Epitypification and phylogeny of *Colletotrichum acutatum* J.H. Simmonds

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Simmonds introduced *Colletotrichum acutatum* in 1965, validated in 1968, with a broad concept, as demonstrated by the selection of several type specimens from a range of hosts. This has created some confusion in the species concept and identification of *C. acutatum*. There are no viable ex-type cultures of *C. acutatum* and furthermore there are no existing cultures of *C. acutatum* on *Carica papaya* from the type locality in south-east Queensland. The application of molecular phylogenetic studies to isolates of *C. acutatum* is only meaningful if the taxonomy is stable and species are properly named. In order to clarify the species concept of *C. acutatum*, an isolate of *Colletotrichum acutatum* from *Carica papaya* from Yandina in Southeast Queensland (Australia) is designated as an epitype. A detailed morphological description is provided. Phylogenies based on a combined ITS and beta-tubulin gene analysis indicate that *C. acutatum* bears close phylogenetic affinities to *C. gloeosporioides* and *C. capsici*. Results also indicate that *C. acutatum* is monophyletic and there is a close relationship between the epitype and other Australian *C. acutatum* isolates from *Carica papaya.* Molecular data, however did not provide further evidence to properly elucidate the taxonomic affinities of *C. acutatum* especially the holotype and epitype. Our studies indicate that given the complexity of the genus *Colletotrichum*, there is a need to check previously described type specimens and redesign neotypes where necessary in order to clarify taxonomic uncertainties.

Key words: *Carica papaya*, *Colletotrichum acutatum*, epitype, plant pathogen, taxonomy

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Introduction

The taxonomy of *Colletotrichum acutatum* is ambiguous (Sreenivasaprasad and Talhinhas, 2005). The broad putative host range has created problems for plant pathologists who need to identify specific plant pathogens for disease management. Molecular techniques have provided additional data for solving problems in fungal identification, and have been applied to taxonomically difficult genera including *Fusarium* (O'Donnell *et al*., 1998, Maxwell *et al*., 2006), *Pestalotiopsis* (Jeewon *et al*., 2002, 2003a,b, 2004, Lui *et al*., 2007), *Mycosphaerella* and its anamorphs (Crous *et al*., 2000; Mancini *et al*., 2005), and to a lesser extent to *Colletotrichum* (Sreenivasaprasad *et al*., 1996; Moriwaki *et al*., 2002; Du *et al*., 2005; Photita *et al*., 2005; Than *et al*., 2007). Gene sequence data is increasingly used to solve taxonomic problems from species level through to higher taxonomic levels. However these studies are generally flawed by the fact the type species have not been studied.

Table 1. The type specimens of *Colletotrichum acutatum* (Simmonds 1968)

QDPI&F Plant Disease Log Book No.	BRIP (ex BRIU) Specimen type	IMI accession no	Geographic location/ date of collection/ collector	Host plant
16741B1	BRIP 4693 (ex BRIU 2437) Microscope slide **Isotype**	IMI 117617 **Holotype**	Ormiston*, Brisbane, Queensland, Australia/ J.H.Simmonds / 1 Oct. 1965	*Carica papaya*
16741B	Not held in BRIP (ex BRIU 2431) **Isoparatype**	IMI 117618 **Paratype**	Ormiston*, Brisbane, Queensland, Australia/ J.H.Simmonds / 1 Oct. 1965	*Carica papaya*
16741A - pink type	Not held in BRIP (ex BRIU 2432) **Isoparatype**	IMI 117619 **Paratype**	Ormiston*, Brisbane, Queensland, Australia/ J.H.Simmonds / 1 Oct. 1965	*Carica papaya*
16633D – pink type	BRIP 49837 (ex BRIU 2435) Dried culture plates **Isoparatype**	IMI 117620 **Paratype**	Ormiston*, Brisbane, Queensland, Australia/ J.H.Simmonds / 5 Jul. 1965	*Carica papaya*
16633A	BRIP 49838 (ex BRIU 2434) Dried culture plates **Isoparatype**	IMI 117621 **Paratype**	Ormiston*, Brisbane, Queensland, Australia/ J.H.Simmonds / 5 Jul. 1965	*Carica papaya*
11711A	BRIP 4684, BRIP 11083 (ex BRIU 2433) Dried culture plates and living culture **Isoparatype**	IMI 117622 **Paratype**	Eight Mile Plains, Brisbane, Queensland, Australia/ J.H.Simmonds / 14 Jul. 1955	*Capsicum frutescens*
16738C – pink type	BRIP 4697 (ex BRIU 2436) Dried culture plates **Isoparatype**	IMI 117623 **Paratype**	Nambour, Queensland, Australia/ K.G.Pegg / 1 Oct. 1965	*Delphinium* sp.
13483-0	BRIP 11084 Non-viable lyophilized culture **Topotype**	-	Ormiston*, Brisbane, Queensland, Australia/ J.H.Simmonds / 29 Jan. 1959	*Carica papaya*

Further more, species in the taxonomically different genera are often identified without reference to the type specimens (e.g. characterization of causal agents from chilli anthracnose in Thailand by Than *et al*., 2007) and one must question whether the taxon was correctly identified. No matter how many strains of fungi are sequenced, unless the type material is examined, taxonomic implications are speculative and conclusions are weak because there is no certainty that the strains used represent the type of the species or genus. In order to stabilize the application of the species names, it is necessary to examine and sequence types (preferably the holotype or isotypes) if they exist. If this is not possible then it may be necessary to examine paratypes or even to designate an epitype that clearly represents the type species.

When Simmonds (1965) initially published the name *Colletotrichum acutatum* he omitted to designate a type specimen. In order to rectify this Simmonds (1968) listed a holotype (IMI 117617) and six paratypes (IMI 117618-117623) with the corresponding isotype and six isoparatypes grouped together (BRIU 2431-2437). Importantly, Simmonds' type specimens were selected from three hosts: the holotype and four paratypes from *Carica papaya*, and a paratype from each of *Capsicum frutescens* and *Delphinium ajacis*. Unfortunately this was not made clear when he listed

the type specimens (Simmonds, 1968). All of the specimens were dried agar cultures. The BRIU specimens were subsequently incorporated into herbarium BRIP. All of these type specimens have been examined during this study and corresponding IMI and BRIP accession numbers were tabulated (Table 1). Two of the type specimens (dried cultures) viz. the holo-type (IMI 117617) and the paratype (IMI 117619), both from *Carica* papaya, have been subjected to phylogenetic analysis specifically of the rDNA ITS region (Vinnere *et al*., 2001).

Simmonds (unpublished correspondence with IMI) chose type specimens that showed the range of characters he considered represented the inherent variability of this taxon. Simmonds' (1965) concept of *Colletotrichum acutatum* was that it had conidia that were variable in length and infected a range of hosts, causing particularly serious diseases on papaya and strawberry. The distinguishing morphological character was that the conidia had pointed ends.

In our view, Simmonds' (1965, 1968) broad concept of *Colletotrichum acutatum*, as demonstrated by the selection of several type specimens from a range of hosts, has created uncertainties in the species concept of *C. acutatum*. There are no viable ex-type cultures of *Colletotrichum acutatum* and further there are no viable cultures of *Colletotrichum acutatum* on papaya from the type locality (Queensland Department of Primary Industries & Fisheries, Redlands Research Station, Ormiston, Brisbane, Queensland, Australia).

Article 9.7 of the International Code of Botanical Nomenclature (Vienna Code, 2006) states that "An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name of a taxon. When an epitype is designated, the holotype, lectotype, or neotype that the epitype supports must be explicitly cited." We have consequently chosen an isolate of *C. acutatum* (BRIP 28519) from *Carica papaya* from Yandina in South-east Queensland (Australia) to represent the epitype of *C. acutatum*. In addition, phylogenetic analyses from DNA sequences generated from the rDNA-ITS regions and β-tubulin gene were performed to investigate the relationships with other closely related species.

Description

Colletotrichum acutatum J.H. Simmonds, Queensland J. Agric. Anim. Sci. **25**: 178A (1968).

= *Colletotrichum acutatum* J.H. Simmonds, Queensland J. Agric. Anim. Sci. **22**: 458 (1965), nom. inval.

Holotype: AUSTRALIA, Queensland, Brisbane, Ormiston, Redlands Research Station, on *Carica papaya*, 1 Oct. 1965, J.H. Simmonds. (IMI 117617; **isotype** BRIP 4693 ex BRIU 2437).

Epitype**:** MycoBank: 511272. AUSTRALIA, Queensland, Yandina, on fruit of *Carica papaya*, May 1987, L.M. Coates, specimen of living culture stored at QDPI&F Plant Pathology Herbarium, Indooroopilly, Australia (BRIP 28519); ex-epitype living culture in Biotech Culture Collection, Thailand (BCC 28680), Centraalbureau voor Schimmelcultures, Netherlands (CBS122122), The University of Hong Kong Culture Collection, the University of Hong Kong, Hong Kong (HKUCC 10928), Korean Agricultural Culture Collection (KACC 43258) and International Collection of Microorganisms from Plants (ICMP 17298).

Colonies on PDA circular, raised, at first orange-white, sometimes grey and becoming pale orange with age, aerial mycelia white, dense, cottony without visible conidial masses, reverse bright orange but sometimes yellowish-brown to olive-brown, very slow-growing with growth rate of 2.3-2.6 mm ($\bar{x}$ = 2.5 ± 0.14, n = 5) (Fig. 1). *Sclerotia* absent. *Acervuli* absent in culture. *Setae* absent. *Conidiophores* 3-45 µm long ($\bar{x}$ = 20 ± 13.44, n = 10) × 2-6 µm wide ($\bar{x}$ = 4 ± 1.58, n = 10) hyaline, cylindrical, unicellular common, but sometimes septate, single common, but sometimes aggregated and branched, tapering towards the end, acute at the apex. *Conidiogenous cells* 6-10 µm long ($\bar{x}$ = 8 ± 1.13, n = 10) × 2.5-4 µm wide ($\bar{x}$ = 3 ± 0.42, n = 10) hyaline, ellipsoidal to subglobose, smooth, tapering towards a truncate apex. *Conidia* common in mycelium 7-14 µm long ($\bar{x}$ = 10 ± 1.67, n = 20) × 2.5-3.5 µm wide ($\bar{x}$ = 3 ± 0.18, n = 20), one-celled, gluttulate, hyaline, fusiform with both ends pointed. *Appressoria* in slide cultures 7-15 µm long

Table 2. Sources of isolates used in this study and reference sequences from Gen Bank used in analysis

Culture collection accession number	Gene bank Acc# ITS	β- tubulin	Isolates / source	*Colletotrichum* species	Location	Host
BCC 28680 CBS 122122 ICMP 17298 HKUCC 10928 KACC 43258	EF143974	EF143970	BRIP28519/ Than *et al*., 2007	*C. acutatum*	Yandina, QLD, Australia	*Carica papaya*
-	EF143975	-	BRIP28517/ Than *et al*., 2007	*C. acutatum*	Yandina, QLD, Australia	*Carica papaya*
-	EF143971	EF143967	BRIP4703/ Than *et al*., 2007	*C. acutatum*	Townsville, QLD, Australia	*Fragaria ananassa*
-	EF143972	EF143968	BRIP4704/ Than *et al*., 2007	*C. acutatum*	Forest Glen, QLD, Australia	*Fragaria ananassa*
-	EF143973	EF143969	BRIP11086/ Than *et al*., 2007	*C. acutatum*	Nambour, QLD, Australia	*Fragaria ananassa*
HKUCC 10891	DQ453418	DQ454066	S2/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Fragaria* sp.
HKUCC 10872	DQ453419	DQ454063	S3/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Fragaria* sp.
HKUCC 10873	DQ453420	DQ454062	S4/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Fragaria* sp.
HKUCC 10890	DQ453421	DQ454065	S5/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Fragaria* sp.
HKUCC 10814	DQ453422	DQ454064	S6/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Fragaria* sp.
HKUCC 10871	DQ453423	DQ454067	S7/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Fragaria* sp.
HKUCC 10848	DQ453406	DQ454058	Mj2/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10865	DQ453407	-	Mj3/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10879	DQ453408	DQ454059	Mj4/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10851	DQ453409	DQ454060	Mj5/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10893	DQ453410	DQ454061	Mj6/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10850	DQ453411	DQ454068	Mj9/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10894	DQ453412	DQ454069	Mj10/ Than *et al*., 2007	*C. acutatum*	Chiangmai, Thailand	*Capsicum annuum*
-	AY266405	-	G2/Photita *et al* 2004	*C. acutatum*	Thailand	*Fragaria* sp.
-	AB042301	-	MAFF 306406/ Moriwaki *et al*, 2002	*C. acutatum*	Japan	*Eriobotrya japonica*
-	AF411701	-	IMI 117619(holotype)/Vinnere *et al*, 2001	*C. acutatum*	Australia	*Carica papaya*
-	AJ301921	-	BBA 70349/ Nirenberg *et al*., 2002	*C. acutatum*	Indonesia	*Capsicum* sp.
-	AJ301920	-	BBA 70348/ Nirenberg *et al*., 2002	*C. acutatum*	Indonesia	*Capsicum* sp.
-	AJ748617	-	PR220/ Talhinhas *et al*., 2005	*C. gloeosporioides*	Portugal	*Olea europaea*
-	AJ748616	-	VM206/ Talhinhas *et al*., 2005	*C. gloeosporioides*	Portugal	*Olea europaea*
-	AJ314718	-	8/ Talhinhas *et al*., 2002	*Colletotrichum* sp	Australia	*Fragaria* sp.

Table 2 (continued). Sources of isolates used in this study and reference sequences from Gen Bank used in analysis

Culture collection accession number	Gene bank Acc# ITS	β- tubulin	Isolates / source	*Colletotrichum* species	Location	Host
-	AB255249	-	JP18/Morakotkarn *et al* (unpublished)	*Colletotrichum* sp.	Japan	*Bambusa* sp.
-	AY266381	-	ZE0040/Photita *et al*., 2004	*C. gloeosporioides*	Thailand	*Fragaria* sp.
-	-	AJ748633	CA455/ Talhinhas *et al*., 2002	*C. acutatum*	*United Kingdom*	*Photinia* sp.
-	-	AJ748611	PT186/ Talhinhas *et al*., 2005	*C. acutatum*	*Portugal*	*Olea europaea*
-	-	AJ748608	PT166/ Talhinhas *et al*., 2005	*C. acutatum*	*Portugal*	*Olea europaea*
-	-	AJ748607	PT135/ Talhinhas *et al*., 2005	*C. acutatum*	*Portugal*	*Olea europaea*
-	-	AJ748615	PT 201/ Talhinhas *et al*., 2005	*C. acutatum*	*Portugal*	*Olea europaea*
-	-	AJ748605	PT108/ Talhinhas *et al*., 2005	*C. acutatum*	*Portugal*	*Olea europaea*
-	-	AJ314722	1/ Talhinhas *et al*., 2005	*Colletotrichum* sp	*Portugal*	*Lupinus albus*
-	-	AJ409300	3/ Talhinhas *et al*., 2005	*C. acutatum*	Portugal	*Lupinius albus*
-	-	AJ409296	99/ Talhinhas *et al*., 2005	*C. acutatum*	USA	*Fragaria* sp.
-	-	AJ748636	PD88-673 / Talhinhas *et al*., 2002	*C. acutatum*	Netherlands	*Anemone* sp.
-	-	AJ314716	20/ Talhinhas *et al*., 2002	*Colletotrichum* sp.	Portugal	*Fragaria* sp
-	-	AJ409298	2/ Talhinhas *et al*., 2002	*Colletotrichum* sp.	Portugal	*Lupinus albus*
-	-	AJ300709	15/ Talhinhas *et al*., 2002	*Colletotrichum* sp.	Portugal	*Lupinus albus*
-	-	AJ314712	29 /Talhinhas *et al*., 2002	*Colletotrichum* sp	Portugal	*Lupinus* sp.
-	-	AJ314720	PT232/Talhinhas *et al*., 2002	*C. acutatum*	Portugal	*Olea europaea*
-	-	AJ748632	PD85-694/ Talhinhas *et al*., 2005	*C. acutatum*	Netherlands	*Chrysanthemum* sp.
-	-	AJ748635	PD89-582/Talhinhas *et al*., 2005	*C. acutatum*	Netherlands	*Cyclamen* sp.
HKUCC 10883	DQ453992	DQ454036	Ku2/ Than *et al*., 2007	*C. gloeosporioides*	Ratchaburi, Thailand	*Capsicum annuum*
HKUCC 10864	DQ453995	DQ454030	Ku5/ Than *et al*., 2007	*C. gloeosporioides*	Kanchanaburi, Thailand	*Capsicum annuum*
HKUCC 10889	DQ453996	DQ454034	Ku6/ Than *et al*., 2007	*C. gloeosporioides*	Kanchanaburi, Thailand	*Capsicum annuum*
HKUCC 10860	DQ453401	DQ454043	M1/ Than *et al*., 2007	*C. gloeosporioides*	Chiangmai, Thailand	*Mangifera indica*
HKUCC 10861	DQ453402	-	M2/ Than *et al*., 2007	*C. gloeosporioides*	Chiangmai, Thailand	*Mangifera indica*
HKUCC 10862	DQ453403	-	M3/ Than *et al*., 2007	*C. gloeosporioides*	Chiangmai, Thailand	*Mangifera indica*
HKUCC 10863	DQ453404	DQ454046	M4/ Than *et al*., 2007	*C. gloeosporioides*	Chiangmai, Thailand	*Mangifera indica*
HKUCC 10849	DQ453405	DQ454047	M5/ Than *et al*., 2007	*C. gloeosporioides*	Chiangmai, Thailand	*Mangifera indica*
HKUCC 10876	DQ453427	DQ454050	U10/ Than *et al*., 2007	*C. capsici*	Ubonrachathani, Thailand	*Capsicum annuum*
HKUCC 10867	DQ453428	DQ454051	U12/ Than *et al*., 2007	*C. capsici*	Ubonrachathani, Thailand	*Capsicum annuum*
HKUCC 10852	DQ453414	DQ454056	R5/ Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*

Table 2 (continued). Sources of isolates used in this study and reference sequences from Gen Bank used in analysis

Culture collection accession number	Gene bank Acc# ITS	β- tubulin	Isolates / source	*Colletotrichum* species	Location	Host
HKUCC 10869	DQ453415	DQ454053	R7/ Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10870	DQ453416	DQ454049	R11/ Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10880	DQ453417	-	R12/ Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10857	DQ453988	DQ454047	Ccmj3/ Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10859	DQ453990	DQ454054	Ccmj10/ Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*
HKUCC 10855	DQ453424	DQ454052	Skp4 / Than *et al*., 2007	*C. capsici*	Chiangmai, Thailand	*Capsicum annuum*
-	AY376544	AY376591	STE-U5291/ Lubbe *et al*., 2004	*C. gloeosporioides*	USA	*Fragaria* sp.
-	AY376508	AY376556	STE-U5303/ Lubbe *et al*., 2004	*C. acutatum*	India	*Hevea brasiliensis*
-	AY376526	AY376574	STE-U5304/ Lubbe *et al*., 2004	*C. capsici*	Tanzania	*Arachis hypogaea*
-	AY376510	AY376558	STE-U5292/ Lubbe *et al*., 2004	*Glomerella acutata*	South Africa	*Olea europaea*
-	AY376525	AY376573	STE-U2289/ Lubbe *et al*., 2004	*C. boninense*	-	-
	AJ749679	AJ748636	PD-88-673/ Talhinhas *et al*., 2005	*C. acutatum*	Netherlands	*Anemone* sp.

BRIP: Queensland Department of Primary Industries Plant Pathology Herbarium; CAB: Centraalbureau voor Schimmelcultures, Netherlands; HKUCC: The University of Hong Kong Culture Collection, The University of Hong Kong, Hong Kong; KACC: Korean Agricultural Culture Collection; ICMP: International Collection of Microorganisms from Plants; STE-U: University of Stellenbosch Culture Collection.

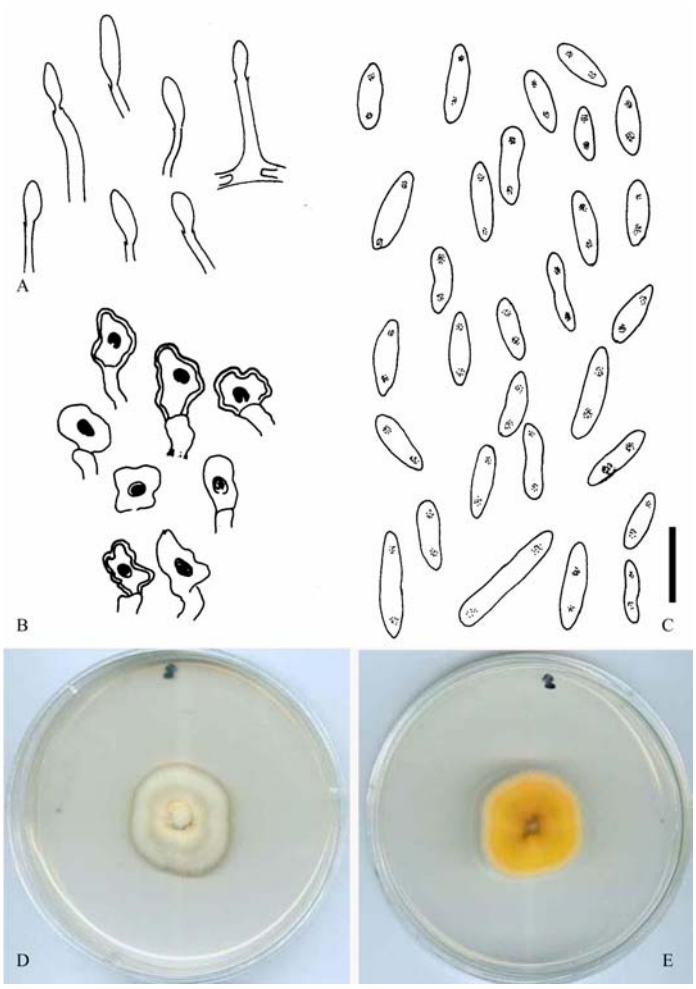

Fig. 1. *Colletotrichum acutatum*. **A.** Conidiogenous cell. **B.** Appressoria. **C.** Conidia. **D & E.** upward and reverse sides of culture on PDA 7-days after inoculation of epitype (BRIP 28519) (Bar = 15 µm).

($\bar{x}$ = 10.5 ± 2.46, n = 20) × 5-8 µm long ($\bar{x}$ = 6 ± 0.84, n = 20), dark brown, ovate, clavate and sometimes irregularly lobed. Supporting hyphae hyaline, branched and septate.

Additional specimens examined: AUSTRALIA, Queensland, Brisbane, Ormiston, Redlands Research Station, on *Carica papaya*, 1 Oct. 1965, J.H. Simmonds (**paratype** in IMI 117618, ex BRIU 2431, not held in BRIP); Queensland, Brisbane, Ormiston, Redlands Research Station, on *Carica papaya*, 1 Oct. 1965, J.H. Simmonds (**paratype** IMI 117619, ex BRIU 2432, not held in BRIP); Queensland, Brisbane, Ormiston, Redlands Research Station, on *Carica papaya*, 5 Oct. 1965, J.H. Simmonds (**paratype** BRIP 49837 ex BRIU 2435, IMI 117620); Queensland, Brisbane, Ormiston, Redlands Research Station, on *Carica papaya*, 5 Oct. 1965, J.H. Simmonds (**paratype** in BRIP 49838 ex BRIU 2434, IMI 117621); Queensland, Brisbane, Eight Mile Plains, on *Capsicum frutescens*, 14 Jul. 1955, J.H. Simmonds (**paratype** BRIP 4684 = BRIP 11083 ex BRIU 2433, IMI 117622); Queensland, Nambour, on *Delphinium ajacis*, 1 Oct. 1965, K.G. Pegg (**paratype** BRIP 4697 ex BRIU 2436, IMI 117623); Queensland, Upper Ross River, Townsville, on *Fragaria ananassa*, May 1971, W. Pont (BRIP 4703); Queensland, Forest Glen, Sep. 1972, R.A. Peterson, (BRIP 4704); Queensland, Nambour, Mar. 1965, K.G. Pegg (BRIP 11086); THAILAND**,** Chiangmai, Mae Jo-Sansai Street, Mae Doo, on *Capsicum annuum*, 24 Sep. 2005, P.P. Than (HKUCC 10848, HKUCC 10865, HKUCC 10879, HKUCC 10851, HKUCC 10893, HKUCC 10850).

Phylogenetic assessment of *Colletotrichum acutatum* epitype

The phylogeny of the epitype and closely related species were investigated using individual and combined sequence analyses of nuclear ribosomal DNA (ITS rDNA) and beta tubulin gene fragments. ITS ribosomal DNA based phylogenies indicate that all *C. acutatum*

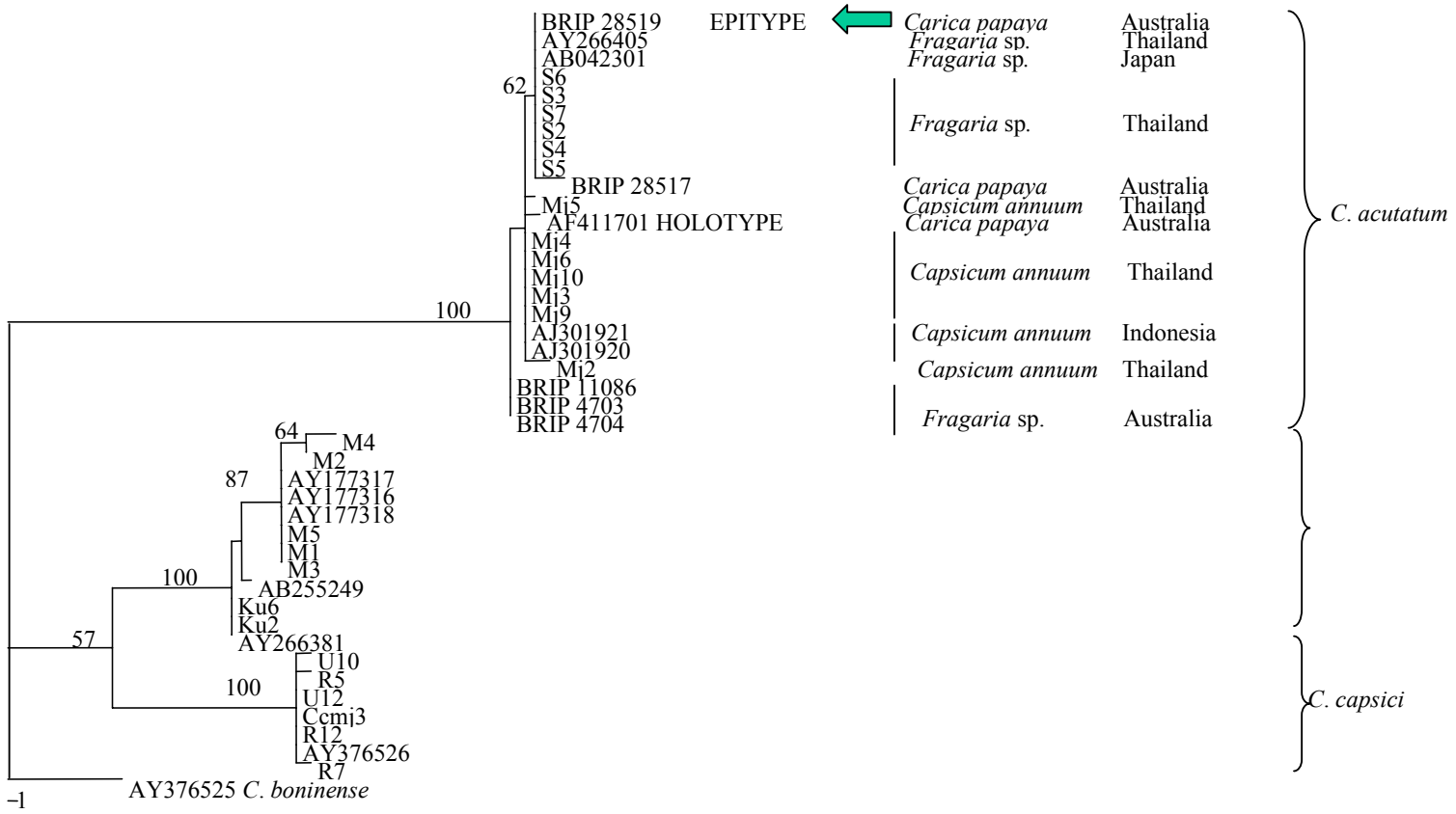

Fig. **2**. Phylogram of one of twelve trees generated from parsimony analysis based on rDNA-ITS sequences of *Colletotrichum acutatum* isolates (TL = 104.00, CI = 0.885, RI = 0.987, RC = 0.873, HI = 0.115). Data were analysed with random addition sequence, weighed parsimony and treating gap as missing data. Values above branching node indicate bootstrap supports obtained from bootstrap analysis with 1000replicates. (Bar = 1 % sequence divergence).

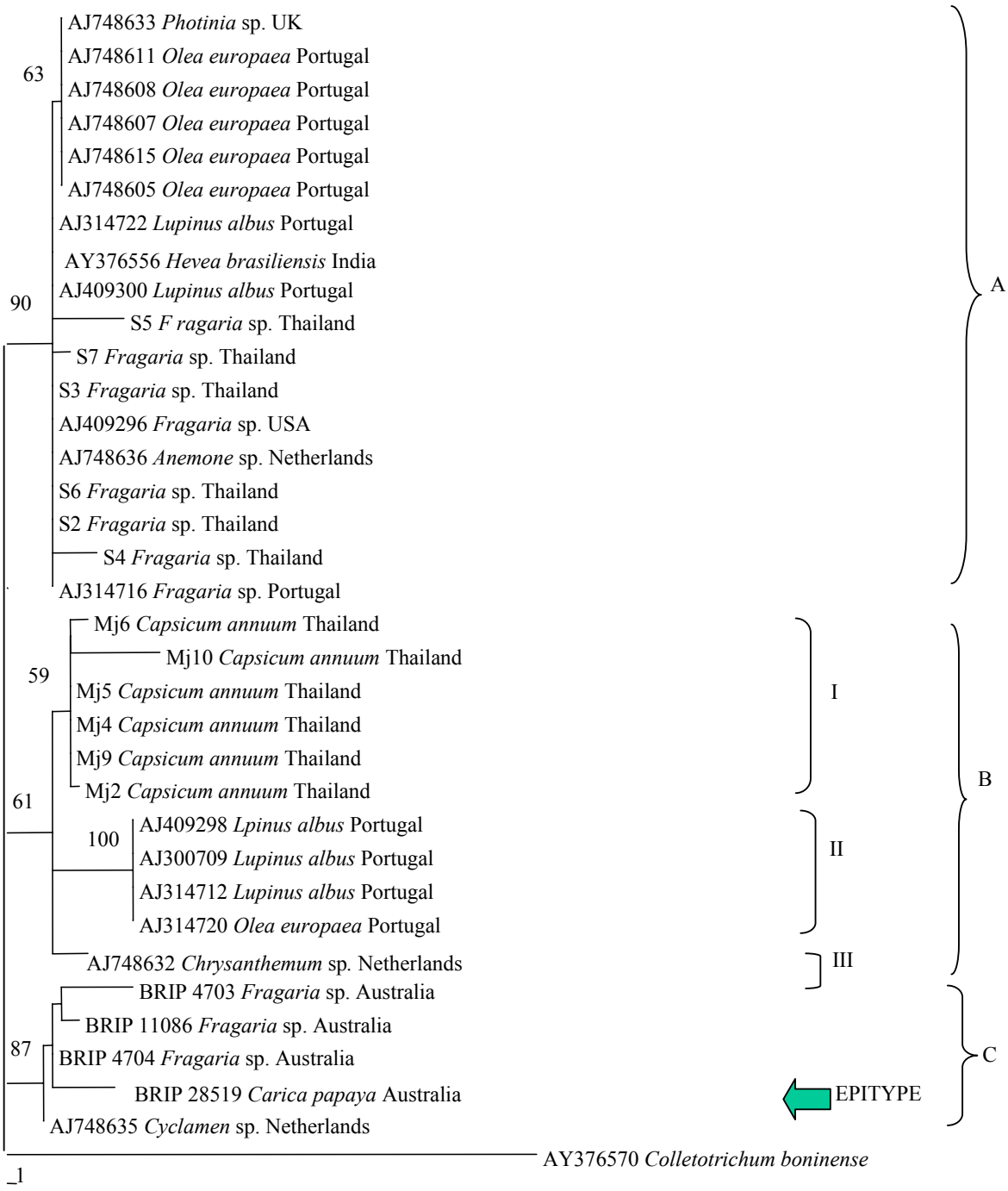

Fig. 3. Phylogram of one of four trees generated from parsimony analysis based on β-tubulin (tub2) sequences of *Colletotrichum acutatum* isolates (TL=104.00, CI=0.885, RI=0.987, RC=0.873, HI=0.115). Data were analysed with random addition sequence, weighed parsimony and treating gap as missing data. Values above branching node indicate bootstrap support obtained from 1000replicates. Tree is rooted with *Colletotrichum boninense*. (Bar = 1 % sequence divergence).

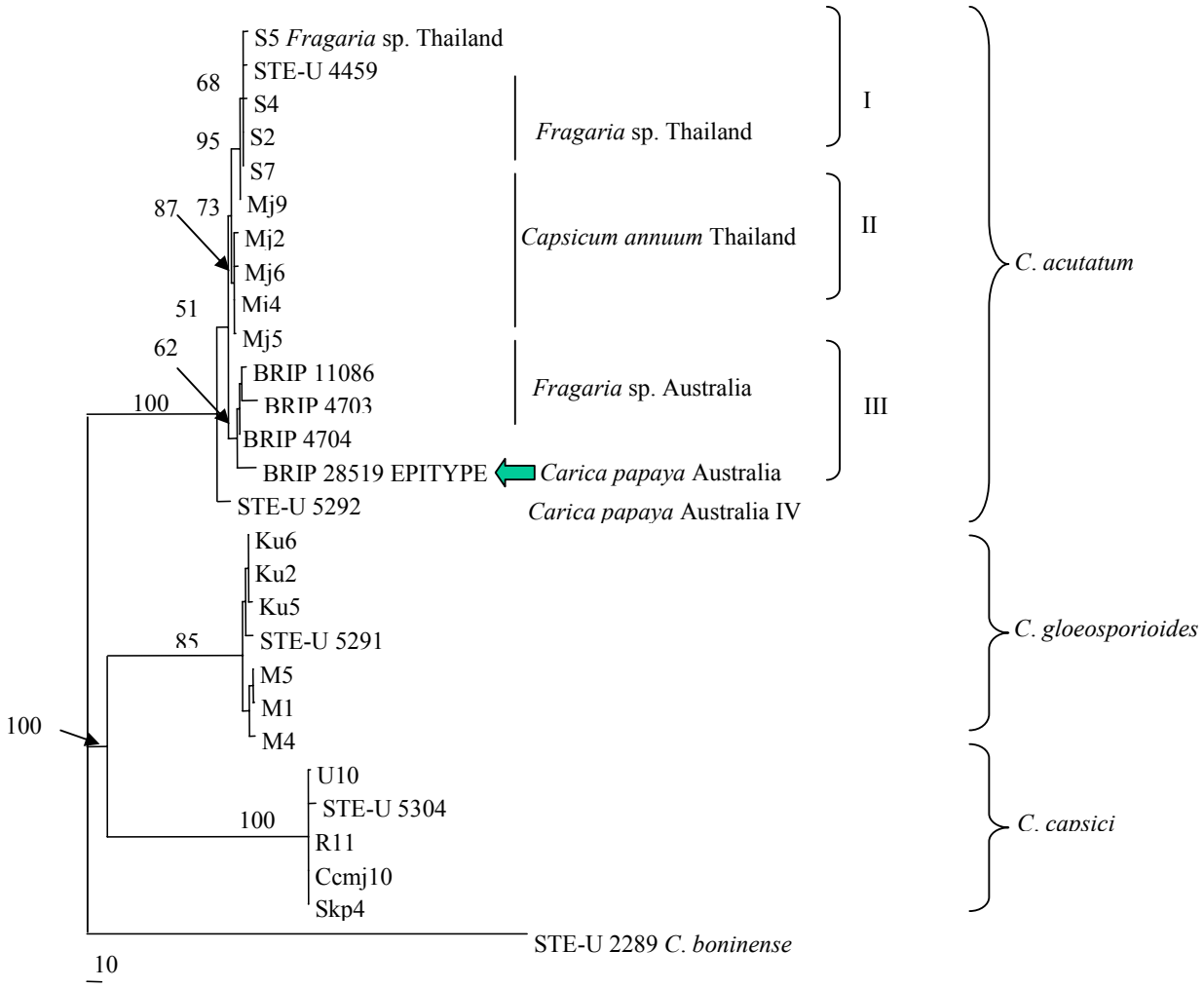

Fig. 4. Phylogram of single tree generated from parsimony analysis based on rDNA ITS and b-tubulin (tub2) sequences of *Colletotrichum acutatum* isolates (TL = 540, CI = 0.830, RI = 0.958, RC = 0.843, HI = 0.120). Data were analysed with random addition sequence, weighed parsimony and treating gap as missing data. Values above branching node indicate bootstrap supports obtained from 1000replicates. (Bar = 10 % sequence divergence).

strains, including the holotype sequence (AF 411701) from *Capsicum*, *Carica papaya* and *Fragaria* hosts constitute a strongly supported monophyletic clade (100% BS, Fig 2). Within this monophyletic clade, there are 3 subclades, albeit poorly supported. In particular it is noted that BRIP 28517 and the holotype are in different subclades although they have been isolated from the same host and region. The basal subclade consists of BRIP 11086, BRIP 4703 and BRIP 4704, which were all isolated from *Fragaria*. Conclusive relationships based on host associations of *C. acutatum* is unwise as there are a number of isolates that have been isolated from *Fragaria*, but they cluster with the epitype which has been isolated from *Carica papaya*. Host fungal relationships based on phylogeny have been discussed in detail in Jeewon *et al*. (2004) and any association between hosts and species within *Colletotrichum* does not seem to be justified at present.

In contrast, phylogeny from partial sequences of beta-tubulin 2-gene analysis depicts a slightly different result (Fig. 3). The epitype clusters with other *C. acutatum*

Australian isolates such as BRIP 11086, BRIP 4703 and BRIP 4704 with relatively high support (87%). There is a similar phylogenetic scenario from the combined dataset (Fig. 4). At present, given the limited taxon sampling, unknown or doubtful identify of other isolates, any conclusive phylogenetic affinities of the epitype could be misleading. Further studies, are necessary to clarify intra-specific phylogenetic relationships of *Colletotrichum acutatum*. The designation of an epitype, available as an ex-epitype culture at BRIP, CBS, HKUCC, KACC and ICMP (Table 2) will help remove ambiguity about the application of the name *C. acutatum*.

The diversity of pathogenic species of *Colletotrichum* in the South East Asian region is largely underexplored (Photita *et al*., 2005). In addition, species are morphologically complex and inter (or infra) species classification has been hampered by either incomplete or inadequate characterization (Cannon *et al*., 2000). A number of previously described species are either in poor condition or cannot provide essential data for molecular documenttation. There is a need to review and redescribe a number of previously described specimens and in many cases, neotypes, lectotypes or epitypes will have to be designated. This has been established in *Botryosphearia* (Phillips *et al*., 2007) and *Diplodia* (Alves *et al*., 2006). This paper is a continuity of our previous effort to revise economically important species of *Colletotrichum* (Shenoy *et al*., 2007; Than *et al*., 2007). In these studies we have identified pitfalls with phylogenetic analyses and previously described type specimens. With the availability of epitypes from recent collections and cultures derived from them, we believe that this is an important direction in the systematics of these fungi and hope that there will be a steady progression in resolving their taxonomy.

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References

Alves, A., Correia, A. and Phillips, A.J.L. (2006). Multigene genealogies and morphological data support *Diplodia cupresis* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. Fungal Diversity 23: 1-15.

Cannon, P.F., Bridge, P.D. and Monte, E. (2000). Linking the past, present, and future of *Colletotrichum* systematics. In: *Colletotrichum: Host specificity, Pathology, and Host-pathogen interaction*. (eds. D. Prusky, S. Freeman and M. Dickman). APS Press, USA: 1-20.

Crous, P.W., Aptroot, A., Kang, J.C., Braun, U. and Wingfield, M. J. (2000). The genus *Mycosphaerella* and its anamorphs. Studies in Mycology 45: 107-121.

Du, M., Schardl, C.L. and Vaillancourt, L.J. (2005). Using mating-type gene sequences for improved phylogenetic resolution of *Colletotrichum* species complexes. Mycologia 97: 641-658.

Jeewon, R., Liew, E.C.Y. and Hyde, K.D. (2002). Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters. Molecular Phylogenetics and Evolution 25: 378-392.

Jeewon, R., Liew, E.C.Y., Simpson, J.A., Hodgkiss, I.J. and Hyde, K.D. (2003a). Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. Molecular Phylogenetics and Evolution 27: 372-383.

Jeewon, R., Cai, L., Zhang. K. and Hyde, K.D. (2003b). *Dyrithiopsis lakefuxianensis* gen et sp. nov. from Fuxian Lake, Yunnan, China and notes on the taxonomic confusion surrounding *Dyrithium*. Mycologia 95: 911-920.

Jeewon, R., Liew, E.C.Y. and Hyde, K.D. (2004). Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. Fungal Diversity 17: 39-55.

Lui, A.R., Xu, T. and Guo, L.D. (2007). Molecular and morphological description of *Pestalotiopsis hainanensis* sp. nov., a new endophyte from a tropical region of China. Fungal diversity 24: 23-26.

Lubbe, C.M., Denman, S., Cannon, P.F., Groenewald, J.Z., Lampretcht, S.C. and Crous, P.W. (2007) Characterization of *Colletotrichum* species associated with diseases of *Proteaceae*. Mycologia 96: 1268-1279.

Mancini, N., Perotti, M., Ossi, C.M., Cavallero, A., Matuka, S., Paganoni, G., Burioni, R., Rama, P. and Clementi, M. (2006). Rapid molecular identification of fungal pathogens in corneal samples from suscepted keratomycosis cases. Journal of Medical Microbiology 55: 1505-1509.

Maxwell, A., Jackson, S.L., Dell, B. and Hardy, G.E. (2005). PCR-identification of *Mycospharella* species associated with leaf diseases of *Eucalyptus*. Mycological Research 109: 992-1004.

Moriwaki, J., Tsukiboshi, T. and Sato, T. (2002). Grouping of *Colletotrichum* species in Japan based on r DNA Sequences. Journal of General Plant Pathology 68: 307-320.

Nirenberg, H.I., Feiler, U., Hagedorn, G. (2002). Description of *Colletotrichum lupini* comb. nov. in modern terms. Mycologia 94:307-320.

O'Donnell, K., Cigilink, E. and Nirenberg, H.I. (1998). Molecular systematics and pylogeography of the *Giberella fujikuroi* species complex. Mycologia 90: 465-493.

Phillips, A.J.L., Crous, P.W. and Alves, A. (2007). *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. Fungal Diversity 25: 141-155.

Photita, W., Taylor, P.W.J., Ford, R., Lumyong, P., McKenzie, H.C. and Hyde, K.D. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. Fungal Diversity 18: 117-133.

Shenoy, B.D., Jeewon, R., Lam, W.H., Bhat, D.J., Than, P.P., Taylor, P.W.J. and Hyde, K.D. (2007). Morpho-molecular characterisation and epitypification of *Colletotrichum capsici* (*Glomerellaceae*, *Sordariomycetes*), the causative agent of anthracnose in chilli. Fungal Diversity 27: 197-211.

Simmonds, J.H. (1965). A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. Queensland Journal of Agricultural and Animal Science 22: 437-459.

Simmonds, J.H. (1968). Type specimens of *Colletotrichum gloeosporioides* var. *minor* and *Colletotrichum acutatum*, Queensland Journal of Agricultural and Animal Science 25: 178A.

Sreenivasaprasad, S., Mills, P., Meehan, B.M. and Brown, A. (1996). Phylogeny and systematics of 18 *Colletotrichum* species based on ribosomal DNA spacer sequences. Genome 39: 499-512.

Sreenivasaprasad, S. and Talhinhas, P. (2005). Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. Molecular Plant Pathology 6: 361-378.

Talhinhas, P., Sreenivasaprasad, S, Neves-Martins, J. and Oliveira, H. (2002). Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupin. Phytopathology 92: 986-996.

Talhinhas, P., Sreenivasaprasad, S, Neves-Martins, J. and Oliveira, H. (2005). Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. Applied and Environmental Microbiology, 71: 2987-2998.

Than, P.P., Jeewon, R., Hyde, K.D., Pongsupasamit, S., Mongkolporn, O. and Taylor, P.W.J. (2007) Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. Plant Pathology Doi: 10.111/j.1365-3059.2007.01782.x.

Vinnere, O., Fatehi, J., Wright, S.A.I. and Gerhardson, B. (2001). The causal agent of anthracnose of *Rhododendron* in Sweden and Latvia. Mycological Research 106: 60-69.