CHAPTER 3

Discovery of the *Eucalyptus* canker pathogen, *Cryphonectria cubensis*, on *Miconia* (Melastomataceae) in Colombia

ABSTRACT

Cryphonectria cubensis is a serious canker pathogen on commercially grown *Eucalyptus* species in the tropics and subtropics. During recent surveys for native hosts of *C. cubensis* in Colombia, a fungus with fruiting structures similar to those of *C. cubensis* was found on native *Miconia theaezans* and *M. rubiginosa,* both members of the Melastomataceae. The morphology of this fungus was studied and DNA sequences were obtained for the ITS1/ITS2 region of the rDNA operon and the β-tubulin genes. Pathogenicity of the fungus was also assessed on various Melastomataceae. Isolates from *M. theaezans* and *M. rubiginosa* grouped together with other South American *C. cubensis* isolates from *Eucalyptus* species and *Syzygium aromaticum.* Fruiting structures on *M. rubiginosa* also resembled those of *C. cubensis* on *E. grandis*. *Cryphonectria cubensis* isolates from *E. grandis* and *M. theaezans* were mildly pathogenic on the various hosts, although *Tibouchina* spp. and *M. rubiginosa* appeared to be more susceptible to *C. cubensis* than a number of *Eucalyptus* clones and *M. theaezans*. The occurrence of *C. cubensis* on native *Miconia* spp. supports the view that this pathogen is native to South and Central America.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is one of the most serious pathogens of *Eucalyptus* spp. (Myrtaceae) in South America (Boerboom & Maas 1970, Hodges *et al.* 1976, Hodges, Geary & Cordell 1979, Hodges 1980), including Colombia (Van der Merwe *et al.* 2001). The associated canker disease has also been reported from other parts of the world with tropical, sub-tropical or temperate climates, mostly Africa (Gibson 1981, Roux *et al.* 2003, Wingfield, Swart & Abear 1989), Southeast Asia (Florence, Sharma & Mohanan 1986, Hodges, Alfenas & Cordell 1986, Sharma, Mohanan & Florence 1985) and Australia (Davison & Coates 1991). In these regions, Cryphonectria canker is most severe in areas with high rainfall and temperature (Boerboom & Maas 1970; Hodges *et al.* 1976, 1979, Sharma *et al.* 1985).

Cankers caused by *C. cubensis* are usually found at the base or lower stems of trees, but may also occur higher up on the trunks (Sharma *et al.* 1985, Hodges *et al.* 1976, 1979). The pathogen kills the cambium and in severe cases, can result in tree death (Sharma *et al.* 1985, Hodges *et al.* 1976, 1979). The only practical management option for the disease is planting resistant *Eucalyptus* species and clones (Alfenas, Jeng & Hubbes 1983, Hodges *et al.* 1976, Sharma *et al.* 1985, Van Heerden & Wingfield 2002).

Until recently, *C. cubensis* has been known only to occur on trees belonging to the Myrtaceae. These hosts are predominantly species of *Eucalyptus* but also include clove (*Syzygium aromaticum* (L.) Merr. & Perry) (Hodges *et al.* 1986) and strawberry guava (*Psidium cattleianum* Sabine) (Hodges 1988). The recent discovery of *C. cubensis* on *Tibouchina urvilleana* (DC). Logn. (Fig. 1a) and *T. lepidota* Baill. (Fig. 1b), which are members of the Melastomataceae native to South America, was thus considered intriguing (Wingfield *et al.* 2001). The report of Wingfield *et al.* (2001) has led to subsequent disease surveys and the discovery of the fungus on ornamental *T. granulosa* in South Africa (Myburg *et al.* 2002a).

The possible origin of *C. cubensis* presents an interesting question that is also important in terms of disease management. One hypothesis is that the pathogen originated on clove, also a member of the Myrtaceae, in Indonesia (Hodges *et al.* 1986). The world-wide distribution of this fungus would then have occurred through the establishment of clove

plantations linked to the spice trade (Hodges *et al.* 1986). The discovery of *C. cubensis* on native *Tibouchina* spp. in South America has, however, raised the alternative hypothesis that *C. cubensis* could have originated in that part of the world (Wingfield *et al.* 2001).

Results from phylogenetic studies, based on DNA sequence for three gene regions (Myburg, Wingfield & Wingfield 1999, Myburg *et al.* 2002b), have shown that *C. cubensis* from South America and Southeast Asia resolve into two distinct phylogenetic sub-clades. This suggests that *C. cubensis* in these areas are different from one another and was not introduced into one area from another. Equally intriguing is the recent discovery based on comparisons of β-tubulin and histone *H3* gene sequences (Myburg *et al.* 2002b), that South African isolates of *C. cubensis* are distinct from those of South American and Southeast Asian origin, and probably represent a distinct taxon.

During recent surveys for *C. cubensis* on native Melastomataceae in Colombia, a fungus resembling *C. cubensis* was found on a number of new hosts in the Melastomataceae. The aim of this study was to identify the fungus based on morphology and DNA sequences. Pathogenicity of the isolates originating from the new hosts was also tested on these hosts and on *E. grandis* W. Hill ex Maiden.

MATERIALS AND METHODS

Symptoms and collection of samples

Disease surveys were conducted in various areas of Colombia with different altitudes and precipitation (Fig. 2). Specimens were collected from *Miconia theaezans* (Bonpl.) Cogn. (Fig. 1c) in a natural forest from the La Selva farm of Smurfit Carton de Colombia near the city Pereira in the Risaralda province. Cankers covered in fruiting structures were also found on *M. rubiginosa* (Bonpl.) DC. trees (Fig. 1d) of different ages on the farm Vanessa, near Timba in the Cauca province. These trees occurred within a *Eucalyptus* plantation where *C. cubensis* has previously been collected.

Disease symptoms on the *Miconia* spp. included branch die-back, and cankers on branches, trunks or the tree bases that often resulted in the death of trees or tree parts.

The cankers were generally associated with physical wounds to branches and stems. Fruiting structures were produced abundantly around the edges of the actively growing canker margins.

Specimens collected from cankers were transported to the laboratory for further analysis. Single conidial isolations were made from the fruiting structures by suspending spore masses in sterile water and plating the resulting suspensions on malt extract agar MEA (20 g/l Biolab malt extract agar). Single germinating conidia were then transferred to fresh MEA plates. Representative isolates have been preserved at 5 °C in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 1). The original bark specimens from whom isolations were made have been deposited (Table 2) in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM).

DNA sequence comparisons

Isolates from *Miconia* spp. and *E. grandis* were included in the DNA sequence comparisons (Table 1). Previously characterised *C. cubensis* isolates from *Eucalyptus* spp. (Myburg *et al.* 2002b) and *S. aromaticum* (Myburg *et al.* 1999, 2003) from different parts of the world were included for comparative purposes. In addition, representative species of *Cryphonectria* and *Endothia*, namely *C. parasitica* (Murr.) Barr, *C. radicalis* (Schw.: Fr.) Barr, *C. nitschkei* (Otth.) Barr, *C. macrospora* (Kobayashi & Ito) Barr and *E. gyrosa* (Schw.: Fr.) Fr. were sequenced by Venter *et al.* (2002). Two *Diaporthe ambigua* Nitschkei isolates were included as outgroup taxa to root the phylogenetic trees.

Isolates for DNA sequence comparisons were grown in Malt Extract Broth (20 g/l Biolab malt extract). DNA was extracted from mycelium as described in Myburg *et al.* (1999). The internal transcribed spacer (ITS) regions ITS1 and ITS2, as well as the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, were amplified using the primer pair ITS 1 and ITS 4 (White *et al.* 1990). Two regions within the β-tubulin gene were amplified with primer pairs Bt1a/Bt1b and Bt2a/Bt2b respectively (Glass & Donaldson 1995). The reaction conditions for amplifying these gene regions were the same as those given by Myburg *et al.* (1999) and Myburg *et al.* (2002b) respectively. PCR products were visualised on 1% agarose (ethidium bromide stained) gels using a UV light.

Purification of PCR products was done using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany).

The purified PCR products were sequenced with the same primers that were used to amplify the respective DNA regions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom) was used to sequence the amplification products on an ABI PRISM 3100™ automated DNA sequencer.

The resulting raw nucleotide sequences were edited using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software. Sequences were manually aligned. Phylogenetic trees were inferred using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 2002). A Templeton Nonparametric Wilcoxon Signed Ranked test (Kellogg, Appels & Mason-Gamer 1996) was applied to the rRNA and β-tubulin gene sequence data sets to determine whether they could be analysed collectively in the parsimony analysis.

A phylogenetic tree was inferred from maximum parsimony (MP) using the heuristic search option with the tree-bisection-reconnection (TBR) branch swapping and MULTREES options (saving all optimal trees) effective. Gaps inserted during manual sequence alignment were treated as fifth character (NEWSTATE) in the heuristic searches. A 1000 replicate bootstrap was executed to assess the confidence levels of the branch nodes of the phylogenetic tree. The sequence data generated in this study have been deposited in GenBank and accession numbers are listed in Table 1.

Morphology

Conidiomata from the bark specimens were rehydrated for one min in boiling water. The structures were then sectioned at –20 °C to a thickness of 12-14 µm with a Leica CM1100 cryostat after embedding them in Leica mountant (Setpoint Premier, Johannesburg, South Africa). Sections were mounted on microscope slides in lactophenol. Structures were also sectioned by hand to observe the morphology of the conidiophores. Twenty measurements, presented as (min-)(mean-SD) – (mean+SD)(-max) µm, of ascospores, asci, conidia and conidiophores suspended in lactophenol and

3% KOH, were taken for the specimens. A measurement range from two structures was obtained for the eustromata and perithecia. Colour notations of Rayner (1970) were used.

Pathogenicity tests

Greenhouse inoculation trials. Three isolates from *E. grandis* in Colombia (CMW 10638, CMW 10639, CMW 10640) and two isolates from *M. theaezans* (CMW 10625, CMW 10626) were screened for pathogenicity on *T. urvilleana* (seven months old) plants in a greenhouse with natural light at ~25 °C. Five trees per isolate were inoculated and an equal number of trees were inoculated with sterile water agar (WA) (20 g/l Biolab agar) plugs as controls. Inoculations were made with a cork borer (9 mm diam). Agar discs of the same size were taken from the edges of actively growing cultures and placed inside the wounds with the mycelium facing downwards. The agar discs were covered with tissue paper moistened with sterile water, and secured with masking tape. The masking tape was removed after ten days.

Trees were inoculated in October 2001 and lesion development was evaluated after four weeks. Lesions were exposed by scraping away the bark and the lengths of the lesions were measured. The most pathogenic isolates from *E. grandis* and *M. theaezans* (CMW 10639 and CMW 10625 respectively) were selected for subsequent field inoculation trials.

In a second greenhouse trial, two isolates from *M. rubiginosa* (CMW 10022 and CMW 10024) were inoculated on *T. urvilleana* and *E. grandis* (clone ZG14), which were 17-24 months old and up to 1.8 m high. A highly pathogenic isolate of *C. cubensis* from South Africa (CMW 2113), used in previous pathogenicity studies (Myburg *et al.* 2002a, Van Heerden & Wingfield 2001, 2002) was included for comparative purposes. Inoculation procedures were the same as those in the first trial and ten trees were inoculated for each of the three isolates and for the negative control using WA discs. Inoculations were done as described above except that a cork borer with a diameter of 6 mm was used. The trial was inoculated in May 2002, and evaluated in June 2002.

Field inoculation trials. The first inoculation trial was conducted at Rancho Grande farm, Restrepo, Valle (76° 30' 49" W and 3° 51' 43" N, 1067 mm/y, 1469 masl). This trial included reciprocal inoculations with isolates from *E. grandis* (CMW 10639) and *M. theaezans* (CMW 10625) selected in the first greenhouse trial. Five tree species were used, namely *T. semidecandra* Cogn. (Fig. 1f), *T. lepidota*, *T. urvilleana*, *M. theaezans* and a clone of *E. grandis* (clone 274). These trees were one year old and were growing in plastic planting containers. Twenty trees of each species were inoculated per isolate, and an equal number of trees were inoculated with WA discs to serve as negative controls. Inoculations were conducted in a similar way to greenhouse inoculations but the diameter of the wound was 4 mm. Trees were inoculated in May 2002 and lesion development was evaluated after twelve weeks. Internal lesion length in the cambium was measured for all field trials.

The second field trial was at the Vanessa farm (Fig. 2), Timba, Cauca province (76° 35' 15" W and 3° 5' 42" N, 3143 mm/y, 2048 msal). Isolate CMW 10022 from *M. rubiginosa*, shown to be pathogenic in the preliminary greenhouse trial, was used. Twenty three-year-old *E. grandis* trees (clone 275), 20 trees from seeds of a cross between *E. grandis* and *E. urophylla* (*E. "urogandis"* clone 212), and 20 *M. rubiginosa* trees were inoculated. The *M. rubiginosa* trees were approximately six years old and formed part of the native vegetation surrounding the commercial plantations. Ten trees of each host were inoculated with MEA to serve as negative controls. The trial was initiated in June 2002 and lesion lengths were measured after 12 weeks in late September 2002. The same inoculation techniques used in greenhouse and other field trials were applied, except that the inoculation wounds were six mm in diameter. The data for the pathogenicity trials were analysed using a one-way Analysis of Variance (ANOVA) with SAS (1990).

RESULTS

DNA sequence comparisons

Amplification of the ITS1, 5.8S and ITS2 rRNA regions as well as the two regions in the β-tubulin gene resulted in PCR products of approximately 600bp and 550bp respectively. The Templeton Nonparametric Wilcoxon Signed Ranked test (Kellogg *et al.* 1996)

showed that the rRNA and the β-tubulin sequence data sets could be combined in the phylogenetic analyses. The combined data set consisted of 32 taxa with the *D. ambigua* isolates as the outgroup (Fig. 3). This data set consisted of 1498 sequence characters of which 886 were constant, 44 were variable parsimony-uninformative and 568 were variable parsimony-informative.

The phylogenetic tree generated from the heuristic search (Fig. 4, tree length = 1198 steps, consistency index/CI = 0.8, retention index/RI = 0.9) resolved the taxa into three clades separately from the outgroup taxa. The largest of the three clades represented *C. cubensis*, while the other two included representative species of *Cryphonectria* (*C. parasitica*, *C. radicalis*, *C. nitschkei* and *C. macrospora)* grouping in the one clade, and *E. gyrosa* in the other (bootstrap support = 100% respectively).

The *C. cubensis* clade represented this fungus isolated from a variety of hosts originating from South America, Southeast Asia and South Africa. All three geographical areas are represented as three well supported clades in the phylogenetic tree (Fig.4). The Southeast Asian group (bootstrap 98%) included *C. cubensis* isolated from clove and *Eucalyptus* species. The South African group is characterised by *C. cubensis* isolated from *E. grandis* (bootstrap 95%). The South American group (bootstrap 72%) include *C. cubensis* isolated from *Eucalyptus* spp. and *S. aromaticum* as reported previously (Myburg *et al.* 1999, 2002b, 2003). Isolates originating from *M. theaezans* (CMW 9980, CMW 9993, CMW 10626, CMW 10639) and *M. rubiginosa* (CMW 9970, CMW 9996, CMW 10022, CMW 10024, CMW 10025, CMW 10026, CMW 10028), grouped within the South American sub-clade.

Morphology

Specimens from *M. rubiginosa* (PREM 57517) had ascomata similar to those of *C. cubensis* found on *E. grandis* in Colombia (PREM 57294). They could be distinguished from conidiomata since only one to three fuscous-black (13''''m), cylindrical necks (380-720 µm long) emerged from the bark (Fig. 5a). Orange (15) stromatic tissue was sometimes visible at the base of the necks (Figs 5a-b). Longitudinal sections revealed umber (15m), *textura porrecta* tissue surrounding the black perithecial necks (Figs 5b-c) and reduced prosenchymatous stromatic tissue present at the base of the neck (Figs 5b,

5d). Asci were fusoid, eight-spored with a refractive apical ring, (19.5-)20.5-24.5(-27.0) µm long and (4.5-)5.0-6.5(-7.0) µm wide (Fig. 5e). Ascopores were fusoid to oval, hyaline with a single septum in the center of the spores, (5.0-)5.5-7.0(-8.5) µm long and 2.0-2.5 µm wide (Figs 5f). The ascomata also resembled those previously described from South America (Bruner 1917, Hodges *et al.* 1979, Hodges 1980) and ascomata previously described from other parts of the world (Heath *et al.* 2003, Myburg *et al.* 2002a, 2003).

Conidiomata of the fungus on *M. rubiginosa* (PREM 57517) were similar in shape to those of *C. cubensis* occurring on *E. grandis* (PREM 57294). Structures were pyriform, superficial and fuscous-black (13''''m) with a single attenuated neck and luteous (19) spore drops or tendrils (Figs 5g-h). The tissue of the conidiomatal base was umber (15m), *textura globulosa* but the neck tissue was *textura porrecta* (Fig. 5i). Conidiophores were branched, and conidiogenous cells enteroblastic phialidic, cylindrical with inflated bases and attenuated apices (Figs 5j-k). Conidia were hyaline, oblong to oval, aseptate, 3.0-4.0 µm long, 1.5-2.0 µm wide (Fig. 5l). These characteristics were also similar to those described previously (Bruner 1917, Hodges *et al.* 1979, Hodges 1980, Myburg *et al.* 2002b, 2003).

A few morphological differences exist between structures on *E. grandis* and *M. rubiginosa*. The stromatic tissues of the ascomata on *E. grandis* were slightly more distinctly erumpent than those on *M. rubiginosa*. Conidiomata on *M. rubiginosa* were much smaller (25-400 µm long in total above surface of bark) than those on *E. grandis* (420-960 µm long in total above surface of bark). Conidiomata on *E. grandis* were also better developed with wide bases (210-420 µm wide above surface of bark) and long, strongly attenuated necks (220-440 µm long), unlike conidiomata on *M. rubiginosa* that had narrow bases (140-260 µm wide above surface of bark) and shorter necks (140-180 µm long).

Pathogenicity tests

Greenhouse inoculations. In the first greenhouse trial (Table 3), inoculation with *C. cubensis* isolates from *E. grandis* (CMW 10638, CMW 10639, CMW 10640) and *M.*

theaezans (CMW 10625, CMW 10626) resulted in lesion formation (Fig. 6). The more pathogenic isolates (CMW 10625, CMW 10638, CMW 10639) were not significantly different from each other (Fig. 6), but differed significantly ($P < 0.0014$) from the control inoculation (Table 3). Isolates CMW 10639 from *E. grandis* and CMW 10625 from *M. theaezans* were chosen for field inoculations (Fig. 6) because they were most pathogenic for each isolate group from a particular host.

In the second greenhouse trial (Table 4), isolates from *M. rubiginosa* (CMW 10022, CMW 10024) and the South African isolate of *C. cubensis* (CMW 2113) resulted in different size lesions (Fig. 7). The South African isolate was more pathogenic on the *E. grandis* clone than the other isolates tested (Fig. 7). This isolate was also less pathogenic on *T. urvilleana* (Fig. 7) than on the *E. grandis* clone. An isolate from *M. rubiginosa* (CMW 10024) was more pathogenic on *E. grandis* than on *T. urvilleana* (Fig. 7) and it was also more pathogenic on *E. grandis* than the other isolate from *M. rubiginosa* (CMW 10022). Isolate CMW 10022 was equally pathogenic on *E. grandis* and *T. urvilleana* (Fig. 7). All isolates produced lesions significantly larger ($P = 0.001$) than the control inoculations (Table 4). Only *E. grandis* trees infected by the South African isolate (CMW 2113) produced epicormic shoots below the inoculation points, indicating that the inoculated stems were being girdled.

Field inoculation trials. In the first field trial (Table 5), lesions were produced on all tree species (*T. urvilleana*, *T. lepidota*, *T. semidecandra*, *M. theaezans*, *E. grandis*) in response to inoculation with isolates CMW 10693 from *E. grandis* and CMW 10625 from *M. theaezans*. The longest lesions were produced on *T. urvilleana* (Fig. 8a) and *T. lepidota* (Fig. 8b), while lesions on *T. semidecandra* (Fig. 8c), although smaller, also differed significantly ($P = 0.001$) from control inoculations (Fig. 9). Lesions on *M. theaezans* (Fig. 8d) and the *E. grandis* clone (Fig. 8e) were only slightly longer than the control inoculations (Fig. 9). Lesions produced by the two isolates (CMW 10639, CMW 10625) were similar in size on each tree species (Fig. 9).

In the second field trial (Table 6), trees of *M. rubiginosa* (Figs 8f, 10) were more susceptible ($P = 0.0001$) to the *C. cubensis* isolate from *M. rubiginosa* (CMW 10022) than the *E. grandis* trees tested (Figs 8g-h, 10). Inoculations with isolate CMW 10022 on

the susceptible *E. grandis* clone 275 and the hybrid clone 212 gave rise to lesions that did not differ from those of the control inoculations (Fig. 10).

DISCUSSION

This study reports on the discovery of the serious *Eucalyptus* pathogen *C. cubensis* on native *Miconia* species (Melastomataceae) in Colombia. Isolates of the fungus from *M. theaezans* and *M. rubiginosa* grouped in the sub-clade that characterises *C. cubensis* occurring in South America, as defined in previous studies (Myburg *et al.* 1999, 2002a, 2002b, 2003, Roux *et al.* 2003). Structures on herbarium specimens linked to these isolates had conidiomata and ascomata typical of *C. cubensis* and spores were similar in size to those previously reported for this fungus (Hodges 1980, Myburg *et al.* 2002b, 2003).

Different host bark and environmental conditions have in the past been shown to result in variable morphology of *C. cubensis* structures (Bruner 1917, Hodges *et al.* 1986, Myburg *et al.* 2003). This complicates morphological comparisons between samples from different hosts. For instance, conidiomata on *M. rubiginosa* were much smaller than those on *E. grandis*, but the isolates originating from the specimens of *M. rubiginosa*, were shown to be identical to those from *E. grandis* based on DNA sequences. These differences observed between the conidiomata on *M. rubiginosa* and *E. grandis*, complicates identification. DNA sequences should thus accompany morphological identifications to verify identifications.

Native Melastomataceae in Colombia differed in their susceptibility to *C. cubensis* in the field inoculation trials. In the field trial where five different host species were tested, *T. urvilleana* and *T. lepidota* were highly susceptible to the two isolates of *C. cubensis*. This is in contrast to *M. theaezans* that was highly tolerant to infection. *Tibouchina semidecandra* was less susceptible than the other two species of *Tibouchina*, but more susceptible than *M. theaezans*.

Results of the different pathogenicity trials suggest that in the field, *C. cubensis* is more pathogenic on *M. rubiginosa* than on *E. grandis*. It was previously suggested that *C. cubensis* could have an origin in South America on native Melastomataceae (Wingfield

et al. 2001). It is generally believed that pathogens are less pathogenic on their native hosts than exotic species (Leppik 1970, Newhouse 1990). Therefore, the *E. grandis* clones used in the trials were expected to be more susceptible to *C. cubensis* than *M. rubiginosa*. However, these commercially grown clones have been subjected to intensive selection for resistance to disease over the past few years. It is thus possible that the clones or seed lots chosen for these trials have high degrees of tolerance to the pathogen. The fact that disease is not commonly seen on native Melastomataceae might also imply that the artificial inoculation techniques used to test pathogenicity, breach barriers that limit infection under natural conditions.

In this study we have shown that *C. cubensis* from South America occurs on *M. theaezans* and *M. rubiginosa*, two species of a genus not previously known as a host of the pathogen. The other recently recognised native hosts of the fungus in this country are *Tibouchina* spp. (Wingfield *et al.* 2001). The first discovery of *C. cubensis* on *M. theaezans*, was in native vegetation far removed from *Eucalyptus* plantations. It thus seems likely that *C. cubensis* occurs naturally on this host. In the case of *M. rubiginosa*, the trees were felled during the establishment of a *Eucalyptus* compartment. The *M. rubiginosa* trees, however, recovered and *C. cubensis* was found on these trees, as well as on the *Eucalyptus* trees in the adjacent compartment. It is unclear in which direction *C. cubensis* spread in this case, although it most likely was already present on *M. rubiginosa*. Further studies will be required to resolve this question.

Members of the Melastomataceae are common in South America, Central America, the Caribbean islands and Hawaii (Everett 1981). The occurrence of *C. cubensis* on species belonging to this family supports the hypothesis that the fungus occurred widely through South and Central America and the Caribbean prior to the commercial planting of *Eucalyptus* species. Detailed population studies will shed more light on the origin or origins of *C. cubensis*, and its movement throughout the world.

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Table 1. Isolates included in this study.

Isolate number[a]	Species identity	Host	Origin	GenBank accession numbers
CMW 2113	*Cryphonectria cubensis*	*Eucalyptus grandis*	South Africa	AF 046892, AF 273067, AF 273462
CMW 62	*C. cubensis*	*E. grandis*	South Africa	AF 292041, AF 273063, AF 273458
CMW 8755	*C. cubensis*	*E. grandis*	South Africa	AF 292040, AF 273064, AF 273459
CMW 8757	*C. cubensis*	*Eucalyptus*	Venezuela	AF 046897, AF 273069, AF 273464
CMW 8758	*C. cubensis*	*Eucalyptus*	Venezuela	AF 046898, AF 273068, AF 273463
CMW 9970	*C. cubensis*	*Miconia rubiginosa*	Colombia	AY 214291, AY 214219, AY 214255
CMW 9996	*C. cubensis*	*M. rubiginosa*	Colombia	AY 214292, AY 214220, AY 214256
CMW 10022	*C. cubensis*	*M. rubiginosa*	Colombia	AY 262389, AY 262393, AY 262397
CMW 10024	*C. cubensis*	*M. rubiginosa*	Colombia	AY 262390, AY 262394, AY 262398
CMW 10025	*C. cubensis*	*M. rubiginosa*	Colombia	AY 214293, AY 214221, AY 214257
CMW 10026	*C. cubensis*	*M. rubiginosa*	Colombia	AY 214294, AY 214222, AY 214258
CMW 10028	*C. cubensis*	*M. rubiginosa*	Colombia	AY 214295, AY 214223, AY 214259
CMW 9980	*C. cubensis*	*M. theaezans*	Colombia	AY 214297, AY 214225, AY 214261
CMW 9993	*C. cubensis*	*M. theaezans*	Colombia	AY 214298, AY 214226, AY 214262
CMW 10625	*C. cubensis*	*M. theaezans*	Colombia	---
CMW 10626	*C. cubensis*	*M. theaezans*	Colombia	AY 262392, AY 262396, AY 262400
CMW 10639	*C. cubensis*	*M. theaezans*	Colombia	AY 263419, AY 263420, AY 263421
CMW 10775	*C. cubensis*	*Syzygium aromaticum*	Brazil	AY 084003, AY 084015, AY 084027

CMW 10776	*C. cubensis*	*S. aromaticum*	Brazil	AY 084004, AY 084016, AY 084028
CMW 10777	*C. cubensis*	*S. aromaticum*	Brazil	AY 084005, AY 084017, AY 084029
CMW 8756	*C. cubensis*	*E. marginata*	Indonesia	AF 046896, AF 273077, AF 375606
CMW 2632	*C. cubensis*	*E. marginata*	Australia	AF 046893, AF 273078, AF 375607
CMW 3839	*C. cubensis*	*S. aromaticum*	Indonesia	AF 046904, AY 084011, AY 084023
CMW 1651	*C. parasitica*	*Castanea dentata*	USA	AF 046901, AF 273074, AF 273467
CMW 1652	*C. parasitica*	*C. dentate*	USA	AF 046902, AF 273075, AF 273468
CMW 10455	*C. radicalis*	*C. dentate*	Italy	AF 452113, AF 525705, AF 525712
CMW 10477	*C. radicalis*	*C. dentate*	Italy	AF 368328, AF 368347, AF 368346
CMW 10463	*C. macrospora*	*Castanopsis cuspidata*	Japan	AF 368331, AF 368351, AF 368350
CMW 10518	*C. nitschkei*	*Quercus sp.*	Japan	AF 452118, AF 525706, AF 525713
CMW 10435	*Endothia gyrosa*	*Q. palustris*	USA	AF 368325, AF 368337, AF 368336
CMW 10442	*E. gyrosa*	*Q. palustris*	USA	AF 368326, AF368339, AF368338
CMW 5288	*Diaporthe ambigua*	*Malus domestica*	South Africa	AF 543817, AF 543819, AF 543821
CMW 5587	*D. ambigua*	*M. domestica*	South Africa	AF 543818,AF 543820,AF 543822

[a] CMW refers to the culture collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Table 2. Specimens used in morphological comparisons.

Identity	Herbarium no.[a]	Linked culture[b]	Host	Origin	Date	Collector
Cryphonectria cubensis	PREM 57294	CMW 10639[c]	*Eucalyptus grandis*	Vanessa, Colombia	2000	M. J. Wingfield
C. cubensis	PREM 57517	CMW 2357 CMW 9996 CMW 10025 CMW 10026 CMW 10028 CMW 10022 CMW 10024	*Miconia rubiginosa*	Vanessa, Colombia	2001	C. A. Rodas

[a] PREM, National Collection of Fungi, Pretoria, South Africa.

[b] CMW refers to the culture collections of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.

[c] Isolate CMW 10639 did not originate from PREM 57294, but were collected from the same location.

Table 3. One way ANOVA analysis for lesion length measurements of Colombian *Cryphonectria cubensis* isolates from *Eucalyptus grandis* (CMW 10638, CMW 10639, CMW 10640), *Miconia theaezans* (CMW 10625, CMW 10626) and a negative control inoculated on *Tibouchina urvilleana* seedlings in the greenhouse.

Source	SS	df	MS	F	Pr > F
Isolate	33267.36	5	6653.47	5.66	0.0014
Error	28219.6	24	1175.81		

R-Square = 0.541047

CV = 55.63578

Table 4. One way ANOVA analysis for lesion length measurements of Colombian *Cryphonectria cubensis* isolates from *Miconia rubiginosa* (CMW 10022 and CMW 10024), a South African *C. cubensis* isolate (CMW 2113) and a negative control inoculated on *Tibouchina urvilleana* and *Eucalyptus grandis* (clone ZG14) seedlings in the greenhouse.

Source	SS	df	MS	F	Pr > F
Host	310624.68	7	44374.95	39.58	0.0001
Error	80714.3	72	1121.03		

R-Square = 0.793748

CV = 35.99711

Table 5. One way ANOVA analysis for lesion length measurements of Colombian *Cryphonectria cubensis* isolates from *Eucalyptus grandis* (CMW 10639), *Miconia theaezans* (CMW 10625) and a negative control inoculated on one-year-old *Tibouchina semidecandra*, *T. lepidota*, *T. urvilleana*, *M. theaezans* and *E. grandis* (clone 274) seedlings in Colombia.

Source	**SS**	**df**	**MS**	**F**	**Pr > F**
Isolate	380936.00	2	190468.00	65.59	0.0001
Host	645144.98	4	161286.24	55.54	0.0001
Isolate*Host	349986.03	8	43748.12	15.07	0.0001
Error	792756.03	273	2903.86		

R-Square = 0.629927

CV = 95.18900

Table 6. One way ANOVA analysis for lesion length measurements of a Colombian *Cryphonectria cubensis* isolate from *Miconia rubiginosa* (CMW 10022) and a negative control inoculated on three-year-old *Eucalyptus grandis* trees (clone 275), trees from a *E. grandis* and *E. urophylla* cross (*E. urograndis* 212) and six-year-old *M. rubiginosa* trees in Colombia.

Source	**SS**	**Df**	**MS**	**F**	**Pr > F**
Isolate	43156.02	2	21578.01	60.43	0.0001
Host	58174.31	2	29087.15	81.50	0.0001
Isolate*Host	24.37	1	24.37	0.07	0.7945
Error	29622.25	83	356.89		

R-Square = 0.728547

CV = 53.02294

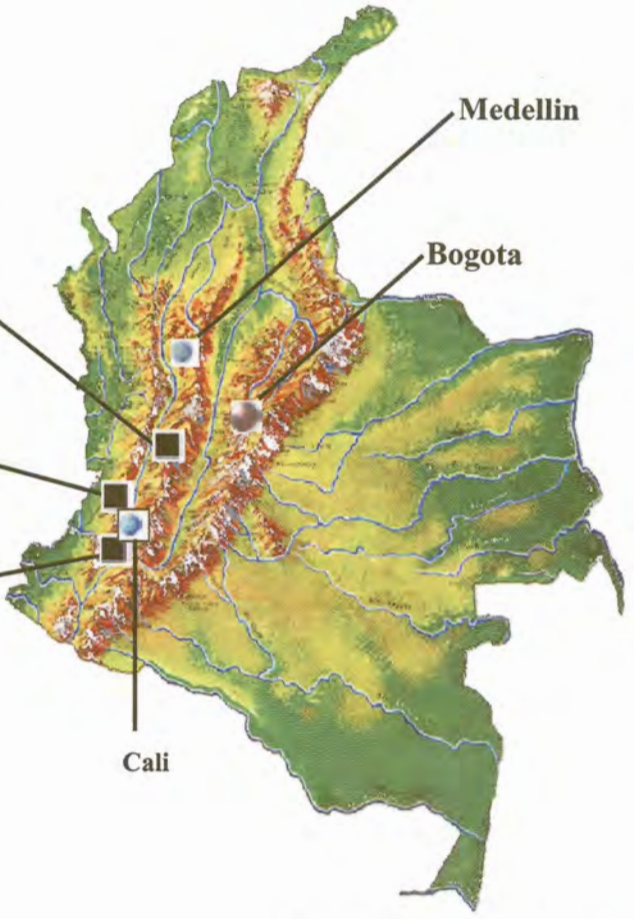
Fig. 1. Native Melastomataceae on which *Cryphonectria cubensis* was found and that were used in pathogenicity trials. **(a)**. *Tibouchina urvilleana*. **(b)**. *T. lepidota*. **(c)**. *Miconia theaezans*. **(d)**. *M. rubiginosa*. **(e)**. *T. semidecandra*.

Fig. 2. Map of Colombia showing co-ordinates, altitude and precipitation of the locations where *Cryphonectria cubensis* was discovered on various Melastomataceae, and where field trials were conducted.

Discovery of *Miconia theaezans*
Selva, Pereira, Risaralda
2048 masl, 3143 mm/y
75^0 35' 34" W and 4^0 47' 26" N

First field inoculation trial
Rancho Grande farm, Restrepo, Valle,
2102 masl, 2414 mm/y
76^0 26' 39" W and 4^0 1' 51" N

Discovery of *Miconia rubiginosa*
and second field inoculation trial
Vanessa, Timba, Cauca
1000 masl, 2365 mm/y
76^0 35' 15" W and 3^0 5' 42" N



Source: Instituto Geografico Agustin Codazzi

Fig. 3. Raw sequence data of the two regions sequenced within the β-tubulin gene (designated as β-tub 1a/1b and β-tub 2a/2b) and the ITS1, conserved 5.8S and ITS2 regions of the rDNA operon. The start of each region is indicated above the alignment. The exon regions of the β-tubulin gene, as well as the conserved 5.8S region of the rDNA operon, are indicated in red. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "-". Bases matching those of **CMW 2113** are indicated with a ".".

```
[                10        20        30        40        50        60        70        80        90]
[ β-tub 1a/1b →  .         .         .         .         .         .         .         .         .]
CMW 2113         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACGGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG ATGGCTGCCT
CMW 62           .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 8755         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 8756         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 2632         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 3839         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 8758         GA........ .......... ..A....... .......C.. .......... .......... .......... .......... ..........
CMW 8757         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 9970         .......... .......... .......... .......C.. ..TA...... .......... .......... .......... ..........
CMW 9996         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10025        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10026        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10028        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 9980         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 9993         .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10626        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10022        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10024        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10639        NNNNNNNNNN NNNG...... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10775        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10776        .......... .......... .......... .......C.. .......... .......... .......... .......... ..........
CMW 10777        .......... .......... .......... .......C.. .......... ........A. .......... .......... ..........
CMW 1651         .......... .......... .......... .CC.A..C.. C......... .......... .......... .......... ..........
CMW 1652         .......... .......... .......... .CC....C.. C......... .......... .......... .......... ..........
CMW 10455        .......... .......T.. .......... .CC....C.. T......... .......... .......... .......... ..........
CMW 10477        .......... .......T.. .......... .CC....C.. T......... .......... .......... .......... ..........
CMW 10463        .......... .......T.. .......... .CC....C.. T......... .......... .......... .......... ..........
CMW 10518        .......... .......T.. .......... .CC.A..C.. T......... .......... .......... .......... ..........
CMW 10435        .......... ......TT.. .......... .C.....C.. C......... .......... .......... .......... .....C....
CMW 10442        .......... ......TT.. .......... .C.....C.. C......... .......... .......... .......... .....C....
CMW 5288         .......... C......... ........T. .C.....C.. .......C.C .......... .......... .......... .....C....
CMW 5587         .......... C......... ........T. .C.....C.. .......C.C .......... .......... .......... .....C....
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[                100       110       120       130       140       150       160       170       180]
[                .         .         .         .         .         .         .         .         .]
CMW 2113    CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC CCCCGCCCCT CGCGCCTCGG GGCGCCTCGG CCGAAGCTCG
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... ..A..A.... ..........
CMW 8756    .......... .......... .......... .......... .......... .......... .......... ..A..A.... ........T.
CMW 2632    .......... .......... .......... .......... .......... .......... .......... ..A..A.... ........T.
CMW 3839    .......... .......... .......... .......... .......... .......... .......... ..A..A.... ........T.
CMW 8758    .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 8757    .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 9970    .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 9996    .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10025   .......... .......... .......... .......G.. .......... T......... .......... A.A..A.... ........T.
CMW 10026   .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10028   .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 9980    .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 9993    .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10626   .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10022   .......... .......... .......... ...A...... .......... T......... .......... A.A..A.... ........T.
CMW 10024   .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10639   .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10775   .......... .........T .....A.... .......... .......... T.......T. .........A A.A..A.... ........T.
CMW 10776   .......... .......... .......... .......... .......... T......... .......... A.A..A.... ........T.
CMW 10777   .......... .........T .......... .......... .......... T......... ........G.. A.A..A.... ........T.
CMW 1651    .......... .......... .........A. ....T..... ..........T TT.TTGT.T. T--------- ---------T ..TCGCAAGT
CMW 1652    .......... .......... .........A. ....T..... ..........T TT.TTG..T. T--------- ---------T ..TCGCAAGT
CMW 10455   .......... .......... .......... ....T..... ..........G GTTTTTTTT. TT.TT..TTC CC.CTTG.CT ..TCGCAAGT
CMW 10477   .......... .......... .......... ....T..... ..........G GTTTTTTTT. TT.TT..TTC CC.CTTG.CT ..TCGCAAGT
CMW 10463   .......... T......... .........A. ....T..... ..........T TT.TATA... T--------- ---------- -.TCGCAAGC
CMW 10518   .......... .........T .........T. ....T..... ..........T TT.T.TGT.. TCT------- ---------- ..TCGCAGGC
CMW 10435   .......... ......C..T .......... ....T..... ..........G GT-TC....C ACACA.C..C T.G-.GC.TT TG.GG.GCT.
CMW 10442   .......... ......C..T .......... ....T..... ..........G GT-TC....C ACACA.C..T T.G-.GC.TT TG.GG.GCT.
CMW 5288    .......... T..T...... .......... ....T..... ..........- ---------- GAGCATCT-- -----.CACA .GACCCAAGT
CMW 5587    .......... T..T...... .......... ....T..... ..........- ---------- GAGCATCT-- -----.CACA .GACCCAAGT
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[                 190        200        210        220        230        240          250        260        270]
[                  .          .          .          .          .          .           .          .          .]
CMW 2113     TCTGCTAACC CTCATCGTC- ---------- ---------- ---------- ---------- ---------- CAGCCGTGGC AAGGTCTCCA
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     ....T....T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 9996     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    .........T ..T....... .......... .......... .......... .......... .......... ..T....... ..........
CMW 10026    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    .......G.T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10024    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .........T ..T....... .......... .......... .......... .......... .......... .......... ..........
CMW 1651     C---------T .GAC-GAA.G TCTTG....G GCTGTTTGGC TAACCCTGTC TTTCTCTCTT CCCCTTCT.C T........T ..........
CMW 1652     C---------T .GAC-GAA.G TCTTG....G GCTGTTTGGC TAACCCTGTC TTTCTCTCTT CCCCTTCT.C A........T ..........
CMW 10455    .---------T .GAT-AAAGT CGTCTCT..G GCTTGTTTGC TAACC.TGTT TCTCTCCCCC CCCCCCCAAC .......... ..........
CMW 10477    .---------T .GAT-AAAGT CGTCTCT..G GCTTGTTTGC TAACC.TGTT TCTCTCCCCC CCCCCCCAAC .......... ..........
CMW 10463    C---------T .GAT-GAA.A TCTCG....G GCTTCTTGGC TAACCCCACG TTTCTCTCTT TC...CT..C T.......... ..........
CMW 10518    C---------T .CAT-GAA.A TCTTG....G GCTTTTTGGC TAACCCCATG TTTCTCTCTT TCCCCCTT.C T.......... .G........
CMW 10435    ..A.GGCTTG T.TT.T.CTG ACCCC..... .......... TATCCCTC.. .......... .........C -.......... ..........
CMW 10442    ..A.GGCTTG T.TT.T.CTG ACCCC..... .......... TATCCCTC.. .......... .........C -.......... ..........
CMW 5288     ---------- --GT.T.CGC GCTGACACTG TCTT...... .......... .......... .........C T........A ..........
CMW 5587     ---------- --GT.T.CGC GCTGACACTG TCTT...... .......... .......... .........C T........A ..........
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```
[                280       290       300       310       320       330       340       350       360]
[                .         .         .         .         .         .         .         .         .]
CMW 2113    TGAAGGAGGT CGAGGACCAG ATGCGCAACG TCCAGAGCAA GAACTCGTCC TACTTCGTCG AGTGGATCCC CAACAACGTC CAGACCGCCC
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... T......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... T......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... T......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... T......... ........T. .......... .......... ........A. .......... .......... ..........
CMW 8757    .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 9970    .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 9996    .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10025   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10026   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10028   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 9980    .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 9993    .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10626   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10022   .......... T......... ........T. .......... .......... .......... .......... ........A.. ..........
CMW 10024   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10639   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10775   .......... T......... ........T. .........G .......... .......... ..G....... .......... ..........
CMW 10776   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 10777   .......... T......... ........T. .......... .......... .......... .......... .......... ..........
CMW 1651    .......A.. .......... .......... .......... .......... .......... .......... .......T.. ..........
CMW 1652    .......A.. .......... .......... .......... .......... .......... .......... .......T.. ..........
CMW 10455   .......... A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10477   .......... A......... .......... .......... .......... .......... .......... .......... ..........
CMW 10463   .......... .......... .......... .......... .......... .......... .A........ .......... ..........
CMW 10518   .......... .......... ..........T .......... .......... .......... .A........ .......... ..A.......
CMW 10435   .......... .........A .......... .......... .......... .......... .......... .......... ..........
CMW 10442   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5288    .......... .......... .......... .......... .......... .......... .A.....T.. .......... ..........
CMW 5587    .......... .......... .......... .......... .......... .......... .A.....T.. .......... ..........
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[                370        380        390        400        410        420        430        440        450]
[                .          .          .          .          .          .          .          .          .]
CMW 2113    TCTGCTCCAT CCCCCCCAAG GGTCTCAAGA TGTCCTCCAC CTTTGTTGGC AACTCCACTG CCATCCAGGA GCTCTTCAAG CGTATCGGCG
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025   .G........ .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022   .......... .......... .......... ..A....... .......... .......... .......... .......... ..........
CMW 10024   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651    .......... T......... ..C....... .......G.. ......C... .........C. .......... .......... ...G......
CMW 1652    .......... T......... ..C....... .......G.. ......C... .........C. .......... .......... ...G......
CMW 10455   .......... .......... ..C....... .......... ......C... .........C. .......... .......... ...G......
CMW 10477   .......... .......... ..C....... .......... ......C... .........C. .......... .......... ...G......
CMW 10463   .......... .......... ..C....... .......T.. ......C... .........C. .......... .......... ...G......
CMW 10518   .......... ......T... ..C....... .......T.. ......C... .........C. .......... .......... ...G......
CMW 10435   .......... .....:.G.. .......... .......... .......... .....T..C. .......... ...T...... ..CG.T....
CMW 10442   .......... ........G. .......... .......... .......... .....T..C. .......... ...T...... ..CG.T....
CMW 5288    .G.....G.. ...T...... .......... .......T.. ...C.....T .....G.... .T......... ...G...... ...G......
CMW 5587    .G.....G.. ...T...... .......... .......T.. ...C.....T .....G.... .T......... ...G...... ...G......
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[                460       470       480       490       500       510       520       530       540]
[                .         .         .         .         .  β-tub 2a/2b      .         .          .]
CMW 2113     AGCAGTTCAC TGCTATGTTC CGTCGCAAGG CTTTCTTGCA TTGGTACACT GGCAAACCAT CTCTGGCGAG CACGGCCTCG ACAGCAA-TG
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... C......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... C......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651     .......T.. C..C...... ..G....... .......... .......... .......... ...C...... .......... ..........
CMW 1652     .......T.. C..C...... ..G....... .......... .......... .......... ...C...... .......... ..........
CMW 10455    .......... C..C...... .......... .......... ......T... .......... ...C...... .......... ......G...
CMW 10477    .......... C..C...... .......... .......... ......T... .......... ...C...... .......... ......G...
CMW 10463    .......... C..C...... .......... .......... .......... .......... ...C...... ..T..T.... ..........
CMW 10518    .......... C..C...... .......... .......... .......... .......... ...C...... ..T....... ..........
CMW 10435    .......... C..C...... .......... .C........ .........C .......... ...C...... .......... .....G....
CMW 10442    .......... C..C...... .......... .C........ .........C .......... ...C...... .......... .....G....
CMW 5288     .......... ...C...... A.G....... .......... .......... .......... .......... .....T.... ..........
CMW 5587     .......... ...C...... A.G....... .......... .......... .......... .......... .....T.... ..........
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[                 550       560          570         580         590         600         610         620       630]
[                  .         .            .           .           .           .           .           .        .]
CMW 2113     GCGTGTACGT ---ACCCTCC TGTTGCACCA GGCGG----- ---------- -CGCGCCTC- --GAGCTT-C CC-GCTGACC A-CTGCACAG
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .......... .......... ..C....... .......... .......... .......... .......... .......... ...C......
CMW 2632     .......... .......... ..C....... .......... .......... .......... .......... .......... ...C......
CMW 3839     .......... .......... ..C....... .......... .......... .......... .......... .......... ...C......
CMW 8758     .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 9970     .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 9996     .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10025    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10026    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10028    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 9980     .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 9993     .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10626    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10022    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10024    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10639    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... ..C....... .......... .......... .......... .......... .......... ..........
CMW 1651     .......... ........AT CT------.GG CTT--CCCAA G.CAAGACAG A....A..T. ........T. .T........ ...CA..T..
CMW 1652     .......... ........AT CT------.GG CTT--CCCAA G.CAAGACAG A....A..T. ........T. .T........ ...CA..T..
CMW 10455    .......T.. ......G.G. CT.ACAC.GG CTTT.CCCAG A.CAAGACAG A.......T. .......CT. .TT....... .A.CA.....
CMW 10477    .......T.. ......G.G. CT.A-....G .CTTTGCCCA GACAAGACAG A.......T. .......CT. .TT....... .A.CA.....
CMW 10463    .......T.. ........A. CT-------G .CTT-CCCCA G.CAAGATAG A....A..T. GT......T. .T.......G ...CA..T..
CMW 10518    .......T.. .....T..A. CT-------G .CTT-CCCCA G.CAAGATAG A....A..T. GT...AC.T. .T......T. ...CA..T..
CMW 10435    .......T.. TGT.....A. ..C..----- -----CCCGG CC.......G A......... .G.GC.C.T. ..C....... ...C......
CMW 10442    .......T.. TGT.....A. ..C..----- -----CCCGG CC.......G A......... .G.GC.C.T. ..C....... ...C......
CMW 5288     .......... ......TCGT ATC------- -----CCCTG CCCACTGGTC T..TC.TCTC CCTC.GC.TG G..A.....A ..........
CMW 5587     .......T.. ......TCGT ATC------- -----CCCTG CCCACTGGTC T..TC.TCTC CCTC.GC.TG G..A.....A ..T.......
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[                640       650       660       670       680       690       700       710       720]
[                .         .         .         .         .         .         .         .         .]
CMW 2113    CTACAACGGC ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TC-TGT---- ----CGG--G AC-CA-GGCT
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .T........ .......... .......... .......... .......... .......... .......T..
CMW 2632    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651    .......... .......... .......... .......... .........T .......... ....TAT... ........GT. .T...A.-..
CMW 1652    .......... .......... .......... .......... .........T .......... ....TAT... ........GT. .TA..A.-..
CMW 10455   ......T... .......... .......... .......... .......... .......... ..C.TATCAT CCAT...GT. .....A....
CMW 10477   ......T... .......... .......... .......... .......... .......... ..C.TATCAT CCAT...GT. .....A....
CMW 10463   .......... .......... .......... .......... .......... .......... ....TAT... ........GT. ..........
CMW 10518   .......... .......... .......... .......... .......... .......... ....TAT... ........GT. ..........
CMW 10435   .......... .......... .......... .......... .......... .......... ...-TAT... ....G..G.. .........C
CMW 10442   .......... .......... .......... .......... .......... .......... ...-TAT... ....G..G.. .........C
CMW 5288    T......... ..T....... .......... .......... .......... ........A. ..AACAGCCA CGTCGTCAAT T.AA.TTTGA
CMW 5587    T......... ..T....... .......... .......... .......... ........A. ..AACAGCCA CGTCGTCAAT C.AA.TTTGA
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[                730       740       750       760       770       780       790       800       810]
[                .         .         .         .         .         .         .         .         .]
CMW 2113     GGCGCGTCA- --------TC CCGCCCGCGA ACCCCCTGTG CGT-----GA CCGAGCTCCC G--------- --------CT GACGCGCTCC
CMW 62       ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    ..G..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651     CAA.-C.T-C ACCTCGGC-A A.C...C.CC C..TTTCCG. G.CCTT.... .TTCTGGTAT AGGCGAGCTT CC.TCTT... .........T.
CMW 1652     ACAAGC.TCC ACCTGGGCCA A.C...C.CC C..TTTCCG. G.CCTTCT.. .TTCTGGTAT AGGCGAGCAT CC.TCTT... .........T.
CMW 10455    CAA..A..CA TCTCGACC.T GG....C.C. .......C.C .C-....... .TTCTGG.AT AGGCGAAGTT CCCTCTTT.. .........TT
CMW 10477    CAA..A..CA TCTCGACC.T GG....C.C. .......C.C .C-....... .TTCTGG.AT AGGCGAAGTT CCCTCTTT.. .........TT
CMW 10463    C.A..A..CA TCTCAACCC. ..C...CTCC CAAAT.CCG. GCCCCTC... .TTCTGG.AT AGGCGAGCTT CC.TCTT... .........T.
CMW 10518    C.A..A..CA TCTCAGCCCA ..C.TGTTCC ---------- ---.CTC.C. .TTCTGGTA. AGGCGAGCTT CC.TCTT... .........T.
CMW 10435    CTG------. .....GCG.G G.C..GC.CG CGG...CTG. ---..CGT.. .......... A......... .......... ..........
CMW 10442    CTG------. .....GCG.G G.C..GC.CG CGG...CTG. ---..CGT.. .......... A......... .......... ..........
CMW 5288     CAAC.TA.GG CA.....-- -T.GTTT--- ---------- .CCGCCGTCG .---------- -CAAGGCCTT G......... A....AT.TA
CMW 5587     CAAC.TA.GG CA.....-- -T.GTTT--- ---------- ..CGCCGTCG .---------- -CAAGGCCTT G......... A....AT.TA
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[                820       830       840       850       860       870       880       890       900]
[                .         .         .         .         .         .         .         .         .]
CMW 2113    -TGTCACAGG CCTCCGGCAA CAAGTATGTC CCCCGCGCCG TCCTCGTCGA TCTCGAGCCC GGCACCATGG ACGCCGTCCG TGCCGGCCCC
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .........T .......... .......... .........T .......... .......... ..........
CMW 2632    .......... .......... .........T .......... .......... .........T .......... .......... ..........
CMW 3839    .......... .......... .........T .......... .......... .........T .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651    T....-.... .......... .........T .......... .......... .......... ..T....... .T....... C..T......
CMW 1652    T....-.... .......... .........T .......... .......... .......... ..T....... .T....... C..T......
CMW 10455   T....-.... .T....C... .........T .......... .......... .........G ..T....... .T....... C..........
CMW 10477   T....-.... .T....C... .........T .......... .......... .........G ..T....... .T....... C..........
CMW 10463   T.A..-.... .....A.... .........T ........A. .......... .......... ..T....... .T....... C..........
CMW 10518   T.A..-.... .....A.... .........T ........T. .......... .......... ..T....... .......... C..........
CMW 10435   .....-.... .......... .......... .......... .......... .......... ..T....... .......... C..........
CMW 10442   .....-.... .......... .......... .......... .......... .......... ..T....... .......... C..........
CMW 5288    TC.C.-.... .......... .........G ..T....... .......... .......... ..T....... .......... ......T...
CMW 5587    TC.C.-.... .......... .........G ..T....... .......... .......... ..T....... .......... ......T...
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[                910       920       930       940       950       960       970       980       990]
[                .         .         .         .      ITS 1 →      .         .         .         .]
CMW 2113    TTCGGCCAGC TGTTCCGCCC CGACAACTTC GTCTTCGGCC AGTCCCCCAG ATACCCTTTG TGAACTTATA -CCTTTTTAT CGTTGCCTCG
CMW 62      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022   .......... .......... .......... .......... .......... ...G...... .......... ......G...
CMW 10024   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639   .......... .......... .......... .......... .......... ........G. .......... .......... ..........
CMW 10775   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777   .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651    ..T..T.... .......T.. .......... .....T.... .......... .......... .......... ...A..A..... ..........
CMW 1652    ..T..T.... .......T.. .......... .....T.... .......... .......... .......... ...A..A..... ..........
CMW 10455   ..T..T.... .......T.. .......... .....T.... .......... .......... .......... ....A..... ..........
CMW 10477   ..T..T.... .......T.. .......... .....T.... .......... .......... .......... ....A..... ..........
CMW 10463   ..T..T.... .......T.. .......... .....T.... .......... .......... ......C... ....A..... ..........
CMW 10518   ..T..... .......T.. .......... .....T.... .......... .......... ......C... ....A..... ..........
CMW 10435   .......... .......... .......... .......... .......... .......... .......... ....A..... ..........
CMW 10442   .......... .......... .......... .......... .......... .......... .......... ....A..... ..........
CMW 5288    .......... .......... .......... .......... .......... .A........ .......... ....----.. ..........
CMW 5587    .......... .......... .......... .......... .......... .A........ .......... ....----.. ..........
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[                    1000       1010       1020       1030       1040       1050       1060       1070     1080]
[                    .          .          .          .          .          .          .          .          .]
CMW 2113      GCGCCGAGCC --GGGAGTGC TCTTCTGTGC ---------- ---------- --------TC CCC------- ---------- ------CACC
CMW 62        .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993      .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775     .........T .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651      ....T..... TCT..G.G-- ---------.G GGTTGGCGAA GGCAGATTTT CTTCCTTC.. ....TCCCTC CCCCCCCT.. ..CTTC....
CMW 1652      ....T..... TCT..G.G-- ---------.G GGTTGGCGAA GGCAGATTTT CTTCCTTC.. ....TCCCTC CCCCCCCT.. ..CTTC....
CMW 10455     ....T..... CG...G--AG GGAAAAAAAA AAAAAAAAGG GGGGAAATTC TGTTTCCCCT TTTC.TTTTT CCCCCCCTTC CCCTTCAT..
CMW 10477     ....T..... C....G.GAG GGAAAAAAAA AAAAAAAAGG GGGGAAATTT GTTTCCCCCT TTTTTTTTTT CCCCCCCTTC CCCTTTAT..
CMW 10463     ....T..... CC...G.G.A .T..T--GAG AGAGTC..TC TCTCTCCTTC CTTC.....-- -T.GC..... .....CTTCT ACC.------
CMW 10518     ....T..... CC...G.G.A .T..T--GAG AGAGTC..TC TCTCTCCTTC CTTC.....-- -T.GC..... .....CTTCT C.---------
CMW 10435     ....T.....- ..T..G.GC- ---------- .......... ....ACTCTC CTGTG..CC. ...C...... .....ACCGT GCAAGCG---
CMW 10442     ....T.....- ..T..G.GC- ---------- .......... ....ACTCTC CTGTG..CC. ...C...... .....ACCGT GCAAGCG---
CMW 5288      ...AA.GC.G GCC------- ---------- .......... .......... .......... .......... .....ACCGA GGCCCCTTGG
CMW 5587      ...AA.GC.G GCC------- ---------- .......... .......... .......... .......... .....ACCGA GGCCCCTTGG
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[                  1090       1100       1110       1120       1130       1140       1150       1160     1170]
[                  .          .          .          .          .          .          .          .          .]
CMW 2113     GCGCAAGCAG TG------GA GCAGGCCCGC CGGCGGCCCA CCAAACTCTT TGTTTTTAGA A-CGTATCTC TTCTGAGTGT TTATAACAAA
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .T........ .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .T........ .......... .......... .......... ..........
CMW 1651     .T....A.G. .TGTTGGG.. .......... .......... .T........ ........T. ...C...... ........AC A..A.-....
CMW 1652     .T....A.G. .TGTTGGG.. .......... .......... .T........ ........T. ...C...... ........AC A..A.-....
CMW 10455    .T.T..AATC G.GTGCTG.. .....G.... .......... TT........ ..G.....A. .A.C...... ..T.....T- -..A..A...
CMW 10477    .G.A..AATC G.GGGCTG.. A.......C. .......... TT.....T.. ........A. ...C.....T ..T.....T- -..A..A...
CMW 10463    .T....A.G. .TGTT..G.. .......... .......... .......... ........T. ...C...... ........AC A.T...A...
CMW 10518    .T....A.G. .TGTT..G.. .......... .......... .......... ........T. ...C...... ........AC A.T...A...
CMW 10435    .T.------- --........ .......... .......... .......... .......... C......... C......... ...C..A..-
CMW 10442    .T.------- --........ .......... .......... .......... .......... C......... C......... ...C..A..-
CMW 5288     .AA...--- --........ ....-..... ........A. .......... -....C.TAG T.-.A..--- C.....--- -..A..A...
CMW 5587     .AA...--- --........ ....-..... ........A. .......... -....C.TAG T.-.A..--- C.....--- -..A..A...
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[                1180       1190       1200       1210       1220       1230       1240       1250     1260]
[                .    5.8S →          .          .          .          .          .          .          .]
CMW 2113     CAAA--TGAA TCAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651     A...AA.... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1652     A...AA.... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10455    A...A..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10477    A...A..... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10463    A...A..... .......... .......... .......... .......... ....A..... .......... .......... ..........
CMW 10518    A...A..... .......... .......... .......... .......... ....A..... .......... .......... ..........
CMW 10435    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10442    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5288     ..T.AA.... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5587     ..T.AA.... .......... .......... .......... .......... .......... .......... .......... ..........
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[                  1270        1280        1290        1300        1310        1320        1330        1340     1350]
[                  .           .           .           .           .           .           .           ITS 2 →    .]
CMW 2113     GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CCGCTGGAAT TCCAGCGGGC ATGCCTGTTC GAGCGTCATT TCAACCCTCA
CMW 62       .......... .......... .......... .......... ..CG...... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... ..CG...... .......... .......... .......... ..........
CMW 8756     .......... .......... .......... .......... ..CG...... .......... .......... .......... ..........
CMW 2632     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 3839     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8758     .......... .......... .......... .......... ..CG...... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9996     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10024    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651     .......... .....G.... .......... .......... .......... .......... ....-..... .......... ..........
CMW 1652     .......... .....G.... .......... .......... .......... .......... ....-..... .......... ..........
CMW 10455    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10477    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10463    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10518    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10435    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10442    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 5288     .......... .......... .......... .......... ..T....T.. ...G.A.... .......... .......... ..........
CMW 5587     .......... .......... .......... .......... ..T....T.. ...G.A.... .......... .......... ..........
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[                  1360       1370       1380       1390       1400       1410       1420       1430     1440]
[                  .          .          .          .          .          .          .          .          .]
CMW 2113     AGCCTGGCTT GGTGTTGGGG CACTACCTGT TC-ACAGCGG GTAGGCCCTG AAATTTAATG GCGGGCTCGC TAAGACTCTG AGCGTAGTAG
CMW 62       .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8755     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8756     .......... .......... ....G..... .TT....... .......... .......G.. .......... .......... .A........
CMW 2632     .......... .......... ....G..... .TT....... .......... .......G.. .......... .......... ..........
CMW 3839     .......... .........N ....G..... .TT....... .......... .......G.. .......... .......... ..........
CMW 8758     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 8757     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9970     .......... .......... .......... .......... .......... .......... .......... .N........ ..........
CMW 9996     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10025    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10026    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10028    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9980     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 9993     .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10626    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10022    .......... .......... .......CT. .......... .......... .......... .......... .......... ..........
CMW 10024    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10639    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10775    .......... .......... .......... .T........ ..G....... .......... .......... .......... ..........
CMW 10776    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 10777    .......... .......... .......... .......... .......... .......... .......... .......... ..........
CMW 1651     ...T...... .......... T......C.. AA.--.A... -......... .......G.. .......... .......... ...C......
CMW 1652     ...T...... .......... T......C.. AA.--.A... -......... .......G.. .......... .......... ..........
CMW 10455    ...T.A.... .......... ......TC.. AA.--.A... -......... ....C..G.. .......... .......... ..........
CMW 10477    ...T.A.... .......... ......TC.. AA.--.A... -......... ....C..G.. .......... .......... ..........
CMW 10463    .......... .......... T......C.. CA.--.A... -......... .......G.. .......... .......... ..........
CMW 10518    .......... .......... T......C.. CA.--.A... -......... .......G.. .......... .......... ..........
CMW 10435    .......... .......... .......... A..--.A... -......... .......G.. .......... .......... ..........
CMW 10442    .......... .......... .......... A..--.A... -......... .......G.. .......... .......... ..........
CMW 5288     .......... ....A..... ....G.T.CC GAG.GG.A.- -C........ ....C..G.. ...A...... C.G...C.C. ..........
CMW 5587     .......... ....A..... ....G.T.CC GAG.GG.A.- -C........ ....C..G.. ...A...... C.G...C.C. ..........
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[                    1450        1460        1470        1480        1490  1500]
[                    .           .           .           .           .        .]
CMW 2113     TTTTTAT--- ---CACCTCG CTTTGGAA-G GATTAGCGG- TGCTCTTGCC GTAAAACC
CMW 62       .......... .......... .......... .......... .......... ........
CMW 8755     .......... .......... .......... .......... .......... ........
CMW 8756     .......... .......... .......... .....CGA.. ...C...... ........
CMW 2632     .......... .......... .......... .......... .......... ........
CMW 3839     .......... .......... .......... .......A.. .......... ........
CMW 8758     .......... .......... .......... .......... .......... ........
CMW 8757     .......... .......... .......... .......... .......... ........
CMW 9970     .......... .......... .......... .......... .......... .....C..
CMW 9996     .......... .......... .......... .......... .......... ........
CMW 10025    .......... .......... .......... .......... .......... ........
CMW 10026    .......... .......... .......... .......... .......... ........
CMW 10028    .......... .......... .......... .......... .......... ........
CMW 9980     .......... .......... .......... .......... .......... ........
CMW 9993     .......... .......... .......... .......... .......... ........
CMW 10626    .......... .......... .......... .....T.... .......... .......T
CMW 10022    .......... .......... .......... ......NNN. NNNNNNNNNN NNNNNNNN
CMW 10024    .......... .......... .......... .......... .......... .......T
CMW 10639    .......... .......... .......... .......... .......... ........
CMW 10775    .......... .......... .......... .......... .......... ........
CMW 10776    .......... .......... .......... .......... .......... ........
CMW 10777    .......... .......... .......... .......... .........G ........
CMW 1651     .....T.TTC TTCA...... .......... .........T .......... ........
CMW 1652     .....T.TTC TTCA...... .......... .........T .......... ........
CMW 10455    .....T.TTC TTC....... ........A. .........T ....T..... ........
CMW 10477    .....T.TTC TTC....... ........A. .........T ....T..... ........
CMW 10463    .....T-... ..CA...... .......... .........T .......... ........
CMW 10518    .....T-... ..CA...... .......... .........T .......... ........
CMW 10435    ..--...... .......... .......... .........T -......... ........
CMW 10442    ..--...... .......... .......... .........T -......... ........
CMW 5288     ..A-...... ...---.... ..CC....G. CCC.G....T -..C..-... ..T.....
CMW 5587     ..A-...... ...---.... ..CC....G. CCC.G....T -..C..-... ..T.....
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Fig. 4. The phylogenetic tree (tree length = 1198 steps, consistency index/CI = 0.8, retention index/RI = 0.9) generated from a combined data set comprising ribosomal and β-tubulin gene sequences. Confidence levels of the tree branch nodes >50% are indicated and were determined by a 1000 replicate bootstrap analysis. Isolates sequenced in this study are bolded. Host species for *C. cubensis* are indicated in capital letters. The *Diaporthe ambigua* isolates were used as the outgroup taxa.

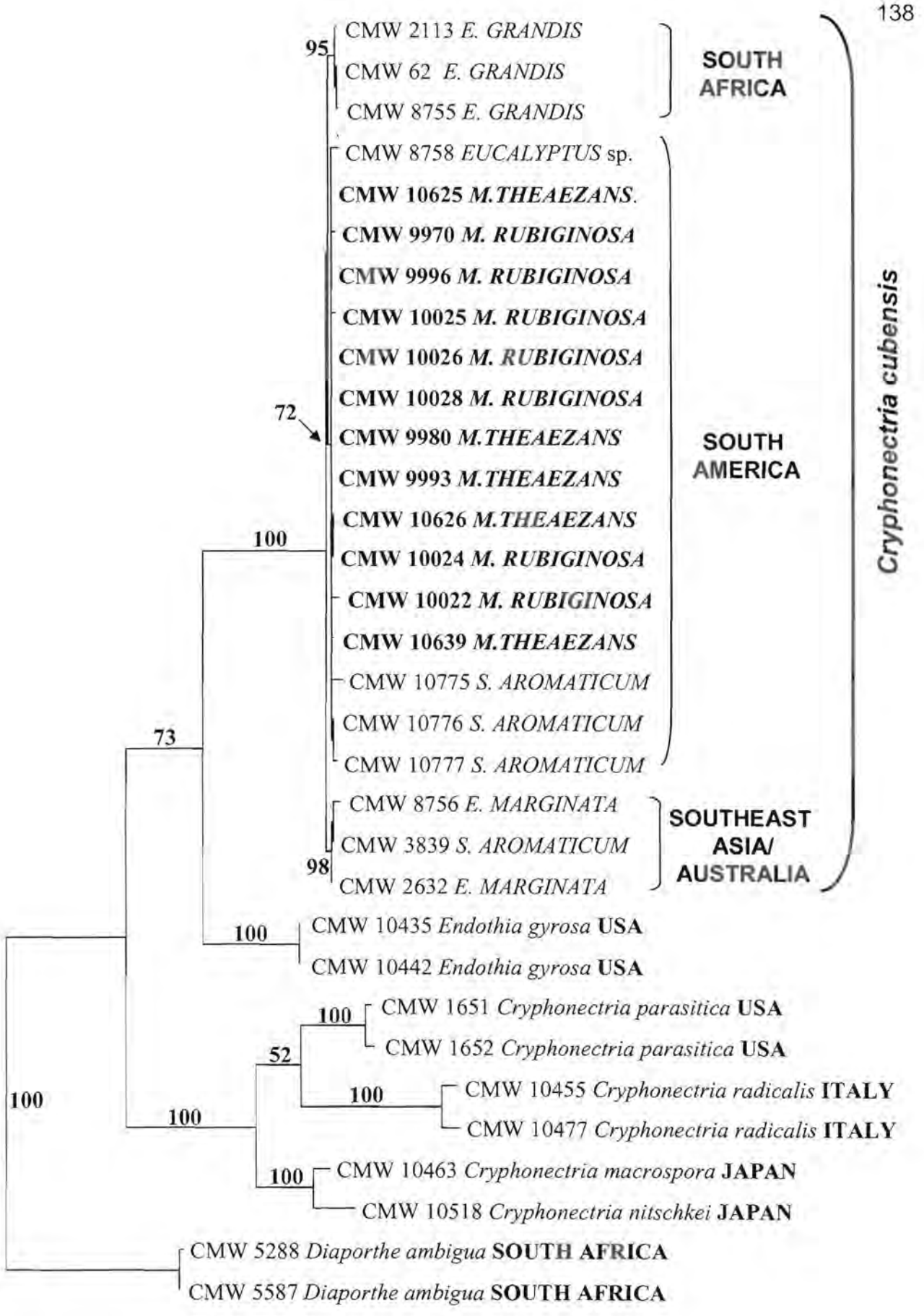

Fig. 5. Light micrographs of *Cryphonectria cubensis* on *Miconia rubiginosa* in Colombia. **(a)**. Perithecial neck and orange stromatic tissue (arrow) on bark. **(b)**. Vertical section through ascoma. **(c)**. Perithecial neck and surrounding tissue (arrow). **(d)**. Stromatic tissue of ascoma. **(e)**. Ascus. **(f)**. Ascospores. **(g)**. Conidioma on bark. **(h)**. Vertical section through conidioma. **(i)**. Tissue of the conidiomal base and neck (arrow). **(j)**. Conidiophores. **(k)**. Enteroblastic phialidic conidiogenous cell (arrow). **(l)**. Conidia. Bars a-b, g-h = 100 µm; c-d, i = 20 µm; e-f, j-l = 10 µm.

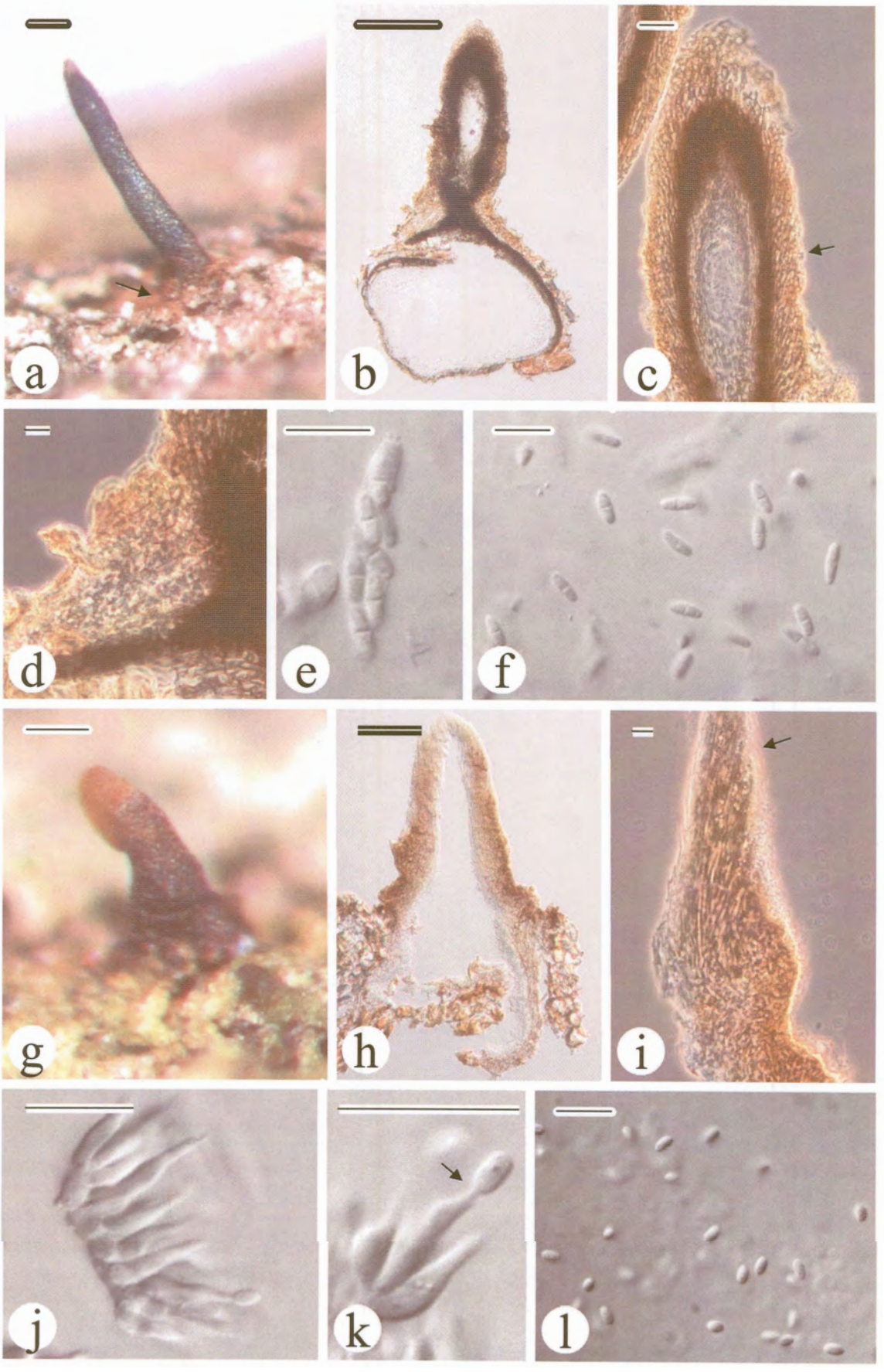

Fig. 6. Results of inoculation trial with isolates of *Cryphonectria cubensis* from *Miconia theaezans* (CMW 10625, CMW 10626) and *Eucalyptus grandis* (CMW 10640, CMW 10638, CMW 10639) from Colombia, and a negative control. Inoculations were done in a greenhouse on seven-month-old *Tibouchina urvilleana*. Mean length of lesions is shown with 95% confidence limits.

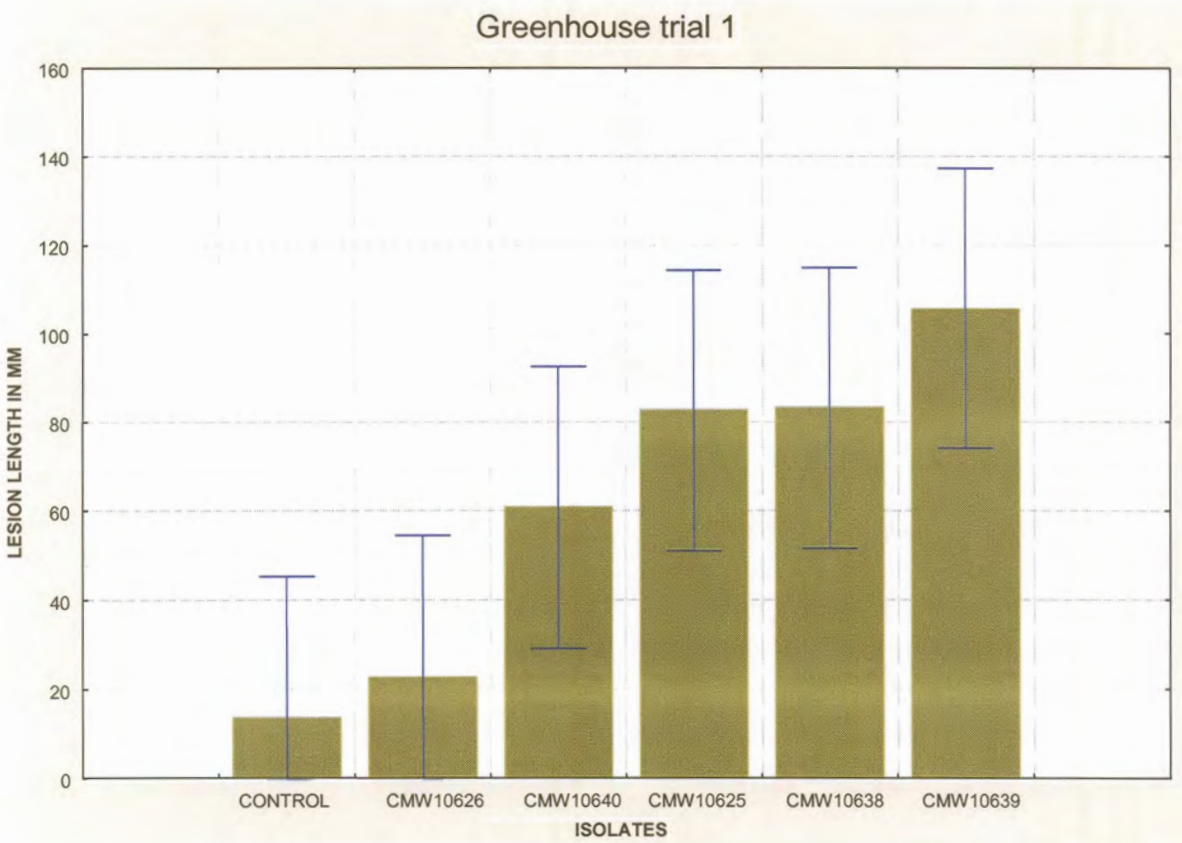
Greenhouse trial 1
LESION LENGTH IN MM
160
140
120
100
80
60
40
20
0
CONTROL
CMW10626
CMW10640
CMW10625
CMW10638
CMW10639
ISOLATES

Fig. 7. Results of inoculation trials in the greenhouse with isolates of *Cryphonectria cubensis* from *Miconia rubiginosa* (CMW 10022, CMW 10024) and a negative control. Inoculations were done on one-year-old *Tibouchina urvilleana* and a ZG14 clone of *Eucalyptus grandis*. A *C. cubensis* isolate from *E. grandis* in South Africa (CMW 2113) was also included. Mean length of lesions is shown with 95% confidence limits.

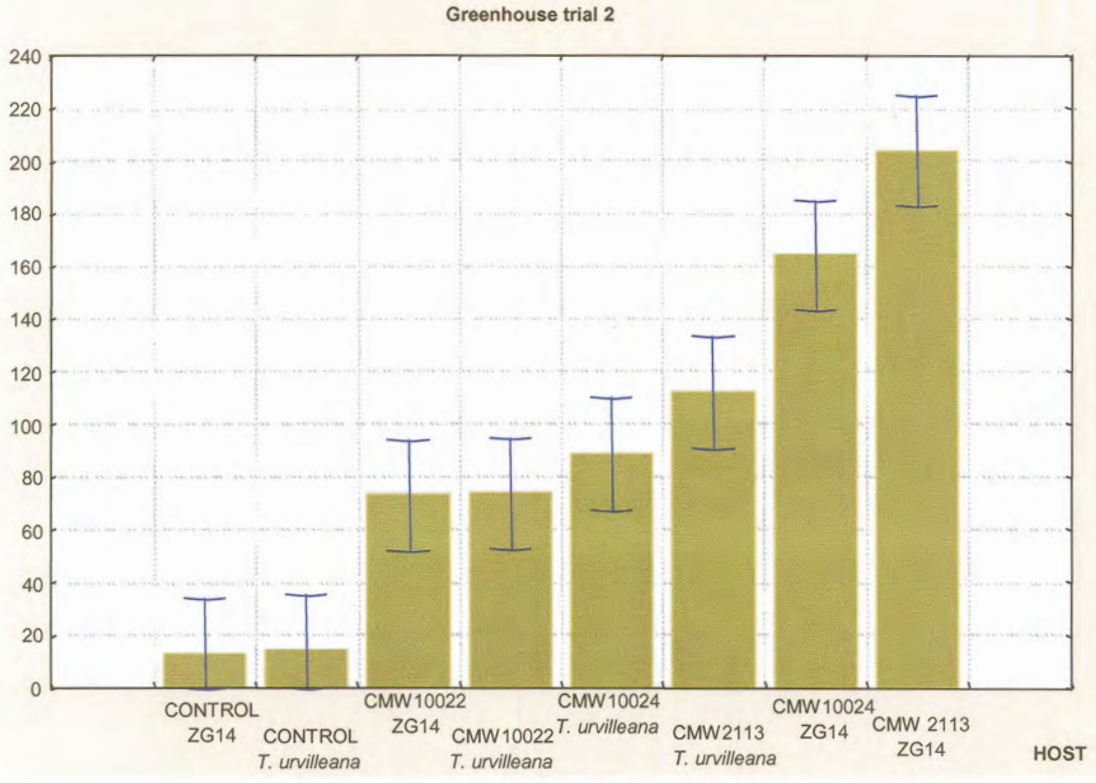

Fig. 8. Lesions produced by isolates of *Cryphonectria cubensis* from *Miconia theaezans* (CMW 10625) and *M. rubiginosa* (CMW 10022) on various hosts inoculated in field trials in Colombia. Control inoculations are indicated with a "c". **(a)**. Lesions on *Tibouchina urvilleana* inoculated with isolate CMW 10625. **(b)**. Lesions on *T. lepidota* inoculated with isolate CMW 10625. **(c)**. Lesions on *T. semidecandra* inoculated with CMW 10625. **(d)**. Lesions on *M. theaezans* inoculated with CMW 10625. **(e)**. Lesions on an *Eucalyptus grandis* clone (274) inoculated with CMW 10625. **(f)**. Lesions on *M. rubiginosa* inoculated with CMW 10022. **(g)**. Lesions on an *E. grandis* clone (275) inoculated with CMW 10022. **(h)**. Lesions on a cross between *E. grandis* and *E. urophylla* (*"E. urograndis"* 212) inoculated with isolate CMW 10022.

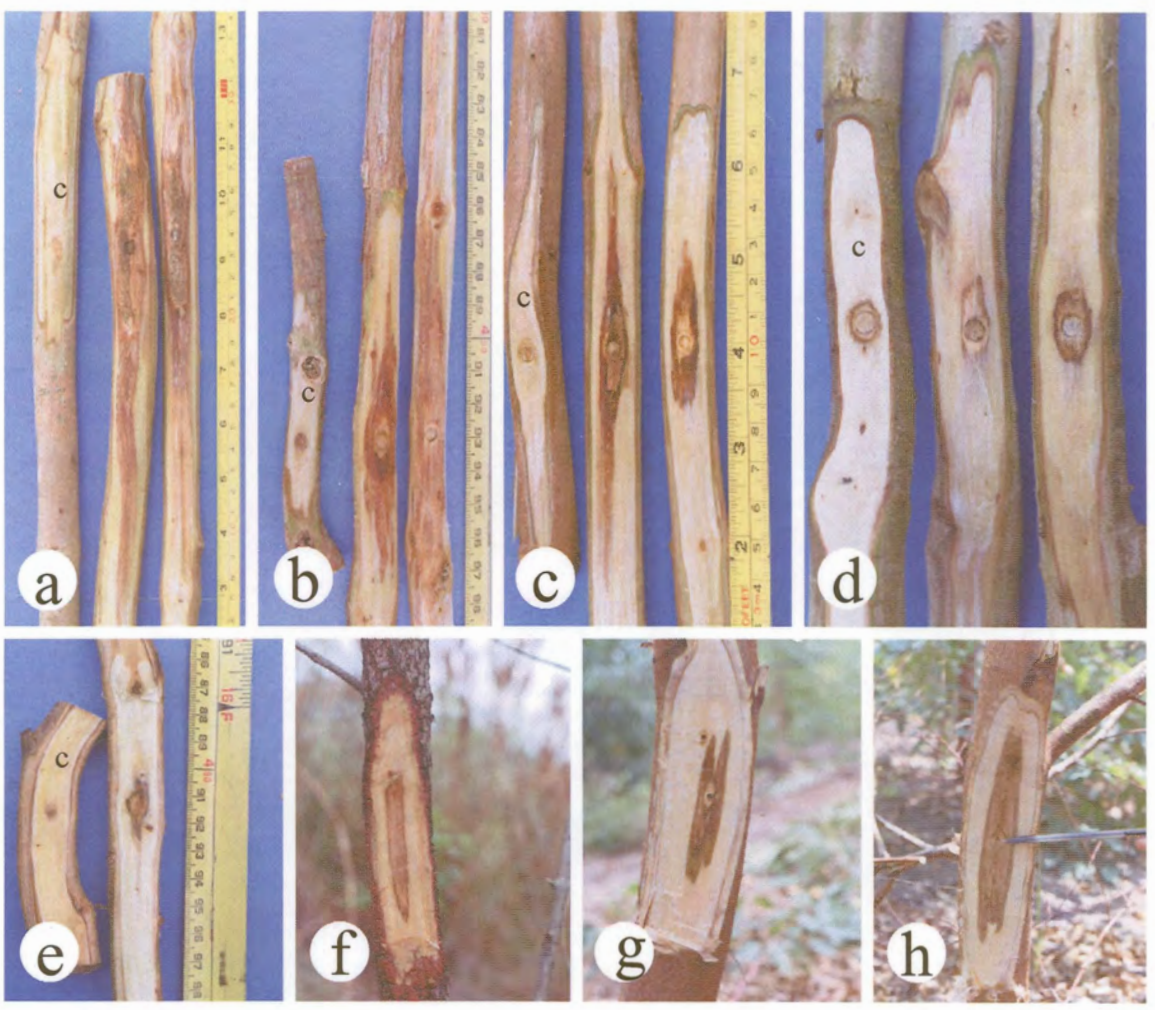

Fig. 9. Results of inoculation trials with isolates of *Cryphonectria cubensis* from *Miconia theaezans* (CMW 10625) and *Eucalyptus grandis* (CMW 10639) from Colombia, and a negative control. The field inoculations were done in Colombia on one-year-old *Tibouchina urvilleana*, *T. lepidota*, *T. semidecandra*, *M. theaezans* and an *E. grandis* clone (274). Mean lesion length is shown with 95% confidence limits.

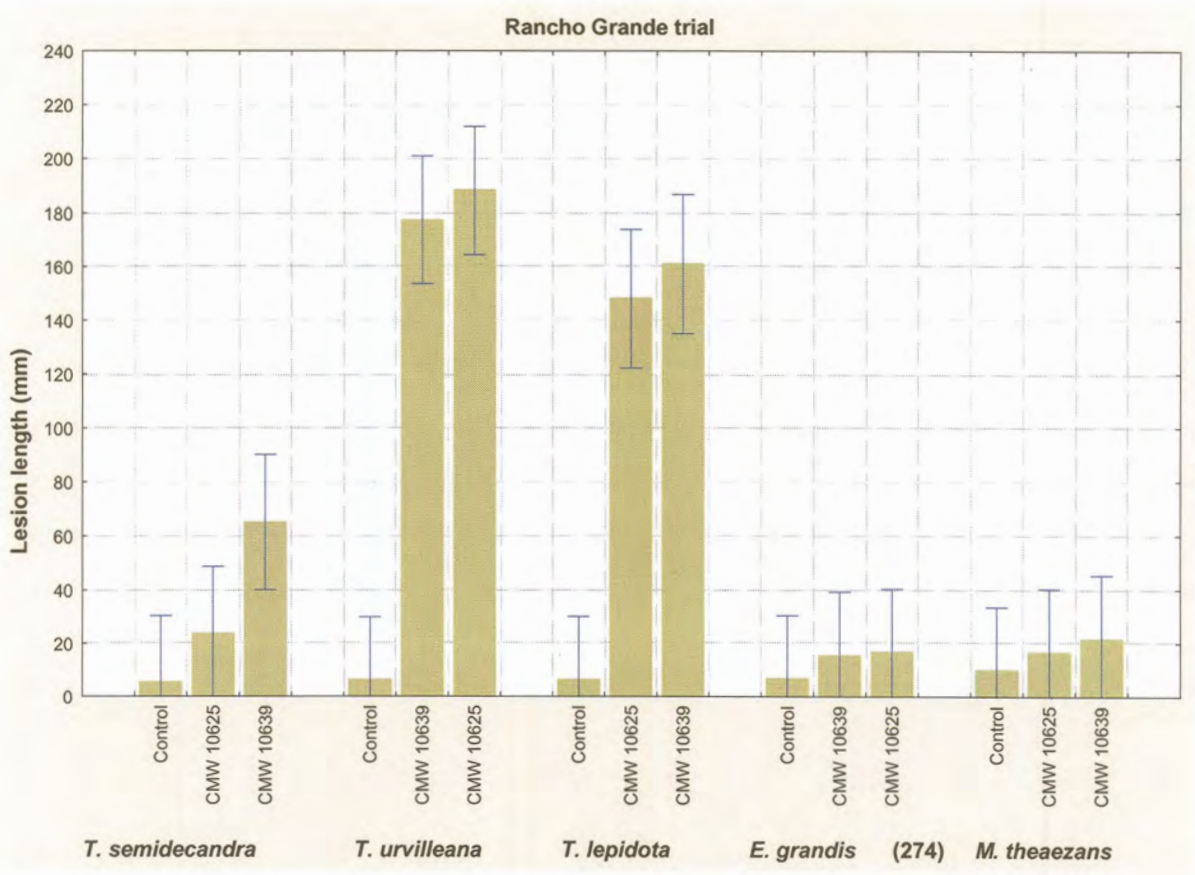

Fig. 10. Results of field inoculation trials with an isolate of *Cryphonectria cubensis* from *Miconia rubiginosa* (CMW 10022) and a negative control. Inoculations were done on six-year-old *M. rubiginosa,* a three-year-old *E. grandis* clone (275) and an *E. urograndis* cross (212). Mean length of lesions is shown with 95% confidence limits.