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Colletotrichum siamense, *C. theobromicola* and *C. queenslandicum* from several plant species and the identification of *C. asianum* in the Northern Territory, Australia

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Abstract Four species, *Colletotrichum asianum*, *C. queenslandicum*, *C. siamense* and *C. theobromicola*, were isolated and identified from several plants species in the Northern Territory, Australia. Some of these fungal associations represent first reports, namely, *C. queenslandicum* on *Passiflora edulis*; *C. siamense* on *Artocarpus heterophyllus*, *Eriobotrya japonica*, *Ficus carica*, *Mentha* sp., *Piper nigrum*, *Rosmarinus officinalis* and *Theobroma cacao*; and *C. theobromicola* on *Coffea canephora* and *C. arabica*. *Colletotrichum asianum* was isolated from mango for the first time in the NT. These collections help provide evidence for the absence of *Colletotrichum* spp. of biosecurity importance in the Northern Territory.

Keywords *Colletotrichum gloeosporioides* species complex · Anthracnose · Plant health surveillance · biosecurity

Introduction

Colletotrichum is an important genus of plant pathogenic fungi that cause postharvest rots and anthracnose on a wide range of fruit, vegetable and ornamental hosts, especially in subtropical and tropical regions (Hyde *et al*. 2009a). Despite *Colletotrichum* spp. being collectively listed in the top 10 fungal pathogens of scientific and economic importance (Dean *et al*. 2012), little is known about their host interactions. There are many accounts of *Colletotrichum* surviving asymptomatically on plant surfaces or as an endophyte within host tissue (Leandro *et al*. 2002; Damm et al. 2013; Talhinhas *et al*. 2011). Epiphytic and endophytic life phases and quiescent infection stages may precede a damaging necrotic phase in which lesions develop (Cannon et al. 2012), or may indicate variable virulence (Rojas *et al*. 2010).

In many cases, the accuracy of historical data about *Colletotrichum* spp. requires scrutiny with regard to host association, putative pathogenicity and pathogen identification (Hyde *et al*. 2009b). The identification of *Colletotrichum* spp. is further complicated by the lack or inaccessibility of many type specimens (Cai et al. 2009) and the occurrence of species complexes that are not easily resolved by morphological and single loci sequence approaches (Crouch *et al*. 2009; Damm et al. 2013; Weir *et al*. 2012). Since Weir *et al*. (2012) resolved the *C. gloeosporioides* species complex into 22 species using multi-locus phylogenies, several other novel species in this complex have been described (Peng *et al*. 2012; Doyle *et al*. 2013; Lima *et al*. 2013; Liu et al. 2013; Peng *et al*. 2013; Udayanga *et al*. 2013).

Accurate identification of host and knowledge of pathogen distributions are essential for making informed decisions in regard to biosecurity and disease management. The aim of this study was to examine isolates of *Colletotrichum* from horticultural plants in the Northern Territory (NT) in order to determine the status of species of biosecurity importance. Several species from within the *C. gloeosporioides* species complex were identified from various host plants collected in the NT during plant health surveillance carried out by members of the Northern Australia Quarantine Strategy (NAQS); a national program administered by the Commonwealth of Australia to detect biosecurity threats through targeted surveillance of plant pathogens, weeds and insect pests along coastal areas of northern Australia.

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Table 1 Colony morphology of representative isolates from this study

Species	Colony description [a]	Conidia	Isolate
Colletotrichum asianum	Greyish white with sparse aerial mycelium and abundant dark acervuli towards the centre; reverse tinged salmon; colonies about 7 cm diam.	L: (13) 14.0 – 18.5 (21) W: (4) 4.0 – 5.0 (6)	BRIP 57972a
Colletotrichum theobromicola	Grey with abundant tufted mycelium; abundant acervuli with orange conidial masses towards the centre; reverse grey; colonies covers entire plate	L: (12) 14.0 – 18.0 (21) W: (3.5) 4.0 – 5.0 (5)	BRIP 57984a
Colletotrichum siamense	White with dense cottony aerial mycelium; a few acervuli at the point of inoculation with orange conidial masses; reverse grey to pale brown; covers entire plate	L: (12) 13.5 – 17.5 (18) W: (3) 3.5 – 5.0 (5)	BRIP 57976a
Colletotrichum queenslandicum	Grey with only very scant aerial mycelium towards the centre; entirely covered with numerous, scattered acervuli that ooze orange conidial masses; covers entire plate.	L: (13) 14.0 – 16.5 (18) W: (4) 3.5 – 6.0 (9)	BRIP 57981a

[a] after 10 d at 25 ° C under 12 h near ultraviolet light/12 h dark

Materials and methods

Sample collection

Samples were collected from hosts targeting symptoms of anthracnose. Samples were collected mostly from a tropical plant nursery at Bees Creek (33 km south east of Darwin), and a garden at Middle Point (65 km south-east of Darwin); with additional specimens from Darwin and the Tiwi Islands. Samples were surface sterilised by wiping with ethanol (95 %) soaked wipes (KimTech®, Kimberly Clark, Canberra), and then moist incubated in plastic trays using paper towels soaked with sterile tap water. Plates of water agar (WA) supplemented with rifamipicin (Sigma, St Louis, Missouri) at 10 μg. L^{-1} were inoculated with a sterile needle touched onto conidial masses indicative of *Colletotrichum* that had formed on moist incubated plant tissue. Hyphal tip cultures were then maintained on agar plates of half-strength potato dextrose agar (½ PDA) (Difco™, Becton Dickinson, North Ryde, NSW). Pure cultures were deposited at the Plant Pathology Herbarium, Dutton Park, Queensland (BRIP).

Molecular identification

Mycelia were scraped off 2 wk old cultures on PDA and macerated with 0.5 mm glass beads (Daintree Scientific, St. Helens, Tasmania, Australia) in a Tissue Lyser (Qiagen, Hilden, Germany). Genomic DNA was extracted with the Gentra Puregene DNA Extraction kit (Qiagen) according to the manufacturer's instructions. The primers V9G (De Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of the ribosome genes. The primers T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) were used to amplify part of the beta-tubulin (BT) gene. All gene regions were amplified with the Phusion High-Fidelity PCR Master Mix (New England Biolab, Ipswich, MA, USA). The PCR products were purified and sequenced by Macrogen Incorporated (Seoul, Korea) on the 3730*xl* DNA Analyser (Applied Biosystems, Foster City, USA) with the amplifying primers. The ITS and BT sequences from the BRIP isolates were compared against the GenBank nucleotide database using BLASTn. A direct comparison was also made against ITS or BT sequences from ex-type cultures to verify the identification.

Morphological examination

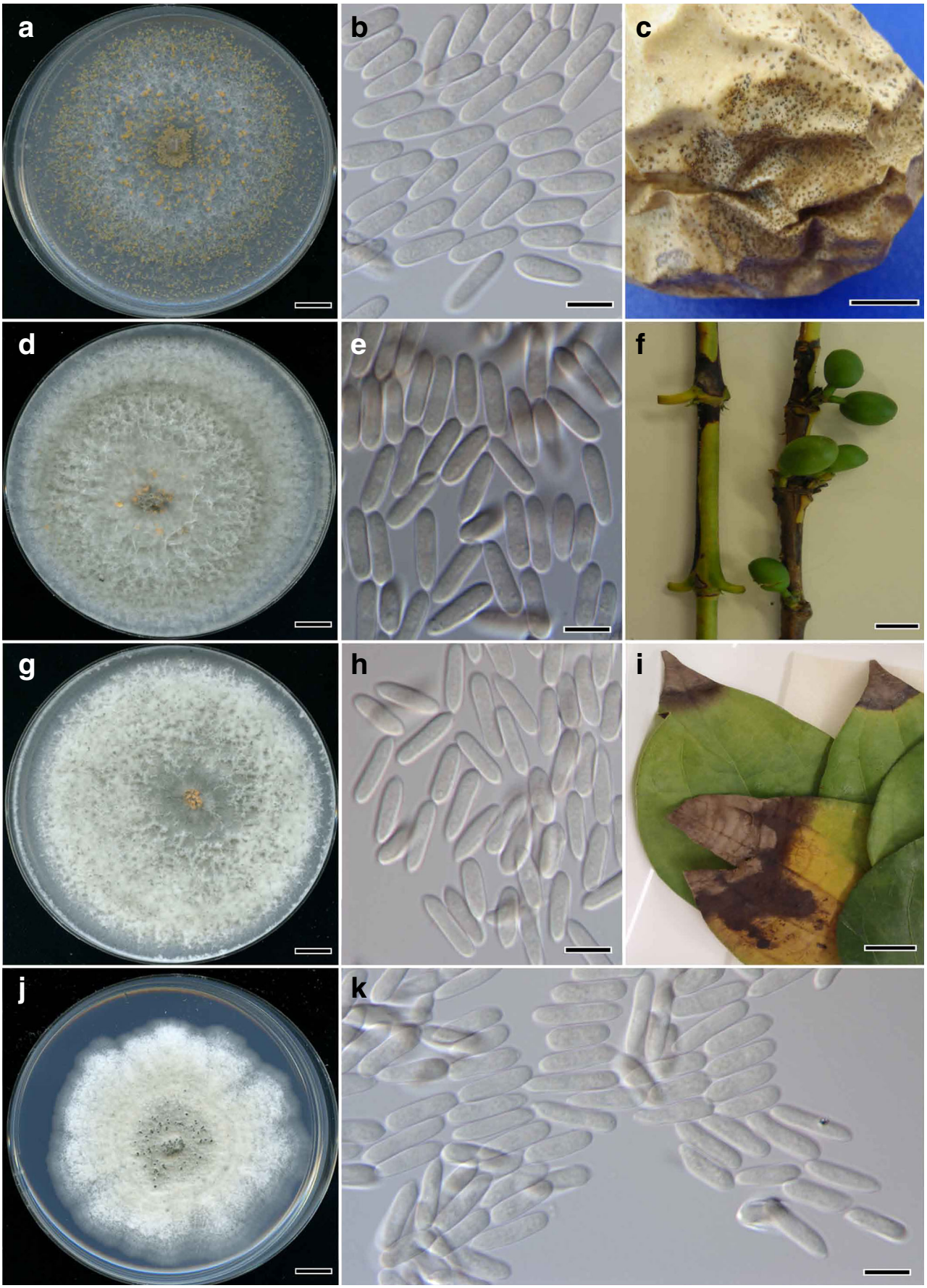
Morphological data was collected from representative cultures of the four species: Conidia were measured after 10 days incubation at 25 ° C under 12 h near ultraviolet light/12 h dark (Smith 2002) to encourage sporulation. Ranges were expressed as (min.–) mean-SD – mean+SD (–max.) with values rounded to 0.5 μm. Means and standard deviations (SD) were made from at least 20 measurements. Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast.

Results

Sample collection and morphology

Colletotrichum asianum, *C. queenslandicum*, *C. siamense* and *C. theobromicola* are recorded for the first time from the Northern Territory, Australia. The four species are briefly described from symptoms (Table 1) and morphology (Fig. 1).

Fig. 1 Morphology and conidia of isolates in this study after 10 d incubation at 25 ° C under 12 h near ultraviolet light/12 h dark. **a b** *Colletotrichum queenslandicum* (BRIP57981a) morphology and conidia from **c** *Passiflora edulis*; **d- e** *Colletotrichum theobromicola* (BRIP57984a) morphology and conidia from **f** *Coffea arabica*; **g- h** *Colletotrichum siamense* (BRIP57976a) morphology and conidia from **i** *Piper nigrum*; **j-k** *Colletotrichum asianum* (BRIP57972a) morphology and conidia from *Mangifera indica*. Scale bar for **a d g j c f i**=100 mm; **b e h k**=10 μm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Colletotrichum theobromicola was recorded from *Coffea arabica* for the first time, and *C. queenslandicum* was recorded from *Passiflora edulis* for the first time. *Colletotrichum siamense* was isolated for the first time from eight plant species: *Coffea canephora*, *Piper nigrum*, *Theobroma cacao*, *Ficus carica*, *Artocarpus heterophyllus*, *Eriobotrya japonica*, *Mentha* sp. and *Rosmarinus officinalis*. A summary of *Colletotrichum* isolates identified in this study is listed in Table 2.

Molecular Identification

One isolate was identified as *C. asianum* based on a 100 % identity to GenBank FJ972612 (ITS sequence of the ex-type strain of *C. asianum* CBS 130418). One isolate was identified as *C. queenslandicum* based on a 99 % identity to GenBank JX010414 (BT sequence of the ex-type strain of *C. queenslandicum* ICMP 1778). Thirteen isolates were identified as *C. siamense* based on a 99–100 % identity to GenBank JX010404 (the BT sequence of the ex-type strain of *C. siamense* CBS 130417). Finally, two isolates were identified as *C. theobromicola* based on a 99 % identity to GenBank JX010294 (ITS sequence of the ex-type strain of *C. theobromicola* CBS 124945). The BRIP accession numbers and isolate details are listed in Table 2.

Discussion

Most of the isolates in this study were identified as *C. siamense*, which has been reported from China, USA, the African continent, Vietnam (Weir *et al.* 2012) and Thailand (Prihastuti *et al.* 2009) on a wide range of hosts. In Australia, *C. siamense* has been recorded in New South Wales and Queensland (Weir *et al.* 2012) but has not been previously reported from the NT. *Colletotrichum siamense* is thought to be geographically diverse with a wide host range (Weir *et al.* 2012). In this study *C. siamense* was collected numerous times at both sites southwest of Darwin and from a wide range of hosts. Of the 20 isolates of *Colletotrichum* identified in this study, 16 were *C. siamense* isolated from 10 of the 12 hosts examined. A majority of these hosts did not exhibit signs of disease, and consequently *C. siamense* is likely a common and widespread saprobe or endophyte.

Colletotrichum siamense is a recently described species (Prihastuti *et al.* 2009) in the *C. gloeosporioides* species complex and very little is known about its ecology and epidemiology. Consequently, it is difficult to ascertain whether these plant associations are actually first records for *C. siamense*. It is interesting to note that BLAST comparison of BT gene sequence data of some *Colletotrichum* spp. from Phoulivong *et al.* (2010) matched that of *C. siamense* (Weir *et al.* 2012). Clarifying historical records may identify many other host associations for *C. siamense*.

Table 2 A list of isolates examined in this study

Species	Collection no.[a]	Host	Symptom	Locality [b]	GenBank no.	
					ITS [c]	**BT [d]**
C. asianum	BRIP 57972a	*Mangifera indica*	Stem dieback	Bees Creek	KF877314	
C. queenslandicum	BRIP 57981a	*Passiflora edulis*	Fruit (postharvest)	Darwin		KF877320
C. siamense	BRIP 57967b	*Artocarpus heterophyllus*	Asymptomatic leaf	Middle Point		KF877321
C. siamense	BRIP 57977a	*Artocarpus sericicarpus*	Asymptomatic leaf	Bees Creek		KF877322
C. siamense	BRIP 57970a	*Coffea arabica*	Leaf lesion	Bees Creek		KF877323
C. siamense	BRIP 57963a	*Coffea canephora*	Asymptomatic petiole	Middle Point		KF877324
C. siamense	BRIP 57964a	*Coffea canephora*	Asymptomatic stem	Middle Point		KF877325
C. siamense	BRIP 57965a	*Coffea canephora*	Asymptomatic leaf	Middle Point		KF877326
C. siamense	BRIP 57980a	*Eriobotrya japonica*	Asymptomatic leaf	Bees Creek		KF877327
C. siamense	BRIP 57979a	*Ficus carica*	Asymptomatic leaf	Bees Creek		KF877328
C. siamense	BRIP 57975a	*Mentha sp.*	Leaf lesion	Bees Creek		KF877329
C. siamense	BRIP 57976a	*Piper nigrum*	Leaf lesion	Bees Creek		KF877330
C. siamense	BRIP 57978a	*Rosmarinus officinalis*	Asymptomatic leaf	Bees Creek		KF877331
C. siamense	BRIP 57966a	*Theobroma cacao*	Asymptomatic bud	Middle Point		KF877318
C. siamense	BRIP 57966b	*Theobroma cacao*	Asymptomatic leaf	Middle Point		KF877319
C. theobromicola	BRIP 57969a	*Coffea arabica*	Flower lesion	Bees Creek	KF877316	
C. theobromicola	BRIP 57984a	*Coffea arabica*	Leaf lesion	Melville Island	KF877317	

[a] BRIP: Plant Pathology Herbarium, Dutton Park, Queensland, Australia; [b] Locality in the Northern Territory; [c] internal transcribed spacer region; [d] beta-tubulin

Colletotrichum asianum was isolated from *Mangifera indica* with stem dieback and mummified fruit. It was not confirmed if the isolate was the cause of the stem dieback although *C. asianum* is associated with fruit rots (Weir *et al*. 2012). This finding represents the first identification of *C. asianum* in the NT. *Colletotrichum asianum* is commonly recorded on *M. indica* with records from Australia (New South Wales), Columbia, Japan, Panama, Philippines (Weir *et al*. 2012) and Thailand (Phoulivong *et al*. 2010).

Colletotrichum queenslandicum was recorded on *P. edulis* from fruit showing symptoms of postharvest anthracnose. This specimen was collected from a plant that may have been imported from interstate. This species was first isolated from *Carica papaya* in Queensland, where it was originally named *C. gloeosporioides* var. *minor* (Simmonds 1968), but is now accepted as a unique species (Weir *et al*. 2012). *Colletotrichum queenslandicum* has also been reported from Fiji (Weir *et al*. 2012).

Colletotrichum theobromicola was first described from *T. cacao* by Delacroix (1905). As the type specimen was not located, a neotype was proposed by Rojas *et al*. (2010). *Colletotrichum theobromicola* has been recorded in Australia (New South Wales and Queensland), Israel, Mexico, New Zealand, Panama and USA (Weir *et al*. 2012). This species is a known pathogen on *Stylosanthes*, *Fragaria* and *T. cacao* (Rojas *et al*. 2010, Weir *et al*. 2012). In this study, *C. theobromicola* was identified from two plants of *C. arabica* from geographically different locations and associated with plant disease symptoms. This finding represents the first identification of *C. theobromicola* from *Coffea arabica*. Further investigation into the epidemiology of this isolate was beyond the scope of our study, but of note is that one of the host plants had anthracnose-like lesions on the stem. This plant was situated in the shade and sampled near the end of the wet season (Mar.). The second plant exhibited symptoms of anthracnose on leaves, stems and flowers, and was growing in full sun at the end of the dry season (Oct.). Koch's postulates were not completed and currently there is no evidence in the literature that indicates *C. theobromicola* is a pathogen of coffee.

This work has expanded the distribution and host range of four species from the *C. gloeosporioides* species complex; *C. asianum*, *C. queenslandicum*, *C. siamense* and *C. theobromicola*. The identification of these species alone provides background information for biosecurity risk assessment processes. Unravelling the ecology and host interactions of these fungi will eventually lead to a better understanding of the role these species play as pathogens, with consequent improved disease management and biosecurity outcomes.

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