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SHORT RESEARCH NOTE

First record of the *Eucalyptus* stem canker pathogen, *Coniothyrium zuluense* from Hawaii

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Abstract. A new stem canker disease on *Eucalyptus grandis* in Hawaii is recorded. Symptoms are similar to those of *Coniothyrium* canker on *Eucalyptus* in South Africa. A fungus resembling *Coniothyrium zuluense* was found on lesions and analysis of ITS sequences confirmed this identification. *Coniothyrium* canker is a serious disease of *Eucalyptus* in South Africa and strategies to reduce its impact in Hawaii may be required.

Coniothyrium zuluense causes a serious canker disease of *Eucalyptus*. The disease was first observed on *Eucalyptus grandis* trees in KwaZulu Natal, South Africa, in 1989 and rapidly became a serious constraint to *Eucalyptus* forestry in the area (Wingfield *et al*. 1997). Since its discovery, *C. zuluense* has become widespread in *Eucalyptus* growing areas of South Africa, where it has affected the productivity of many new clones, hybrids and species of *Eucalyptus*. At the time of its discovery, *Coniothyrium* canker of *Eucalyptus* was known only in South Africa. The disease was then discovered in 1996 in Thailand infecting *Eucalyptus camaldulensis* (Van Zyl 1999). More recently, the disease has been found in Mexico (Roux *et al*. 2002).

Coniothyrium canker is typified by the occurrence of small, necrotic spots (Fig. 1*a*, *b*) on the young green stems of trees (Wingfield *et al*. 1997). These coalesce to form larger cankers that eventually girdle susceptible trees. Severely affected trees display reduced height growth and wood quality is seriously degraded due to the presence of kino pockets. Infections on the stem make it difficult to peel the bark from trees and this leads to increased costs in preparation of the wood before pulping (Van Zyl *et al*. 2002*a*; Wingfield *et al*. 1997).

During the course of a recent survey of *Eucalyptus* diseases in Hawaii, symptoms typical of those of *Coniothyrium* canker were observed on *Eucalyptus grandis* trees growing in the vicinity of Hilo. Morphology of the conidia associated with lesions suggested that the causal agent was *C. zuluense*. However, this fungus has very few morphological characteristics on which to base definitive identifications. Differences in colour were found in the morphology of colonies generated from single conidia ranging from yellow-olive to green-olive on top. The colonies also had a granular appearance with irregular margins (Fig. 1*c, d*). There were, however, no obvious differences in the morphology of these colonies compared with those of *C. zuluense* from South Africa. Previous studies (Van Zyl *et al*. 1997) have shown that isolates of *C. zuluense* differ markedly in morphology and that DNA sequence comparisons are required to identify this fungus with certainty (Van Zyl 1999; Van Zyl *et al*. 2002*b*). The aim of this study was thus to use sequence data derived from the ITS regions of Hawaiian isolates for identification and also to determine the relatedness of these isolates with those occurring elsewhere in the world.

Single-conidial isolates were made from pycnidia produced on necrotic lesions on the bark of trees and these are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (UP), Pretoria, South Africa. Isolates were grown on 2% malt-extract agar (MEA) in Petri dishes at 25°C for 30 days. Mycelium was scraped from the cultures, freeze dried, immersed into liquid nitrogen and ground to a fine powder for DNA extraction. The pulverised mycelium was incubated at 80°C for 10 min and at 60°C for 1 h with 1 mL of extraction buffer DEB (200 mM Tris-HCl pH 8, 150 mM NaCl, 25 mM EDTA pH8, 0.5% SDS) then centrifuged at 13000 rpm for 15 min to remove cellular debris. The supernatant (750 μL) was transferred to a new tube and purified with three phenol/chloroform washes, followed by one final wash with chloroform. Deoxyribonucleic acid was precipitated over night at –20°C with 0.7% isopropanol. After centrifugation, the DNA pellet was washed with 70% ethanol and resuspended in 100 μL of

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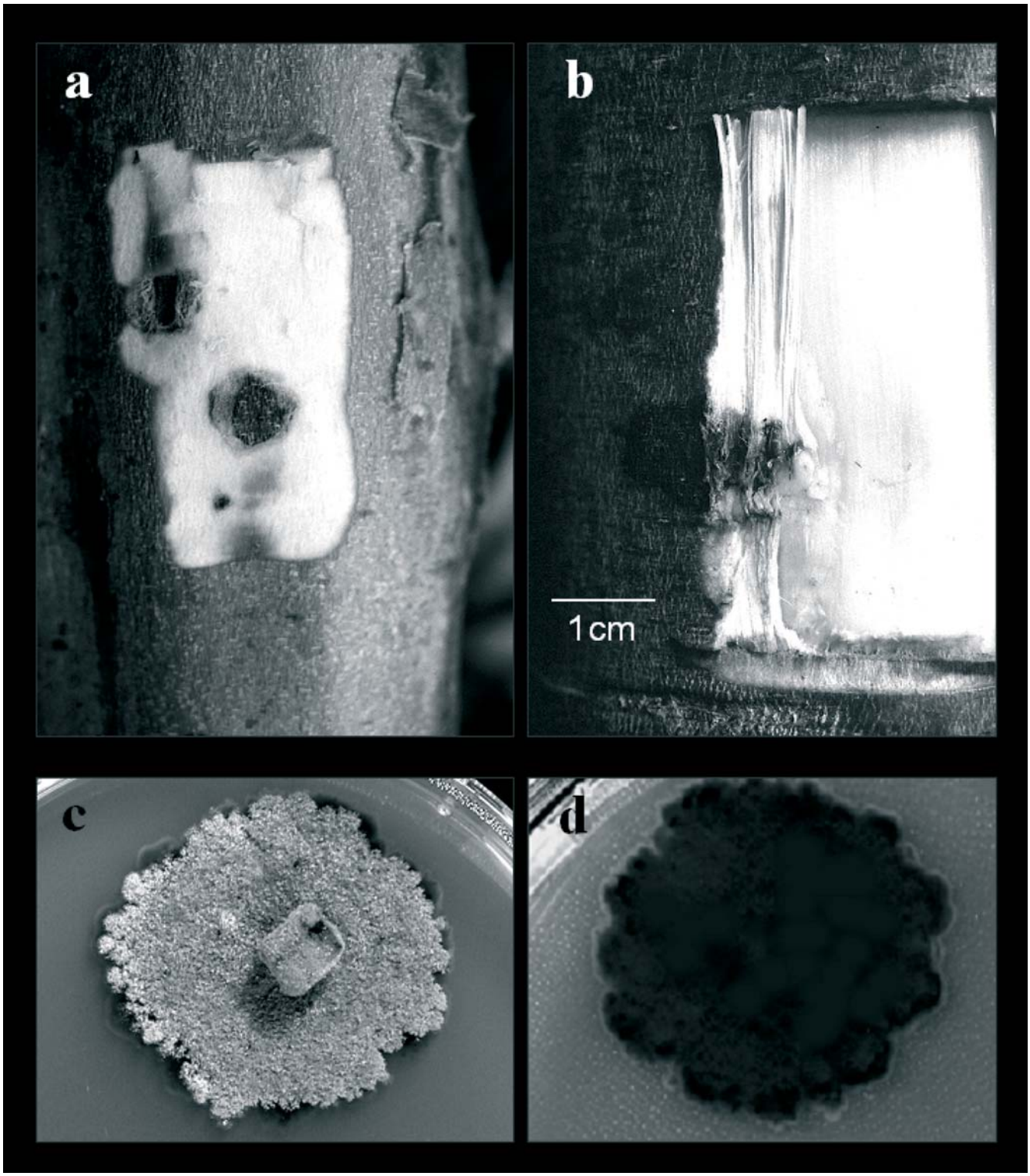

Fig. 1. Typical lesions associated with *Coniothyrium* canker and colony morphology. (*a*) Young stem showing the characteristic spindle-shaped lesions and kino exudation. (*b)* Lesion from which bark has been removed to show kino pockets and malformation of the wood. (*c*) Isolate of *C. zuluense* from Hawaii on 2% MEA plate, top view. (*d*) Characteristic dark colour of *C. zuluense*, reverse side. Scale bar is applicable to all pictures in the figure.

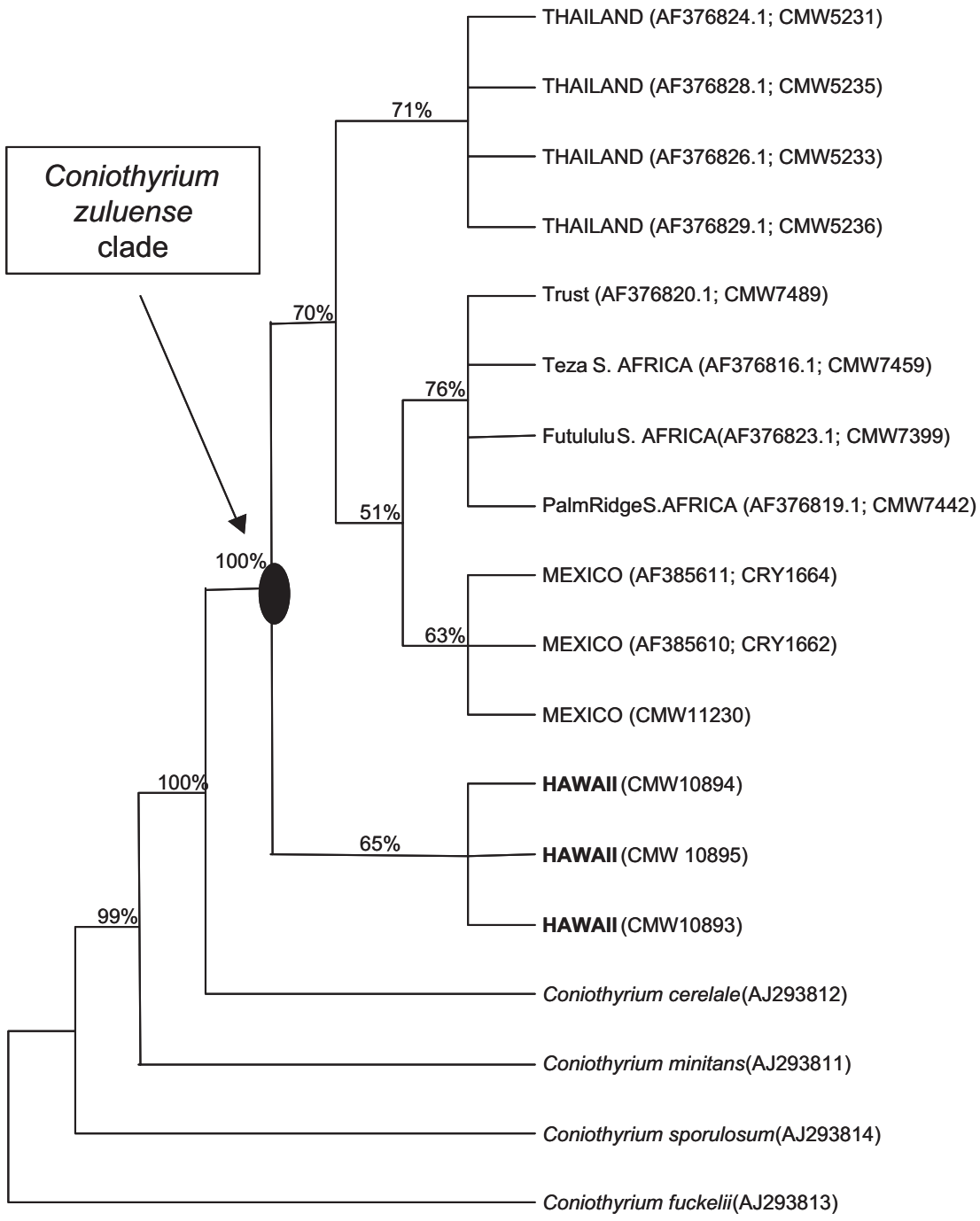

Fig. 2. Bootstrap consensus parsimony tree obtained based on the analysis of the ITS1, 5.8S RNA gene and ITS2 using the heuristic search option (Length = 183, CI = 0.989, RI = 0.994, HI = 0.011). GenBank and FABI-UP accession numbers are given beside locality names. *Coniothyrium cereale, C. manitans, C. sporulosum* and *C. fuckeli* were used as outgroups. The arrow indicates the node defining the *C. zuluense* group.

distilled water with 5 μL RNAse A (2.5 uM). Partial sequence of the internal transcribed spacer (ITS) 1 region, the rRNA gene 5.8S, and the ITS 2 region was amplified by PCR using the following primers: ITS1: 5′ TCC GTA GGT GAA CCT GCG G and ITS4: 5′ GCT GCG TTC TTC ATC GAT GC (White *et al*. 1990).

Deoxyribonucleic acid sequences for Hawaiian isolates were compared with sequences for *C. zulense* from South Africa, Thailand and Mexico (Van Zyl *et al*. 2002*b*). Sequences of other *Coniothyrium* species, *C. fuckelii* (AJ293813), *C. minitans* (AJ293811), *C. sporulosum* (AJ293814) and *C. cereale* (AJ293812) were included as outgroups. These sequences were aligned using Clustal X (Thompson *et al*. 1997) with the final alignment adjusted through visual inspection. Parsimony and distance analyses were carried out using PAUP* 4.0b10 (Swofford 2002). Gaps generated in the alignment were treated as missing data. Pairwise distances were estimated using the Kimura with two parameters model (Kimura 1980) and neighbourjoining was used as grouping algorithm (Saitou and Nei 1987). One thousand bootstrap replicates were done in each case to assess the statistical support of nodes in the phylogenetic trees.

Equivalent topologies were obtained by distance and parsimony reconstruction methods. A single most parsimonious tree (Fig. 2) was obtained using a heuristic search. The tree length was 183 steps with a consistency index (CI) of 0.989 and a retention index (RI) of 0.994. A major *C. zuluense* group was separated from the other *Coniothyrium* species with 100% bootstrap support. Smaller internal clades were obtained within this major *C. zuluense* group. *C. zuluense* isolates from South Africa resided in a group that was distinct from *C. zuluense* isolates from Mexico and Thailand, although there was a closer association with Mexican isolates than with those from Thailand. This is consistent with the previous findings of Roux *et al*. (2002). The Hawaiian isolates also constituted a separate branch within the main *C. zuluense* clade (Fig. 2).

The fact that *C. zuluense* isolates from Hawaii form an independent lineage is consistent with previous DNA sequence comparisons for this fungus. Thus, isolates from Mexico, Thailand and South Africa all reside in a discrete larger group but within clades closely linked to area of origin (Roux *et al*. 2002). More extensive collections of the fungus and DNA sequences from a number of different gene regions will be required to determine whether these groupings represent taxonomic entities or not. Such studies will also enhance our knowledge of the possible origin and worldwide distribution of this important pathogen.

C. zuluense is one of the most important pathogens of *Eucalyptus* in South Africa (Wingfield *et al*. 1997). Hawaii has a growing and impressive *Eucalyptus* planting program and the discovery of the fungus and the canker disease associated with it in Hawaii could be cause for concern. Assuming that the fungus behaves in a manner similar to that in South Africa, this disease could complicate tree growing in Hawaii. However, significant progress has been made in reducing the impact of *C. zuluense* in South Africa through tree breeding and selection of disease tolerant clones (Van Zyl *et al*. 2002*a*). Thus, if *Coniothyrium* canker becomes serious in Hawaii, it should be possible to reduce its impact in a similar manner.

Acknowledgements

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