

Cytospora species (Ascomycota, Diaporthales, Valsaceae): introduced and native pathogens of trees in South Africa

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Abstract. *Cytospora* spp. (anamorphs of *Valsa* spp.) are common inhabitants of woody plants and they include important stem and branch canker pathogens. Isolates of these fungi were collected from diseased and healthy trees in South Africa. They were identified based on morphology and DNA sequence homology of the intertransgenic spacer ribosomal DNA. South African isolates were compared with isolates collected in other parts of the world, and they represented 25 genetically distinct sequences residing within the populations of 13–14 known species and three unique lineages. Several species are new records for South Africa, doubling previous reports of these fungi from the country. Similarities between South African isolates of *Cytospora* from non-native *Eucalyptus*, *Malus*, *Pinus*, *Populus*, *Prunus* and *Salix* species and isolates from Australia, Europe or America suggest that the fungal pathogens were imported with their hosts as endophytes. Isolates from indigenous *Olea* and *Acacia* appear to represent native populations. Host shifts were evident, including populations on *Eucalyptus* that also occurred on *Mangifera*, *Populus*, *Sequoia*, *Tibouchina* and *Vitex*. Isolates related to *Valsa kunzei* represent the first report of a *Cytospora* species on the widely cultivated timber tree, *Pinus radiata*. An identification key to *Cytospora* species in South Africa is included.

Additional keywords: forestry, genetic tree, key, phylogeny.

Introduction

Species of *Cytospora* Ehrenb. cause cankers and dieback on many genera of hardwoods and coniferous trees, but rarely on herbaceous plants. Sinclair *et al.* (1987) and Farr *et al.* (1989) list over 85 species of woody hosts susceptible to *Cytospora* canker, which is sometimes referred to as *Valsa* canker, Leucostoma canker or Perennial canker. The pathogens infect the inner bark, which is also referred to as the bark periderm. In hardwoods, the adjacent sapwood is discoloured. In conifers, no discoloration of the adjacent cambium is observed even though the fungus can be isolated from nearby xylem (Schoeneweiss 1983). Hydraulic conductivity of the xylem vessels in the new wood is also disrupted (Chang *et al.* 1991), causing wilting and death of infected branches or trees. *Cytospora* cankers are especially destructive on *Prunus* spp. in commercial orchards, on *Picea* spp. and *Acer* spp. in the landscape, and on *Populus* spp. in forestry.

Cytospora spp. are the anamorphs of the ascomycete genus *Valsa* Fr. Fruiting bodies consist of stromata (conidiomata) that usually contain either labyrinthine chambers or clusters

of pycnidia, having filamentous conidiophores and allantoid hyaline conidia. In moist conditions, the conidia exude from the fruiting bodies in gelatinous matrices, usually as yellow, orange, red or pallid tendrils. *Cytospora* spp. are numerous and may or may not have a known sexual form (teleomorph) in the genus *Valsa*.

Some *Cytospora* spp. are important plant pathogens but plant pathologists have usually been unable to precisely identify isolates to species based solely on morphology of the asexual fruiting bodies. Accurate identification of *Cytospora* spp. using morphological features has long been recognised as difficult. This is discussed in the monograph of Spielman (1983) where she states that the anamorph of *Valsa ambiens* Fr. is not distinguishable from anamorphs of many other species of *Valsa*. Défago (1935) also discussed the inadequacy of using purely morphological features for practical species delimitation.

The genus *Cytospora* was described by Ehrenberg (1818). Tulasne and Tulasne (1863) were the first to postulate that *Valsa* and *Cytospora* were two forms of the same

organism. Modern concepts of these fungi began with the work of von Höhnelt (1919) and Nannfeldt (1932) that separated species based on centrum characteristics. They removed many species that belonged to different genera and reduced many names to synonymy based on studies of type specimens. Grove (1935) and Gutner (1935) published useful compilations of descriptions of the known species of *Cytospora*. The most recent monographic works include those of Kobayashi (1970) for species in Japan, Gvritishvili (1982) and Vasilyeva (1994) for species in the former Union of Soviet Socialist Republics (USSR), Spielman (1983, 1985) for North American species on hardwoods and Adams *et al.* (2005) for species on *Eucalyptus*. These works include the description of new species and the amalgamation of many other species based on morphology and, in latter works, DNA sequence homology. The *Index Fungorum* (Kirk *et al.* 2004) lists 541 species of *Cytospora* and 549 species of *Valsa*. Most species are believed to be synonyms. For example, Spielman (1985) accepted only five species on hardwoods in North America.

Thorough reviews of the history of classification and nomenclature of the anamorphic genus *Cytospora* and the holomorphic genus *Valsa*, and their segregates, have been published (Spielman 1983, 1985; Adams *et al.* 2005). Currently, *Cytospora* contains the anamorphs of the genera *Valsa*, *Leucostoma* (Nitschke) Höhn., *Valsella* Fuckel and *Valseutypella* Höhn. These four genera have been accommodated in the single genus *Valsa* by Adams *et al.* (2005), and three were previously treated as *Valsa* by Vasilyeva (1994). Based on preliminary phylogenetic analyses (Adams *et al.* 2002, 2005; Castlebury *et al.* 2002), the species in the four genera are treated as *Valsa* spp. in the present study.

Cytospora spp. can be highly virulent and destructive pathogens on cultivated *Prunus* (Biggs 1989) and *Populus* species (Kepley and Jacobi 2000). However, on many tree species these fungi are considered facultative wound parasites that attack weakened trees. It has also long been argued that some might be strictly saprobic on dying trees (Christensen 1940). Pathogenicity may be a species-specific character but it is also a character that is particularly difficult to determine.

Cytospora spp. have been shown to exist in healthy plant tissues and they are encountered during isolations from sound bark, xylem and leaves of many tree species (Bills 1996). They are endophytes in bark, xylem and leaves (Fisher *et al.* 1993). The term endophyte is used here in the sense of Chapela (1989) to refer to a fungus inhabiting the internal environment of a living plant. One of the earliest demonstrations of the endophytic nature of *Cytospora* is that of Christensen (1940). He demonstrated that saplings of *Populus*, *Salix* and *Sorbus aucuparia*, when carefully surface sterilised, become parasitised by *Cytospora* when samples are excised, coated in hot wax and incubated in a cold room

for a few weeks. *Cytospora* spp. are dominant in the xylem and also occur in the leaves of *E. nitens* and *E. grandis* from two distinct geographic and climatic areas of South Africa (Smith *et al.* 1996). Similarly, they are the dominant fungi in the xylem of *E. globulus* in Uruguay, where they are confined to the xylem (Bettucci and Saravay 1993). The nature of *Cytospora* appears to fit the definition of Petrini (1991) of latent pathogens existing as symptomless endophytic infections. Chapela (1989) refers to *Cytospora* spp. as xylotropic endophytes. He argues that previous studies of endophytes in trees have not excluded the possibility that the isolated fungi are derived from spores on the bark whereas the methods used in his study exclude that possibility (Chapela and Boddy 1988). Xylotropic endophytes differ from general endophytes in the characteristic of growing into secondary xylem when wood dries.

Doidge (1941, 1950) and Doidge *et al.* (1953) compiled the first lists of the *Cytospora* and *Valsa* species in South Africa. The work reported six species including *C. australiae* Speg. on *E. viminalis*; *C. sacchari* E.J. Butler on *Saccharum officinarum*; *C. verrucula* Sacc. and Berl. (teleomorph *Valsa verrucula* Nitschke) on *Acacia* sp.; *V. leucostoma* Fr. [anamorph *C. leucostoma* Sacc., 'sexual fruiting bodies rarely found', syn. *Leucostoma persoonii* (Nitschke) Höhn.] on *Prunus salicina* and hybrids; *Malus sylvestris*; *V. salicina* (Pers.) Fr. (anamorph *C. fugax* Fr., syn. *C. xanthosperma* Fr.) on *Salix capensis* and other *Salix* spp.; and *V. sordida* Nitschke [anamorph *C. chrysosperma* (Pers.) Fr., 'sexual fruiting bodies rarely found'] on *Populus balsamifera*, *Populus canescens* and other *Populus* spp.

Doidge (1950) and Doidge *et al.* (1953) also reported on two species of *Valsa* and one of *Cytospora* that were transferred to other genera: *C. foliicola* Lib. = *Ceuthospora foliicola* Lib.; *V. infinitissima* Kalchbr. and Cooke = *Peroneutypella infinitissima* (Kalchbr. and Cooke) Doidge; and *V. stellulata* Fr. = *Eutypella stellulata* (Fr.) Sacc. Representative specimens of *C. leucostoma*, *E. stellulata*, *P. infinitissima* and *V. sordida* from South Africa were deposited in the national herbaria of South Africa (PREM) and of the United States of America (USA; BPI).

After Doidge *et al.* (1953), Gorter (1977, 1981, 1982) updated the index of plant pathogens in South Africa and added *C. eucalypticola* Van der Westh. (teleomorph *V. fabianae* G.C. Adams, M.J. Wingf. and Jol. Roux). This species was reported to cause cankers on *Eucalyptus* spp. (van der Westhuizen 1965a, 1965b). The most recent host index of South African plant diseases (Crous *et al.* 2000) reduced the list of species to five recognising *C. australiae* on *E. viminalis*; *C. chrysosperma* on *P. balsamifera*; *C. eucalypticola* on *E. saligna* and *E. cloeziana*; *C. leucostoma* on *Prunus armeniaca*, *P. avium*, *P. persica*, *P. salicina* and *M. sylvestris*; and *C. sacchari* on *S. officinarum*. *Valsa salicina* and *V. verrucula* were excluded.

The Plant Protection Research Institute (PPRI) in Pretoria, South Africa, maintains cultures of unidentified *Cytospora* spp. on *Acacia nilotica*, *E. camaldulensis*, *E. grandis*, the fruits of *Olea europaea* and *Mangifera indica*. Another culture from *Quercus robur* has been lost. The national herbaria of South Africa (PREM) in Pretoria, South Africa, contains specimens from South Africa of *C. australiae*, *C. eucalypticola*, *V. leucostoma*, '*V. salicina*' [as *C. salicis* (Corda) Rabenh.] and *V. sordida*. The objective of this study is to identify species of *Cytospora* in South Africa based on DNA-based characterisation and morphology. In this way, we aim to increase the understanding of geographical and host range relationships, and to discuss possible origins of the pathogens in South Africa.

Materials and methods

Strains sequenced

Cytospora and *Valsa* isolates from South Africa were collected from diseased trees, dead branches and living leaves primarily during 1999–2001. Older cultures were obtained from the culture collections of PPRI and the Forestry and Agricultural Biotechnology Institute, University of Pretoria (CMW). A total of 33 isolates of *Cytospora* from South Africa were selected for DNA sequence comparisons (Tables 1 and 2). Sequences of additional isolates from other parts of the world, geographic regions and culture collections were included in the analyses. Many, but not all, of the sequences have been used in previous studies (Adams *et al.* 2005). Most cultures were accompanied by herbarium specimens, which could be examined (Tables 1 and 2). Several reference cultures were obtained from the Centraalbureau voor Schimmelcultures (CBS), the American Type Culture Collection (ATCC) and the International Mycological Institute CABI Biosciences (IMI) collections, and were sequenced. These included cultures deposited by experts in *Valsa* taxonomy including Défago (1935, 1942) and Spielman (1985).

Axenic cultures of *Cytospora* spp. were obtained by cutting fruiting bodies in half horizontally and applying a drop of water to an exposed locule. Following swelling of the gelatinous matrix, some of the spore mass was lifted and streaked across the surface of an agar medium. Cultures were also isolated from cambium at the margins of cankers using standard methods, including the brief flaming of branch surfaces followed by cutting to expose the canker margin and isolation of infected tissue pieces. Acidified malt extract agar (1.25% malt extract, 100 mg/L tetracycline, pH 5) was used to culture the fungi. Actively growing colonies were purified by excising single terminal cells from individual hyphae. A terminal cell was transferred to an agar plate and subsequent cultures were derived from the hyphal tip cell.

Microscopy

For examination of pycnidia and perithecia on bark, samples were washed for 30 min in 2% potassium hydroxide (KOH), rinsed with water and placed in fixative (2% formaldehyde, 2% glutaraldehyde in 0.025 M phosphate buffer, pH 7.4) for 1 day. The specimens were then soaked 2–3 h in 5% hydrochloric acid (HCl) and neutralised in buffer. Specimens were then placed in osmium (1% OsO₄ in 0.025 M phosphate buffer) for 3 h, followed by dehydration in a graded ethanol series. Specimens were transferred from 100% ethanol to 100% propylene oxide, then to a graded series of resin/propylene oxide solutions. Resin concentrations were 25, 50, 75, 87 and 100%, and each stage was infiltrated for ~12 h. The resin was a mixture of Poly/Bed 812® (PolyScience, Inc., Warrington, PA, USA), Araldite epoxy resins and dodecylsuccinic anhydride hardener in the proportions 5 : 4 : 12. The

resin was cured at 60°C for 2 days, and sections for light microscopy were cut with a glass knife at approximately 0.75 mm with a thickness of 2–3 µm. Slides were stained with 1% toluidine blue at 100°C and mounted with the same resin used for embedding.

Identification of species

Species were identified based on morphological features of the sexual fruiting bodies on natural substrates. When sexual fruiting bodies were absent, species were identified based on their relative DNA homology to specimens from sexual fruitings. In some instances, isolates were identified to putative species based on DNA homology to cultures derived from sexual fruiting bodies and deposited by other scientists. Additionally, isolates of the distinctive species *C. pruinosa* (Fr.) Sacc. and *C. umbrina* (Bonnord.) Sacc. were identified based on the morphology of the locules in the conidiomata. These two species of *Cytospora* have identifiable asexual fruiting bodies (von Höhnelt 1914, 1917, 1928; Gvritishvili 1982; Spielman 1985).

DNA extraction and amplification

Genomic DNA was extracted from mycelium of the isolates (Tables 1, 2). Isolates were grown in 25 mL of 2% malt extract broth at room temperature for 5–10 days. Mycelia were then harvested by vacuum filtration through miracloth (Calbiochem-Novobiochem Corp., La Jolla, CA, USA), lyophilised, and stored at –20°C. DNA was extracted from lyophilised mycelium by a cetyltrimethyl-ammonium bromide (CTAB) method (Hallen *et al.* 2003).

PCR amplification and sequencing protocols

Approximately 2.5 ng of the total genomic DNA was used per 100 µL reaction mixture for PCR amplification (White *et al.* 1990). Primers used in the amplifications of the isolates included intertransgenic spacer regions ITS1, ITS2, ITS3, and ITS4 for the ITS ribosomal DNA (White *et al.* 1990). The cycling reactions were performed in a DNA Thermal Cycler (Perkin-Elmer, Norwalk, CT) or a similar machine using standard protocols. Two hundred microlitres of each PCR product were purified using the DNA-binding resin and protocol of the Wizard PCR Preps DNA purification system (Promega Corp., Madison, WI, USA). Sequencing was performed using a *Taq* DyeDeoxi Terminator™ cycle system, the ABI Catalyst 800 and the ABI Prism 373A or 377 fluorescence sequencer (PE Applied Biosystems, Foster City, CA, USA). Sequencing reactions were carried out using the Big Dye fluorescent labelling sequencing kit (PE Applied Biosystems). Amplified double-stranded PCR products were sequenced independently along both strands with the primers listed above.

DNA data analysis

DNA sequences of *Cytospora* isolates from South Africa and other regions have been deposited and accessioned in the National Center for Biotechnology Information, GenBank (AF192314–21, AF260263–6, AY347316–80 and DQ243789–812; Tables 1 and 2). The ITS region sequences of all strains were aligned with ClustalX 1.81 (Thompson *et al.* 1994, 1997) then visually proofread. Sequences were analysed as uniformly weighed unordered characters, and as interleaved blocks of aligned sequence. A *Cytospora*-like fungus, which was morphologically a *Cytospora* but had a sequence more closely homologous to those of *Diaporthe* Nitschke, served as the outgroup. This fungus is more closely related to *Valsa* than are other species of *Diaporthe* (Adams *et al.* 2005). Ambiguities in alignment of short segments of sequence were tested experimentally for their effect on topology and bootstrap indices. No segments were excluded from the analyses.

Sequences were compared in maximum parsimony analysis (Swofford and Maddison 1987) with a total of 143 taxa. The analysis was computed with PAUP version 4.0b10 (Swofford 2003) using heuristic searches with the tree bisection-reconnection (TBR) branch-swapping

Table 1. South African taxa investigated in this study
Taxa in the cladograms (Fig. 1) that are not in Tables 1 and 2 are in Adams *et al.* (2002, 2005)

Taxon	Isolate citation	Geographic origin in South Africa	Host	Herbarium specimen ^A	Culture ^B	GenBank ^C
<i>Valsa cinereostroma</i>	Adams <i>et al.</i> (2005)	South Africa	<i>Mangifera indica</i>	MSC 388710	CBS 116830, PPRI 6767, CMW 5265	AF260267
<i>Valsa</i> aff. <i>cinereostroma</i> ' <i>C. australia</i> ' sensu <i>P. Crous</i>	Crous <i>et al.</i> (1990)	White River	<i>Eucalyptus grandis</i> Clone HL&L	PREM 50454	CBS 116831, STE-U67, CMW 6501	AY347376
<i>Valsa</i> aff. <i>cinereostroma</i> ' <i>C. australiae</i> ' sensu <i>P. Crous</i>	Crous <i>et al.</i> (1990)	White River	<i>E. grandis</i>	MSC 388709	CBS 116832, STE-U68, CMW 6502	AY347376
<i>Valsa</i> aff. <i>cinereostroma</i> ' <i>C. australia</i> ' sensu <i>P. Crous</i>	Crous <i>et al.</i> (1990)	Wartburg	<i>E. nitens</i>	MSC 388707	CBS 116833, STE-U129, CMW 6503	AY347376
<i>Valsa</i> aff. <i>cinereostroma</i>		Amsterdam	<i>E. nitens</i>	MSC 388858	CBS 118087, CMW 1514	AY347378
<i>Valsa ceratosperma</i> sensu lato	Adams <i>et al.</i> (2005)	Bloemfontein	<i>Jacaranda acutifolia</i>	MSC 387070		
<i>Valsa cypri</i>		South Africa	Fruit of <i>Olea</i>		PPRI 6334, CMW 5264	DQ243789
<i>Valsa cypri</i>		Sterkfontein cave	<i>Olea europaea</i> v. <i>africana</i>	MSC 384995	CBS 118555, CMW 5280	DQ243790
<i>Valsa fabianae</i> , <i>C. eucalypticola</i>	Type of anamorph	Tzaneen	<i>E. saligna</i>	PREM 42543		
<i>Valsa fabianae</i> , <i>C. eucalypticola</i>	Adams <i>et al.</i> (2005)	New Castle	<i>E. dunnii</i>	MSC 380697	CBS 116851	AY347360
<i>Valsa fabianae</i> , <i>C. eucalypticola</i>	Adams <i>et al.</i> (2005)	KwaMbonambi	<i>E. saligna</i>	MSC 380718	CBS 116853	AY347369
<i>Valsa fabianae</i> , <i>C. eucalypticola</i>		Seven Oaks	<i>E. grandis</i>	MSC 388713	CMW 940	AF260265
<i>Valsa kunzei</i>		Eastern Cape	<i>Pinus radiata</i>	MSC 384994	CBS 118556, CMW 5257	DQ243791
<i>Valsa leucostoma</i>	Adams <i>et al.</i> (2002)	Nelspruit	<i>Prunus persica</i>	MSC 368341	CBS 118557, CMW 5259	AF191180
<i>Valsa malicola</i>		Mountain View, Beaufort West	<i>Malus domestica</i>	MSC 384993	CBS 118559, ATCC.MYA 3056	DQ243792
<i>Valsa germanica</i>		Mountain View, Beaufort West	<i>Populus</i> sp.	MSC 384992	CBS 118560	DQ243793
<i>Valsa nivea</i>		Belfast, Mpumalanga	<i>Populus canescens</i>	MSC 384991	CMW 5274	DQ243794
<i>Valsa nivea</i>		Belfast, Mpumalanga	<i>Populus canescens</i>	MSC 384996		DQ243794
<i>Valsa nivea</i>		Bloemfontein	<i>Populus simonii</i>		CBS 118561, CMW 5273	DQ243795
<i>Valsa nivea</i>		Mountain View, Beaufort West	<i>Malus domestica</i>	MSC 384990	CBS 118562	DQ243796
' <i>Valsa salicina</i> '		Brakpan	<i>Salix</i> sp.	PREM 205469		
<i>Valsa sordida</i>		Pretoria	<i>Salix</i> sp.	MSC 380717	CMW 5269	AY347324
<i>Valsa sordida</i>		New Castle	<i>Salix</i> sp.	MSC 387071		AY347324
<i>Valsa sordida</i>		Pretoria	<i>Populus nigra</i> cv. 'italica'	MSC 384988	CBS 118563	DQ243797
<i>Cytospora chrysosperma</i> S. lato ' <i>C. australiae</i> ' sensu Doidge	Adams <i>et al.</i> (2005)	Wellington	<i>E. viminalis</i>	PREM 13072		
<i>Cytospora</i> aff. <i>austromontana</i>		Hermanus, Cape	<i>E. grandis</i>	MSC 388706	CBS 116822, PPRI 5926	AY347379
<i>Cytospora variostromatica</i>	Adams <i>et al.</i> (2005)	Waterkloof, Pretoria	<i>E. camaldulensis</i> leaves	MSC 388705	CBS 118086, PPRI 5297	AF260264
<i>Cytospora variostromatica</i>		KwaMbonambi	<i>E. grandis</i>		CMW 1237	AF260263
<i>Cytospora variostromatica</i>		KwaMbonambi	<i>E. grandis</i>		CMW 1238	AF260263
<i>Cytospora variostromatica</i>		Allemanskraal dam	<i>Populus deltoides</i>	MSC 388711	CBS 118564, CMW 5270	DQ243798
<i>Cytospora variostromatica</i>		KwaMbonambi	<i>E. grandis</i>	MSC 388712	CBS 116860, CMW 1240	AF260263
<i>Cytospora</i> aff. <i>variostromatica</i>		dam near Nelspruit	<i>Populus balsamifera</i>	MSC 384989	CBS 118565, CMW 5250	DQ243799
<i>Cytospora</i> sp. putative species 1	Smith <i>et al.</i> (1996)	Piet Retief	<i>E. grandis</i>		CMW 5357	AF192318
<i>Cytospora</i> sp. putative species 1	Smith <i>et al.</i> (1996)	KwaMbonambi	<i>E. grandis</i>		CMW 5358	AF192319
<i>Cytospora</i> sp. 'Uncertain lineage'		South Africa	<i>Acacia nilotica</i>		CBS 118566, PPRI 6421, CMW 5255	DQ243800

^AAccession numbers with the prefix MSC are from the mycology herbarium of the Beal-Darlington herbarium at Michigan State University, East Lansing, MI, USA; and PREM are in the National Mycological Herbarium in Pretoria, South Africa.

^BAccession numbers with the prefix PPRI are from the culture collection of the Plant Protection Research Institute, Agriculture Research Center, Pretoria, South Africa; CMW are from the culture collection of M. J. Wingfield at the Tree Protection Cooperative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; STE-U are from the culture collection of Pedro Crous at Stellenbosch University, Stellenbosch, South Africa; and ATCC are from the American Type Culture Collection, Manassas, Virginia, USA.

^CGenBank sequences are by the authors and Adams *et al.* (2005).

algorithm. The tree with the greatest natural logarithm (LN) likelihood was selected from among the equally most parsimonious (MP) trees, using the Kishino–Hasegawa test (Kishino and Hasegawa 1989). To develop a consensus tree, 1000 heuristic searches (Hedges 1992) were performed by bootstrapping (Felsenstein 1985). Confidence intervals for branches on the consensus tree were inserted into the selected MP tree.

Because at least one clade appeared to be evolving at an accelerated rate, based on branch lengths, maximum likelihood analysis was

employed because of its lower sensitivity to unequal rates of evolution (Felsenstein 1981). The maximum likelihood model of substitution for the 143 taxon dataset was computed using Modeltest 3.04 (Posada and Crandall 1998). The number of taxa slowed calculation of a maximum likelihood tree using PAUP to an unreasonable period. Therefore, the likelihood model, TrN + I + G, was used in Bayesian phylogenetic analysis with MrBayes version 3.0b4 (Ronquist and Huelsenbeck 2002). The Markov chain Monte Carlo convergence acceleration technique of Metropolis coupling was employed (Metropolis–Hastings algorithm;

Table 2. Reference taxa used in this study
Taxa in the cladograms (Fig. 1) that are not in Tables 1 and 2 are in Adams *et al.* (2002, 2005)

Taxon	Isolate citation or Ex-type	Geographic origin	Host	Herbarium specimen ^A	Culture ^B	GenBank ^C
<i>Valsa abietis</i> sp. complex 2	Défago (1942)	Switzerland	<i>Abies alba</i>		CBS 185.42	AY347336
<i>Valsa abietis</i> sp. complex 1 (syn. <i>Valsa weiriiana</i>)	Adams <i>et al.</i> (2002)	British Columbia, Canada	<i>Pseudotsuga menziesii</i>	MSC 384998	CBS 118567	AF192551
<i>Valsa abietis</i> sp. complex 1 (syn. <i>Valsa weiriiana</i>)	Adams <i>et al.</i> (2002)	British Columbia, Canada	<i>Chamaecyparis</i> sp.	MSC 384999	CBS 118092	AF192550
<i>Valsa ambiens</i>	Défago (1942)	Switzerland	<i>Taxus baccata</i>		CBS 191.42	AY347330
<i>Valsa ambiens</i> subsp. <i>leucostomoides</i>	Spielman (1985)	New Jersey, USA	<i>Acer rubrum</i>	CUP 069132	CBS 116809, ATCC 52279	AY347339
	Spielman (1985)	New York and Illinois, USA	<i>Acer</i> spp.	CUP 060133 CUP 060137 CUP 060135	CBS 118089 ATCC 52281 CBS 116810 CBS 153.29	AY347346 AY347347 AY347348 AY347337
<i>Valsa auerswaldii</i>		USSR	<i>Eucalyptus grandis</i> × <i>tereticornis</i>	MSC 368317	CBS 116811, CMW 5260	AF192315
<i>Valsa brevispora</i>		Tchitanga, Republic of Congo	<i>Taxus baccata</i>		CBS 192.42	AY347333
<i>Valsa ceratophora</i>	Défago (1942)	Switzerland	<i>Prunus persica</i>	MSC 378869	CBS 118568, ATCC 62910 ATCC 64877	AF191171 AF191170
<i>Valsa cincta</i>	Adams <i>et al.</i> (2002)	Canada	<i>Malus domestica</i>		CBS 118091	AF191169
<i>Valsa cincta</i>	Adams <i>et al.</i> (2002)	Michigan, USA	<i>Malus domestica</i>	MSC 368327	CBS 117081, CMW 5700	AY347377
<i>Valsa cincta</i>	Adams <i>et al.</i> (2002)	Chile	<i>Eucalyptus globulus</i>	MSC 375220	CBS 201.42	DQ243801
<i>Valsa cinereostroma</i>	Ex-type		<i>Syringa</i> sp.	CBS H-19178	CBS 200.42	DQ243801
<i>Valsa cypri</i>	Défago (1942)	Switzerland	<i>Fraxinus excelsior</i>	CBS H-19176	CBS 116815	AY347340
<i>Valsa cypri</i>	Défago (1942)	Switzerland	<i>Sequoia sempervirens</i>	MSC 380713		
<i>Valsa eucalypti</i>		Mt. Tamalpais, California, USA				
<i>Valsa eugeniae</i>	Sivanesan and Holliday (1970)	Tanzania	<i>Eugenia</i> sp.		CBS 118569, IMI 044946	AY347344
<i>Valsa eugeniae</i>	Sivanesan and Holliday (1970)	Western Malaysia	<i>Eugenia aquea</i>		CBS 116817, IMI 062499	AY347345
<i>Valsa fabianae</i> , <i>C. eucalypticola</i>	Ex-type	Tasmania, Australia	<i>Eucalyptus nitens</i>	DAR 43948	CBS 116840, ATCC 96150	AY347358
<i>Valsa friesii</i>	Défago (1942)	Switzerland	<i>Abies alba</i>		CBS 194.42	AY347328
<i>Valsa germanica</i>	Défago (1942)	Switzerland		CBS H-19163	CBS 195.42	AY347325
<i>Valsa kunzei</i> var. <i>piceae</i>	Proffer and Hart (1988)	Michigan, USA	<i>Picea pungens</i>	MSC 380720	CBS 118093, ATCC 64880	AY347320
<i>Valsa kunzei</i> var. <i>piceae</i>	Proffer and Hart (1988)	Michigan, USA	<i>Picea pungens</i>	MSC 380721	CBS 118094, ATCC 64881	AY347320
<i>Valsa leucostoma</i> f. sp. <i>instittiae</i>	Défago (1942)	Switzerland	<i>Prunus instittia</i> var. <i>italica</i>		CBS 263.34	AF191175
<i>Valsa mali</i>	Adams <i>et al.</i> (2002)	Japan	<i>Malus domestica</i>	CBS H-19166	CBS 376.29	AF191186
<i>Valsa malicola</i>	Adams <i>et al.</i> (2002)	Michigan, USA	<i>Malus domestica</i>	MSC 384997	CBS 118570, ATCC.MYA-3055	DQ243802
<i>Valsa massariana</i>	Adams <i>et al.</i> (2002)	Switzerland	<i>Sorbus aucuparia</i>	CBS H-14029	CBS 133.76	AF191173
<i>Valsa myrtigena</i>	Ex-type	Hilo, Hawaii, USA	<i>Tibouchina urvilleana</i>	MSC 380715	CBS 116843	AY347363
<i>Valsa nivea</i>	Défago (1942)	Switzerland	<i>Populus nigra</i>	CBS H-19169	CBS 259.34	AF191174
<i>Valsa parapersoonii</i> comb. nov.	Ex-type	Michigan, USA	<i>Prunus serotina</i>	MSC 375221	CBS 116845	AF191181
<i>Valsa pini</i>	Défago (1942)	Switzerland	<i>Pinus sylvestris</i>		CBS 197.42	AY347332
<i>Valsa pini</i>		New York, USA	<i>Pinus strobus</i>		CBS 224.52	AY347316
<i>Valsa salicina</i>	Défago (1942)	Switzerland	<i>Salix</i> sp.	CBS H-19181	CBS 203.42	AY347323
<i>Valsa sordida</i>		United Kingdom	<i>Populus tremula</i>		CBS 197.50	AY347322

(Continued next page)

Table 2. continued

Taxon	Isolate citation or Ex-type	Geographic origin	Host	Herbarium specimen ^A	Culture ^B	GenBank ^C
<i>Valsa subclipeata</i>		Netherlands	<i>Rhododendron ponticum</i>	CBS H-11544	CBS 117.67	AY347331
<i>Valsa translucens</i>	Défago (1942)	Switzerland	<i>Salix</i> sp.		CBS 152.42	AF191182
<i>Valsa viridistroma</i>	Wehmeyer (1936)	Georgia, USA	<i>Cercis canadensis</i>		CBS 202.36	AY452120
comb. nov.						
<i>Valsa viridistroma</i>		USA	<i>Cercis canadensis</i>		CBS 249.54	AY452119
comb. nov.						
<i>Valsella melostoma</i>	Adams <i>et al.</i> (2002)	Michigan, USA	<i>Malus domestica</i>	MSC 375216	CBS 118572	AF191184
<i>Valseutypella multicolis</i>	Ex-type	Spain	<i>Quercus ilex</i> subsp. <i>rotundifolia</i>		CBS 105.89, ATCC 96780	DQ243803
<i>Cytospora abyssinica</i>	Ex-type	Wondo Genet, Ethiopia	<i>Eucalyptus globulus</i>	MSC 380700	CBS 116189, CMW 10181	AY347353
<i>Cytospora abyssinica</i>		Wondo Genet, Ethiopia	<i>Eucalyptus globulus</i>		CBS 117605, CMW 10179	AY347352
<i>Cytospora abyssinica</i>		Wondo Genet, Ethiopia	<i>Eucalyptus globulus</i>	MSC 380702	CBS 117004, CMW 10178	AY347354
<i>Cytospora acaciae</i>		Mallorca, Spain	<i>Ceratonia siliqua</i> fruit	CBS H-11495	CBS 468.69	DQ243804
African-Mediterranean lineage						
<i>Cytospora australiae</i>	Type	Argentina	<i>Eucalyptus globulus</i>	LPS 31746		
<i>Cytospora austromontana</i>	Ex-type	Perisher V, NSW, Australia	<i>Eucalyptus pauciflora</i>	MSC 380693	CBS 116820, CMW 6735	AY347361
<i>Cytospora berkeleyi</i>	Ex-type	Palo Alto, California, USA	<i>Eucalyptus globulus</i>	MSC 380710	CBS 116823	AY347350
<i>Cytospora carbonacea</i>		Germany	<i>Ulmus campestris</i>		CBS 219.54	DQ243805
<i>Cytospora cedri</i>		Italy			CBS 196.50	AF192311
<i>Cytospora decorticans</i>		Netherlands	<i>Fagus sylvatica</i>		CBS 116.21	AY347335
<i>Cytospora diatrypelloidea</i>	Ex-type	Orbost, Vic., Australia	<i>Eucalyptus globulus</i>	MSC 380719	CBS 116826, CMW 8549	AY347368
<i>Cytospora disciformis</i>	Ex-type	Uruguay	<i>Eucalyptus grandis</i>	MSC 368323	CBS 116827, CMW 6509	AY347374
<i>Cytospora erobotryae</i>	Ex-type	Saharapur, India	<i>Eriobotrya japonica</i>		CBS 116846, IMI 36523	AY347327
<i>Cytospora eucalyptina</i>	Adams <i>et al.</i> (2005)	Cali, Colombia	<i>Eucalyptus grandis</i>	MSC 375217	CBS 116853, CMW 5882	AY347375
<i>Cytospora eutypelloides</i>		England	<i>Prunus armeniaca</i>		IMI 140798	DQ243806
<i>Cytospora harti</i>		Netherlands	<i>Populus</i> sp.		CBS 195.49	DQ243807
<i>Cytospora minuta</i>		Norway	<i>Fraxinus americana</i>		CBS 134.25	DQ243808
<i>Cytospora mougeotii</i>			<i>Picea abies</i>		CBS 198.50	AY347329
<i>Cytospora nitschkii</i>		Wondo Genet, Ethiopia	<i>Eucalyptus globulus</i>	MSC 380699	CBS 117606, CMW 10184	AY347355
<i>Cytospora nitschkii</i>		Wondo Genet, Ethiopia	<i>Eucalyptus globulus</i>	MSC 380701	CBS 116854, CMW 10180	AY347356
<i>Cytospora putative</i> sp. 1	Ex-type	Thailand	<i>Eucalyptus camaldulensis</i>	MSC 388704	CBS 116861	AF192320
<i>Cytospora rhodophila</i>		Germany	<i>Rosa</i> sp.		ATCC 38695	DQ243809
<i>Cytospora ribis</i>		Netherlands	<i>Ribes rubrum</i>		CBS 187.36	DQ243810
<i>Cytospora sacchari</i>		India	<i>Saccharum officinarum</i>		CBS 160.33	DQ243811
<i>Cytospora tritici</i>		Monheim, Germany	<i>Triticum aestivum</i>	CBS H-11545	CBS 827.84	DQ243812
<i>Cytospora valsoides</i>	Ex-type	Sumatra, Indonesia	<i>Eucalyptus grandis</i>	MSC 380717	CBS 117003, CMW 4309	AF192312
<i>Cytospora variotromatica</i>	Ex-type	Orbost, Vic., Australia	<i>Eucalyptus globulus</i>	MSC 380695	CBS 116858, CMW 6766	AY347366
<i>Cytospora-like fungus</i>	Adams <i>et al.</i> (2005)	Indonesia	<i>Eucalyptus urophylla</i>	MSC 380704	CMW 460	AF192313
<i>Cytospora-like fungus</i>	Adams <i>et al.</i> (2005)	Indonesia	<i>Eucalyptus urophylla</i>	MSC 380703	CBS 117015, CMW 461	AF192316

^A Accession numbers with the prefix MSC are from the mycology herbarium of the Beal-Darlington herbarium at Michigan State University, East Lansing, MI, USA; CUP are from the mycology herbarium of Cornell University, Ithaca, NY, USA; DAR are in the herbarium of the Department of Agricultural Researchs, CSIRO, Australia; CBS H are in the herbarium of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, but were not available for this study; and LPS are from the Colecciones Micológicas, Universidad Nacional de la Plata, Instituto de Botánica C. Spegazzini, La Plata, Argentina.

^B Accession numbers with the prefix CMW are from the culture collection of M. J. Wingfield at the Tree Protection Cooperative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; ATCC are from the America Type Culture Collection, Manassas, Virginia, USA; and IMI are from the culture collection of the International Mycological Institute, CABI Bioscience, Egham, Surrey, United Kingdom.

^C GenBank sequences are by the authors and Adams *et al.* (2002, 2005), except AY452119 and AY452120 (Myburg *et al.* 2004).

Larget and Simon 1999; Huelsenbeck and Ronquist 2001). Three million searches were performed, with a burn-in of 100 000 discarded trees, to calculate the posterior probability distributions for branches on the Bayesian maximum likelihood consensus (BML) tree. The MP and the BML trees were displayed using TreeView (Page 1996).

Results and taxonomy

Phylogenetic analysis of the dataset of 25 South African taxa with unique sequence (from 33 isolates) and 118 taxa from other parts of the world (total 143 taxa) is reflected in two cladograms (Fig. 1A, B). Names in italics in the cladograms are of host genus and of identified fungal species. The length of the DNA sequence for each isolate, inclusive of introduced gaps to permit alignment of the entire set, was 617 nucleotides with 221 parsimony-informative characters.

The MP tree chosen for presentation had 1218 steps, LN = -8038.02, and consistency index of 0.416, retention index of 0.830, and a re-scaled consistency index of 0.346. The BML consensus tree had a mean tree length of 20.258 (variance = 1.2452) and estimated LN = -7435.31 (arithmetic mean) once the posterior probability reached $P = 0.000$ and the cumulative posterior probability reached $P = 1.000$.

Based on phylogenetic analyses of the ITS sequences, *C. sacchari* represents the root of the gene tree lineage that includes *Cytospora* anamorphs of *Valsa* inclusive of the former genera *Leucostoma* Höhn., *Valsella* Fuckel and *Valseutypella* Höhn. (Fig. 1). The *Cytospora* isolates from South Africa reside in several clades, representing 14 or more species and lineages marked by vertical bars and numbered in bold in Fig. 1. Ten described *Cytospora* spp. found in South Africa shown in bold numbering in Fig. 1 include the following from the top of the cladogram: **3.** *V. leucostoma* on *Prunus* and *Malus* (Smit and Adams 1999; Adams *et al.* 2002); **4.** *V. nivea* Fuckel (anamorph *C. nivea* Sacc.) on *Populus* (Hayova and Minter 1998b); **5.** *C. variostromatica* G.C. Adams and M.J. Wingf. (Adams *et al.* 2005) on *Eucalyptus* and *Populus*; **7a, b.** *V. cypri* (Tul.) Tul. and *C. Tul* (anamorph *C. pruinosa*) on *Olea europaea* stems and fruits (Hayova and Minter 1998f); **8.** *V. kunzei* var. *piceae* Waterman [anamorph *C. kunzei* var. *piceae* Waterman, syn. *C. halesiae* Ellis and Everh., syn. *Leucostoma kunzei* (Fr.) Höhn.] on *Pinus radiata* (Waterman 1955); **9.** *V. malicola* (anamorph *C. schulzeri* Sacc. and P. Syd.) on *Malus* (Hayova and Minter 1998g); and *V. germanica* Nitschke (anamorph *C. germanica* Sacc.), on *Populus* (Kobayashi 1970); **10.** *V. sordida* (anamorph *C. chrysosperma*) on *Populus* and *Salix* (Hayova and Minter 1998i); **11.** *V. cinereostroma* G.C. Adams and M.J. Wingf. on *Mangifera* fruit (Adams *et al.* 2005); and **14.** *V. fabianae* (anamorph *C. eucalypticola*) on *Eucalyptus* (Adams *et al.* 2005).

In addition to these 10 species, *Valsa ceratosperma* (Tode) Maire *sensu lato* [anamorph *C. sacculus* (Schwein.) Gvrit.] was seen in South Africa on *Jacaranda* (Hayova and

Minter 1998e) but could not be amplified and sequenced, and *C. sacchari* was reported as common in South Africa on *Saccharum officinarum* (Sivanesan 1983) but was not seen in this study. Of these 12 species, nine are well known (Kobayashi 1970; Barr 1978; Spielman 1985; Hayova and Minter 1998a, 1998b, 1998c, 1998d, 1998e, 1998f, 1998g, 1998h, 1998i), and three (*C. variostromatica*, *V. cinereostroma*, *V. fabianae*) are recently described (Adams *et al.* 2005). The information on South African isolates and species is summarised in Table 1.

In addition to the 12 described species, other South African isolates reside in several unique lineages that represent unresolved taxa or putative new species. There are five unique lineages of *Cytospora* present among the South African isolates. Numbered in bold from the top of the cladogram (Fig. 1A), these are referred to as; **1.** Putative species 1, **2.** 'Uncertain lineage' on *Ceratonia* and *Acacia*, **6.** *C. aff. variostromatica* on *Populus*, **12.** *V. aff. cinereostroma* on *Eucalyptus* and **13.** *C. aff. austromontana* on *Eucalyptus*. Three (Putative species 1, *V. aff. cinereostroma* and *C. aff. austromontana*) have been characterised and discussed previously by Adams *et al.* (2005). The 'uncertain lineage' is evident in the first cladogram, Fig. 1A, where Bayesian analysis groups the Spanish isolate of *C. acaciae* on *Ceratonia* with the South African isolate from *A. nilotica* (Table 1). However, in cladogram Fig. 1B, parsimony analysis does not group the representative *C. acaciae* with the South African isolate from *A. nilotica* (see **2a, b**). *C. aff. variostromatica* is discussed and informally described below.

South African isolates fall into clades in the phylogenetic trees that we interpret, tentatively, as species complexes. The isolates in the *V. malicola/V. germanica* species complex include one with the morphology of *C. schulzeri*, the anamorph of *V. malicola*, and a second with the morphology of *C. germanica*. Reference isolates of *V. malicola* and *V. germanica* are also included in the complex. Thus, we list the South African isolates as two taxa while recognising them as belonging to the same species complex based on ITS sequence homology. South African isolates in the *V. cypri* species complex are referred to, tentatively, as *V. cypri* despite the fact that they differ significantly (99% bootstrap confidence level) from the European and North American reference isolates. Many of the reference isolates of well known species also cluster in strongly supported clades with reference isolates of other less well known taxa (based on ITS sequence). These clades are labelled as species complexes named for the best known species in the group.

The unique lineages, the described species, and species complexes with South African representatives are discussed below in relation to the cladograms (Fig. 1), and in the order in which they appear beginning at the top of the phylogenetic trees. South African species represented only by herbarium specimens and not in the phylogenetic trees

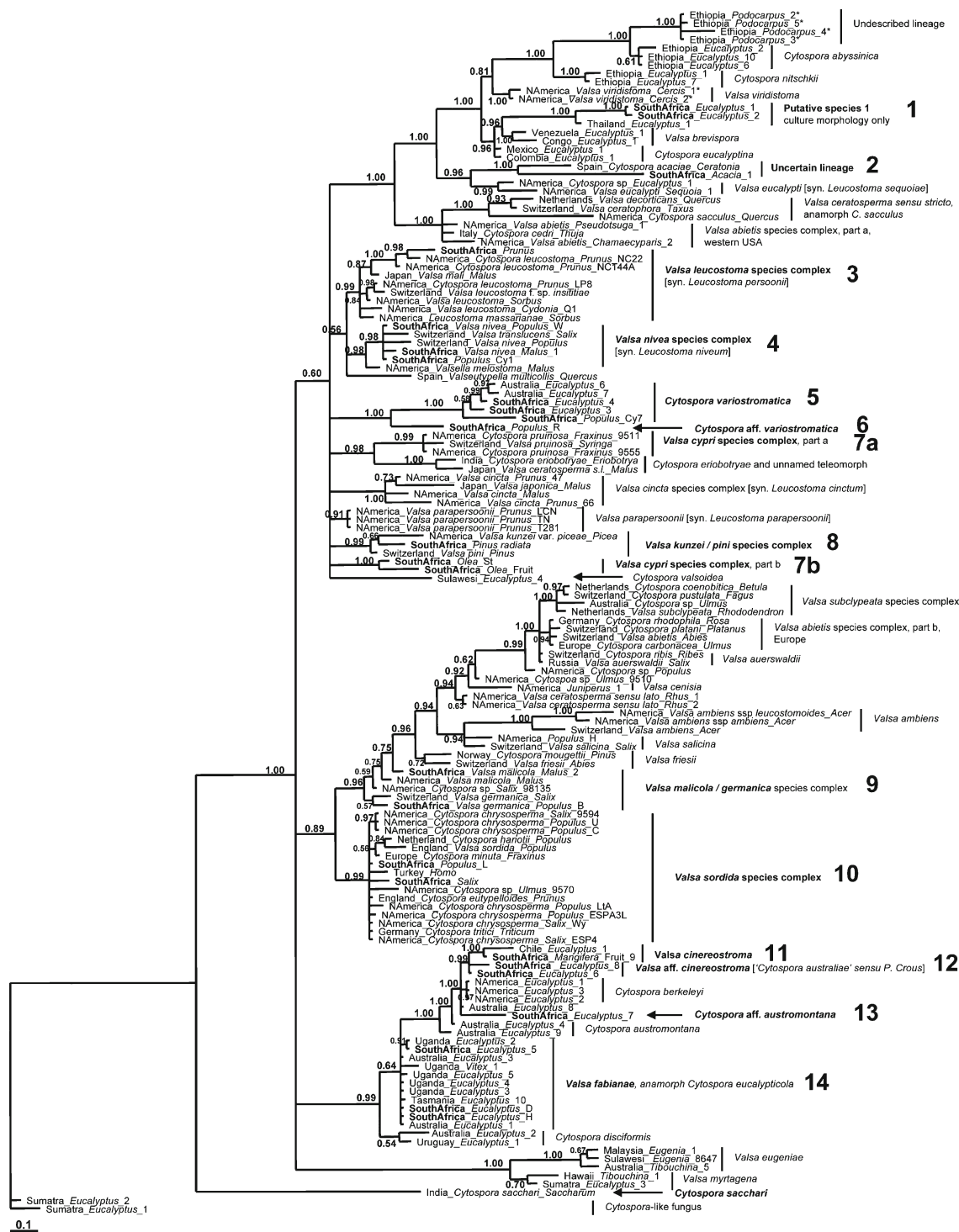


Fig. 14. Cladograms of South African *Cytospora* species in relation to other *Cytospora* species and their *Valsa*, *Leucostoma*, *Valsella* and *Valseutypella* teleomorphs. The species relationships were inferred from Bayesian analysis of DNA sequence of the ITS region of the nuclear rDNA operon. The single gene tree represented is the Bayesian consensus tree calculated using the Metropolis–Hastings algorithm and three million searches, following the burn-in of 100 000 discarded trees. Isolates from South Africa are in bold. Names in regular fonts reflect country of origin followed by host in italics and a number representative of a unique DNA sequence that corresponds to one or more isolates given in Tables 1 and 2 (i.e. Ethiopia *Eucalyptus*-2). Branch lengths correspond to inferred genetic distances with the units of the bar equalling the number of expected nucleotide substitutions. Numbers at nodes are posterior probability distributions. Sequences are the authors' or from Adams *et al.* (2002, 2005) except those marked with an asterisk. Scale bar: 10 nucleotide substitutions.

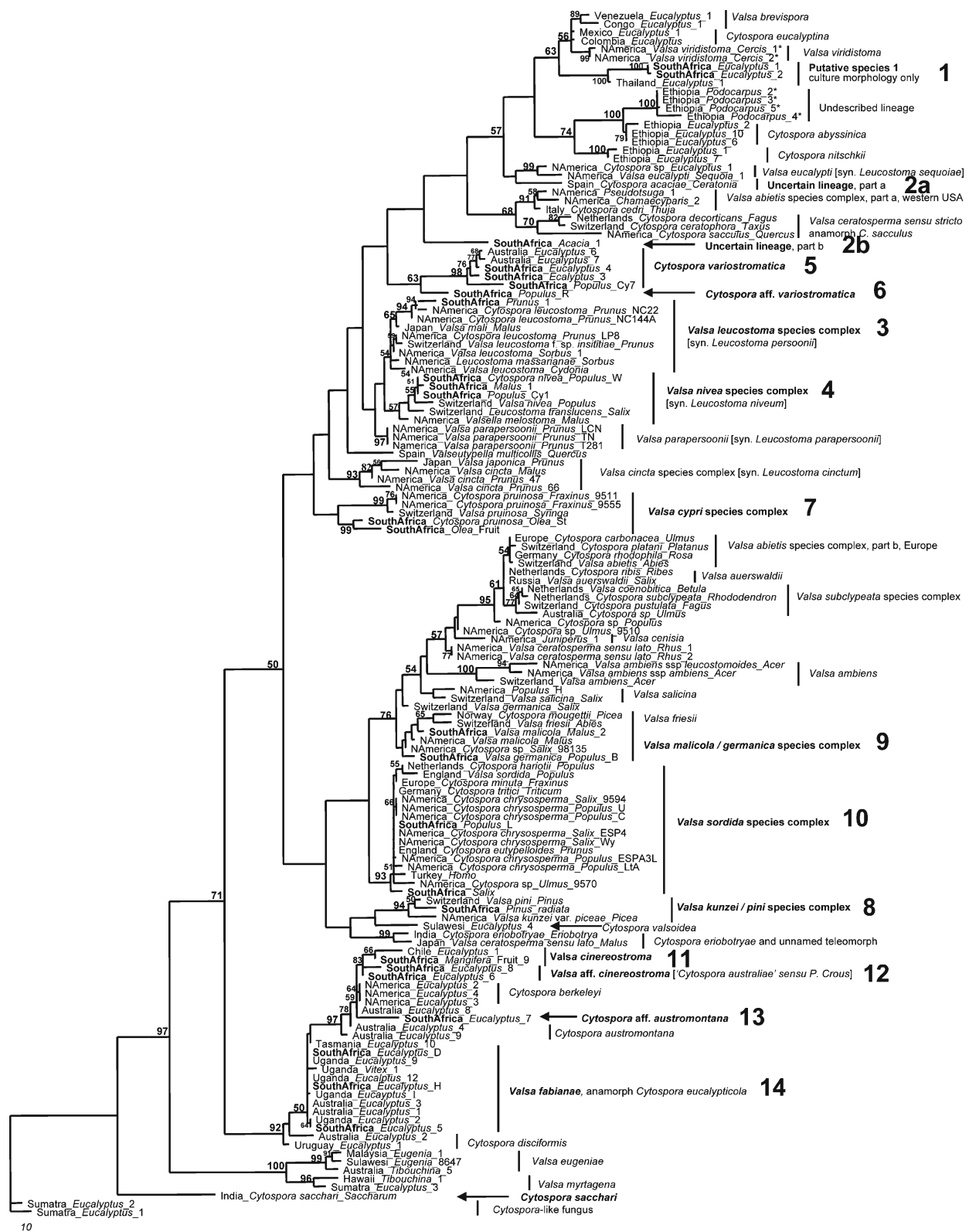


Fig. 1B. Cladograms of South African *Cytospora* species in relation to other *Cytospora* species and their *Valsa*, *Leucostoma*, *Valsella* and *Valseutypella* teleomorphs. The single gene tree represented is from one of the most parsimonious trees with the greatest log likelihood. Branch lengths correspond to inferred genetic distances with the units of the bar equalling the number of nucleotide substitutions. Numbers at nodes are percent bootstrap support (1000 replications). Scale bar: 0.1 nucleotide substitutions per site.

are also discussed. The abbreviation 'aff.', which stands for 'affinity to', is used to refer to isolates in the phylogeny that are closely related by DNA sequence to, but just outside the clade of, a described species.

1. Putative species 1 (Adams *et al.* 2005)

Adams *et al.* (2005) describes the cultural morphology of the isolates in this clade, but culture morphology is not sufficient to identify or describe any *Cytospora* species. This clade includes a group of *Cytospora* isolates collected as part of the study of Smith *et al.* (1996) from leaves of *E. grandis* in Tzaneen and *E. camaldulensis* in Mtubatuba. The isolates were collected as endophytes in green leaves. The South African strains are closely related to an isolate, CBS 116861 (MSC 388704), from Thailand on *E. camaldulensis*. No teleomorph is known for this species.

Hosts

Eucalyptus grandis, *E. camaldulensis*.

Distribution

Piet Retief, Mpumalanga and KwaMbonambi, KwaZulu-Natal, South Africa; Thailand.

Specimens examined

South Africa, Mpumalanga, Piet Retief, endophyte in leaf of *E. grandis*, April 1994, H. Smith, living culture CMW 5357; KwaZulu-Natal, KwaMbonambi, from stem canker on *E. grandis*, April 1994, H. Smith, living culture CMW 5358; **Thailand**, on dead branches of *E. camaldulensis*, September 1996, M.J. Wingfield (MSC 388704), and living culture CBS 116861.

2. Uncertain lineage

The relationship of the South African isolate PPRI 6421 from *Acacia nilotica* could not be resolved by parsimony analysis (Fig. 1B) of the ITS sequence. Bayesian analysis (Fig. 1A) placed it firmly (1.00 posterior probability) with *C. acaciae* Oudem., but such contradictory conclusions are the artefact of insufficiently informative data. The latter species is represented by a strain (CBS468.69) from fruit of *Ceratonia siliqua* (family *Fabaceae*) collected in Mallorca, Spain. Perhaps related isolates may be found that occur on other hosts native to the Mediterranean region and contiguous to Africa. We would speculate that the South African isolate may be representative of an African population common on *Acacia* (family *Mimosaceae*). More extensive collections from *Acacia*, *Ceratonia*, and other hosts are needed, and should improve our understanding of similar isolates. Morphology on natural substrates is required for characterisation. No teleomorphs are reported for these collections.

Hosts

Acacia nilotica, *Ceratonia siliqua*.

Distribution

South Africa; Mallorca, Spain.

Specimens examined

South Africa, on fruit of *A. nilotica*, 1995, C. Roux, living culture CBS 118566; **Spain**, Mallorca, Can Pastilla on dried fruit of *C. siliqua*, June 1969, H.A. van der Aa, living culture of *C. acaciae* Oudem., CBS 468.69.

3. *Valsa leucostoma* species complex (anamorph *Cytospora leucostoma*)

South African strains of this species are typical and closely related to populations present in North Carolina, USA, based on sequence homology (Adams *et al.* 2002). Smit and Adams (1999) and others have reported recently on studies of this species in South Africa (as *Leucostoma persoonii*). The teleomorph has not been seen in collections from South Africa.

Teleomorph not seen. Anamorph separate from teleomorph stromata, discrete. Conidiomatal stroma immersed in bark, erumpent, labyrinthine, leucocytosporoid (resembling *Leucocytospora* Höhn., locule subdivided by invaginations and delimited by a conceptacle), up to 2 × 1.5 mm diam., conceptacle grey to black. Discs white to light grey, furfuraceous, formed of amorphous material, nearly flat to convex, lenticular, up to 1000 × 500 µm, 1 ostiole per disc. Locules multi-chambered, subdivided by invaginations into irregular chambers sharing common walls. Conidiophores hyaline, unbranched or occasionally branched at the base, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, 9–17 × 0.7–1 µm. Conidia hyaline, eguttulate, allantoid, aseptate, (4.5–) 5–7 × 1–1.5 µm.

Host

Prunus persica.

Distribution

Mpumalanga, Nelspruit, South Africa; worldwide.

Specimens examined

South Africa, Mpumalanga, Nelspruit, on dead branches of *P. persica*, October 1998, G. Adams and J. Roux (MSC 368341), and living culture CBS 118557.

4. *Valsa nivea* species complex (anamorph *Cytospora nivea*), Fig. 2

This species is not listed in Crous *et al.* (2000), but herbarium specimens are present at PREM. It is a ubiquitous species on *Populus* worldwide and would be expected in South Africa because the host is commonly grown and has been distributed as vegetative material. To the best of our knowledge, this is the first report of the species occurring on

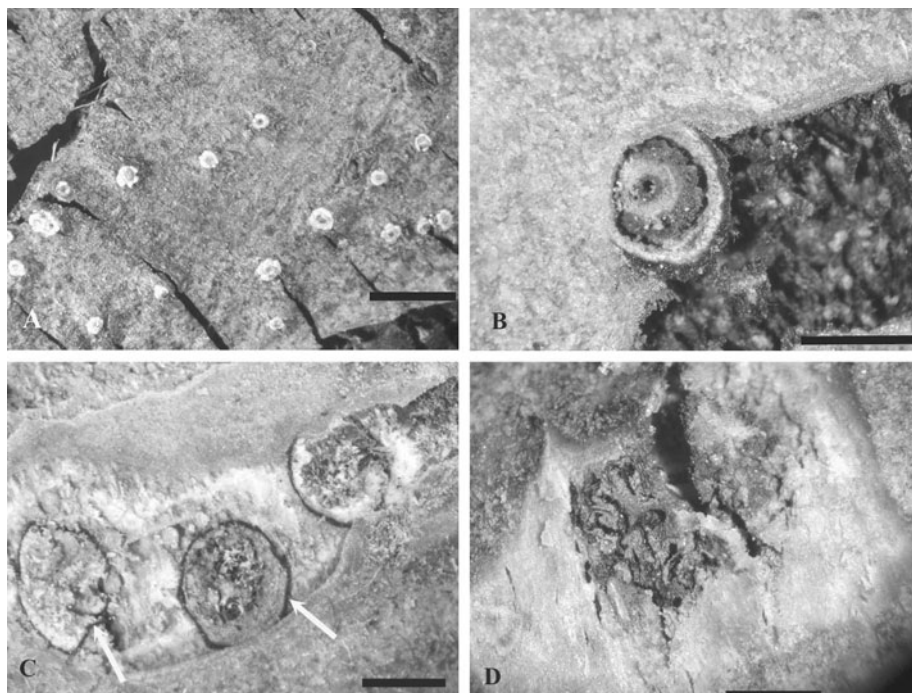


Fig. 2. Morphology of *Cytospora nivea* the anamorph of *Valsa nivea* from *Malus domestica* and *Populus canescens* of South Africa. (A) Habit of conidiomatal discs on twig. (B) Transverse sections through a disc showing a conceptacle delimiting the furfuraceous stroma of the disc. (C) Transverse sections through two conidiomata show the whitish stroma surrounding the chambers and the dark conceptacles (white arrows) delimiting the conidiomata. (D) Transverse section through a conidioma shows the labyrinthine chambers and shared walls. Scale bars: (A, C, D) 1 mm, (B) 0.5 mm.

Malus. A species was collected from Beaufort West on an apple tree that was coinfecting with *V. malicola* on adjacent branches. The teleomorph was not seen in collections from South Africa. Although *V. nivea* has not been directly reported from *Malus*, *Valsella melostoma* does occur on *Malus* and differs from *V. nivea* only in having more than eight ascospores per ascus. The sequence data presented in this study support the view that *V. melostoma* is probably a multispored variant of *V. nivea*. von Petrak (1919, 1969), Müller and von Arx (1973) and Adams *et al.* (2005) did not believe that the polysporous nature of *Valsella* was sufficient to separate species of *Valsella* from *Leucostoma*. von Petrak (1969) argued that *Valsella polyspora* Nitschke and *Valsella adhaerens* Fuckel were polysporous forms of *Valsa auerswaldii*, and that *Valsella salicis* Fuckel, *Valsella fertilis* (Nitschke) Sacc. and *Valsella nigro-annulata* Fuckel were polysporous forms of *Valsa translucens* (De Not.) Ces. and De Not. Species previously placed in *Leucostoma* and *Valsella* are referred to herein as leucostomoid *Valsa*, and polysporous leucostomoid *Valsa* species, respectively (Adams *et al.* 2005).

Teleomorph not seen. Anamorph separate from teleomorph stromata, discrete. Conidiomatal stromata immersed in bark, erumpent, labyrinthine leucocytosporoid, (0.6–)1.4–2.0 mm diam., conceptacles dark. Discs white,

nearly flat to convex, circular, up to 0.2 mm diam., furfuraceous, of amorphous material, 1–(4) ostiole per disc. Ostioles beige to light grey. Locules multi-chambered, subdivided by invaginations into irregular chambers sharing common walls. Conidiophores hyaline, branched at base and above the base 15–25 × 1 µm inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, 8–10 × 1.2 µm. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, (6–)7(–7.5) × 1.2 µm.

Hosts

Malus domestica, *Populus canescens*, *Populus simonii* and other *Populus* spp.

Distribution

Beaufort West, Western Cape, Mpumalanga, Belfast, and Bloemfontein, Free State, South Africa.

Specimens examined

South Africa, Western Cape, Beaufort West, on dead branches of *Malus domestica*, 23 October 2000, J. Roux (MSC 384990), and living culture CBS 118562; Free State, Bloemfontein, on cankered branches of *Populus simonii*, 1996, G. H. Zhao, living culture

CBS 118561; Mpumalanga, Belfast, on cankered branches of *P. canescens* (MSC 384991, MSC 384996), and living culture CMW 5274.

5. *Cytospora variostromatica*

Adams *et al.* (2005) describe and illustrate *C. variostromatica*. Conidiomatal stromata are highly variable on a single specimen and can be unilocular, cytosporoid (locule subdivided by invaginations) and lamyelloid (resembling *Lamyella* Fr., locules undivided, clustered with multiple ostioles converging at the disc) with ostioles converging to a shared disc. South African representatives of *C. variostromatica* are isolated from declining *Populus deltoides* at the Allemanskraal dam as well as from *E. camaldulensis* in Pretoria. The species also occurs on *Eucalyptus* in Australia. Presence on *Populus* might be the result of opportunistic colonisation of senescent tissues because the isolate was not virulent compared with South African isolates of *V. nivea* and *V. sordida* when dormant cuttings were inoculated (Adams unpublished data). Therefore, presence on *Populus* might be a result of 'host jumping' from an exotic *Eucalyptus* (Slippers *et al.* 2005). We speculate that this species is probably native to Australia and usually found on *Eucalyptus*. The teleomorph is unknown for this species.

Teleomorph unknown. Conidiomatal stromata immersed in bark, erumpent, unilocular, cytosporoid, and lamyelloid, medium grey, circular to ovoid, 0.3–0.7(–1) mm diameter, 1–10 locules in entostroma below the discs. Discs dark grey, nearly flat, circular to lenticular, 0.1 × 0.1 mm to 0.3 × 0.9 mm diam., occasionally forming compound discs, furfuraceous, of amorphous material, with 1–10 ostioles. Ostioles dark grey, not above the disc surface, surrounded by medium grey entostroma. Locules globose, from solitary undivided, compressed vertically, to solitary multi-chambered, subdivided by invaginations, to occasionally multiple undivided commonly in groups of two and often clustered and compressed laterally. Dark brown walls of indefinable textura type. Conidiophores hyaline

with 1–4 branches, with up to four verticillate phialides, 12–15 × 1–1.5 µm inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices with minute collarettes, 7–8.5 × 1 µm. Conidia hyaline, eguttulate, allantoid, aseptate, (4.5–)5(–5.5) × 1 µm.

Hosts

Populus deltoides, *E. camaldulensis*, *E. globulus*.

Distribution

Allemanskraal dam, Free State and Pretoria, Gautang, South Africa; Orbost, Victoria, and Kyogee, New South Wales, Australia.

Specimens examined

South Africa, Free State, Allemanskraal dam on cankered stems of *Populus deltoides*, 1996, G. H. Zhao (MSC 384989), and living culture CBS 118564; Gautang, Pretoria on dead leaves of *E. camaldulensis*, 1995, C. Roux (MSC 388705), and living culture CBS 118086 (PPRI 5297); **Australia**, Victoria, Orbost, Toslarree on dead branches of *E. globulus*, September 2000, M.J. Wingfield (MSC 380695, **holotype** of *Cytospora variostromatica*), living ex-type culture CBS 116858.

6. *Cytospora* aff. *variostromatica*, Fig. 3

The relationship of this fungus to *C. variostromatica* is not well supported and the designation *C. aff. variostromatica* is tentative. The conidioma is delimited by a dark conceptacle. The conidia are larger and the locules more labyrinthine than in the *C. variostromatica* specimens we have studied (Adams *et al.* 2005). The fungus probably is representative of an undescribed species common in South Africa. Abundant fruiting of this taxon was present on dead branches of *Populus balsamifera* near the dam at Nelspruit, Mpumalanga, but teleomorphs were not present. The fungus is apparently unrelated to known species (such as *V. nivea* and *V. sordida*) that occur on *Populus* in the Northern Hemisphere based on our current knowledge and collections.

Teleomorph not seen. Conidiomatal stromata immersed in bark, erumpent, labyrinthine leucocytosporoid, medium

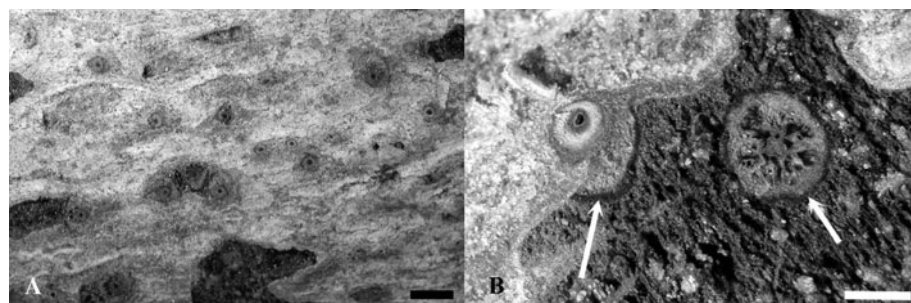


Fig. 3. Morphology of *Cytospora* aff. *variostromatica* from *Populus balsamifera* of South Africa. (A) Habit of conidiomatal discs on twig. (B) Transverse sections through two conidiomata show the whitish stroma surrounding the ostiole (left), the multi-chambered labyrinthine locule, and the dark conceptacles delimiting the conidiomatal stromata (white arrows). Scale bars: 1 mm.

grey, circular to ovoid, 1–2 mm diam., locules in entostroma below the discs, conceptacles dark. Discs light grey to whitish, nearly flat, circular, 0.3–0.5 mm diam., furfuraceous, of amorphous material, with one ostiole per disc. Ostioles reddish brown to grey, level with disc surfaces, surrounded by light grey to whitish entostroma. Locules multi-chambered, subdivided by invaginating walls, sharing common walls. Conidiophores hyaline, branched at the base and mid-way, $14\text{--}15 \times 1\text{--}1.5 \mu\text{m}$ inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes, $5\text{--}10 \times 1 \mu\text{m}$. Conidia hyaline, eguttulate, allantoid, aseptate, $6\text{--}(7.5) \times 1 \mu\text{m}$.

Host

Populus balsamifera.

Distribution

Nelspruit, Mpumalanga, South Africa.

Specimens examined

South Africa, Mpumalanga, Nelspruit, near the dam on dead branches of *Populus balsamifera*, October 1998, G. Adams and J. Roux (MSC 384989), and living culture CBS 118565.

7. *Valsa cypri* species complex (anamorph *Cytospora pruinosa*), Figs 4 and 5

Valsa cypri is ubiquitous on *Fraxinus*, *Syringa*, and *Olea* worldwide. The morphology of conidiomata in South African collections agrees with the morphology of *C. pruinosa*, the anamorph of *V. cypri*. *Cytospora pruinosa* is so distinctive in morphology that it had been used in erecting the monospecific genus *Cytophoma* Höhn. However, *Cytophoma* was submerged in *Cytospora* and is a synonym (Gvritishvili 1982; Adams *et al.* 2005). In the South African specimens, however, the ring-like (or wing-like in transverse section) ectostromatal collar around the ostiole is absent or not readily discerned. The conidiomata formed in thin bark (epidermis) of the current year's branches, and the collars might form only in differentiated bark. The phylogenetic relationship of the South African isolates on *O. europaea* is more that of a sister species to *V. cypri* than part of the populations collected from *Fraxinus* in North America and *Syringa* in Europe. The isolates could be representative of a closely related and undescribed species. The *Cytospora* infections on twigs appeared to be associated with insect-caused wounding.

Teleomorph not seen. Anamorph discrete. Conidiomatal stromata absent. Conidiomata immersed in epidermis, erumpent, cytophomoid (resembling *Cytophoma* Höhn., solitary locule undivided by invaginations) (0.5–)0.6(–1.2) mm diam. Ectostromatal collar absent or not apparent, entostromata absent. Discs dark grey, nearly flat, circular, 100–150 μm diam., discrete ostioles.

Ostioles dark grey externally, light grey internally. Locules uniloculate, undivided by invaginations. Conidiophores hyaline, branched at base and commonly branched above the base, $20\text{--}25 \times 1.5 \mu\text{m}$ inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, $10\text{--}15 \times 1.5 \mu\text{m}$. Conidia hyaline, eguttulate, allantoid, aseptate, $5\text{--}6 \times 1.2 \mu\text{m}$.

Host

Olea europaea (syn. *Olea europaea* var. *africana*).

Distribution

Sterkfontein Cave, Krugersdorp, Gauteng, South Africa.

Specimens examined

South Africa, Gauteng, Krugersdorp, Sterkfontein Cave parking lot, on dead twigs of *O. europaea* at locations of insect feeding injury, October 1998, G.C. Adams and J. Roux (MSC 384995), and living culture CBS 118555; on fruit of cultivated *O. europaea*, date and collector unknown, living culture PPRI 6334.

8. *Valsa kunzei* species complex (anamorph *Cytospora kunzei*): *Valsa kunzei* var. *piceae* (anamorph *Cytospora kunzei* var. *piceae*), Figs 6 and 7

A South African specimen on *Pinus radiata* is morphologically most similar to *V. kunzei* var. *piceae* and has ostiolar discs with unusually numerous tightly compacted ostioles. Additionally, the anamorph is scarce and less labyrinthine than North American specimens of var. *piceae* on *Picea*. The South African specimen is closely related by DNA homology to reference strains of *V. pini* (Alb. and Schwein.) Fr. (anamorph *C. pini* Desm.) (Urban 1957) and *V. kunzei* var. *piceae* (anamorph *C. kunzei* var. *piceae*) from Europe and North America, respectively. It was present on dead branches of *Pinus radiata*, Eastern Cape, and has densely clustered ostioles whereas the morphology of the teleomorph of *V. pini* is characterised by perithecial ostioles forming in a ring around the periphery of the disc. A conceptacle is present but it is light and difficult to see around the ascostroma of the South African specimen. The phylogenetic analysis supports the inference that *V. kunzei* and *V. pini* are possibly morphological variants of one species. Alternatively, the ITS sequence may be inadequate for distinguishing two closely related species and proposing a synonymy may be premature. *Valsa pini* occurs on timber species of *Pinus*, but it has not been reported on *Pinus radiata*. This is the first report of a *Valsa* sp., or a *Cytospora* sp., on *Pinus radiata*, a timber species planted worldwide.

Ascostromata immersed in bark, erumpent, circular to ovoid, (1.2–)2.0(–2.5) mm diam., leucostomoid, 10–50 perithecia arranged circinate in beige entostromata

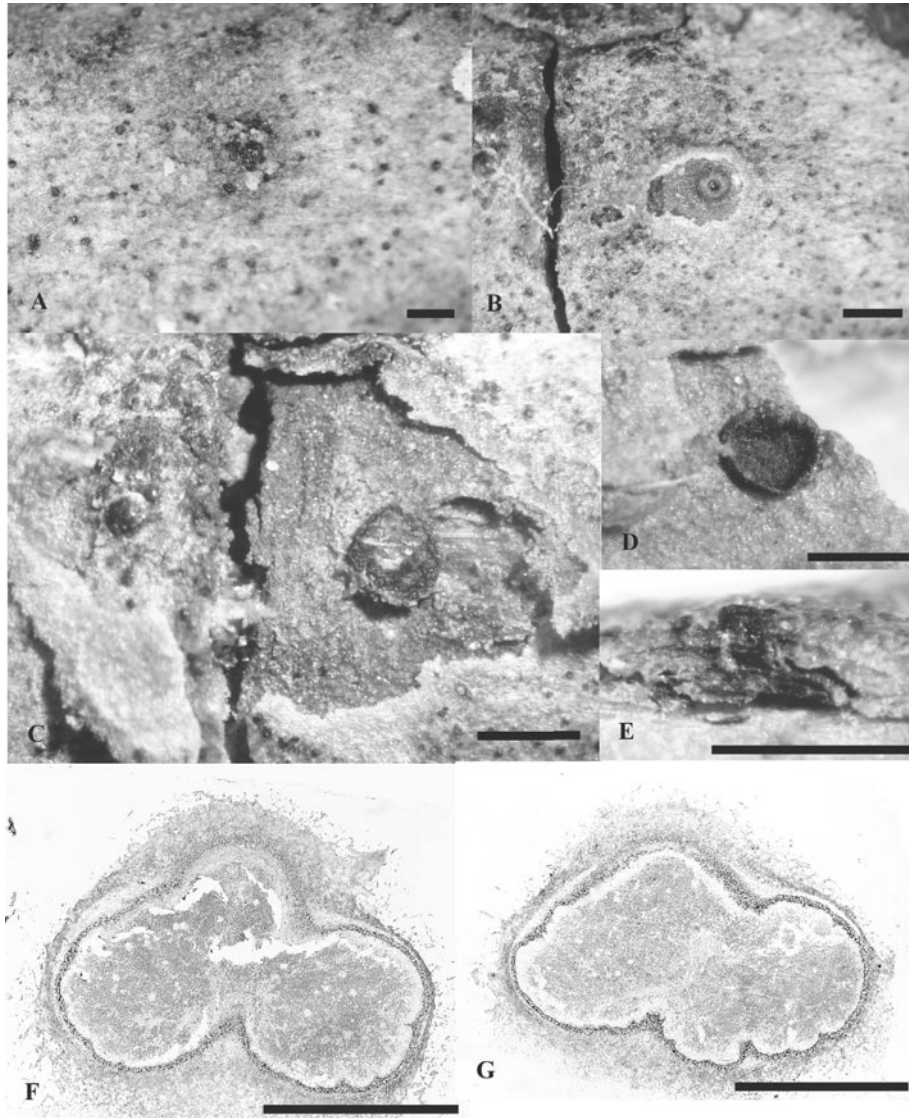


Fig. 4. Morphology of *Cytospora pruinosa* the anamorph of *Valsa cypri* from *Olea* of South Africa. (A) Habit of conidiomatal discs on bark surface. (B) Disc cut transversely. (C) Conidioma lifted out of embedded position in wood. (D) Underside of conidioma exposed by lifting bark. (E) Longitudinal cut through ostiolar disc of conidioma. (F, G) Transverse microtome sections through conidiomata show single locules with absence of invaginations and chambers. Scale bars: (A, B) 250 µm, (C–E) 1 mm, (F, G) 0.5 mm.

below the discs, conceptacles dark, often obscure. Discs usually obscured by tightly packed ostiolar necks, when apparent pale brown to beige, flat, circular to ovoid, (0.2–)0.5(–0.8) mm diam., 10–50 ostioles. Ostioles laterally inserted, dark brown, at the same level as the disc surface, clustered in centres of discs, (40–)50(–60) µm diam. Perithecia globose, 0.3–0.4 mm diam., laterally inclined, surrounded with white to beige entostromata of loose amorphous material, walls dark brown, of textura epidermoidea. Asci free, clavate to elongate obovoid, 20–30 × 5–7 µm, with a refractive chitinous ring in the

non-amyloid apical apparatus, 8-spored. Ascospores biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, 6.5–7 × 1–1.5 µm.

Anamorph interspersed amongst ascostromata, discrete. Conidiomatal stromata immersed in bark, erumpent, rosette to labyrinthine leucocytosporoid, 0.8–1 mm diam., conceptacles dark but often obscured. Discs dark grey, nearly flat, circular, up to 0.2 mm diam., one ostiole per disc. Ostioles dark grey, furfuraceous. Locules multi-chambered, subdivided by invaginations into regular radially arranged chambers sharing common walls. Conidiophores hyaline,

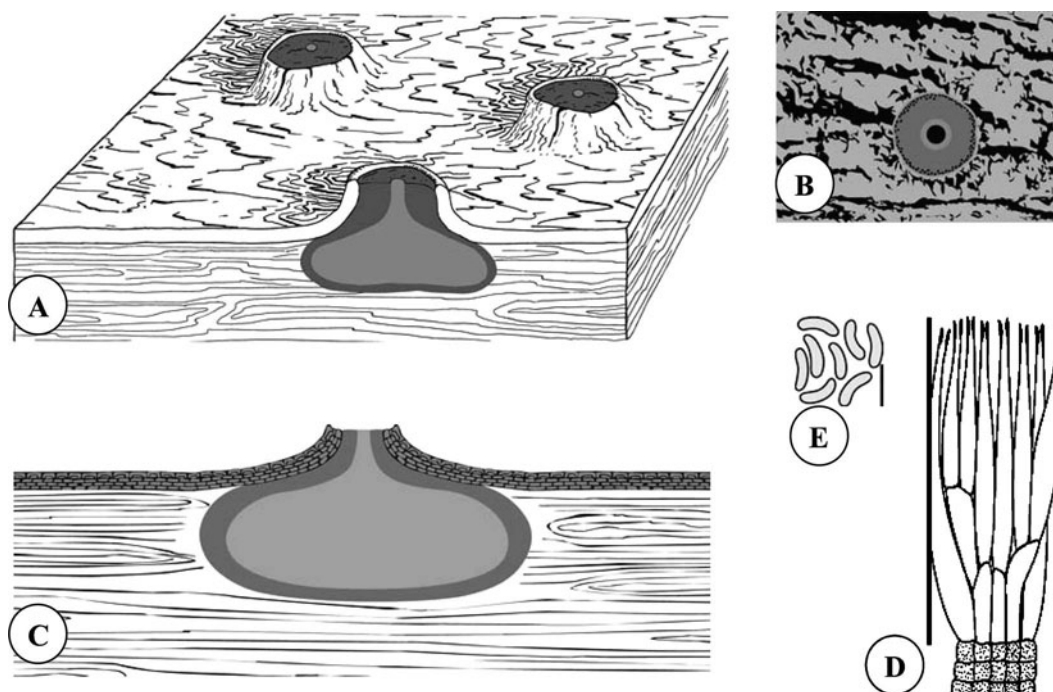


Fig. 5. Illustrations of *Cytospora pruinosa* the anamorph of *Valsa cypri*. (A) Habit sketch. (B) Ostiolar disc erumpent from bark. (C) Transverse section through conidiomatal stroma in plant. (D) Conidiophores in hymenium. (E) Conidia. Scale bars: (D) 25 μm , (E) 5.5 μm .

branched at base and above the base, $15\text{--}25 \times 1 \mu\text{m}$ inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, $8\text{--}10 \times 1 \mu\text{m}$. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, $4\text{--}4.5 \times 0.9 \mu\text{m}$.

Host

Pinus radiata.

Distribution

Eastern Cape, South Africa; USA.

Specimens examined

South Africa, Eastern Cape, on dead branches of *Pinus radiata*, 18 February 1999, J. Roux (MSC 384994), and living culture CBS 118556; **USA**, Michigan, East Lansing, on dying branches of *Picea pungens*, 1987, T. Proffer (MSC 380720, MSC 380721), and living cultures CBS 118093 and CBS 118094, respectively.

9. The *Valsa malicola*/*Valsa germanica* species complex: 9, pro parte. *Valsa malicola* (anamorph *Cytospora schulzeri*), Figs 8 and 9

The Bayesian analysis groups a Beaufort West collection from *Populus* with the reference strain of *V. germanica* (CBS 195.42); however, the support is weak (0.57 posterior probability). The parsimony analysis does not group the

two isolates together, rather it groups the Beaufort West strain with *V. malicola* collections from North America (MSC 384997) and South Africa (CBS 118559) collected on *Malus*, and the North American strain (98135) collected on *Salix*. The two strains on *Malus* are from specimens exhibiting the distinguishing feature of multiple ostioles forming a ring within the disc of a conidioma. This feature is characteristic of *C. schulzeri*, the anamorph of *V. malicola*. The strains from *Populus* and *Salix* are from specimens exhibiting one central ostiole in a whitish disc. This feature is more characteristic of *C. germanica*. Both phylogenetic analyses group the North American strain from *Salix* with *V. malicola* despite the differences in ostiole arrangements. From these results, we have come to believe that the two species, *V. malicola* and *V. germanica*, represent a species complex that varies in morphology when occurring on different hosts. Unfortunately, teleomorphs are not present in the collections from *Populus* and *Salix* and they could have provided additional morphological information for the argument. Hubbes (1960) and Spielman (1985) considered *V. germanica* a synonym of *V. sordida* based on morphological studies but our phylogeny disagrees with that synonymy. Additionally, Spielman (1983) considered *V. malicola* a synonym of *V. ambiens* (Pers.) Fr. and our phylogeny disagrees with that synonymy.

Teleomorph not seen. Anamorph separate from teleomorph stromata, discrete. Conidiomatal stromata

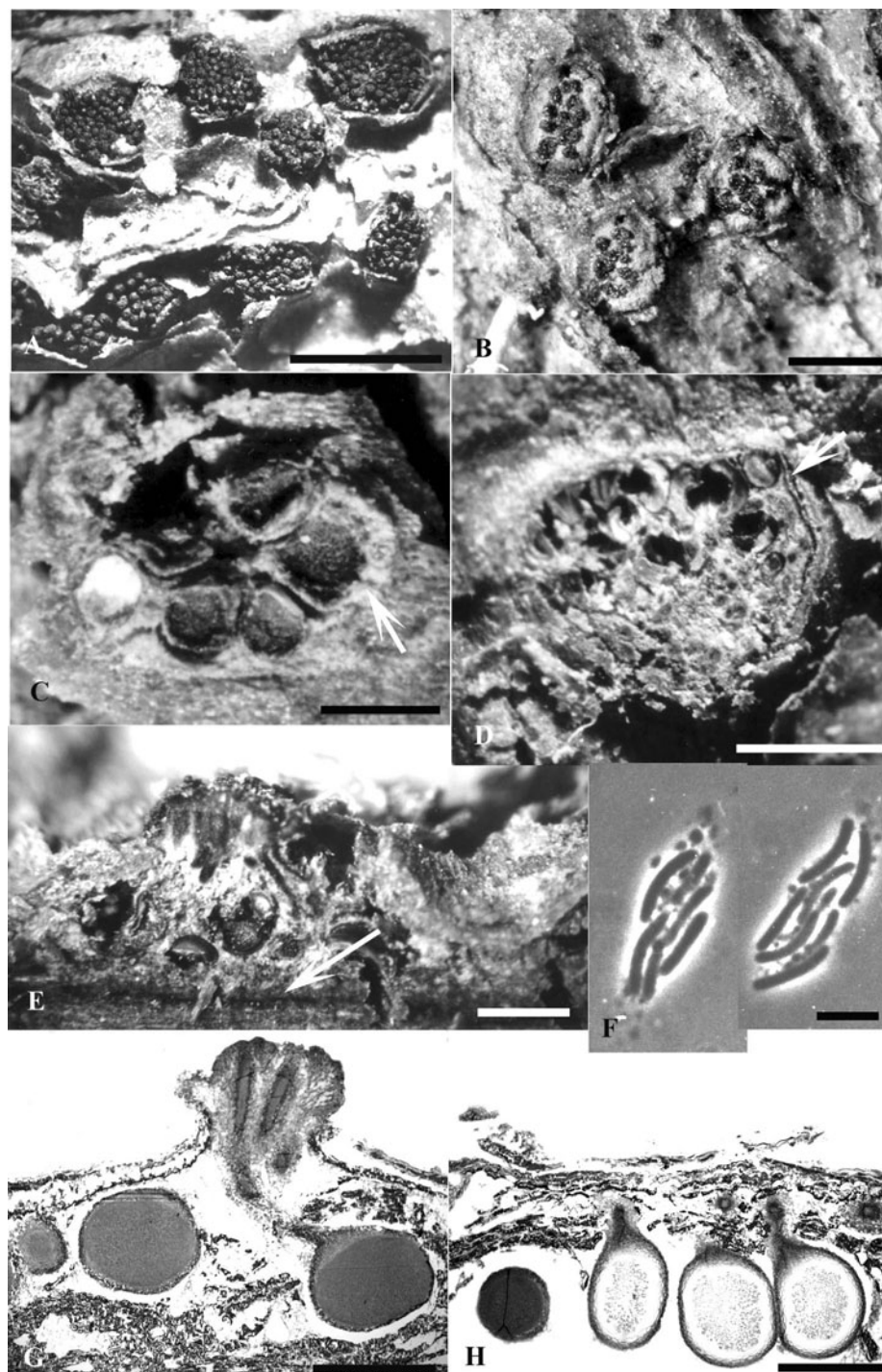


Fig. 6. Morphology of *Valsa kunzei* var. *piceae*. (A) Average habit of ostiolar discs of ascostromata shows dense compacted ostioles obscuring discs. (B) Rare habit of ostiolar discs with light brown discs and centrally arranged ostioles. (C) Transverse section through small ascostroma below disc shows circinate arrangement of a few globose perithecia surrounded in entostroma (white arrow). (D) Transverse sections through larger ascostromata below discs show compacted perithecia and ascostromata delimited by thin conceptacle (white arrow). (E) Longitudinal section through ascostroma shows tightly adhering ostiolar necks in disc, perithecia in entostroma delimited from wood by a dark conceptacle (white arrow). (F) Two asci, each with apical ring apparatus stained with cotton blue, and eight ascospores. (G, H) Longitudinal microstome sections through ascostromata show arrangement of perithecia. Scale bars: (A) 1 mm, (B, C) 0.5 mm, (D, E) 1 mm, (F) 5 μ m, (G, H) 300 μ m.

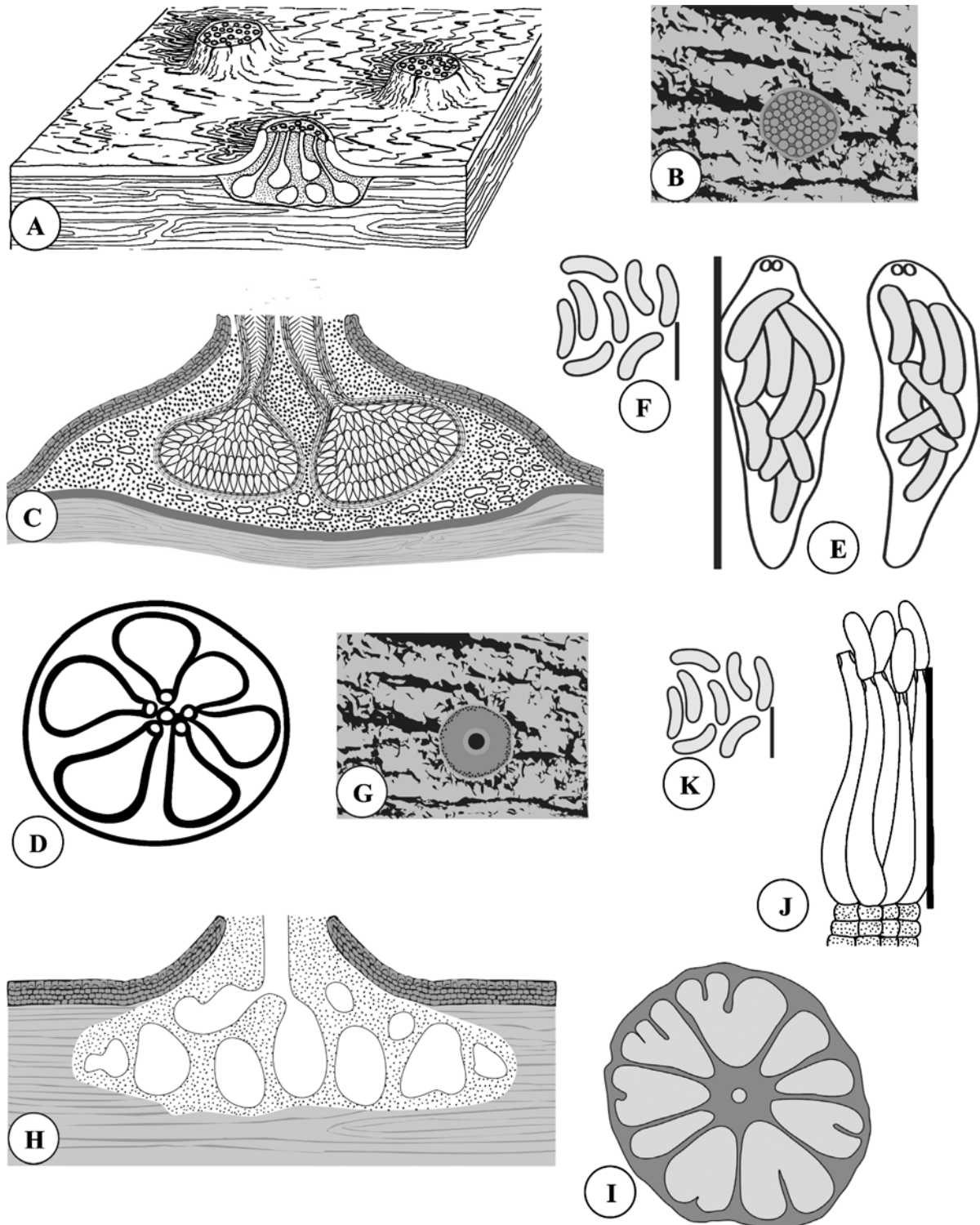


Fig. 7. Illustrations of *Valsa kunzei* var. *piceae*. (A) Habit sketch. (B) Ostiolar discs of teleomorph erumpent from bark. (C) Longitudinal section through ascostroma in plant. (D) Transverse section through ascostroma. (E) Asci. (F) Ascospores. (G) Ostiolar disc of anamorph erumpent from bark. (H) Longitudinal section through conidiomal stroma in plant. (I) Transverse section through conidiomal stroma. (J) Conidiophores in hymenium. (K) Conidia. Scale bars: (E) 25 μm , (F) 7 μm , (J) 20 μm , (K) 4.5 μm .

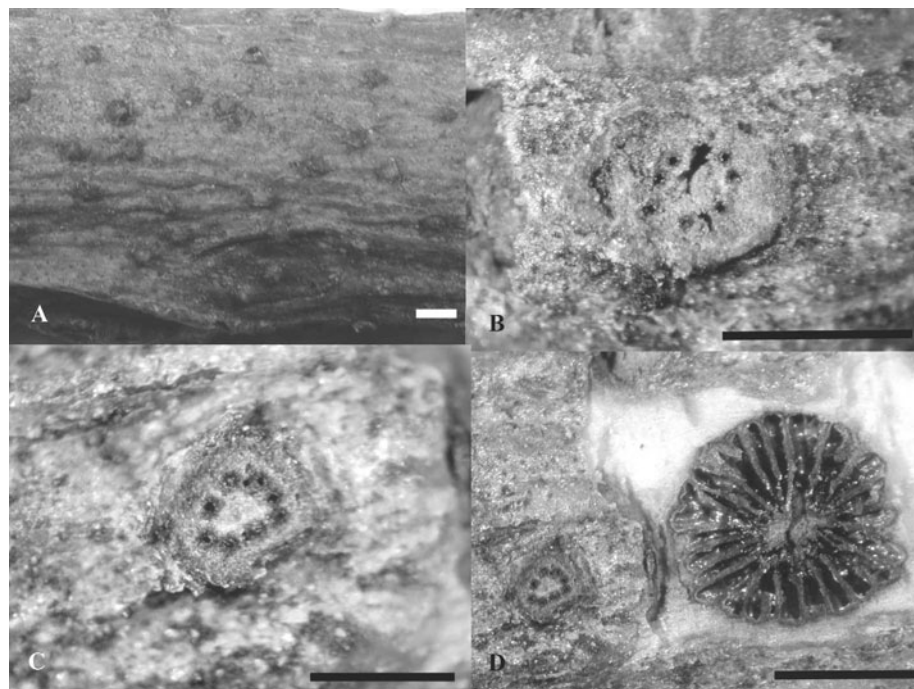


Fig. 8. Morphology of *Cytospora schulzeri* the anamorph of *Valsa malicola* from *Malus* of South Africa. (A) Habit of conidiomatal discs on twig. (B) Disc shows presence of circinate ostioles. (C) Transverse section through disc shows light-brown stroma tissue around and inside the circle of ostioles. (D) Transverse section through conidioma shows locule divided radially into about 23 chambers. Scale bars: (A, D) 1 mm, (B, C) 0.5 mm.

immersed in bark, erumpent, rosette to labyrinthine cytosporoid with regular radially arranged chambers, circular 1.4–1.5 mm diam. Discs light to medium brown, nearly flat, circular, up to 0.5 mm diam., with 5–10 ostioles per disc. Ostioles dark brown, circinate, at the same level as the disc surface, about 70 µm diam. Locules multi-chambered, subdivided by entire invaginations into regular radially arranged chambers sharing common walls. Conidiophores hyaline, branched at bases and above the bases, 17–20 × 1.5 µm inclusive of phialides, phialides in whorls, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, (7–)10(–12) × 1 µm. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, (4–)6 × 0.9 µm.

Host

Malus domestica.

Distribution

Beaufort West, Western Cape, South Africa; worldwide.

Specimens examined

South Africa, Western Cape, Beaufort West, on dead branches of *Malus domestica*, 23 October 2000, J. Roux (MSC 384993), and living culture CBS 118559; **USA**, Michigan, Grand Haven, Fruitridge road, on dead

branches of *Malus domestica*, 1993, G. Adams (MSC 384997), and living culture CBS 118570; Colorado, Fort Collins, Ansel Waltrous Campground on cankered stems of multistemmed *Salix* sp., April 1998, J. Kepley and W. Jacobi, living culture 98135.

9. The *Valsa malicola/Valsa germanica* species complex: 9, pro parte. *Valsa germanica* (anamorph *Cytospora germanica*), Fig. 10

Teleomorph not seen. Anamorph separate from teleomorph stromata, discrete. Conidiomatal stromata immersed in bark, erumpent, labyrinthine cytosporoid, circular to ovoid 0.7–1.4 mm diam. Discs whitish, nearly flat, circular, 0.3–0.4 mm diam., with 1(–2) ostiole per disc. Ostioles light grey, at the same level as the disc surface, about 150 µm diam. Locules multi-chambered, subdivided by invaginations into irregularly arranged chambers sharing common walls. Conidiophores hyaline, branched at bases and above the bases, 10–15 × 1 µm inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, 5–7 × 1 µm. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, 5 × 1 µm.

Hosts

Populus spp., *Salix* sp.

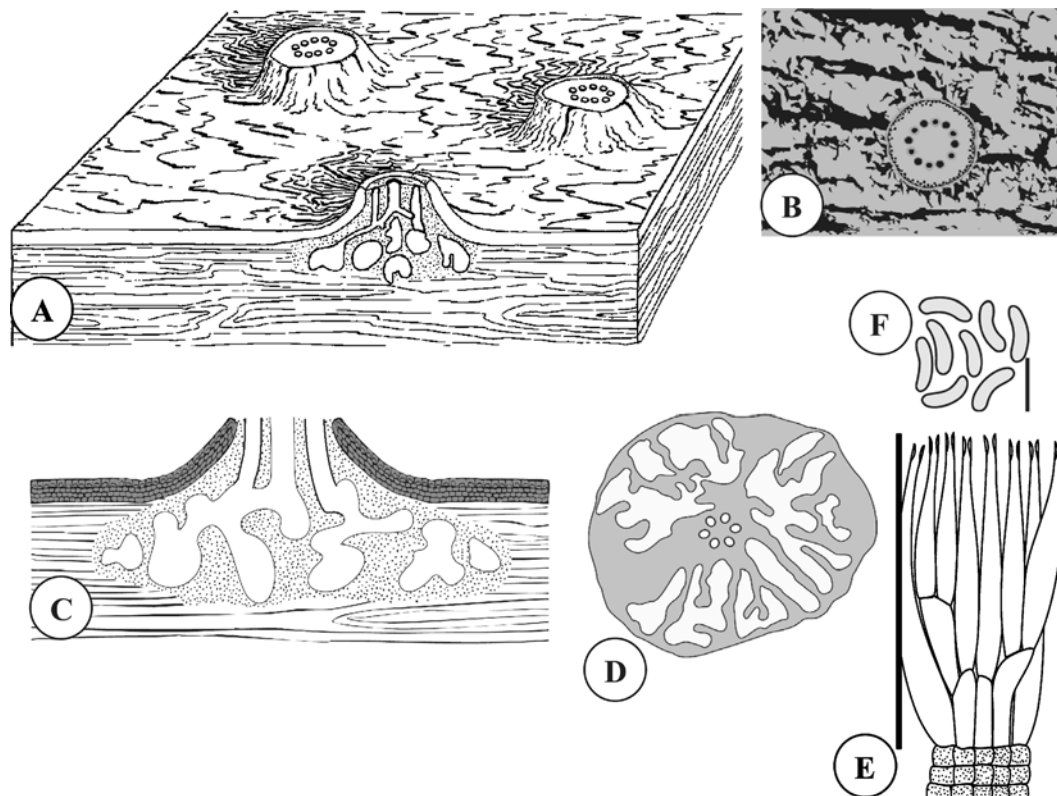


Fig. 9. Illustrations of *Cytospora schulzeri* the anamorph of *Valsa malicola*. (A) Habit sketch. (B) Ostiolar disc erumpent from bark. (C) Longitudinal section through conidiomatal stroma in plant. (D) Horizontal cross section through conidiomatal stroma. (E) Conidiophores in hymenium. (F) Conidia. Scale bars: (E) 19 μ m, (F) 6 μ m.

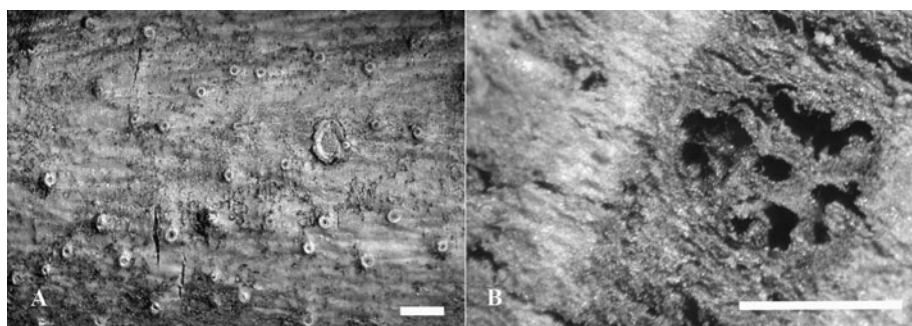


Fig. 10. Morphology of the anamorph of *Valsa germanica* from *Populus* of South Africa. (A) Habit of conidiomatal discs on twig. (B) Transverse sections through conidiomata show labyrinthine chambers. Scale bars: 1 mm.

Distribution

Beaufort West, Western Cape, South Africa; worldwide.

Specimens examined

South Africa, Western Cape, Beaufort West, on dead branches of *Populus* sp., 23 October 2000, J. Roux (MSC 384992), and living culture CBS 118560.

10. *Valsa sordida* species complex (anamorph *Cytospora chrysosperma*), Fig. 11

Valsa sordida [anamorph *C. chrysosperma* (Pers.) Fr.] was present on *Populus* and *Salix* in South Africa. The teleomorph was present in collections from *Salix* but was not seen on *Populus*. In the cladograms several *Cytospora* species with unknown sexual states are grouped

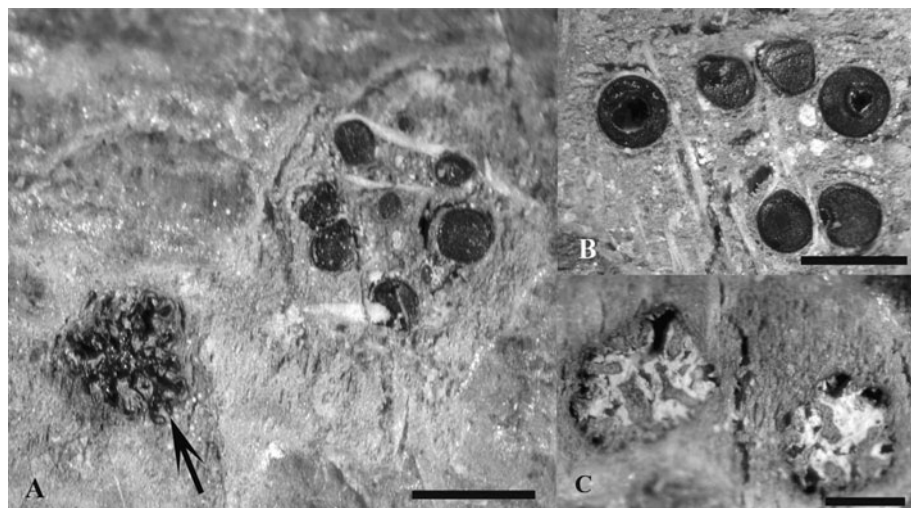


Fig. 11. Morphology of *Valsa sordida* including the teleomorph and anamorph. (A) Transverse sections through conidioma (arrow) and ascostroma on bark of *Salix*. (B) Transverse section through ascostroma below disc shows widely spaced globose perithecia surrounded in entostroma. (C) Transverse sections through two conidiomatal stomata show labyrinthine chambers with shared walls in stomata. Scale bars: (A–C), 1 mm.

within the *V. sordida* clade, such as *C. hariatii* Briard on *Populus*, *C. minuta* Thüm. on *Fraxinus*, *C. triticum* Punith. on *Triticum*, and *C. eutypelloides* Sacc. on *Prunus*. Grouping could result from failure of the ITS sequence to sufficiently resolve the separate species. More likely, the species are representatives of the population diversity and morphological variation within *V. sordida*. The range of hosts on which *V. sordida* has been reported includes the *Salicaceae* only; however, here the range would be extended to include *Ulmus*, *Triticum*, *Fraxinus*, *Prunus* and, remarkably, even *Homo sapiens* (Kalkanci *et al.* 2006). Other herbarium specimens of *Cytospora* collected in South Africa have similar morphology to *C. chrysosperma*, see under *C. chrysosperma* s. lat.

Ascostromata immersed in bark, erumpent, circular to ovoid 1.4–2.0 mm diam., euvalloid, 7–13 perithecia, not crowded, arranged circinate in grey entostromata below the discs. Discs white, flat, circular to ovoid, 0.5–0.6 mm diam., 3–13 ostioles. Ostioles laterally inserted, dark grey, at the same level as the disc surface, in a ring (circinate) around periphery of discs, about 60 µm diam.. Perithecia globose, 0.3–0.6 mm diam., laterally inclined, surrounded with grey entostromata of amorphous material, walls dark brown, of textura epidermoidea. Asci free, clavate to elongate obovoid, (40–)50(–60) × 8–12 µm, with a refractive chitinous ring in the non-amyloid apical apparatus, 6–8-spored. Ascospores biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, 10–13 × 2.0 µm.

Anamorph interspersed amongst ascostromata, discrete. Conidiomatal stomata immersed in bark, erumpent, labyrinthine cytosporoid, 1–2 mm diam. Discs white to light

grey, nearly flat, circular, 0.4–0.5 mm diam, one ostiole per disc. Ostioles dark grey. Locules multi-chambered, subdivided by invaginations into irregular chambers sharing common walls. Conidiophores hyaline, branched at base, about 15 × 1 µm inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, (6–)8–10 × 1 µm. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, 4–5 × 1 µm.

Hosts

Salix spp., *Populus nigra* v. 'italica' and other *Populus* spp.

Distribution

Pretoria, Gauteng and New Castle, KwaZulu-Natal, South Africa; worldwide.

Specimens examined

South Africa, Gauteng, Pretoria, Hatfield, on dead branches of *Salix* sp., December 1998, G. Adams and J. Roux (MSC 380717), and living culture CMW 5269; Gauteng, Pretoria, Hatfield, on dead branches of *Populus nigra* v. 'italica', 2 November 1998, G. Adams and J. Roux (MSC 384988), and living culture CBS 118563; KwaZulu-Natal, New Castle, on dead branches of *Salix* sp., October 1998, G. Adams and J. Roux (MSC 387071).

11. *Valsa cinereostroma* (*Cytospora* anamorph not named)

South African isolate PPRI 6767 is placed in the species *V. cinereostroma* based on ITS sequence homology. This isolate is from mango fruit and only a culture exists. The teleomorph and anamorph of *V. cinereostroma* is described

and illustrated on *Eucalyptus* in Adams *et al.* (2005) from Chile, South America, where it occurs on *Eucalyptus*. Further sampling from *Eucalyptus* and *Mangifera* in South Africa might recover related strains. A comparison of the South African strain to *Cytospora mangifera* G.P. Agarwal and N.D. Sharma (described from India) should be pursued when a culture becomes available of the latter.

Anamorph discrete. Conidiomatal stromata immersed in bark, erumpent, rosette to labyrinthine leucocytoporoid, up to 1.5 mm diam., conceptacles dark. Discs dark brown, nearly flat, circular, up to 0.5 mm diam., discrete ostioles. Ostioles chalky pale grey, furfuraceous. Locules multi-chambered, subdivided by invaginations into regular to irregular radially arranged chambers sharing common walls. Conidiophores hyaline, branched at base, up to four branches above the base $9\text{--}15 \times 1 \mu\text{m}$, inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, $(7\text{--})8\text{--}(10) \times 1 \mu\text{m}$. Conidia hyaline, eguttulate, elongate-allantoid, aseptate $(5\text{--})5.5\text{--}(6) \times 1 \mu\text{m}$.

Hosts

Mangifera indica, *Eucalyptus globulus*.

Distribution

South Africa; Chile.

Specimens examined

South Africa, from fruit of *Mangifera indica*, 1998, C. Roux (MSC 388710), and living culture CBS 116830 (PPRI 6767); **Chile**, on dead branches of *Eucalyptus globulus*, March 2000, M.J. Wingfield (MSC 375220, **holotype** of *Valsa cinereostroma*), and ex-type living culture CBS 117081.

12. *Valsa* aff. *cinereostroma* (*Cytospora* anamorph not named)

Several isolates collected from *E. nitens* and *E. grandis* near Wartburg, White River and Amsterdam, South Africa may have affinities to *V. cinereostroma* and may represent part of the diversity within this species. The one herbarium specimen PREM 50454 on a *Eucalyptus* twig is sparse on fruiting bodies and the teleomorph is not present. Cultures were obtained from P. Crous (Centraalbureau voor Schimmelcultures and University of Stellenbosch) who described the anamorph (Crous *et al.* 1990). This species is unique in having rosette leucocytoporoid conidiomata and large conidia on *Eucalyptus*. The morphology of this species is further described in Adams *et al.* (2005), including culture characteristics.

Teleomorph not observed. Conidiomatal stromata immersed in bark or in leaves, variably erumpent, rosette cytoporoid. Locules subdivided by invaginations into six or more regular radially arranged chambers sharing

common walls. Conidiophores hyaline, branched at base, up to four branches above the base $9\text{--}15 \times 1 \mu\text{m}$, inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, $(7\text{--})8\text{--}(10) \times 1 \mu\text{m}$. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, variable, $(3.5\text{--})5.5\text{--}(6) \times 0.9\text{--}1 \mu\text{m}$ on the natural material, but uniform $(5\text{--})5.5\text{--}(6) \times 1 \mu\text{m}$ *in vitro*.

Hosts

Eucalyptus grandis, *Eucalyptus nitens*.

Distribution

Amsterdam, Wartburg, and White River, Barberton, South Africa.

Specimens examined

South Africa, Barberton, White River, Jessivale State Forest on twig from *Eucalyptus grandis* Clone HLandL, December 1988, P.W. Crous (PREM 50454, as '*Cytospora australiae* Speg.'), and living cultures CBS 116831; White River on twig from *Eucalyptus grandis*, December 1988, P.W. Crous (MSC 388709), and living culture CBS 116832; Wartburg on twig from *Eucalyptus nitens*, December 1988, P.W. Crous (MSC 388707), and living culture CBS 116833; Amsterdam on twig from *Eucalyptus nitens*, 1998, M.J. Wingfield (MSC 388858), and living culture CBS 118087.

13. *Cytospora* aff. *austromontana*

A single isolate, PPRI 5926, from *Eucalyptus* in Hermanus, Western Cape, South Africa shows relationship to *C. austromontana* G.C. Adams and M.J. Wingf. from Australia, and *C. berkeleyi* G.C. Adams from California, USA, based on DNA homology. These species have distinct morphologies but share similar ITS sequence homology and more gene sequences are needed to determine whether distinct species can be readily delimited. The Hermanus isolate is referred to as *C. aff. austromontana* based on its similar morphology to *C. austromontana* in culture, as described in Adams *et al.* (2005). Teleomorphs were not found with the specimen, and it is now known only from culture.

Teleomorph unknown. Conidiomatal stromata unavailable on natural substrate; globose, glabrose, 0.4 mm diam. *in vitro*. Locules cytoporoid, subdivided by invaginations into chambers sharing common walls. Conidiophores unbranched to branched at the base. Conidiogenous cells embedded in a continuous gelatinous matrix, subcylindrical phialides, tapering to the apices, with minute collarettes, $6\text{--}10 \times 1 \mu\text{m}$. Conidia hyaline, eguttulate, allantoid, aseptate, $(3\text{--})4\text{--}5 \times 0.8\text{--}0.9 \mu\text{m}$.

Host

Eucalyptus sp.

Distribution

Hermanus, Western Cape, South Africa.

Specimen examined

South Africa, Western Cape, Hermanus, on dead twigs of *Eucalyptus* sp., ca. 1995, C. Roux (MSC 388706), and living culture CBS 116822 (PPRI 5926).

14. *Valsa fabianae* (anamorph *Cytospora eucalypticola*)

The type locality of the anamorph, *C. eucalypticola*, is Tzaneen, South Africa. The anamorph of this species is well distributed in South Africa and occurs on several species of *Eucalyptus*. The anamorph is also found on *Vitex doniana* in Uganda and on *Eucalyptus* in Australia. The teleomorph has been found in Uganda and Tasmania, Australia on *Eucalyptus*. The anamorph description has been emended and illustrated with the teleomorph in Adams *et al.* (2005).

Anamorph usually interspersed amongst teleomorphs, discrete. Conidiomatal stromata immersed in bark, erumpent, unilocular, lamyelloid, and rosette cytosporoid, usually solitary and unilocular deep in bark, 0.15–0.5 mm diam.; when on surfaces of bark the stromata become rich medium brown, hemispherical, rosette cytosporoid, and up to 0.6 mm diam. Discs if present dark to medium brown or grey, convex to nearly flat, circular to ovoid, 0.1–0.3 mm diam., (1)–2–4(–7) laterally inserted ostioles. Ostioles medium brown, 40–80 µm diam., noticeably thinner than perithecial beaks, level to 0.15–0.3 mm above the disc surfaces. Locules globose, simple undivided when deep in the bark, rarely 1–2 incomplete invaginations, or rosette cytosporoid when on the surfaces with (2)–6(–7) uniform regular radially arranged chambers, 100–200 µm diam., sharing common walls. In culture, conidiomata rosette cytosporoid with 3–4 regular, radially arranged chambers sharing common walls. Conidiophores hyaline, unbranched or occasionally branched at base, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, (6.5–)10(–14) × 1–1.5 µm. Hymenial elements rarely interspersed amongst the conidiogenous cells, elongate to subcylindrical, inflated. Conidia hyaline, eguttulate, allantoid, aseptate, (3.5–)4(–4.5) × 1 µm.

Hosts

E. saligna, *E. grandis*, *E. dunnii*.

Distribution

Northern Transvaal, Tzaneen; KwaMbonambi, Newcastle; KwaZulu-Natal and Seven Oaks, South Africa; Esperance Valley, Tasmania, Australia.

Specimens examined

South Africa, Northern Transvaal, Tzaneen, Westfalia Estates on dead branches of *Eucalyptus saligna* in plantations, 31 March 1964,

G.C. van der Westhuizen (PREM 42543 **holotype** of *Cytospora eucalypticola*); KwaZulu-Natal, Homeleigh plantation house, on large fallen branch of *Eucalyptus saligna*, 1999, G.C. Adams (MSC 380718), and living culture CBS 116852; KwaMbonambi, New Castle, Normandine plantation on bark of advancing canker on *Eucalyptus dunnii* stressed by extreme drought and frost damage, 22 June 1999, J. Roux and G.C. Adams (MSC 380697), and living culture CBS 116851; **Australia**, Tasmania, Esperance Valley, Geeveston, DFR trial, teleomorph and anamorph on cankers of *Eucalyptus nitens*, 4 June 1987, K.M. Old (DAR 43948, **holotype** of *V. fabianae*), and ex-type living culture CBS 116840.

Cytospora sacchari

The ITS sequence for *C. sacchari* is of an isolate from India. Despite an attempt to locate *C. sacchari* in South Africa, only an unrelated Diaporthaceous anamorph was found on *Saccharum officinarum* with symptoms of the disease. The species is, however, commonly encountered in South Africa by plant pathologists working with sugarcane. Cultures and herbarium specimens are needed for study but could not be located for this study.

Not represented in the phylogenetic trees

Valsa ceratosperma, *s. lat.* (anamorph *Cytospora sacculus*, *s. lat.*) Fig. 12

The anamorph of *V. ceratosperma*, *C. sacculus*, occurs in South Africa because a specimen (MSC 387070) from *Jacaranda* has the distinctive morphological features of the conidioma, the multiple locules with independent walls converging into a single shared ostiole (Fig. 2). This characteristic morphology is referred to as torsellipsoid, which had been the basis of the former genus *Torsellia* Fr., now a synonym of *Cytospora* (Barr 1978). The torsellipsoid morphology is particularly difficult to distinguish on the *Jacaranda* specimen because of the crowded and compressed condition of the independent locules. The unique morphology has been considered sufficient to designate such a specimen as *C. sacculus* (Spielman 1985) prior to our studies (Adams *et al.* 2005). We have found it useful to designate such a specimen as *C. sacculus s. lat.* because phylogenetic analysis reveals that several species share the torsellipsoid morphology (Adams *et al.* 2005). Use of *sensu lato* is the conservative choice for designation of the specimen from *Jacaranda* because no amplifiable DNA is available for sequencing. The teleomorph is not present on the specimen.

Valsa verrucula (anamorph *C. verrucula*) has been considered a synonym of *V. ceratosperma* (Hubbes 1960). A report (Doidge 1950) of *C. verrucula* on *Acacia* from South Africa likely refers to another example of *V. ceratosperma s. lat.* The report mentions a record at the Royal Botanical Gardens at Kew but no specimen. Unfortunately, the absence of a specimen prevents re-evaluation of this report, which is necessary, because *V. verrucula* is a poorly understood species usually found on *Salix* in Europe.

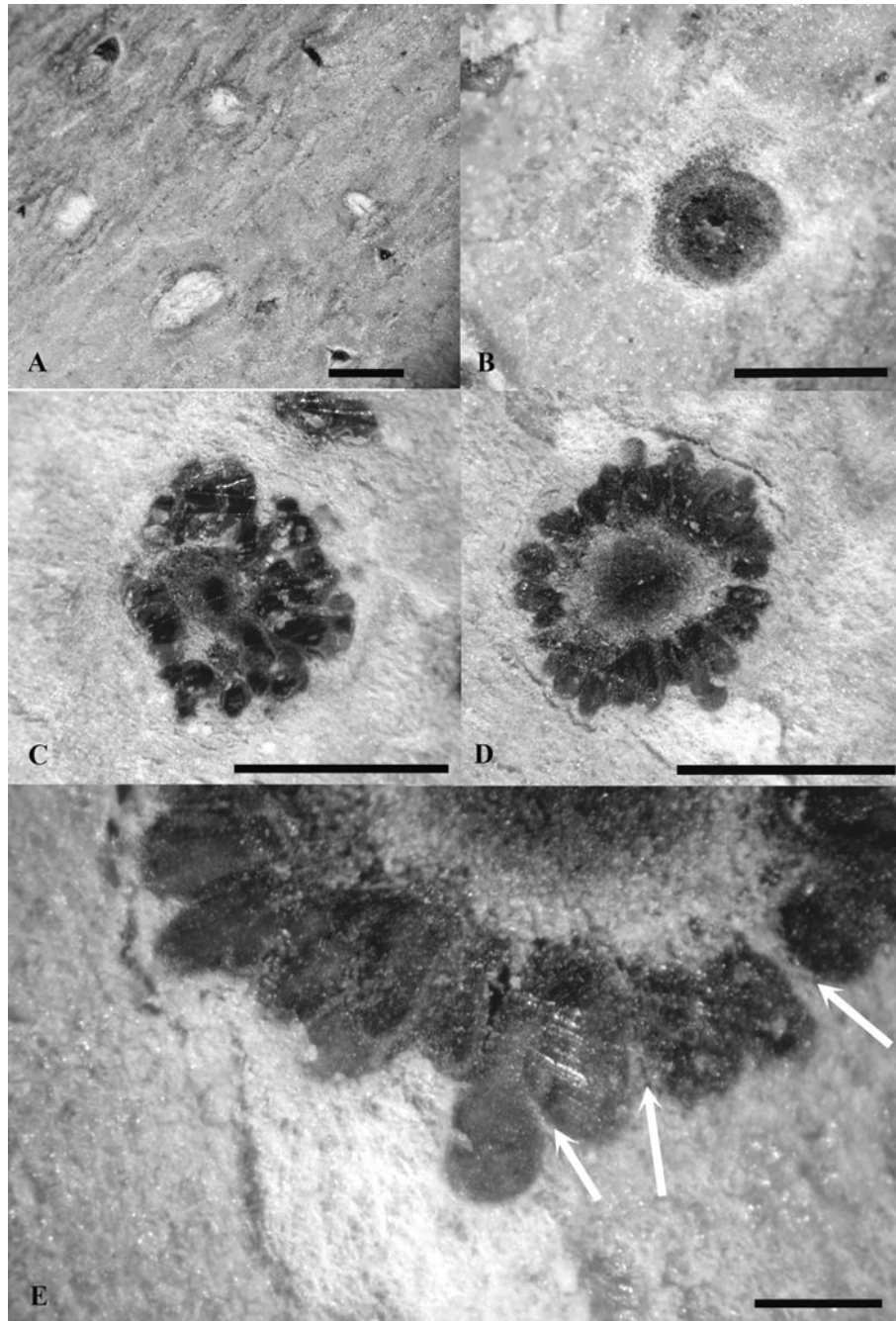


Fig. 12. Morphology of *Cytospora sacculus* the anamorph of *Valsa ceratosperma* s. lat. from *Jacaranda* of South Africa. (A) Habit of conidiomatal discs (dark areas) on twig. (B) Transverse section through disc just below bark surface shows brown stroma tissue around one ostiole. (C, D) Transverse sections through two conidiomata show the multiple locules. (E) Magnified view of locules shows presence of separate walls (white arrows) around each independent locule. Scale bars: (A, C, D) 1 mm, (B) 0.4 mm, (E) 100 μ m.

Teleomorph not seen. Anamorph separate from teleomorph stromata, discrete. Conidiomatal stromata immersed in bark, erumpent, discoid, convex, torsellioid, 1.2–1.4 mm up to 1.5 mm diam. Discs light brown to brown,

nearly flat to convex, circular, 0.2–0.3 mm diameter with one ostiole. Ostioles brown, 40–80 μ m diam., level with disc surfaces, surrounded by brown stroma. Conidioma of multiple locules united at the shared ostiole. Locules

tear-shaped to elongate ovoid, 10–30 simple undivided, about $80 \times 600 \mu\text{m}$ diam., not sharing common walls, walls of textura epidermoidea, not surrounded by entostromata, each locule with an ostiole converging towards the disc to one shared ostiole per disc. Conidiophores hyaline, branched at base and midpoint, $15\text{--}18 \times 1\text{--}1.5 \mu\text{m}$ inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, with minute collarettes and periclinal thickenings, $8\text{--}10 \times 1 \mu\text{m}$. Conidia hyaline, eguttulate, allantoid, aseptate, $5\text{--}(6) \times 1 \mu\text{m}$.

Host

Jacaranda acutifolia.

Distribution

Bloemfontein, Free State, South Africa.

Specimens examined

South Africa, Free State, Bloemfontein, on dead branches of *Jacaranda acutifolia*, 10 October 1988, W.J. Swart (MSC 387070).

Cytospora chrysosperma s. lato and ‘*Cytospora australiae*’

A herbarium specimen, PREM 13072, collected in Wellington, South Africa on dead branches of *E. viminalis* is identified by R. Taylor in 1919 as *C. australiae* Speg. No culture is available for DNA amplification. This species is different from the type specimen of *C. australiae* (LPS 31746), which has torselliod conidiomata (Adams *et al.* 2005) whereas the Wellington specimen has labyrinthine cytosporoid conidiomata. The morphology of the latter specimen (PREM 13072) is illustrated in Adams *et al.* (2005) who identifies it as *Cytospora chrysosperma*, *sensu lato*. It has larger and more complex stromata and smaller conidia than *V. aff. cinereostroma*, but larger conidia than other species on *Eucalyptus* in South Africa. Therefore, despite reports of *C. australiae* in South Africa, the species has not yet been verified as present. Cultures of *C. australiae* from Argentina have not been located.

Teleomorph unknown. Conidiomatal stromata immersed in bark, erumpent, rosette to labyrinthine cytosporoid, medium grey, discoid, circular to ovoid, $500\text{--}900 \mu\text{m}$ diam., with a large multi-chambered locule. Discs absent. Ostioles medium grey, prominent, hemispherical ($65\text{--}75\text{--}(96) \mu\text{m}$ diam. Locules complex multi-chambered, subdivided frequently by invaginations, chambers irregular (regular at perimeter), sharing common walls, $500\text{--}900 \mu\text{m}$ diam., with discrete ostioles. Conidiophores hyaline, unbranched or occasionally branched at the bases, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, collarettes minute $(6.5\text{--}7)(\text{--}9) \times (0.7\text{--}1)(\text{--}1.5) \mu\text{m}$. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, $(3.5\text{--}4)(\text{--}4.5) \times 0.8 \mu\text{m}$.

Host

Eucalyptus viminalis.

Distribution

Wellington, South Africa.

Specimens examined

South Africa, Wellington: on dead branch of *E. viminalis*, 1919, R. Taylor (PREM 13072, ‘*Cytospora australiae* Speg.’); **Argentina**, Buenos Aires: Recoleta, on dead twigs of *Eucalyptus globulus*, 18 Feb. 1880, C. Spegazzini (LPS 31746, **holotype** of *C. australiae*).

‘*Valsa salicina*’ (anamorph ‘*Cytospora fugax*’), Fig. 13

The herbarium specimen, PREM 20469, from South Africa was listed by Doidge (1950) as *Valsa salicina*. It possesses only the anamorph and had been re-labelled at PREM as ‘*C. salicis*’. Unfortunately, the morphology of the conidiomatal stromata is not distinguishable from that of many other anamorphs, such as *V. germanica*, *V. sordida* or other *Cytospora* found on *Salix* (Grove 1935). The disc of the South African specimen is pale brown as is the ectostroma, and it, therefore, only superficially resembles the described anamorphs of *V. salicina*, which have white discs

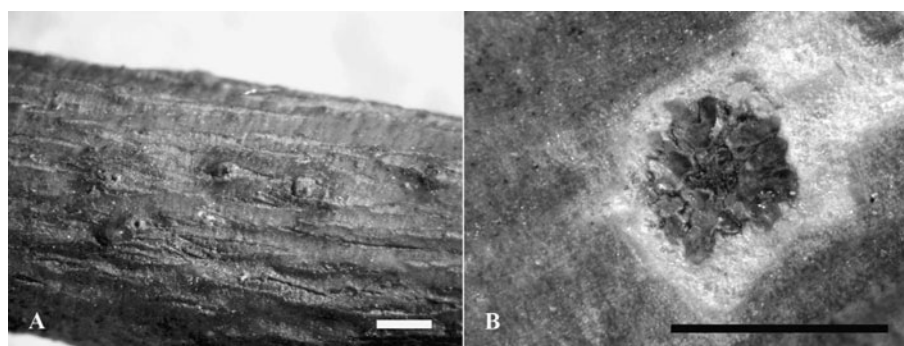


Fig. 13. Morphology of ‘*Valsa salicina*’ on flowering *Salix* sp. of South Africa. (A) Habit of conidiomatal discs on bark surface. (B) Transverse section through conidioma. Scale bars: 1 mm.

(Hayova and Minter 1998*h*). Also, no DNA is available for use in phylogenetic study. The presence of *V. salicina*, therefore, needs further confirmation for South Africa.

Teleomorph not seen. Anamorph separate from teleomorph stromata, discrete. Conidiomatal stromata immersed in bark, erumpent, labyrinthine cytosporoid, circular to ovoid, about 0.8 mm diameter. Discs light brown, nearly flat, circular, 0.25–0.3 mm diam., with one ostiole per disc. Ostioles medium brown, at the same level as the disc surface, about 100 µm diam. Locules multi-chambered, subdivided by invaginations into irregularly arranged chambers sharing common walls. Conidiophores hyaline, branched at bases and above the bases, about 15 × 1 µm inclusive of phialides, embedded in a continuous gelatinous matrix. Conidiogenous cells subcylindrical phialides, tapering to the apices, minute collarettes, periclinal thickenings minute, 6–10 × 1 µm. Conidia hyaline, eguttulate, elongate-allantoid, aseptate, (4–)5(–6) × 1 µm.

Host

Salix sp.

Distribution

Brakpan, Gauteng, South Africa.

Specimens examined

South Africa, Gauteng, Brakpan, on flowering twigs of *Salix* sp., 8 November 1925, Colonel Bottomley (PREM 20469).

Discussion

From the results of this study, we have been able to identify many formerly collected specimens and have added numerous new records of *Valsa* spp. or their *Cytospora* anamorphs to those presently known from South Africa. Identification of *Cytospora* species using DNA sequence homology comparisons has made it possible to recognise many species and unique lineages of these fungi in South Africa. Names can be applied to most of the species based on either morphology or the estimated relationship between an unidentified strain and a statistically supported clade of isolates, which contain morphologically identifiable strains or reference strains. Because morphology has been a weak and often inadequate means of identifying *Cytospora* species (Spielman 1985; Adams *et al.* 2005), the present study based on sequences for a single gene has substantially elevated identifications over past reports. It has also provided a reproducible method for recognising distinct species or species aggregates in *Cytospora*. In this regard, studying pathogens using DNA sequence comparisons has substantially increased our ability to recognise species and species complexes that have previously been impossible to resolve.

Many of the well known Northern Hemisphere species appear to be more complex than assumed, based on the

ITS rDNA phylogeny. These are treated temporarily as species complexes in this study, and much work is needed to better understand and delineate them. A more thorough evaluation of the species concepts will necessitate analysis of congruent phylogenetic trees based on sequences from multiple genes. The species complexes most in need of study include *V. malicola/V. germanica*, the two disparate parts (parts a and b) of *V. abietis* and the two parts of *V. cypri*. The other species complexes labelled on the cladograms are often groupings of a well known species and several rarely reported species, particularly rarely reported anamorphic species. In these clades, the rarely reported species probably represent fruiting bodies of the common species exhibiting morphological extremes. For example in the *V. sordida* species complex, the rarely reported anamorphic species, *C. eutypelloides*, *C. tritici*, *C. minuta* and *C. hariotii*, are likely described from morphological forms resulting from host or environmental influences. Similarly, in the *V. kunzei/V. pini* species complex, the two commonly encountered species, *V. kunzei* and *V. pini*, may be described from extremes in the morphological variation within one species. Further morphological studies comparing type specimens of the various species clustered within a species complex should increase our understanding of the flexibility of some characters. Other species complexes, such as the *V. malicola/V. germanica* and the *V. cypri* species complexes, might be species in the process of diverging into two species. Population-genetic studies may help resolve these complexes.

Common Northern Hemisphere species that have not been found in South Africa include *V. abietis* Fr. (anamorph *C. abietis* Sacc.) (Kobayashi 1970), *V. ambiens* (Pers.) Fr. subsp. *ambiens* Spielman (anamorph *C. annulata* Ellis and Everh.) (Hayova and Minter 1998*c*), *V. ambiens* subsp. *leucostomoides* (Peck) Spielman (anamorph *C. leucosperma* Fr.) (Hayova and Minter 1998*d*) and *V. cincta* Fr. [anamorph *C. cincta* Sacc., syn. *Leucostoma cinctum* (Fr.) Höhn.] (Hayova and Minter 1998*a*; Adams *et al.* 2002).

The *Cytospora* species recorded from South Africa including those treated in this study are described predominantly from non-native tree species including *Eucalyptus*, *Malus*, *Pinus*, *Populus*, *Prunus* and *Salix*. In many instances, ITS sequences of the pathogens show homology with *Cytospora* species from the Northern Hemisphere on *Malus*, *Pinus*, *Populus*, *Prunus* and *Salix*. *Cytospora* isolates from South Africa on *Eucalyptus* are similar to isolates from Australia, California, Chile, Indonesia, Thailand and Uganda. The absence of reports of these species on native trees in South Africa, combined with our understanding of a common endophytic habit, suggests that these species were introduced on exotic trees with seed or vegetative propagation materials.

Many of the fungi recognised in the present study were most likely introduced into South Africa during the establishment of agriculture and forestry industries. The

history of horticulture, pomology and forestry in the Southern Hemisphere includes a long period of establishment of exotic species rather than the use of native species by Europeans. Tree species were often imported for food production, particularly species of *Citrus*, *Malus*, *Mangifera*, *Olea*, *Persea*, *Prunus* and others. Exotic tree species were imported as ornamentals for landscapes and gardens including species of *Tibouchina* Aubl. Other exotic species were imported for fibre and timber such as *Acacia*, *Eucalyptus*, *Pinus* and *Populus*. The potential for the movement of plant pathogens and other pests on such plant material has long been recognised (Wingfield *et al.* 2001; Wingfield 2003; Gryzenhout *et al.* 2004; Slippers *et al.* 2005). Importation of soil and potted plants has generally been restricted except when a valued species required a non-native mycorrhizal fungus for successful establishment. Introduction of plant material has been restricted to importation of seed and healthy vegetative propagation material, usually hardwood cuttings. Concerns over introduction of foreign pathogens have led to government establishment of regulatory agencies that regulate shipping, inspection and quarantine issues. Regulatory agencies have not been aware of the endophytic nature of canker causing pathogenic fungi such as *Cytospora* species. Although restricting and destroying tree material showing canker disease symptoms, healthy material carrying latent pathogens has routinely passed through inspection. A heightened awareness of the latent and endophytic nature of many leaf and stem pathogens has become widespread only since the 1980s, and particularly following the publication of treatises such as those of Redlin and Carris (1996).

In South Africa and much of the Southern Hemisphere, forestry has been dominated by large international companies. Planting of trials to evaluate performance of diverse genetic material of *Eucalyptus*, *Pinus* and *Populus* species is a common practice of the forestry industry with trials often reproduced on different continents. Provenance planting and seed orchards for selection and increase of superior genetic material for breeding programs are often long-term assets. Breeding programs lead to large-scale vegetative propagation of desirable genotypes and these genotypes are sometimes shared internationally. An endophytic fungus in *Eucalyptus* or *Populus* may thus inadvertently be transported internationally during numerous instances and in multiple directions.

Plant pathologists are concerned that pathogens such as species of *Cytospora* might move from an introduced host to a native host or vice versa. *Valsa nivea* apparently has moved from *Populus* to *Malus* in South Africa. *Cytospora* lineages on *Eucalyptus* (family *Myrtaceae*) may have moved to or from *Mangifera* (family *Anacardiaceae*), *Populus* (family *Salicaceae*), *Sequoia* (family *Taxodiaceae*), *Tibouchina* (family *Melastomataceae*) and *Vitex* (family *Verbenaceae*). Because *Cytospora* species cause disease on

plants under stress, movement of a pathogen to a new host may reflect the vulnerability of a host's defensive systems under stress or the severity of the stress. Stress is particularly common to trees planted outside their native range, provenance or climate zone (Schoeneweiss 1975). Fisher *et al.* (1993) suggest that *Eucalyptus* outside their native habitat become infected with less host-specific fungi and Espinosa-García and Langenheim (1990) demonstrate this in *Sequoia*. The *V. cypri* isolates from *Olea* might represent a new species that could be native because *O. europaea* [synonym, *O. europaea* var. *africana* (Mill.) P. Green] is native to South Africa. The *Cytospora* on *Acacia*, discussed as of uncertain lineage, might also be native to Africa.

Acknowledgements

The herbarium of the National Collection of Fungi (PREM) and the culture collection (PPRI) of the Biosystematics division: Mycology, Agricultural Research Council, provided essential resources required to undertake this study. We thank Marlene Cameron for help with the illustrations. Funding support in the form of a fellowship to G. C. Adams from the National Research Foundation of South Africa is gratefully acknowledged. Additionally, we thank the members of the Tree Protection Co-operative Programme and the THRIP initiative of the Department of Trade and Industry, South Africa, for financial support.

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Received 20 April 2006, accepted 29 June 2006