Distribution of Chrysoporthe Canker Pathogens on *Eucalyptus* and *Syzygium* spp. in Eastern and Southern Africa

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ABSTRACT

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Chrysoporthe cubensis and *C. austroafricana*, collectively known as *Cryphonectria cubensis* in the past, are important canker pathogens of *Eucalyptus* spp. worldwide. Previous studies have suggested that *Chrysoporthe austroafricana* occurs only in South Africa, whereas *C. cubensis* occurs in Australia, Cameroon, Tanzania, Democratic Republic of Congo, Republic of Congo, Southeast Asia, and South, Central, and North America. In South Africa, *C. austroafricana* is a pathogen on nonnative *Eucalyptus* (Myrtaceae) and *Tibouchina* (Melastomataceae) spp., both residing in the order Myrtales. Recently, the fungus also has been found on native *Syzygium cordatum* trees in the country, leading to the hypothesis that it is native to Africa. In contrast, *C. cubensis* is thought to have been introduced into Africa and is known only on non-native *Eucalyptus* spp. and *S. aromaticum* (clove) in four countries. The aim of this study was to consider the distribution of *Chrysoporthe* spp. on non-native *Eucalyptus* spp. as well as on native Myrtales in southern and eastern Africa. Isolates were collected from as many trees as possible and characterized based on their morphology and DNA sequence data for two gene regions. Results show, for the first time, that *C. cubensis* occurs in Kenya, Malawi, and Mozambique on non-native *Eucalyptus* spp. *C. austroafricana* was found for the first time in Mozambique, Malawi, and Zambia on non-native *Eucalyptus* spp. and native *S. cordatum*. The known distribution range of *C. austroafricana* within South Africa also was extended during these surveys.

Additional keywords: *Cryphonectria*, disease, forestry

Species of *Chrysoporthe* previously treated in the genus *Cryphonectria* (12) are important canker pathogens of *Eucalyptus* spp. grown in plantations in both tropical and subtropical areas worldwide. They have been reported in South and Central America (2,15,16,32), Southeast Asia (8,18,22,29), Australia (6,23), North America (15,22), and Africa (10,18,27,28,40,42). The disease with which *Chrysoporthe* spp. are associated has been known as Cryphonectria canker in the past, and leads to the girdling of stems, wilting, and death of infected trees (4,15,17,29). The cankers can occur at the bases of the stems or are found higher up on the trunks (4,15,16,29). The disease caused by *Chrysoporthe* spp. has been managed successfully by breeding for disease-tolerant *Eucalyptus* hybrids in some countries, such as Brazil and South Africa (1,9,33,39,43). It is, however, still considered a major constraint to the successful establishment of *Eucalyptus* plantations and is regarded as a high priority disease.

The genus *Chrysoporthe* includes two economically important species, *Chrysoporthe cubensis* (Bruner) Gryzenh. & M. J. Wingf. and *C. austroafricana* Gryzenh. & M. J. Wingf., which are pathogenic to *Eucalyptus* spp. These species previously were treated in the genus *Cryphonectria* and, collectively, were known as *Cryphonectria cubensis* (Bruner) Hodges (3,12). Recognition of *Chrysoporthe* spp. as distinct from those of the genus *Cryphonectria* emerged from comparisons of DNA sequence data and clear morphological differences. The most notable morphological differences distinguishing *Chrysoporthe* from *Cryphonectria* spp. are the limited stromatic development in the ascostromata, long and black perithecial necks, and black, pyriform, and superficial conidiomata in species of the former genus (12).

Within *Chrysoporthe* spp., morphological and phylogenetic differences have been observed between isolates from South Africa and those occurring in the in other parts of the world (12,21). Comparisons of DNA sequence data based on multiple gene regions have shown that *C. cubensis* isolates reside in three well-supported phylogenetic groups (21). One of these encompasses South African isolates; another includes isolates from Southeast Asia, East Africa, and Australia; and a third group represents isolates from South America, North America, Central Africa, and West Africa (21). Slightly larger asci and rounded ascospores are found in the South African isolates compared with smaller asci and tapered ascospores in isolates occurring in other parts of the world (12). These differences support the treatment of the South African fungus as a distinct species known as *C. austroafricana* (12). Although isolates from Southeast Asia, East Africa, and Australia (Southeast Asian group) group separately from the South American, North American, Central African, and West African (South American group) isolates in phylogenetic comparisons, morphological differences have not been observed between the two groups (12,21). For the present, they are treated collectively as representing *C. cubensis* (12,21).

C. austroafricana has been reported only from South Africa, where it is considered to be one of the most important pathogens in non-native plantation-grown *Eucalyptus* spp. (5,42). Before its recognition as a distinct species, this fungus was thought to have been introduced into South Africa (34). Discovery of *C. austroafricana* on the non-native ornamental tree *Tibouchina granulosa* Cogn. (20) provided further support for the view that the fungus had been introduced into South Africa. However, the fungus recently has been discovered causing stem and branch cankers on native *Syzygium cordatum* Hachst. and *S. guineense* (Willd.) D.C (13). This has given rise to the alternative view that *C. austroafricana* is native to Africa and has undergone a host jump to non-native *Eucalyptus* and *Tibouchina* spp. (13,30). This might then also imply that the fungus would occur in countries neighboring South Africa. Native members of Myrtaceae similar to those in South Africa occur in these countries, as do non-native *Eucalyptus* spp., which have been used to establish plantations and woodlots for many years.

C. cubensis is known from Africa and several other regions of the world (12). In Africa, the fungus has been reported only from non-native hosts, namely from *Eucalyptus* spp. in the Republic of Congo (26,28), Democratic Republic of Congo (DRC) (19), and Cameroon (10), as well as

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from *S. aromaticum* (L.) Merr. & Perry. (Clove) on Unguja Island, Zanzibar (Tanzania) (18,22,24). It has been hypothesized that this pathogen was introduced to the African continent (12). Its occurrence in West and Central Africa as well as on the eastern seaboard of Africa would suggest that it occurs on non-native plantation-grown *Eucalyptus* trees in other parts of Africa.

Recently, Roux et al. (27) reported the occurrence of *Chrysoporthe* spp. from several eastern and southern African countries where *Chrysoporthe* spp. previously were not known. Their study greatly expanded the known geographic distribution of this genus of canker pathogens in Africa. However, the study by Roux et al. (27) focused only on non-native plantation-grown *Eucalyptus* spp., and isolates were not identified to species level. The aim of this study was, first, to determine the identity of the isolates collected by Roux et al. (27). A second aim was to conduct additional surveys of native hosts, especially *Syzygium* spp. in South Africa and other southern and eastern African countries.

MATERIALS AND METHODS

Collection of isolates. Surveys of indigenous species residing in the order Myrtales growing in the wild as well as non-native *Eucalyptus* spp. grown in plantations were conducted in Kenya, Malawi, Mozambique, South Africa, Tanzania, and Zambia (Fig. 1). Sampling involved selecting the trees and a subsequent search for disease symptoms. On *Syzygium* spp., dying branches and stem cankers were the symptoms of interest. On *Eucalyptus* spp., cracks, cankers on the stems, and swollen bases provided the best indications that *Chrysoporthe* spp. might be present (Fig. 2A and B). After the detection of fruiting structures (Fig. 2C and D), pieces of wood and bark were scraped or cut off from symptomatic trees, placed in brown paper bags, and labeled for subsequent laboratory study and isolation.

Pieces of wood bearing fungal fruiting bodies were placed in moist chambers to induce spore production. Fungal fruiting bodies were identified using standard microscope techniques (12). Isolations were made by lifting spore drops from fruiting structures. Single spore cultures were made by suspending spores (ascospores or conidia) in sterile distilled water. Spore suspensions were spread onto the surface of 2% malt extract agar (MEA) (malt extract at 20 g/liter and agar at 15 g/liter; Biolab, Midrand, South Africa) containing 100 mg of streptomycin sulfate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in petri dishes. These were incubated overnight and germinating single spores were selected and transferred to fresh plates. Resulting cultures were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (Table 1), where cultures were preserved on agar slants and sterile distilled water. One isolate from Cameroon that was obtained from the Centraalbureau voor Schimmelcultures (CMW 14852 and CBS 101281) also was included in this study.

Fig. 1. Map of Africa showing different countries where surveys were conducted. Arrows or crosses indicate general areas within each country where samples were collected.

DNA sequence comparisons. Representative isolates were selected from each host and geographic area and used for DNA sequence comparisons (Table 1). For each isolate, actively growing mycelium from one MEA plate per isolate was scraped from the surface of the agar using a sterile scalpel and transferred to a 1.5-µl Eppendorf tube. Excess liquid was removed from the tubes by centrifugation at 12,000 rpm for 1 min. DNA was extracted using a modification of the protocol described by Gryzenhout et al. (12). DNA concentrations were estimated visually on a 1% agarose gel using known concentrations of lambda DNA under UV illumination.

The polymerase chain reaction (PCR) was used to amplify β-tubulin 1, β-tubulin 2, and rDNA (internal transcribed spacer [ITS] 1, 5.8S and ITS 2) regions (11,38). The reactions were done in a volume of 25 µl comprising 2 ng of DNA template, 800 µM dNTPs, 0.15 µM each primer, 5 U/µl of Taq polymerase (Roche Diagnostics, Mannheim, Germany), and sterile distilled water (17.4 µl). The PCR reactions were carried out on a thermal cycler (Master cycle; Perkin-Elmer Corporation, Wellesley, MA) consisting of an initial denaturation step at 94ºC for 2 min, followed by 30 amplification cycles consisting of 1 min at 92ºC and 30 s of annealing at 56 to 60ºC, depending on the primer pair used. The PCR products were visualized under UV light on a 2% agarose gel containing ethidium bromide to determine the presence or absence of bands. The PCR products were purified using the High Pure PCR product purification kit according to the manufacturer's protocol (Roche Diagnostics, Mannheim, Germany).

The sequencing reactions (10 µl) consisted of 5× dilution buffer, 4.5 µl of H_2O, DNA (50 ng of PCR product), 10× reaction mix, and one of either reverse or forward primers that were used in the PCR reactions at approximately 2 pmol/µl. The PCR sequencing product was cleaned using Sephadex G-50 beads following the manufacturers' protocol (Sigma-Aldrich, Amersham Biosciences Limited, Sweden). The products were sequenced in both directions using the Big Dye Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an ABI Prism 3100 DNA sequencer (Applied Biosystems).

The gene sequences were analyzed and edited using Sequence Navigator (version 1.0.1; Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences were aligned with those in the TreeBASE dataset (S 1211, M 2095) obtained from Gryzenhout et al. (12). Phylogenetic analysis was performed using the software package Phylogenetic Analysis Using Parsimony (PAUP) version 4.01b (31). Phylogenetic analyses first were done for each gene region separately and then for a combined data set of the ITS and β-tubulin 1 and 2 gene regions. This was preceded by a partition homogeneity test to determine the similarity and combinability of the data from the two gene regions (14).

The most parsimonious trees were obtained with heuristic searches using stepwise addition and tree bisection and reconstruction (TBR) as the branch swapping algorithms. All equally parsimonious trees were saved and all branches equal to zero were collapsed. Gaps were treated as fifth character. Bootstrap replicates (1,000) were done on consensus parsimonious trees (7). Three *Cryphonectria* spp., namely *C. parasitica* (Murrill) M. E. Barr, *C. nitschkei* (G. H. Otth) M. E. Barr, and *C. macrospora* (Tak. Kobay. & Kaz. Itô) M. E. Barr were used as the outgroup taxa to root the trees (12).

RESULTS

Collection of isolates. *Chrysoporthe* samples were collected from Kenya, Malawi, Mozambique, South Africa, and Zambia from both non-native *Eucalyptus* spp. and native *Syzygium* spp. *Eucalyptus* spp. had typical symptoms of canker caused by *Chrysoporthe* spp. Swollen bases and cankers were evident on trees in most of the areas surveyed (Fig. 2). The majority of symptoms on *Eucalyptus* spp. were characterized by swollen basal cankers (Fig. 2A). However, in one plantation in South Africa and one compartment in Malawi near Mt. Mulanje, cankers were found higher on the tree stems, similar to those observed in South American and Asian countries (Fig. 2B). Symptoms on *Syzygium* spp. consisted mostly of cankers on dying branches and stems. Both sexual and asexual structures were encountered in all the areas surveyed and on both host genera considered.

In Zambia, most samples collected were from *Eucalyptus* trees (20 trees) near Kitwe and a few *Syzygium* trees from Kitwe and Chati. In Mozambique, more than 100 *S. cordatum* trees were sampled

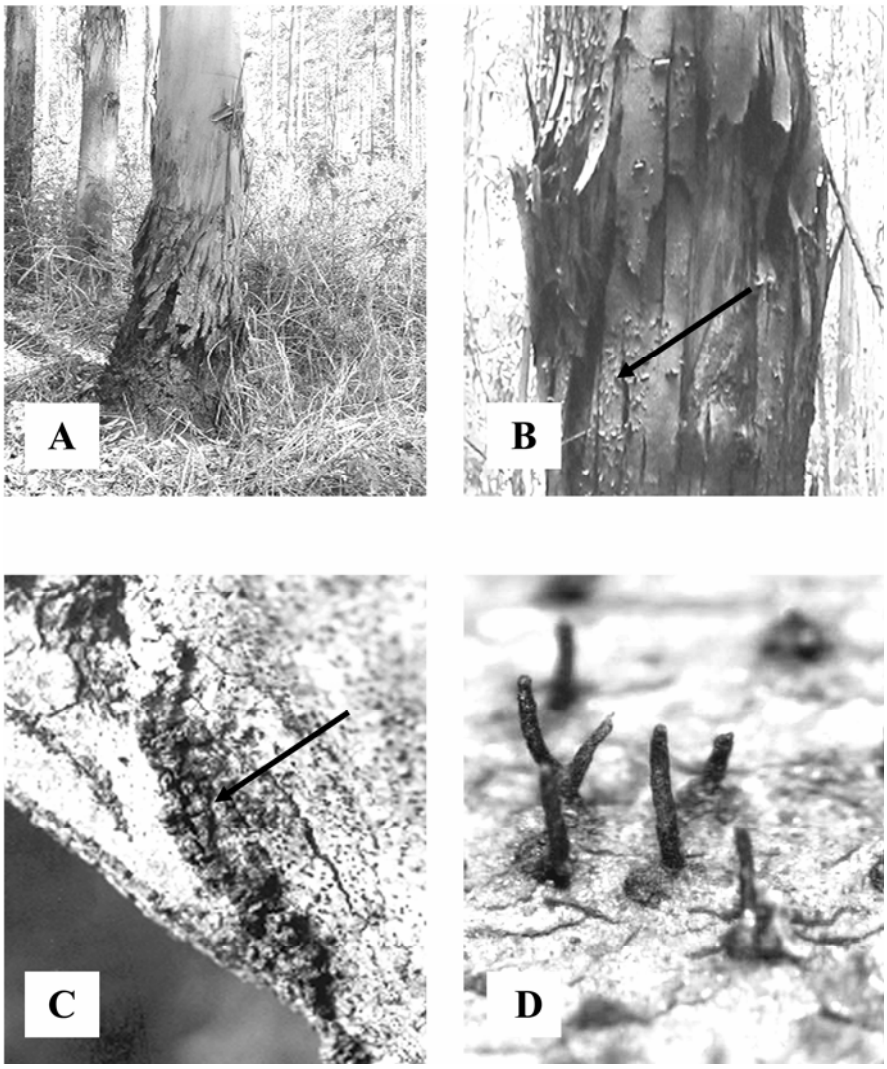

Fig. 2. Signs and symptoms of *Chrysoporthe* infection on *Eucalyptus* spp. and *Syzygium cordatum*. **A,** Canker caused by *Chrysoporthe* sp. on *Eucalyptus* sp. **B,** Fruiting structures of *Chrysoporthe* sp. on the bark of *Eucalyptus* sp. **C,** Fruiting structures of *Chrysoporthe* sp. on *S. cordatum* (figure provided by R. N. Heath, Forestry and Agricultural Biotechnology Institute, University of Pretoria). **D,** Ascomata of a *Chrysoporthe* sp.

over a wide area (Maputo, Gaza, Inhambane, and Sofala provinces), and more than 100 *Eucalyptus* trees were sampled in the Chimoio and Manica areas. In Kenya, more than 50 *Eucalyptus* trees were sampled. Although surveys included *Eucalyptus* spp. in several areas of Kenya, the disease was found only near the coastal town of Malindi. Both *Eucalyptus* and *Syzygium* spp. were surveyed in Tanzania (Njombe area), but no *Chrysoporthe* spp. were obtained from trees in this area. In Malawi, surveys were conducted in several areas, but the disease was found only in the Mt. Mulanje area, on both *Eucalyptus grandis* and *S. cordatum*. The distribution of *Chrysoporthe* spp. also was extended in South Africa, with isolates collected from *S. cordatum* in the Port Edward and Umzinto areas. Although other *Syzygium* spp., *Heteropyxis* spp., and a limited number of *Eugenia* spp. also were surveyed and sampled in South Africa, no *Chrysoporthe* spp. were found on these trees.

DNA sequence comparisons. Sequences were obtained for both the ITS rDNA and β-tubulin 1 and β-tubulin 2 gene regions. The β-tubulin regions were approximately 500 bp, whereas the ITS rDNA amplified was approximately 558 bp in size. Results of the partition homogeneity test showed that all sequences could be aligned for both regions (P value = 0.13). The aligned sequences of the combined regions generated 1,439 characters of equal weight, with 1,194 constant characters, of which 101 were parsimony uninformative and 144 were parsimony informative. In all, 100 most parsimonious trees were retained. A consensus tree (70% majority rule) with a length of 318, a consistency index of 0.945, and retention index of 0.959, was computed (Fig. 3).

Isolates from Kenya, Malawi, and Mozambique that were collected from *Eucalyptus* spp. grouped with *C. cubensis* isolates from Southeast Asia and formed a distinct clade (96% bootstrap). Isolates from *Eucalyptus* and *Syzygium* spp. from Malawi (CMW17098, 17101, 17110, and 17115), Mozambique (CMW1902, 13929, and 13926) and Zambia (13877 and 13976) grouped with *C. austroafricana* isolates from South Africa, collected from *Eucalyptus, Tibouchina,* and *Syzygium* spp. Isolates from the newly sampled areas in South Africa, including those collected from stem cankers from KwaMbonambi (CMW 13878 and 13879), also grouped in this clade (94% bootstrap). An isolate from

Table 1. Isolates included in this study[a]

Isolate no.	Alternative isolate no.	Species identity	Host	Origin	Collector	GenBank accession nos.[b]
CMW 1856	…	*Chrysoporthe cubensis*	*Eucalyptus* sp.	Kauai, Hawaii	NA	AY 083999, AY 084010, AY 084022
CMW 8756	…	*C. cubensis*	*Syzygium aromaticum*	Indonesia	M. J. Wingfield	AF 046896, AF 273077, AF 285165
CMW 3839	…	*C. cubensis*	*S. aromaticum*	Indonesia	M. J. Wingfield	AF 046904, AY 084011, AY 084023
CMW 11288	CBS 115736	*C. cubensis*	*Eucalyptus* sp.	Indonesia	M. J. Wingfield	AY 214302, AY 214230, AY214266
CMW 11289	CBS 115737	*C. cubensis*	*Eucalyptus* sp.	Indonesia	M. J. Wingfield	AY 214303, AY 214231, AY 214267
CMW 10774	…	*C. cubensis*	*S. aromaticum*	Zanzibar	C. S. Hodges	AF 492130, AF 492131, AF 492132
CMW 2631	…	*C. cubensis*	*E. marginata*	Australia	E. Davison	AF 543823, AF543824, AF523825
CMW 10671	CBS 115752	*C. cubensis*	*Eucalyptus* sp.	Rep. Congo	J. Roux	AF 254219, AF 254221, AF 254223
CMW 10453	CBS 505.63	*C. cubensis*	*Eucalyptus* sp.	DRC	E. Davison	AY063476, AY063478, AY063480
CMW 10639	CBS 115747	*C. cubensis*	*E. grandis*	Colombia	C. A. Rodas	AY 263419, AY 263420, AY 263421
CMW 8757	…	*C. cubensis*	*Eucalyptus* sp.	Venezuela	M. J. Wingfield	AF 046897, AF 273069, AF 273464
CMW 10777	…	*C. cubensis*	*S. aromaticum*	Brazil	C. S. Hodges	AY 084005, AY 084017, AY 084029
CMW 10778	CBS 115755	*C. cubensis*	*S. aromaticum*	Brazil	C. S. Hodges	AY 084006, AY 084018, AY 084030
CMW 9432	CBS 115724	*C. cubensis*	*E. grandis*	Mexico	M. J. Wingfield	AY 692321, AY 692324, AY 692323
CMW 13915[c]	…	*C. cubensis*	*Eucalyptus* sp.	Mozambique	G. Nakabonge	DQ246552, DQ246575, DQ246552
CMW 13912[c]	…	*C. cubensis*	*Eucalyptus* sp.	Mozambique	G. Nakabonge	DQ246554, DQ246577, DQ246554
CMW 13883[c]	…	*C. cubensis*	*Eucalyptus* sp.	Mozambique	G. Nakabonge	DQ246553, DQ246576, DQ246553
CMW 13944[c]	…	*C. cubensis*	*Eucalyptus* sp.	Kenya	J. Roux	DQ246550, DQ246573, DQ246550
CMW 13949[c]	…	*C. cubensis*	*Eucalyptus* sp.	Kenya	J. Roux	DQ246551, DQ246574, DQ246551
CMW 14774[c]	…	*C. cubensis*	*Eucalyptus* sp.	Malawi	J. Roux	DQ246555, DQ246578, DQ246555
CMW 14769[c]	…	*C. cubensis*	*Eucalyptus* sp.	Malawi	J. Roux	DQ246556, DQ246579, DQ246556
CMW 14852[c]	CBS 101281	*C. cubensis*	*Eucalyptus* sp.	Cameroon	NA	DQ246557, DQ246580, DQ246557
CMW 62	…	*C. austroafricana*	*E. grandis*	South Africa	M. J. Wingfield	AF 292041, AF 273063, AF 273458
CMW 2113[d]	CBS 112916	*C. austroafricana*	*E. grandis*	South Africa	M. J. Wingfield	AF 046892, AF 273067, AF 273462
CMW 9327	CBS 115843	*C. austroafricana*	*Tibouchina granulosa*	South Africa	M. J. Wingfield	AF 273473, AF 273060, AF 273455
CMW 9328	…	*C. austroafricana*	*T. granulosa*	South Africa	M. J. Wingfield	AF 292040, AF 273064, AF 273458
CMW 13902[c]	…	*C. austroafricana*	*S. cordatum*	Mozambique	G. Nakabonge	DQ246572, DQ246595, DQ246572
CMW 13926[c]	…	*C. austroafricana*	*S. cordatum*	Mozambique	G. Nakabonge	DQ246571, DQ246594, DQ246571
CMW 13929[c]	…	*C. austroafricana*	*Eucalyptus* sp	Mozambique	G. Nakabonge	DQ246570, DQ246593, DQ246570
CMW 14561[c]	…	*C. austroafricana*	*S. cordatum*	South Africa	G. Nakabonge	DQ246559, DQ246582, DQ246559
CMW 14562[c]	…	*C. austroafricana*	*S. cordatum*	South Africa	G. Nakabonge	DQ246560, DQ246583, DQ246560
CMW 13878	…	*C. austroafricana*	*Eucalyptus* sp.	South Africa	J. Roux	DQ246566, DQ246589, DQ246566
CMW 13879	…	*C. austroafricana*	*Eucalyptus* sp.	South Africa	J. Roux	DQ246567, DQ246590, DQ246567
CMW 13977[c]	…	*C. austroafricana*	*Eucalyptus* sp.	Zambia	J. Roux	DQ246569, DQ246592, DQ246569
CMW 13976[c]	…	*C. austroafricana*	*S. cordatum*	Zambia	J. Roux	DQ246568, DQ246591, DQ246568
CMW 17098[c]	…	*C. austroafricana*	*S. cordatum*	Malawi	J. Roux	DQ246561, DQ246584, DQ246561
CMW 17096[c]	…	*C. austroafricana*	*S. cordatum*	Malawi	J. Roux	DQ246565, DQ246588, DQ246565
CMW 17101[c]	…	*C. austroafricana*	*Eucalyptus* sp.	Malawi	J. Roux	DQ246562, DQ246585, DQ246562
CMW 17110[c]	…	*C. austroafricana*	*Eucalyptus* sp.	Malawi	J. Roux	DQ246563, DQ246586, DQ246563
CMW 17115[c]	…	*C. austroafricana*	*Eucalyptus* sp.	Malawi	J. Roux	DQ246564, DQ246587, DQ246564
CMW 10790	…	*Cryphonectria parasitica*	*Quercus serrata*	Japan	M. Kusunoki	AF 140243, AF 140253, AF 140255
CMW 10518	CBS 112919	*C. nitschkei*	*Quercus* sp.	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713
CMW 10463	CBS 112920	*C. macrospora*	*Castanopsis cupsidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

[a] CMW refers to the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, and CBS refers to Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NA = not available; Rep. Congo = Republic of Congo and DRC = Democratic Republic of Congo.

[b] GenBank accession numbers are sequence data of the β-tubulin 1 and 2 (primers Bt1a/1b and Bt2a/2b) and internal transcribed spacer (ITS; primers ITS1 and 4) regions.

[c] Isolates sequenced in this study.

[d] Ex-type cultures.

Cameroon (CMW 14852) that was obtained from the CBS and isolates from DRC and Congo that were included in this analysis grouped together with the South American isolates of *C. cubensis* (94% bootstrap).

DISCUSSION

This study has greatly increased our knowledge of the distribution of two of the most important *Eucalyptus* pathogens currently known. The geographic range of *Chrysoporthe* spp. on native *Syzygium* spp. in eastern and southern Africa also has been expanded considerably. We have shown that *C. austroafricana* causes cankers at the base and higher up on stems of *Eucalyptus* trees in South Africa and Malawi, which is contrary to prior knowledge. Likewise, the sexual state of this fungus has been shown to be equally as abundant as the asexual state in countries north of South Africa, contrary to the situation in southern Africa, where the asexual state predominates (34).

C. austroafricana was known previously only from South Africa on non-native *Eucalyptus* spp. (42), *T. granulosa* (20), and native *S. cordatum* and *S. guineense* (13). Results of this study have shown that the fungus is also present in Malawi, Mozambique, and Zambia on both non-native *Eucalyptus* spp. and native *S. cordatum*. The fungus is widespread in Mozambique and was collected from the southern (Maputo) and central (Chimoio) parts of the country, stretching over a distance of about 1,200 km. Surveys in Zambia were limited to one area and *C. austroafricana* was common on *Eucalyptus* trees in plantations near the town of Kitwe. On *Syzygium* sp., the fungus was found in the same area but only on one tree. In Malawi, *C. austroafricana* was collected from one area (Mt. Mulanje) from both *Eucalyptus* spp. and native *S. cordatum*. The occurrence of *C. austroafricana* in Malawi, Mozambique, and Zambia suggests that the fungus also might be present in other East African countries, such as Tanzania and Zimbabwe.

C. austroafricana recently has been suggested to be native to Africa (13). Our results, showing that the fungus has a wide geographic distribution in southern and eastern Africa on both non-native and native trees, support this hypothesis. This wide distribution and the absence of *C. austroafricana* from other continents, despite extensive surveys, suggests that the fungus is limited to southern Africa. In this respect, it represents a potentially important threat to Myrtaceae elsewhere in the world. The fungus causes a canker disease, which results in reduced growth rates, reduced coppicing, and death of infected

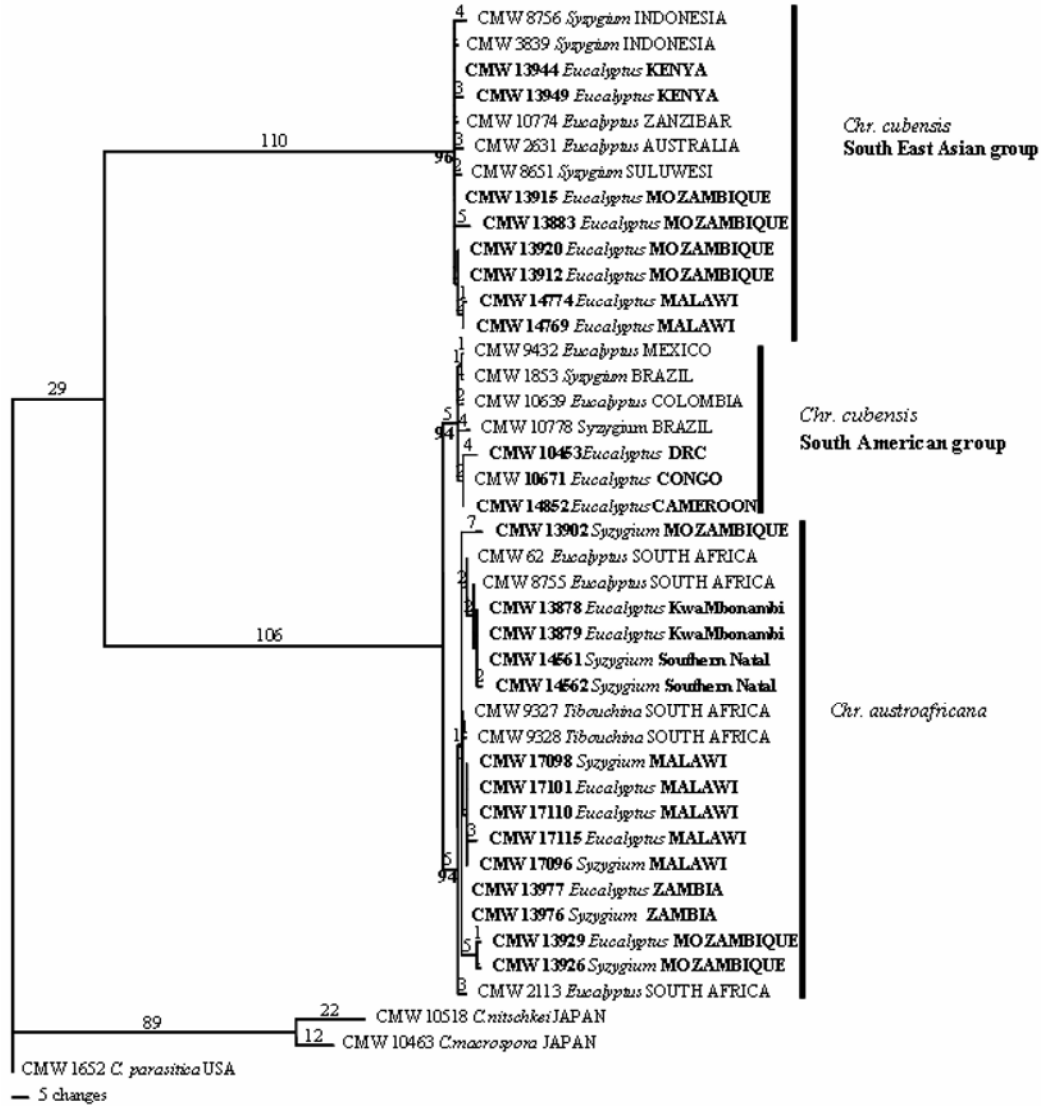

Fig. 3. Phylogenetic tree generated from internal transcribed spacer rDNA and β-tubulin gene sequence data (tree length = 318, consistency index = 0.945, retention index = 0.959) showing relatedness of the isolates of *Chrysoporthe* spp. collected from African countries. Branch lengths are indicated above the branches and bootstrap values above 50% are indicated below the branches. *Cryphonectria parasitica, C. nitschkei*, and *C. macrospora* were used as outgroups. Isolates collected in this study and isolates included in the study from Republic of Congo, Democratic Republic of Congo (DRC), and Cameroon are in bold.

Eucalyptus trees (42). On native *Syzygium* trees, it is found primarily on dead or dying branches (13). Limited studies by Roux et al. (26) and Rodas et al. (25) have shown that *C. austroafricana* isolates from South Africa are more virulent than *C. cubensis* isolates. Therefore, the introduction of *C. austroafricana* isolates to other continents could have serious negative impacts on commercial forestry and biodiversity.

It initially was believed that *C. austroafricana* causes only basal cankers on the stems of *Eucalyptus* spp. in South Africa, whereas *C. cubensis* gives rise to both basal cankers and cankers higher up on the stems of trees in Southeast Asia and South America (5,34,42,40). During the course of our surveys, *C. austroafricana* was isolated from cankers up to 3 m above ground level on *Eucalyptus* trees in the KwaZulu/Natal province (KwaMbonambi) in South Africa and in Malawi. This symptom clearly is less common than it is with *C. cubensis* elsewhere in the world. It is highly possible that environmental factors have an influence on areas of infection on the stems.

One of the early indications that *C. austroafricana* and *C. cubensis* might represent different fungi was the fact that cankers of the former fungus typically are covered with asexual structures (pycnidia), whereas those of the latter fungus more typically bear sexual perithecia (34,41,42). During the present surveys, both sexual and asexual structures of *C. austroafricana* commonly were found on *Eucalyptus* spp. as well as on native *Syzygium* spp. in Malawi, Mozambique, and Zambia. In South Africa, the sexual state of this fungus is abundant on native *Syzygium* spp. but not on *Eucalyptus* (13). Thus, this characteristic might be associated with environmental factors such as temperature and humidity, which are lower in South Africa than more northern African countries.

C. cubensis has been known in Africa since the early 1960s, where it has been recorded on *Eucalyptus* spp. and *S. aromaticum* (10,19,22). Our surveys have extended the geographic range of the fungus to include Kenya, Malawi, and Mozambique, where it occurs on *Eucalyptus* spp. Phylogenetic analyses showed that *C. cubensis* from Kenya, Mozambique, and Malawi groups in the same subclade as *C. cubensis* from Tanzania and Southeast Asia, but separate from isolates from South America, the Republic of Congo, DRC, and Cameroon. This suggests that East African isolates could have been introduced from Asia. This finding should now be tested at the population biology level. It might raise clues as to how the pathogen has moved around the world and provide knowledge that will reduce the risks of future introductions into new areas.

The question regarding the origin of *C. cubensis* remains to be resolved. A previous view has been that the fungus originated in Indonesia on native *S. aromaticum* (18). An alternative hypothesis has been that the fungus originated on native plants in South America (40,41). There have been more recent reports of *C. cubensis* from native plants in South America (25), adding support to the view that this area could represent the origin of the fungus. Population biology studies using vegetative compatibility groups (VCGs) on *C. cubensis* isolates from South America (Venezuela and Brazil) and Southeast Asia (Indonesia) have shown that a large number of VCGs occur in each country (36,37). This suggests either a high level of outcrossing within the populations or well-established native populations in both areas. The fact that the Indonesian population is also highly diverse, together with the clear phylogenetic distinction between Asian and South American isolates, supports suggestions that these two groups of *C. cubensis* isolates might represent distinct species (12,21).

The results of this study, combined with previous findings (18,19,22,27,28,42), show that *Chrysoporthe* spp. have a wide distribution in Africa. In East Africa, all isolates of *C. cubensis* collected reside in the Southeast Asian group defined for the fungus. In contrast, all isolates from West and Central Africa reside in the South American clade of *C. cubensis.* In Mozambique, South Africa, and Zambia, only *C. austroafricana* is present. The populations of isolates collected in the surveys presented in this study will make it possible to consider the origin of *Chrysoporthe* spp. on the African continent and to better understand how these fungi are moving within the region.

The knowledge generated in this study is important to *Eucalyptus* plantation managers. For example, disease caused by *C. austroafricana* in South Africa is managed largely through the planting of disease-tolerant clones (33,35). However, our study shows that, within 2,000 km, *C. cubensis* also occurs, and this is a pathogen against which South African *Eucalyptus* stock has not been tested. Thus, future outbreaks of canker caused by *Chrysoporthe* spp. in South Africa should be carefully monitored. Countries in Central and West Africa, Asia, Australia, and South America, where *C. austroafricana* is still unknown, should also take note of the potential threat of the fungus in their areas.

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