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Characterization of *Colletotrichum* species associated with diseases of Proteaceae

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Abstract: *Colletotrichum* spp. are known to occur on and cause diseases of Proteaceae, but their identities are confused and poorly understood. The aim of the present study thus was to identify accurately the *Colletotrichum* spp. associated with diseases of cultivated Proteaceae. *Colletotrichum* spp. associated with proteaceous hosts growing in various parts of the world were identified based on morphology, sequence data of the internal transcribed spacer region (ITS-1, ITS-2), the 5.8S gene, and partial sequences of the β-tubulin gene. Four species of *Colletotrichum* were found to be associated with Proteaceae. *Colletotrichum gloeosporioides,* a cosmopolitan species known to occur on numerous hosts, was isolated from *Protea cynaroides* cultivated in South Africa and Zimbabwe, and from a *Leucospermum* sp. in Portugal. A recently described species, *C. boninense* was associated with Zimbabwean and Australian Proteaceae but also occurred on a *Eucalyptus* sp. in South Africa. This represents a major geographical and host extension for the species and a description of the African strains is provided. *Colletotrichum crassipes* was represented by a single isolate obtained from a *Dryandra* plant in Madeira. *Colletotrichum acutatum* was isolated from *Protea* and *Leucadendron* in South Africa as well as from other hosts occurring elsewhere. A pathologically distinct population of this species was found to occur on *Hakea* in South Africa. This population is described as *C. acutatum* f. sp. *hakeae,* and its relationship with other strains of *C. acutatum* is discussed. Contrary to earlier literature reports linking *C. gloeosporioides* to anthracnose of Proteaceae, the present study has shown that several distinct species of *Colletotrichum* are associated with different diseases of this crop, which has serious implications for quarantine and disease control practices.

Key words: β-tubulin, *Colletotrichum acutatum, C. acutatum* f. sp. *hakeae, C. boninense, C. crassipes, C. gloeosporioides, Glomerella acutata, G. cingulata,* ITS, systematics

INTRODUCTION

Members of the plant family Proteaceae are indigenous to Australia, South Africa, Central America, South America, southeastern Asia and the southwestern Pacific Islands (Rebelo 1995). Some members of the Proteaceae are valuable commercially and sought after as cut flowers. Certain species increasingly are being cultivated, and global trade in fresh cut-flower proteas and germplasm is growing. Many species of South African Proteaceae are cultivated in Australia, the Azores, Canary Islands, Chile, Israel, Madeira, New Zealand, Portugal, Spain, USA (California, Hawaii) and Zimbabwe. Some Australian Proteaceae (e.g. species of *Banksia* L.f. and *Telopea* R.Br.) similarly are cultivated in countries other than Australia (Crous et al 2000).

One of the factors limiting commercial production of Proteaceae is damage caused by pests and diseases (Knox-Davies 1981, Wright and Saunderson 1995, Crous et al 2004). Some pathogens cause significant losses in the field and in nurseries. Others damage the appearance of blooms, and although they are not debilitating pathogens they are considered important for aesthetic reasons. Furthermore, many pathogens associated with members of the Proteaceae are regarded as actionable quarantine organisms and can

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result in rejection of consignments at the point of import due to contravention of phytosanitary regulations (Crous et al 2000, Taylor 2001).

Among the most devastating fungal pathogens of Proteaceae are *Colletotrichum* spp., causing seedling damping off, shepherd's crook (anthracnose), pruning wound die-back, leaf lesions and stem dieback (Knox-Davies 1981, Knox-Davies et al 1986, von Broembsen 1989, Crous et al 2004). Disease occurrence in cultivated fields tends to be sporadic and is mediated by climatic conditions suitable for disease development and high inoculum levels. Successful infection of Proteaceae is favored by moderate (20–25 C) temperatures and humid conditions (Forsberg 1993). Young tissues are most affected, often displaying the shepherd's crook symptom or leaf necrosis. Nursery conditions often are conducive to disease development, and young plant material is especially susceptible to infection. Thus, losses in nurseries occur annually.

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. is the only *Colletotrichum* species reported to date to infect members of the Proteaceae. This pathogen has been recorded from most areas where Proteaceae are cultivated. Proteaceae hosts include *Banksia, Grevillea* R.Br. ex Knight, *Hakea* Schrad. & J.C.Wendl., *Leucospermum* R.Br., *Leucadendron* R.Br., *Protea* L., *Serruria* Salisb., and *Telopea* (Greenhalgh 1981, Morris 1982, Benic 1986, Knox-Davies et al 1986, von Broembsen 1989, Forsberg 1993, Taylor 2001, Moura and Rodrigues 2001).

Trends in gross morphological characteristics of isolates recently obtained from species of the Proteaceae suggested that more than one species of *Colletotrichum* might occur on this host family. However, the identification of *Colletotrichum* spp. based on morphological features has been beset by confusion since earliest times. The main impediments to identification are the culture medium and light conditions that influence the production of conidiomata, and the variation in the color of the mycelia and the shape and size of the conidia (Sutton 1980, Nirenberg et al 2002). Preliminary molecular data supported the hypothesis that several species of *Colletotrichum* could be pathogens of Proteaceae. The aim of the present study thus was to identify the *Colletotrichum* spp. associated with diseases of Proteaceae cultivated in different parts of the world.

MATERIALS AND METHODS

Isolates.—Forty-eight isolates were examined during this study, as well as their hosts and origins (TABLE I). For comparison, reference strains of several well known species of *Colletotrichum* were included. Isolates were obtained from these sources: the University of Stellenbosch culture collection (STE-U), the culture collection of the Biocontrol Unit of the Plant Protection Research Institute, Agricultural Research Council in South Africa; CABI Bioscience (IMI) in the UK; the University of Arkansas Department of Plant Pathology; and from infected plant material sampled at various nurseries in the western Cape of South Africa. The sampled nursery material was surface disinfested in 1% sodium hypochlorite for 2 min, 70% ethanol for 1 min and rinsed in distilled water. Infected tissues were plated onto 2% potato-dextrose agar (PDA, Biolab, Midrand, South Africa) amended with 0.04 g/L streptomycin.

Phylogenetic analysis.—DNA was extracted from the fungal cultures according to Lee and Taylor (1990), and the ITS and β-tubulin regions were amplified (Kang et al 2001). 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 gene was amplified with primers T1 (O'Donnell and Cigelnik 1997) and βt-2b (Glass and Donaldson 1995). The PCR products were stained with ethidium bromide and observed under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK). Amplification products were purified following the recommended protocol of the NucleoSpin Extract 2 in 1 Purification Kit (Macherey-Nagel GmbH, Germany), and PCR primers were used to sequence both strands of the purified products with the ABI PRISM BigDye Terminator version 3.0 Cycle Sequencing Ready reaction Kit (PE Biosystems, Foster City, California) according to the manufacturer's instructions. Resulting fragments were analyzed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut).

Both the ITS and β-tubulin sequences were assembled with Sequence Alignment Editor version 2.0a11 (Rambaut 2002), from which a consensus sequence was created. These sequences together with retrievals from GenBank were aligned with Clustal W (Thompson et al 1994). Manual improvement of the final alignment based on visual inspection was made where necessary. Sequences of *Botryosphaeria ribis* Grossenb. & Duggar, *Botryosphaeria parva* Pennycook & Samuels and *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & de Not were used as outgroups for both the ITS and β-tubulin data. Neighbor joining analysis was performed with PAUP* version 4.0b10 (Swofford 2000) on the separate and combined 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. Resulting trees were printed with TreeView version 1.6.6 (Page 1996). A partition homogeneity test (Farris et al 1994) was conducted in PAUP (Swofford 2000) to examine the possibility of a joint analysis of the different datasets.

Morphology.—Isolates were incubated at 25 C under near-ultraviolet (NUV) light with 12 h light/dark cycles. Cultures were transferred to PDA, carnation leaf agar (CLA) (Fisher et al 1982), and synthetic nutrient-poor agar (SNA) containing filter paper (Gams et al 1998) to stimulate sporu-

TABLE I. Isolates, hosts and origins

Anamorph/teleomorph	Accession no.	Other culture collection no.	GenBank no. ITS	GenBank no. β-tub	Host	Origin
C. acutatum	STE-U 5122	CBS 112994	AY376497	AY376545	*Leucospermum* sp.	South Africa
C. acutatum	STE-U 164	CBS 112980	AY376498	AY376546	*Pinus radiata*	South Africa
C. acutatum	STE-U 160	CBS 112979	AY376499	AY376547	*Pinus radiata*	South Africa
C. acutatum	STE-U 162	CBS 112981	AY376500	AY376548	*Pinus radiata*	South Africa
C. acutatum	STE-U 4448	CBS 112990	AY376501	AY376549	*Leucadendron* (cv. Safari Sunset)	South Africa
C. acutatum	STE-U 4460	CBS 113006	AY376502	AY376550	*Protea cynaroides*	South Africa
C. acutatum	STE-U 4452	CBS 112992	AY376503	AY376551	*Protea magnifica*	South Africa
C. acutatum	STE-U 4456	CBS 113002	AY376504	AY376552	*Protea repens*	South Africa
C. acutatum	STE-U 4457	CBS 113003	AY376505	AY376553	*Protea* sp.	South Africa
C. acutatum	STE-U 4458	CBS 113004	AY376506	AY376554	*Protea* sp.	South Africa
C. acutatum	STE-U 4459	CBS 113005	AY376507	AY376555	*Protea* sp.	South Africa
C. acutatum	STE-U 5303	IMI 383015; CBS 112989	AY376508	AY376556	*Hevea brasiliensis*	India
C. acutatum	STE-U 5287	A 38; CBS 112995	AY376509	AY376557	Apple	USA
C. acutatum[a]	STE-U 5292	ATCC 56816; IMI 117617; CBS 111296	AY376510	AY376558	Papaya	Australia
C. acutatum f. sp. *hakeae*	STE-U 4471	CBS 112658	AY376511	AY376559	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4467	CBS 113009	AY376512	AY376560	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4466		AY376513	AY376567	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4470	CBS 112759	AY376514	AY376561	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4465		AY376515	AY376562	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4462	CBS 113007	AY376516	AY376563	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4463	CBS 113008	AY376517	AY376564	*Hakea gibbosa*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4461	CBS 112761	AY376518	AY376565	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4469	CBS 112993	AY376519	AY376566	*Hakea sericea*	South Africa
C. acutatum f. sp. *hakeae*	STE-U 4468	CBS 112760	AY376520	AY376568	*Hakea sericea*	South Africa
C. boninense	STE-U 194	CBS 110779	AY376521	AY376569	*Eucalyptus* sp.	South Africa
C. boninense	STE-U 3000	CBS 112762	AY376522	AY376570	*Leucospermum* sp.	Australia
C. boninense	STE-U 2998		AY376523	AY376571	*Leucospermum* sp.	Australia
C. boninense	STE-U 2290		AY376524	AY376572	*Protea cynaroides*	Zimbabwe
C. boninense	STE-U 2289	CBS 112982	AY376525	AY376573	*Protea cynaroides*	Zimbabwe
C. capsici	STE-U 5304	IMI 56173; CBS 113117	AY376526	AY376574	*Arachis hypogaea*	Tanzania
C. caudatum	STE-U 5300	IMI 196464; CBS 113172	AY376527	AY376575	*Cymbopogon martinii*	India
C. coccodes	STE-U 5301	IMI 61249; CBS 112987	AY376528	AY376576	*Lycopersicon esculentum*	Zimbabwe
C. crassipes	STE-U 5302	IMI 359911; CBS 112988	AY376529	AY376577	*Dryas octopetala*	Switzerland
C. crassipes	STE-U 4445	CBS 112984	AY376530	AY376578	*Dryandra* sp.	Madeira
C. dematium	STE-U 5299	IMI 80025; CBS 127.57	AY376531	AY376579	*Peperomia* sp.	Unknown
C. gloeosporioides	STE-U 4295	IMI 356878; CBS 953.97	AY376532	AY376580	*Citrus* sp.	Italy
C. gloeosporioides	STE-U 2291	CBS 112983	AY376533	AY376581	*Protea cynaroides*	Zimbabwe
C. gloeosporioides	STE-U 5297	IMI 266803; CBS 112986	AY376534	AY376582	*Citrus* sp.	Belize
C. gloeosporioides	STE-U 4450	CBS 112991	AY376535	AY376583	*Leucospermum* (cv. Hugh Gold)	Portugal
C. gloeosporioides	STE-U 4455	CBS 113192	AY376536	AY376584	*Protea cynaroides*	South Africa
C. gloeosporioides	STE-U 4454	CBS 113001	AY376537	AY376585	*Protea cynaroides*	South Africa

TABLE I. Continued

Anamorph/teleomorph	Accession no.	Other culture collection no.	GenBank no. ITS	GenBank no. β-tub	Host	Origin
C. gloeosporioides	STE-U 4453	CBS 113000	AY376538	AY376586	*Vitis vinifera*	South Africa
C. graminicola	STE-U 5298	IMI 84302; CBS 113173	AY376539	AY376587	*Zea mays*	Zimbabwe
C. kahawae	STE-U 5295	IMI 319424; CBS 112985	AY376540	AY376588	*Coffea arabica*	Kenya
C. orbiculare	STE-U 5296	IMI 368075; CBS 113171	AY376541	AY376589	*Xanthium spinosum*	Argentina
C. sublineolum	STE-U 5293	IMI 372541; CBS 112997	AY376542		*Sorghum bicolor*	Ethiopia
C. truncatum	STE-U 5294	IMI 217517; CBS 112998	AY376543	AY376590	*Arachis hypogaea*	Gambia
Glomerella cingulata	STE-U 5291	IMI 324985; CBS 113010	AY376544	AY376591	*Fragaria* sp.	USA

[a] STE-U 5292 is the ex-type strain of *C. acutatum*.

lation and facilitate identification. Morphological observations were made from structures mounted in lactic acid. The 95% confidence intervals of conidial measurements were derived from at least 30 observations at 1000× magnification. Slide cultures (Riddell 1950) were made to stimulate the production of appresoria. Reference cultures were established from single-conidium isolates obtained from CLA plates. Cultures of each isolate were maintained on McCartney bottles containing either PDA or malt-extract agar (MEA) and sterile paraffin oil. Cultures are maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U) in South Africa, at CABI Bioscience (IMI) in the UK and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

Cultural studies.—Six isolates of *C. boninense* as well as *C. acutatum* f. sp. *hakeae* were selected for cultural studies. Colony colors were described from isolates incubated at 25 C under NUV light for 10 d according to the designations of Rayner (1970). Growth rates and cardinal temperature requirements for growth were determined for isolates plated onto PDA in 90 mm Petri dishes and incubated in the dark for 7 d at seven temperature regimes, 5–35 C at 5 degree intervals. Three plates were used for each isolate at each temperature. Radial mycelial growth was measured for each plate and the mean calculated at each temperature to determine the growth rates for each species.

RESULTS

Phylogenetic analysis.—For ITS sequences, approximately 550 bases were determined for the 48 isolates (TABLE I) and added to the alignment. The manually adjusted alignment of the ITS nucleotide sequences contained 87 taxa and 542 characters including alignment gaps (data not shown). Approximately 700 bases of the β-tubulin gene were determined for the isolates and added to the alignment. The manually adjusted alignment of the β-tubulin nucleotide sequences contained 50 taxa and 455 characters including alignment gaps (data not shown). Because the use of outgroups with β-tubulin sequences generated by a different primer combination resulted in shorter sequence lengths, the complete sequences generated for the *Colletotrichum* isolates in this study could not be used for the phylogenetic analysis.

The result of the partition homogeneity test ($P = 0.006$, where $P \geq 0.05$ was taken as significantly incongruent) indicated that it was not possible to combine the different datasets, which therefore were analyzed separately. New sequences were deposited in GenBank (TABLE I) and the alignments in TreeBASE (SN1583).

The phylogram obtained from ITS data delimited three clades concerning *Colletotrichum* species associated with Proteaceae (FIG. 1). The first clade had 100% support and included the ex-type strain of *Colletotrichum acutatum* J.H. Simmonds (STE-U 5292) as well as GenBank sequences of *C. lupini* (Bondar) Nirenberg, Feiler & Hagedorn (AJ301975, AJ301968). Within this clade, four well supported groups were observed: the first group (76% support) contained the *C. acutatum* ex-type strain as well as three isolates from South African *Protea* (STE-U 5122, 4460, 4448), the *forma specialis* from *Hakea* (STE-U 4469, 4462, 4465, 4461, 4463, 4468, 4470, 4467, 4471, 4466) and *Pinus* (STE-U 162, 164, 160); the second group (96% support) contained an isolate from apple (STE-U 5287) and a *C. acutatum* sequence from GenBank (AF207793); the third group (65% support) contained an isolate from *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (STE-U 5303), South African Pro-

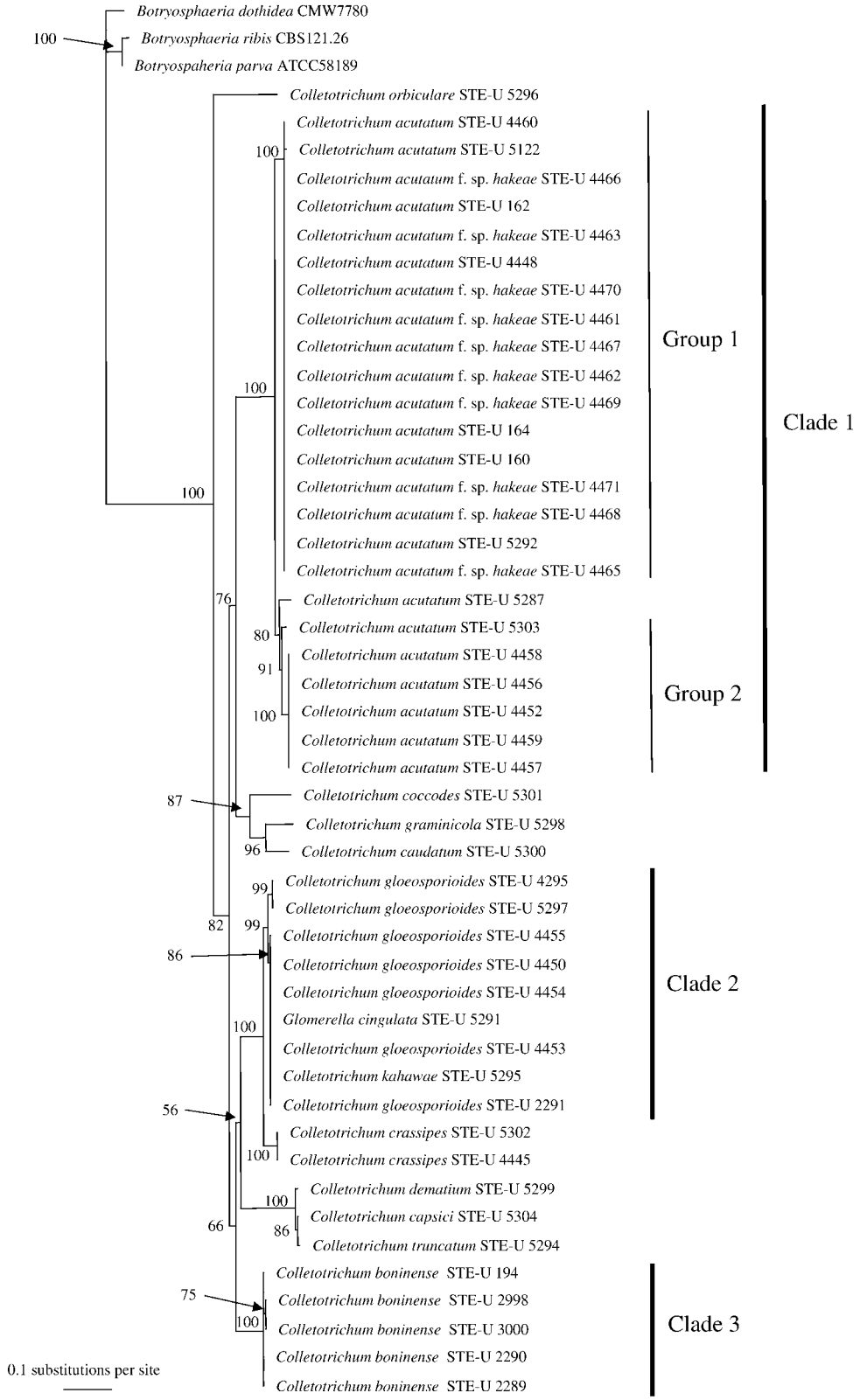


FIG. 1. Phylogram obtained from a neighbor joining analysis of the ITS1, 5.8S rDNA and ITS2 sequence data of *Colletotrichum* isolates from Proteaceae. The tree was rooted to three *Botryosphaeria* species. Branch support is based on 1000 bootstrap replicates and is shown at the nodes. The bar represents 0.1 substitutions per site.

teaceae isolates (STE-U 4457, 4452, 4459, 4456, 4458), as well as three *C. acutatum* sequences obtained from GenBank (AF411765, AF081292, AF090853); and the fourth group (96% support) contained two *C. lupini* sequences from GenBank (AJ301975, AJ301968). The second clade (69% support) was identified as *C. gloeosporioides*. The Proteaceae isolates in this clade originated from Portugal (STE-U 4450), South Africa (STE-U 4454 and 4455) and Zimbabwe (STE-U 2291). This clade also contained isolates of *C. kahawae* J.M. Waller & Bridge (STE-U 5295), *Glomerella cingulata* (Stonem.) Spauld & H. Schrenk (STE-U 5291, AF411769, AF411774, AF411764), *C. gloeosporioides* (AJ311882, STE-U 5297, AJ311883) and a single isolate from *Vitis vinifera* L. (STE-U 4453). Two strains of *C. gloeosporioides* from the type host *Citrus* (STE-U 5297 and STE-U 4295) formed a well supported group within this clade (95% support), as did sequences obtained from GenBank (AF411774, AJ311882, AF411764) and isolates STE-U 4453 and 5291 (83% support). *Colletotrichum crassipes* (Speg.) Arx (STE-U 5302), a GenBank sequence of supposedly *Glomerella cingulata* (AF411775), and one isolate obtained from *Dryandra* R.Br. in Madeira (STE-U 4445) formed a well supported (86% support) clade sister of the second clade.

The third clade (100% support) consisted of two groups, the first of which (98% support) contained Proteaceae isolates from Zimbabwe (STE-U 2290, 2289) and Australia (STE-U 2998, 3000), a South African isolate from *Eucalyptus* (STE-U 194), two GenBank sequences (AJ301974, AB076800), and sequences of *C. boninense* J. Moriwaki, Toy. Sato & T. Tsukiboshi (AB051402, AB051405). The second group (94% support) in this clade also contained two *C. boninense* sequences (AB051400, AB051406) as well as a GenBank sequence of a *Colletotrichum* sp. (AJ301939). The third clade formed a sister clade (96% support) to a clade containing isolates of *C. truncatum* (Schwein.) Andrus & W.D. Moore (STE-U 5294, AJ301945, AF451906, AF451899), *C. dematium* (Pers.) Grove (STE-U 5299) and *C. capsici* (Syd.) E.J. Butler & Bisby (STE-U 5304).

The phylogram obtained from the β-tubulin data (FIG. 2) showed the same three major clades as observed in the ITS phylogram. A well supported *C. acutatum* clade emerged (Clade 1: 100% support), but no support was obtained for groups containing the *Hakea* and *Pinus* isolates (Group 1). However, the third *C. acutatum* group observed in the ITS tree was supported (Group 2: 80% support) in the β-tubulin tree, with the isolates from Proteaceae (STE-U 4458, 4456, 4452, 4459, 4457) forming a subgroup with a 100% support. The *C. gloeosporioides* clade also was well supported (Clade 2: 100% support), and showed the same topology as the ITS clade. The two strains of *C. gloeosporioides* from *Citrus* (STE-U 5297 and STE-U 4295) also formed a group within this clade (99% support). As with the ITS tree, *C. crassipes* STE-U 5302 and an isolate from *Dryandra* (STE-U 4445) formed a clade (100% support) sister of this one. The third well supported clade (100% support) contained the isolate from *Eucalyptus* from South Africa (STE-U 194) and the Proteaceae isolates (STE-U 2290, 2289, 3000, 2998) of *C. boninense*.

TAXONOMY

Four species of *Colletotrichum* and one *formae specialis* were identified in the present study, of which *C. boninense* and *C. acutatum* f. sp. *hakeae* are treated. The other species are discussed only briefly because they form part of a larger revision of *Colletotrichum* species, which will link them to authentic specimens and cultures. For the present, thus, these names are used as by recent authors, based on deposited DNA sequences.

Colletotrichum acutatum f. sp. hakea FIGS. 3–5

Conidiomata with masses of orange conidia. Setae developing in a dense layer around conidiomata, 60–100 μm long, 3- to 8-septate, medium brown at the base, pale brown at the bluntly rounded apex, tapering from a base 3–5 μm diam, to an apex 1.5–2 μm diam. Conidiophores branched below, at times pigmented in the lower part, or reduced to single hyaline conidiogenous cells. Conidiogenous cells subcylindrical, hyaline, smooth, tapering toward a truncate apex with visible periclinal thickening, 12–20 × 3–4 μm. Conidia hyaline, smooth, guttulate, fusoid to naviculate (widest in the upper third), with acutely rounded apex and subtruncate base with a distinct abscission scar; on SNA conidia tend to be naviculate, or to have more bluntly rounded apices, becoming clavate; (9–)11–13(–16) × (3–)4 μm (average 12.5 × 4 μm). Appresoria medium brown, ovoid to clavate, 6–13 × 4–5 μm, 0(–1)-septate. Colonies on SNA with moderate, appressed, white aerial mycelium; on PDA with moderate fluffy aerial mycelium and few aerial conidia. Colonies on SNA with moderate, appressed, white aerial mycelium; on PDA with moderate fluffy aerial mycelium and few aerial conidia; rosy buff (13″f) with vinaceous buff (17‴d) centers on the surface, underneath saffron (15d) with olivaceous gray (21⁗i) centers. Cardinal temperatures for growth were minimum 5 C, opt 25 C, maximum 30 C. No growth was recorded at 35 C. The mean daily growth rate at 25 C was 10.2 mm/d.

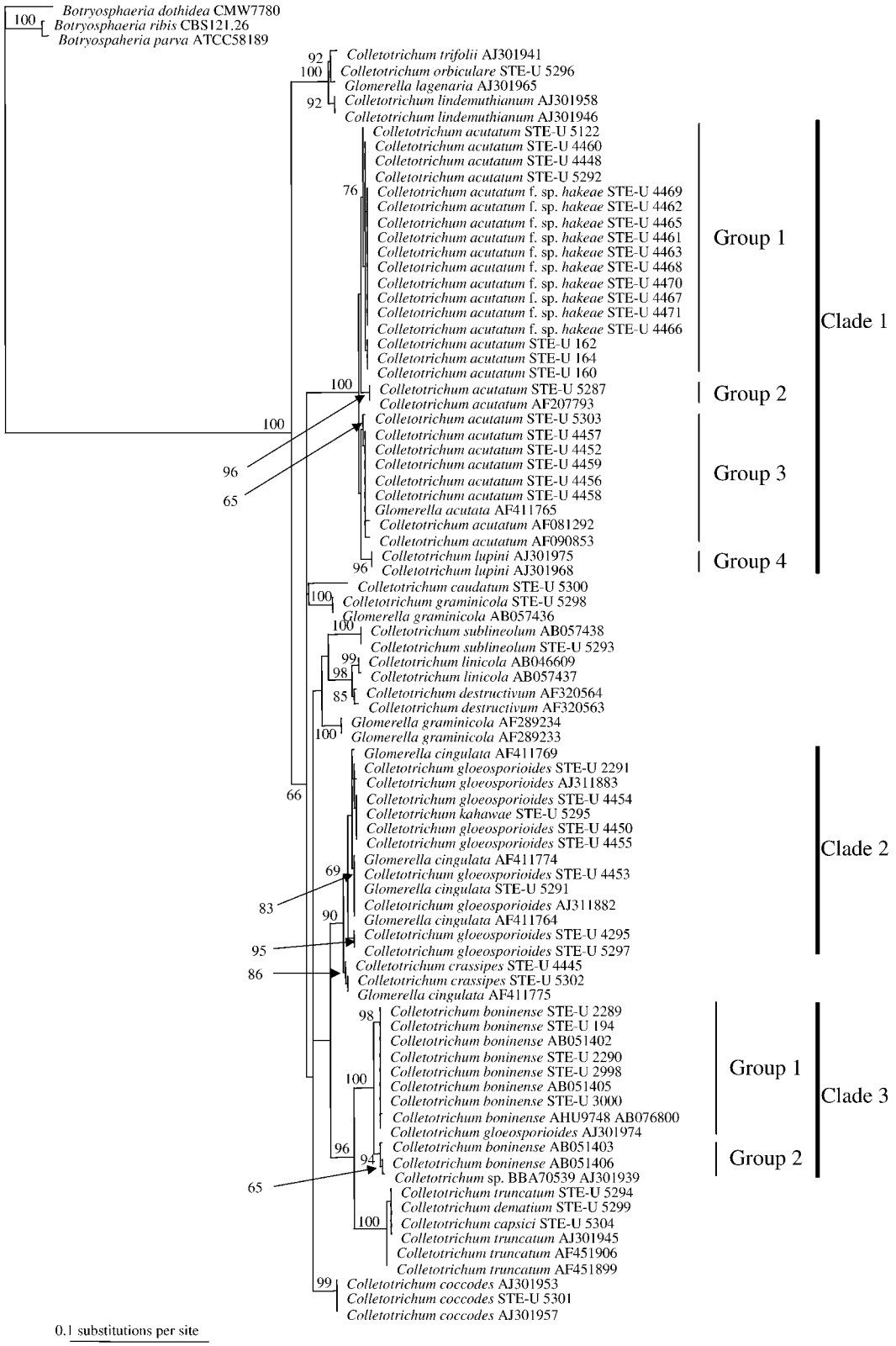


FIG. 2. Phylogram obtained from a neighbor joining analysis of the alignment of sequences from part of the β-tubulin gene of *Colletotrichum* isolates from Proteaceae. The tree was rooted to three *Botryosphaeria* species. Branch support is based on 1000 bootstrap replicates and is shown at the nodes. The bar represents 0.1 substitutions per site.

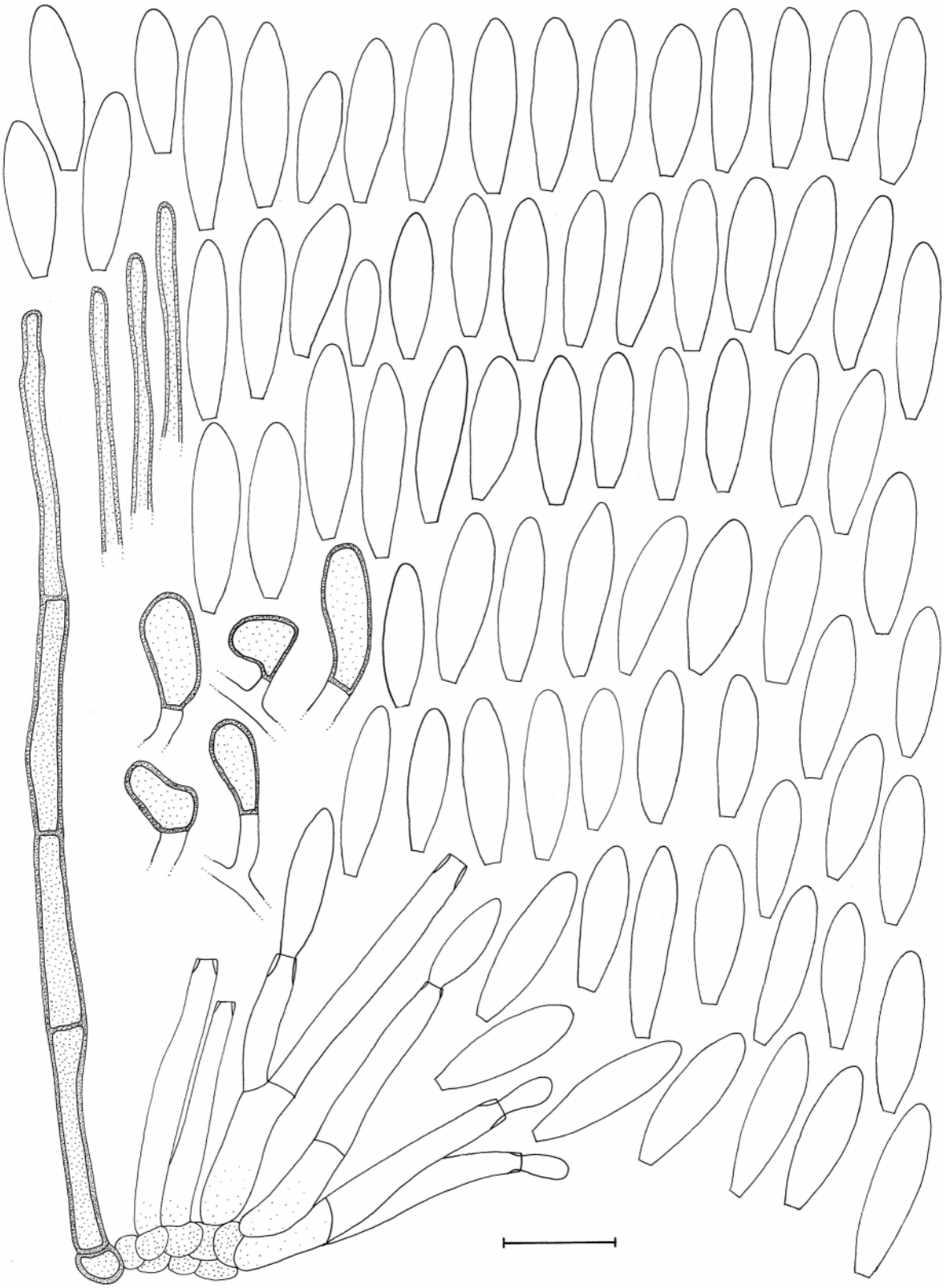
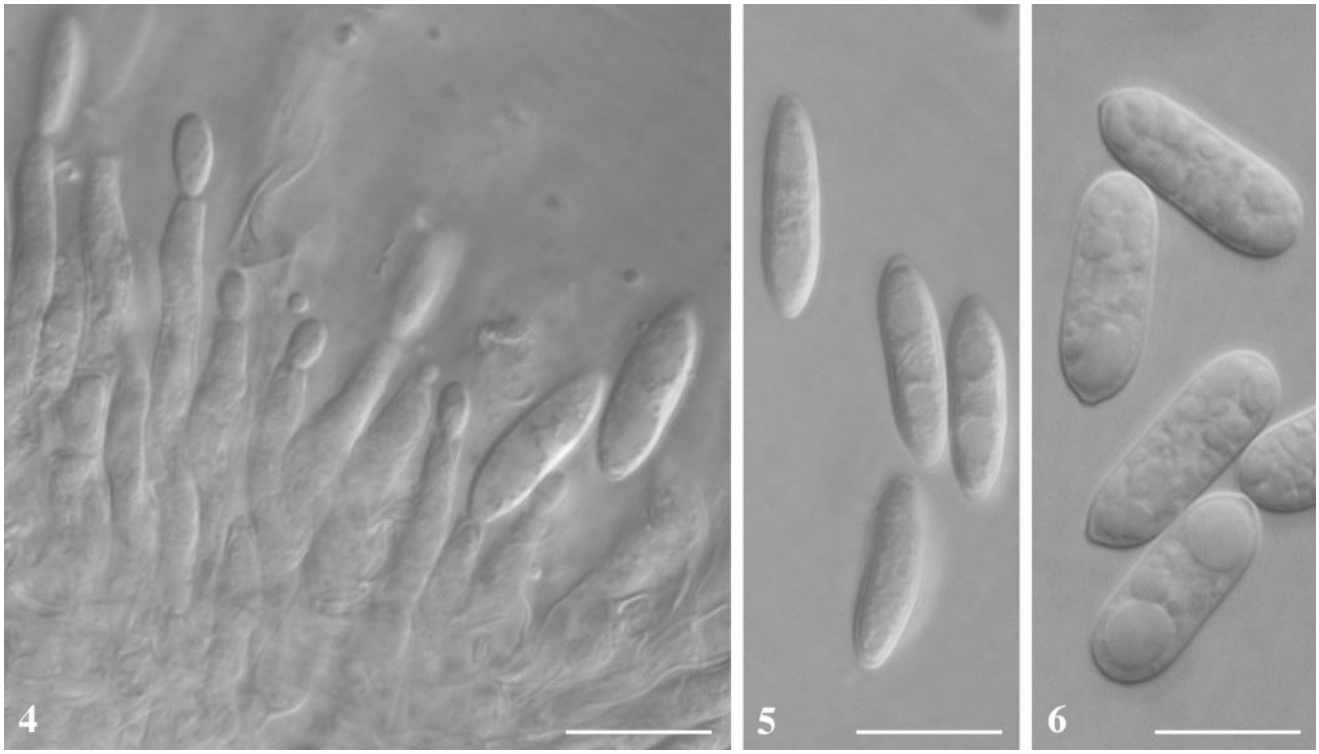


FIG. 3. *Colletotrichum acutatum* f. sp. *hakeae* (STE-U 4461). Setae, conidiophores, conidia and appresoria. Bar = 10 μm.



FIGS. 4–6. Conidiophores and conidia of *Colletotrichum* spp. 4–5. *Colletotrichum acutatum* f. sp. *hakeae* (STE-U 4461). 6. *Colletotrichum boninense* (STE-U 194). Bar = 10 μm.

Colletotrichum boninense J. Moriwaki, Toy. Sato & T. Tsukiboshi, Mycoscience 44: 48, 2003. FIGS. 6–7

Conidiomata with masses of orange conidia. Setae 75–140 μm long, 3- to 5-septate, medium brown at the base, pale brown at the bluntly rounded apex, tapering from a base 4–6 μm wide, to an apex 1.5–2 μm wide. Conidiophores irregularly branched, frequently with a pigmented lower half. Conidiogenous cells subcylindrical to obovoid, to fusoid, or irregular, hyaline, smooth, generally tapering from the lower part toward a truncate apex with visible periclinal thickening, 10–25 × 3–5 μm. Conidia hyaline, smooth, guttulate, subcylindrical with bluntly rounded ends and visible abscission scar, at times tapering inconspicuously to a slightly wider apex, or appearing slightly constricted in the middle of the conidium, (14–)15–16(–18) × 5–6 μm (average 15 × 6 μm). Appresoria medium brown, ovoid to irregularly lobed, 9–11 × 6–8 μm, 0–1-septate. Colonies on SNA with sparse, white aerial mycelium; on PDA with moderate gray aerial mycelium and few aerial conidia. Colonies on SNA with sparse, white aerial mycelium; on PDA with moderate gray aerial mycelium and few aerial conidia; brown vinaceous (5‴m) with rosy buff (13″f) centers on the surface, and brown vinaceous (5‴m) underneath. Cardinal temperatures for growth were minimum 10 C, opt 25 C, maximum 30 C. No growth was recorded at 35 C. The mean daily growth rate at 25 C was 8.1 mm/d.

DISCUSSION

We made an attempt to characterize and distinguish the *Colletotrichum* species associated with species of Proteaceae. Because morphological identification of *Colletotrichum* spp. is hampered by phenotypic variation (Nirenberg et al 2002), it was essential to link the morphological descriptions to molecular data. Although *C. gloeosporioides* is the only *Colletotrichum* species reported to infect Proteaceae, preliminary data led us to suspect that more than one species could be involved; and this suspicion was confirmed in our study.

Four species of *Colletotrichum* (*C. acutatum, C. boninense, C. crassipes, C. gloeosporioides*) and a *forma specialis* (*C. acutatum* f. sp. *hakeae*) were found to be associated with diseased Proteaceae. No obvious correlation could be observed between host specificity and symptom type among the species recognized, with the exception of *C. acutatum* f. sp. *hakeae* from *Hakea,* a host to which these isolates appear to be highly specific (Morris 1982). Of these taxa, *C. boninense* and *C. acutatum* f. sp. *hakeae* are described fully and illustrated, while the other species await

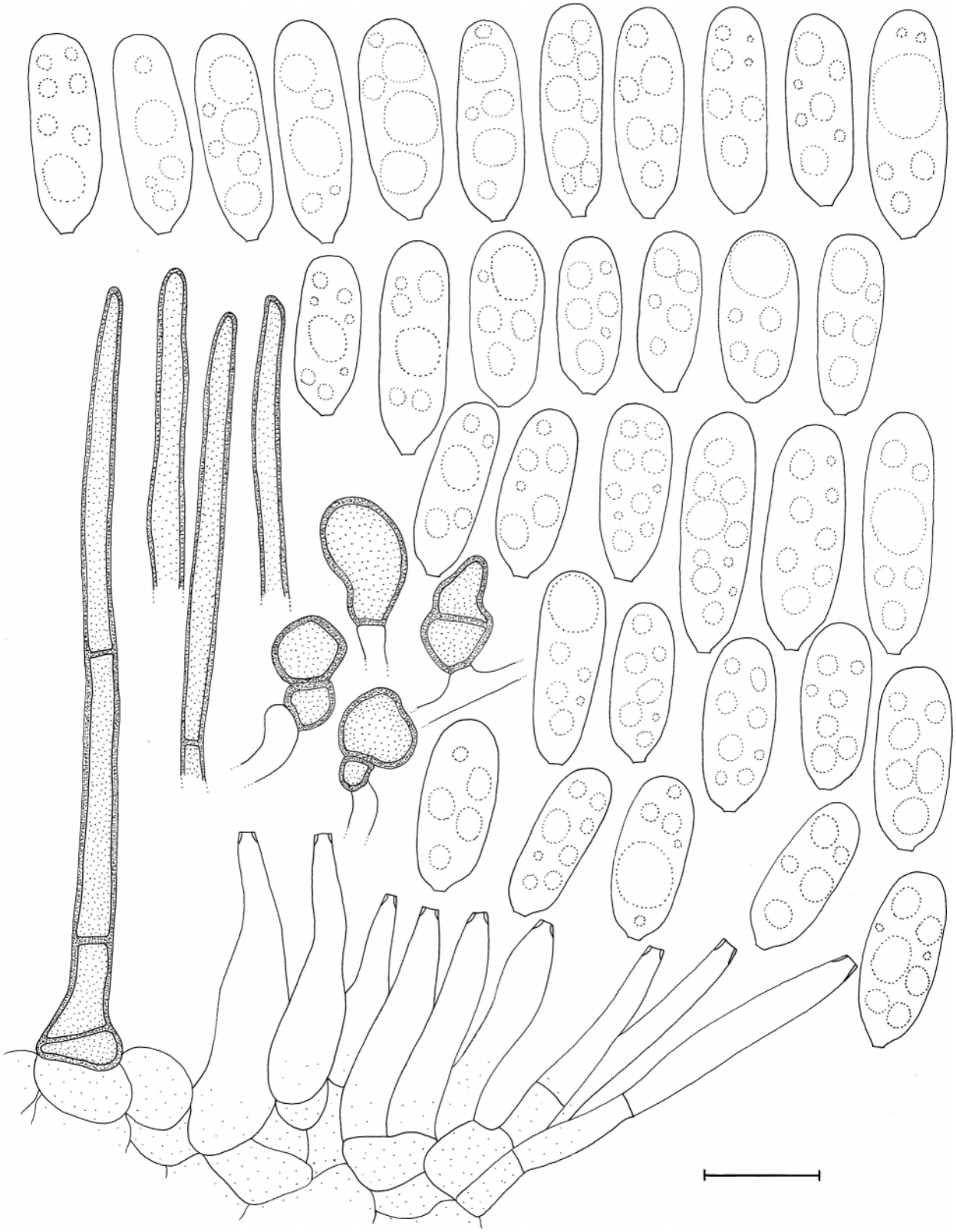


FIG. 7. *Colletotrichum boninense* (STE-U 194). Setae, conidiophores, conidia and appresoria. Bar = 10 μm.

treatment elsewhere, along with a designation of epitype specimens and cultures.

Colletotrichum acutatum is known to have a wide host range and geographic distribution (Dyko and Mordue 1979), and our data also confirm that it occurs on species of *Protea, Leucadendron* and *Leucospermum* in South Africa. This is the first report of *C. acutatum* on Proteaceae, a host family on which it appears to be a serious pathogen. Various subgroups were delineated within the *C. acutatum* clade, which correlate with previous findings (Lardner et al 1999, Johnston and Jones 1997). The characterization of the population from *Hakea* is of special importance to South Africa, because it is used as a biological control agent of *Hakea* (Morris 1982). The latter plant originates in Australia but is considered a noxious weed in South Africa that is spreading through the indigenous fynbos vegetation. The biocontrol agent is sold as a specific strain of the ''*C. gloeosporioides*'' complex (Morris 1982).

Colletotrichum gloeosporioides was confirmed from *Protea cynaroides* (L.) L. growing in South Africa and Zimbabwe and from a *Leucospermum* sp. in Portugal, but it also has been reported to occur on other Proteaceae elsewhere (Greenhalgh 1981, Benic 1986, Knox-Davies et al 1986, von Broembsen 1989, Forsberg 1993, Taylor 2001, Moura and Rodrigues 2001). In view of the data presented here, previous reports of this species must be treated with circumspection. A relatively unknown species, *C. boninense* was found to be associated with Zimbabwean and Australian Proteaceae but also occurred on a *Eucalyptus* sp. in South Africa. This species until recently was treated as part of *C. gloeosporioides* complex (Moriwaki et al 2003).

Colletotrichum crassipes was represented by a single isolate obtained from a *Dryandra* plant in Madeira. A more comprehensive study of the *Colletotrichum* spp. occurring on Proteaceae in Australia, Madeira and Zimbabwe would be required to reveal the importance and distribution of *C. crassipes* and especially *C. boninense,* which until now has been reported only from Japan (Moriwaki et al 2003). The pathogenicity of these species to Proteaceae is being evaluated. Once pathogenicity and more representative global distribution data are available, a re-assessment of the phytosanitary significance of these species can be made.

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