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First record of *Colletogloeopsis zuluense* comb. nov., causing a stem canker of *Eucalyptus* in China

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ABSTRACT

Coniothyrium zuluense causes a serious canker disease of *Eucalyptus* in various parts of the world. Very little is known regarding the taxonomy of this asexual fungus, which was provided with a name based solely on morphological characteristics. In this study we consider the phylogenetic position of *C. zuluense* using DNA-based techniques. Distance analysis using 18S and ITS regions revealed extensive sequence divergence relative to the type species of *Coniothyrium*, *C. palmarum* and species of *Paraconiothyrium*. *Coniothyrium zuluense* was shown to be an anamorph species of *Mycosphaerella*, a genus that includes a wide range of *Eucalyptus* leaf and stem pathogens. Within *Mycosphaerella* it clustered with taxa having pigmented, verruculose, aseptate conidia that proliferate percurrently and sympodially from pigmented conidiogenous cells arranged in conidiomata that vary from being pycnidial to acervular. The genus *Colletogloeopsis* is emended to include species with pycnidial conidiomata, and the new combination *Colletogloeopsis zuluense* is proposed. This is also the first report of the pathogen from China where it is associated with stem cankers on *Eucalyptus urophylla*.

Introduction

Coniothyrium Corda 1840 represents a large genus of asexual fungi that produce conidia in pycnidia. It is one of the oldest genera of coelomycetes and includes more than 800 species, with *C. palmarum* representing the type (Corda 1840). Sutton (1971, 1980) clarified the generic concepts for *Coniothyrium*, limiting it to species in which conidia arise from the percurrent proliferation of conidiogenous cells. Thus, *Coniothyrium* is characterized by having unilocular, immersed, ostiolate, thin-walled and dark brown pycnidia. Conidia are brown, ellipsoidal to cylindrical, formed on percurrently proliferating conidiogenous cells.

In the strict sense, *Coniothyrium* should represent anamorphs of *Leptosphaeria* that are morphologically and phylogenetically similar to *C. palmarum*, the type species of *Coniothyrium* (Crous 1998). *C. zuluense* would thus be expected to represent a member of this group. In contrast, a recent study in which ITS sequence data were used to confirm a record of *C. zuluense* from Ethiopia, has suggested that this fungus is related to species of *Mycosphaerella* (Gezahgne *et al.* 2005). This, together with the importance of the disease has led us to re-evaluate the taxonomic status of *C. zuluense*.

C. zuluense causes a very serious stem canker disease on *Eucalyptus* in South Africa, from where it was originally described (Wingfield *et al.* 1997; van Zyl 1999). Since then, it has

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become one of the most serious pathogens of plantation-grown *Eucalyptus* spp. in the world. In recent years, *Coniothyrium* stem canker has been recorded on *Eucalyptus* spp. in Thailand (van Zyl 1999; van Zyl *et al.* 2002), Mexico (Roux *et al.* 2002), Hawaii (Cortinas *et al.* 2004) Vietnam (Old *et al.* 2003), Ethiopia and Uganda (Gezahgne *et al.* 2003), Argentina (Gezahgne *et al.* 2004) and Uruguay, (M. J. Wingfield, unpubl.). It is thus intriguing that the fungus is not known from Australia, the area of origin of *Eucalyptus*. While *C. zuluense* might be present on *Eucalyptus* spp. where they are native, but sufficiently unimportant to be noted, it could also have originated on trees related to *Eucalyptus* elsewhere in the world. This would be similar to the case of the pathogens causing the important Cryphonectria canker of *Eucalyptus* (Burgess & Wingfield 2002; Wingfield 2003)

Coniothyrium species have very few useful morphological characteristics of taxonomic relevance. Recognition of species has been based on the morphology of the single-celled conidia including wall ornamentation, pigmentation and size (Taylor & Crous 2001). These characteristics have been shown to be insufficient to differentiate between species where various features overlap. This has been especially problematic in the case of *C. zuluense*, in which cultures are highly variable in texture, colour and growth and they also vary markedly in their pathogenicity to clones of *Eucalyptus* (Wingfield *et al.* 1997; van Zyl 1999). These apparent differences led van Zyl (1999) to believe that *C. zuluense* might encompass more than one taxon. Thus, isolates from South Africa and Thailand were compared based on sequences of the ITS region, but these were found to represent a single phylogenetic species despite their extensive phenotypic variation (van Zyl *et al.* 1997).

During the course of surveys of *Eucalyptus* plantations in Africa, South and Central America, and South-East Asia, a large collection of *C. zuluense* cultures have become available to us. These also include a recent collection of isolates from lesions resembling those of Coniothyrium canker on the stems of *Eucalyptus urophylla* trees in China. The aim of this study was primarily to reconsider the taxonomic position of *C. zuluense* as a member of the genus *Coniothyrium*, based on a large global collection of isolates. A secondary objective was to identify the fungus suspected to represent *C. zuluense*, collected from lesions on *Eucalyptus* stems in China.

Materials and methods

Isolates and DNA extraction

Single conidial cultures were established from pycnidia of *Coniothyrium zuluense* collected from host material. The contents of single pycnidia were diluted in sterile distilled water and spread on the surface of 2 % malt extract agar (MEA) plates. After 24 h, germinating conidia were transferred to new MEA plates and these were incubated for 25 d at 25 °C. All cultures used in this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (CMW), University of Pretoria, and a representative set has been deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht (Table 1).

After 25 d, mycelium was scrapped from the Petri dishes, freeze dried, frozen in liquid nitrogen and ground to a fine powder. DNA was then extracted using a phenol-chlorophorm protocol for which details are described by Cortinas *et al.* (2004).

PCR and sequencing

A list of isolates and DNA sequences considered in this study are presented in Table 1. Two regions of the ribosomal DNA operon were amplified by PCR for 27 isolates. The partial small nuclear ribosomal subunit (18S) was amplified with the primers NS3: 5′ GCA AGT CTG GTG CCA GCA GCC and NS4: 5′ CTT CCG TCA ATT CCT TTA AG (White *et al.* 1990). Partial amplification of the internal transcribed spacer 1, the 5.8S ribosomal RNA gene and the complete internal transcribed spacer 2 (ITS1, 5.8S, ITS2) was achieved using the primers ITS1: 5′ TCC GTA GGT GAA CCT GCG G and ITS4: 5′ GCT GCG TTC TTC ATC GAT GC (White *et al.* 1990). All the PCR reactions were performed in 25 μl total volume including 1 μl of genomic DNA from 1:50 dilutions, 1 U Taq polymerase, 10 pmol of each primer, 0.8 mM of each dNTPs, 1 × Taq buffer and 2 mM $MgCl_2$. Cycling conditions were as follows: initial denaturation at 96 °C for 2 min, followed by 10 cycles of 30 s at 95 °C, 30 s at 54 °C, 1 min at 72 °C and 25 cycles of 30 s at 95 °C, 30 s at 56 °C, 1 min at 72 °C, with 5 s extension after each cycle. A final elongation step was carried out for 7 min at 72 °C. PCR amplicons were visualized under UV light on a 1 % agarose gel and then purified by gel filtration through Sephadex G-50 (Sigma S5897) followed by vacuum drying.

Sequencing reactions were performed in 10 μl with 2 μl of purified PCR product, 10 pmol of the same primers used in the PCR, 2 μl 5 × dilution buffer and using the ABI Prism Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). PCR conditions were: 25 cycles of 10 s at 96 °C; 4 s at 50 °C; 4 min at 60 °C. Sequencing products were purified by gel filtration through Sephadex G-50 (Sigma S5897) followed by vacuum drying and electrophoresis using an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems).

Phylogenetic analyses

In addition to the sequence data derived in this study, sequences were extracted from GenBank (Table 1). Alignments were carried out using Clustal under MEGA 3 (Kumar *et al.* 2004). Where necessary, alignments were adjusted manually. All sequences generated in this study have been deposited in GenBank and the accession numbers are shown in Table 1 (marked with *).

Distance analyses were conducted using MEGA 3.0 (Kumar *et al.* 2004). Pairwise distances were estimated using the Kimura with two parameters model (Kimura 1980). Neighbour-joining was used as grouping algorithm (Saitou & Nei 1987) to reconstruct the trees. Gaps generated in the alignment were treated as missing data. One thousand bootstrap replicates were done in each case to assess the statistical support of nodes in the phylogenetic trees (values indicated on the branches).

Table 1 – Fungal isolates and DNA sequences used for SSU and ITS analyses

Culture numbers	Name	Origin	18S GenBank Acc. number	ITS GenBank Acc. number
Strain AA6	*Alternaria alternata*	Canada	U05194	
CPC 4572	*Alternaria malorum*	USA	AY251131	
CPC 4303	*Cercospora oryzae*		AY251103	
CPC 3955	*Cercospora zebrina*	Canada	AY251104	
CPC 3687	*Cladosporium staurophorum*	Colombia	AY251121	
ATCC 200938	*Cladosporium staurophorum*			AF393723
CBS 672.68	*Coniothyrium cereale*			AJ293812
CBS 859.71	*Paraconiothyrium minitans*			AJ293810
CMW 5283, CBS 758.73	*Coniothyrium palmarum*	Israel	DQ240002[a]	DQ240000[a]
CBS 218.68	*Paraconiothyrium sporulosum*			AJ293814
CMW 15833 (CRY 1662)	*Coniothyrium zuluense*	Mexico		AF385610, DQ239988[a]
CMW 15834 (CRY 1664)	*Coniothyrium zuluense*	Mexico	DQ240022[a]	AF385611, DQ239987[a]
CMW 4507	*Coniothyrium zuluense*	Thailand	DQ240024[a]	
CMW 5236	*Coniothyrium zuluense*	Thailand		AF376829, DQ239989[a]
CMW 5235	*Coniothyrium zuluense*	Thailand		AF376828, DQ239990[a]
CMW 7449	*Coniothyrium zuluense*	South Africa	DQ240021[a]	DQ239976[a]
CMW 7479	*Coniothyrium zuluense*	South Africa	DQ240020[a]	DQ239982[a]
CMW 7468	*Coniothyrium zuluense*	South Africa		DQ239983[a]
CMW 7442	*Coniothyrium zuluense*	South Africa		AF376819, DQ239978[a]
CMW 7452	*Coniothyrium zuluense*	South Africa		DQ239977[a]
CMW 7488	*Coniothyrium zuluense*	South Africa		DQ239975[a]
CMW 7489	*Coniothyrium zuluense*	South Africa		AF276820, DQ239980[a]
CMW 7426	*Coniothyrium zuluense*	South Africa		DQ239979[a]
CMW 7459	*Coniothyrium zuluense*	South Africa		AF376816, DQ239981[a]
CMW 13328	*Coniothyrium zuluense*	South Africa	DQ240018[a]	DQ239974[a]
CMW 13324	*Coniothyrium zuluense*	South Africa	DQ240019[a]	AY738214
CMW 6857	*Coniothyrium zuluense*	Vietnam	DQ240023[a]	DQ239986[a]
CMW 6860	*Coniothyrium zuluense*	Vietnam		DQ239985[a]
CMW 15957	*Coniothyrium zuluense*	China	DQ240017[a]	DQ239962[a]
CMW 15968	*Coniothyrium zuluense*	China		DQ239965[a]
CMW 15961	*Coniothyrium zuluense*	China		DQ239961[a]
CMW 15966	*Coniothyrium zuluense*	China		DQ239963[a]
CMW 15078	*Coniothyrium zuluense*	China	DQ240016[a]	DQ239966[a]
CMW 15958	*Coniothyrium zuluense*	China		DQ239964[a]
CMW 15087	*Coniothyrium zuluense*	China		DQ239967[a]
CBS 171.93	*Discosphaerina fagi*	UK	AY016342	
CPC 1535	*Dissoconium dekkeri*	Netherlands	AY251101	
CBS 642.86	*Leptosphaeria bellynckii*			AF439458
ATCC 42652	*Leptosphaeria bicolor*		U04202	
CBS 244.64	*Leptosphaeria congesta*			AF439460
CBS 591.86	*Leptosphaeria typharum*			AF439465
CMW 13704, CBS 110499	*Mycosphaerella ambiphylla*	Australia	DQ240005[a]	AY725530, DQ239970[a]
CMW 11255,	*Mycosphaerella colombiensis*	Colombia	DQ240011[a]	AF309612, DQ239993[a]
CMW 3279, CPC 936	*Mycosphaerella cryptica*	Australia	DQ240003[a]	AF309623, DQ239971[a]
CPC 355	*Mycosphaerella cryptica*	Chile		AF309622
CMW 3042, CPC 801	*Mycosphaerella crystallina*	South Africa	DQ240009[a]	AF309611, DQ239997[a]
CMW 5165, CPC 850	*Mycosphaerella ellipsoidea*		DQ240014[a]	DQ239994[a]
CMW 4942, CPC 760	*Mycosphaerella heimii*	Madagascar		AF309606, DQ239992[a]
CMW 5223, CPC 1362	*Mycosphaerella irregulariramosa*	South Africa	DQ240012[a]	AF309608, DQ239991[a]
CBS 652.85	*Mycosphaerella latebrosa*	Netherlands	AY251114	
CMW 5150, CPC 935	*Mycosphaerella marksii*	Australia	DQ240008[a]	AF309588, DQ239998[a]
CMW 4940, CPC 1214	*Mycosphaerella molleriana*	Portugal	DQ240004[a]	AF309619, DQ239969[a]
CPC 4661	*Mycosphaerella nubilosa*	Spain	AY251120	AY725570
CMW 6210	*Mycosphaerella nubilosa*	Australia	DQ240006[a]	AF449095, DQ239999[a]
CMW 13333, CBS 113265	*Mycosphaerella punctiformis*	Netherlands	AY490775,DQ240010[a]	AY490763, DQ239996[a]
CPC 3837	*Mycosphaerella sp.*	Venezuela	AY251116	
CMW 5348, CPC 1346	*Mycosphaerella suttoniae*	Indonesia	DQ240007[a]	AF309621, DQ239972[a]
CMW 11558, Strain A-1-7	*Mycosphaerella vespa*	Australia		DQ239968[a]
Strain Brun/ 1/ 5	*Mycosphaerella vespa*	Australia	AY110906	AY045497
Strain B/ 3/ 2/ 1	*Mycosphaerella vespa*	Australia		AY045500
CMW 5164, CPC 1232	*Mycosphaerella lateralis*	Zambia		AF309624
CMW 5565	*Ophiostoma quercus*	Ecuador	AY351901	AY351899
CBS 102207	*Paraphaeosphaeria pilleata*	USA	AF250821	
CPC 3688	*Passalora fulva*	Netherlands	AY251109	AY251069

(*continued on next page*)

Table 1 – (continued)

Culture numbers	Name	Origin	18S GenBank Acc. number	ITS GenBank Acc. number
CPC 5121	*Phaeoramularia hachijoense*	USA	AY251100	
CMW 11687	*Phaeophleospora eucalypti*	New Zeland	DQ240015[a]	DQ230001[a]
CPC 1454	*Phaeophleospora eugeniae*		AF309613	
CPC 4195	*Ramularia sp.*		AY251112	
CPC 658	*Septoria tritici*	South Africa	AY251117	
CPC 1488	*Trimmatostroma macowanii*	South Africa	AY260096	

a GenBank entries generated in this study. CPC = Culture collection of Pedro Crous, housed at CBS (Culture collection of Centraalbureau voor Schimmelcultures). CMW = Culture collection at FABI.

The most parsimonious (MP) trees were generated using PAUP v. 4.0b10 (Swofford 2002). For parsimony analyses, heuristic searches were used with the steepest descent option and the TBR swapping algorithm. The characters were equally weighted and treated as unordered. Statistical support of the nodes in the trees was tested with 1000 bootstrap replicates. GenBank AY351901 and AY351899 sequences of *Ophiostoama quercus* (*Ophiostomatales*) were included as outgroups for 18S and ITS analyses, respectively.

Morphology

Growth characteristics of the *Coniothyrium*-like isolates from *Eucalyptus* in China were observed after 25 d. Colours were described following the notations of Rayner (1970). General morphological features were examined microscopically. Pycnidia-like masses from cultures were mounted on slides in 5 % lactic acid.

Results

Phylogenetic analyses

SSU sequences

A total of 565 bp characters of the 18S ribosomal gene were compared amongst 43 taxa corresponding to *Mycospharellaceae*, *Leptosphaeriaceae* and *Ophiostoma quercus* used as outgroup. The reconstructed distance tree (Fig 1) showed that the type species of *Coniothyrium*, *C. palmarum*, grouped with members of *Leptosphaeria* (*Leotosphaeriaceae*, *Pleosporales*). Isolates of *C. zuluense* from South Africa and China grouped distant from *C. palmarum* with species of *Mycosphaerella*. Furthermore, isolates of *C. zuluense* clustered to a subclade of *Mycosphaerella* including the leaf pathogenic species of *Eucalyptus*: *M. molleriana*, *M. vespa*, *M. ambyphylla*, *Phaeophleospora eucalypti*, *M. nubilosa*, *M. cryptica* and *M. suttoniae*.

ITS sequences

After alignment of the ITS region, 535 characters were compared corresponding to 56 taxa. The range of taxa comprised *Mycosphaerellaceae* and *Leptophaeriaceae* and *O. quercus* included as outgroup. Additionally, the number of representatives of *C. zuluense* was increased. The reconstructed tree (Fig 2) showed *C. palmarum* grouping with other *Coniothyrium* species belonging in *Leptosphaeria*. The sub-grouping of *C. zuluense* in the ITS tree had high statistical support. The sequences of *C. zuluense* were located within a *Mycosphaerella* cluster including *M. molleriana*, *M. vespa*, *M. ambiphylla*, *P. eucalypti*, *M. cryptica*, *M. nubilosa* and *M. suttoniae*. The topology of the most parsimonious trees and consensus trees was equivalent to the topology obtained by distance-reconstructed trees (data not shown). The DNA sequences of newly acquired isolates from China clustered within the *C. zuluense* cluster.

Characteristics of cultures from China

Cultures of *Coniothyrium zuluense* from China have a variety of surface colony colours ranging from olive-grey, greenish glaucous to a greyish olive with feathery margins. Cultures varied from greenish to brownish in reverse, to darkly so, with dark brown submerged mycelium. Some of the cultures developed white mycelial rings close to the margins. Aerial mycelium was moderate, and varied from white to pinkish in colour.

Morphology

The pathogen causing stem lesions on *Eucalyptus* was originally described as a new species of *Coniothyrium* based on its pigmented conidia that arose from percurrently proliferating conidiogenous cells that were formed in pycnidia. From the present as well as other phylogenetic studies (Crous *et al.* 2004; Lennox *et al.* 2004), it is clear that *C. zuluense* clusters with a complex of species that have fusoid to ellipsoidal pigmented conidia, that develop percurrently and (or) sympodially from pigmented conidiogenous cells, arranged in conidiomata that vary from being more pycnidioid to acervuloid. In previous studies, species of *Mycosphaerella* forming acervuli were placed in the anamorph genus *Colletogloeopsis* (Crous & Wingfield 1997), while those that were formed in pycnidia have been placed in *Phaeophleospora* (Crous *et al.* 2004).

In phylogenetic studies focusing on *Mycosphaerella* and its anamorphs (Crous *et al.* 2000, 2001a, 2004; Crous, Kang & Braun 2001b), it became clear that many of the anamorph morphologies have evolved more than once in *Mycosphaerella*, and that anamorph morphology is phylogenetically less informative in *Mycosphaerella* than previously suspected (Crous 1998). From the present study it is clear that *C. zuluense* is not congeneric with the *Leptosphaeriaceae*, and thus needs to be accommodated in an anamorph genus of *Mycosphaerella*. Previous *Coniothyrium*-like anamorphs of *Mycosphaerella* have been accommodated in *Phaeophleospora* (Crous *et al.* 2004). However, the type species of *Phaeophleospora*, *P. eugeniae*, has scolecosporous, multiseptate conidia, and clusters distant from the *C. zuluense* subcluster (P. W. Crous, unpubl.). In contrast, *C. zuluense* always clusters in the same clade as *Colletogloeopsis nubilosum* and *Co. molleriana*, which are morphologically

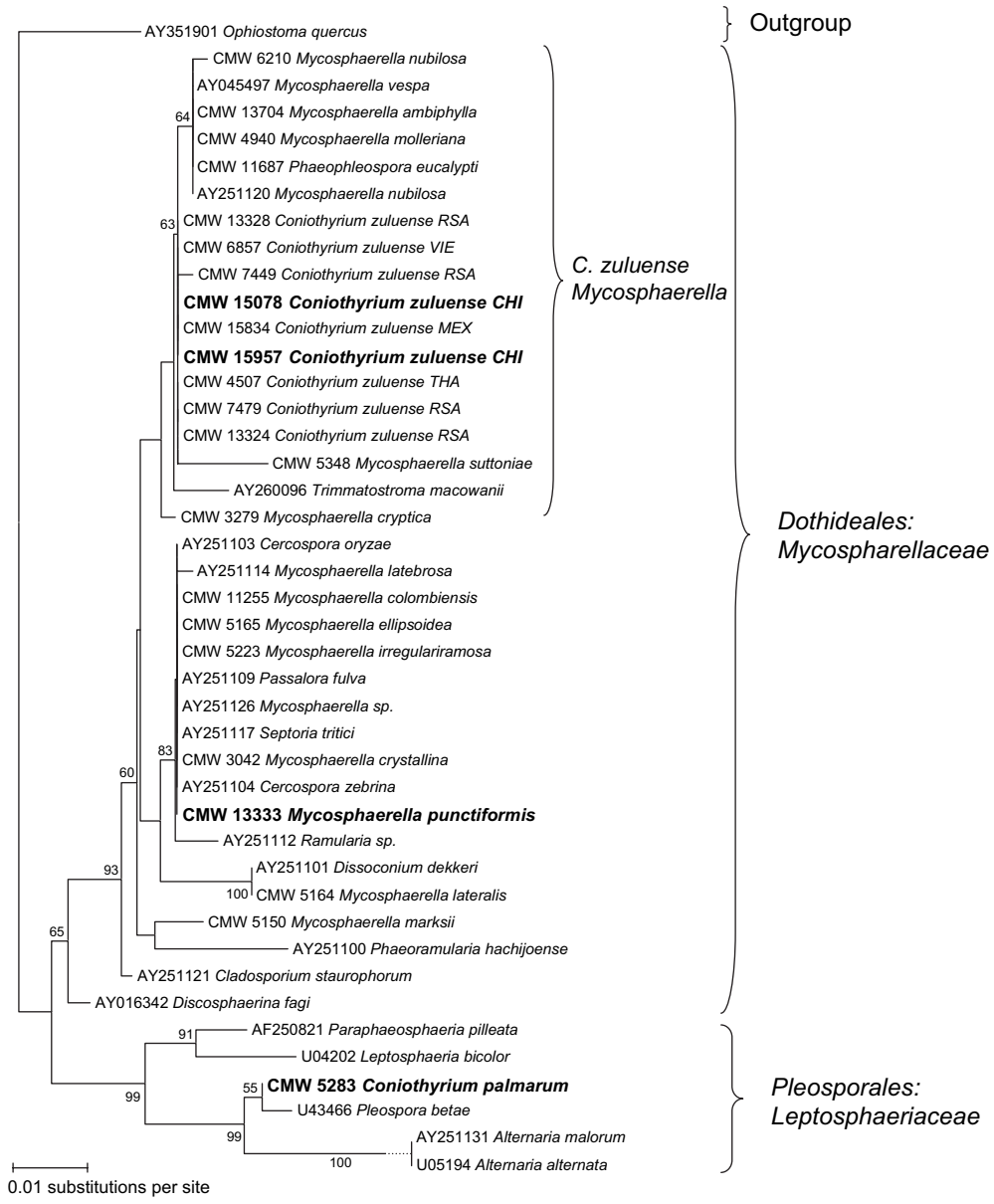

Fig 1 – Small subunit 18S rRNA gene phylogram using Kimura with the two parameters nucleotide substitution model and neighbour-joining. Bootstrap support values from 1000 replicates are shown at nodes. Only values of 60 % or higher are included and *Ophisotoma quercus* is used as outgroup. RSA = South Africa; VIE = Vietnam; CHI = China; THA = Thailand; MEX = Mexico.

similar to *Coniothyrium zuluense* except that they tend to form acervuloid conidiomata and not pycnidia. Within *Mycosphaerella*, conidiomatal structure has been observed to vary, and to be less important in generic circumscription (Crous *et al.* 2001a, b). For this reason, we have chosen to emend the generic circumscription of *Colletogloeopsis* to accommodate species with pycnidia. This is consistent with the observation that the transition between pycnidia and acervuli is rather subtle, and has been seen to frequently develop in the same species, depending on the age of the material (Verkley *et al.* 2004b). Furthermore, *Colletogloeopsis nubilosum*, which forms acervuli on host tissues, has also been observed to form pycnidia in agar when sporulating in culture (crous unpubl. data). For these reasons we do not introduce a new genus for *Coniothyrium zuluense*, but rather emend the description of *Colletogloeopsis* to accommodate this fungus.

Taxonomy

Colletogloeopsis Crous & M.J. Wingf., *Can. J. Bot.* **75**: 668 (1997).

Mycelium internal and external, consisting of pale brown, septate, branched hyphae, smooth to finely verruculose. *Conidiomata* acervuloid to pycnidioid, immersed to erumpent, dark brown to black. *Conidiogenous cells* arising from the upper cells of a stroma, or superficial hyphae (when cultivated), doliiform to subcylindrical, or somewhat irregular, subhyaline to pigmented, smooth to verruculose, proliferating sympodially and percurrently. *Conidia* single, aseptate, rarely 1-septate,

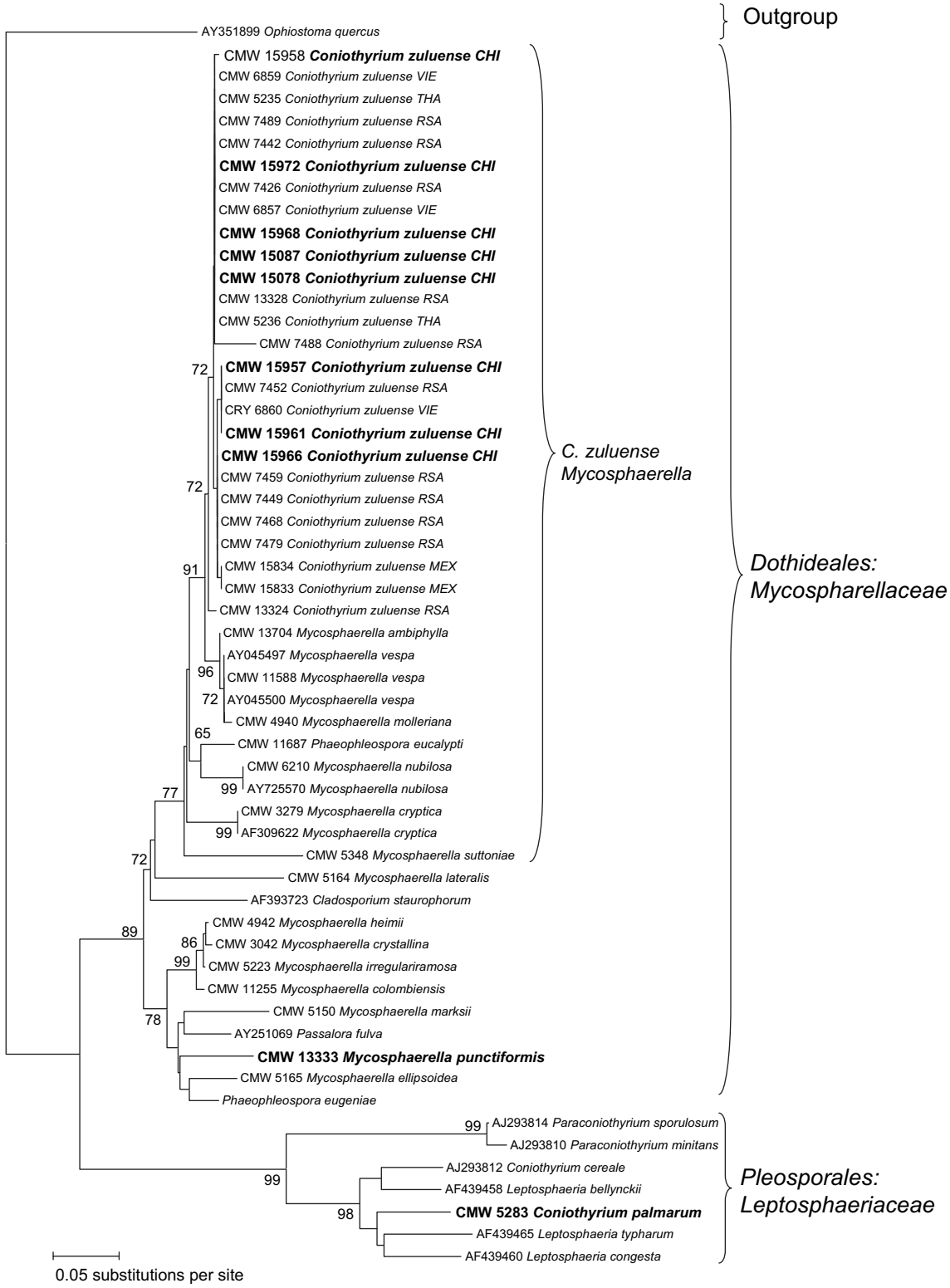

Fig 2 – Phylogram obtained from ITS sequencing data gene using the Kimura with two parameters nucleotide substitution model and neighbour-joining. Bootstrap support values from 1000 replicates are shown at nodes. Only values of 65 % or higher are included and *Ophisotoma quercus* is used as outgroup. RSA = South Africa; VIE = Vietnam; CHI = China; THA = Thailand; MEX = Mexico.

pigmented, smooth to verruculose, fusoid to subcylindrical to ellipsoidal, straight to slightly curved, apex obtuse, base truncate to subtruncate, frequently with a marginal frill.

Teleomorph: *Mycosphaerella*.

Type species: *C. nubilosum* Crous & M.J. Wingf. 1997.

Colletogloeopsis zuluense (M.J. Wingf., Crous & T.A. Cout.) M.N. Cortinas, M.J. Wingf. & Crous, **comb. nov.**

Basionym: *Coniothyrium zuluense* M.J. Wingf., Crous & T.A. Cout., *Mycopathologia* **136**: 142 (1997).

Discussion

By utilising a large number of isolates of the fungal stem pathogen that has been known as *Coniothyrium zuluense*, we have been able to confirm preliminary findings that this fungus is an anamorph of *Mycosphaerella*. This result has emerged not only from a global collection of isolates of the fungus, but also using analysis of both the 18S and ITS regions of the ribosomal DNA operon. Although the fungus is known only in its anamorph state, if its sexual state were to be found, this would clearly be a species of *Mycosphaerella*.

The genus *Coniothyrium* is typified by *Coniothyrium palmarum* that is a member of *Leptosphaeria* (*Leptosphaeriaceae*, *Pleosporales*). Corlett (1991) reported several *Coniothyrium* species as possible anamorphs of *Mycosphaerella*. However, this possibility was not further explored due to the established link between *Coniothyrium* and *Leptosphaeria* (Crous 1998). Nevertheless, Milgate *et al.* (2001) reported the link between *Mycosphaerella vespa* and an anamorph, which they identified as *Coniothyrium ovatum*. Clearly, several links between probable *Coniothyrium*-like anamorphs and species of *Mycosphaerella* are known from the literature. The recent circumscription of *Coniothyrium* (Lennox *et al.* 2004; Verkley *et al.* 2004a) makes this genus unavailable for *Coniothyrium*-like anamorphs residing in *Mycosphaerella*. In the past this situation has been resolved by describing these anamorphs in *Phaeophleospora* (Crous *et al.* 2004). This situation is no longer tenable, however, as the type species of *Phaeophleospora*, *P. eugeniae*, clusters well apart from the *Coniothyrium*-like anamorphs, which reside in a clade with species of *Colletogloeopsis*. By emending the generic circumscription of the latter genus, we have provided a suitable home for the *Coniothyrium*-like anamorphs of *Mycosphaerella*.

Coniothyrium zuluense constitutes a demonstrated link between *Coniothyrium*-like anamorphs and *Mycosphaerella*. This raises the possibility that other *Coniothyrium* species on *Eucalyptus*, such as *C. eucalypticola* Sutton and *Coniothyrium kallangurense* are also anamorphs of *Mycosphaerella*. Cultures of these fungi are currently not available and their transfer to *Colletogloeopsis* must await further study.

In addition to re-considering the generic placement of *Coniothyrium zuluense*, this study has provided the first firm evidence that the fungus has entered areas of *Eucalyptus* propagation in China. Plantation forestry in China is rapidly expanding, and now exceeds more than 1.3 M ha, mostly *Eucalyptus urophylla*, *E. grandis* and their hybrids (Minsheng 2003). Areas such as Guandong Province where *Colletogloeopsis zuluense* was discovered have a hot humid climate that is ideally suited to infections by the fungus. Although the disease has not reached serious levels in China, the occurrence of *C. zuluense* in that country deserves serious consideration.

Records of the stem canker disease caused by *C. zuluense* have rapidly increased in number since its first discovery in South Africa in 1988. The origin of this pathogen remains unknown. After its first discovery, Wingfield *et al.* (1997) speculated that it might have originated on native *Myrtaceae*. This was primarily based on the fungus not being known to occur in any other country of the world. *C. zuluense* is now known from many countries where eucalypts are being cultivated (van Zyl 1999; Roux *et al.* 2002; van Zyl *et al.* 2002; Gezahgne *et al.* 2003; Old *et al.* 2003; Cortinas *et al.* 2004). Thus, *C. zuluense* in China could have originated in any one of these countries, or alternatively it could be native on *Eucalyptus* in the centre of origin of these trees, but not yet discovered there. The significant damage that *C. zuluense* causes to *Eucalyptus* propagation justifies further studies on its biology and population genetics. Such studies would give rise to management options for the canker disease and enhance understanding of its origin, which would also contribute to efforts to breed and select resistant trees.

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