al. 2004). In warm humid climates, susceptible varieties can experience complete loss if the pathogen is left uncontrolled (Hoover et al. 2011). Sequences of reference isolates of this species are available in GenBank but it has not yet been epitypified.

Phyllosticta ardisiicola Motohashi, I. Araki & C. Nakash., Mycoscience 49 (2008)

Hosts: Ardisia crenata (Myrsinaceae)

Disease symptoms: Leaf spot

Distribution: Japan

Note: This species was introduced as new species based on its morphological differences with taxa from related hosts (Motohashi et al. 2008). Molecular data should be included in future work to confirm the status with closely related species.

Phyllosticta aspidistricola Motohashi, I. Araki & C. Nakash., *Mycoscience* **49**: 138–146 (2008)

Hosts: Aspidistra elatior (Liliaceae)

Disease symptoms: Leaf spot

Distribution: Japan

Note: This species were introduced as new species base on its morphological differences with taxa from related hosts (Motohashi et al. 2008). Molecular data should be included in future work to confirm the status with closely related species. *Phyllosticta beaumarisii* A.P. Paul & M.D. Blackburn, Australas. Pl. Path. 15: 41 (1986)

Spermatial state: Leptodothiorella sp.

Hosts: Muehlenbeckia adpressa (Nyctaginaceae)

Disease symptoms: leaf spots with distinctive necrotic lesions

Distribution: Australia

Note: The disease is prevalent during autumn and winter. Mature diseased leaves age and abscise when a new flush of growth occurs in spring. Pathogenicity testing has shown that *P. beaumarisii* is the causal agent of the disease as compared to other species of *Phyllosticta* associated with the host (Paul and Blackburn 1986). Although Yip (1987) have provided the full description and illustration of the species, molecular data is needed in future studies to confirm its status as distinct species.

Phyllosticta bifinariae O.L. Pereira, C. Glienke & Crous, Persoonia 26: 52 (2011)

Hosts: Bifrenaria harrisoniae (Orchidaceae)

Disease symptoms: Leaf spot

Distribution: Brazil

Notes: This isolate was originally thought to be representative of *P. capitalensis* but was found to be ecologically and phylogenetically distinct and a pathogen of *Bifrenaria harrisoniae* (Glienke et al. 2011).

Phyllosticta brazilianiae D. Stringari, C. Glingke & Crous, Persoonia 26: 47–56 (2011)

Hosts: Mangifera indica (Anacardiaceae)

Disease symptoms: Symptomless endophyte

Distribution: Brazil

Note: The species is ecologically distinct from *P. anacardiacearum* being an endophyte, and failing to induce leaf spots despite repeated inoculation on mango (Glienke et al. 2011). Molecular data has also shown that it is distinct from other closely related species.

Phyllosticta capitalensis Henn., Hedwigia 48: 13 (1908)

Hosts: *Phyllosticta capitalensis* was originally described on *Stanhopea* (*Orchidaceae*) from Brazil by Hennings (1908) although now thought to occur in wide range of hosts.

Disease symptoms: leaf spots (when cause disease) Distribution: Worldwide

Distribution: Worldwide Note: *Phyllosticta capitalensis* is the most recently proposed name for the entities that were formally incorrectly referred to as *Guignardia mangiferae* (Baayen et al. 2002; Glienke et al. 2011). The taxon is frequently isolated as an endophyte and has a wide host range and geographic distribution. Okane et al. (2001) identified an endophytic *Phyllosticta* strain in ericaceous plants from Japan, *as Phyllosticta capitalensis*, describing the teleomorph as a new species, *G. endophyllicola*. Baayen et al. (2002) recognized the common endophytic species associated with

a wide host range of plants based on ITS sequence

similarities, which was similar to G. endophyllicola in morphology. Although several names were available for this species, they opted to call the species G. mangiferae (a pathogen 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 the same clade in their study. A comprehensive study of endophytic and pathogenic Phyllosticta species on Citrus was carried out by Glienke et al. (2011). Their combined phylogenetic tree revealed the P. capitalensis sensu lato clade to be genetically distinct from a reference isolate of G. mangiferae isolated from India. Several names were available for this clade, the oldest being P. capitalensis. Glienke et al. (2011) therefore, suggested 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 analyzed to fully resolve the morphological variation still observed within this clade.

Phyllosticta citriasiana Wulandari, Crous & Gruyter, Fungal Diversity 34: 31 (2009).

Hosts: Citrus maxima (Rutaceae)

Disease symptoms: tan spots (produces shallow lesions with a small central grey to tan crater usually with dark brown margin on fruits)

Distribution: Asia (China, Thailand, Vietnam)

Notes: The tan spot symptom usually appears after the fruit has started to ripen and sometimes it can occur after harvest. Combined gene analysis, morphological and culture based characters were employed to distinguish the species from *P. citricarpa* and other species considered (Wulandari et al. 2009). Recent studies on endophytic and pathogenic species of *Phyllosticta* from citrus in different regions of the world shows that the morphological, cultural and biochemical characters for species were consistent with the results of phylogenetic analysis of related taxa (Glienke et al. 2011; Wang et al. 2011). A specific primer pair Pca8/ITS4 was also designed and selected, and a PCR protocol was used to detect *P. citriasiana* in recent study (Wang et al. 2011).

Phyllosticta citribraziliensis C. Glienke & Crous, Personia 26: 54 (2011)

Hosts: Citrus limon (Rutaceae)

Disease symptoms: Symptomless endophyte

Distribution: Brazil

Notes: This species is closely related to *P. spinarum* but phylogenetically distinct. Also *P citribraziliensis* is morphologically distinguished in having larger conidia, a thick mucilaginous sheath surrounding its conidia and branched conidiophores.

Phyllosticta citricarpa (McAlpine) van der Aa, Stud. Mycol. 5: 40 (1973)

Teleomorph: Guignardia citricarpa Kiely

Hosts: Citrus aurantius, C. limon, C. delicoisa, C. reticulata, C. sinensis (Rutaceae)

Disease symptoms: Black spot of citrus, foliar and fruit diseases, premature fruit drop

Distribution: Asia, Africa, Australia, USA (Florida)

Notes: P. citricarpa causes foliar and fruit disease of Citrus spp. G. citricarpa (anamorph P. citricarpa) which causes Citrus Black Spot is regulated as a quarantine pest in the European Union and the USA (Wang et al. 2011). This pathogen can infect the rind of Citrus fruit causing disease lesions (Kiely 1948a). Serious infection near the pedicel of the developing fruit possibly will lead to premature fruit drop (Baayen et al. 2002). The first report of Black spot on Citrus orchards was near Sydney, Australia and it was described as Phoma citricarpa McAlpine (McAlpine 1899). The teleomorph was described as Guignardia citricarpa Kiely (Kiely 1948b). van der Aa (1973) classified the anamorph as Phyllosticta citricarpa (McAlpine) Van der Aa. The species was recollected from Australia and an epitype was designated and the distinctiveness from P. citriasiana was confirmed (Glienke et al. 2011).

Phyllosticta citrichinaensis H.X. Wang, K.D. Hyde & H.Y. Li, Fungal Diversity (2011)

Hosts: Citrus spp. (*Rutaceae*)

Distribution: China

Disease symptoms: small grey, red-brown or brown spots and freckles on leaves, melanose like black spots on fruits

Notes: This taxon has been isolated as an endophyte and is also weak pathogen (Wang et al. 2011). *P. citrichinaensis* differs from the other four *Phyllosticta* species associated with citrus in its morphological, cultural and biochemical characteristics.

Phyllosticta convallariae Pers., Traité sur les Champignons Comestibles (Paris): 148 (1818)

Hosts: *Polygonatum* spp., *Convallaria* (*Convallariaceae*), *Maianthemum* (*Liliaceae*).

Disease symptoms: Red leaf spot

Distribution: Asia, Europe

Note: This is the generic type of *Phyllosticta* and has been clearly designated in Donk (1968). This species causes reddish-brown leaf spots on its host and has a *Leptodothiorella* spermatial state (http://www.uni-graz.at/ ~oberma/fungi-of-austria/phyllosticta-convallariae.html; available online 19 Sep, 2011) and needs recollecting and epitypifying. An earlier name was found in *Phyllosticta cruenta* (van der Aa 1973) but this needs confirmation based on sequencing collections from the original host.

Phyllosticta cruenta (Fr.) J.J. Kickx, Fl. Crypt. Flandres 1: 412 (1867)

Teleomorph: *Guignardia reticulata* (DC.: Fr.) Aa Hosts: *Polygonatum* spp., *Convallaria* (*Convallariaceae*), *Maianthemum* (*Liliaceae*).

Disease symptoms: Leaf spot

Distribution: Asia, Europe

Notes: *P. cruenta* is the earlier name for type of *Phyllosticta convallariae* and in older literature, it was considered to be an intermediate form between *Phyllostic-tina* and *Dothiorella* (van der Aa and Vanev 2002). This taxon should be recollected and epitypified as its distinctiveness from *P. convallariae* needs confirmation.

Phyllosticta cussoniae Cejp, Bothalia 10(2): 341 (1971) Teleomorph: *Guignardia cussonia* Crous

Host: Cussonia spp.

Disease symptoms: On leaves causing a prominent leaf spot.

Distribution: South Africa

Notes: In the phylogenic tree presented in Glienke et al. (2011) the isolates of this species clusters in a distinct clade and appears to represent a distinct taxa. Representative isolates were obtained from South Africa by P.W. Crous and designated as epitype (Glienke et al. in prep.).

Phyllosticta dioscoreae Cooke, Grevillea 6(no. 40): 136 (1878)

Teleomorph: *Guignardia dioscoreae* A.K. Pande, Sydowia 22(5–6): 367 (1969) [1968]

Host: Dioscorea spp. (Dioscoreaceae)

Disease symptoms: Leaf spot

Distribution: Africa (South Africa), Asia, Australia, South America (Brazil), USA.

Notes: An isolate identified as *Phyllosticta dioscoreae* from *Hibiscus rosa-sinensis* has been reported to produce the anti-cancer compound taxol (Kumaran et al. 2009b), but the species was identified on basis of morphological characters and therefore needs confirmation with molecular data. This species commonly causes leaf spots on *Dioscorea* spp. with its *Guignardia* state usually being produced (N. Wulandari, pers. comm.) and should be recollected and epitypified to establish if it is a distinct species.

Phyllosticta fallopiae Motohashi, I. Araki & C. Nakash., Mycoscience 49 (2008)

Hosts: Fallopia japonica

Disease symptoms: leaf spots

Distribution: Japan

Note: This species were introduced as new species base on its morphological differences with taxa from related hosts therefore molecular data are needed in future studies to establish its uniqueness (Motohashi et al. 2008).

Phyllosticta hypoglossi (Mont.) Allesch., Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1(6): 163 (1898)

Hosts: Living leaves and dead cladodes *Ruscus spp*. (*Liliaceae*)

Disease symptoms: Occurring on living and dead cladodes and stems, though distinct spots not reported,

Distribution: Asia, Europe (France, Italy, Portugal, Turkey, Ukraine)

Notes: The spermatial state is a *Leptodothiorella* sp. The taxon was originally described from France, and the sequenced isolates used by Glienke et al. (2011) collected from Italy could potentially be used for epitypification.

Phyllosticta kerriae Motohashi, I. Araki & C. Nakash., Mycoscience 49 (2008)

Teleomorph: Unknown

Hosts: Kerria japonica

Disease symptoms: leaf spots

Distribution: Japan

Note: This species were introduced as new species base on its morphological differences with taxa from related hosts therefore the molecular based re evaluation is needed to establish its uniqueness (Motohashi et al. 2008).

Phyllosticta minima (Berk. & M.A. Curtis) Underw. & Earle, Bulletin of the Alabama Agricultural Experiment Station 80: 168 (1897)

Hosts: Acer spp. (Maples) (Sapindaceae)

Disease symptoms: Leaf spot (black or purple eye spot). Distribution: Asia (China), North America

Notes: This pathogen has been reported as a common causative agent of ornamental *Acer* spp. (http://www.unce. unr.edu/publications/files/ho/2005/fs0547.pdf). *Phyllosticta gallarum* has been recorded causing similar leaf spots on *Caragana* spp. (http://www.unl.edu/nac/diseasetrees/chap4. pdf). Recollection and taxonomic re evaluation is needed to confirm if this species can be differentiated from closely related species.

Phyllosticta musarum (Cooke) Aa, *Stud. Mycol.* 5: 72 (1973)

Teleomorph: Guignardia musae Racib.

Hosts: Musa spp. (Musaceae).

Disease symptoms: Leaf spot, fruit spot, banana freckle, banana black spot

Distribution: Widespread

Notes: The pathogen (*Phyllosticta musarum* or the sexual state *Guignardia*) infects leaves and also affects the external appearance of the fruit, decreasing its quality and marketability. Severe infections of the disease may cause premature death of the older leaves on some banana cultivars. Preinfection studies shows that the pathogen seems to penetrate directly through the epidermal cuticle layer of the host by forming appressoria and infection pegs (Pu et al. 2008). Wulandari et al. (2010) investigated the problem of the occurrence of the species epithet ("musae") on separate occasions related to sexual state based on herbarium specimens and fresh collectios and distingushed there different species including taxonomic novelties. However the *Phyllosticta/Guidnardia* species from banana

needs to re evaluated based on morphological and molecular approach in future studies.

Phyllosticta owaniana G. Winter, Hedwigia 24: 31 (1885)

Host: *Brabejum stellatifolium (Proteaceae)* Disease symptoms: leaf spot Distribution: South Africa

Notes: *P. telopeae* Yip has been reported from *Telopea* speciosissima (*Proteaceae*) and is distingushed from *P. owaniana* by its larger conidia and much longer appendages. Both of the species being accepted in van der Aa and Vanev (2002). The sequences of the type of *P. owaniana* has been used in phylogenetic analysis but as the outgroup which shows the species to be significantly different from other *Phyllosticta* species. An epitype will be designated based on fresh collections (Glienke et al., in prep).

Phyllosticta solitaria Ellis & Everh., Proc. Acad. nat. Sci. Philad. 47: 430 (1895)

Hosts: *Malus* spp., *Crataegus* spp. (*Rosaceae*). *Pyrus* spp.

Disease symptoms: Leaf spot, fruit blotch, twig canker.

Distribution: Asia (India, China), Africa (Zimbabwe, South Africa), Europe (Greece), North America (USA), South America (Brazil).

Notes: Although the teleomorph is unknown, Guba (1925) have noticed the fructification on fallen leaves in spring (van der Aa and Vanev 2002). *P. solitaria* causes a serious blotching of apples which reduces fruit quality and also known as quarantine pest by EPPO (http://www.eppo.org/QUARANTINE/fungi/Phyllosticta_solitaria/PHYSSL_ds. pdf). The ability of the fungus to withstand long periods of cold storage should be noted in quarantine purposes. Reevaluation of the pathogen and epitypification is needed in future studies.

Phyllosticta sphaeropsoidea Ellis & Everh., Bull. Torrey bot. Club 10(7): 97 (1883)

Teleomorph: Guignardia aesculi (Peck) V.B. Stewart

Hosts: Aesculus spp. (Hippocastanaceae).

Disease symptoms: leaf blotch (disease known as buckeye blotch or horse chestnut blotch), black rot, brown leaf margin and necrotic tissue

Distribution: Asia, Europe, North America

Notes: *Guignardia aesculi* (sexual stage), initiates leaf infections in early spring, while *P. sphaeropsoidea* (asexual stage) perpetuates infections during the summer. Infections from both stages combine to cause horse chestnut leaf blotch (Gillman 2005; Pastricakova 2004). Recollection from various host species of *Aesculus* and various geographical locations are needed to establish the uniqueness of the taxa with molecular data.

Phyllosticta spinarum (Died.) Nag Raj & M. Morelet, Bull. Soc. Sci. nat. Arch. Toulon et du Var 34(219): 12 (1978) Hosts: Juniperus sp, Chamaecyparis pisifera, Platycladus orientalis (Cupressaceae), Hedera helix (Araliaceae)

Disease symptoms: none reported, presumed endophyte Distribution: Europe (Germany, France, Italy), USA

Notes: This was originally described from *Juniperus* sp. in Germany while the isolates sequenced in Glienke et al. (2011) were from *Chamaecyparis pisifera* and *Hedera helix* (from France and Italy). The endophytic isolate putatively identified as *P. spinarum* from *Platycladus orientalis* is known to produce novel secondary metabolites (Wijeratne et al. 2008).

Phyllosticta vaccinii Earle, Bull. Torrey bot. Club 24: 31 (1897)

Teleomorph: Guignardia vaccinii Shear

Hosts: Vaccinium spp. (Ericaceae).

Disease symptoms: Blast or blight of flowers and young fruits; early rot of fruits in storage

Distribution: Asia (China), North America

Notes: Weidemann et al. (1982) listed the anamorph of *Guignardia vaccinii* as *Phyllosticta elongata*, but van der Aa (2002) lists both *Phyllosticta elongata* and *Phyllosticta vaccinii* as anamorphs. A strain of this species (CBS165.86) has been sequenced by Duong (2008) and appears to be a distinct species in phylogenetic analysis. However, future work is needed to establish the uniqueness of taxa.

Concluding remarks

Species recognition criteria in *Phyllosticta* and the sexual state *Guignardia* have evolved from morphological criteria to phylogenetic species concepts that involves DNA sequence data derived from type material. However as compared to the number of accepted species in previous revisionary treatments, there is a lack of molecular-based studies considering the wide range of hosts and various geographic locations. We therefore recommend the recollection of taxa, and epitypification where possible. The number of resolved names in the genus would be updated with ongoing projects and the sexual states should be linked accordingly to establish operational biological species.

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