A taxonomic re-assessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov.

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Shivas, R.G. and Tan, Y.P. (2009). A taxonomic re-assessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. Fungal Diversity 39: 111-122.

A comparative morphological and molecular analysis of the ITS and β-tubulin regions of 48 Australian isolates identified as *Colletotrichum acutatum*, with published descriptions and DNA sequence data from Australia and overseas, revealed three distinct groups. One group contained the holotype and two paratypes of *C. acutatum*, whereas a recently designated epitype was found to be non-conspecific with the holotype. Two new species, *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov., are described and illustrated with the latter having as holotype the non-conspecific epitype of *C. acutatum*. These three taxa corresponded with phylogenetic groupings of *C. acutatum* previously reported by other authors.

Key words: *Colletotrichum*, phylogeny, taxonomy, β-tubulin, ITS

Article Information
Received 11 November 2009
Accepted 22 November 2009
Published online 9 Decemebr 2009
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Introduction

Colletotrichum acutatum J.H. Simmonds ex J.H. Simmonds is a major pathogen with a worldwide distribution causing diseases commonly known as anthracnose on a wide range of plants, including legumes, vegetables, small fruits and perennial tree crops, in more than 40 plant families (Walker *et al*., 1991; Johnston and Jones, 1997; Sreenivasaprasad and Talhinhas, 2005; Hyde *et al*., 2009). *Colletotrichum acutatum* was first described from Australia (Simmonds, 1965, 1968), where it has been reported as causing diseases on avocado, papaya, strawberry, tomato (Sim-monds, 1965), grapes (Melksham *et al*., 2002; Whitelaw-Weckert *et al*., 2007), olive (Spooner-Hart *et al*., 2007), *Acacia* (Golzar, 2009) and almond (McKay *et al*., 2009). There are at least three infraspecific forms of *C. acutatum*, *viz*. *C. acutatum* f. sp. *pineum* Dingley & J.W. Gilmour, which causes terminal crook disease of pine seedlings (Dingley & Gilmour, 1972); *C. acutatum* f. sp. *hakeae* Lubbe, Denman, P.F. Cannon, J.Z. Groenew., Lampr. & Crous, which is one of the most devastating fungal pathogens of *Proteaceae* in South Africa, where it is used as a biological control agent of weedy *Hakea* (Lubbe *et al*., 2004); and *C. acutatum* var. *fioriniae* Marcelino & Gouli, which is parasitic on a scale insect (Marcelino *et al*., 2008).

C. acutatum is morphologically differentiated from other species of *Colletotrichum* by its conidia, which have pointed ends (Simmonds, 1965). A teleomorph, *Glomerella acutata* Guerber & J.C. Correll (2001) has been described. Further, a variety of this teleomorph, *G. acutata* var. *fioriniae* Marcelino & Gouli, has been described for entomopathogenic strains found on scale insects in north-eastern USA (Marcelino *et al*., 2008).

When Simmonds (1965) first described *Colletotrichum acutatum* he omitted to designate a type specimen and rectified this (Simmonds, 1968) by listing a holotype (IMI 117617) and six paratypes (IMI 117618-117623) held in herbarium IMI with corresponding isotype and six isoparatypes now held in herbarium BRIP. Simmonds' (1965, 1968)

Table 1. Identity and origin of the living cultures of *Colletotrichum* examined in this study.

BRIP accession number	Host	Symptom	Locality	Year	GenBank no.	
					ITS	β-tub
C. acutatum **(group A[a] or A5[b])**						
27048	*Mangifera indica*	Fruit rot	Ayr, Qld	1993	GU183326	GU183307
52652	*Ranunculus* sp.	Seedling collapse	Clayton South, Vic	1989	GU183349	GU183308
52653	*Anemone* sp.	Stem spot/discolouration	Geelong, Vic	1976	GU183350	GU183309
52656	*Anemone* sp.	Leaf lesion/stem distortion	Geelong, Vic	1977	GU183353	GU183310
52690	*Pistacia vera*			1989	GU183355	GU183311
52691	*Fragaria x ananassa*		Wanneroo, WA	1988	GU183356	GU183312
52692	*Olea europaea*	Fruit spot	Kalamunda, WA	1991	GU183357	GU183313
52695	*Boronia megastigma*		Mt Barker, SA	2004	GU183360	GU183314
C. fioriniae **(group C[a] or A3[b])**						
20127	*Persea americana*	Fruit rot	Brisbane, Qld	1989	GU183320	GU183268
28761	*M. indica*	Stem endophyte	Yarwun, Qld	1994	GU183333	GU183269
29284	*P. americana*	Fruit rot	Mt Tamborine, Qld	2002	GU183335	GU183270
29285	*P. americana*	Fruit rot	Mt Tamborine, Qld	2002	GU183336	GU183271
52335	*P. americana*	Fruit rot	Pemberton, WA	2008	GU183346	GU183272
52336	*P. americana*	Fruit rot	Pemberton, WA	2008	GU183347	GU183273
52656	*Anemone* sp.	Leaf lesion	Geelong, Vic	1977	GU183361	GU183274
52696	*Acacia acuminata*	Leaf and stem blight	Manjimup, WA	2008	GU183362	GU183267
52697	*Actinidia chinensis*			1991	GU183320	GU183268
C. simmondsii **(group D[a] or A2[b])**						
4684	*Capsicum frutescens*		Brisbane, Qld	1955	GU183315	GU183275
4703	*F. × ananassa*		Townsville, Qld	1971	GU183316	GU183276
4704	*F. × ananassa*	Fruit rot	Forest Glen, Qld	1972	GU183317	GU183277
11086	*F. × ananassa*		Nambour, Qld	1965	GU183318	GU183278
19776	*Carica papaya*	Fruit anthracnose	Yandina, Qld	1987	GU183319	GU183279
24124	*Nephelium lappaceum*	Fruit rot	Kamerunga, Qld	1989	GU183321	GU183280
24191	*A. chinensis*	Stem endophyte	Mt Tamborine, Qld	1991	GU183322	GU183281
24197	*A. chinensis*	Fruit rot	Mt Tamborine, Qld	1991	GU183323	GU183282
24243	*Litchi chinensis*	Fruit anthracnose	Atherton Tableland, Qld	1992	GU183324	GU183283
24246	*L. chinensis*	Fruit anthracnose	Atherton Tableland, Qld	1992	GU183325	GU183284
28420	*Cyphomandra betacea*	Fruit rot	Mt Tamborine, Qld	1987	GU183327	GU183285
28487	*Averrhoa carambola*	Fruit rot	Qld	1987	GU183328	GU183286
28517	*C. papaya*	Fruit anthracnose	Yandina, Qld	1987	GU183329	GU183287
28518	*C. papaya*	Fruit anthracnose	Yandina, Qld	1987	GU183330	GU183288

Table 1 (continued). Identity and origin of the living cultures of *Colletotrichum* examined in this study.

BRIP accession number	Host	Symptom	Locality	Year	GenBank no.	
					ITS	β-tub
28519	*C. papaya*	Fruit anthracnose	Yandina, Qld	1987	GU183331	GU183289
28533	*P. americana*	Fruit anthracnose	Qld	1986	GU183332	GU183290
28832	*M. indica*	Fruit rot	Ayr, Qld	1993	GU183334	GU183291
39473	*L. chinensis*	Pepper spot	Byron Bay, NSW	2003	GU183337	GU183292
48724	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183338	GU183263
48726	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183339	GU183294
48729	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183340	GU183295
48731	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183341	GU183296
48734	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183342	GU183297
48737	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183343	GU183298
48761	*L. chinensis*	Fruit anthracnose	Mena Creek, Qld	2003	GU183344	GU183299
52651	*Vaccinium corymbosum*	Fruit rot	Knoxfield, Vic	1987	GU183345	GU183300
52654	*F. × ananassa*		Scoresby, Vic	1976	GU183348	GU183301
52655	*F. × ananassa*	Fruit rot	Silvan, Vic	1955	GU183351	GU183302
52657	*Lycopersicon esculentum*	Fruit rot	Tweed Heads, NSW	1980	GU183352	GU183303
52693	*F. × ananassa*		Wanneroo, WA	1992	GU183354	GU183304
52694	*F. × ananassa*		Baldivis, WA	1981	GU183358	GU183305

[a]Group as described by Lardner *et al.* (1999)
[b]Group as described by Sreenivasaprasad and Talhinhas (2005)

broad concept of *C. acutatum* is demonstrated by the selection of several type specimens from a range of hosts, *viz*. *Carica papaya*, *Capsicum frutescens* and *Delphinium ajacis*. It has subsequently been shown that these type specimens have variable morphological and molecular characteristics (Vinnere *et al*., 2002; Than *et al*., 2008) and represent a species complex. Further confusion has arisen from one of Simmonds' paratype cultures (coll. no. 16633D), which in 1967 was sent to New Zealand, where it was deposited as ICMP 1783 (page 225, Guerber and Correll, 2001) and subsequently deposited in the USA as ATCC 56816 and the Netherlands as CBS 294.67. This culture is ex-paratype (IMI 117620) and not ex-holotype (see Table 1 in Than *et al*., 2008). Comparison of the published ITS sequences of these two cultures, holotype (IMI 117617) and paratype (ATCC 56816), revealed that they differed by one base pair (Vinnere *et al*., 2002; Farr *et al*., 2006).

Vinnere *et al*. (2002) also showed by molecular analysis of the dried cultures of the holotype and one of the paratypes (IMI 117619) of *C. acutatum* that there was nucleotide divergence between these isolates. Than *et al*. (2008) proposed a more recently collected isolate as an epitype culture (BRIP 28519), from the same host and location as the holotype, to allow precise application of the name *C. acutatum*.

Several authors have identified distinct molecular groups within *C. acutatum*. Lardner *et al*. (1999) first identified seven groups based upon morphology and RAPD banding patterns. Later, Sreenivasaprasad and Talhinhas (2005) reorganised *C. acutatum* into eight groups (A1 to A8) based upon ITS sequences of isolates published in ten papers (Lardner *et al*., 1999; Yang and Sweetingham, 1998; Freeman *et al*., 2001; Martínez-Culebras *et al*., 2002; Nirenberg *et al*., 2002; Afanador-Kafuri *et al*., 2003; Guerber *et al*., 2003; Martínez-Culebras *et al*., 2003; Lubbe *et al*., 2004). Whitelaw-Weckert *et al*. (2007) used multigene phylogenies to group Australian isolates of *C. acutatum* from grapes and several other hosts into the groups of Sreenivasaprasad and Talhinhas (2005), and proposed a new group, A9. Some authors have proposed that the high genetic divergence in populations of *C. acutatum* indicated that it should be divided into distinct species (Vinnere *et al*., 2002; Guerber *et al*., 2003; Sreenivasaprasad and Talhinhas, 2005). The morphologically and genetically distinct groups of *C. acutatum* described by Johnston and Jones (1997) and Lardner *et al*. (1999) also support its division into separate species. In this paper we report the genetic relationships found amongst Australian isolates of *C. acutatum*, and for-mally establish two new species. The location and status of type specimens and ex-type cultures are given.

Materials and methods

DNA extraction, amplification, sequencing and data analyses

The isolates (Table 1) were grown on potato dextrose agar. The mycelia were scrapped off the agar plates and macerated with 0.5mm glass beads (Daintree Scientic) in a TissueLyser (Qiagen). Total DNA was then extracted with the Gentra Puregene DNA Extraction kit (Qiagen) according to the manufacturer's instructions.

The ITS and β-tubulin regions were amplified with the Phusion High-Fidelity PCR Master Mix (Finnzymes). The ITS1 region, 5.8S rRNA gene, and the ITS2 region of the nuclear-encoded ribosomal RNA gene were amplified with primers ITS1 and ITS4 (White *et al*., 1990), and part of the β-tubulin-2 gene (exons 2 to 6) was amplified with primers T1 (O'Donnell and Cigelink, 1997) and βt-2b (Glass and Donaldson, 1995). The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions, and sequenced by either the Australian Genome Research Facility (Brisbane, Australia) or by Macrogen Incorporated (Seoul, Korea) on the AB 3730*xl* capillary sequencer (Applied Biosystems).

Both the ITS and β-tubulin sequences were assembled with Vector NTi (Invitrogen) and deposited into GenBank. These sequences (Table 1), together with published sequences retrieved from GenBank (Table 2), were aligned with ClustalW as implemented by MEGA4 (Tamura *et al*., 2007). Sequences of *Colletotrichum caudatum*, *Colletotrichum coccodes*, and *Colletotrichum graminicola* were used as outgroups for both the ITS and β-

Table 2. GenBank sequences used in phylogenetic analyses.

Isolate	Host	Locality	GenBank no.	
			ITS	β-tub
***C. acutatum* (group A[a] or A5[b])**				
IMI 117617 (holotype)	*C. papaya*	Australia	AF411700	
IMI 117619 (paratype)	*C. papaya*	Australia	AF411701	
IMI 117620 (paratype)	*C. papaya*	Australia	FJ788417	FJ788419
PT227	*Olea* sp.	Portugal	AJ749694	AJ748618
PD90-443	*Phlox* sp.	Netherlands	AJ749671	AJ748627
***C. fioriniae* (group C[a] or A3[b])**				
EHS58 (holotype)	*Fiorinia externa*	USA	EF464594	EF593325
EHS48	*F. externa*	USA	EF464593	EF593322
CA318	Magnolia	UK	AJ749677	AJ748634
STE-U 5287	*Malus* sp.	USA	AY376509	AY376557
***C. simmondsii* (group D[a] or A2[b])**				
STE-U 4452	*Protea magnifica*	South Africa	AY376503	AY376551
STE-U 5303	*Hevea brasiliensis*	India	AY376508	AY376556
PT135	*Olea* sp.	Portugal	AJ749683	AJ749607
DAR28076[c]	*Vitis* sp.	Australia	DQ991740	
DAR76888[c]	*Vitis* sp.	Australia	DQ991743	
DAR75574[c]	*Vitis* sp.	Australia	DQ991738	
***Colletotrichum acutatum* (group B[a] or A4[b])**				
JG05	*Ceanothus* sp.	France	AJ300557	AJ409302
PT169	*Olea* sp.	Portugal	AJ749685	AJ748609
PT248	*Olea* sp.	Portugal	AJ749698	AJ748622
Colletotrichum coccodes				
STE-U 5301	*Lycopersicon* sp.	Zimbabwe	AY376528	AY376576
Colletotrichum caudatum				
STE-U 5300	*Cymbopogon* sp.	India	AY376527	AY376575
Colletotrichum graminicola				
STE-U 5298	*Zea mays*	Zimbabwe	AY376539	AY376587

[a]Group as described by Lardner *et al.* (1999)
[b]Group as described by Sreenivasaprasad and Talhinhas (2005)
[c]Group A9 as proposed by Whitelaw-Weckert *et al.* (2007)

tubulin data. UPGMA analysis was performed with MEGA4 on separate datasets using the Kimura-2-parameter substitution model. Alignment gaps were treated as missing character states, and all characters were unordered and of equal weight. The resulting tree was evaluated with 1000 bootstrap replications to test the clade stability.

Results

Phylogenetic analysis

For ITS sequences, approximately 500 bases were determined for the isolates and added to the alignment. The alignment included published sequences, retrieved from GenBank, which had been classified into the *C. acutatum* groups of Sreenivasaprasad and Talhinhas (2005), including the holotype (IMI 117617, Genbank accession: AF411700) and two paratypes (IMI 117619 and IMI 117620, Genbank accessions: AF411701 and FJ788417, respectively) of *C. acutatum*, as well as the holotype of *C. fioriniae* (EHS58, Genbank accession: EF464594). The phylogram obtained from the ITS data delineate the Australian isolates into *C. acutatum* groups A2, A3, and A5 of Sreenivasaprasad and Talhinhas (2005) (Fig. 1).

Approximately 750 bases of the β-tubulin-2 gene were determined for the isolates. The alignment of the β-tubulin sequences

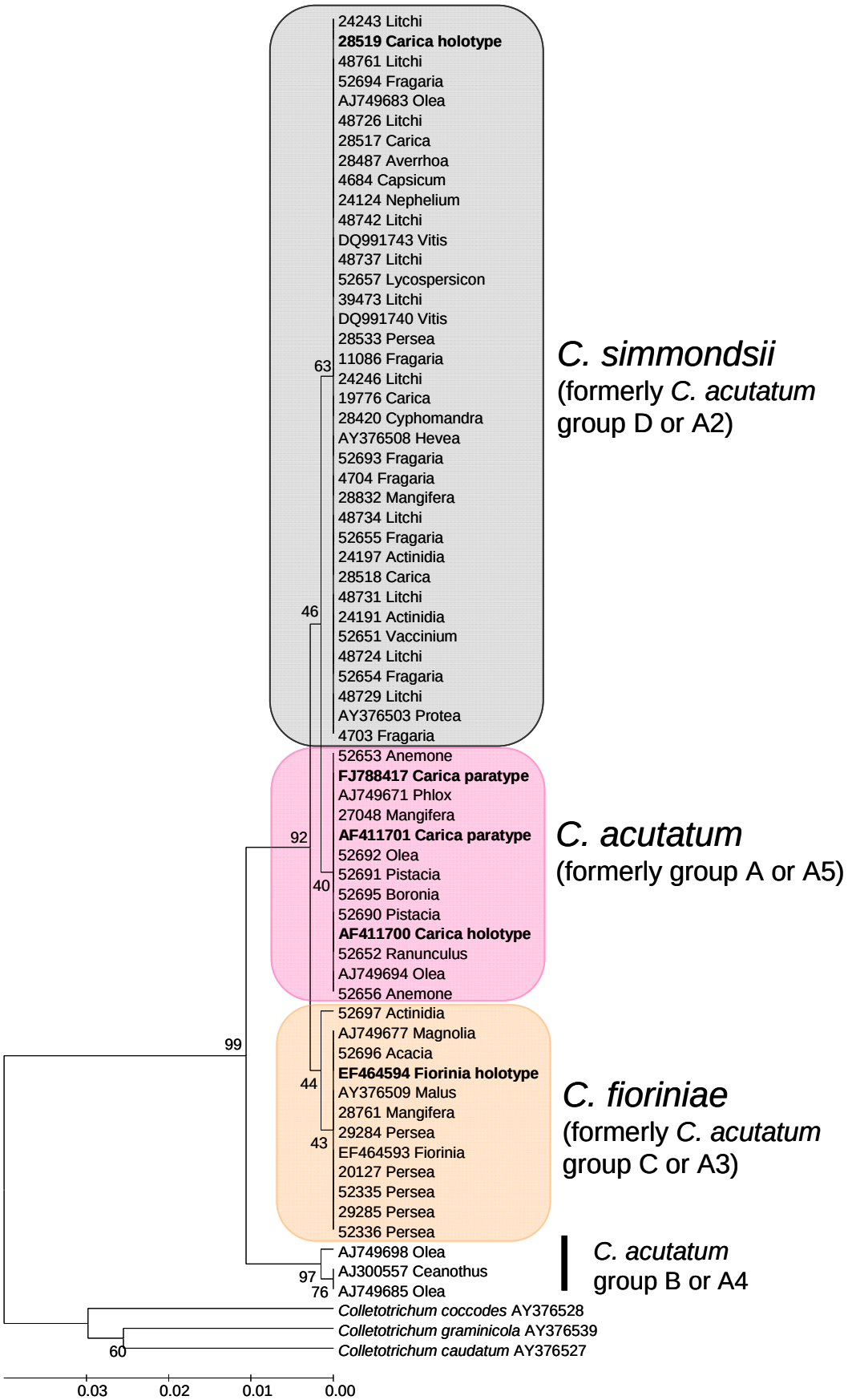


Fig. 1. UPGMA tree derived from the ITS1, 5.8S rRNA, and ITS2 sequences of BRIP isolates and published sequences. The tree was rooted to *Colletotrichum graminicola*, *Colletotrichum caudatum*, and *Colletotrichum coccodes*. Branch support is based on 1000 bootstrap replicates and is shown at the nodes. The bar represents 0.001 substitutions per site. The clade which represents *C. acutatum* is coloured in pink, whilst the newly proposed *C. simmondsii* and *C. fioriniae* are coloured grey and apricot, respectively.

included sequences from the same isolates used in the analysis of ITS, with the exception of the *C. acutatum* holotype (IMI 117617) and one of the paratypes (IMI117619), and the DAR isolates from *C. simmondsii* (Table 2). The phylogram obtained from the β-tubulin data were consistent with the ITS groupings (Fig. 2), but with improved resolution.

Taxonomy

Australian isolates of *C. acutatum* could be separated into three groups based upon cultural characteristics as well as molecular analysis of the ITS and β-tubulin sequence data. These groups corresponded with previously characterised phylogenetic groups of *C. acutatum* (Lardner *et al*., 1999; Sreenivasaprasad and Talhinhas, 2005; Whitelaw-Weckert *et al*., 2007). Taxonomy, plant pathology and biosecurity are best served if these three groups are treated as separate species. Vinnere's *et al*. (2002) sequence analysis of the holotype specimen (IMI 117617, GenBank accession number AF411700) and one of the paratype specimens (IMI 117619, GenBank accession number AF411701) from old herbarium specimens of *C. acutatum* showed that they belong to group A of Lardner *et al*. (1999) and A5 of Sreenivasaprasad and Talhinhas (2005). The type of *C. acutatum* var. *fioriniae* sits within *C. acutatum* group C (A3) and we consider this variety should be raised to species status. The third group D (A2 including A9) is described as a new species.

Colletotrichum acutatum J.H. Simmonds ex J.H. Simmonds, Queensland Journal of Agricultural Science 25: 178A (1968)

For detailed descriptions of *C. acutatum* based on type material see Guerber and Correll (2001) and Vinnere *et al*. (2002). Isolates of *C. acutatum* have conidia with pointed ends (Fig. 11) and often develop carmine pigments in the agar without flecking (Figs 9-10), although the formation of these pigments is not an independently reliable characteristic to define the species.

Holotype: Australia, Queensland, Ormiston, Redlands Research Station, on *Carica papaya*, 1 Oct. 1965, J.H. Simmonds, IMI 117617 – dried culture; isotype BRIP 4693 – microscope slide; paratype *loc. id*., on *Carica papaya*, 1 Oct. 1965, J.H. Simmonds, IMI 117619 – dried culture; paratype *loc. id*., on *Carica papaya*, 5 July 1965, J.H. Simmonds, IMI 117620 (ICMP 1783, ATCC 56816, CBS 294.67) – living culture, BRIP 49837 – dried culture.

Teleomorph: *Glomerella acutata* Guerber & J.C. Correll, Mycologia 93: 225 (2001). Holotype: as dried perithecia produced by crossing strains ATCC 56816 and ATCC MYA-662 of *C. acutatum*, in FH; isotype in PDD, as dried and liquid-preserved perithecia.

Colletotrichum fioriniae (Marcelino & S. Gouli) R.G. Shivas & Y.P. Tan, **comb. et stat. nov.**

MycoBank: 515411

Teleomorph: *Glomerella fioriniae* (Marcelino & Gouli) R.G. Shivas & Y.P. Tan, comb. et stat. nov.

Basionym: *Colletotrichum acutatum* var. *fioriniae* Marcelino & Gouli, in Marcelino, Giordano, Gouli, Gouli, Parker, Skinner, TeBeest and Cesnik, *Mycologia* 100: 362 (11 Aug. 2008). – Type on mummified adult *Fiorinia externa* insect, Ward Pound Ridge Reserve, New York, U.S.A., 2005, J.A.P. Marcelino & S. Gouli (EHS58, University of Vermont, Department of Plant and Soil Science, Entomology Research Laboratory, Worldwide Collection of Entomopathogenic Fungi, Burlington, Vermont, U.S.A.; ex-type living culture preserved in 10% glycerol at –80° C; MycoBank MB507440). Marcelino *et al*. (2008) produced *G. acutata* var. *fioriniae* in some self-fertile isolates as well as some cross-fertile isolates of *C. fioriniae*.

Morphologically similar to *Colletotrichum acutatum*; differs by having colonies on PDA that are grey cottony with aerial mycelium in compact tufts (Fig. 3), and in reverse pale brownish pink with dark flecking (Fig. 4). Conidiomata sparse with masses of orange conidia. Sclerotia absent. Setae absent. Conidia narrowly elliptical, 9 – 15 x 3.0 – 4.5 μm, smooth, hyaline, pointed at both ends (Fig. 5). Distinguished from other taxa by its cultural appearance and ITS sequence.

Hosts and disease: Australia: leaf and stem blight of *Acacia acuminata*, fruit rot of *Persea americana*, endophyte in *Mangifera indica*; U.S.A.: endophyte in 28 other species

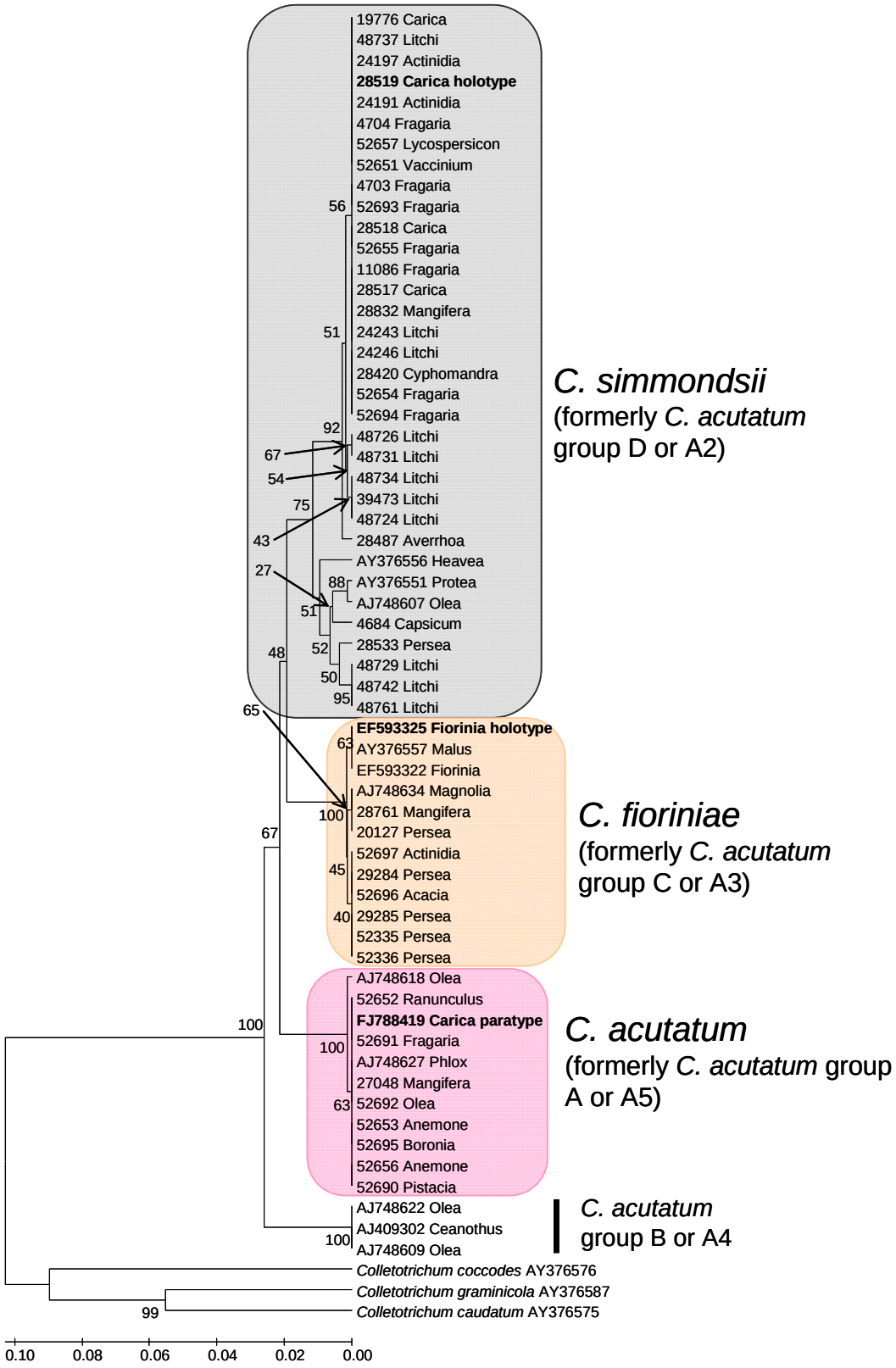
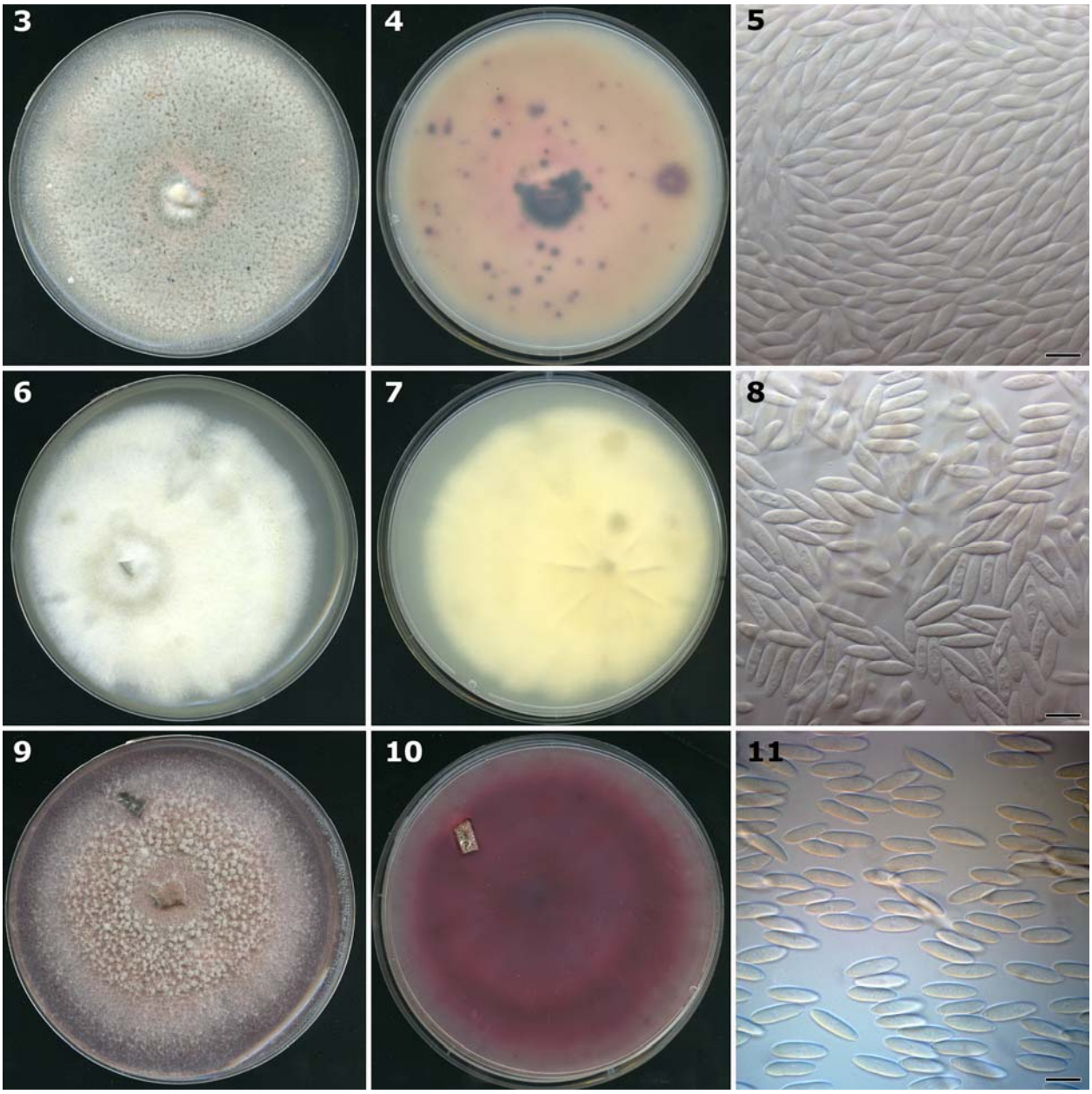


Fig. 2. UPGMA tree derived from the β-tubulin-2 gene sequences of BRIP isolates and published sequences. The tree was rooted to *Colletotrichum graminicola*, *Colletotrichum caudatum*, and *Colletotrichum coccodes*. Branch support is based on 1000 bootstrap replicates and is shown at the nodes. The bar represents 0.001 substitutions per site. The clade which represents *C. acutatum* is coloured in pink, whilst the newly proposed *C. simmondsii* and *C. fioriniae* are coloured grey and apricot, respectively.



Figs 3-5. *Colletotrichum fioriniae* after 4 weeks on PDA. 3. Mycelial colony (BRIP 52696). 4. Plate in reverse (BRIP 52696). 5. Conidia (BRIP 52696). **Figs 6-8.** *Colletotrichum simmondsii* after 4 weeks on PDA. 6. Mycelial colony (BRIP 28519). 7. Plate in reverse (BRIP 28519). 8. Conidia (BRIP 4704). **Figs 9-11.** *Colletotrichum acutatum* after 4 weeks on PDA. 9. Mycelial colony (BRIP 52652). 10. Plate in reverse (BRIP 52652). 11. Conidia (BRIP 52652). Bars = 10µm.

of plants, entomopathogenic on elongate hemlock scale (*Fiorinia externa*).

Colletotrichum simmondsii R.G. Shivas & Y.P. Tan, **sp. nov.** Figs 6-8

Etymology: named after John (Jack) H. Simmonds (1901-1992), an eminent Australian plant pathologist who first named *Colletotrichum acutatum*.

Morphologia est similis *Colletotricho acutato*; differt quod habet colonias in PDA quae sunt cinereae gossypinae sine caespitibus, et, reversae, pallidae cinereae ad pallidas aurantiacas, interdum cum maculis fuscis. *Conidiomata* abundantia cum massis conidiorum aurantiacorum. Sclerotia absunt. Setae absunt. *Conidia* subcylindracea, 10 – 16 x 3.5 – 4.5 µm, levia, hyalina, basi angusta, apice rotundato vel angusto. Differunt ab aliis taxis specie culta et ITS ordine.

Typus: Australia, Queensland, Yandina, on *Carica papaya*, May 1987, *L.M. Coates*, BRIP 28519 (epitype of "*Colletotrichum acutatum*" *sensu* Than *et al*. 2008, *non* Simmonds 1968), holotype; ITS sequence GenBank GU183331, MycoBank MB515420; ex-type living culture in BRIP 28519, BIOTEC Culture Collection, Thailand (BCC 28680), CBS 122122, HKUCC 10928, ICMP 17298, KACC 43258.

Morphologically similar to *Colletotrichum acutatum*; differs by having colonies on PDA that are grey cottony and in reverse pale grey to pale orange sometimes with dark flecking. *Conidiomata* abundant with masses of orange conidia. Sclerotia absent. Setae absent. *Conidia* subcylindrical, 10 – 16 x 3.5 – 4.5 µm, smooth, hyaline, narrowed at the base, rounded or narrowed at the apex (Fig. 8). Distinguished from other taxa by its cultural appearance (Figs. 6 and 7) and ITS sequence.

Known hosts: endophyte in *Actinidia chinensis*; fruit rot of *Capsicum frutescens*, *Carica papaya*, *Cyphomandra betaceae*, *Fragaria* × *ananassa*, *Litchi chinensis*, *Lycopersicon esculentum*, *Mangifera indica*, *Nephelium lappaceum*, *Persea americana*, *Vaccinium corymbosum*.

Specimens examined: AUSTRALIA (Table 1). The earliest collection of *C. simmondsii* that we examined was one from *Capsicum frutescens* taken in 1955 by J.H. Simmonds who determined it as *C. acutatum* (BRIP 4684). Based on DNA sequence data the epitype (BRIP 28519) of *C. acutatum* designated by Than *et al*. (2008) is not conspecific with the holotype of *C. acutatum*. Thus it becomes available to be used as the holotype of *C. simmondsii*. This is allowed because an epitype is not the type of a name, it is something designated to support or amplify the interpretation of a holotype, lectotype or neotype. We have selected BRIP 28519 as the holotype of *C. simmondsii* in preference to the earlier 1955 Simmonds' collection as this avoids any possible confusion as to which taxon Than's *et al*. (2008) epitype now represents. This is important as further new taxa morphologically and molecularly similar to *C. acutatum* may be found.

Discussion

There is no need, and neither is it possible, to designate a new epitype for *C. acutatum* in light of the establishment of *C. simmondsii*. The primary purpose of the original epitype was to stabilise the taxonomy of *C. acutatum*, particularly in light of Simmonds' broad concept as demonstrated by the selection of several type specimens for different hosts. The partial DNA sequences extracted by Vinnere *et al*. (2002) from the holotype and one of the paratypes are sufficient to characterise *C. acutatum* as belonging to the same group as another of its paratypes which we have sequenced (Figs 1 and 2). This particular paratype (IMI 117620) of *C. acutatum* is widely distributed as a living culture, which will facilitate future multi-locus phylogenetic analysis of this species.

The most reliable means of distinguishing *C. acutatum* from *C. fioriniae* and *C. simmondsii* is by their ITS sequence data. The cultures of these species can also be differentiated by the pigments produced by cultures in PDA as seen in reverse, *viz*. intense carmine red without flecking (*C. acutatum*, Figs 9-10), pale pink with flecking (*C. fioriniae*, Figs 3-4) and pale orange, yellow or without pigments, with or without flecking (*C. simmondsii*, Figs 6-7). Cultural characteristics are influenced by many factors and are indicative but not reliable for species identification. The pointed conidia of each of these species are morphologically similar (Figs 5, 8 and 11) and this character is totally unreliable for differentiation of these three taxa.

The DNA sequence analysis showed that *C. acutatum* group B (A4) formed a phylogenetically distinct group and may represent a new species. However without Australian isolates of *C. acutatum* group B (A4) we were unable to complete a taxonomic assessment of this group.

The non-conspecificity of the holotype and epitype of *C. acutatum* and the description of two new species from this complex, illustrate the need for careful molecular analyses to help establish species boundaries in *Colletotrichum*. Multigene loci sequence data can provide a more reliable assessment (Cai *et al*., 2009) and future studies to distinguish taxa in species complexes may need to incorporate more genes. All isolates of *Colletotrichum sense lato* (both future and past) will need carefully examination to ensure the correct usage of species names. The host range and host specificity of *C. fioriniae* and *C. simmondsii* is not clear. Whitelaw-Weckert *et al*. (2007) used *in vitro* infection to show that there was a lack of host specificity between isolates of *C. acutatum sensu lato* (corresponding to *C. acutatum*, *C. fioriniae* and *C. simmondsii*) and further that there was potential for cross infection between horticultural crops. It is our hope that recognition of these phylogenetically distinct groups as separate species will facilitate studies into the biology of these fungi, especially their pathogenicity, distribution, host

range and specificity. This in turn will allow better plant health management and biosecurity decisions to be made.

Acknowledgements

We wish to thank Dr Peter R. Johnston (Herbarium PDD, New Zealand) and Prof. Kevin D. Hyde (Mae Fah Luang University, Thailand) for their helpful advice and comments. Mr Don Barrett (Brisbane, Australia) is thanked for translation of the Latin diagnosis. We are especially grateful to those who provided us with isolates, namely Jay Anderson (QPIF), Dr James Cunnington (VPRI, Victoria), Jan Dean (QPIF), Nuccia Eyres (Department of Agriculture and Food, Western Australia), Julie McClements (Department of Agriculture and Food, Western Australia) and Sharon Morely (VPRI, Victoria).

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