Species of Colletotrichum on Agavaceae

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ABSTRACT

Species of Colletotrichum cause diseases on a wide range of hosts, frequently infecting plants in the Agavaceae (monocotyledons: Liliales). Three species of Colletotrichum restricted to the Agavaceae were detected through morphological studies of specimens and molecular sequence analyses of the LSU of the nu-rDNA and the ITS region of the nu-rDNA from cultures. Colletotrichum agaves on Agave is fully described and illustrated. Colletotrichum dracaenophilum is described as a new species for isolates having long conidia and occurring on Dracaena sanderiana from China. Colletotrichum phormii and Glomerella phormii are determined to be the correct scientific names for the asexual and sexual states, respectively, of a species commonly referred to as C. rhodocyclum and G. phacidiomorpha occurring mainly on Phormium. In addition, C. gloeosporioides and C. boninense were isolated from plants in the Agavaceae. All species of Colletotrichum described on Agavaceae were evaluated based on type specimens. A key to the five species of Colletotrichum on Agavaceae is included. This paper includes one new species, Colletotrichum dracaenophilum, and three new combinations, Colletotrichum phormii, Glomerella phormii, and Phaeosphaeriopsis phacidiomorpha.

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Introduction

Members of the genus Colletotrichum cause diseases on a number of host plants. These diseases, often referred to as anthracnose, include black spot of strawberry and key lime anthracnose (C. acutatum), anthracnose of tomato fruit (C. coccodes), sorghum red stalk rot (C. graminicola), coffee berry disease (C. kawahae), anthracnose of beans (C. lindemuthianum) and many others (Holliday 2000). According to Sutton (1992:1) species of Colletotrichum are especially important as the cause of preharvest and postharvest problems in the tropics ‘as latent or quiescent infections’. Some plant pathogenic species of Colletotrichum are of quarantine significance because they could be introduced into countries where the disease does not occur. Species of Colletotrichum are also frequently encountered as endophytes of tropical woody plants (Arnold et al. 2003; Lu et al. 2004; Promputtha et al. 2005) as well as herbaceous and monocotyledonous plants (Photita et al. 2004, 2005). The taxonomy and phylogenetics of most species of Colletotrichum have not been studied, which limits accurate knowledge of the distribution and biology of these species, as well as the ability to identify individual taxa.

Historically, it was assumed that species of Colletotrichum were host-specific. New species were described when this fungus was encountered on a new host plant genus resulting in more than 660 names published in Colletotrichum (Index Fungorum http://www.indexfungorum.org/Names/Names.asp). About 600 of these were considered synonyms of C. gloeosporioides by Arx (1957) based on his morphological examination of type specimens. In later studies some of
these synonyms have proven to be distinct species. A useful key for the identification of species of Colletotrichum was published by Sutton (1980) who distinguished 21 species and species-groups based primarily on characteristics of the conidia, appressoria, setae, sclerotia, and colonies produced under standardized conditions combined with plant host. Later Sutton (1992) listed 39 accepted species in Colletotrichum each with a short description but no key. At present about 40 species are accepted in the genus Colletotrichum (Kirk et al. 2001), of which 25 are represented in GenBank. However, most species of Colletotrichum have not been studied in culture or using molecular, biochemical or genetic techniques. Neither of the two previously described species on Agavaceae included in this paper was accepted by Sutton (1992).

Recent molecular systematics research on the genus Colletotrichum suggests that some taxa are host specific while others are not. Using multiple gene phylogenies, RFLPs, and mating tests, some easily recognizable morphological species have been shown to consist of genetically distinct groups. For example, within C. acutatum seven distinct molecular groups could be identified (Guerber et al. 2003). Matting compatibility was determined within one clade that included isolates from a wide range of hosts and geographic origins but also occurred between two phylogenetically distinct clades, one of which included only isolates from Australia and New Zealand that cause fruit rots and terminal crook disease of pine seedlings. Within the C. acutatum complex, a new species, C. lupini, with one variety C. lupini var. setosum, has recently been recognized (Nirenberg et al. 2002) that occurs only on Lupinus. This species was originally considered to be C. gloeosporioides (Elmer et al. 2001). Other species are being segregated within the C. gloeosporioides complex, such as C. kahawae, cause of coffee berry disease (Lubbe et al. 2004). Based on isolates from diseased leaves of eight different plant species in Japan, Colletotrichum boninense (Moriwaki et al. 2003) was described and distinguished both morphologically and by sequences of the ITS region of the nu-rDNA repeat. Two of the eight groups of Colletotrichum isolated as endophytes from tropical trees were considered to be related to C. boninense (Lu et al. 2004). Thus, some species of Colletotrichum may function as both plant pathogens and endophytes (Photita et al. 2004, 2005).

Plants belonging to the Agavaceae (monocotyledons: Liliidae) intercepted at US ports of entry are frequently infected with species of Colletotrichum. At present the United States is prohibiting the entry of Phorgrim tenax (New Zealand flax) infected with a long-spored species of Colletotrichum that has been called C. rhodocyclum. Additional species of Colletotrichum having conidia greater than 20 μm have been encountered on living plants of Dracaena sanderiana (lucky bamboo) from China. Two-gene molecular analyses of all available Colletotrichum species were undertaken in this study in order to clarify the identity and number of species of Colletotrichum known on the Agavaceae. Five species were isolated from plants in the Agavaceae, including one new species, C. dracaenophilum. Three of these species are described, illustrated and discussed, and a key to the five species of Colletotrichum on Agavaceae is provided.

Materials and Methods

Morphological/cultural characterization

Cultures were obtained from acervuli and perithecia in leaves of specimens intercepted at ports of entry in the US. A small number of conidia were transferred from the host substrata to sterile water in a hanging drop slide, mixed, and spread on a plate of corn meal dextrose agar (Difco, Detroit, MI) plus antibiotics (CMD-A, 0.2 % neomycin and 0.2 % streptomycin). After incubation overnight at room temperature, single or multiple germinating conidia were transferred to new CMD-A and potato dextrose agar (PDA, Difco, Detroit, MI) plates for processing. Additional cultures were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht.

For microscopic examination infected plant material was rehydrated and mounted in 3 % potassium hydroxide. Sections of acervuli ca 10 μm thick were obtained with a freezing microtome and mounted in lactic acid with cotton blue. Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with bright-field and fluorescence illumination. Calcofluor was used as the fluorescent dye. Photographs and measurements of microscopic features were taken using an Olympus Q5 digital camera (Olympus, Tokyo) and ImagePro software (Media Cybernetics, Silver Spring, MD).

The description of cultural characteristics was based on isolates grown at 25 °C, under NUV plus fluorescent light on PDA for 6 d. Colours were described using terms in Raynor (1970). Sterilized alfalfa stems (Medicago sativa) on water agar were inoculated with each isolate and maintained under the same conditions listed above in order to obtain dried cultures as herbarium specimens. Slide cultures using PDA were made for each isolate (Malloch 1981) and examined for the development of appressoria.

Parent specimens from which cultures were derived, as well as dried cultures were deposited at the US National Fungus Collections (BPI), Beltsville, Maryland. Living cultures were deposited at the CBS.

DNA isolation, sequencing and analyses

Provenance of cultures used for sequencing analyses is listed in Table 1. Mycelium for DNA extractions was cut with a sterile scalpel from actively growing cultures on PDA and extracted with the UltraClean Plant DNA Isolation Kit as per the manufacturer’s instructions (MoBio Laboratories, Inc., Solana Beach, CA). Individual genes were amplified by PCR in 25 μl reactions with 12.5 μl of PCR Master Mix (Promega, Madison, WI), 1.25 μl each of 10 μM primers and 10 μl diluted (10- to 100-fold) DNA template using the following primers: LSU nu-rDNA LR0R and LR7 (Vilgalys & Hester 1990); ITS rDNA ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Standard cycling parameters with a 55 °C annealing temperature were used for both gene regions. The PCR products were cleaned with Montage PCR Centrifugal Filter Devices (Millipore Corp., Billerica, MA) according to the manufacturer’s protocol and sequenced as described in Rossman et al. (2004). Sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov) and are listed in Table 1.
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1 AR, Amy Rossman; ATCC, American Type Culture Collection; BPI, US National Fungus Collections; CBS, Centraal Bureau voor Schimmelcultures; FAU, Francis A. Uecker; GJS, Gary J. Samuels; MCA, M. Catherine Aime; MEP, Mary E. Palm.
Sequencing reactions were edited and contiguous sequences were assembled in Sequencher v.4.1.4 (Gene Codes Corp., Ann Arbor, MI). Sequences obtained were blasted in GenBank to confirm identity as Colletotrichum spp. Plectosphaerella cucumerina was chosen as an outgroup as this fungus represents the closest known relative to Glomerella/Colletotrichum (Huhndorf et al. 2004). Sequence alignments were constructed by eye in Se-Al v2.0a11 (http://evolve.zoo.ox.ac.uk) for each gene and then concatenated in PAUP v.4.0b10 (Swofford 2002). Sequences were selected from GenBank for those Colletotrichum and outgroup taxa for which both ITS and LSU sequences were available from the same isolate. These were: Colletotrichum sp. AY539806 and AY539807, C. circinans AJ301955, C. coccodes AB105970 and AB06153, C. dematium AJ301954, C. destructivum AF320562 and AB106148, C. fuscum AB105966 and AB106161, C. higginsianum AB105957 and AB105956, C. lindemuthianum AJ301958, C. linicola AB057437 and AB106150, C. sublineolum AJ301978, C. trifolii AJ301941, C. truncatum AJ301985, Glomerella cingulata AF543786, G. glycis AB057435 and AB106160, G. lagenarium AJ301965, and Plectosphaerella cucumerina AF176952 and AF176953. Data analyses followed Rossman et al. (2004). The dataset consisted of 70 isolates aligned across 1272 bp. A total of 86 bp were excluded from analyses due to ambiguities in alignment. MP analyses were conducted in PAUP as heuristic searches with 100 random addition replicates and tree bisection-reconnection branch swapping. Support for the branching topologies was evaluated by BS analysis derived from 1000 replicates with ten random addition replicates each. ML analyses were conducted by the quartet puzzling method (Strimmer & von Haeseler 1996) in PAUP with 10 K puzzling steps; transition: transversion ratio = 2.

Results

Five different species of Colletotrichum were isolated from plant hosts in the Agavaceae. Three of these species appear to occur only on specific genera in the Agavaceae, viz. C. agaves on Agave, C. dracaenophilum on Dracaena, and C. phormii on Phormium, as indicated by the phylogenetic analyses (see below in Results). These three species are described and illustrated in the taxonomy section of this paper. Two additional species having a wide host range were identified from Dracaena. Two isolates of Colletotrichum from Dracaena were identified as C. boninense based on both molecular and morphological characteristics. The following specimens of C. boninense were examined: China: on living leaves of Dracaena sanderiana, APHIS interception Hoboken New Jersey, 30 Apr. 2001, P. Milicia, det. M. Feinstein, isol. AR 3658 = CBS 118193 (BPI 748487); 25 Oct. 2001, P. Milicia & M. Feinstein, isol. AR 3751 = CBS 118774 (BPI 871833). One ascospore isolate was obtained from a specimen of Glomerella cingulata on Dracaena that grouped with other sequences of G. cingulata including the homothallic isolates studied by Uecker (1994). The following specimen of G. cingulata was examined: Indonesia: on Dracaena, APHIS interception Miami Florida 223164, 4 Mar. 2002, H. Ruiz, det. A.Y. Rossman, isol. from ascospores AR 3788 = CBS 118192 (BPI 871497).

Phylogenetic analyses

Fifty-five new sequences from isolates of Colletotrichum/Glomerella were analysed along with 17 sequences selected from GenBank. A comprehensive dataset was assembled that includes both ITS and LSU sequences from 27 of the estimated 40 species of Colletotrichum (Kirk et al. 2001). Of the 1185 characters included, 970 were constant, 111 variable characters were parsimony uninformative, and 104 variable characters were parsimony informative. Four equally parsimonious trees of length 402 were found by MP; CI = 0.61; RI = 0.87. Both MP and ML methods uncovered the same major lineages (Fig 1).

Taxonomy

Colletotrichum agaves Cavara, Fungi Longobardiae Exsiccati No. 100 (1892).

Leaf lesions circular, chlorotic, with or without a brown margin, usually 2–4 cm diam, up to 6 cm diam or more, several on one leaf, occasionally becoming confluent, eventually causing death of plant tissue that becomes purplish to dark brown. Acrervuli epiphyllous, scattered or forming concentric rings, distinct to confluent, developing beneath epidermis, becoming erumpent with age, often surrounded by remnants of epidermis, globose to oblong, in longitudinal section of hyaline to dark brown cells forming textura angularis, 0.10–1 mm wide, 200–400 μm tall, brown to black, producing masses of pale orange conidia. Setae numerous, projecting beyond hymenial surface, brown to black, thick-walled, septate, straight to irregularly crooked, tapering to acute or rounded apices, 60–211 × 5–8 μm. Conidiophores dark brown to hyaline arising from upper cells of acervuli. Conidigenous cells hyaline, enteroblastic, 18.5–24 × 3.5–7 μm, cylindrical, perincinal thickenings moderate, channel narrow to broad. Conidia hyaline, (17.5–) 19.0–30.5 (–33) × 5–8 (–9.5) μm (mean = 23.5 × 6.5 μm, sd length = 3 μm, width = 0.5 μm, n = 146), aseptate, cylindrical or expanding to a broadly rounded apex, straight or slightly curved, with one or two guttules.

Colonies 3.7–4.2 cm diam, rosy buff to sepia, peach at margin, margin smooth, broadly wavy, reverse rosy buff to peach, profuse sporulation on agar and alfalfa twigs, acervuli similar to above, open acervuli each with numerous black setae. Appressoria not seen. All other characters as on natural substrata.

Host range: On numerous species of Agave as well as the closely related genus Furcraea, including F. macrophylla Baker (wild siala).

Geographic distribution: Known from warm temperate and tropical regions including southern Europe, the Neotropics, Cyprus (Georgiou & Papadopoulos 1957); Africa (Kenya in Nattrass 1961); and the US (FL, MO in greenhouses, MS in Parris 1959). Although no specimens were located and examined, C. agaves has also been reported from Asia, including China (Tai 1979), Korea (Cho & Shin 2004), the Philippines (Teodoro 1937) and Taiwan (Anonymous 1979).

Type specimens examined: Italy: Pavia: Ticinum, in botanical garden, on decaying leaves of cultivated Agave americana,
Fig 1 – MP tree derived from combined ITS and LSU sequence matrix. Nodes with moderate support by bootstrapping (50–70 %) and quartet puzzling (50–70 %) are of medium thickness. Nodes with both strong bootstrapping support (>70 %) and quartet puzzling reliability (>70 %) are indicated by heavily blackened branches. Support values are given above supported branches: the number before the slash represents bootstrapping support values from MP analyses; the number after the slash represents quartet puzzling reliability scores for that node. Isolates from Agavaceae are indicated in bold type.
Cavara (BPI specimen in bound exsiccati – lectotypus hic designatus; additional isolectotype specimens BPI 397134 and BPI 397135).


Notes: Colletotrichum agaves causes a foliar disease of agave plants as first documented by Hedgcock (1905) who reported...
plants dying from this fungus in greenhouses at the Missouri Botanical Garden, St Louis. This disease was also discussed by Barthelet (1942) in Italy. Petrak (1942) reported it on plants growing outdoors in Florida.

C. agaves is easily distinguished from other species on Agavaceae by the numerous black setae that develop throughout the conidiogenous region in acervuli. Acervuli often develop in concentric rings in necrotic areas on the upper surface of living leaves of Agave. Acervuli of Colletotrichum from Agave developing on alfalfa stems in culture appear to be morphologically the same as C. agaves present on the type and other specimens. In addition after 6 d on PDA, the growth rate of C. agaves (3.7–4.2 cm) is faster than the slow-growing C. phormii (2.5–3.4 cm) but slower than C. dracaenophilum (6–6.7 cm). Arx (1957) listed C. agaves among the several hundred synonyms of C. gloeosporoides while Hughes (1958) considered C. agaves a synonym of C. coccodes. The molecular data for C. agaves show this species to be a distinct taxon, separate from both C. gloeosporoides and C. coccodes (Fig 1). No teleomorph is known for this species.

**Excluded and doubtful names of Colletotrichum on Agave**

Gloeosporium agaves Syd. & P. Syd., Ann. Mycol. 5: 362 (1907). No conidia were found on the immature type specimen. This name was considered to be a synonym of Colletotrichum carpoporoides by Arx (1957).

*Specimen examined: Brazil: Sao Paulo: Campinas, on Agave sp., Apr. 1898, F. Noark, comm. F. Sydow, ex herb Pazschke (B).*  
Gloeosporium macropus was considered a possible synonym of C. agaves by Hedgcock (1905) but this species is reported only on hosts other than Agave. The type specimen was not examined.

*Colletotrichum dracaenophilum* D. Farr & M. E. Palm, *sp. nov.*

_Fig 3A–F_

*MycoBank no.: MB 510231*

*Etym.: From the host name, Dracaena, and -philum, Greek for loving.*

Acervuli nigri, globosi vel irregularim elongati. Setae sparse in hymenium, nigrae, 143–187 × 5–6.5 μm, laeves, 3–6 septatae. Cellulae conidiogenae hyalinae, heteroblastae, 18–32 × 4–8 μm. Conidia (20–)22–34–(38) × (5.5–)6.5–9.5(–10) μm (mean = 29 × 8.5, n = 190, sd length = 3.2, latitudo = 0.96), late clavata v. cylindrica, hyalina.  

Acervuli on dying stems, numerous in discoloured areas, developing subepidermally, surface exposed through irregularly splitting of epidermal tissue that folds back, acervuli black, globose to irregularly elongate, often coalescing, 210–630 μm in longest dimension. Cells at acervular surface mainly 5–6 μm diam forming textura prismaticata, radially oriented toward outer edge. Setae sparse, scattered in hymenium, black, 143–187 × 5–6.5 μm, straight to slightly irregularly rounded, becoming narrower and hyaline at rounded apex, smooth, 3–6 septate. Conidiogenous cells hyaline, enteroblastic, 18–32 × 4–8 μm, pericellic thickenings moderate, channel narrow to broad. Conidia (20–)22–34–(38) × (5.5–)6.5–9.5(–10) μm (mean = 29 × 8.5, sd length = 3.5 μm, width = 0.9 μm, n = 190), broadly clavate to cylindrical, frequently slightly curved, hyaline, guttulate.

Colonies 5–6.7 cm diam, pale pink except dark where sporulating, margin pale pink, reverse speckled from profuse sporulation, sparse aerial mycelium, rosy buff to saffron in centre, rosy buff to saffron in reverse, except for presence of dark acervuli, paler at even margin; immature, white acervuli developing at margin. Acervuli on alfalfa stems the same as on natural substrata except slightly smaller, 140–420 μm in longest dimension, separate, and superficial.

_Host range: On stems of Dracaena sanderiana and Dracaena sp._

*Geographic distribution: Known only from China._

_Notes: C. dracaenophilum is distinguished from all other species of Colletotrichum known from Agavaceae by the long conidia that average more than 28 μm long. In culture, C. dracaenophilum is the fastest growing of the three long-spored species of Colletotrichum on Agavaceae. Although Dracaena sanderiana is native to western Africa, this host is now cultivated throughout the world especially as ‘lucky bamboo’ in small containers._

**Excluded names of Colletotrichum on Dracaena**

_**Colletotrichum dracaenae**_ Allesch., in Rabenhorst, Rabenh. _Krypt._ Fl. 7: 560 (1902).

The type specimen of this taxon was examined. The conidia were originally described as 14–18 × 5–7 μm, which agrees with our examination of the specimen in which the conidia were 14.5–18.5 × 5–8.5 μm (mean = 16 × 6.5 μm, sd length = 0.5 μm, width = 0.5 μm, n = 72). This is much smaller than conidia on the specimens from China described above. Based on a morphological examination of the type specimen, Arx (1957) considered _C. dracaenae_ to be a synonym of _C. gloeosporoides_ and our observations agree with that conclusion.

*Specimen examined: Germany: Munich, in the greenhouse of the botanical garden, on faded leaves of Dracaena latifolia, Apr. 1895, J. E. Weiss (M-009006)._  

The fruiting bodies on the type specimen of this later homonym have a very thin subhymenial layer that is only one or two layers thick. This is unlike those of _Colletotrichum_ spp.

*Specimen examined: Sri Lanka: Peradeniya, on leaves of Dracaena sanderiana, May 1904, T. Petch 6775 (K(M) 125641 — holotype of _C. dracaenae_ Petch, hom. illeg._).

_**Colletotrichum dracaenicola**_ Sacc. & Trot, _Syll. Fung._ 22: 1205 (1913)._
Colletotrichum on Agavaceae


The type specimen of C. dracaenica could not be located, although requested from NAP and FORUN, nor is it housed at PAD (Gola 1930). Based on the description in Saccardo, this species has conidia 12–19 × 2–7 μm that are much smaller than those of C. dracaenophilum.


The type specimen for this name could not be located, however, based on the description in Saccardo, this species has conidia that are 5–12 × 2.5–3.5 μm, much smaller than those of C. dracaenophilum. An exsiccati specimen at BPI identified as this species was immature.


Colletotrichum phormii (Henn.) D.F. Farr & Rossman, comb. nov.


Teleomorph: Glomerella phormii (J. Schröt.) D.F. Farr & Rossman, comb. nov.


On lower surfaces of living leaves, acervuli black, 280–420 μm long, no setae observed, scattered, subepidermal, developing in large, elliptical discolored areas, lesions (0.2–) 2.0–3.7 × 0.5–0.9 cm, with generally well-defined, white to brown margin, broad to narrow pale green outer region, and brown to black in inner region, acervuli not splitting widely, producing pale orange cirrhus of conidia. On upper surface acervuli less conspicuous, not in discolored areas, developing below epidermis, erumpent through epidermis that remains attached, surface sunken. Acervuli of a palisade of 2–5, dark cells subtending conidiogenous cells. Conidiogenous cells cylindrical, 13–26 × 4–5 μm. Conidia cylindrical to long fusiform, gradually tapering to broadly truncate base, apex rounded, straight to slightly curved, (16.5–)19–29.5(–31) × 4–7.5 μm (mean = 24.5 × 5.5 μm, sd length = 2.5 μm, width = 0.5 μm, n = 69). Appressoria terminal or lateral, at first obpyriform, becoming irregularly lobed, 9–12 × 6–11 μm.

Ascomata on upper and lower surface of leaves in large, elliptical, discoloured areas similar to those bearing acervuli, with or without a narrow, black margin, subepidermal, sometimes partially erumpent, solitary, scattered to crowded or aggregated, black, shiny when exposed, globose to ellipsoid, flattened. Ascomatal walls of thin-walled, brown cells, 9–15 μm diam. Paraphyses sparse, inflated, hyaline. Ascii unitunicate, narrowly clavate with a rounded apex and short stipe, 56–70 × 15–20 μm, with an indistinct apical ring in immature asci, 8-spored, obliquely seriate. Ascospores hyaline, non-septate, ellipsoidal, 15–22 × 4.5–6 μm.

Colonies 2.5–3.5 cm diam, slightly to thickly fluffy, vinaceous buff to hazel, slightly paler at margin, margin even, olivaceous grey toward a centre with slimy conidial masses, olivaceous buff to hazel, slightly paler at margin, margin even, olivaceous grey toward a centre with slimy conidial masses, olivaceous grey toward a centre with slimy conidial masses, olivaceous grey toward a centre with slimy conidial masses.

Type specimens examined: Germany: Hortus Berolinensis: Kalthaus, on Phormium tenax, Apr. 1889 (B 70 005220—holotype of Fusarium phormii; BPI-isotype); Breslau, Bot. Garten im Warmhause, on decaying leaf tips of P. tenax, on or before Mar. 1894 (Pilze Schlesiens: Type of Physalospora phormii not located).


Notes: C. phormii is the best known of the long-spored species of Colletotrichum on Agavaceae with most reports under the name C. rhodocyclum following the thorough study of this species by Kinghorn (1936). Since that report relatively little has been published about this disease. C. phormii (as C. rhodocyclum or the teleomorph name G. phacidiomorpha) was reported to cause a disease on Phormium tenax cultivated as a commercial crop in Devonshire, England in the spring of 1934 and later that year. Kinghorn (1936) published the only account of both the disease and the development of the fungus. Perithecia were reported to develop on leaves in 3–4 weeks; however, no perithecia developed in culture during this study. Of the three long-spored species of Colletotrichum specific to Agavaceae, C. phormii is the slowest growing in culture.

In reviewing the nomenclature of this species it was discovered that the description of the basionym Cryptosporium rhodocyclum, initially cited as ‘Montagne in litt.‘, was not validly published until Almeida & Camara did so in 1909, the date from with this epithet originates. Thus, the earliest basionym for this species is Fusarium phormii Henn. 1899.
Key to species of Colletotrichum on Agavaceae

1 Conidia generally less than 20 μm long .............................................................................................................. 2
Conidia generally longer than 20 μm long .................................................................................................................. 3

2(1) Conidia 12–17 × 3.5–5 μm, base truncate ............................................................................................................ gloeosporioides
Conidia 13–15.5 × 5–6 μm, base with a scar-like hilum .......................................................................................... boninense

3(1) Conidia averaging longer than 28 μm; colonies relatively fast-growing, 6–6.7 cm diam on PDA in 6 d. On Dracaena spp. .............................................................................................................................. draenophyllum
Conidia averaging less than 28 μm long; colonies slow-growing, less than 5 cm diam on PDA in 6 d ......................... phormii

4(3) Conidia 19–29.5 × 4–7 μm; setae generally lacking; colonies 3–3.2 cm on PDA in 6 d. On Phormium spp. ...................... phormii
Conidia 19–30.5 × 5.0–8.5 μm; setae abundant; colonies 3.5–4.5 cm on PDA in 6 d. On Agave and Furcraea spp. ............. agaves

and that epithet is herein transferred to Colletotrichum. One additional synonym, Gloeosporium phormii Sacc. 1915, is based on a specimen collected in Malta. Later Saccardo (1931) recognized the synonymy of his name with that of Fusarium phormii when he cited this name as G. phormii (Henn.) Sacc. listing the specimen from Malta as well as Henning’s type specimen from the Berlin Botanical Garden in Germany. The later homonym of Gloeosporium phormii (Henn.) Wollenw. should perhaps be listed as G. phormii (Henn.) Bubák. Wollenweber’s Fusarium delineatum autographicum was published in 1916, however, it was not possible to determine the precise date of publication. Bubák’s combination was published on 10 October 1916 (Bubák 1916), thus it is assumed that Wollenweber’s new combination is earlier than that of Bubák. In addition, this authorship is accepted by Kirk et al. [Index Fungorum (http://www.indexfungorum.org/Names/Names.asp) under Gloeosporium phormii], although von Arx (1957) attributed this new combination to Bubák. The question of authorship in 1916 is moot because this new combination was preceded by Saccardo’s publication of the same name in 1915.

The sexual state of Colletotrichum phormii was first described as Physalospora phormii, a name that is herein transferred to the genus Glomerella and serves as the name for the teleomorph of C. phormii. This name had previously been placed in the monotypic genus Hypostegium Theiss. 1916, thus Hypostegium is a later synonym of Glomerella Schrenk & H. SpaULD. 1903. The teleomorph of C. phormii was regarded as Glomerella phacidiomorpha by Kinghorn (1936) but an examination of the basionym of that name reveals that Sphaeria phacidiomorpha does not refer to a Glomerella (see below).

Excluded names of Colletotrichum phormii and Glomerella phormii:
Phyllosticta haematocycla was listed as a synonym of C. phormii as C. rhodocyclum by Almeida and Camara (1909). However, this name is now recognized as Phoma haematocycla (Berk.) Aa & Boerema (in de Gruyter et al. 1993) based on an examination of the type specimen of Phyllosticta haematocycla and is accepted in Boerema et al. (2004).

Phaeosphaeriopsis phacidiomorpha (Ces.) D.F. Farr & M.E. Palm, comb. nov.

Synonyms: Sphaeria phacidiomorpha Ces., Fungi Europaei no. 2337 (1876).
Species examined: Italy: Horto Botanico Neapolitano, on leaves of Phormium tenax, Cesati, Rabenhorst, Fungi Europaei 2337 (BPI bound-lectotype of Sphaeria phacidiomorpha lectotypus hic designatus).

The teleomorph of Colletotrichum phormii has often been referred to as G. phacidiomorpha following Kinghorn (1936). The basionym Sphaeria phacidiomorpha was described in Rabenhorst’s Fungi Europaei (1876) and listed in Rabenhorst (1878). However, an examination of the type specimen of Sphaeria phacidiomorpha reveals that this taxon is not a Glomerella. Although appearing macroscopically similar to Glomerella, this fungus has bitunicate, obpyriform asci surrounded by abundant, filiform, branched pseudoparaphyses with four- to five-septate, hyaline ascospores that become distinctly constricted at one of the middle septa and yellow at maturity (Fig 4G–I). The conidia described as cylindric, hyaline, 3 μm long agree with the M. phaeosporioides-like anamorphs of Phaeosphaeriopsis (Camara et al. 2003). Based on our examination of the type specimen, Sphaeria phacidiomorpha is placed in Phaeosphaeriopsis in the Dothideomycetes, unrelated to Glomerella/Colletotrichum.

Discussion

Fungi in the genus Colletotrichum are present in soil and on plants as pathogens and endophytes throughout the world. Of the 40 recognized species, about 25 are represented by sequences in GenBank, although not all have ITS sequences. About 500 plant species are reported to be hosts of species of Colletotrichum with most plants said to be infected with C. gloeosporioides. Recent publications have addressed the systematics of species of Colletotrichum but often only taxa on one plant host are included, e.g. Fragaria (Martinez Culebras et al. 2003; Xiao et al. 2004), Rhododendron (Vinnere et al. 2002), or only one species of Colletotrichum is treated (Guerber et al. 2003; Horvath & Vargas, 2004). Many of the species defined by Sutton (1980) have yet to be sequenced and their concepts clarified.

Fig 4G–I
Of the five species of Colletotrichum on Agavaceae, three were restricted to specific genera of Agavaceae while the other two species have broad host ranges. Both Colletotrichum agaves and C. dracaenophilum appear to be host-specific taxa that are distinct within the genus Colletotrichum. Neither of these taxa is closely related to any other species included in this molecular analysis of Colletotrichum species.

Based on the molecular sequence data presented here, C. phormii is most closely related to C. acutatum (Fig 1). Originally described as a rotting-fungus genus, C. acutatum is now known from a variety of plants (Farr et al. 2005; Peres et al. 2005). Many older reports of C. gloeosporioides as a plant pathogen may actually refer to C. acutatum. C. acutatum is one of the most troublesome pathogens of strawberries along with C. gloeosporioides (Xiao et al. 2004). Within what is increasingly regarded as the C. acutatum species complex (Guerber et al. 2003; Peres et al. 2005; Talhinhas et al. 2002), one species has been recognized as host-specific, namely C. lupini. C. phormii also constitutes a distinct and host-specific species within the C. acutatum lineage. Conidia of C. phormii (19–29 × 4–7 μm) are morphologically distinct from those of C. acutatum (8–16 × 2.5–4 μm), which are considerably shorter and narrower than those of C. phormii. In addition, colonies of C. acutatum on PDA often produce bright pink to reddish pigments, which are not produced by C. phormii. C. phormii is also morphologically distinct from C. lupini that produces conidia measuring 12.5–15 × 4–45 μm (Nirenberg et al. 2002), considerably smaller than those of C. phormii.

C. boninense was described recently as a segregate of the C. gloeosporioides complex (Moriwaki et al. 2003) and is known from a diversity of plant species including monocotyledonous, dicotyledonous, herbaceous and woody plants. It is differentiated from C. gloeosporioides by conidial length/breadth ratio. C. boninense has relatively broad conidia 13–15.5 × 5–6 μm with length/breadth 1.8–3.3, a hilum-like conidial base, and cream to orange-coloured colonies on PDA (Moriwaki et al. 2003).

One ascospore isolate of Glomerella cingulata, the sexual state of C. gloeosporioides, from Dracaena fell within the C. gloeosporioides group as expected, allied with two other sexual state isolates. The C. gloeosporioides-complex, including G. cingulata, includes strains from diverse hosts that function as both endophytes and as plant pathogens causing leaf spots. All strains of C. gloeosporioides from Theobroma included in this study were isolated as endophytes as reported by Arnold et al. (2003) while the strains from Pueraia (kudzu) are being studied as potential biocontrol agents of this invasive weed (C. Boyette, pers. comm.). A thorough study of relationships among fungi in the C. gloeosporioides-complex is needed.

The sexual states of species of Colletotrichum are placed in the holomorph genus Glomerella, although relatively few species are known to produce the sexual state. At present only five species with a Glomerella state are recognized by Kirk et al. (2001), although 95 species names in Glomerella have been published. Glomerella produces solitary, uniloculate perithecia usually immersed in the substrata and becoming partially erumpent. The centrum development of G. cingulata was carefully described by Uecker (1994) who noted that this was similar to that of Plectosphaerella cucumerina (Uecker 1993), the species most closely related to Glomerella. In Glomerella, unbranched true paraphyses develop from the perithecial base among the ascogenous hyphae but dissolve as the asci develop and thus may not be evident in mature perithecium. The asci are unitunicate, generally lacking an apical ring, although the apex of immature asci often appears thickened. Ascospores are uniseriate, non-septate, although often guttulate, hyaline, generally ellipsoidal, and smooth-walled. Species of Glomerella/Colletotrichum are placed in the Glomerellaceae. Phylogenetic placement of this family has not been adequately determined although species of Glomerella/Colletotrichum seem to be closely allied with Plectosphaerella/Plectosporium in the Phyllachorales (Huhndorf et al. 2004). Within Glomerella/Colletotrichum few morphological distinctions between taxa can be made based on the sexual state alone. Morphological variability among species of Glomerella/Colletotrichum is generally more evident in the asexual state as is the case with other ascomycetes, e.g., Botryosphaeria/Fusisococcum/Diplodia (Alves et al. 2004); Calonectria/Cylindrocladum (Crous 2002); and Hypocrea/Trichoderma (Chaverri & Samuels 2003).

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