A modern account of the genus Phyllosticta

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Conidial states of Guignardia are in the genus Phyllosticta. In accordance to nomenclatural decisions of IBC Melbourne 2011, this paper validates species that were in Guignardia but are now accepted in Phyllosticta. The conclusions are arrived based on molecular analyses and morphological examination of holotypes of those species previously described in the genus Guignardia. Thirty-four species of Phyllosticta, viz. P. ampelicida (Engelm.) Aa, P. aristolochiicola R.G. Shivas, Y.P. Tan & Grice, P. bifrenariae O.L. Pereira, Glienke & Crous, P. braziliniae O.L. Pereira, Glienke & Crous, P. candeloflamma (J. Fröhlich & K.D. Hyde) Wulandari, comb. nov., P. capitalensis Henn., P. cavendishii M.H. Wong & Crous, P. citriasiana Wulandari, Gruyter & Crous, P. citribraziliensis C. Glienke & Crous, P. citricarpa (McAlpine) Aa, P. citrichinaensis X.H. Wang, K.D. Hyde & H.Y. Li, P. clematidis (Hsieh, Chen & Sivan.) Wulandari, comb. nov., P. cruenta (Fr.) J. Kickx f., P. cussoniae Cejp, P. ericarum Crous, P. garciniae (Hino & Katumoto) Motohashi, Tak. Kobay. & Yas. Ono., P. gaultheriae Aa, P. hostae Y.Y. Su & L. Cai, P. hubeiensis K. Zhang, Y.Y. Su & L. Cai, P. hymenocallidicola Crous, Summerell & Romberg, P. hypoglossi (Mont.) Allesch., Rabenh., P. ilicis-aquifolii Y.Y. Su & L. Cai, P. korthalsellae A. Sultan, P.R. Johnst, D.C. Park & A.W. Robertson, P. maculata Wong & Crous, P. morindae (Petr. & Syd.) Aa, P. musarum (Cooke) Aa, P. muscadinii (Luttr.) Wulandari, comb. nov., P. owaniana G. Winter, P. partenocissi K. Zhang, N. Zhang & L. Cai, P. philoprina (Berk. & M.A. Curtis) Aa, P. schimae Y.Y. Su & L. Cai, P. spinarum (Died.) Nag Raj & M. Morelet, P. styracicola Zhang, Y.Y. Su & L. Cai, and P. vaccinii Earle are accepted in this study based on examination of type material of "Guignardia" species, including three new combinations. It is anticipated that other species of *Phyllosticta* will be accepted, following future molecular studies.

Key words – accepted species – Dothideomycetes – molecular phylogeny – monograph – plant diseases – saprobes – taxonomy

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Introduction

Species of Guignardia Viala & Ravaz (Botryosphaeriales) produce simple brown, thin-walled ascomata, containing bitunicate and fissitunicate asci and variously shaped, hyaline, unicellular ascospores, with or without mucilaginous sheaths or appendages (Sivanesan 1984, Wulandari et al. 2010a,b, 2011, Glienke et al. 2011). The ascomata contain sparse, cellular pseudoparaphyses. Several species of Guignardia have been linked to Phyllosticta asexual states via cultural studies or observation of co-occurence of both states in close proximity on the host (van der Aa 1973, van der Aa & Vanev 2002, Motohashi et al. 2008a,b, 2010, 2011). Another asexual state, Leptodothiorella, is known in some species (Wulandari et al. 2010, Su & Cai 2012, Zhang et al. 2013b). There are 340 epithets listed under Guignardia in Index Fungorum (2013). Unfortunately, there is no monographic work on the genus Guignardia so far and one generally relies on publications containing described species on the hosts (Hyde 1995) or those introduced as new species (van der Aa 1973, Punithalingham 1974, Sivanesan 1984, Hyde 1995, Okane et al. 2001, 2003, van der Aa & Vanev 2002, Motohashi et al. 2008a,b, Wulandari et al. 2009, Motohashi et al. 2010, Wikee et al. 2011, Glienke et al. 2011, Wong et al. 2012, Su & Cai 2012, Zhang et al. 2013b).

Phyllosticta is the name given to the asexual states of Guignardia (van der Aa 1973, Sivanesan 1984, Hyde 1995, Wulandari et al. 2010a,b), which are phytopathogens producing brown, thin-walled conidiomata, simple, containing round to oval, hyaline, unicellular conidia. The distinctive character Phyllosticta is the thin mucilaginous sheath surrounding the spore and an apical appendage (van der Aa 1973, van der Aa & Vanev 2002). Species of Guignardia and Phyllosticta are found associated with leaf spots of numerous plants and even isolated as endophytes (Okane et al. 2008, Glienke et al. 2011). A few species are saprobic (Wang et al. 2008). Because of their phytopathogenic nature, Phyllosticta species have been studied more than the Guignardia states. van der Aa (1973) has provided a detailed account of the genus Phyllosticta and accepted 30 species in the

genus. van der Aa & Vanev (2002) updated the taxonomy of the genus and accepted 154 species. The species are differentiated largely on host specificity and conidial characters.

The International Botanical Congress held in Melbourne, Australia, in 2011, made efforts to bring an end to dual nomenclature of fungi (McNeill et al 2012, Hawksworth et al. 2011). It was resolved that the connected sexual and asexual states will have only one name to represent the genus or species which previously had two (or more) names. Thus, it is required to make a choice of name between Guignardia or Phyllosticta as these genera have been unequivocally linked by cultural and molecular data (Glienke et al. 2011, Wikee et al. 2013, Zhang et al. 2013b). In this paper, we use Phyllosticta over Guignardia, as suggested in recent publications (Glienke et al. 2011, Su & Cai 2012, Wong et al. 2012, Wikee et al. 2013, Zhang et al. 2013a,b) and the latter is treated as a synonym of Phyllosticta. The genus Phyllosticta is placed in the family Phyllostictaceae in Botryosphaeriales (Liu et al. 2012).

The reasons for using *Phyllosticta* over "Guignardia" are:

- 1. Phyllosticta was introduced by Persoon (1818) and is an earlier name than Guignardia introduced by Viala & Ravaz (1892). The earlier name takes priority (see International Code for Nomenclature of Algae, Fungi and Plants;
 - http://www.iapttaxon.org/nomen/main.php?page=title).
- 2. There are more *Phyllosticta* names in various indices, i.e. Index Fungorum, Species Fungorum, MycoBank and GenBank, than *Guignardia*.
- 3. The records of *Phyllosticta* in search engines, such as Google Scholar are higher.
- 4. *Phyllosticta* is an important genus of plant pathogens and therefore important in quarantine. For example, *P. citricarpa* causes citrus black spot (CBS) and is an important quarantinable species in Europe.
- 5. *Phyllosticta* is often used in agricultural reference books (van der Aa & Vanev 2002).

In this paper, "Guignardia" species are described based on holotypes. In all, 127 specimens of "Guignardia" from 31 herbaria have been examined, including the type material of many taxa. Descriptions and illustrations of eight accepted species of Phyllosticta are documented based holotypes and annotations of 26 accepted species of *Phyllosticta* are provided based on published literature. A key to all species of Phyllosticta is not provided at present because identification based is morphology and, for some species, on the phylogeny.

Materials & Methods

The holotypes of 127 species named "Guignardia" were obtained on loan from herbaria (B, BPI, BRIP, CBS, DAR, F, FH, G, K, KRA, LE, MA, NTU, NY, PAV, PDD, PH, PREM, SIENA, TNS, TUR, W, YAM, and ZT) (Holmgren & Holmgren 1998). Ascomata were rehydrated, carefully dissected under a microscope and mounted in lactic acid. Twenty ascospores and conidia were measured. Hand sections were made to determine and illustrate the wall of ascomata. An Olympus microscope with an attached camera lucida apparatus was used for drawing. Photomicrographs were made on a Nikon eclipse 80i with an EOS 450D Nikon camera. All line drawings were prepared by the first author. Morphological characters are recorded as in Wulandari et al. (2009).

Results

Thirty-four presently accepted species of *Phyllosticta* are annotated, based on recent publications and examination of type material of "Guignardia" species. It is anticipated that many other species of *Phyllosticta* will be accepted in the future following molecular studies.

Taxonomy

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

Possible synonymys

Caudophoma B.V. Patil & Thirum., Sydowia 20: 36 (1968) [1966]

Guignardia Viala & Ravaz, Bull. Soc. Mycol. Fr. 8: 63 (1892)

Laestadiella Höhn., Ann. Mycol. 16: 50 (1918)

Leptasteromella Petr., Sydowia 20: 235 (1968) [1966]

Leptodothiorella Höhn., Hedwigia 60: 173, 175 (1918)

Leptodothiorella Aa, Stud. Mycol. 5: 13 (1973)

Leptophacidium Höhn., Sber. Akad. Wiss. Wien, Math. naturw. Kl., Abt. 1 127: 331 [3 repr.] (1918)

Macrophyllosticta Sousa da Câmara, Anais Inst. Sup. Agron. Univ. Téc. Lisboa 3: 36 (1929)

Montagnellina Höhn., Sber. Akad. Wiss. Wien, Math. naturw. Kl., Abt. 1 121: 387 [49 repr.] (1912)

Myriocarpa Fuckel, Jb. Nassau. Ver. Naturk. 23–24: 116 (1870) [1869–70]

Pampolysporium Magnus, Verh. Zool.-Bot. Ges. Wien 50: 444 (1900)

Phyllosphaera Dumort., Comment. Bot.: 86 (1822)

Phyllostictina Syd. & P. Syd., Ann. Mycol. 14: 185 (1916)

Polysporidium Syd. & P. Syd., Ann. Mycol. 6: 528 (1908)

Phytopathogenic, causing leaf spots, endophytes or rarely saprobic on plants. Sexual states: Ascomata small, pseudothecia, solitary to aggregated, scattered, uniloculate, immersed in host tissue or becoming erumpent, globose to subglobose, dark brown, ostiolate, with or without an ostiolar papilla. Peridium thin, composed of thick-walled pseudoparenchymatous cells towards exterior, with inner wall cells thin and hyaline. Pseudoparaphyses absent pseudothecia, sometimes present as tubular cells in immature ascomata. Asci 8-spored, bitunicate, fissitunicate, clavate to cylindrical, with a short pedicel, with or without an ocular chamber. Ascospores seriate, hyaline, 1-celled, ovoid, ellipsoidal to rhomboidal, straight or curved, wide in the middle, guttulate, with distinct mucilaginous appendages at one or both ends which in some disappear or remain as remnants at spore ends. Asexual state: Conidiomata epiphyllous, small, pycnidial, gregarious, immersed, reddish brown to dark brown, uniloculate, subglobose, papillate, ostiolate. Wall of conidioma thick in culture, continuous with epidermal or subepidermal stroma, composed of several layers, with cells of the outer layers thick-walled, dark brown, isodiametric or slightly flattened; inner layers with thin-walled cells. Conidiogenous cells holoblastic, hyaline, short, cylindrical. Conidia rarely 1-celled, very 2-celled, broadly ellipsoidal to subglobose or obovate, rounded at both ends, occasionally truncate at the base, slightly indented at the apex, hyaline, smoothwalled, coarsely granular, surrounded by a slime layer, usually with a hyaline, flexuous, narrowly cylindrical, mucilaginous apical appendage. Appendage often disappearing when mounted in water or other reagents. Spermogonia pycnidial, numerous, crowded on faded leaf spots with unbordered necrotic spots on the leaf, epi- or amphiphylous, uniloculate, subglobose, sometimes embedded multiloculate stroma, black, papillate, ostiolate. Wall of spermogonium, continuous with subepidermal stroma, consisting of several layers of cells; the cells of the outer layers thick-walled, dark brown, slightly flattened; inner layers thin-walled, hyaline, flattened. Spermatiogenous cells holoblastic, hyaline, cylindrical to narrowly flattened. Spermatia 1celled, hyaline, dumbbell-shaped to cylindrical, bluntly rounded at both ends.

Accepted species in Phyllosticta

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

- ≡ *Naemospora ampelicida* Engelm., Trans. Acad. Sci., St Louis 2: 165 (1863)
- = *Phyllosticta ampelopsidis* Ellis & G. Martin, in G. Martin, J. Mycol 2: 14 (1886)
- = *Phyllosticta labrusca* Thüm., Die Pilze des Weinstockes: 189 (1878)
- = *Phyllosticta vitea* Sacc., in P. Sydow, Syll. Fung. 12: 564 (1896)
- = *Phyllosticta viticola* Thüm., Die Pilze des Weinstockes: 188 (1878)
- = *Phyllosticta vulpinae* Allesch., Rabenh. Kryptog.-Fl. 1, 6: 98 (1898); illeg. name (Art. 52)

Host – *Vitis riparia* (Vitaceae)

Phyllosticta ampelicida is the causal agent of black rot in grapes and one of the destructive fungal pathogens in North viticulture. Engelman American (1861)species as Naemospora described this ampelicida without designating a type. van der Aa (1973) re-described and transferred the fungus to *Phyllosticta*, recording it as asexual state of Guignardia bidwellii (Ellis) Viala & Ravaz (van der Aa & Vanev 2002). Neotypifying this species using a strain from ATCC, Zhang et al. (2013b) concluded that P. ampelicida is not only the asexual state of G. bidwellii but also host specific on Vitis sp. Zhang et al. (2013b) observed that P. ampelicida (ATCC 200578) morphologically similar to the protologue of *P*. ampelicida as described by Engelman (1863) but distinct from P. bidwellii (CBS 111645) in host, morphology and distribution.

Phyllosticta parthenocissi, which occurs on Parthenocissus sp. (Vitaceae) differs from P. ampelicida (Zhang et al. 2013b) in size of conidia. Cultural characteristics, aerial mycelium and multi-gene phylogenies (ITS, EF, actin and GPDH) also show that P. ampelicida is distinct from P. parthenocisii (Zhang et al. 2013b).

Phyllosticta aristolochiicola R.G. Shivas, Y.P. Tan & Grice, Persoonia 29: 154 (2012)

Host – on leaves of *Aristolochia acuminata* (Aristolochiaceae)

aristolochiicola Phyllosticta associated with leaf spots and shot-hole disease of Aristolochia clematitis and A. sempervirens in Oueensland, Australia (Shivas & Alcorn 1996). Phyllosticta ariestolochia Tassi is a replacement name for P. tassiana Saccardo & Traverso occurring on Aristolochia (Aristolochiaceae). homonym a illegitimate name (van der Aa & Vanev 2002). A GenBank nucleotide blast result for ITS, showed that P. aristolochiicola is most similar to P. cordilinophylla (97% similarity), while LSU sequence data showing this species to be close to P. abietis and P. minima (99% similarity). This species forms a distinct clade from P. abietis and P. minima (Crous et al. 2012a).

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

Host – *Bifrenaria harrisoniae* (Orchidaceae)

Previously reported as a pathogen on *Bifrenaria harrisoniae* (Silva et al. 2008), this species is also recorded from healthy leaves of orchids in Brazil (Glienke et al. 2011). *Phyllosticta bifrenariae* is distinct from *P. capitalensis* and *P. aplectri* (other species of *Phyllosticta* recorded on orchids) in that the conidia of *P. bifrenariae* are larger (10–16 × 7–9 µm) than those of *P. capitalensis* [(10–)11–12(–14) × (5–)6–7 µm] and *P. aplectri* (5–8 × 4–6 µm). Glienke et al. (2011) reported that *P. bifrenariae* is phylogenetically distinct from other *Phyllosticta* species, forming a separate clade with 97% bootstrap support.

Phyllosticta braziliniae O.L. Pereira, Glienke & Crous, Persoonia 26: 53 (2011)

Host – *Mangiferae indica* (Anacardiaceae)

Several species of *Phyllosticta* have been recorded from *Mangifera indica* (Glienke et al. 2011, van der Aa 1973, van der Aa & Vanev 2002). *Phyllosticta anacardiacearum* is a replacement name of *Phyllostictina mangiferae* (van der Aa 1973). van der Aa & Vanev (2002) reported *P. mortonii* and *P. mangiferae* as excluded species of *Phyllosticta*. The conidia of *P. braziliniae* (8–10–11(12.5) x (5-)6(-7) µm) differ in size from *P. capitalensis* $[(10-)11-12(-14)\times(5-)6-7$ µm)].

Without causing any leaf spot disease on mango, *P. braziliniae* is ecologically distinct from *P. anacardiacearum*. Phylogenetically *P. braziliniae* is distinct from other *Phyllosticta* species with 97% bootstrap value (Glienke et al. 2011).

Phyllosticta candeloflamma (J. Fröhlich & K.D. Hyde) Wulandari, **comb. nov.** Fig. 1–2 MycoBank: MB 805537

= Guignardia candeloflamma J. Fröhlich & K.D. Hyde, Mycol. Res. 99: 110 (1995) MycoBank: MB 363083

Leaf spots $1-2.7 \times 0.6-3$ mm, ellipsoidal, pale brown, with thin, darker brown border, zonate in the centre, round to ellipsoidal, medium brown, containing numerous ascomata. Ascomata 50–130 μ m

50-80 μm high, amphigenous, sometimes hyphogenous, black, globose to immersed subglobose, in leaf tissues. coriaceous, solitary to clustered, ostiolate, with ostiole as black dots at the centre. Peridium 17.5–31.25 μm wide, composed of 2–3 layers of cells textura angularis and pigmented outwardly and around the ostiole, paler in the innermost laver. Pseudoparaphyses observed. Asci 50–90 × 19–25 μ m (\bar{x} = 68 × 22 μ m, n = 20), 8-spored, bitunicate, with an ocular chamber 3–7 µm high, clavate to broadly clavate, rounded at the apex, where the diam. is 14–25 µm, tapering gradually to a 6–7 \times 3–10 µm long pedicel attached to the basal peridium. Ascospores $17-22 \times 8-11 \, \mu m \, (\bar{x} =$ $20 \times 9 \mu \text{m}$, n = 20), biseriate to triseriate, ellipsoidal, hyaline to greenish, 1-celled, coarse-guttulate, smooth-walled, with polar mucilagenous sheath at both side ends, with sheath 3.3-13.2 µm wide extended at the base up to 15 µm long to candle-flame-shape.



Fig. 1 – *Phyllosticta candeloflamma* (BRIP 20472, holotype) a. Leaf spots. b. Ascomata on the host surface. c. Section of ascoma. d. Peridium comprising one strata of *textura angularis* cells; note cells with thickened brown cell walls at the ostiolar region. e–f. Asci. g–i. Ascospores. – Scale bars a = 5 mm, b = $100 \, \mu m$, c–d = $15 \, \mu m$, e–i = $10 \, \mu m$.

Host – On living leaves of *Pinanga* sp. (Arecaceae)

Known distribution – Australia, Indonesia.

Material examined – Australia, Queensland, Smithfield, Australia, on leaves of *Pinanga* sp., 12 February 1992, K.D. Hyde, (BRIP 20472, holotype); Indonesia, Irian Jaya, on leaves of *Pinanga* sp., March 1992, K.D. Hyde (BRIP 20398, isotype).

Phyllosticta candeloflamma can form zonate spots on Pinanga leaves. This species is distinct from other species of Phyllosticta in having a polar mucilaginous sheath at each end of the ascospores with the basal appendage extending to a candle-flame-shaped appendage.

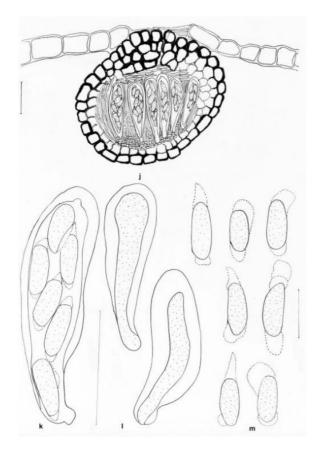


Fig. 2 – *Phyllosticta candeloflamma* (BRIP 20472, holotype) line drawing. j. Section of ascoma (darkened area are fungal cells, in the arrowed region). k. Mature ascus. l. Immature asci. m. Ascospores. – Scale bars $j = 50 \mu m$, $k - 1 = 25 \mu m$, $n = 20 \mu m$.

Phyllosticta capitalensis Henn., Hedwigia 48: 13 (1908) Fig. 3–4 = Guignardia aleuritis (Vassiljevsky)

Aa (1973), Stud. Mycol. 5: 88 (1973)

- ≡ *Melanops aleuritis* Vassiljevsky, Notulae Systematicae Section Cryptogamica Instituti Botanici Academiae Scientiarum U.S.S.R. 5: 9 (1940)
- = Guignardia allamandae A.K. Pande & V.G. Rao, J. Econ. Taxon. Bot. 14: 158 (1990)
- = *Phyllosticta concinna* (Syd.) Aa, Stud. Mycol. 5: 49 (1973)
- = *Guignardia concinna* (Syd.) Aa, Stud. Mycol. 5: 49 (1973)
- = *Melanops concinna* Syd., Annls Mycol. 24: 372–373 (1926)
- = Guignardia endophyllicola Okane, Nakagiri & Tad. Ito, Can. J. Bot. 79: 103 (2001)
- = Guignardia eucalyptorum Crous, South Africa J. Bot. 59: 605 (1993)
- = *Phyllosticta eucalyptorum* Crous, South Africa J. Bot. 59: 605 (1993)
- = *Guignardia smilacis* A.J. Roy, Indian Phytopath. 20: 348 (1968)
- = Guignardia graminea Lobik, Material on flora and fauna observations of Terek region 25
- = Guignardia pipericola F. Stevens, Trans. Illinois State Acad. Sci. 10: 183 (1917)
- = Guignardia smilacinae (Dearn. & House) Dearn. & House, New York State Museum Bull..266: 73 (1925).
- = Guignardia bumeliae J.H. Mill. & G.E. Thomps., Mycologia 32: 4 (1940)
- = *Guignardia helicteres* F. Stevens, Trans. Illinois State Acad. Sci. 10: 183 (1917)
- = Guignardia festiva (Syd.) Sacc., Sylloge Fung. 24(2): 783 (1928)
- = Guignardia foeniculata (Mont.) Arx & E. Mull, Beitr. Kryptfl. Schweiz 11(no. 1): 48 (1954)
- = Guignardia cocogena (Cook) Punith., Mycol. Pap. 136: 21 (1974)
- = Guignardia capsici Punith., Mycol. Pap. 136: 13 (1974)
- = Guignardia castanopsidis W.H. Hsieh, Chi Y. Chen & Sivan., Mycol. Res. 101: 901 (1997)
- = *Guignardia cocoicola* Punith., Mycol. Pap. 136: 15 (1974)
- = Guignardia codiae Thaung, Trans. British Mycol. Soc. 66: 107 (1976)
- = Guignardia coffeae Punith. & B.S. Lee, in Punithalingam, Mycol. Pap. 149: 19 (1981)
- = Guignardia dyerae Punith. & P.H. Wong, in Punithalingam, Mycol. Pap. 149: 28 (1981)
- = Guignardia perseae Punith., Mycol. Pap. 136: 43 (1974)
 - = Guignardia pini Sivan., Trans. British

Mycol. Soc. 73: 169 (1979)

= Guignardia heveae Syd. & P. Syd., Ann. Mycol. 14: 360 (1916)

= Guignardia miconia Seixas & R.W. Barreto, in Seixas, Barreto & Killgore, Mycologia 99: 103 (2007)

= Guignardia musicola Wulandari, L. Cai & K.D. Hyde, Crypto. Mycol. 31: 412 (2010)

= *Guignardia musae* F. Stevens, Bull. Bernice P. Bishop Museum, Honolulu, Hawaii 19: 101 (1925)

= Guignardia stephensii Wulandari & K.D. Hyde, in Wulandari, To-anun, Cai, Abd-Elsalam & Hyde, Crypto. Mycol. 31: 406 (2010)

= Guignardia musae F. Stevens, Bull. Bernice P. Bishop Museum, Honolulu, Hawaii 19: 101 (1925)

Ascomata 93–125 µm diam., 100–125 um high, present on the upper surface of the leaves, black, globose to subglobose, immersed to semi-immersed in leaf tissues or growth media. Peridium 13-25 µm wide, with one strata of 2–3 layers of cells textura angularis with thickened brown walls around the ostiole. Pseudoparaphyses not observed. Asci 31–50 × 8–13 µm ($\bar{x} = 41 \times 10$ µm, n = 20), 8-spored, bitunicate, cylindrical to cylindro-clavate, rounded at the apex, where the diameter is 5-11 µm, tapering gradually to a $4-6 \times 3-5$ µm long pedicel attached to the basal peridium. Ascospores $11-13 \times 3-4 \mu m$ ($\bar{x} = 13 \times 3 \mu m$, n = 20), uniseriate to biseriate, ellipsoidal wide in the middle, obtrullate, hyaline to greenish, 1celled, coarse-guttulate, smooth-walled, with or without mucilaginous pads at the ends.

Hosts – As saprobe on dead leaves of Aleurites fordii, A. moluccana (= A. triloba), A. cordata, A. montana (Euphorbiaceae), on fallen leaf of Smilax aspera (Smilacaceae), on dead stems of Atropis distans (Poaceae), on fallen leaves of Bumelia lycioides (Sapotaceae), on stellate dead leaves of Vagnera (Convallariceae, formerly Alliaceae); endophyte Rhododendron pulchrum (Ericaceae); leaf spots on living leaves of Allamanda cathartica (Apocynaceae), Cordia ferruginae (Boraginaceae), Eucalyptus dives (Myrtaceae), Helicteres (Sterculiaceae), Heptapleurum venulosum (Schefflera bengalensis) (Araliaceae), Piper sp. (Piperaceae), and Sumbaviopsis albicans (Euphorbiaceae) (Table 1).

Known distribution – Brazil, Brunei Darussalam, Carribean, Costa Rica, Dominican Republic, Eastern Cape, Georgia, Germany, Hong Kong, India, Indonesia, Italy, Jamaica, Japan, Malaysia, Myanmar, Netherlands, New Zealand, Panama, Pakistan, Philippines, Puerto Rico, South Africa, Sri Lanka, Taiwan, Thailand, Tasmania, China, UK, USA, Venezuela, Virgin Islands.

Material examined - Georgia, Batumi, on leaves of Aleurites fordii, 24 September 1935, V.L. Vassiljevsky, (LE 34833 lectotype of G. aleuritis, designated here), ibid. (LE 34834, LE 34835, paratypes of G. aleuritis). ibid., Island Sheldobai on stems of Atropis distans, 15 July 1925, D.K. Volgunov, V.L. Komarov Botanical Institute. Prof. Popov Street 2. Saint Petersburg 197376, Russia (LE 122701, from the holotype of G. graminea). India, Dhobighat, Raniket, Almora UP, on leaves of Smilax aspera, 3 July 1963, A.J. Roy, Eidgenossische Technische Hochschule, Zurich (ETH, holotype of G. smilacis); ibid., Pune, Maharashtra, on leaves of Allamanda cathartica, February 1978, B.R.D. Yadav, Herbarium Cryptogamie Ajrekar Mycological Herbarium (AMH 4049, holotype of G. allamandae). Costa Rica (La Caja bei), 6 January 1925, H. Sydow (BPI 1110937, holotype of G. concinna). Japan, on living leaves of *Rhododendron* pulchrum "Ohmurasaki" as endophytic cluture (IFO-H 12230, holotype of G. endophyllicola). South Africa, Tzaneen, Northern Transvaal, on leaves of Eucalyptus dives, 27 March 1991, P.W. Crous, (PREM 51291, holotype of G. eucalyptorum), USA, New York, Karner, on leaves of Vagnera stellata, April, C.H. Peck, (DAOM 4710, holotype of G. smilacinae); Georgia, Milledgeville, on fallen leaves of Bumelia lycioides, 20 March 1939, George E. Thompson & Julian H. Miller, Julian H. Miller Mycological Herbarium, Georgia Museum of Natural History, University of Georgia (GA 8357, holotype of G. bumelliae, NY, isotype of G. bumelliae). Pakistan, Lahore, Botanical garden on leaves of Heptapleurum venulosum, 25 July 1960, S. Ahmed (IMI 83132, holotype of G. foeniculata). Puerto Rico, on leaves of Piper sp., 22 September 1913, F.L. Stevens

 Table 1 Synopsis of species synonymized under Phyllosticta capitalensis

Species	Host plants (and mode of life)	Ascomata (μm)	Peridium (μm)	Ascospore shape*	Asci (μm)	Ascospores (μm)
Guignardia	Aleurites fordii	93–125 × 100–125	13–25	A	31–50 ×	11–13 ×
aleuritis	(dead leaves)				8–13	3–4
G. allamandae	Allamanda cathartica (leaves pathogen)	90–150 × 94–100	12.5–15	A	41–67.5 × 12.5–15	15–20 × 6.6
G. bumeliae	Bumelia lycioides (dead leaves)	70–100 × 70–90	20–25	В	44–76 × 10–24	15–19 × 7–8
G. cephalotaxi	Cephalotaxi drupaceae (living leaves)	70–160 × 100–160	17–20	C to B	45–80 × 13–17	11–18 × 5–9
G. concinna	Cordia verruginae (living leaves)	70–100 × 94–100	12.5–15	С	50–80 × 18–24	14–20 × 7.5–10
G. endophyllicola	Rhododendron pulchrum (healthy leaves)	118–156 × 8–139	23–39	A	38–54 × 14–15	9–16 × 4–5
G. eucalyptorum	Eucalyptus dives (weak leaf pathogen)	$60-175 \times 58-213$	25–30	A	45–112 × 11–17	11–16 × 3–6
G. festiva	Sumbaviopsis albicans (living leaves)	150–200 × 170–200	15–20	C to B	35–75 × 15–17	13–18 × 6–8
G. foeniculata	Heptapleurum venulosum (living leaves)	$80-130 \times 70-120$	15–20	A	50–94 × 8–20	11–16 × 4–6
G. graminea	Atropis distans (dead leaves)	88–138 × 88–138	19–25	В	33–60 × 6–15	9–15 × 3–6
G. helicteres	Helicteres sp. (living leaves)	70–100 × 94–100	12.5–15	В	50–94 × 8–20	14–18 × 5–9
G. pipericola	Piper sp. (dead leaves)	$100-150 \times 150-200$	15–20	A	73–95 × 16–18	15–20 × 6–13
G. smilacinae	Vagnera stellata (dead leaves)	150–200 × 88–100	30–55	В	39–75 × 10–16	10–19 × 5–13
G. smilacis	Smilax aspera (dead leaves)	56–94 × 38–56	7.5–12.5	A	41–94 × 11–18	11–17 × 4–6
G. sterculiae	Sterculia foetidae (dead leaves)	113–162 × 108–155	13–25	С	56–106 × 19–25	15–21 × 8–13

A = ellipsoidal swollen in the middle and obtrullate, B = obtrullate, C = clavate

No. 4998 (ILL 9747, isotype of *G. pipericola*); ibid., Barcelona, on leaves of *Helicteres jamaicensis*, 10 August 1915, F.L. Stevens, Herbarium C.E. Chardon No. 1366, University Illinios Herbarum 9260 (BPI 1366, isotype of *G. helicteres*); ibid., on leaves of *Helicteres*, 7 July 1924, F.L. Stevens, Cornell University, Insular Experiment Station (NY 2615, holotype of *G. helicteres*). Philippines, Palawan Island, Palawan Province, Brooks Point (Addison Peak), on living leaves of *Sumbaviopsis albicans*, February 1911, A.D.E. Palmer, Philippine Island Plants No. 12637 (F 10766, from the holotype of *G. festiva*, BPI 598372, in poor condition).

Phyllosticta capitalensis is described here based on the holotype specimen of G. aleuritis from dead leaves of Aleurites fordii, and other collections, viz. G. allamandae from Allamanda cathartica, G. endophyllicola from healthy leaves of Rhododendron sp., G. eucalyptorum as a weak pathogen on leaves of Eucalyptus dives, G. graminea from dead leaves of Atropis distans, G. smilacinae from dead leaves of Vagnera stellata, G. smilacis from dead leaves of Smilax aspera, and other species are synonymized under P. capitalensis as ascospores are ellipsoidal in shape and swollen in the middle.

A synopsis of the synonyms is given in Table 1. Five authentic specimens Guignardia aleuritis (= "Melanops aleuritica" (sic) Vassil.) have been examined and none of these handwritten labels read epithet as "aleuritidis" and designated as "sp. nov.". Nevertheless, curator of LE (Dr Vadim Melnik, pers. comm.) is convinced that this material is what Vassiljevsky based his description of the new taxon (Vassiljevsky 1940). We designate the best of these specimens, LE 34833 as the lectotype to represent the taxon. Fresh collections from Thailand also have shown that capitalensis is generally found Euphorbiaceae.

Phyllosticta capitalensis occurs on many tropical plants as an endophyte (Bayen et al 2002, Glienke et al. 2011, Motohashi et al. 2011, Okane et al. 1998, 2001, 2003, Photita et al 2001, Wikee et al. 2011, 2013, Wulandari et al. 2011), as a weak pathogen (Crous et al. 1993, Glienke et al. 2011, Thongkantha et al. 2008) or as a saprobe following senescence of

host tissue as in *Ficus* leaves (Wang et al. 2008), *Castanopsis acuminatissima*, *Smilax aspera* (Roy 1967) and *Aleurites fordii* (Vassiljevsky 1940).

There have been several molecular studies on *Phyllosticta capitalensis* (Baayen et al. 2002, Everett & Rees-George 2006, Glienke et al. 2011, Motohashi et al. 2008a,b, 2009, 2010, Pandey et al. 2004, Peres et al. 2007, Okane et al. 2001, 2003, Rodrigues et al. 2004, Sultan et al. 2011, Wang et al. 2012, Wickert et al. 2012, Wikee et al. 2011, 2013, Wong et al. 2012, Wulandari et al. 2009, 2012), the latest being by Wikee et al. (2013) which all show the wide host range of this endophytic species and its synonyms.



Fig. 3 *Phyllosticta capitalensis* (PREM 51291, holotype of *Guignardia eucalyptorum*) on CLA media. a. Ascomata (arrowed). b. Ascomata on weed tissue in the media. c–d. Peridium comprising one strata of 2–3 layers of cells *textura angularis* without thickened brown cells. e–g. Asci. h–j. Ascospores – Scale bars: a–b = 3 mm, c–d = 10 μm, e–g = 15 μm, h–j = 5 μm.

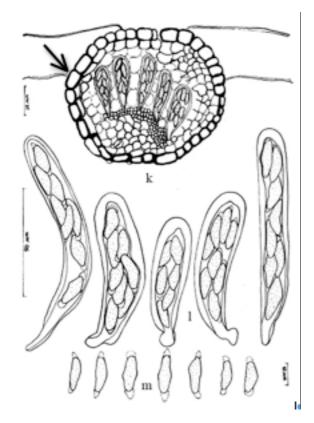


Fig. 4 *Phyllosticta capitalensis* (PREM 51291, holotype of *Guignardia eucalyptorum*) line drawing. k. Section of ascoma (arkened area fungal cells in arrowed region). l. Asci. m. Ascospores – Scale bars: $k = 25 \mu m$, $l = 50 \mu m$, $m = 10 \mu m$.

Phyllosticta cavendishii M.H. Wong & Crous, Fungal Diversity 56: 177 (2012)

Host - Musa sp.

Wong et al. (2011) stated that the morphology of *P. cavendishii* is different from *P. musarum*, *P. stevenshii*, *P. maculata* and *P. capitalensis* also occurring on Musaceae. Conidia of *P. cavendishii* are smaller (12–)13–16(–17) µm × 8–9(–10) µm than *P. maculata* (15–)16–19(–21) µm × (9–)10–12(–13) µm. Further, a maximum parsimonious tree showed *P. cavendishii* in a separate clade from other *Phyllosticta* species with 88% bootstrap support (Wong et al. 2011). *P. cavendishii* occurs on *Musa* cv. Cavendish and other cultivars.

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

Host – Citrus maxima (Rutaceae) Four species of Phyllosticta occur on Citrus spp.: P. citricarpa, P. citriasiana, P. citrichinaensis and P. capitalensis (Kiely 1948, Wulandari et al. 2009, Zhang et al. 2011). P. citriasiana differs from these other species in morphology and phylogeny. P. citriasiana found in Thailand occurs on Citrus maxima. Phyllosticta citricarpa forms a yellow zone in oatmeal agar whereas P. citriasiana does not. The conidia of *P. citriasiana* are larger (10– $12-14(-16) \mu m \times (5-)6-7(-8) \mu m$ than those of P. citricarpa (9–10 \times 6–7 µm). Although the species is pathogenic to Citrus maxima, this has not been included in EPPO most wanted list (EPPO/CABI Phylogenetically, this species clusters in a separate clade to other *Phyllosticta* species with 93% bootstrap support (Wulandari et al. 2009).

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

Host − *Citrus* sp. (Rutaceae)

P. citribraziliensis occurs on healthy leaves of *Citrus* sp. in Brazil (Glienke et al. 2011). This species is distinct in having larger conidia (8–)10–12(–13) μ m × 6–7(–8) μ m than the type of *P. spinarum* (8–)9.8(–12) μ m × (6–)6.6(–7) μ m. Phyllogenetically, this species forms a separate clade from *P. spinarum* with 68% bootstrap support (Glienke et al. 2011).

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

≡ *Phoma citricarpa* McAlpine, Fungus Diseases of Citrus trees in Australia: 21 (1899)

= Guignardia citricarpa Kiely, Proc. Linn. Soc. N.S.W. 73: 259 (1948)

Host – *Citrus sinensis* (Rutaceae)

So known from far Australia. morphologically and phylogenetically citricarpa is different from P. citribraziliensis also described from Citrus (Glienke et al. 2011). Unfortunately, the culture that we examined was in poor condition. P. citricarpa has larger conidia than P. capitalensis, $9-10 \times$ 6–7 µm vs. 8–10 × 4–5 µm. P. citricarpa forms a yellow hallow in oatmeal agar medium. P. citricarpa is closely related to P. citriasiana with 93% bootstrap support. P. citricarpa is an important quarantine plant pathogen (EPPO/CABI 1997).

Phyllosticta citrichinaensis X.H. Wang, K.D. Hyde & H.Y. Li, Fungal Diversity 52: 215 (2011)

Host – *Citrus maxima* (Rutaceae)

Wang et al. (2011) stated that P. citrichinaensis differs from other Phyllosticta species described from Citrus spp. based on conidia dimension, cultural characteristics and phylogeny. P. citrichinaensis (8–12 × 6–9 µm) has larger conidia than P. citribraziliensis (8–12 × 6–7 µm). P. citrichinaesis has a paler appearance in culture as compared to P. citriasiana. The maximum growth temperature of P. citrichinaensis (30–35°C) is somewhat intermediate between P. citricarpa (30–36°C) and P. citriasiana (30–33°C). The phylogenetic tree for combined three genes (ITS, actin and TEF1) showed a distinctive clade from P. citribraziliensis with 68% bootstrap support.

Phyllosticta clematidis (W.H. Hsieh, Chi Y. Chen & Sivan.) Wulandari, **comb. nov.** Fig. 5 MycoBank: MB 805538

= Guignardia clematidis W.H. Hsieh, Chi Y. Chen & Sivan., Mycol. Res. 101: 901 (1997) MycoBank: MB 437714

Pathogen causing discoloration on tip of the leaf and the leaf becomes tan coloured: the area of infection wider in the vein and in the middle of leaf; paler coloured region and around the infection with numerous ascomata. Ascomata 160-180 μm diam., 130-140 μm high, superficial on the leaves, black, globose to subglobose, sometimes immersed in plant tissues, coriaceous, solitary to clustered, ostiole inconspicuous. Peridium 15-20 µm wide, comprising one strata of 2-3 layers of cells textura angularis with thickened brown walls. Pseudoparaphyses not observed. Asci 60–112 × 19–29 µm ($\bar{x} = 91 \times 25 \text{ µm}, n = 20$), 8-spored, bitunicate, ocular chamber 1–5 µm high, broadly cylindrical to cylindro-clavate, rounded at the apex, where the diam. is 12.5-22.5 μ m, tapering gradually to a 4–6 \times 2.5– 37.5 µm long pedicel attached to the basal peridium. Ascospores $12.5-25 \times 5-11 \, \mu m \, (\overline{x} =$ $19 \times 8 \mu m$, n = 20), biseriate or occasionally overlapping triseriate, obtrullate, hyaline to greenish, 1-celled, coarse-guttulate, smoothwalled, without a mucilaginous sheath.

Host – Causing leaf spot of *Clematis* hayatai (Ranunculaceae)

Known distribution – Taiwan.

Material examined – Taiwan, Piluchi, on leaves of *Clematis hayatai*, 28 April 1995, W.H. Hsieh (IMI 369599, holotype)

The main character that distinguishes this species from all other *Phyllosticta* sexual states is the ascospore appendage (Fig. 6, arrowed). The ascospores inside the asci are diamond shaped and when released they are broadly fusiform. *P. clematidis* has larger conidia $(12.5-25 \times 5-11 \ \mu m)$ than *P. capitalensis* $(15-17 \times 5-6 \ \mu m)$ (Glienke et al. 2011).

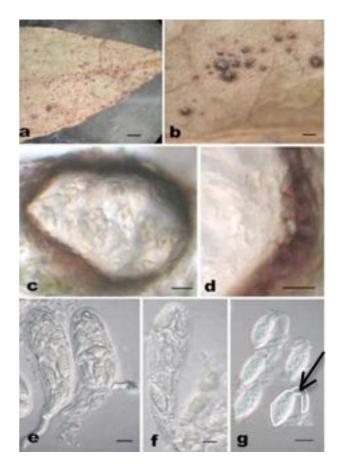


Fig. 5 – *Phyllosticta clematidis* (IMI 369599, holotype) a. Leaf spot. b. Ascomata on the host surface. c–d. Peridium comprising one strata of 2–3 layers of cells *textura angularis* with thickened brown walls. e–f. Asci. g. Ascospores with an appendage (arrowed). – Scale bars a = 3 mm, b = 100 μ m, c–d = 16 μ m, e–f = 29 μ m, g = 11 μ m.

Phyllosticta cruenta (Fr.) J. Kickx f., Mém. Acad. R. Sci. Lett. Arts. Brux. 23: 22 (1849)

Fig. 6

≡ *Sphaeria cruenta* Fr., Syst. Mycol. 2: 581 (1823)

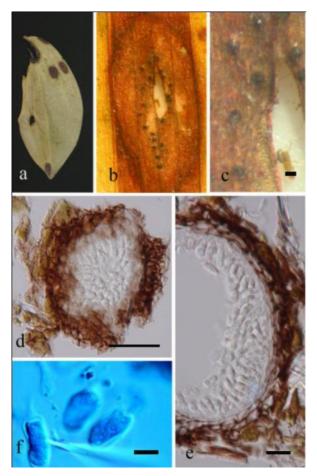


Fig. 6 – *Phyllosticta cruenta* (MA 8727, holotype). a–b. Leaf spot. c. Pycnidia. d. Peridium comprising one strata of 2–3 layers of cells *textura angularis* with thick brown walls. e. Conidiogenous cells. f. Conidia. – Scale bars – b = 200 μ m, c = 100 μ m, d = 30 μ m, e = 15 μ m, f = 6 μ m.

= *Guignardia reticulata* (DC.) Aa, Stud. Mycol. 5: 23 (1973)

= *Phyllosticta convallariae* Pers., Traité sur les Champignons Comestibles (Paris): 148 (1819); inval. name (Art. 32. 1)

Pycnidia 143–170 μ m diam., 88–136 μ m high, on the surface of the leaf, black, globose to subglobose, partly immersed in leaf tissues, coriaceous, solitary to clustered, ostiolate, with ostioles as black dots in the centre, often growing together with ascomata. Peridium 11–23 μ m wide composed of 2–3 layers of cells *textura angularis* pigmented in the outmost layers around ostiole, with inner wall layer cells paler. Conidiogenous cells 6–14 \times 2–3 μ m ($\bar{x}=9\times2~\mu$ m, n = 20), holoblastic, determinate, discrete, rarely integrated, hyaline, cylindrical to doliiform,

arising from the cells lining the pycnidial locule. Conidia $10\text{--}15 \times 4\text{--}6~\mu\text{m}$ ($\overline{x}=12\times 5~\mu\text{m}$, n = 20), hyaline to greenish, 1-celled, coarse-guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by 1–2 μm thick mucilaginous sheath which persists at maturity and in some conidia with a single, 2–6 μm long, hyaline, curved or straight apical appendage.

Host – Causing leaf spot of *Polygonatum multiflorums* (Liliaceae).

Known distribution – Romania.

Material examined – Romania, Caraş-Severin, on leaves of *Polygonatum multiflorum*, 8 June 1976, G. Negrean, Herbarium Mycologicum Romanicum No. 2728, Herbarium Horticulture Botany Matrit (MA 8727, holotype).

The conidial dimensions of the holotype ($10-15 \times 4-6 \mu m$) differ from that of CBS 858.71 ($12-21 \times 5-10 \mu m$) examined by van der Aa. van der Aa (1973) described two species occurring on *Polygonatum* spp., i.e *P. subeffusa* and *P. cruenta*.

Phyllosticta cussoniae Cejp, Bothalia 10: 342 (1971) Fig. 7

= Guignardia cussoniae Crous, South Africa J. Bot. 59: 606 (1993)

Pycnidia 160-180 µm diameter, 100-160 µm high, on the surface of the leaf, black, globose to subglobose, partly immersed in leaf tissues, coriaceous, solitary to clustered, ostiolate, with ostioles as black dots in the centre, often growing together with ascomata. Peridium 15–31 µm wide composed of two to three layers of cells, arranged in a textura angularis and pigmented in the outer layers around ostiole, paler inner layer. Conidiogenous cells $6-14 \times 2-3 \mu \text{m}$ ($\overline{x} = 9 \times 2$ μ m, n = 20), holoblastic, determinate, discrete, rarely integrated, hyaline, cylindrical to doliiform, arising from the cells lining the pycnidial locule. Conidia 8–15 \times 4–6 µm (\bar{x} = 11×5 µm, n = 20), hyaline to greenish, 1celled. coarse-guttulate, smooth-walled, globose, ellipsoidal, clavate or obclavate, with an obtuse apex, sometimes truncate at the base, surrounded by 2–5 μ m ($\bar{x} = 3 \mu$ m, n = 20) thick mucilaginous sheath which persists at maturity and in some conidia with a single, 612 μ m ($\bar{x} = 10 \mu$ m, n = 20) long, hyaline, curved or straight apical appendage.

Host – Causing leaf spot of *Cussonia umbellifera* (Araliaceae).

Known distribution – South Africa.

Material examined – South Africa, Eastern Transvaal, Schagen, Nelspruit district, on living leaf of *Cussonia umbellifera*, 25 December 1933, L.C.C. Liebenberg (PREM32821, holotype).

Conidia of *P. cussoniae* (8–15 \times 4–6 μ m) are smaller than those of *P. capitalensis* (8–15(–18) \times 6–10 μ m). Phylogenetically, *P. cussoniae* segregates into a distinct clade from other species of *Phyllosticta*, such as *P. bifrenariae*, *P. citricarpa*, *P. citriasiana*, *P. citribraziliensis*, *P. spinarum* and *P. hypoglossi* with 98% bootstrap support (Glienke et al. 2011).



Fig. 7 – *Phyllosticta cussoniae* (PREM 32821, holotype). a, b. Pycnidia. c–d. Peridium comprising one strata of 2–3 layers of cells *textura angularis* with an apex of thickened brown walls. e. Conidiogenous cells. f–g.

Conidia. – Scale bars $b = 200 \mu m$, $c = 100 \mu m$, $d = 30 \mu m$, $e-f = 15 \mu m$, $f = 6 \mu m$.

Phyllosticta ericarum Crous, Persoonia 28: 212 (2011)

Host – *Erica gracilis* (Ericaceae)

Phyllosticta ericae, P. ericarum and P. gaultheriae were described from Ericaceae (van der Aa 1973, van der Aa & Vanev 2002, Crous 2011). P. ericae is identical to P. pyrolae based on morphological characters. P. pyrolae is similar to P. ericae described from Erica carnea (Ericaceae). Okane et al. (2001) showed that P. pyrolae is distinct from 14 strains from Ericaceae based on ITS sequence data. Conidia of *P. ericarum* ((10–)11–12(–14) \times (5–)6–7 µm)) are slightly longer than those of P. pyrolae $(4.5-7.5 \times 4-9 \mu m)$. This species also has smaller conidia than P. capitalensis. Blast results data revealed that P. ericarum is closely related to Guignardia philoprina in ITS sequence data and P. hymenocallidicola in LSU sequence data (Crous et al. 2012).

Phyllosticta garciniae (I. Hino & Katum.) Motohashi, Tak. Kobay. & Yas. Ono, Mycoscience 51: 94 (2010) Fig. 8

= Guignardia garciniae I. Hino & Katum., Bull. Fac. Agric. Yamaguti Univ. 16: 607

Ascomata 175–250 µm diam., 250–375 μm high, on the upper surface of the leaves, black, slightly brown to black, globose to subglobose, semi-immersed to semi immersed in leaf tissues. Peridium 55–125 µm wide, one strata of 2-4 layers of cells textura angularis with thickened brown walls around ostiole. Pseudoparaphyses not observed. Asci 50–95 × $16-21 \mu m (\bar{x} = 16 \times 21 \mu m, n = 20), 8$ -spored, bitunicate, cylindrical to cylindro clavate, rounded at the apex, where the diam. is 10-16 μm , tapering gradually to a 6–19 \times 6–8 μm long pedicel attached to the basal peridium. Ascospores $13-21 \times 6-10 \mu m$ ($\bar{x} = 17 \times 8 \mu m$, n = 20), uniseriate to biseriate, ellipsoidal, widest 2/5 near the apex (obtrullate), hyaline to greenish, 1-celled, coarse-guttulate, smoothwalled, with mucilaginous sheath at one end.

Host – On living leaves of *Garcinia* spicata (Clusiaceae).

Known distribution – Japan.

Material examined – Japan, Ryukyu Island on leaves of *Garcinia spicata*, 24 June 1961, Hino & Katumoto, Herbarium laboratory of Plant Pathology Faculty of Agriculture Yamaguti University Number 21744, Japan (TFM 21744, holotype of *Guignardia garciniae*).

P. garciniae is distinct from other Phyllosticta species in that ascomata are black with beak whitest on the top, a peridium more than 50 μm thick, and a polar pad at one side of the ascospores. Motohashi et al. (2010) found the asexual stages of this fungus. This is a good species in the genus because of its unique ascomata, peridium and ascospore features.

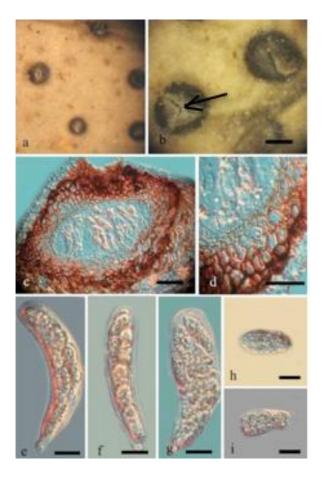


Fig. 8 – *Phyllosticta garciniae* (TFM 21744, from the holotype of *Guignardia garciniae*). a–b. Appearance of ascomata on the host surface with withest on the top (arrowed). c–d. Peridium comprising one strata of 2–3 cells *textura angularis* with thickened brown walls. e–g. Asci. h–i. Ascospores with mucilaginous sheath (arrowed). – Scale bars b = 250 μm, c, e–i = $10 \mu m$, d = $20 \mu m$.

Phyllosticta gaultheriae Aa, Stud. Mycol. 5: 56 (1973)

= Guignardia gaultheriae Aa, Stud. Mycol. 5: 56 (1973)

Host – Gaultheria procumbens (Ericaceae)

van der Aa (1973) stated that P. gaultheriae is comparable to P. pyrolae in regard to conidial size (4–9 × 4–7 μ m in P. gaultheriae and 4.5–7.5 × 4–9 μ m in P. pyrolae). They differ only in apical appendage that reduces into an inconspicuous sheath. Su & Cai (2012) also mentioned the variable and distinct conidial dimension of P. gaultheriae and P. ilicis-aquifolii. Phylogenetically P. gaultheriae is distinct from P. bidwellii (CBS 447.70 strain) and P. ilicis-aquifolii (Sultan et al. 2011, Su & Cai 2012, Zhang et al. 2013b).

Phyllosticta hostae Y.Y. Su & L. Cai, Persoonia 28: 79 (2012)

Host – *Hosta plantaginia* (Liliaceae)

P. hostae is the only *Phyllosticta* species on *Hosta plantaginia*. Conidia of *P. hostae* (8–15(–18) × 6–10 μm) are smaller than those of *P. hypoglossi* (8–15 × 5–9 μm). Phylogenetic analyses by maximum parsimony tree and combined four genes (ITS, EF, actin and GPDH) showed that this species is distinct and in a separate subclade from *P. cussoniae*, *P. hypoglossi*, *P. citribraziliensis* and *P. spinarum* with 99% bootstrap value (Su & Cai 2012).

Phyllosticta hubeiensis K. Zhang, Y.Y. Su & L. Cai, Mycol. Progress 12 (On Line article) (2013)

Host – *Viburnum odoratissimum* (Adoxaceae)

van der Aa (1973) stated that the conidial dimension of P. hubeiensis (10–14.5 × 6–9 µm) is smaller than P. concentrica (8–20 × 4–14 or 10–15 × 7–10 µm). The conidiogenous cells and spermatia also differ in size (van der Aa 1973). This species is special in that its conidia fail to detach from the conidiogenous cells. Phylogenetically P. hubeiensis is closely related to P. concentrica (previously described as the asexual state of Guignardia philoprina) and P. gaultheriae in ITS sequences data (Zhang et al. 2013a).

Phyllosticta hymenocallidicola Crous, Summerell & Romberg, Persoonia 27: 139 (2011)

Host – *Hymenocallis littoralis* (Amaryllidaceae)

P. hymenocallidicola is the only species that occurs on Hymenocallis littoralis as P. hymenocallidis was found to be Phoma narcissi (van der Aa 1973). Conidia of P. hymenocallidicola $[(8-)9-10(-11)\times(6-)6.5-7$ µm] are smaller than those of P. capitalensis $[8-15(-18)\times6-10$ µm)]. P. hymenocallidicola is phylogenetically distinct from other Phyllosticta species that have been submitted to GenBank (Crous et al. 2011, Glienke et al. 2011). Crous et al. (2011) showed that P. hymenocallidicola is close to P. owaniana based on mega BLAST using ITS sequences.

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

- = *Macrophoma hypoglossi* (Mont.) Berl. & Voglino, Atti Soc. Veneto-Trent. Sci. Nat. 10(1): 192 (1886)
- = *Phoma hypoglossi* (Mont.) Sacc., Syll. Fung. 3: 162 (1884)
- = *Phyllostictina hypoglossi* (Mont.) Petr. & Syd., Beih. Reprium nov. Spec. Regni veg.: 203 (1927)
- ≡ *Sphaeropsis hypoglossi* Mont., Annls Sci. Nat., Bot., sér. 3, 12: 307 (1849)

Host – *Ruscus hypoglossum* (Liliaceae)

The conidial dimensions and other characters show differences from other *Phyllosticta* species occurring on Liliaceae. *P. hypoglossi* has bigger conidia and a longer appendage sheath compared to *P. yuccae*, i.e., $7.5-15.4 \times 6-9.5 \mu m$ vs. $8-15(-18) \times 6-10 \mu m$ and $4-15 \mu m$ vs. $10-35 \mu m$ for *P. hypoglossi*. This species forms a distinct clade from other *Phyllosticta* species (Glienke et al. 2011, Su & Cai 2012).

Phyllosticta ilicis-aquifolii Y.Y. Su & L. Cai, Persoonia 28: 81 (2012)

Host – *Ilex aquifolium* (Aquifoliaceae)

P. ilicis-aquifolii is characterized by its large conidia and long mucoid sheath or appendage. The conidial dimensions of *P. ilicis-aquifolii* (4–9 × 4–7 μ m) are smaller than *P. gaultheriae* (10–18 × 6–9 μ m). The

spermatial state and conidiogenous cells also differ in shape and dimensions. *P. ilicis-aquifolia* has grey to leaden grey growth in culture with an irregular margin, whereas *P. gaultheriae* has greenish to black cultures with a lobed margin (van der Aa 1973). *P. ilicis-aquifolii* is phylogenetically close to *P. gaultheriae* based on ITS sequences with 94% bootstrap support. Phylogenetic tree generated from DNA combined sequences alignment showed that this species is closely related to *P. gaultheriae* and *P. yuccae* with 100% bootstrap support (Su & Cai 2012).

Phyllosticta korthalsellae A. Sultan, P.R. Johnst., D.C. Park & A.W. Robertson, Stud. Mycol. 68: 241 (2011)

Host – *Korthalsella lindsayi* (Viscaceae)

This is first report of a *Phyllosticta* from *Korthalsella* from New Zealand. Based on ITS sequence data, it was inferred that *P. korthalsella* is related to *P. eugeniae* and *Guignardia bidwellii* with 76% bootstrap support (Sultan et al. 2011).

Phyllosticta maculata Wong & Crous, Fungal Diversity 56: 180 (2012) Fig. 9

= Guignardia musae Racib., Bull. Int. Acad. Sci. Lett. Cracovie, Cl. sci.math. nat. Sér. B, sci. nat. 3: 388 (1909)

Ascomata 100–125 µm high, 75–150 um diam., on upper and lower surface of leaf and on banana fruit skin, globose to subglobose, black, semi-immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, with ostiole as black centrally located dots. Peridium 12.5–20 um wide, upper part composed of compressed brownish, thinwalled cells, 1–4 cells thick, lower part hyaline, composed of flattened, dark brown cells. darkest around the ostiole. Asci 49–105 \times 16– 28 µm ($\bar{x} = 74 \times 21$ µm, n = 20), 8-spored, bitunicate, broadly cylindro-clavate, rounded at the apex, where the diam. is 8–21 µm, tapering gradually to a 5–10 \times 5–10 μ m long pedicel attached to the basal peridium, ocular chamber 3–8 µm high. Ascospores $20–25 \times 8–13$ µm (\bar{x} = $22 \times 10 \mu m$, n = 20), uniseriate or occasionally overlapping biseriate, clavate to oblong, hyaline to greenish, 1-celled, coarseguttulate, smooth-walled, without

mucilaginous appendages.

Host – on living leaves causing freckle leaves of *Musa* spp. (Musaceae)

Known distribution – American Samoa, Australia, Indonesia, Malaysia, Papua New Guinea.

Material examined – Indonesia, Bogor, on leaves of *Musa acuminata*, Raciborski, (KRA 063561, from holotype of *Guignardia musae*).

This species was recently reidentified by Wong et al. (2012). The new name "maculata" was derived from the commonly caused freckle diseases worldwide that occurs on *Musa* spp. Four other species of *Phyllosticta* are known on Musaceae, *P. musae* Racib. (Raciborski 1909), *P. musarum*, *P. capitalensis* and *P. cavendishii* (Wong et al. 2012). Phylogenetically, *P. maculata* forms a distinct clade, from other *Phyllosticta* occuring on Musaceae, and is close to *P. vaccinii* and *P. cavendishii*, with 73% based on LSU sequence and 88% on ITS sequence bootstrap support (Wong et al. 2012).

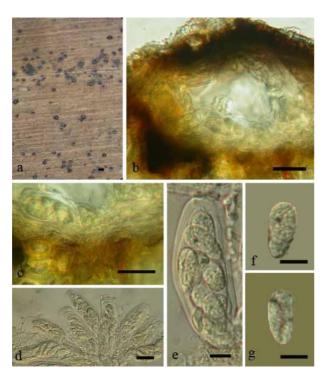


Fig. 9 – *Phyllosticta maculata* (KRA 063561, from holotype of *Guignardia musae*) a. Ascomata on the host surface. b–c. Peridium comprising sterile tissue of plant cells. d–e. Asci. f–g. Ascospores. – Scale bars: $a=100 \mu m$, $b-c=20 \mu m$, $d=30 \mu m$, $e-g=10 \mu m$.

Phyllosticta morindae (Petr. & Syd.) Aa, Stud. Mycol. 5: 69 (1973)

- = *Guignardia morindae* (Koord.) Aa, Stud. Mycol. 5: 69 (1973)
- = *Phyllostictina morindae* Petr. & Syd., Feddes Repert., Beih. 42: 200 (1927)
- = *Physalospora morindae* Koord., Verh. K. Akad. Wet., tweede sect. 13(4): 190 (1907)

Host – *Morinda citrifolia* (Rubiaceae)

The type is not available in BO (Mien A. Rifa'i pers. comm.). *Phyllosticta morindae* is distinct from other species in having smaller conidia than *P. capitalensis* and *P. maculata*.

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

- = *Macrophoma musae* (Sacc.) Berl. & Voglino, Atti Soc. Veneto-Trent. Sci. Nat. 10(1): 187 (1886)
- = *Phoma musae* Sacc., Syll. Fung. 3: 163 (1884)
- = *Sphaeropsis musarum* Cooke, Grevillea 8: 93 (1880)

Host - Musa sp. (Musaceae)

 $P.\ musarum$ is morphologically distinct from other species of Phyllosticta occurring on banana (Musaceae). It occurs only on leaves of banana types AAB and ABB. $P.\ musarum$ differs from $P.\ cavendishii$ by its conidial dimensions and phylogenetic affinities. $P.\ musarum$ has bigger conidia $[(12-)13-16(-20)\times (7-)9-10(-11)\ \mu m]$ than $P.\ cavendishii$, $[(12-)13-16(-17)\ \times\ 8-9(-10)\ \mu m]$. Phylogenetic data showed that $P.\ musarum$ is closely related to $P.\ cavendhishii$ with 63% bootstrap support.

Phyllosticta muscadinii (Luttr.) Wulandari, **comb. nov. et stat. nov.** Fig. 10–11 MycoBank: MB 805539

= Guignardia bidwellii f. musacadinii Luttr., Phytopathology 36: 913 (1946) MycoBank: MB 346168

Pathogenic, leaf spots, irregular to regular with a red brown border and cream centre with numerous ascomata and pycnidia. Ascomata visible on the surface of the leaf as black dot, $70-100 \mu m$ diam., $94-100 \mu m$ high, black, globose to subglobose, immersed in plant tissue, coriaceous, solitary to clustered,

ostiolate, with ostioles as black dots in the centre. Peridium 12.5–15 µm wide, comprising one strata of 2-6 layers of cells textura angularis with thickened brown walls. Pseudoparaphyses tubular. Asci $50-80 \times 18-35$ $\mu m \ (\bar{x} = 77 \times 15 \ \mu m, \ n = 20), \ 8$ -spored, bitunicate, with an ocular chamber 1–2 μm high, broadly cylindrical to cylindric-clavate, rounded at the apex, where the diam. is 13-18 μ m, tapering gradually to a 7.5–16 \times 2–6 μ m very long pedicel attached to the basal peridium. Ascospores $14-20 \times 7.5-10 \, \mu m$ ($\overline{x} =$ $15 \times 7 \mu m$, n = 20), biseriate or occasionally overlapping uniseriate, ovate to oblong, hyaline to greenish, 1-celled, coarse guttulate, smoothwalled, without a hyaline mucilaginous sheath at the base. Pycnidia 50–100 µm diam., 70–100 um high, black, globose to subglobose, immersed in plant tissues, coriaceous, solitary to clustered, ostiolate, ostioles as a black dots in the centre. Peridium 10–15 µm wide, comprising one strata of 1-2 layers of cells textura angularis with thickened brown walls. Conidiogenous cells short cylindrical 16-41 × 11–15 µm ($\bar{x} = 24 \times 13$ µm, n = 20). Conidia $7-10 \times 4-7 \; \mu \text{m} \; (\bar{x} = 8 \times 6 \; \mu \text{m}, \; n = 20), \; \text{oblong},$ 1-celled, containing coarse guttules, smoothwalled, 1-2 µm wide mucilaginous sheath and 3–6 (4) µm long appendage.

Host – Causing leaf spots of *Muscadinia rotundifolia* (Vitaceae)

Known distribution – USA.

Material examined – USA, on overwintered leaves of *Muscadinia rotundifolia*, Experiment Spalding Co., 15 April 1945, E.S. Luttrell, Georgia Cryptogams (BPI 598288, isotype).

Phylogenetically there is a species complex of *Phyllosticta* in Vitaceae. Zhang et al. (2013b) mentioned that *P. ampelicida*, *P. bidwellii* and *P. parthenocissi* form three distinct clades. This finding is similar to Witch et al. (2012) who also showed that more than two species occur on Vitaceae (Witch et al. 2012, Zhang et al. 2013b). *P. muscadinii* is a good species based on pathogenicity tests (Luttrell 1946). Furthermore *P. muscadinii* has smaller conidia than *P. ampelicida* and *P. parthenocissi*.

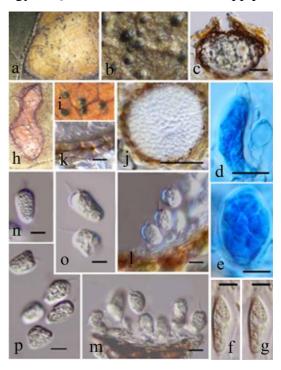


Fig. 10 – *Phyllosticta muscadinii* (BPI 598288, isotype). a, h. Leaf spot. b, i. Ascomata on the host surface of fruits. c–j. Peridium comprising one strata of 2–4 layers of cells of *textura angularis* with thickened brown walls. d–e. Asci with pseudoparaphyses. f–g. Ascospores. i. Pycnidia on the host surface. j–k. Section of pycnidium. l–m. Conidiogenous cells. n–p. Conidia. – Scale bars: c = 50 μm, d–e = 25 μm, f–g = 10 μm, j = 100 μm, k–m = 15 μm, n–p = 7 μm.

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

Host – *Brabejum stellatifolium* (Proteaceae)

Phyllosticta owaniana is a distinct species and forms a separate clade in the genus (Wulandari et al. 2009, Glienke et al. 2012, Zhang et al. 2013). It differs from P. capitalensis by its shorter and wider conidia $(10-14\times7-8.5~\mu m$ for P. owaniana and $15-17\times5-6~\mu m$ for P. capitalensis) (van der Aa 1973, van der Aa & Vanev 2002, Glienke et al. 2011).

Phyllosticta partenocissi K. Zhang, N. Zhang & L. Cai, Mycologia 105(4): 1030 (2013)

Host – Parthenocissus quinquefolia (Vitaceae)

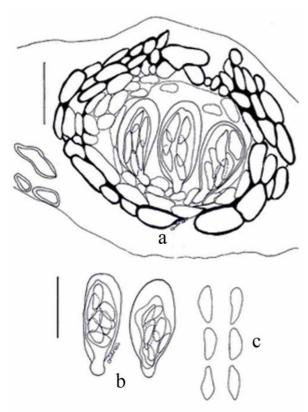


Fig. 11 – *Phyllosticta muscadinii* (BPI 598288, isotype) line drawing. a. Section of ascoma on the leaf. b. Asci. c. Ascospores. – Scale bars = $25 \mu m$.

Morphologically this fungus is distinct from other species of Phyllosticta occurring on Vitaceae. The dimensions of conidia differ from P. ampelicida (Zhang et al. 2013). Phyllosticta parthenocissi was described as a new species and a replacement name for strain CBS 111645, previously described Guignardia bidwellii. The maximum parsimony tree showed that P. parthenocissi associated with Parthenocissus is a distinct clade from that of seven strains from Japan associated with Vitis sp. with 55% bootstrap support (Zhang et al. 2013b).

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

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

= *Guignardia ilicis* (Jacz.) Schrantz, Bull. Trimmest. Soc. Mycol. Fr. 76: 327 (1961)

= Guignardia rhodorae (Cook) B.H. Davis, Mycologia 38: 48 (1946)

Host – *Taxus baccata* (Taxaceae)

This species is phylogenetically distinct from other species (Wulandari et al. 2009, Wikee et al. 2011, Sultan et al. 2011, Zhang et al. 2013b). *Phyllosticta philoprina* is distinct from *P. concentrica* (Motohashi et al. 2009), which was previously synonymised as the asexual state of *Guignardia philoprina* (van der Aa 1973).

Phyllosticta schimae Y.Y. Su & L. Cai, Persoonia 28: 80 (2012)

Host – *Schima superba* (Theaceae)

Phyllosticta schimae is distinct from *P. parthenocissi* based on morphology and phylogeny (Su & Cai 2012). *Phyllosticta schimae* has longer but narrower conidia (7–13 \times 4–7 μ m) than those of *P. parthenocissi* (7.5–10 \times 6–9 μ m). Maximum parsimony derived from four sequences (ITS, EF, GDPH and actin) showed that *P. shimae* is closely related to *P. parthenocissi* (strain CBS 111645) with 51% bootstrap support (Zhang et al. 2013b).

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

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

Host – *Juniperus* sp. (Cupressaceae)

Phyllosticta spinarum has smaller conidia lacking a mucoid sheath as compared to *P. citribraziliensis*. Phyllosticta spinarum is phylogenetically distinct from other species in maximum parsimony tree when using analysis of ITS, EF, actin and GDPH gene sequences (Glienke et al. 2011). It forms a separate clade from *P. citribraziliensis* with 68% bootstrap support (Glienke et al. 2011).

Phyllosticta styracicola K. Zhang & L. Cai, Mycological Progress 12: 551 (2013)

Host – *Styrax grandiflorus* (Styracaceae)

Phyllosticta styracicola is a distinct species with conidia $9.5-13\times6.5-9$ μm, which are smaller than *P. schimae* $(7-13\times4-7$ μm) and longer than *P. parthenocissi* $(7.5-10\times6-9$ μm). *Phyllosticta styracicola* clustered in a separate clade from *P. schimae* and *P. parthenocissi* with 87% bootstrap support (Zhang et al. 2013a).

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

- ≡ *Phyllosticta vaccinii* (Earle) Shear, Mycologia 15: 131 (1923)
- = *Phyllosticta sparsa* Bonar, Mycologia 20: 296 (1928)
- = *Phyllosticta vaccinii* Demaree & Wilcox, Phytopathology 37: 292 (1947)

Host – *Vaccinium macrocarpon* (Ericaceae)

Polymorphism exists in P. vaccinii and this species has already been epitypified by Zhang et al. (2013b). The parsimony tree presented in Zhang et al. (2013b) indicates that Guignardia vaccinii Shear is not the same biological species as Phyllosticta vaccinii as they fall into different clades in sequence analysis (Zhang et al. 2013b). Guignardia vaccinii Shear is morphologically phylogenetically distinct from Phyllosticta vaccinii, which was previously recorded as the asexual state of Guignardia vaccinii. This anamorph and teleomorph connection was proposed by Shear in 1907 (Zhang et al. 2013b). Weidemann et al. (1982) regarded P. elongata as the anamorph state of G. vaccinii, this connection need to be clarified. The clade E in the paper published by Zhang et al. (2013b), known as the ex-type of Guignardia vaccinii (CBS 126.22) forms a separate clade from ATCC 46255 epitypified as *P. vaccinii* by Zhang et al. (2013b).

Discussion

Species of Phyllosticta were named mainly on morphological characters and molecular phylogeny based on recent publications. Some species are distinguished because of their unique appendages, as in P. candeloflamma and P. clematidis. Phyllosticta garciniae has an unusual thick peridium in the ascomata. Both sexual and asexual states were present on the P. muscadinii specimen examined. The material of P. candeloflamma had only the sexual state whereas only asexual state was present on P. cussoniae. Phyllosticta capitalensis was recorded on numerous tropical plants. Guignardia mangiferae is a genetically and morphologically distinct species from previously recorded Phyllosticta species on Mangiferae indica. The name P. mangiferae has already been used and it is necessary to

choose another name as and when more isolates become available; thus the name of G. *mangiferae* is still available as a valid name (Glienke et al. 2011).

Pure cultures from single spore isolations of several *Phyllosticta* species are not available and therefore gene sequence data and analyses have not been possible for many species. It is important to recollect and restudy the taxonomy and phylogeny of important old species.

There are several problems, which inhibited taxonomic work during the course of this study, and are noted below:

- 1. Low numbers of ascomata in the voucher holotype specimens.
- 2. The low quality of holotype specimens; some specimens were in poor condition.
- 3. Need for epitypifying many species.
- 4. The need for a polyphasic approach and multi-gene phylogenies to accurately resolve species (Wang et al. 2012, Zhang et al. 2013b). A single gene that can resolve species well is needed.
- 5. Need for cultures of each species for obtaining sequences for phylogenetic analyses. Single spore isolation of many *Phyllosticta* speies is not possible (Crous et al. 2009) and attempts to isolate from ascomata or pycnidia may be necessary (Zhang et al. 2009c).

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