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FULL PAPER

Keiichi Motohashi · Junji Nishikawa · Mitsuteru Akiba
Chiharu Nakashima

Studies on the Japanese species belonging to the genus *Phyllosticta* (1)

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Abstract As the first report of monographic studies of the genus *Phyllosticta* in Japan, four new species, *Phyllosticta disanthi* on *Disanthus cercidifolius*, *P. hoveniicola* on *Hovenia dulcis*, *P. ligustricola* on *Ligustrum obtusifolium,* and *Phyllosticta alliacea* on *Allium fistulosum*, are described and illustrated. A new teleomorphic state of *P. alliorum*, *Guignardia alliacea*, is confirmed.

Key words *Guignardia alliacea · Phyllosticta alliacea · Phyllosticta disanthi · Phyllosticta hoveniicola · Phyllosticta ligustricola*

Introduction

The genus *Phyllosticta* (teleomorph: *Guignardia* Viala & Ravaz) was established by Persoon in 1818. Many species of *Phyllosticta* have been known as the causal fungi of leaf spot diseases of various plants (van der Aa and Vanev 2002). Genus *Phyllosticta* is also known as the endophytic fungi in a wide range of host plants, including Ericaceae (Baayen et al. 2002; Okane et al. 2001, 2003; Petrini 1986; Petrini et al. 1991).

Plant diseases caused by species of *Phyllosticta* are reported worldwide. According to the world list of the genus *Phyllosticta* (van der Aa and Vanev 2002) and other related literature, 238 species of *Phyllosticta* sensu lato are recorded on herbal and arboreal plants in 192 genera belonging to 84 families in Japan. Of these, 87 species of *Phyllosticta* were newly established by Japanese mycologists (Fukui 1936, 1942; Hara 1916, 1918, 1920, 1925, 1927, 1930, 1931a, 1931b, 1938, 1959; Hori 1913; Katsuki 1950a,b; Kawamura 1913; Kobayashi 1974, 1977; Kobayashi and Chiba 1961; Kobayashi and Okamoto 2003; Kobayashi and Onuki 1990; Miura 1928, 1957, 1962; Miyake 1909; Miyake and Hara 1910; Naito 1940, 1952; Sawada 1916, 1918, 1943, 1950a,b, 1958, 1959; The Phytopathological Society of Japan 2000; Togashi 1936a,b; Tsuruda 1915). Recently, some new leaf spot diseases caused by the species of *Phyllosticta* were reported in Japan on *Nandina domestica* Thunb. and *Pachysandra terminalis* Siebold & Zucc. (Takeuchi and Horie 1998). Similarly, leaf spot disease (leaf blight) of *Hamamelis japonica* Siebold & Zucc, which is suspected to be caused by a species of *Phyllosticta*, has a wide range of distribution and is rapidly spreading, causing severe damage (Kawabe 2003; Kawabe and Onozato 2004; Kawabe et al. 2002; Yoshida and Kobayashi 1999, 2000, 2001).

Saccardo (1878, 1884) revised the generic concept of *Phyllosticta*. The revision of Saccardo was based on perithecia subepidermal, lenticular, thin membranous, perforated by an ostiole, punctifom, areolate on leaf or rarely on stem, as well as small, ovoid to oblong, aseptate, hyaline to greenish, conidia. However, most subsequent investigators described the species based on the concept as "All leaf inhabiting pycnidial fungi with hyaline, one-celled conidia should be classified in *Phyllosticta*, whereas morphologically comparable fungi on stem in *Phoma*" (van der Aa and Vanev 2002). The genus concept, however, was reconstructed by van der Aa and collaborators (van der Aa 1973; van der Aa and Vanev 2002). The concept was called *Phyllosticta* sensu stricto, following "foliicolous or ramulicolous, stromatic conidiomata, conidiogenesis holoblastic, conidia one-celled (only exceptionally two-celled), hyaline, often 10–20 × 5–10 μm, globosal, roundish or ellipsoidal, usually filled with greenish guttules, surrounded by a slime layer and provided with an apical extracellular (non-cellular) appendage, conidial base usually truncate." In addition, they referred to the connection between the genus *Phyllosticta* and the teleomorph *Guignardia* Viala & Ravaz, and defined that the species of *Phyllosticta* sensu stricto was

K. Motohashi (✉) · C. Nakashima
Graduate School of Bioresources, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
Tel. +81-59-231-9638; Fax +81-59-231-9450
e-mail: 505D401@m.mie-u.ac.jp

J. Nishikawa
Kakegawa Research Center, Sakata Seed Corporation, Shizuoka, Japan

M. Akiba
Kyushu Research Center, Forestry and Forest Products Research Institute, Kumamoto, Japan

restricted to the anamorph state of the teleomorph *Guignardia*. Hence, the new genus concept of *Phyllosticta* (van der Aa 1973; van der Aa and Vanev 2002) was widely accepted among mycologists as well as phytopathologists.

However, there still is controversy for the species concept. The species of teleomorph *Guignardia* and its anamorph are host specific, with a host range confined to species of a single host genus or some allied host genera within a single family, from the results of inoculation tests (Luttrell 1946, 1948; Reusser 1964; Stewart 1916), whereas some endophytic *Phyllosticta* species have a wide host range. For example, Okane et al. (2001, 2003) isolated endophytic *Phyllosticta* cultures from 67 plant species in 54 genera of 38 families. All these cultures were identified as a single species, *Phyllosticta capitalensis* Henn., based on morphology and sequence analysis of ribosomal DNA internal transcribed spacer regions. Baayen et al. (2002) revealed that *Guignardia citricarpa* Kiely (anamorph: *P. citricarpa* (McAlpine) Aa) was different from nonpathogenic species *Guignardia mangiferae* A.J. Roy isolated from citrus, based on cultural characteristics, growth rate, and thickness of mucoid sheath surrounding the conidial wall. Also, they differed in nucleotide sequence data, although the size of their conidia was the same. On the other hand, delimitation of the species of *Phyllosticta* in modern taxonomy through adopted phylogenetic relationships is not yet available. Hence, in this study, we applied van der Aa's species concept (van der Aa 1973; van der Aa and Vanev 2002) wherein the species epithet was given on the basis of fungus morphology on diseased leaf of host plant, cultural characteristics, and connection with teleomorph species.

In this article, four new species of *Phyllosticta* and a new species of *Guignardia* with *Phyllosticta* anamorph are described.

Materials and methods

Specimens for microscopic observation were made by hand-sectioning the material and mounting the section with Shear's fluid (Chupp 1940). Monoconidial isolation was done on sugi needle decoction agar (Ito et al. 1952) with modifications [100 g needles and green shoots of sugi (*Cryptomeria japonica*) boiled for 30 min in 1000 ml tap water; the decoction was filtered and then 40 g agar was added]. For each living culture, a single germinated conidium was transferred onto oatmeal agar (Difco Oat Meal Agar; Becton, Dickinson, Sparks, MD, USA). All the dried specimens were deposited at the Herbarium of Forest Pathology, Forestry and Forest Products Research Institute (TFM: FPH), Tsukuba, Ibaraki Prefecture, Japan and Mycological Herbarium, Laboratory of Plant Pathology, Mie University (MUMH), Tsu, Mie Prefecture, Japan. Living cultures were deposited in the Microbiological Genebank, National Institute of Agrobiological Sciences (MAFF), Tsukuba, Ibaraki Prefecture, Japan or Biological Resource Center, the National Institute of Technology and Evaluation (NBRC), Kisaradzu, Chiba Prefecture, Japan.

Results and discussion

Phyllosticta disanthi Motohashi & C. Nakash., sp. nov. Fig. 1a,b

Maculae folii vivi, orbiculares, ellipticae vel irregulariter rotundatae, cinereae vel pallide brunneae, 2–10 mm diametro, marginem atro-brunneae. Pycnidia epiphylla, subglobosa vel globosa, 69–98 × 74–98 µm. Paries pycnidii ex cellulis depressus vel irregularibus 2–5-stratosis composi-

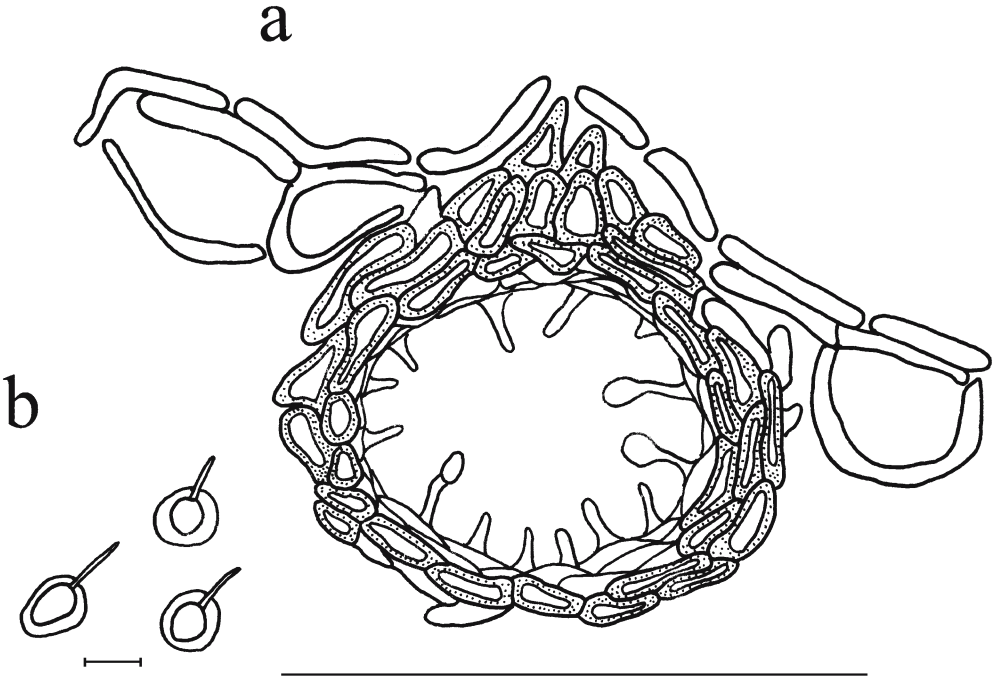

Fig. 1. *Phyllosticta disanthi* on *Disanthus cercidifolius*. **a** Pycnidium. **b** Conidia. *Bars* **a** 100 µm; **b** 10 µm

tus, brunneus vel fuscus, circa ostiolum fuscus. Cellulae conidiogenae holoblasticae, hyalinae, cylindricae, conicae vel lageniformes, 2.5–9.8 × 1.2–2.5 µm. Conidia continua, globosa, elliptica vel obovata, primo basi truncata, posterius utrinque rotundata, 6.1–12.3 × 4.9–7.4 µm, strato mucoso circumdantia, guttulas numerosas continentia, apice appendice 4.9–9.8 µm longa praedita.

Type specimens: On *Disanthus cercidifolius* Maxim., Kurokami, Kumamoto-shi, Kumamoto Prefecture, Japan, 7 October 2005, collected by K. Motohashi, C. Nakashima, and T. Akashi (holotype, TFM:FPH-7835) (isotype, MUMH 10449).

Leaf spots orbicular, ellipsoid, or irregularly rotundate, light gray to pale brown, 2–10 mm in diameter, surrounded by dark brown border. Pycnidia epiphyllous, subepidermal, subglobose to globose, 69–98 × 74–98 µm. Pycnidial wall composed of depressed or irregular cells in 2–5 layers, brown to dark brown, darker around ostiole, hyaline or paler toward the conidiogenous region. Conidiogenous cells holoblastic, hyaline, cylindrical, conical, or lageniform, 2.5–9.8 × 1.2–2.5 µm. Conidia unicellular, globose, ellipsoid, or obovoid, truncate at the base when young, later rounded at both ends, 6.1–12.3 × 4.9–7.4 µm, surrounded by a slimy layer, containing numerous minute guttules, with a slender and short apical appendage 4.9–9.8 µm long.

Host: *Disanthus cercidifolius* Maxim. (Hamamelidaceae; "Marubanoki" in Japanese).

Note: The species belonging to the genus *Phyllosticta* sensu stricto has not yet been reported on the plant genus *Disanthus* (Hamamelidaceae). Seven species of the genus *Phyllosticta* sensu lato are recorded on Hamamelidaceae: these are *P. bucklandiae* Shreem., *P. hamamelidis* Peck, *P. hamamelidis* Cooke ex G. Martin (later homonym of *P. hamamelidis* Peck), *P. hamamelidis* Miura (later homonym of *P. hamamelidis* Peck), *P. liquidambaricola* Sawada, *P. liquidambaris* C.C. Chen, and *P. liquidambaris-formosanae* J.K. Bai & G..Z. Lu (van der Aa and Vanev 2002; Bai et al. 2003). Among these, *P. hamamelidis* Peck on *Hamamelis* spp. differs from *P. disanthi* in lacking an apical appendage, although *P. hamamelidis* Peck belongs to the genus *Phyllosticta* sensu stricto because of morphology and dimensions (van der Aa 1973). *Phyllosticta liquidambaricola* on *Liquidambar formosana* Hance is also included in the *Phyllosticta* sensu stricto from its original description and figures. However, the small size of its pycnidia (65–83 × 57–65 µm) and conidia (6.5–9 × 5–6.5 µm) lacking the apical appendage are discriminately different from the presently described species. *Phyllosticta bucklandiae* was transferred to the genus *Phoma* as a synonym of *Phoma pomorum* Thüm. var. *pomorum* (van der Aa and Vanev 2002). Similarly, *P. liquidambaris-formosanae* should be excluded from *Phyllosticta* sensu stricto, because its original description and figures (conidiogenous cells lining the cavity, ampulliform, 2–5 × 1.5–2 µm; conidia ellipsoid to fusiform, unicellular, hyaline, 5–7 × 2–2.5 µm) are typical for the species of *Phoma*. According to van der Aa and Vanev (2002), *P. hamamelidis* Cooke ex G. Martin (conidia oval or cylindrical, 2–3 × 1.5 µm), *P. hamamelidis* Miura (conidia globose, about 2 µm diameter), and *P. liquidambaris* (conidia globose, elliptical, or cylindrical, 14.3–17.1 × 6.6–7.6 µm) should be excluded from the genus *Phyllosticta* sensu stricto based on their size and shape of conidia. *P. hamamelidis* Cooke ex G. Martin and *P. hamamelidis* Miura should be classified into *Asteromella* Pass. & Thüm. (spermatial state of *Mycosphaerella*) or small-spored coelomycetous genus. *Phyllosticta liquidambaris* should be classified into large-spored coelomycetous genus. Therefore, *P. disanthi* is described as a new species on *Disanthus* plants.

Phyllosticta hoveniicola Motohashi & C. Nakash., sp. nov. Fig. 2a,b

Maculae folii vivi, ellipticae vel irregulariter rotundatae, pallide brunneae vel brunneae, 5–15 mm diametro, marginem atro-brunneae. Pycnidia epiphylla, subglobosa vel globosa, 61–86 × 61–93 µm. Paries pycnidii ex cellulis depressus vel irrergularibus 1–3-stratosis compositus, brunneus vel fuscus, circa ostiolum fuscus. Cellulae conidiogenae holoblasticae, hyalinae, cylindricae vel conicae, 3.7–9.8 × 1.2–

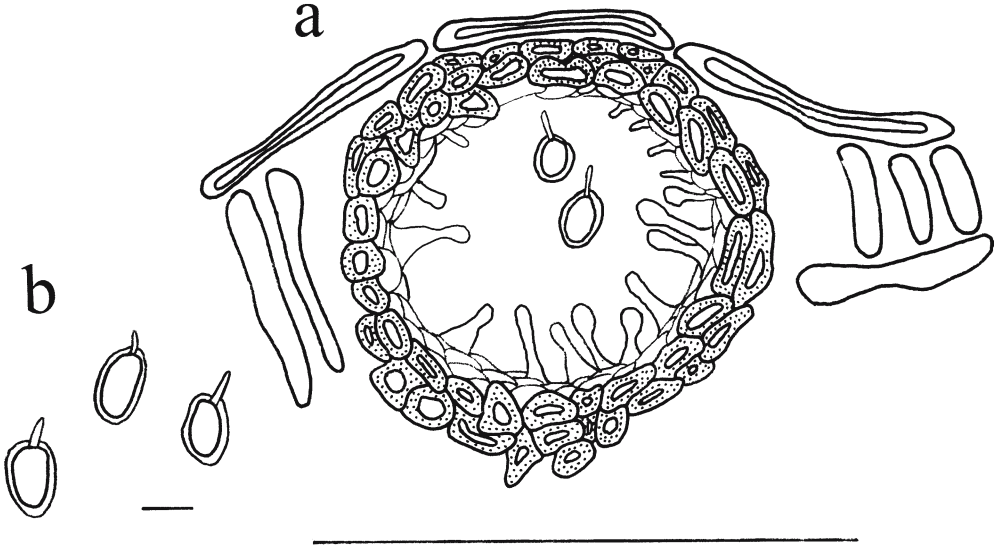

Fig. 2. *Phyllosticta hoveniicola* on *Hovenia dulcis*. **a** Pycnidium. **b** Conidia. *Bars* **a** 100 µm; **b** 10 µm

2.5 µm. Conidia continua, globosa, elliptica vel obovata, primo basi truncata, posterius utrinque rotundata, 8.6–12.3 × 6.1–8.6 µm, strato mucoso circumdantia, guttulas numerosas continentia, apice appendice 2.5–7.4 µm longa praedita.

Type specimens: On *Hovenia dulcis* Thunb., Kurokami, Kumamoto-shi, Kumamoto Prefecture, Japan, 7 October 2005, collected by K. Motohashi, C. Nakashima, and T. Akashi (holotype, TFM:FPH-7833) (isotype, MUMH 10447).

Leaf spots ellipsoid or irregularly rotundate, pale brown to brown, 5–15 mm in diameter, surrounded by a dark brown border. Pycnidia epiphyllous, subepidermal, subglobose to globose, 61–86 × 61–93 µm. Pycnidial wall composed of depressed or irregular cells in 1–3 layers, brown to dark brown, darker around ostiole, hyaline or pale, and flattened toward the conidiogenous region. Conidiogenous cells holoblastic, hyaline, cylindrical, or conical, 3.7–9.8 × 1.2–2.5 µm. Conidia unicellular, globose, ellipsoid, or obovoid, truncate at the base when young, later rounded at both ends, 8.6–12.3 × 6.1–8.6 µm, surrounded by a slime layer, containing numerous minute guttules, with slender and short apical appendage 2.5–7.4 µm long.

Host: *Hovenia dulcis* Thunb. (Rhamnaceae; "Kenponashi" in Japanese).

Note: On *Hovenia* plants, *Phyllosticta hoveniae* Gucevič had been recorded. However, it was treated as a synonym of *Phoma exigua* Desm. var. *exigua* by van der Aa et al. (2000) based on morphological characteristics of the type specimen. Our fungus, however, belongs to the genus *Phyllosticta* sensu stricto based on its stromatic conidiomata, cylindrical or conical conidiophores, conidia with slime layer and apical appendage, and holoblastic conidiogenesis. Consequently, it is described as a new species of *Phyllosticta* sensu stricto on *Hovenia* plants.

Phyllosticta ligustricola Motohashi & C. Nakash., sp. nov. Fig. 3a,b

Maculae folii vivi, orbiculares vel ellipticae, saepe concentrice zonales, cinereae vel pallide brunneae, 3–6 mm diametro, marginem atro-brunneae. Pycnidia amphigena, subglobosa vel globosa, 86–100 × 74–98 µm. Paries pycnidii ex cellulis depressus vel irrergularibus 1–3-stratosis compositus, brunneus vel fuscus, circa ostiolum fuscus. Cellulae conidiogenae holoblasticae, hyalinae, cylindricae, conicae vel lageniformes, 4.9–12.3 × 1.2–2.5 µm. Conidia continua, globosa, elliptica, obovata, primo basi truncata, posterius utrinque rotundata, 7.4–14.7 × 4.9–7.4 µm, strato mucoso circumdantia, guttulas numerosas continentia, apice appendice 4.9–9.8 µm longa praedita.

Type specimens: On *Ligustrum obtusifolium* Siebold & Zucc., Kurokami, Kumamoto-shi, Kumamoto Prefecture, Japan, 7 October 2005, collected by K. Motohashi, C. Nakashima, and T. Akashi (holotype, TFM:FPH-7834) (isotype, MUMH 10448) (ex-type cultures, MAFF 240053 and NBRC 102256).

Leaf spots orbicular to ellipsoid, often extended with concentric rings, light gray to pale brown, 3–6 mm in diameter, surrounded by a dark brown border. Pycnidia amphiphyllous, subepidermal, subglobose to globose, 86–100 × 74–98 µm. Pycnidial wall composed of depressed or irregular cells in 1–3 layers, brown to dark brown, darker around ostiole, hyaline or paler, and flattened toward the inside. Conidiogenous cells holoblastic, hyaline, cylindrical, conical, or lageniform, 4.9–12.3 × 1.2–2.5 µm. Conidia unicellular, globose, ellipsoid, or obovoid, truncate at the base when young, later rounded at both ends, 7.4–14.7 × 4.9–7.4 µm, surrounded by a slime layer, containing numerous minute guttules, with slender and short apical appendage 4.9–9.8 µm long.

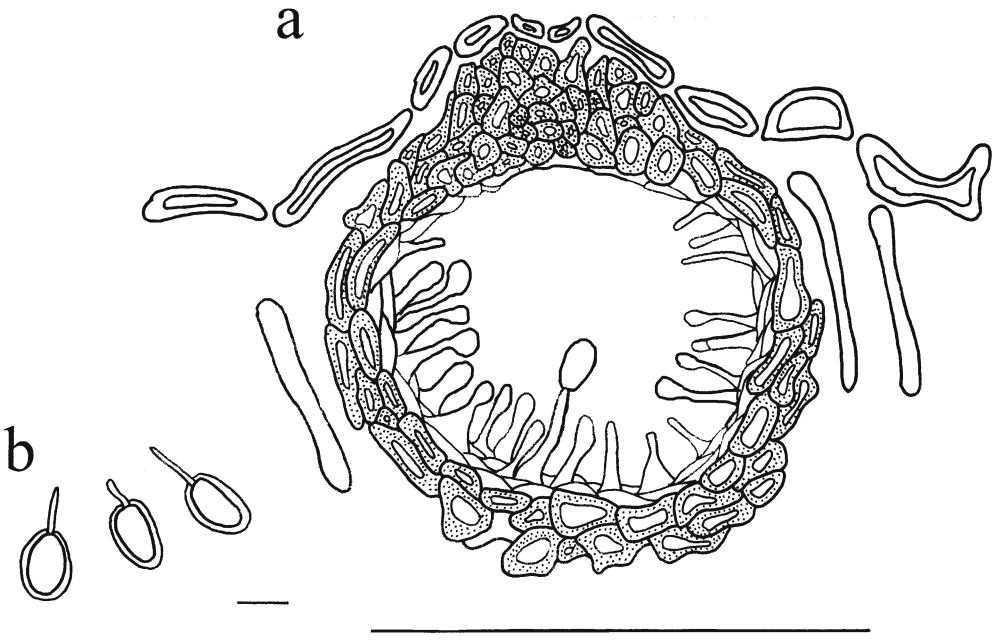

Fig. 3. *Phyllosticta ligustricola* on *Ligustrum obtusifolium*. **a** Pycnidium. **b** Conidia. *Bars* **a** 100 µm; **b** 10 µm

Host: *Ligustrum obtusifolium* Siebold & Zucc. (Oleaceae; "Ibotanoki" in Japanese).

Note: Four species of *Phyllosticta* sensu lato have been reported from *Ligustrum*: *P. ibotae* Nann., *P. ligustri* Sacc., *P. ligustrina* Sacc. & Speg., and *P. ovalifolii* Brunaud. Of these, *P. ligustrina* was treated as a synonym of *Coniothyrium ligustri* Brunaud (van der Aa and Vanev 2002). *Phyllosticta ibotae* differs from *Phyllosticta ligustricola* in its large pycnidia (780–840 µm) and ostioles (100–130 µm). According to van der Aa and Vanev (2002), *P. ligustri* [a fungus as described in the diagnosis was not found on the holotype specimen, excepting *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Coniothyrium ligustri*, and an *Asteromella* fungus] and *P. ovalifolii* (conidia oblong, 8–10 × 3 µm) were excluded from the genus *Phyllosticta* sensu stricto. We also accept these treatments and propose a new species, *P. ligustricola*.

Guignardia alliacea Motohashi, Jun. Nishikawa & C. Nakash., sp. nov. Fig. 4a–c

Coloniae in agaro decocto tuberorum viridi-griseae, postea nigrae, marginem undulatae; reversum nigrum. Ascocarpia subglobosa, solitaria, 86–135 × 105–159 µm. Paries ascocarpii multistratosus, brunneus vel fuscus. Asci subclavati vel cylindrici, 74–98 × 9.8–12.3 µm, octospori, crassi-bitunicati. Ascosporae distichae, hyalinae, unicellulares, multiguttulatae, fusiformes vel ellipsoideae, 17.2–22.1 × 4.9–7.4 µm, ad medium inflatae, apice utrique rotundatae et appendice obturamentiformi gelatinosa haerentes.

Type specimens: On *Allium fistulosum* L. inoculated with anamorphic stage *Phyllosticta alliacea*, Kakegawa-shi, Shizuoka Prefecture, Japan, 2 February 2006, collected by J. Nishikawa (holotype, TFM:FPH-7861) (isotype, MUMH 10464) (ex-type culture, MAFF 240062).

Colonies on potato dextrose agar growing at 10°–35°C (optimum at 28°–30°C), greenish-gray, later black, with undulate and dark brown margin; reverse black. Ascocarps subglobose, solitary, 86–135 × 105–159 µm, without pseudoparaphyses, ascocarpic wall composed of many layers of brown to dark brown compressed cells. Asci subclavate to cylindrical, 74–98 × 9.8–12.3 µm, 8-spored, wall thickened and bitunicate. Ascospores distichous, hyaline, unicellular, multiguttulate, fusiform to ellipsoid, wider at the midregion, 17.2–22.1 × 4.9–7.4 µm; both ends rounded with gelatinous caps, germinating at 10°–35°C (optimum at 15°–20°C).

Anamorph: ***Phyllosticta alliacea*** Motohashi, Jun. Nishikawa & C. Nakash., anam. nov. Fig. 5a,b

Pycnidia epiphylla, subglobosa vel globosa, saepe aggregata, 77–130 × 91–155 µm. Paries pycnidii ex cellulis depressus vel irregularibus 3–5-stratosis compositus, brunneus vel fuscus. Cellulae conidiogenae holoblasticae, hyalinae, cylindricae, 4.9–11 × 1.2–3 µm. Conidia continua, globosa, elliptica, obovata, primo basi truncata, posterius utrinque rotundata, 7.4–12.3 × 4.9–7.4 µm, strato mucoso circumdata, guttulas numerosas continentia, apice appendice 4.9–9.8 µm longa praedita.

Type specimens: Anamorphic fungi on *Allium fistulosum* L., Kakegawa-shi, Shizuoka Prefecture, Japan, 14 July 2004, collected by J. Nishikawa (holotype, TFM:FPH-7859) (isotype, MUMH 10443) (ex-type cultures, MAFF 240044 & NBRC 102248).

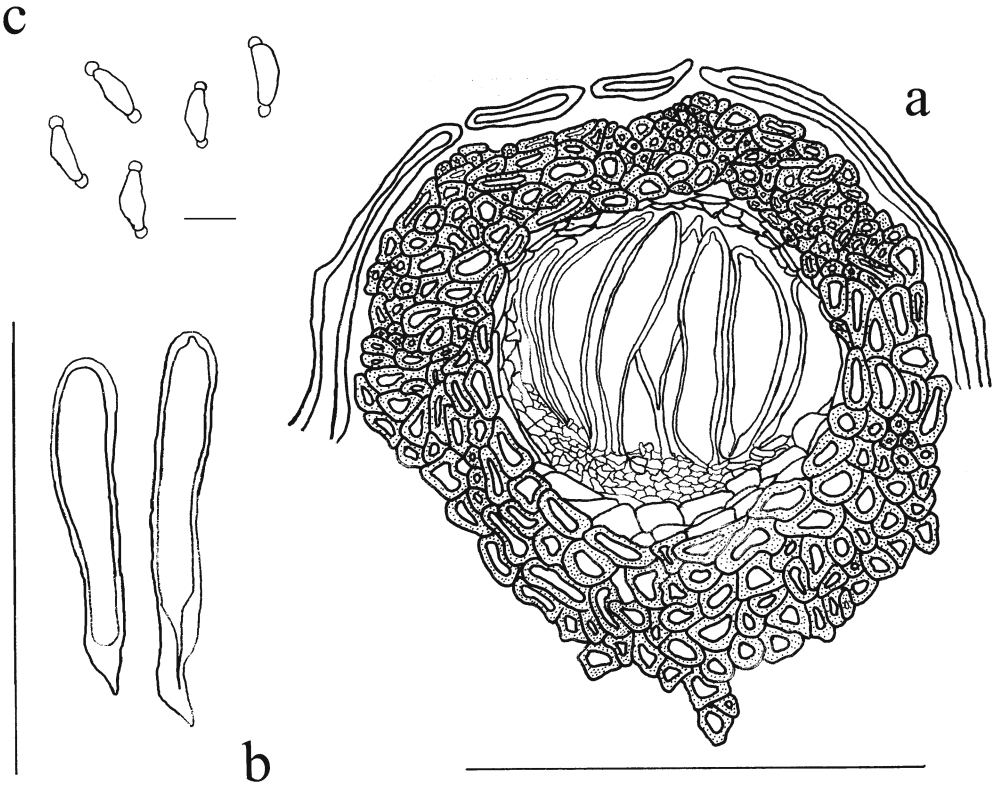

Fig. 4. *Guignardia alliacea* on *Allium fistulosum*. **a** Ascocarp. **b** Asci. **c** Ascospores. *Bars* **a, b** 100 µm; **c** 10 µm

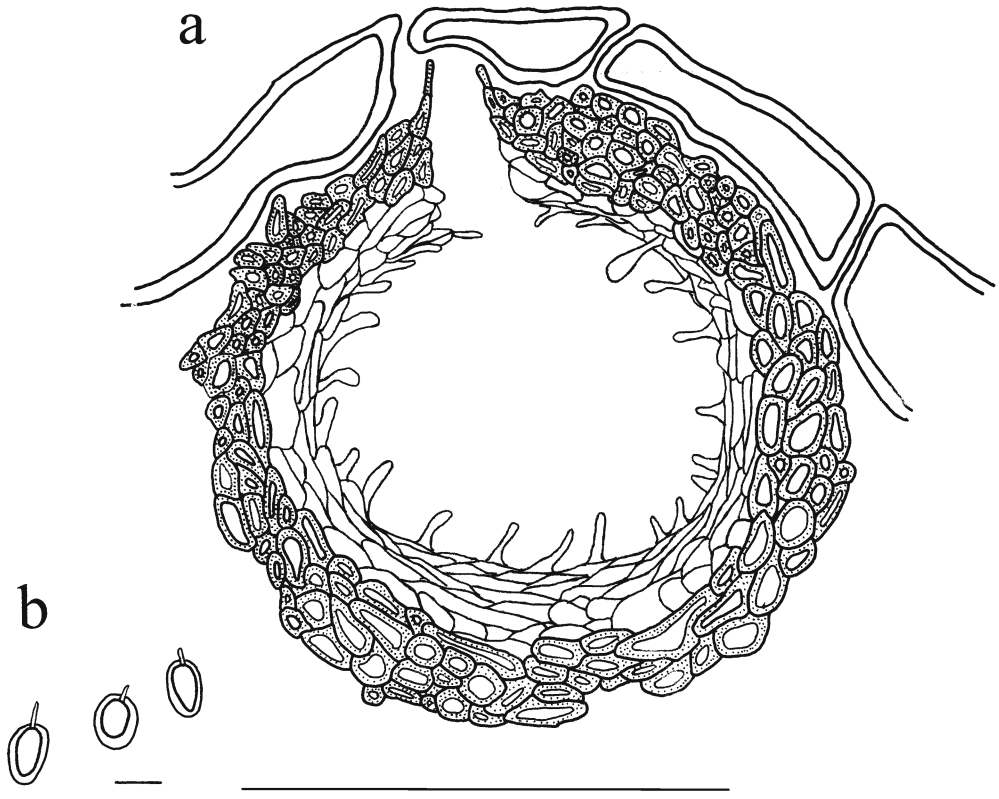

Fig. 5. *Phyllosticta alliacea* on *Allium fistulosum*. **a** Pycnidium. **b** Conidia. *Bars* **a** 100 µm; **b** 10 µm

Leaf spot pale yellow at leaf tip at the beginning, later circular to irregular, finally turn black. Pycnidia epiphyllous, subepidermal, subglobose to globose, often aggregated, 77–130 × 91–155 µm. Pycnidial wall 3–5 layers, composed of depressed or irregular cells, brown to dark brown, hyaline or paler toward the inside. Conidiogenous cells holoblastic, hyaline, cylindrical, 4.9–11 × 1.2–3 µm. Conidia unicellular, globose, ellipsoid, or obovoid, truncate at the base when young, later rounded at both ends, 7.4–12.3 × 4.9–7.4 µm, surrounded by a slime layer, containing numerous fine guttules, with apical appendage, 4.9–9.8 µm long, germinating at 10°–35°C (optimum at 20°C) in water.

Host: *Allium fistulosum* L. (Liliaceae; "Negi" in Japanese).

Disease name: Phyllosticta leaf blight ("Kokumon-hagare-byo" in Japanese).

Note: Unknown *Phyllosticta* species was found on *Allium fistulosum* in Shizuoka Prefecture in year of 2004–2005, with pycnidia on blackish region leaf spots. In our inoculation experiment, young seedlings of *Allium fistulosum* sprayed with conidial suspension showed leaf blight symptom in 5 days and formation of a *Guignardia* fungus on inoculated leaves after 10 days. Single ascospore culture from the *Guignardia* fungus formed conidiomata of *Phyllosticta* in reisolated cultures. These *Phyllosticta* were identical to each other based on morphological characteristics, and the teleomorph–anamorph relationship of the *Guignardia* and *Phyllosticta* species was confirmed in this study.

Six species of the genus *Phyllosticta* sensu lato have been recorded on *Allium*, namely, *P. allii* Baudyš, *P. allii* Tehon & E. Y. Daniels (later homonym of *P. allii* Baudyš), *P. allii* Rothers (later homonym of *P. allii* Baudyš), *P. alliicola* Lobik, *P. allii-rotundi* Lobik, and *P. cepae* Verwoerd & du Plessis. van der Aa et al. (2000) treated *P. alliicola* as a synonym of *Phoma exigua* var. *exigua* from reexamination of the type specimen. *Phyllosticta allii-rotundi* was transferred to *Asteromella allii-rotundi*, which was regarded as the spermatial state of the genus *Mycosphaerella* (van der Aa and Vanev 2002). Likewise, van der Aa and Vanev (2002) pointed out that *P. allii* Tehon & E.Y. Daniels (conidia ovoid, 7.5 × 3.7 µm), *P. allii* Rothers (conidia ovoid, 5.6 × 2.8 µm), and *P. cepae* (conidia ellipsoid to semiovoid, 3.4–7.1 × 1.7–2.2 µm) belong to *Phoma* and not to *Phyllosticta* sensu stricto. They also reported that *P. allii* Baudyš did not belong to *Phyllosticta* sensu stricto based on its description. Moreover, the genus *Guignardia* has never been reported on *Allium*. From these results, the holomorphic *Guignaridia* species including the *Phyllosticta* anamorph on *Allium* was found to be a new species.

As described above, the *Guignardia* teleomorph has not been observed in nature except in the inoculation experiment using the *Phyllosticta* anamorph. In addition, the damage by disease of *Allium* caused by the anamorph is quite serious in the field. Therefore, the authors propose the binary name also for the anamorph for practical use.

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