Fungal Diversity (2013) 61:89–105
DOI 10.1007/s13225-013-0249-2

Species of the *Colletotrichum gloeosporioides* complex associated with anthracnose diseases of *Proteaceae*

Fang Liu · Ulrike Damm · Lei Cai · Pedro W. Crous

Received: 30 April 2013 / Accepted: 11 June 2013 / Published online: 6 July 2013

Abstract Anthracnose disease of *Proteaceae* has in the past chiefly been attributed to infections by *C. acutatum*, *C. boninense* and *C. gloeosporioides*. In the present study, a multi-locus phylogenetic analysis (ACT, CAL, CHS-1, GAPDH, GS, ITS, TUB2) revealed that strains of the *C. gloeosporioides* complex associated with *Proteaceae* belong to at least six species. These include *C. alienum*, *C. aotearoa*, *C. kahawae* (subsp. *ciggaro*), *C. siamense*, and two new taxa, *C. proteae* and *C. grevilleae*. The most economically important pathogen of *Proteaceae* seems to be *C. alienum*, and not *C. gloeosporioides* as previously reported. All taxa associated with *Proteaceae* are morphologically described on different media in culture, except strains of *C. siamense*, which proved to be sterile. Furthermore, *C. populi* is synonymised with *C. aenigma*.

Keywords Ascomycota · *Colletotrichum* · Morphology · Phylogeny · Systematics

Introduction

The *Proteaceae* is a family of the *Proteales* in the *Rosidae* which developed approximately 96 million years ago, representing one of the most prominent plant families of the Southern Hemisphere, in southern Africa, Asia, Australia, Central and South America, especially in areas with long dry seasons (Crous et al. 2004a). The majority of genera, however, are found in Australia and South Africa (Taylor et al. 2001c). Given their beauty, unique appearance and relatively long shelf life, some members of the *Proteaceae* have been sought-after for the export market being commercially valuable as cut flowers (Crous and Palm 1999). Many species of South African *Proteaceae* are cultivated in Australia, the Azores, Chile, France, Israel, New Zealand, Portugal (including Madeira Island), Spain (including Canary Islands), Thailand, USA (California, Hawaii) and Zimbabwe. Some Australian *Proteaceae* species are also cultivated in countries other than Australia (Crous et al. 2000).

One of the factors limiting commercial production of *Proteaceae* is damage caused by fungal diseases (Knox-Davies 1981; Wright and Saunderson 1995; Crous et al. 2004a). Some pathogens were even considered as actionable quarantine organisms (Crous et al. 2000). *Colletotrichum* spp. belong to the most devastating fungal pathogens of *Proteaceae*, causing seedling damping off, shepherd's crook, anthracnose, leaf lesions, pruning wound dieback and stem dieback (Knox-Davies 1981; Knox-Davies et al. 1986; Von Broembsen 1989; Crous et al. 2004a).

Colletotrichum gloeosporioides was previously regarded as the only *Colletotrichum* species to infect species of the *Proteaceae* (Baxter et al. 1983). Based on morphology, sequence data of the internal transcribed spacer region (ITS) and partial sequences of the Beta-tubulin gene (TUB2), Lubbe et al. (2004) differentiated four species of *Colletotrichum* (*C. acutatum*, *C. boninense*, *C. crassipes*, *C. gloeosporioides*) and one *forma specialis* (*C. acutatum f. sp. hakeae*) associated with diseased *Proteaceae*. An additional strain identified as *C. gloeosporioides* based on ITS and 28S rDNA gene (LSU) sequence data was included in the study of Marincowitz et al. (2008a).

Recently, systematic studies of *Colletotrichum* species complexes have started to employ a polyphasic approach to

F. Liu · L. Cai (✉)
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
e-mail: mrcailei@gmail.com

F. Liu · P. W. Crous
Department of Biology, Microbiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

U. Damm · P. W. Crous
CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

P. W. Crous
Laboratory of Phytopathology, Wageningen University and Research Centre (WUR), Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

species identification, emphasizing multi-locus phylogeny in conjunction with recognisable phenotypic characters (Cai et al. 2009; Damm et al. 2009, 2012a, b; Rojas et al. 2010; Liu et al. 2011; Weir et al. 2012). Using this approach, many cryptic and new species associated with *Proteaceae* have been revealed, e.g. *C. acutatum*, *C. australe*, *C. fioriniae*, *C. nymphaeae* and *C. simmondsii* in the *C. acutatum* species complex (Damm et al. 2012a) and *C. boninense* and *C. karstii* in the *C. boninense* species complex (Damm et al. 2012b). However, in the recent revision of the *C. gloeosporioides* species complex (Weir et al. 2012), only one strain from *Proteaceae* (*Banksia*, series *Dryandra*) was included. The aim of the present study is therefore to reassess the identification of strains associated with *Proteaceae* that belong to the *C. gloeosporioides* species complex.

Materials and methods

Isolates

Isolates previously identified as *C. gloeosporioides* and related strains associated with *Proteaceae* obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands, were used for morphological and phylogenetic analyses and presented in Table 1. Type specimens of the species newly described here are located in the fungarium of the CBS. Descriptions of new species are based on an examination of ex-type cultures.

Morphological analysis

Agar plugs (5-mm-diam) were taken from the periphery of actively growing cultures and transferred to the centre of 9-cm-diam Petri dishes containing 2 % potato dextrose agar (PDA; Difco) or synthetic nutrient-poor agar medium (SNA; Nirenberg 1976) amended with double-autoclaved stems of *Anthriscus sylvestris* placed onto the agar surface. Cultures were incubated at 20 °C under near UV light with a 12 h photoperiod for 10 d. Colony characters and pigment production on PDA and SNA were noted after 10 d. Colony colours were rated according to Rayner (1970). Growth rates were measured after 7 and 10 d.

Conidia were taken from acervuli and mounted in lactic acid. Cultures were examined periodically for the development of perithecia. Ascospores were described from perithecia crushed in lactic acid. Appressoria on hyphae were observed on the reverse side of colonies grown on SNA plates. At least 30 measurements per structure were noted and observed with a Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination. Range measurements were made according to methods described by Liu et al. (2012).

Phylogenetic analysis

Genomic DNA of the isolates was extracted using the method of Damm et al. (2008). Eight loci including the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a partial sequence of the actin (ACT), chitin synthase 1 (CHS-1), beta-tubulin (TUB2), calmodulin (CAL), histon3 (HIS3) and glutamine synthetase (GS) gene were amplified and sequenced using the primer pairs ITS1F (Gardes and Bruns 1993)+ITS4 (White et al. 1990), GDF1+GDR1 (Guerber et al. 2003), ACT-512F+ACT-783R (Carbone and Kohn 1999), CHS-79F+CHS-354R (Carbone and Kohn 1999), T1 (O'Donnell and Cigelnik 1997)+Bt-2b (Glass and Donaldson 1995), CL1+CL2A (O'Donnell et al. 2000) or CL1C+CL2C (Weir et al. 2012), CYLH3F+CYLH3R (Crous et al. 2004c) and GSF1+GSR1 (Stephenson et al. 1997), respectively. The PCR protocols were performed as described by Damm et al. (2009). Some isolates occasionally gave two bands (GS and TUB2), which were then amplified using a touchdown PCR program (Zhou et al. 2006). The DNA sequences generated with forward and reverse primers were used to obtain consensus sequences using MEGA5 (Tamura et al. 2011), and subsequently aligned using MAFFT v.6 (Katoh and Toh 2010), and the alignments edited manually using MEGA5.

A maximum parsimony analysis was performed on the multi-locus alignment (ACT, CAL, CHS-1, GAPDH, GS, ITS, TUB2) using PAUP v.4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded from all analyses. Unweighted parsimony (UP) analysis was performed. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability was assessed in a bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa.

A second phylogenetic analysis using a Markov Chain Monte Carlo (MCMC) algorithm was conducted to generate trees with Bayesian posterior probabilities in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003). Nucleotide substitution models were determined using MrModeltest v.2.3 (Nylander 2004) for each gene region and included in the analyses. Two analyses of four MCMC chains were run from random trees for 10 million generations and sampled every 1000 generations. The first 25 % of trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining trees.

Sequences derived in this study were deposited in GenBank (Table 1), the concatenated alignment in TreeBASE (www.treebase.org) (S13708), and taxonomic novelties in MycoBank (Crous et al. 2004b).

Table 1 Strains of *Colletotrichum* studied in this paper with details about host/substrate and location, and accession numbers of reference sequences from GenBank

Species	Accession number[a]	Host	Locality	GenBank accessions							
				ITS	GAPDH	CAL	ACT	CHS-1	GS	TUB2	HIS3[c]
C. aenigma	ICMP 18608[b]	*Persea americana*	Israel	JX010244	JX010044	JX009683	JX009443	JX009774	JX010078	JX010389	–
C. aenigma	ICMP 18686	*Pyrus pyrifolia*	Japan	JX010243	JX009913	JX009684	JX009519	JX009789	JX010079	JX010390	–
C. aenigma(syn. *C. populi*)	HMBFU 191[b]	*Poplar* sp.	China	AB632347	JN211081	–	JN184704	–	JN211101	JN862898	–
C. aenigma	HMBFU 141	*Poplar* sp.	China	AB632350	JN211083	–	JN184708	–	JN211103	JN862901	–
C. aenigma	HMBFU 173	*Poplar* sp.	China	AB632349	JN211084	–	JN184707	–	JN211104	JN862900	–
C. aenigma	HMBFU 163	*Poplar* sp.	China	AB632348	JN211082	–	JN184706	–	JN211102	JN862899	–
C. aeschynomenes	ICMP 17673[b], ATCC 201874	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009721	JX009483	JX009799	JX010081	JX010392	–
C. alatae	CBS 304.67[b], ICMP 17919	*Dioscorea alata*	India	JX010190	JX009990	JX009738	JX009471	JX009837	JX010065	JX010383	–
C. alatae	ICMP 18122	*Dioscorea alata*	Nigeria	JX010191	JX010011	JX009739	JX009470	JX009846	JX010136	JX010449	–
C. alienum	ICMP 12071[b]	*Malus domestica*	New Zealand	JX010251	JX010028	JX009654	JX009572	JX009882	JX010101	JX010411	–
C. alienum	IMI 313842, ICMP 18691	*Persea americana*	Australia	JX010271	JX010018	JX009664	JX009580	JX009754	JX010074	JX010385	–
C. alienum	**CBS 111982, CPC 2925**	***Grevillea sp.***	**Australia**	**KC297069**	**KC296998**	**KC296952**	**KC296932**	**KC296975**	**KC297021**	**KC297091**	**KC297034**
C. alienum	ICMP 18621	*Persea americana*	New Zealand	JX010246	JX009959	JX009657	JX009552	JX009755	JX010075	JX010386	–
C. alienum	**CBS 133930, CPC 5204, JT1118**	***Protea cynaroides***	**Portugal**	**KC297076**	**KC297000**	**KC296957**	**KC296938**	**KC296982**	**KC297023**	**KC297096**	**KC297038**
C. alienum	**CBS 132883, CPC 16168**	***Serruria* sp.**	**South Africa**	**KC297077**	**KC297006**	**KC296958**	**KC296937**	**KC296983**	**KC297025**	**KC297097**	**KC297041**
C. alienum	**CBS 132880, CPC 2926**	***Grevillea* sp.**	**Australia**	**KC297075**	**KC297005**	**KC296959**	**KC296939**	**KC296981**	**KC297022**	**KC297099**	**KC297042**
C. alienum	**CBS 122687, CPC 13164, PREM 59587, CMW 22211**	***Leucospermum* sp.**	**South Africa**	**KC297074**	**KC296999**	**KC296956**	**KC296931**	**KC296980**	**KC297027**	**KC297095**	**KC297037**
C. alienum	**CBS 115183, STE-U 5226**	***Leucadendron* sp., cv. 'High Gold'**	**Portugal**	**KC297073**	**KC297004**	**KC296955**	**KC296936**	**KC296979**	**KC297024**	**KC297094**	**KC297036**
C. alienum	**CBS 113001, STE-U 4454, B 5678.1**	***Protea cynaroides***	**South Africa**	**KC297071**	**KC297002**	**KC296954**	**KC296934**	**KC296977**	**KC297026**	**KC297092**	**KC297040**
C. alienum	**CBS 112991, STE-U 4450, JT 1168.1**	***Leucadendron* sp., cv. 'High Gold'**	**Portugal**	**KC297070**	**KC297001**	**KC296953**	**KC296933**	**KC296976**	**KC297028**	**KC297098**	**KC297039**
C. alienum	**CBS 113192. STE-U 4455, B 5712.2**	***Protea cynaroides***	**South Africa**	**KC297072**	**KC297003**	**KC296964**	**KC296935**	**KC296978**	**KC297029**	**KC297093**	**KC297035**
C. aotearoa	**CBS 111962, STE-U 2901**	***Knightia* sp.**	**New Zealand**	**KC297061**	**KC296992**	**KC296948**	**KC296926**	**KC296969**	**KC297015**	**KC297084**	**KC297050**
C. aotearoa	**CBS 114139, STE-U 2902**	***Knightia* sp.**	**New Zealand**	**KC297063**	**KC296994**	**KC296949**	**KC296927**	**KC296972**	**KC297019**	**KC297087**	**KC297055**
C. aotearoa	**CBS 114140, STE-U 2900**	***Knightia* sp.**	**New Zealand**	**KC297067**	**KC296996**	**KC296950**	**KC296928**	**KC296973**	**KC297016**	**KC297088**	**KC297053**
C. aotearoa	**CBS 111965, STE-U 2897**	***Knightia* sp.**	**New Zealand**	**KC297062**	**KC296995**	**KC296947**	**KC296924**	**KC296970**	**KC297018**	**KC297085**	**KC297051**
C. aotearoa	**CBS 111971, STE-U 2898**	***Knightia* sp.**	**New Zealand**	**KC297068**	**KC296993**	**KC296946**	**KC296925**	**KC296971**	**KC297017**	**KC297086**	**KC297052**
C. aotearoa	ICMP 18548	*Coprosma* sp.	New Zealand	JX010206	JX009961	JX009609	JX009854	JX009445	JX010114	JX010425	–
C. aotearoa	ICMP 18537[b]	*Coprosma* sp.	New Zealand	JX010205	JX010005	JX009611	JX009564	JX009853	JX010113	JX010420	–
C. aotearoa	ICMP 18535	*Dacrycarpus dacrydioides*	New Zealand	JX010201	JX009968	JX009617	JX009545	JX009766	JX010107	JX010423	–
C. aotearoa	ICMP 17326	*Podocarpus totara*	New Zealand	JX010202	JX010049	JX009616	JX009578	JX009768	JX010106	JX010422	–
C. aotearoa	**CBS 132448, CPC 17784**	***Banksia marginata***	**Australia**	**KC297064**	**KC296997**	**KC296951**	**KC296921**	**KC296974**	**KC297020**	**KC297089**	**KC297054**
C. asianum	IMI 313839, ICMP 18696	*Mangifera indica*	Australia	JX010192	JX009915	JX009723	JX009576	JX009753	JX010073	JX010384	–
C. asianum	ICMP 18580[b], CBS 130418	*Coffea arabica*	Thailand	FJ972612	JX010053	FJ917506	JX009584	JX009867	JX010096	JX010406	–
C. boninense	MAFF 305972[b], ICMP 17904, CBS 123755	*Crinum asiaticum* var. *sinicum*	Japan	JX010292	JX009905	JQ005674	JX009583	JX009827	–	JQ005588	–
C. clidemiae	ICMP 18706	*Vitis* sp.	USA	JX010274	JX009909	JX009639	JX009476	JX009777	JX010128	JX010439	–

Table 1 (continued)

Species	Accession number[a]	Host	Locality	GenBank accessions							
				ITS	GAPDH	CAL	ACT	CHS-1	GS	TUB2	HIS3[c]
C. clidemiae	ICMP 18658[b]	*Clidemia hirta*	USA	JX010265	JX009989	JX009645	JX009537	JX009877	JX010129	JX010438	–
C. cordylinicola	ICMP 18579[b], MFLUCC 090551	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470238	HM470235	JX009864	JX010122	JX010440	–
C. fructicola	CBS 125397, ICMP 18646	*Tetragastris panamensis*	Panama	JX010173	JX010032	JX009674	JX009581	JX009874	JX010099	JX010409	–
C. fructicola	ICMP 18581[b], CBS 130416	*Coffea arabica*	Thailand	JX010165	JX010033	FJ917508	FJ907426	JX009866	JX010095	JX010405	–
C. gloeosporioides	CORCG5	*Vanda* sp.	China	HM034809	HM034807	HM034803	HM034801	HM034805	–	HM034811	–
C. gloeosporioides	IMI 356878[b], ICMP 17821, CBS 112999	*Citrus sinensis*	Italy	JX010152	JX010056	JX009731	JX009531	JX009818	JX010085	JX010445	–
C. grevilleae	**CBS 132879[b], CPC 15481, DISTEF.GREV.Z**	***Grevillea* sp., root and collar rot**	**Italy**	**KC297078**	**KC297010**	**KC296963**	**KC296941**	**KC296987**	**KC297033**	**KC297102**	**KC297056**
C. horii	ICMP 10492[b], NBRC 7478	*Diospyros kaki*	Japan	GQ329690	GQ329681	JX009604	JX009438	JX009752	JX010137	JX010450	–
C. horii	ICMP 17968	*Diospyros kaki*	China	JX010212	GQ329682	JX009605	JX009547	JX009811	JX010068	JX010378	–
C. horii	ICMP 12942	*Diospyros kaki*	New Zealand	GQ329687	GQ329685	JX009603	JX009533	JX009748	JX010072	JX010375	–
C. kahawae* subsp. *ciggaro	**CBS 112984, ICMP 17932, STE-U 4445, JT 1096**	***Banksia* sp.**	**Portugal**	**KC297059**	**KC296989**	**KC296944**	**KC296923**	**KC296966**	**KC297014**	**KC297082**	**KC297048**
C. kahawae* subsp. *ciggaro	**CBS 115194, STE-U 5196, JT1096**	***Banksia* sp.**	**Spain**	**KC297060**	**KC296991**	**KC296945**	**KC296920**	**KC296968**	**KC297011**	**KC297083**	**KC297049**
C. kahawae subsp. *ciggaro*	ICMP 18539[b]	*Olea europaea*	Australia	JX010230	JX009966	JX009635	JX009523	JX009800	JX010132	JX010434	–
C. kahawae subsp. *ciggaro*	CBS 237.49, ICMP 17922	*Hypericum perforatum*	Germany	JX010238	JX010042	JX009636	JX009450	JX009840	JX010120	JX010432	–
C. kahawae* subsp. *ciggaro	**CBS 111861, STE-U 2191**	***Leucospermum* sp.**	**USA**	**KC297057**	**KC296988**	**KC296942**	**KC296922**	**KC296965**	**KC297012**	**KC297080**	**KC297046**
C. kahawae* subsp. *ciggaro	**CBS 114499, STE-U 2192**	***Leucospermum* sp.**	**USA**	**KC297058**	**KC296990**	**KC296943**	**KC296919**	**KC296967**	**KC297013**	**KC297081**	**KC297047**
C. kahawae subsp. *kahawae*	IMI 319418[b], ICMP 17816	*Coffea arabica*	Kenya	JX010231	JX010012	JX009642	JX009452	JX009813	JX010130	JX010444	–
C. kahawae subsp. *kahawae*	IMI 301220, ICMP 17811	*Coffea arabica*	Malawi	JX010233	JX009970	JX009641	JX009555	JX009817	JX010131	JX010430	–
C. musae	IMI 52264, ICMP 17817	*Musa sapientum*	Kenya	JX010142	JX010015	JX009689	JX009432	JX009815	JX010084	JX010395	–
C. musae	CBS 116870[b], ICMP 19119	*Musa sp.*	USA	JX010146	JX010050	JX009742	JX009433	JX009896	JX010103	HQ596280	–
C. nupharicola	CBS 469.96, ICMP 17938	*Nuphar lutea* subsp. *polysepala*	USA	JX010189	JX009936	JX009661	JX009486	JX009834	JX010087	JX010397	–
C. nupharicola	CBS 470.96[b], ICMP 18187	*Nuphar lutea* subsp. *polysepala*	USA	JX010187	JX009972	JX009663	JX009437	JX009835	JX010088	JX010398	–
C. nupharicola	CBS 472.96, ICMP 17940	*Nymphaea ordorata*	USA	JX010188	JX010031	JX009662	JX009582	JX009836	JX010089	JX010399	–
C. proteae	**CBS 132882[b], CPC 14859**	***Protea* sp.**	**South Africa**	**KC297079**	**KC297009**	**KC296960**	**KC296940**	**KC296986**	**KC297032**	**KC297101**	**KC297045**
C. proteae	**CBS 134301, CPC 14860**	***Protea* sp.**	**South Africa**	**KC842385**	**KC842379**	**KC842375**	**KC842373**	**KC842377**	**KC842381**	**KC842387**	**KC842383**
C. proteae	**CBS 134302, CPC 14861**	***Protea* sp.**	**South Africa**	**KC842386**	**KC842380**	**KC842376**	**KC842374**	**KC842378**	**KC842382**	**KC842388**	**KC842384**
C. psidii	CBS 145.29[b], ICMP 19120	*Psidium* sp.	Italy	JX010219	JX009967	JX009743	JX009515	JX009901	JX010133	JX010443	–
C. queenslandicum	ICMP 1778[b]	*Carica papaya*	Australia	JX010276	JX009934	JX009691	JX009447	JX009899	JX010104	JX010414	–
C. queenslandicum	ICMP 18705	*Coffea* sp.	Fiji	JX010185	JX010036	JX009694	JX009490	JX009890	JX010102	JX010412	–
C. salsolae	ICMP 19051[b]	*Salsola tragus*	Hungary	JX010242	JX009916	JX009696	JX009562	JX009863	JX010093	JX010403	–
C. siamense	**CBS 112983, STE-U 2291, JT 814**	***Protea cynaroides***	**Zimbabwe**	**KC297065**	**KC297007**	**KC296961**	**KC296929**	**KC296984**	**KC297030**	**KC297100**	**KC297043**
C. siamense	**CBS 113199, STE-U 2290, JT 813**	***Protea cynaroides***	**Zimbabwe**	**KC297066**	**KC297008**	**KC296962**	**KC296930**	**KC296985**	**KC297031**	**KC297090**	**KC297044**
C. siamense	ICMP 18578[b], CBS 130417	*Coffea arabica*	Thailand	JX010171	JX009924	FJ917505	FJ907423	JX009865	JX010094	JX010404	–

Table 1 (continued)

Species	Accession number[a]	Host	Locality	GenBank accessions							
				ITS	GAPDH	CAL	ACT	CHS-1	GS	TUB2	HIS3[c]
C. siamense (syn. *C. jasmini-sambac*)	CBS 130420, ICMP 19118	*Jasminum sambac*	Vietnam	HM131511	HM131497	JX009713	HM131507	JX009895	JX010105	JX010415	–
C. siamense	GZAAS 5.09538	*Murraya* sp.	China	JQ247632	JQ247608	JQ247597	JQ247656	–	JQ247620	JQ247645	–
C. siamense (syn. *C. hymenocallidis*)	CBS 125378, ICMP 18642	*Hymenocallis americana*	China	JX010278	JX010019	JX009709	GQ856775	GQ856730	JX010100	JX010410	–
C. siamense	GZAAS 5.09506[b]	*Murraya* sp.	China	JQ247633	JQ247609	JQ247596	JQ247657	–	JQ247621	JQ247644	–
C. theobromicola	CBS 124945[b], ICMP 18649	*Theobroma cacao*	Panama	JX010294	JX010006	JX009591	JX009444	JX009869	JX010139	JX010447	–
C. theobromicola	CBS 142.31, ICMP 17927	*Fragaria×ananassa*	USA	JX010286	JX010024	JX009592	JX009516	JX009830	JX010064	JX010373	–
C. theobromicola	ICMP 17957, CBS 124251, MUCL 42294	*Stylosanthes viscosa*	Australia	JX010289	JX009962	JX009597	JX009575	JX009821	JX010063	JX010380	–
C. ti	ICMP 4832[b]	*Cordyline* sp.	New Zealand	JX010269	JX009952	JX009649	JX009520	JX009898	JX010123	JX010442	–
C. ti	ICMP 5285	*Cordyline australis*	New Zealand	JX010267	JX009910	JX009650	JX009553	JX009897	JX010124	JX010441	–
C. tropicale	ICMP 18672, MAFF 239933	*Litchi chinensis*	Japan	JX010275	JX010020	JX009722	JX009480	JX009826	JX010086	JX010396	–
C. tropicale	CBS 124949[b], ICMP 18653	*Theobroma cacao*	Panama	JX010264	JX010007	JX009719	JX009489	JX009870	JX010097	JX010407	–
C. viniferum	GZAAS 5.08601[b], CBS 130643, yg1	*Vitis vinifera*, cv. 'Shuijing'	China	JN412804	JN412798	JQ309639	JN412795	–	JN412787	JN412813	–
C. viniferum	GZAAS 5.08608, CBS 130644, gg4	*Vitis vinifera*, cv. 'Hongti'	China	JN412802	JN412800	JQ412782	JN412793	–	JN412784	JN412811	–
C. viniferum	GZAAS 5.08614, gg9	*Vitis vinifera*, cv. 'Shuijing'	China	JN412805	JN412797	JN412783	JN412794	–	JN412789	JN412810	–
C. xanthorrhoeae	BRIP 45094[b], ICMP 17903, CBS 127831	*Xanthorrhoea preissii*	Australia	JX010261	JX009927	JX009653	JX009478	JX009823	JX010138	JX010448	–
G. cingulata "f. sp. *camelliae*"	ICMP 10643	*Camellia×williamsii*	UK	JX010224	JX009908	JX009630	JX009540	JX009891	JX010119	JX010436	–
G. cingulata "f. sp. *camelliae*"	ICMP 18542	*Camellia sasanqua*	USA	JX010223	JX009994	JX009628	JX009488	JX009857	JX010118	JX010429	–
G. cingulata "f. sp. *camelliae*"	ICMP 10646	*Camellia sasanqua*	USA	JX010225	JX009993	JX009629	JX009563	JX009892	JX010117	JX010437	–

[a] *CBS* Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; *ATCC* American Type Culture Collection, Manassas, VA, USA; *ICMP* International Collection of Microorganisms from Plants, Auckland, New Zealand; *MAFF* MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; *HMBFU* Mycological Herbarium of Beijing Forestry University, Beijing, China; *STE-U* Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; *GZAAS* Guizhou Academy of Agricultural Sciences, Guizhou Province, China; *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai Thailand

[b] ex-holotype, ex-epitype or ex-neotype cultures

[c] HIS3 gene was not used in multilocus phylogenetic analysis

Strains and sequences generated in this paper are in bold, other sequences were obtained from Prihastuti et al. (2009), Yang et al. (2009, 2011), Phoulivong et al. (2010), Weir and Johnston (2010), Wikee et al. (2011), Damm et al. (2012b), Li et. al. (2012), Peng et al. (2012, 2013) and Weir et al. (2012)

Results

Phylogeny

The phylogenetic analysis included 86 strains with *Colletotrichum boninense* (MAFF 305972) as outgroup. The dataset of seven genes (ACT, CAL, CHS-1, GAPDH, GS, ITS, TUB2) comprised 3745 characters including the alignment gaps, of which 967 characters were parsimony-informative, 505 parsimony-uninformative and 2251 constant. Parsimony analysis resulted in 121 equally parsimonious trees, and one of them (Length=2394, CI=0.765, RI=0.924, RC=0.706) is shown in Fig. 1. The Bayesian tree confirmed the tree topology of the trees obtained with maximum parsimony.

The isolates from *Proteaceae* studied here (indicated with red squares) belong to six clades (Fig. 1). Nine strains clustered with *C. alienum*, six strains with *C. aotearoa* and four strains with *C. kahawae* subsp. *ciggaro*. Three isolates from *Protea* sp. identified as *C. proteae* form a clade on a long branch (100/1.00), which was basal to the top part of the phylogeny formed by 12 closely related species that correspond to the clade addressed as the Musae clade by Weir et al. (2012). A single strain lineage, representing *C.*

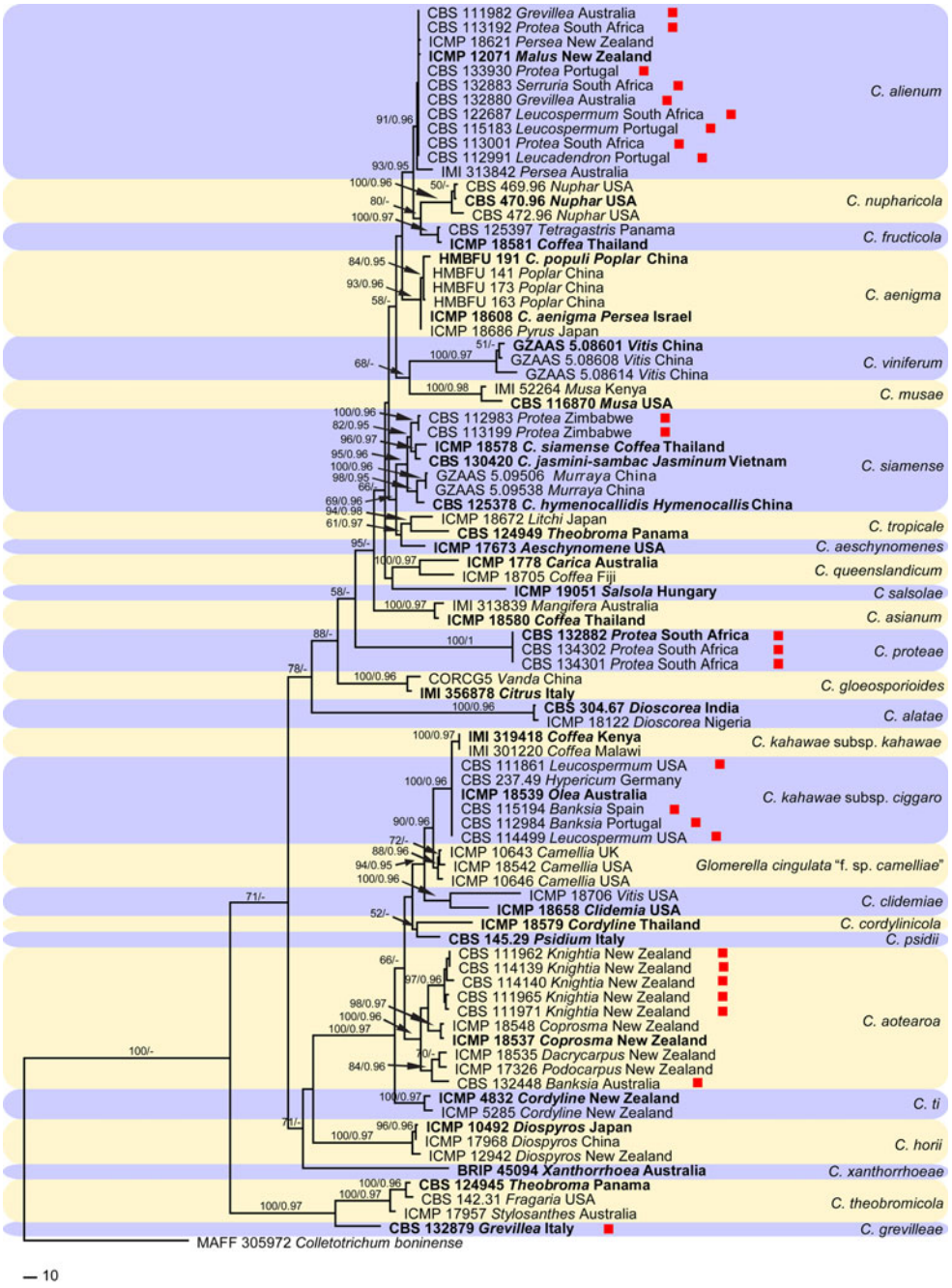

Fig. 1 One of 121 equally parsimonious trees obtained from a heuristic search of combined ACT, CAL, CHS-1, GAPDH, GS, ITS and TUB2 gene sequences of 85 isolates from the *Colletotrichum gloeosporioides* species complex and one outgroup *C. boninense*. Bootstrap support values (1000 replicates) above 50 % and Bayesian posterior probability values above 0.95 are shown at the nodes. Ex-type cultures are emphasised in bold, and include the taxonomic name as originally described. Strain number is followed by host and country of origin. Isolates associated with *Proteaceae* are marked with a *red square*

grevilleae, formed a sister lineage to *C. theobromicola*; the two species form a clade that is basal to all other species in the *C. gloeosporioides* complex. Two strains from *Proteaceae* clustered with the ex-type strains of *C. siamense* (ICMP 18578), *C. jasmine-sambac* (CBS 130420), *C. hymenocallidis* (CBS 125378) and strains from *Murraya* in China (Fig. 1). The ex-type strain of *C. populi* (HMBFU 191) and other authentic cultures of *C. populi* grouped with the ex-type strain of *C. aenigma* (ICMP 18608) and also formed a well-supported monophyletic lineage.

Taxonomy

Based on results of the multigene phylogeny, the 25 *Colletotrichum* strains from *Proteaceae* hosts studied belong to six species within the *C. gloeosporioides* complex, including two species that proved to be new to science. In addition, two synonymies of recently described species were recognised. All species associated with *Proteaceae* are characterised and illustrated below, except for *C. siamense*.

Colletotrichum aenigma B. Weir & P.R. Johnst., Stud. Mycol. 73: 135 (2012)

= *Colletotrichum populi* C.M. Tian & Z. Li, Mycotaxon 120: 283 (2012)

Descriptions of this species are provided by Li et al. (2012) and Weir et al. (2012).

Notes: *Colletotrichum aenigma* and *C. populi* were both described recently (Li et al. 2012; Weir et al. 2012). Their ex-holotype cultures however belong to the same terminal clade (Fig. 1). There is only one base pair difference in the ITS and one difference in the GS sequences between *C. aenigma* and *C. populi*. Furthermore, the GAPDH, ACT and TUB sequences are identical; CAL and CHS-1 sequences were not generated by Li et al. (2012) and therefore not included in this study. Since *C. aenigma* was published online on 21 Aug. 2012 prior to *C. populi* (28 Sep. 2012), *C. populi* is regarded as a synonym of *C. aenigma*.

Colletotrichum alienum B. Weir & P.R. Johnst., Stud. Mycol. 73: 139 (2012) Fig. 2

On PDA: Vegetative hyphae hyaline to medium brown, usually smooth-walled, sometimes verrucose, septate, branched. Chlamydospores not observed. Conidiomata not developed, conidiophores formed directly on hyphae of the aerial mycelium. Setae not observed. Conidiophores rarely observed, hyaline to pale brown, simple or septate, sometimes branched. Conidiogenous cells hyaline to pale brown, cylindrical, up to 62.5 μm long, apex 1–1.5 μm diam. Conidia hyaline, aseptate, smooth-walled, cylindrical, both ends rounded, 13.5–16.5×4–5.5 μm, mean±SD=14.7±0.8×4.7±0.4 μm, L/W ratio=3.1.

Sexual morph developed on PDA. Ascomata globose, sometimes obpyriform, brown to black, 150–500 μm diam, usually covered by aerial mycelium, ostiolate, neck brown, outer wall composed of flattened angular cells, 5–13 μm diam. Interascal tissue composed of paraphyses, thin-walled, hyaline, septate, with rounded apex. Asci cylindrical, 50–89.5×8–10.5 μm, 8-spored. Ascospores uni- or biseriately arranged, hyaline, aseptate, smooth-walled, allantoid with rounded ends, 9.5–21.5×3–5.5 μm, mean±SD=16.6±3.0×4.2±0.6 μm, L/W ratio=4.0.

On SNA: Asexual and sexual morph not observed. Appressoria not observed on the undersurface of the medium, but appressoria-like structures that possibly function as chlamydospores were observed within the medium. These are single, aseptate, smooth-walled, brown, globose, obovoid or irregular, 11–21.5×5–9 μm.

On *Anthriscus* stem: Asexual morph not observed. Ascomata globose, brown to black, covered by aerial mycelium, outer wall composed of flattened angular cells, 6–13 μm diam. Interascal tissue composed of paraphyses, thin-walled, hyaline, septate, 2.5–4.5 μm diam, the apex rounded. Asci cylindrical, 50–84.5×5–12.5 μm, 8-spored. Ascospores uni- or biseriately arranged, hyaline, aseptate, smooth-walled, allantoid or fusiform with rounded to slightly acute ends, 15–22.5×4–5.5 μm, mean±SD=19±2×4.7±0.4 μm, L/W ratio=4.0.

Culture characteristics: Colonies on PDA low convex with entire margin, entirely covered with dense, whitish aerial mycelium, surface olivaceous grey with white margin; reverse iron grey to greenish black with white margin; colony diam 76–78 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, filter paper and *Anthriscus* stem covered with sparse whitish mycelium; colony diam 70–72 mm in 7 d, > 90 mm in 10 d.

Materials examined: **AUSTRALIA**, New South Wales, on *Grevillea* sp., 1999, P.W. Crous, culture CBS 111982=CPC 2925; on *Grevillea* sp., 1999, P.W. Crous, culture CBS 132880=CPC 2926. **PORTUGAL**, on *Leucadendron* sp., cv. 'High Gold', Apr. 2000, S. Denman, culture CBS 115183=STE-U 5226 (strain described); on *Leucadendron* sp., cv. 'High Gold', Apr. 2001, J.E. Taylor, culture CBS 112991=STE-U 4450=JT 1168.1; Madeira Island, Florialis Estate, on *Protea cynaroides*, 2 Jan. 2002, S. Denman, culture CBS 133930=CPC 5204. **SOUTH AFRICA**, Western Cape Province, on *Serruria* sp., 4 Jan. 2009, K. Bezuidenhout, culture CBS 132883=CPC 16168; Caledon, on *P. cynaroides*, 1 Jun. 2001, S. Denman, culture CBS 113192=STE-U 4455=B 5712.2; Caledon, on *P. cynaroides*, Jun. 2001, S. Denman, culture CBS 113001=STE-U 4454=B 5678.1; Betty's Bay, on leaf litter of *Leucadendron* sp., 26 Jun. 2000, S. Marincowitz, culture CBS 122687=CPC 13164=PREM 59587=CMW 22211=SL 587.

Notes: Strains CBS 113001, CBS 112991 and CBS 113192 were previously identified as *C. gloeoporioides* based on ITS and Beta-tubulin sequences (Lubbe et al. 2004). Strain CBS 122687 was also identified as *C. gloeosporioides* based on ITS and LSU sequences (Marincowitz et al. 2008a). However, the multi-locus

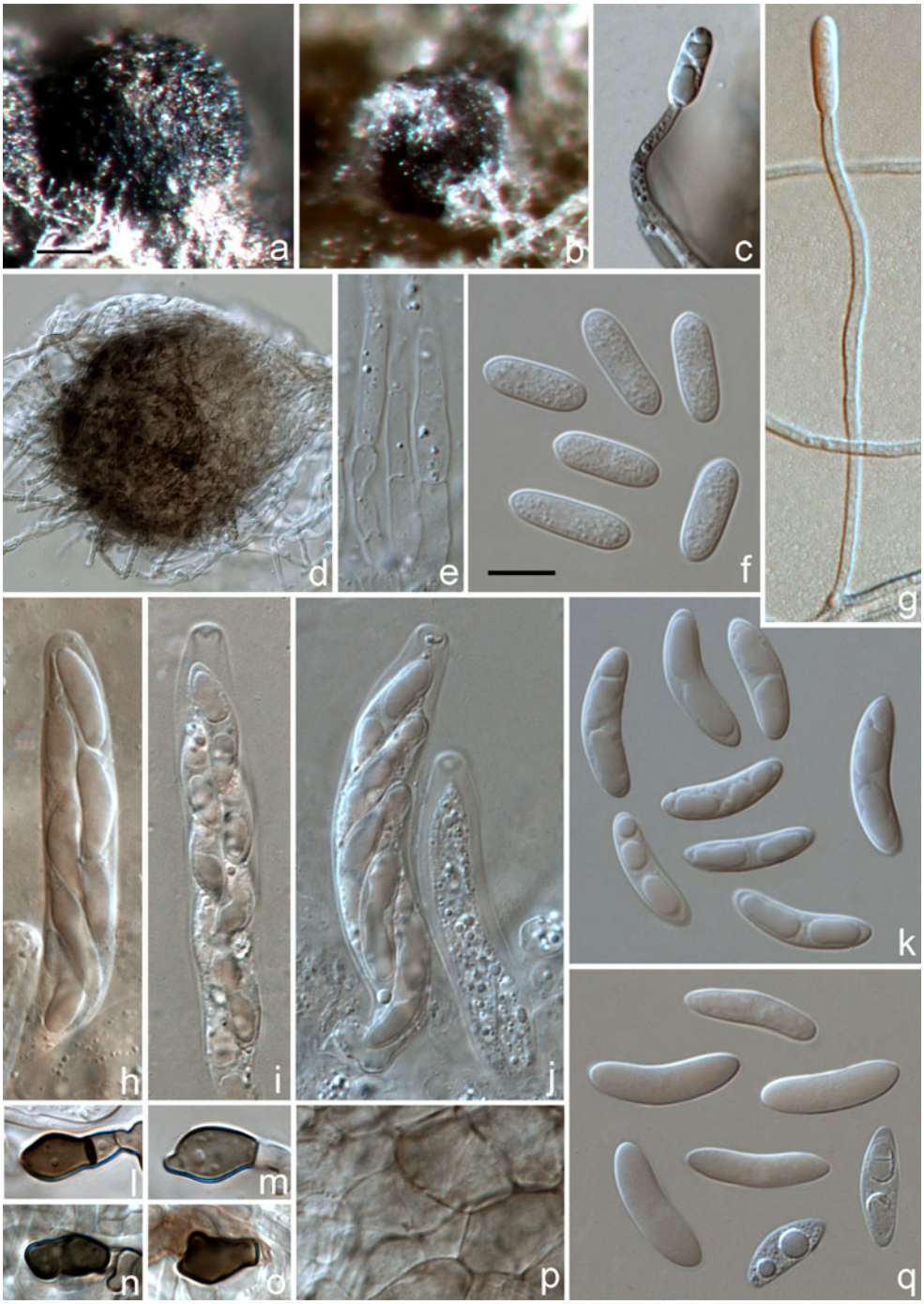

Fig. 2 *Colletotrichum alienum* (from strain CBS 115183). **a**–**b**, **d**. Ascomata; **c**, **g**. Conidiophores; **e**. Paraphyses; **f**. Conidia; **h**–**j**. Asci; **k**, **q**. Ascospores; **l**–**o**. Appressoria-like structures; **p**. Outer surface of peridium. a, c–h, k, p. from PDA; b, i–j, q. from *Anthriscus* stem; l–o. from SNA. a–b. DM; c–q. DIC.—Scale bars: a=100 μm; f=10 μm; a applies to a–b; f applies to c–q

phylogenetic analysis in this study showed that these strains clustered together with the ex-type culture of *C. alienum* (ICMP 12071) (Fig. 1). This species is common on members of *Proteaceae* in Australia, South Africa and Europe.

Colletotrichum aotearoa B. Weir & P.R. Johnst., Stud. Mycol. 73: 139 (2012) Fig. 3

On PDA: Vegetative hyphae hyaline to pale brown, smooth-walled, septate, branched. Chlamydospores not observed. Conidiomata acervular, conidiophores and setae either directly formed from hyphae or on a cushion of roundish hyaline cells. Setae pale to dark brown, smooth-walled to verruculose, 1–3-septate, 40–110 μm long, base inflated or cylindrical, 3.5–6 μm diam, tip more or less acute. Conidiophores hyaline, septate, branched. Conidiogenous cells hyaline, cylindrical to ampulliform, 8.5–16×3–5.5 μm, apex 1.5–2.5 μm diam. Conidia hyaline, aseptate, smooth-walled, cylindrical, both ends rounded, contents sometimes with guttulae, 11–21.5(−28)×4–6 μm, mean±SD=15.5±3.8×5.0±0.5 μm, L/W ratio=3.1.

On SNA: Chlamydospores not observed. Conidiomata acervular. Setae pale to dark brown, smooth-walled to

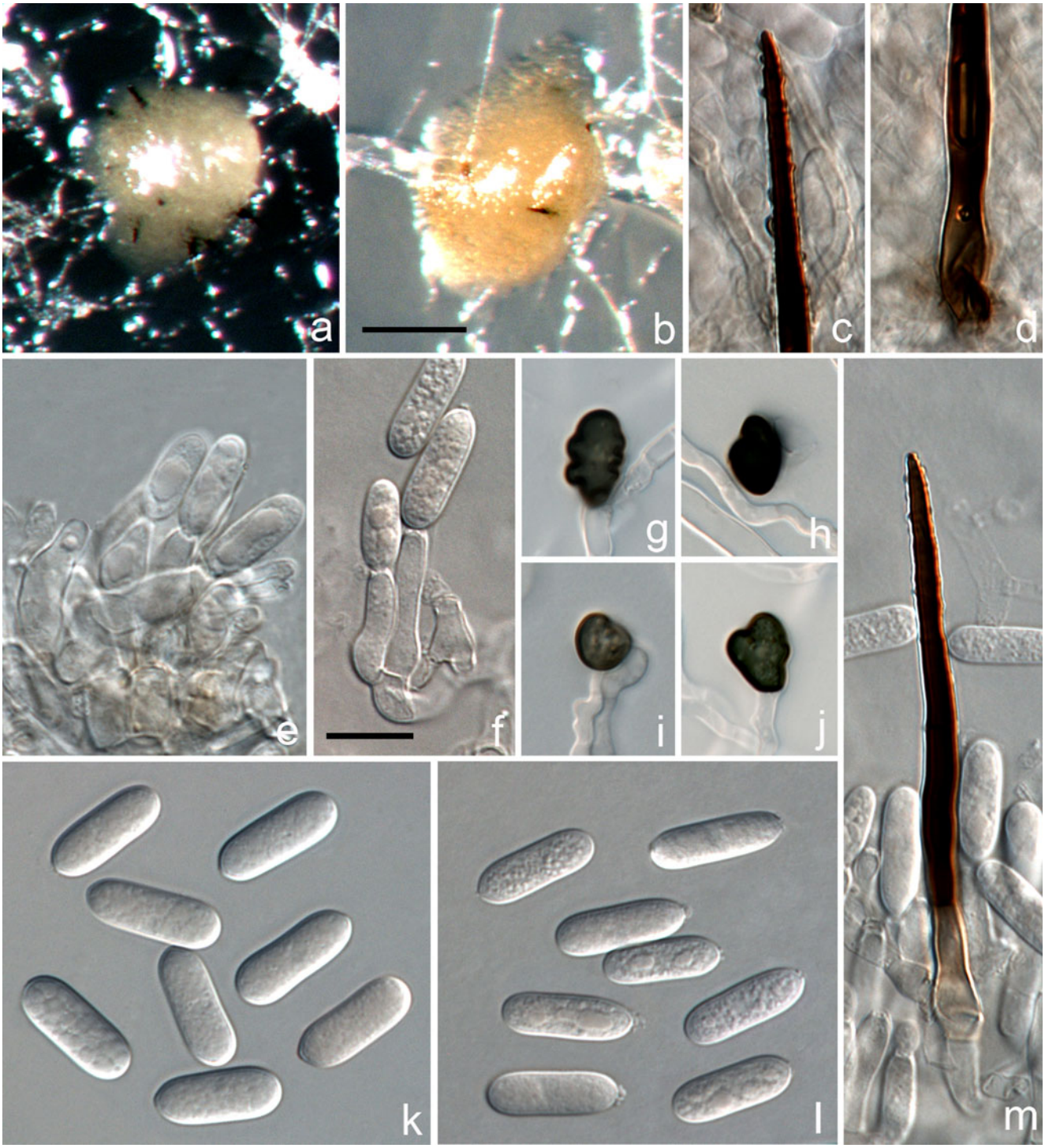

Fig. 3 *Colletotrichum aotearoa* (from strain CBS 114140). **a**–**b**. Acervuli; **c**, **d**, **m**. Setae; **e**–**f**. Conidiophores; **k**, **l**. Conidia; **g**–**j**. Appressoria. a, c–e, k. from PDA; b, f–j, l–m. from SNA. a–b. DM; c–m. DIC.—Scale bars: b=100 μm; f=10 μm; b applies to a–b; f applies to c–m

verruculose, 1–3-septate, 59–66 μm long, base cylindrical or inflated, 3.5–5.5 μm diam, tip±acute. Conidiophores hyaline to pale brown, septate, branched. Conidiogenous cells hyaline to pale brown, cylindrical or ampulliform, 9–21 μm long, apex 1.5–2.5 μm diam. Conidia hyaline, aseptate, smooth-walled, cylindrical, both ends rounded, contents with small guttulae, 12.5–16×4–5 μm, mean±SD=14.0±0.8×4.5±0.3-μm, L/W ratio=3.1. Appressoria not observed in strain CBS 114140, appressoria of strain CBS 111971 medium to dark brown, solitary, aseptate, circular, ellipsoidal or irregular in outline, crenate or slightly lobed at edge, 6.5–12×6–7.5 μm, mean±SD=9.2±1.4×6.3±0.6 μm, L/W ratio=1.5.

Culture characteristics: Colonies on PDA low convex with entire margin, olivaceous grey to greenish black, conidial masses salmon; reverse greenish grey to greenish black; colony diam 76–80 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, umber, white to buff pigment, *Anthriscus* stem and medium covered with salmon conidial masses; colony diam 65–67 mm in 7 d, > 90 mm after 10 d.

Materials examined: **AUSTRALIA**, Victoria, Victoria Valley road, Dunkeld, on *Banksia marginata*, 17 Oct. 1999, I. Pascoe, culture CBS 132448=CPC 17784=VPRI 41610A. **NEW ZEALAND**, Buried Village, on *Knightia* sp., 1999, P.W. Crous, culture CBS 114140=STE-U 2900 (strain described); Buried Village, on *Knightia* sp., 1999, P.W. Crous, culture CBS 111971=STE-U 2898; Buried Village, on *Knightia* sp., 1999, P.W. Crous, culture CBS 111965=STE-U 2897; Buried Village, on *Knightia* sp., 1999, P.W. Crous, culture CBS 114139=STE-U 2902; Buried Village, on *Knightia* sp., 1999, P.W. Crous, culture CBS 111962=STE-U 2901.

Notes: *Colletotrichum aotearoa* was recently described and reported on a wide host range in New Zealand, and was assumed to be a native species (Weir et al. 2012). However, results of this study report this species in Australia as well and extend its host range to *Banksia* and *Knightia*. Based on ITS sequence comparison, Weir et al. (2012) suggested the possible occurrence of this species on *Boehmeria* in China (GenBank records GQ120479 and GQ120480, Wang et al. 2010), which would need to be confirmed with DNA sequence data from additional gene loci.

Colletotrichum grevilleae F. Liu, Damm, L. Cai & Crous, **sp. nov. Fig. 4**

MycoBank MB 802496

Etymology: Referring to the host genus, *Grevillea*.

On PDA: Vegetative hyphae hyaline to medium brown, usually smooth-walled, sometimes verrucose, septate, branched. Chlamydospores not observed. Conidiomata not observed, conidiophores formed directly on hyphae of the aerial mycelium. Setae not observed. Conidiophores hyaline to pale brown, simple or septate, sometimes branched. Conidiogenous cells hyaline to pale brown, cylindrical to ampulliform, straight to flexuous, 11–53×1.5–4 μm, apex 1–1.5 μm diam, collarette rarely observed, 0.5–1.5 μm long. Conidia hyaline, usually aseptate, sometimes becoming 1–3-septate with age, smooth-walled, cylindrical to clavate, both ends rounded, or one end rounded and one end±acute, 7–22.5(−37)×3–6 μm, mean±SD=15.4±6.6×3.9±0.8 μm, L/W ratio=3.9.

On SNA: Chlamydospores not observed. Conidiomata acervular. Setae not observed. Conidiophores hyaline to pale brown, septate, branched. Conidiogenous cells hyaline to pale brown, straight or flexuous, cylindrical to ampulliform, 9.5–27.5×2.5–4.5 μm, opening 1.5–2.5 μm diam. Conidia hyaline, aseptate, smooth-walled, cylindrical, 12.5–17×3.5–5.5 μm, mean±SD=14.5±1.1×4.4±0.3 μm, L/W ratio=3.3. Appressoria not observed.

Culture characteristics: Colonies on PDA low convex with entire margin, surface olivaceous black to dark slate blue with white margin, reverse dark slate blue; colony diam 82–84 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, short sparse white aerial mycelium and buff pigment around *Anthriscus* stem, conidial mass salmon; colony diam 70–74 mm in 7 d, colonial diam>90 mm in 10 d.

Material examined: **ITALY**, Catania, from root and collar rot of *Grevillea* sp., Jan. 2000, G. Polizzi (CBS H-21120 **holotype**, culture ex-type CBS 132879=CPC 15481=DISTEF.GREV.Z).

Notes: Sequence data derived from the ITS region does not separate *C. grevilleae* from *C. theobromicola*, but they can be distinguished based on CAL or GAPDH. Although *C. grevilleae* is only represented by a single isolate, it shows sufficient phylogenetic distance to *C. theobromicola* (Fig. 1). Apart from *C. grevilleae* and *C. alienum* that are treated in this study, there are three *Colletotrichum* species that were previously reported from *Grevillea*. *Colletotrichum acutatum* and *C. fioriniae* that were found on *Grevillea* from Australia and Germany, respectively, are both with a broad host spectrum and belong to the *C. acutatum* species complex (Damm et al. 2012a). Another *Colletotrichum* species, *C. palhinhae*, was described by González Fragoso (1924) as parasite of *Lamproderma echinulatum* (Stemonitida, Amoebozoa) growing on branches and leaves of *Grevillea robusta* in Portugal. However, the conidia of *C. palhinhae* (9–12×1.5–2 μm) are smaller than that of *C. grevilleae*, fusoid and green, and the width of conidia≤2 μm, which is unlikely to be a *Colletotrichum* species in our current understanding.

Colletotrichum kahawae **subsp.** ***ciggaro*** B. Weir & P.R. Johnst., Stud. Mycol. 73: 158 (2012) Fig. 5

On PDA: Vegetative hyphae hyaline to pale brown, smooth-walled, septate, branched. Chlamydospores not observed. Conidiomata acervular, conidiophores formed from a cushion of roundish hyaline cells. Setae absent. Conidiophores hyaline, septate, branched. Conidiogenous cells hyaline, cylindrical, ampulliform or elongate ampulliform, 10.5–13×2–4 μm, apex 1.5–2 μm diam. Conidia hyaline, aseptate, smooth-walled, cylindrical, both ends rounded, 10–14×4–5.5 μm, mean±SD=12.2±0.9×4.7±0.3 μm, L/W ratio=2.6.

On SNA: Chlamydospores not observed. Conidiomata acervular. Setae not observed. Conidiophores hyaline to pale brown, simple or septate, branched or unbranched. Conidiogenous cells hyaline to pale brown, cylindrical or ampulliform, up to 40 μm long, apex 1.5–2.0 μm diam. Conidia hyaline, aseptate, smooth-walled, cylindrical, both ends rounded, 9.5–13.5(−22)×3.5–5.5 μm, mean±SD=12.1±2.0×4.2±0.4 μm, L/W ratio=2.9. Appressoria medium to dark brown, aseptate,

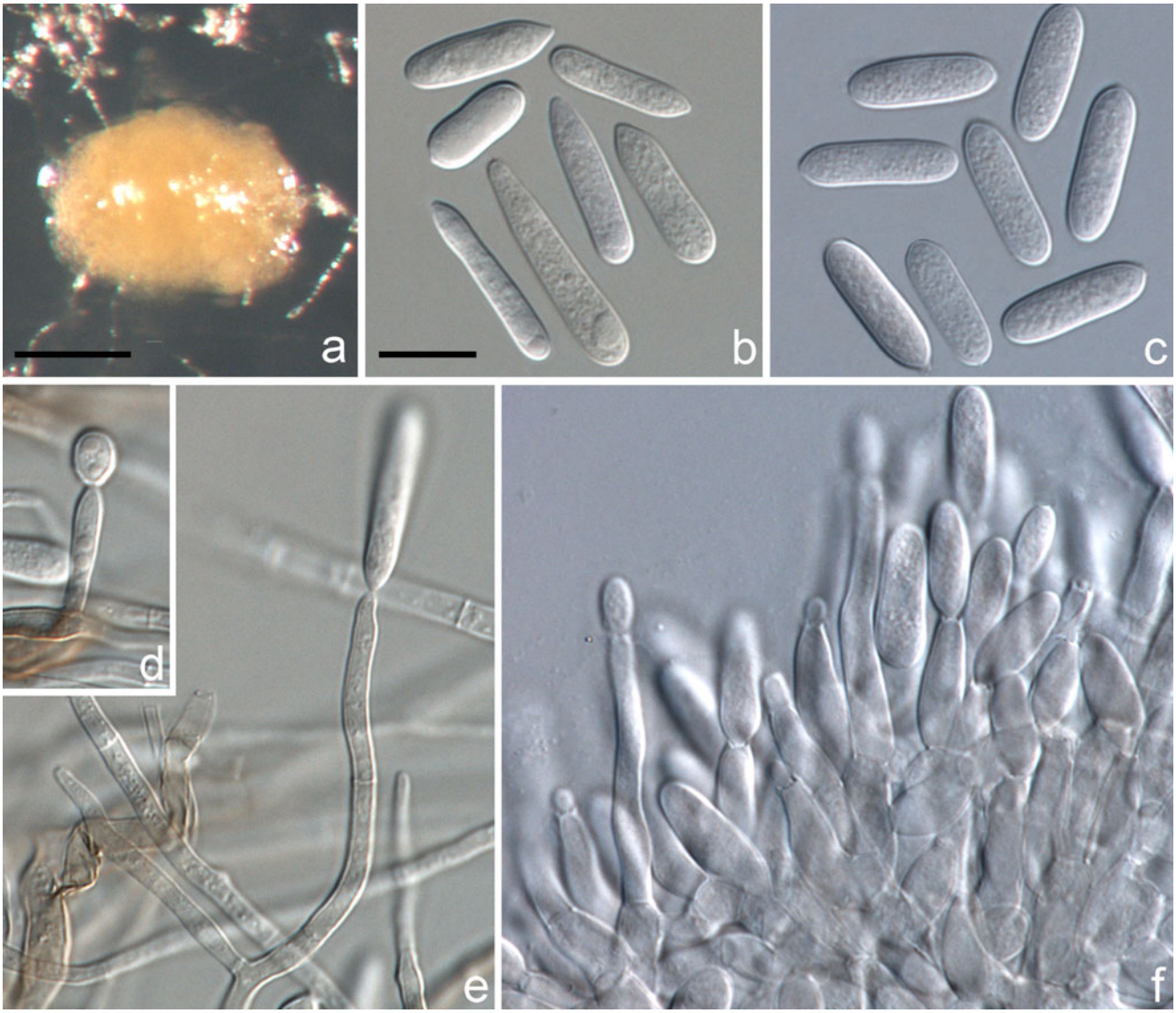

Fig. 4 *Colletotrichum grevilleae* (from ex-holotype strain CBS 132879). **a**. Acervulus; **b**–**c**. Conidia; **d**–**f**. Conidiophores. a, c, f. from SNA; b, d–e. from PDA. a. DM; b–f. DIC.—Scale bars: a=100 μm; b=10 μm; b applies to b–f

solitary or in groups, with a circular, ovoid, ellipsoidal to irregular outline, and crenate or lobed margin, 6–12.5×4.5–8.5 μm, mean±SD=9.0±1.6×6.6±0.8 μm, L/W ratio=1.4.

Culture characteristics: Colonies on PDA low convex with entire margin, surface greenish grey with a whitish margin, conidial masses salmon; reverse greenish grey with a whitish margin; colony diam 76–80 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, filter paper and *Anthriscus* stem covered with whitish to pale mouse grey aerial mycelium, conidial mass on SNA medium pale mouse grey to mouse grey, buff pigment around *Anthriscus* stem; colony diam 72–74 mm in 7 d, > 90 mm in 10 d.

Materials examined: **PORTUGAL**, Madeira Island, on *Banksia* sp., 1 Apr. 2001, J.E. Taylor, culture CBS 112984=STE-U 4445=ICMP 17932. **SPAIN**, Santa de Serra, on *Banksia* sp., 1 Apr. 2002, S. Denman, culture CBS 115194=STE-U 5196. **USA**, Hawaii, on *Leucospermum* sp. cv. 'Safari Sunset', 26 Jan. 1999, P.W. Crous, culture CBS 114499=STE-U 2192; Hawaii, on *Leucospermum* sp. cv. 'Safari Sunset', 26 Jan. 1999, P.W. Crous, culture CBS 111861=STE-U 2191.

Notes: *Colletotrichum kahawae* sensu Waller et al. (1993) was divided into two subspecies, *C. kahawae* subsp. *kahawae* and *C. kahawae* subsp. *ciggaro* (Weir et al. 2012). *Colletotrichum kahawae* subsp. *kahawae* was distinguished from *C. kahawae* subsp. *ciggaro* on the basis of its host range and GS gene sequence to stress the biosecurity importance of the coffee berry pathogen (Weir et al. 2012). Isolates of *C. kahawae* occurring on *Proteaceae* were identified as *C. kahawae* subsp. *ciggaro* according to Weir et al.

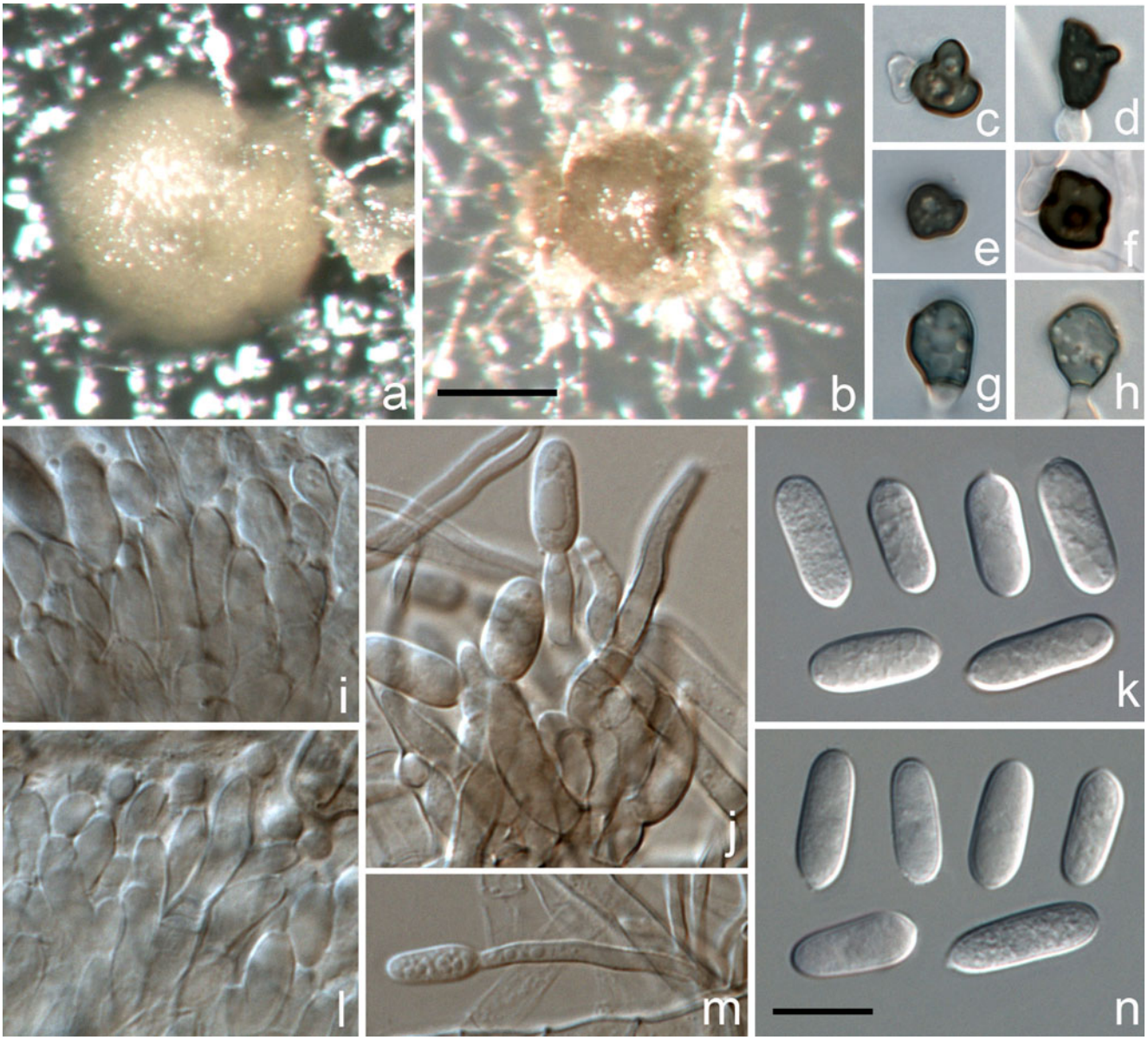

Fig. 5 *Colletotrichum kahawae* subsp. *ciggaro* (from strain CBS 114499). **a**–**b**. Acervuli; **c**–**h**. Appressoria; **i**–**j**, **l**–**m**. Conidiophores; **k**, **n**. Conidia. a, i, k–l. from PDA; b–h, j, m–n. from SNA. a–b. DM; c–n. DIC.—Scale bars: b=100 μm; n=10 μm; b applies to a–b; n applies to c–n

(2012) by the presence of a 22 bp insertion in their GS sequences that is missing in those of *C. kahawae* subsp. *kahawae*.

Strain CBS 112984 included in this study was regarded as *C. crassipes* by Lubbe et al. (2004), probably due to the name tag applied to it and a strain from *Dryas* in Switserland (CBS 112988=IMI 359911) in the CBS and IMI culture collections. *Colletotrichum crassipes* was originally described as *Gloeosporium crassipes* (Spegazzini 1878) from fruits of *Vitis vinifera* in Italy and forms conidia that are larger than those of *C. kahawae*, measuring 20–30×7–8 μm; the two species are therefore unlikely to be conspecific. However, the taxonomic status of *C. crassipes* remains uncertain. Isolates from grapes from the original location of *Gloeosporium crassipes* in Italy are required to serve as epitype to stabilize the application of the name, and to resolve its relationship with other taxa in the genus.

Colletotrichum proteae F. Liu, Damm, L. Cai & Crous, **sp. nov. Fig. 6**

MycoBank MB 802498

Etymology: Referring to the host genus, *Protea*.

On PDA: Vegetative hyphae hyaline to pale brown, smooth-walled, septate, branched. Chlamydospores not observed. Conidiomata acervular, conidiophores formed from a cushion of roundish, hyaline or pale brown cells. Setae rare, only one observed, medium brown, smooth-walled, 1- septate, basal cell pale brown, cylindrical, tip round. Conidiophores hyaline to pale brown, septate, branched. Conidiogenous cells hyaline to

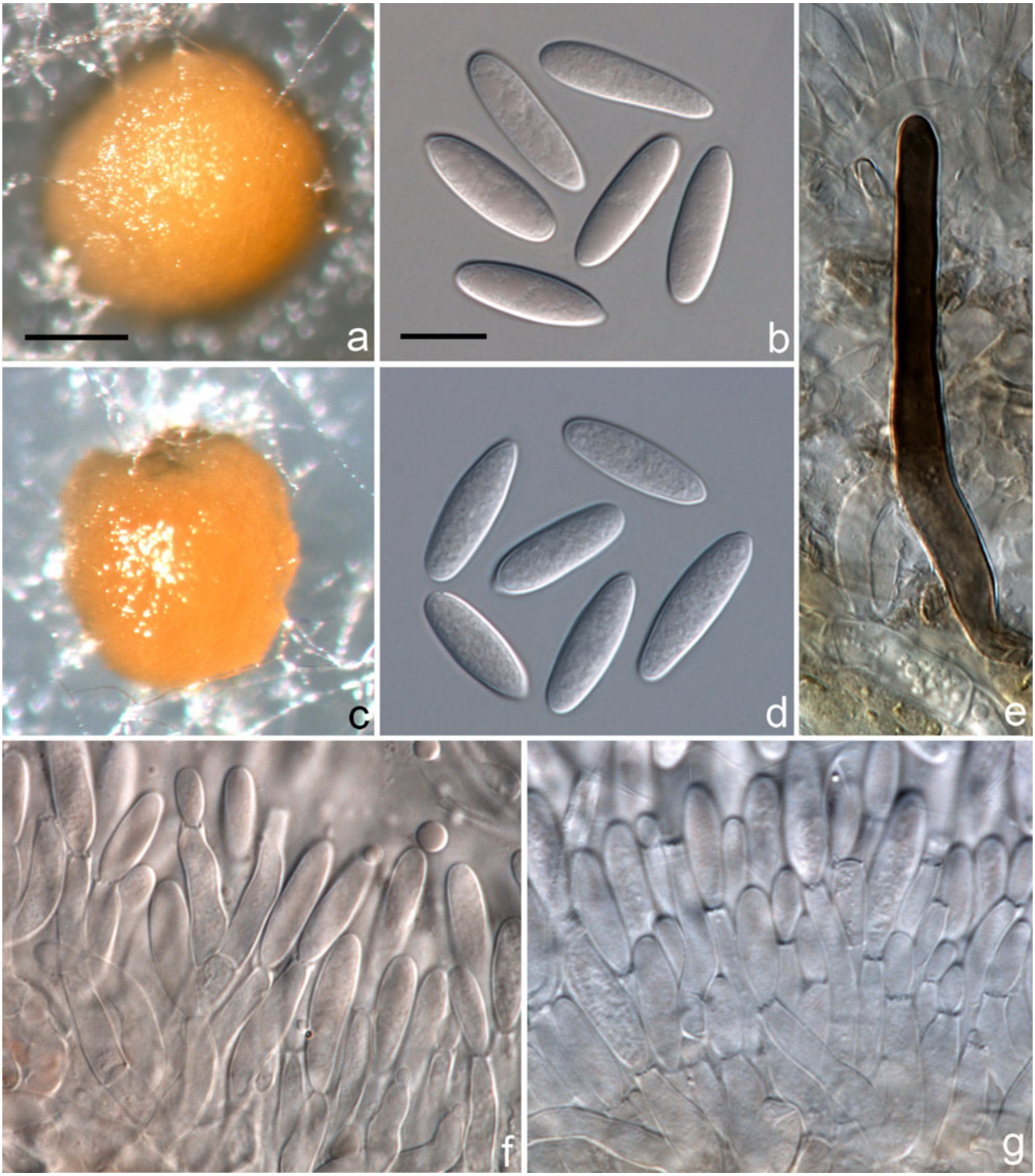

Fig. 6 *Colletotrichum proteae* (from ex-holotype strain CBS 132882). **a**, **c**. Acervuli; **b**, **d**. Conidia; **e**. Seta; **f**–**g**. Conidiophores. a–b, e–f. from PDA; c–d, g. from SNA. a, c. DM; b, d–g. DIC.—Scale bars: a=100 μm; b=10 μm; a applies to a, c; b applies to b, d–g

pale brown, cylindrical, ampulliform to elongate ampulliform, 12.5–19×2–3 μm, apex 1–2 μm diam. Conidia hyaline to pale brown, aseptate, smooth-walled, fusiform to ellipsoidal, 15.5–19×4–5 μm, mean±SD=17.0±0.9×4.6±0.3 μm, L/W ratio=3.7.

On SNA: Chlamydospores not observed. Conidiomata acervular. Setae not observed. Conidiophores hyaline, septate, branched. Conidiogenous cells hyaline, cylindrical, ampulliform to elongate ampulliform, 12.5–20×2.5–4.5 μm, apex 1.5–2.5 μm diam. Conidia hyaline, aseptate,

smooth-walled, fusiform to ellipsoidal, 14.5–18.5×3.5–5.5 μm, mean±SD=16.9±1.1×4.5±0.4 μm, L/W ratio=3.8. Appressoria not observed.

Culture characteristics: Colonies on PDA raised with entire margin, white, sparse aerial mycelium, conidial mass apricot, sepia or brown-vinaceous; reverse white; colony diam 70–74 mm in 7 d, > 90 mm in 10 d. Colonies on SNA flat with entire margin, aerial mycelium lacking, medium buff to honey pigment, conidial mass salmon, apricot, or iron-grey, colony diam 61–65 mm in 7 d, > 90 mm in 10 d.

Materials examined: **SOUTH AFRICA**, Western Cape Province, Tsitsikamma National Park, Nature's Valley, on *Protea* sp., 9 Jan. 2008, P.W. Crous (CBS H-21119 **holotype**, culture ex-type CBS 132882=CPC 14859); Western Cape, Tsitsikamma National Park, Nature's Valley, on *Protea* sp., 9 Jan. 2008, P.W. Crous, culture CBS 134301=CPC 14860; Western Cape, Tsitsikamma National Park, Nature's Valley, on *Protea* sp., 9 Jan. 2008, P.W. Crous, culture CBS 134302=CPC 14861.

Notes: Although the fusiform to ellipsoidal conidia of *C. proteae* are reminiscent of species belonging to the *C. acutatum* complex (Damm et al. 2012a), DNA sequence data demonstrate that this species belongs to the *C. gloeosporioides* species complex. To our knowledge, no species in *C. gloeosporioides* species complex was described on *Protea* before, except two further *Colletotrichum* species treated in this study, which are *C. alienum* and *C. siamense* from *Protea* in South Africa and Zimbabwe, respectively. Another species was identified as *C. boninense* by Lubbe et al. (2004); strains from *Protea cynaroides* in Zimbabwe and *Protea obtusifolia* in Portugal (Madeira Island) proved to be *C. karstii*, belonging to the *C. boninense* species complex (Damm et al. 2012b).

Colletotrichum siamense Prihastuti, L. Cai & K.D. Hyde, Fungal Divers 39: 98 (2009)

A descriptions of this species was provided by Prihastuti et al. (2009).

Materials examined: **THAILAND**, Chiang Mai, Mae Lod Village, on *Coffea arabica* berries, 12 Dec. 2007, H. Prihastuti, ex-holotype culture CBS 130417=ICMP 18578=CGMCC 3.14174=MFLU 090230=BPD-I2. **ZIMBABWE**, on *P. cynaroides*, Mar. 1999, S. Denman, culture CBS 112983=STE-U 2291=JT814; on *P. cynaroides*, Mar. 1999, S. Denman, culture CBS 113199=STE-U 2290=JT813.

Notes: *Colletotrichum jasmini-sambac* and *C. hymenocallidis* were synonymised with *C. siamense* based on a multi-locus (ACT, CAL, CHS-1, GAPDH and ITS) phylogenetic analysis by Weir et al. (2012), while a recent study based on sequence data of ITS, TUB2, DNA lyase (APN2) and an intergenic spacer between the 3' end of the DNA lyase and the mating type locus MAT1-2 (apn2mat/IGS) recognised a further species closely related to *C. siamense*, *C. melanocaulon*, and two unnamed clades (Doyle et al. 2013). A study by Sharma et al. (2013, this issue) based on ApMat sequences of a large set of strains recognised several clades within *C. siamense* suggesting *C. siamense* to be a species complex. In our study, *C. siamense* shows high sequence variability as well. However, more strains need to be included to support a further splitting of *C. siamense*, possible resurrecting *C. jasmini-sambac* and *C. hymenocallidis* and possible recognising the strains from *Protea* as a distinct species.

The name *Colletotrichum murrayae* Gutner was originally applied to isolates associated with *Murraya exotica* (synonym of *M. paniculata*) in Russia (Bondartseva-Monteverde et al. 1936). *Colletotrichum murrayae* Gutner was placed in synonymy with *C. gloeosporioides* by von Arx (1957). Although the name *C. murrayae* Gutner has had not been used since its description, it is still a legitimate name. Recently, Peng et al. (2012) described a new species associated with leaf spots of *Murraya* sp. in China as *C. murrayae* L.J. Peng & K.D. Hyde, using the same epithet as the species published by Bondartseva-Monteverde et al. in 1936. Therefore, *C. murrayae* L.J. Peng & K.D. Hyde is an illegitimate name. Our multi-locus phylogeny (Fig. 1) revealed that the ex-holotype strain of *C. murrayae* L.J. Peng & K.D. Hyde clusters with *C. siamense*, however a further study is needed to verify the taxonomic status of the strains from *Murraya* sp. in China. Another species, *C. exoticum* was described on *Murraya exotica* in India by Pavgi and Singh (1964). Epitypification of *C. murrayae* Gutner and *C. exoticum* is needed and their relationships within the *C. gloeosporioides* species complex remain to be clarified.

Discussion

The name *Colletotrichum gloeosporioides* was originally applied to *Colletotrichum* isolates associated with diseases of *Citrus* from Italy (Penzig 1882). Since then, many morphologically similar species were described on the basis of host association (Hyde et al. 2009). Based on morphological characteristics, von Arx (1957) synonymised around 600 names under *C. gloeosporioides*. *Colletotrichum gloeosporioides* has long been regarded as a species complex comprising many morphologically similar species. It was only after the epitypification of *C. gloeosporioides* (Cannon et al. 2008) however, that phylogenetically distinct species were defined within this complex. An important contribution to the taxonomy of *C. gloeosporioides* species complex was made by Weir et al. (2012), who applied multi-locus phylogenetic analyses to a large number of isolates revealing this complex to consist of at least 23 taxa. Some of the synonyms of *C. gloeosporioides* treated by von Arx (1957) have been found to represent distinct species in other species complexes, e.g. *C. dracaenae* and *C. godetiae* (Damm et al. 2012a, b). Thus far, *C. gloeosporioides* sensu lato isolates from only

approximately 100 host plants have been restudied using multi-locus phylogenetic analyses, the majority of the host plants with anthracnose disease symptoms in von Arx's treatment (von Arx 1957) remain to be recollected and restudied.

Proteaceae cut-flowers are extensively cultivated in South Africa, Zimbabwe, Israel, Australia and New Zealand. However, comprehensive phytosanitary regulations induced by the World Trade Organisation (WTO) restrict the trade of *Proteaceae* cut-flowers (World Trade Organisation 1994; Crous et al. 2000; Taylor et al. 2001a, b). Thus rapid and accurate identification of plant pathogenic fungi is essential to ensure appropriate phytosanitary measures and suitable control strategies (Crous and Groenewald 2005). A large number of fungal pathogens are known to occur on *Proteaceae* (Crous et al. 2004a). The taxonomy of some of these species has changed considerably since they were first reported, of which many have been restudied (Crous et al. 2011). While strains from *Proteaceae* previously identified as *C. acutatum* and *C. boninense* were included in the revisions of the respective species complexes (Damm et al. 2012a, b), the present study is the first to revisit the taxonomy of the *C. gloeosporioides* species complex on *Proteaceae* since the first phylogenetic study on this topic published by Lubbe et al. (2004).

Based on the multi-locus phylogenetic analysis conducted in this study, isolates belonging to the *C. gloeosporioides* complex associated with *Proteaceae* are revealed to belong to six species. Some of the strains included here were previously regarded as *C. gloeosporioides* or *C. crassipes* based on ITS, TUB2 or LSU sequence data (Lubbe et al. 2004; Marincowitz et al. 2008a), but are now shown to belong to *C. alienum*, *C. kahawae* subsp. *ciggaro* or *C. siamense* based on our analysis and recent treatments published on this species complex (Weir et al. 2012).

Together with the seven species in the *C. boninense* and *C. acutatum* species complexes (Damm et al. 2012a, b), there are now 13 *Colletotrichum* species known to be associated with *Proteaceae* hosts. Most of these taxa have a diverse host range. The species so far only known from *Proteaceae* are *C. grevilleae* and *C. proteae*.

This study also revealed a wider host distribution of several species. For example, although *C. aotearoa* was originally reported as having a restricted distribution in New Zealand (Weir et al. 2012), it was found here to also occur in Australia. While *C. alienum* was originally described from New Zealand and Australia, it is shown here to also occur in Africa and Europe.

Various species of *Banksia*, *Grevillea*, *Leucadendron*, *Leucospermum* and *Protea* are commercially cultivated for the cut-flower industry (Crous et al. 2000; Marincowitz et al. 2008b). These plants host relatively high species diversities within the *C. gloeosporioides* complex. *Protea* can be infected by several *Colletotrichum* species, e.g. *C. alienum*, *C. siamense* or *C. proteae*, which are reported from Portugal, South Africa, Spain or Zimbabwe, respectively (Table 1). More strains need to be included to evaluate if the strains from *Protea* identified as *C. siamense* represent a distinct species. Further studies are also needed to determine the identity of the *C. gloeosporioides* isolates which were originally regarded as pathogens of *Protea* in Australia, California, Hawaii and the Madeira Islands (Crous et al. 2004a) and are not included in this study.

Presently *C. aotearoa* has been recorded from *Banksia* and *Knightia* (Australia and New Zealand); *C. kahawae* subsp. *ciggaro* was collected on *Banksia* and *Leucospermum* (Portugal, Spain and USA/Hawaii), *C. proteae* on *Protea* (South Africa), and *C. grevilleae* on *Grevillea* (Italy). As these records are based on random, chance collections, it calls for more detailed surveys to determine their host range and distribution, and relative importance.

Among species in the *C. gloeosporioides* complex, *C. alienum* seems to be the economically most important species in *Proteaceae* cultivation because of its wide host range and pathogenicity. *Colletotrichum alienum* is associated with most of the popular *Proteaceae* cut flowers in South Africa and Europe, such as *Grevillea*, *Leucadendron*, *Leucospermum*, *Protea*, and *Serruria*. Strain CBS 113001 (identified as *C. gloeosporioides* in Lubbe et al. 2004, here identified as *C. alienum*) was shown to be highly virulent to leaves of *Protea* cultivars (Lubbe et al. 2006), thus further collections and pathogenicity tests are necessary to characterise its distribution and importance as a pathogen of other genera in *Proteaceae*.

According to current data, *Colletotrichum* species associated with *Proteaceae* have seldom been isolated from other symptomatic or asymptomatic host plants in South Africa, except *Carica papaya* and *Persea americana* (Weir et al. 2012). Although this could be due to the generally limited number of samples investigated, it could also be due to the limited host range of the species studied. Further studies are thus required to resolve the host range, distribution and pathogenicity of the *Colletotrichum* species reported on *Proteaceae*.

Acknowledgments We thank the curators of the CBS culture collection for kindly supplying isolates for this study. We are also very grateful to Dr. Roger G. Shivas who gave valuable suggestions to our work on *Colletotrichum* taxonomy, during his academic visit to China (funded by NSFC 31110103906). This study was financially supported by the National Natural Science Foundation of China (NSFC 31070020) and the External Cooperation Program of the Chinese Academy of Sciences (GJHZ1310). This research was also supported by the Dutch Ministry of Agriculture, Nature and Food Quality through an endowment of the FES programme "Versterking infrastructuur Plantgezondheid".

References

Baxter AP, Van der westhuizen GCA, Eicker A (1983) Morphology and taxonomy of South African isolates of *Colletotrichum*. S Afr J Bot 2:259–289

Bondartseva-Monteverde VN, Guntner LS, Novoselova ED (1936) The parasitic fungi in the greenhouse of the Botanical Institute of the Academy of Science of the USSR. Trudy Botanicheskogo Instituta Akademii Nauk SSSR II 3:715–801

Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Divers 39:183–204

Cannon PF, Buddie AG, Bridge PD (2008) The typification of *Colletotrichum gloeosporioides*. Mycotaxon 104:189–204

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Crous PW, Groenewald JZ (2005) Hosts, species and genotypes: opinions versus data. Australas Plant Path 34:463–470

Crous PW, Palm ME (1999) Systematics of selected foliicolous fungi associated with leaf spots of Proteaceae. Mycol Res 103:1299–1304

Crous PW, Summerell BA, Taylor JE, Bullock S (2000) Fungi occurring on Proteaceae in Australia: selected foliicolous species. Australas Plant Path 29:267–278

Crous PW, Denman S, Taylor JE, Swart L, Palm ME (2004a) Cultivation and diseases of Proteaceae: *Leucadendron*, *Leucospermum* and *Protea*. Centraalbureau voor Schimmelcultures (CBS), Utrecht

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004b) MycoBank: an online initiative to launch mycology into the 21st century. Stud Mycol 50:19–22

Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hywel-Jones NL (2004c) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Stud Mycol 50:415–430

Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, Bezuidenhout CM, Palm ME, Marincowitz S, Groenewald JZ (2011) Fungal pathogens of Proteaceae. Persoonia 27:20–45

Damm U, Mostert L, Crous PW, Fourie PH (2008) Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. Persoonia 20:87–102

Damm U, Woudenberg JHC, Cannon PF, Crous PW (2009) *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Divers 39:45–87

Damm U, Cannon PF, Woudenberg JHC, Crous PW (2012a) The *Colletotrichum acutatum* species complex. Stud Mycol 73:37–113

Damm U, Cannon PF, Woudenberg JHC, Johnston PR, Weir BS, Tan YP, Shivas RG, Crous PW (2012b) The *Colletotrichum boninense* species complex. Stud Mycol 73:1–36

Doyle VP, Oudemans PV, Rehner SA, Litt A (2013) Habitat and host indicate lineage identity in *Colletotrichum gloeosporioides s. l.* from wild and agricultural landscapes in North America. PLoS One 8:e62394

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microb 61:1323–1330

González Fragoso R (1923, publ. 1924) Contribución a la flora micológica Lusitánica. Boletim da Sociedade Broteriana Ser. II, 2:3–83

Guerber JC, Liu B, Correll JC, Johnston PR (2003) Characterization of diversity in *Colletotrichum acutatum* sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. Mycologia 95:872–895

Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prihastuti H (2009) *Colletotrichum*: a catalogue of confusion. Fungal Divers 39:1–17

Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26:1899–1900

Knox-Davies PS (1981) Comments on fungus diseases of plants indigenous to the South-Western Cape. Veld and Flora 67:88–91

Knox-Davies PS, Van Wyk PS, Marasas WFO (1986) Diseases of proteas and their control in the South-Western Cape. Acta Hortic 185:189–200

Li Z, Liang YM, Tian CM (2012) Characterization of the causal agent of poplar anthracnose occurring in the Beijing region. Mycotaxon 120:277–286

Liu F, Hyde KD, Cai L (2011) Neotypification of *Colletotrichum coccodes*, the causal agent of potato black dot disease and tomato anthracnose. Mycology 2:248–254

Liu F, Hu DM, Cai L (2012) *Conlarium duplumascospora* gen. et. sp. nov. and *Jobellisia guangdongensis* sp. nov. from freshwater habitats in China. Mycologia 104:1178–1186

Lubbe CM, Denman S, Cannon PF, Groenewald JZE, Lamprecht SC, Crous PW (2004) Characterization of *Colletotrichum* species associated with diseases of Proteaceae. Mycologia 96:1268–1279

Lubbe CM, Denman S, Lamprecht SC, Crous PW (2006) Pathogenicity of *Colletotrichum* species to *Protea* cultivars. Australas Plant Path 35:37–41

Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ (2008a) Microfungi occurring on Proteaceae in the fynbos. CBS Biodiversity Series 7. Centraalbureau voor Schimmelcultures, Utrecht

Marincowitz S, Groenewald JZ, Wingfield MJ, Crous PW (2008b) Species of Botryosphaeriaceae occurring on Proteaceae. Persoonia 21:111–118

Nirenberg HI (1976) Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion Liseola. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 169:1–117

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University 2

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Mol Phylogenet Evol 7:103–116

O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E (2000) A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. Mycoscience 41:61–78

Pavgi MS, Singh UP (1964) Parasitic fungi from north India. Mycopathologia 23:188–196

Peng LJ, Yang YL, Hyde KD, Bahkali AH, Liu ZY (2012) *Colletotrichum* species on *Citrus* leaves in Guizhou and Yunnan provinces, China. Cryptogamie Mycol 33:267–283

Peng LJ, Sun T, Yang YL, Cai L, Hyde KD, Bahkali AH, Liu ZY (2013) *Colletotrichum* species on grape in Guizhou and Yunnan provinces, China. Mycoscience 54:29–41

Penzig AGO (1882) Funghi agrumicoli. Contribuzione allo studio dei funghi parassiti degli agrumi. Michelia 2:385–508

Phoulivong S, Cai L, Parinn N, Chen H, Abd-Elsalam KA, Chukeatirote E, Hyde KD (2010) A new species of *Colletotrichum* from *Cordyline fruticosa* and *Eugenia javanica* causing anthracnose disease. Mycotaxon 114:247–257

Prihastuti H, Cai L, Chen H, McKenzie EHC, Hyde KD (2009) Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Divers 39:89–109

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute, Kew, UK

Rojas EI, Rehner SA, Samuels GJ, Van Bael SA, Herre EA, Cannon P, Chen R, Pang J, Wang R, Zhang Y (2010) *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panamá: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102:1318–1338

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Sharma G, Kumar N, Weir BS, Hyde KD, Shenoy BD (2013) The ApMat marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*. Fungal Divers. doi:10.1007/s13225-013-0247-4

Spegazzini CL (1878) Ampelomiceti Italici, ossia enumerazione, diagnosi e storia dei principali parassiti della vite. Rivista Viticolt. Enologia 2:405–411 [nos 7–9]

Stephenson SA, Green JR, Manners JM, Maclean DJ (1997) Cloning and characterisation of glutamine synthetase from *Colletotrichum gloeosporioides* and demonstration of elevated expression during pathogenesis on *Stylosanthes guianensis*. Curr Genet 31:447–454

Swofford DL (2002) PAUP 4.0 b10: Phylogenetic analysis using parsimony (* and other methods), v. 4.0b10. Computer programme. Sinauer Associates, Sunderland

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Taylor JE, Crous PW, Palm ME (2001a) Foliar and stem fungal pathogens of Proteaceae in Hawaii. Mycotaxon 78:449–490

Taylor JE, Crous PW, Swart L (2001b) Foliicolous and caulicolous fungi associated with Proteaceae cultivated in California. Mycotaxon 78:75–103

Taylor JE, Lee S, Crous PW (2001c) Biodiversity in the Cape Floral Kingdom: fungi occurring on Proteaceae. Mycol Res 105:1480–1484

Von Arx JA (1957) Die Arten der Gattung *Colletotrichum* Cda. Phytopathol Z 29:413–468

Von Broembsen SL (1989) *Colletotrichum* die-back. In: Handbook of diseases of cut-flower Proteas. Victoria, Australia: International Protea Association, pp 16–19

Waller JM, Bridge PD, Black R, Hakiza G (1993) Characterization of the coffee berry disease pathogen, *Colletotrichum kahawae* sp. nov. Mycol Res 97:989–994

Wang XX, Wang B, Liu JL, Chen J, Cui XP, Jiang H, Peng XD (2010) First reports of anthracnose caused by *Colletotrichum gloesporioides* on ramie in China. Plant Dis 94:1508

Weir BS, Johnston PR (2010) Characterisation and neotypification of *Gloeosporium kari Hori* as *Colletotrichum horii* nom. nov. Mycotaxon 111:209–219

Weir BS, Johnston PR, Damm U (2012) The *Colletotrichum gloeosporioides* species complex. Stud Mycol 73:115–180

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: White TJ, Sninsky JJ, Gelfand DH, Innin MA (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

Wikee S, Cai L, Pairin N, McKenzie EHC, Su YY, Chukeatirote E, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K, Hyde KD (2011) *Colletotrichum* species from Jasmine (*Jasminum sambac*). Fungal Divers 46:171–182

World Trade Organisation (1994) Agreement on the application of sanitary and phytosanitary measures. World Trade Organisation, Geneva

Wright MG, Saunderson MD (1995) *Protea* plant protection: from the African context to the international arena. Acta Hortic 387:129–139

Yang YL, Liu ZY, Cai L, Hyde KD, Yu ZN, Mckenzie EHC (2009) *Colletotrichum* anthracnose of Amaryllidaceae. Fungal Divers 39:123–146

Yang YL, Cai L, Yu ZN, Liu ZY, Hyde KD (2011) *Colletotrichum* species on Orchidaceae in southwest China. Cryptogamie Mycol 32:229–253

Zhou H, Brockington M, Jungbluth H, Monk D, Stanier P, Sewry CA, Moore GE, Muntoni F (2006) Epigenetic allele silencing unveils recessive RYR1 mutations in core myopathies. Am J Hum Genet 79:859–868