Chapter 2

MYCOSPHAERELLA SPECIES CAUSING LEAF BLOTCH DISEASE ON *EUCALYPTUS* SPP. IN CHILE

ABSTRACT

Chile is one of the largest exporters of wood products produced in exotic plantations of *Pinus radiata* and *Eucalyptus* spp. *Eucalyptus globulus* and *E. nitens* are primarily propagated and these represent the second largest commercial plantation resource, after *P. Radiata*. With the expansion of *Eucalyptus* plantations, new disease problems have emerged in Chile. Of these, *Mycosphaerella* leaf disease (MLD) has recently been recognized as one of the most important threats. Little research has been conducted on *Mycosphaerella* spp. on *Eucalyptus* in Chile and their identity is poorly understood. The aim of this study was, to collect and identity these fungi. Diseased adult and juvenile *E. globulus* and *E. nitens* leaves were collected from plantations in Northern-central and Southern-central Chile. Species of *Mycosphaerella* were identified based on ascospore germination patterns, cultural characteristics and sequence data from the Internal Transcribed Spacer (ITS) region of the ribosomal RNA (rRNA) operon. Results showed clearly that six *Mycosphaerella* spp. are associated with *Eucalyptus* leaf disease in the plantations surveyed. These species include *M. africana, M. grandis, M. lateralis, M. molleriana, M. parkii* and *M. walkeri*. Five of these species, *M. africana, M. grandis, M. lateralis, M. molleriana,* and *M. parkii* represent new records for Chile. Previous suggestions that the important pathogens, *M. cryptica* and *M. nubilosa* are present in Chile have not been confirmed in this study. We are currently considering the importance of the *Mycosphaerella* spp. identified in this study, and this must be investigated in order to develop effective management strategies to reduce their impact.

INTRODUCTION

Chile is one of the largest producers of wood products in the world and this industry depends on rapidly growing exotic *Pinus radiata* D. Don and *Eucalyptus* spp. A total of 11% of the country's land is forested and about 2.1 million ha is utilised for commercial plantations (Lowy 1995, WFI 1999). *Eucalyptus* spp. are the second most commonly planted forest tree species. *Eucalyptus* trees were introduced into Chile in 1823 but commercial planting only started in the 1930's. Currently, there are almost 360 000 ha of *Eucalyptus* spp. established in Chile (Jayawickrama, Schlatter & Escobar 1993, INFOR 2002).

Eucalyptus spp. in Chile were considered relatively healthy for several decades until the outbreaks of leaf blotch and leaf spot disease caused by *Mycosphaerella* spp. This newly recorded problems suggest that pressure due to disease and pest problems is increasing markedly in Chile. Forest protection will clearly need to be augmented to ensure the long-term sustainability of forestry in the country.

Eucalyptus globulus and *E. nitens* are two of the most widely planted species in Chile. *E. globulus* has been damaged by several pathogens and insects in the past, but the most severe damage has been caused by species of *Mycosphaerella* Johanson. These fungi are well known as important pathogens of *Eucalyptus* spp. elsewhere and in some countries, they are considered amongst the most important constraints to *Eucalyptus* propagation (Carnegie 2000, Crous 1998, Dungey *et al.* 1997).

The genus *Mycosphaerella* is one of the largest genera of Ascomycetes. Approximately 2000 species have been described and have generaly been identified based on the hosts that they infect (Corlett 1991, 1995). The taxonomy and identification of *Mycosphaerella* spp. is complicated. Numerous characters, such as host, lesion characteristics, ascospore germination patterns, cultural characteristics and anamorph-teleomorph connections have been used to identify these fungi (Crous 1998, 1999, Crous *et al.* 1999, 2001). However, the wide variation within species and the overlap between them has led to confusion in the identification process (Carnegie 2000, Hunter 2002). Recently, molecular techniques and particularly analysis of DNA sequences have contributed considerably to the identification

of *Mycosphaerella* spp. (Crous *et al.* 1999, Stewart *et al.* 1999, Crous *et al.* 2000, Carnegie *et al.* 2001, Hunter 2002).

The aim of this study was to identify the *Mycosphaerella* species causing damage on *Eucalyptus* plantations in Chile. To achieve this objective, surveys were conducted in *E. globulus* and *E. nitens* plantations located in the Northern-central and Southern-central area of Chile. Samples were then examined and *Mycosphaerella* spp. identified using traditional morphological techniques as well as DNA sequence comparisons.

MATERIALS AND METHODS

Collection of Samples

Between April 2001 and April 2002, leaves showing symptoms of MLD were collected from *Eucalyptus* trees in Chile. These samples were collected from *E. globulus* and *E. nitens* trees located in 24 different plantations. The plantations were located in different regions of the Northern and Southern parts of the forestry region (Figure 1). Fifteen infected leaves were collected from each sampled tree and these included both juvenile and adult leaves. Leaves were stored at 4 °C in brown paper envelopes. The samples were then transported to the laboratory for further identification.

Isolations and preparation of cultures

Five leaves from each sample were chosen for the isolation of *Mycosphaerella* spp. Lesions were excised and placed in Petri dishes with water for two hours, after which they were attached, using double-sided adhesive tape, to the lids of 60 mm Petri dishes over 2% malt extract agar (MEA) and incubated in the dark at room temperature, for 24 hours. After incubation, single germinating ascospores were observed on the agar surface and these were individually transferred onto 2% MEA and incubated at 25 °C under cool white light. Cultures were generally slow growing and required about two months before culture morphology could be described. Isolates were grouped based on colour and mycelial characteristics of cultures on MEA. To produce asexual states, cultures were grown on Carnation Leaf Agar (CLA) [1% water agar with sterilized carnation leaves placed onto

medium] and incubated at 25 °C under ultra-violet light (nuv, 250 nm) as suggested by Crous (1998).

DNA extraction and PCR amplification

Mycelium from pure cultures was scraped from the surface of cultures using a sterile scalpel and freeze dried. The dried mycelium was ground to a fine powder using liquid nitrogen. The method used to isolate DNA was as described by Raeder and Broda (1985), with minor modifications. Extracted nucleic acids were precipitated from the aqueous layer using two volumes of absolute ethanol. The nucleic acids were pelleted using centrifugation and washed with 70% ethanol. The resultant nucleic acid pellet was then dissolved in water and 4 µl RNaseA (10 µg/µl) (Sigma, USA) was added to digest the RNA. The resulting DNA was quantified by UV light visualization after electrophoresis on a 1% agarose gel stained with ethidium bromide. A Beckman DU Series 60 Spectrophotometer (Beckman, Germany) was used to calculate the DNA concentration of all DNA samples.

The Internal Transcribed Spacer (ITS) region of the rRNA operon was targeted for amplification using primers ITS1 (5' – TCC GTA GGT GAA CCT GCG G – 3'), and LR1 (5' – GGT TGG TTT CTT TTC CT – 3'), (White, Bruns & Taylor 1990, Vilgalys and Hester 1990). The PCR reaction mixture consisted of 50 µl total volume reaction and contained PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3) supplied by Roche Diagnostic, South Africa, 2.5 mM of each dNTP (Roche Diagnostic, SA), 0.2 µM of primers ITS1 and LR1 (MWG Biotech, Germany), 2.5 U Taq DNA polymerase (Roche Diagnostic, SA). Sterile deionised water was used to make the reaction volume up to 50 µl.

The PCR reaction consisted of an initial denaturation step at 96 °C for 2 min, followed by 40 cycles of template denaturation at 94 °C for 30 s, primer annealing at 56 °C for 30 s and extension at 72 °C for 1 min. A final elongation of 10 min at 72 °C completed the program. Following the PCR reaction, the amplicons were visualised by electrophoresis on 2 % agarose gels in TAE buffer, stained with ethidium bromide and visualized with UV light. A 100 bp (base pair) molecular marker (Roche Diagnostic, SA) was used to determine the sizes of PCR products. PCR products were purified using the High Pure PCR product Purification Kit (Roche Diagnostics, Germany).

DNA sequencing and phylogenetic analysis

Purified PCR products were sequenced using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems), following the protocols recommended by the manufacturer. One forward primer ITS1 and two reverse primers ITS2 and LR1, were used for the reactions (White *et al.* 1990, Vilgalys & Hester 1990). Sequence reactions were run using Polyacrylamide Gel Electrophoresis (PAGE) on an ABI PRISM™ 377 and capillary electrophoresis on an ABI PRISM™ 3100 Autosequencer (Perkin-Elmer Applied Biosystems).

Sequences were analysed using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied Biosystems, USA). Sequence alignments were conducted manually and gaps were inserted wherever necessary. The phylogenetic analysis of the aligned sequences was determined using Phylogenetic Analysis Using Parsimony (PAUP version 4.0b1) (Swofford 1998). All characters in the analysis were given equal weight and the MULPAR option was effective. The heuristic search function based on parsimony with tree bisection reconnection (TBR) was used to generate a phylogram. Branch supports were calculated by performing a Bootstrap analysis of a 1000 replications on the aligned sequences. All resulting trees were rooted to *Ramulispora anguoides* (Nirenberg) Crous, which has been shown to be an effective outgroup taxon for *Mycosphaerella* (Crous *et al.* 2001, Hunter 2002).

Morphological characteristics

All isolates were characterized based on lesion type, ascospore germination patterns, cultural morphology and anamorph production, following the methodology described by Crous (1998). The characteristics of the lesions were noted and grouped based on their shape, colour, and position of pseudothecia (abaxial or adaxial) on the leaf. Several pseudothecia were taken from each lesion and mounted in lactophenol on glass microscope slides. These were then used for comparisons of characteristics of teleomorph structures.

Ascospore germination patterns were also examined at the time of making cultures. Here, single germinating ascospores were isolated from MEA and then mounted in lactophenol on microscope slides and examined based on the characteristics described by Crous (1998). All of the cultures obtained from isolations were grouped based on morphology and thus in

terms of colour, size after growth at 25 °C on MEA for 60 days and, texture of the cultures. Between two to ten representative cultures were then chosen for further identification using DNA sequencing.

RESULTS

Isolations and preparation of cultures

All leaves used in the identification process were collected from 24 different commercial plantations, including those of *E. globulus* and *E. nitens*. Symptoms were variable depending on host and whether the samples were taken from juvenile or adult leaves (Figure 2). Approximately 360 juvenile and adult leaves with obvious symptoms of MLD were ultimately examined. Most of the *Mycosphaerella* infections were obtained from the Northern-central distribution of the surveyed areas, and from juvenile leaves (Table 1).

A total of 276 cultures were obtained representing 92 isolations from diseased *Eucalyptus* leaves. Of these, 80 sets of leaves were from *E. globulus* and 12 were from *E. nitens*. All isolates included in this study have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative isolates have also been deposited in the Cetraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.

Phylogenetic analysis

Amplification of the ITS region of the rRNA operon resulted in amplification products of approximately 600 bp for all isolates. A total of 19 taxa were used in the phylogenetic analysis the sequence alignment resulted in 655 characters. Of these, 396 were constant, 82 were parsimony-uninformative and 181 parsimony-informative. The analysis produced 4 most parsimonious trees with a length of 438 steps. For all trees the consistency index (CI = 0.822), retention index (RI = 0.874) and the homoplasy index (HI = 0.178) was calculated using the heuristic search option. After 1000 Bootstrap replications a consensus tree was obtained that showed the same topology as the most parsimonious trees generated from the heuristic search (Figure 3).

Phylogenetic analysis showed that isolates from *Eucalyptus* leaves in Chile, represent six species of *Mycosphaerella*. These included *M. africana*, *M. grandis*, *M. lateralis*, *M. molleriana*, *M. parkii*, and *M. walkeri*. The six *Mycosphaerella* species identified in this study were grouped into two clades. The first group includes *M. africana*, *M. grandis*, *M. molleriana*, *M. parkii*, and *M. walkeri* within the larger *Mycosphaerella* clade. These species were isolated from *E. globulus* and *E. nitens*. The second group included only *M. lateralis*, which, lies outside the larger *Mycosphaerella* clade and was isolated only from *E. nitens*.

Morphological characteristics

Symptomatic leaves were prevalent on juvenile foliage of *E. globulus* and exhibited a wide range of colours, often with raised brown to dark brown borders (Figure 4). Black pseudothecia were observed on both abaxial and adaxial leaf surfaces, however, most of them were present on the adaxial surfaces of leaves. Most of the cultures obtained from single germinating ascospores were characterized by slow growth. All cultures were grouped according their morphological characteristics (culture morphology, ascospore germination pattern, identity of anamorph and symptoms on leaves) into 10 different morphotypes. Based on morphological characteristics and phylogenetic analysis, the following species were identified.

Mycosphaerella africana Crous & M.J. Wingf.

Mycosphaerella africana was isolated from two plantations (Raqui Reserva 4 and La Colcha) both located in the Arauco area in the Northern central distribution of *Eucalyptus* plantations. In total, 5 isolates were obtained from juvenile leaves of *E. globulus* trees planted in 1999. Leaf spots were distributed through the lamina and were visible as round to irregular spots, light brown, also surrounded by light brown to brown edges (Figure 5) but not dark borders as described by Crous (1998). Black sub epidermal pseudothecia were observed on both sides of the lamina (Figure 5). Ascopore germination was observed after 24 h on MEA and was characterized according to Crous (1998) as Type G (Figure 5). Culture growth was approximately 30 – 40 mm diam. on MEA after 2 months and white-pink fluffy aerial mycelium was observed (Figure 5). No anamorph fruiting structures were observed in the cultures.

Mycosphaerella grandis Carnegie & Keane

Mycosphaerella grandis was isolated from juvenile and adult leaves on both *E. globulus* and *E. nitens* in plantations at many different locations in Northern and Southern areas of Chile. This species was the predominant species, with 71 isolates that represented 68 % of the total isolations in this study. Leaf spots were amphigenous, distributed irregularly through the lamina (Figure 6). Lesions were light brown, to brown with raised purple to brown borders and irregular in size and shape (Figure 6). Ascospores were similar in shape with Type F germination patterns (Crous 1998) (Figure 6). Cultures had colonies green to dark green (Figure 6) and no anamorph was identified in the cultures.

Mycosphaerella lateralis Crous & M.J. Wingf.

Mycosphaerella lateralis was identified only from juvenile leaves of *E. nitens* in plantations, located at Santa Isabel in Southern-central Chile near Valdivia. Eight isolates in this study represented this species. Leaf spots were amphigenous (Figure 7) and lesions were sub-circular to circular in shape and grey to brown in colour, surrounded by dark-brown borders (Figure 7). Ascospores of this species germinated from both poles, with long germ tubes and were typical Type I patterns as described by Crous (1998), (Figure 7). Colonies were grey to light brown and turned brown with increased incubation. Colonies had a smooth appearance and showed some folding (Figure 7). The anamorph *Uwebraunia lateralis* Crous & M.J. Wingf. was rarely found sporulating on Carnation Leaf Agar after two weeks of incubation at 25 °C under near ultra-violet light.

Mycosphaerella molleriana (Thüm.) Lindau in Engler & Prantl.

Mycosphaerella molleriana was identified from juvenile leaves of *E. globulus* in plantations at Raqui Reserva 4 and San Ricardo, both located in Northern-central area (Arauco). Ten isolates of this species were collected. The species was characterised by amphigenous leaf spots, which were irregular in shape and confluent at the middle and borders of the leaf laminas (Figure 8). Lesions were light brown with raised brown borders, bearing black subepidermal pseudothecia (Figure 8). Ascospore germination was observed after 24 h on MEA and fitted the Type C pattern (Figure 8) of Crous (1998). Aerial hyphae were gray to light green, with fluffy white mycelium towards the margins (Figure 8). The

anamorph *Colletogloeopsis molleriana* Crous & M.J. Wingf. was found sporulating on MEA after two weeks incubation at 25 °C under ultra-violet light.

Mycosphaerella parkii Crous & M.J. Wingf., F.A. Ferreira & Alfenas

Mycosphaerella parkii was identified from juvenile leaves on *E. globulus* only at the Raqui Reserva 4 plantation in the Arauco area. Seven isolates were obtained of this species. Leaf spots were amphigenous and they were irregular in shape (Figure 9). The lesions were light brown at the center and turned light brown towards the raised borders (Figure 9). In most lesions, black aggregated and immersed pseudothecia were seen. Ascospores showed the Type D germination pattern (Crous 1998), (Figure 9). The cultures were grey-green to dark green, with radial cracks and white spots around the border of mycelium (Figure 9). The anamorph *Stenella parkii* Crous & Alfenas was not found in Carnation leaf agar culture.

Mycosphaerella walkeri R.F. Park & Keane

Mycosphaerella walkeri was identified from juvenile leaves of *E. globulus* in the plantations San Ricardo, La Colcha, Lomas de la Madera and Raqui, in the Northern-central forestry area. This *Mycosphaerella* species was also isolated from both juvenile and adult *E. globulus* leaves in the Southern-central area. Leaf spots were amphigenous, round to confluent and irregular, however, most of the lesions observed were circular with purple margins (Figure 10). Ascospore germination was of the Type C described by Crous (1998) and Carnegie (2000), (Figure 10). Cultures had white aerial hyphae, surrounded by dark-green submerged mycelium (Figure 10). The anamorph *Sonderhenia eucalypticola* (A.R. Davis) H. Swart & J. Walker. was frequently isolated from the diseased leaves.

DISCUSSION

Prior to this study, virtually nothing was known regarding the identity of *Mycosphaerella* species associated with leaf disease of *Eucalyptus* spp. in Chile. Those identifications previously reported, were based on symptoms and morphological structures on lesions. Thus, results of this study have provided the first attempt at detailed identification of these important pathogens in Chile.

Results of this study, supported by DNA sequence comparisons have given rise to the identification of six species. These included *M. africana*, *M. grandis*, *M. lateralis*, *M. molleriana*, *M. parkii*, and *M. walkeri*. *M. africana*, *M. grandis*, *M. lateralis*, *M. molleriana*, and *M. parkii* are reported for the first time from Chile.

Mycosphaerella leaf disease caused high levels of defoliation on *Eucalyptus* spp. during the 2000 growth season, in both the Northern-central and Southern-central area of Chile. In the Arauco area (Northern-central) the problem was confined to juvenile leaves, while in the Valdivia area (Southern-central) the problem was recognized from both juvenile and adult leaves of *Eucalyptus* spp. Although the results of the current study have shown that *M. grandis* and *M. walkeri* are present on adult leaves, we now believe that the severe damage observed in the 2000 season was not caused only by MLD. At that time it was suggested that the aggressive *M. cryptica* (Cooke) Hansf. was responsible for the disease (Gonzalez 2000, unpublished report, Mohammed 2001, unpublished report). We have shown clearly that this is not so, and there is no evidence to suggest that *Mycosphaerella* spp. contributed to the defoliation problems. Rather, current views are that the adult leaf loss was mainly due to physiological disturbance, probably linked to frost damage or other environmental factors.

In the Northern-central (Arauco) area of Chile, we were able to identify five species of *Mycosphaerella*. It is impossible to know which of these is more or less important in the defoliation of juvenile *E. globulus* leaves in the area. *Mycosphaerella grandis* was clearly the most predominant species in all areas (68 %). We thus believe that this species is an important component of the *Eucalyptus* leaf disease. *Mycosphaerella grandis* is known only from Tasmania, where it causes leaf disease on *E. grandis*, *E. globulus* and *E. nitens* (Carnegie & Keane 1998). This species showed very similar germination patterns and culture characteristics to *M. juvenis* that is one of the more important species occurring in South Africa (Crous & Wingfield 1996, Crous 1998, Hunter 2002). This identification constitutes the first report of *M. grandis* in Chile as well as South America. Further studies are thus required to evaluate its relative importance.

Mycosphaerella africana, *M. molleriana* and *M. parkii*, were identified only from juvenile leaves on *E. globulus* in the Arauco area. Their presence was infrequent and concentrated in Raqui Reserva 4, La Colcha and San Ricardo. These species were frequently found

together with *M. grandis* and were not easy to distinguish based on the symptoms or type of lesions that they produced on leaves. *Mycosphaerella africana* is known from Colombia (Crous 1998) and its appearance in Chile, which is geographically close to Colombia, is perhaps not surprising. The fungus has also been reported in Portugal, South Africa and Zambia (Crous 1998). There is, however, no evidence that this species is an important pathogen.

Mycosphaerella molleriana has been reported in the USA (California), Portugal and this name has been used by previous authors (Gonzalez & Parra 1994, Muñoz 1999) as present in Chile. However, in the past, this fungus was thought to be a synonym of *M. nubilosa* (Crous, Wingfield & Park 1990), that later was rejected by Crous and Wingfield (1996). Hunter (2002), using molecular techniques, has also shown that this species represents a distinct taxon. *Mycosphaerella nubilosa* is recognised as a major pathogen of *E. globulus* and *E. nitens* (Dick & Gadgil 1983, Park 1988, Milgate *et al.* 2001) but the confusion in the taxonomy of *M. molleriana* and *M. nubilosa* makes it difficult to assess the importance of *M. molleriana*. Thus, *M. molleriana* was reported before, but as a synonym of *M. nubilosa*, so this is the first clear report, which is supported by both morphological and DNA sequence data.

Mycosphaerella parkii has previously been recorded from Brazil, Colombia and Indonesia (Crous 1998). Together with *M. africana* and *M. molleriana*, it appeared infrequently in this study and is unlikely to have been important in defoliation. This species is also reported from Chile for the first time.

Mycosphaerella lateralis, was isolated only from *E. nitens* plantations in the Valdivia area. It was present on juvenile leaves of *E. nitens* but there were no signs of severe defoliation. We, therefore, do not believe that it is particularly important. This species has previously been reported from South Africa and Zambia (Crous 1998) and also from Australia (Maxwell *et al.* 2000). This is the first record of the fungus in South America including Chile.

Mycosphaerella walkeri was common in Chile although it is usually found in the asexual (*Sonderhenia*) state and was not commonly isolated using the technique used to collect *Mycosphaerella* spp. in this study. The fungus causes small discrete spots on the juvenile

and less commonly adult leaves of *E. globulus* and *E. nitens*. It has been reported from Australia, New Zealand, Portugal, Equator, and Colombia, and has also previously been reported from Chile (Crous 1998, Wingfield *et. al* 1995).

Effective management of MLD on *Eucalyptus* in Chile will be important and necessary for the forestry activity in the future. Thus far, losses due to defoliation caused by MLD have not been quantified and this deficiency must be addressed. In order to achieve effective management strategies of this disease, it will also be important to have greater knowledge of the pathogen and rapid screening techniques for the different species. Selection for resistant clones or hybrids should be initiated in the future, however, it is also important to achieve a better understanding related to MLB and its effect on *Eucalyptus* plantations.

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Table 1. *Mycosphaerella* spp. identified from *Eucalyptus* plantations in Chile.

Collected from	Host	Foliage	*Mycosphaerella spp.* identified	Anamorph	Distribution
Arauco Concepcion (Northern-Central area)	*E. globulus*	Juvenile	*M. africana*	Unknown	Colombia, Portugal, South Africa, Zambia
			M. grandis	Unknown	Australia
			M. molleriana	*Colletogloeopsis molleriana*	USA (California), Portugal
			M. parkii	*Stenella parkii*	Brazil, Colombia, Indonesia
			M. walkeri	*Sonderhenia eucalypticola*	Australia, Chile, New Zealand
Valdivia Los Lagos (Southern-Central area)	*E. globulus*	Juvenile	*M. grandis*	Unknown	Australia
			M. lateralis	*Uwebraunia lateralis*	South Africa, Zambia
			M. walkeri	*Sonderhenia eucalypticola*	Australia, Chile, New Zealand
		Adult	*M. grandis*	Unknown	Australia
	E. nitens	Juvenile	*M. lateralis*	*Uwebraunia lateralis*	South Africa, Zambia

Crous (1998) & Carnegie (2000).

Figure 1. Geographic distribution of *Eucalyptus* commercial plantations in Chile where samples were collected.

1: Concepcion and Arauco areas (northern-central)

2: Valdivia area (southern-central)

Figure 2. Commercial plantations of *Eucalyptus globulus* affected by MLD. (**A**) Juvenile foliage (**B**) Adult foliage.

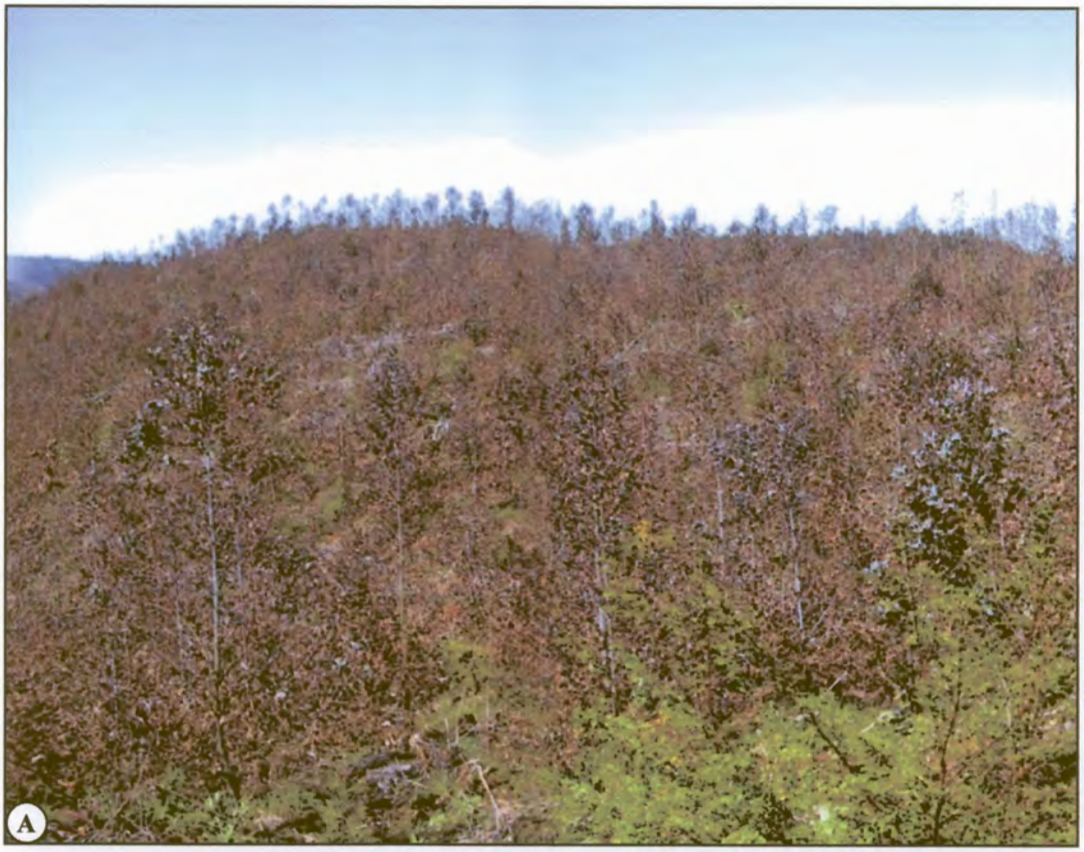

Figure 3. Phylogram based on aligned ITS sequence data from the rRNA operon of *Mycosphaerella* spp. isolated from *E. globulus* and *E. nitens* from Chile (CI = 0.822, RI = 0.874, HI = 0,178) inferred using heuristic and branch swapping options of PAUP version 4.0b1. Bootstrap support of 1000 replications is listed above the branches.

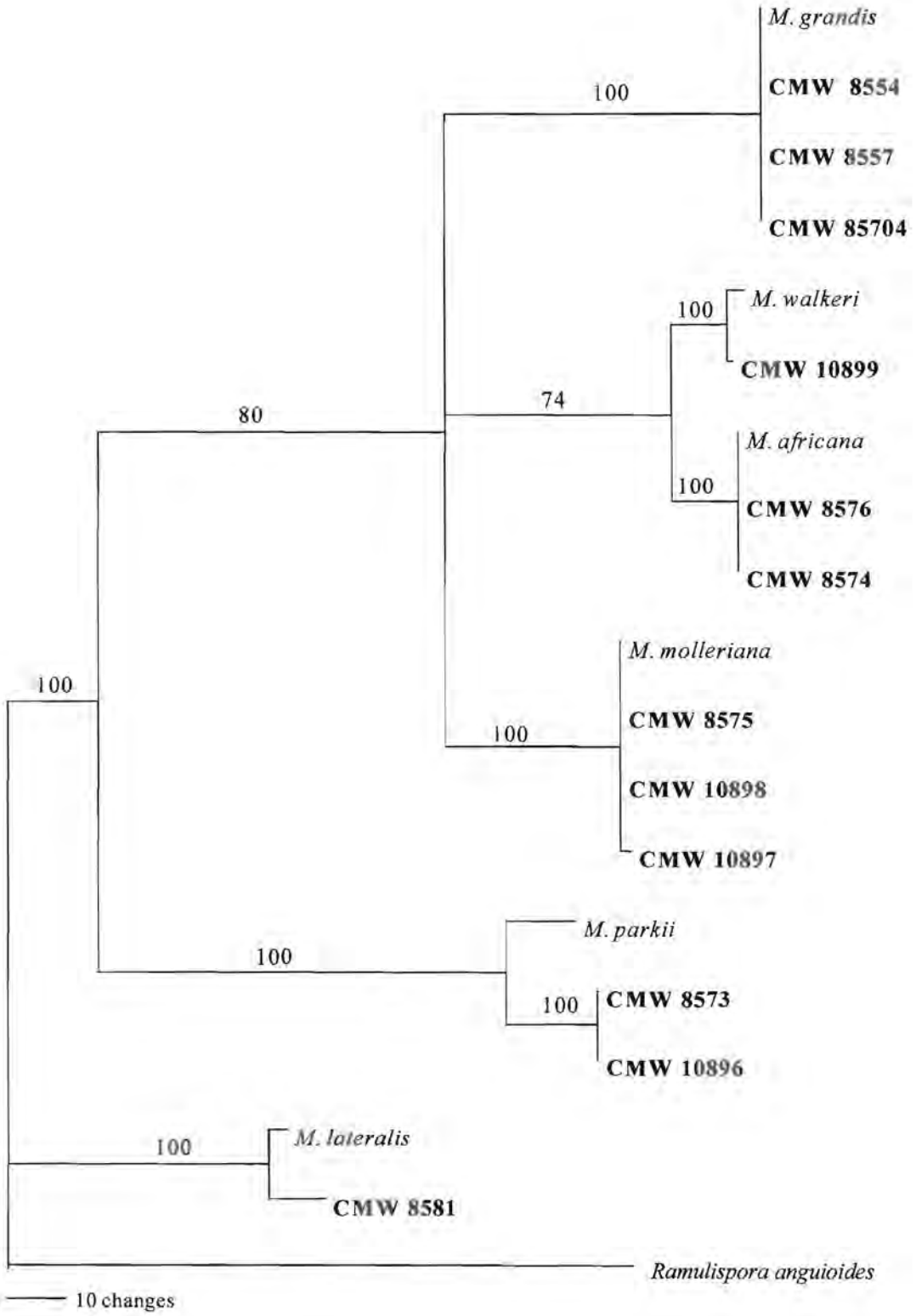
M. grandis
CMW 8554
CMW 8557
CMW 85704
M. walkeri
CMW 10899
M. africana
CMW 8576
CMW 8574
M. molleriana
CMW 8575
CMW 10898
CMW 10897
M. parkii
CMW 8573
CMW 10896
M. lateralis
CMW 8581
Ramulispora anguioides
100
100
74
80
100
100
100
100
100
10 changes

Figure 4. Leaf symptoms associated with infections by *Mycosphaerella* species isolated from Chile. (**A**) Juvenile leaves of *E. globulus* from the Northern-central area (Arauco). (**B**) Adult leaves of *E. globulus* from the Southern-central area (Valdivia).

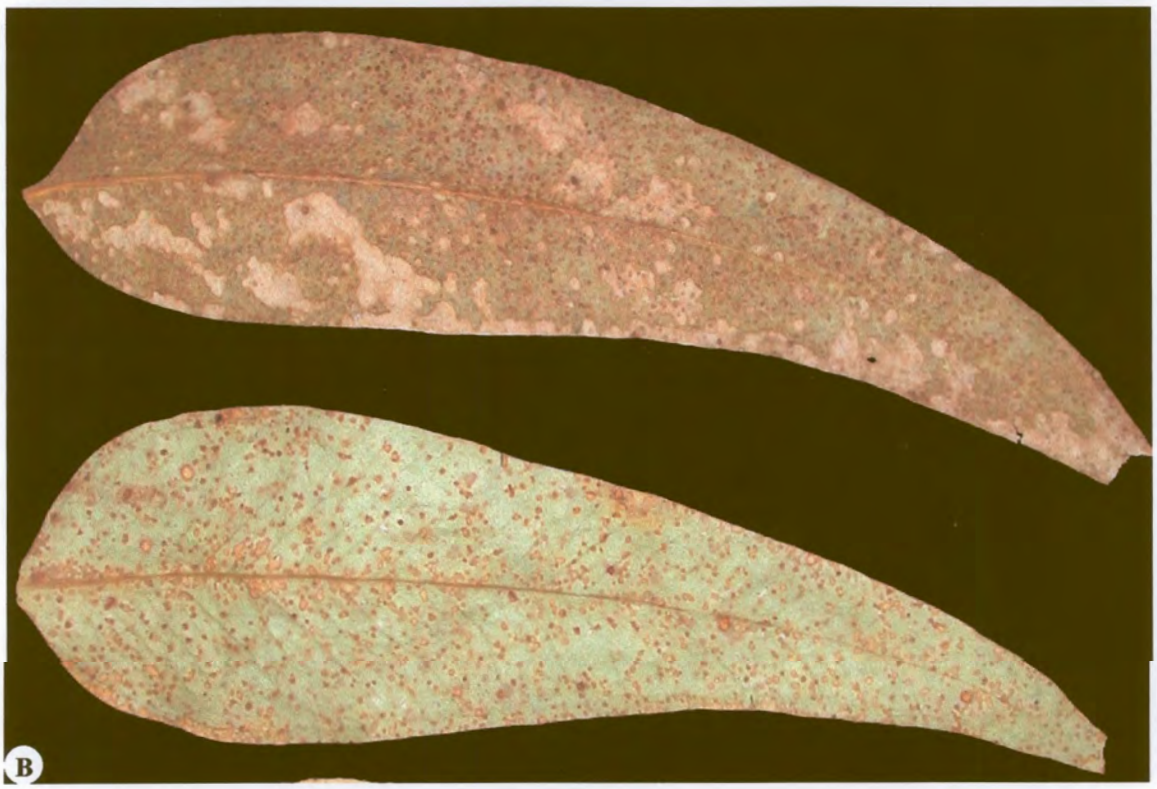

Figure 5. *Mycosphaerella africana*. (**A**) *E. globulus* leaf showing symptoms. (**B**) Lesion of *M. africana*. (C) Ascospore germination pattern. (**D**) Culture of *M. africana*.

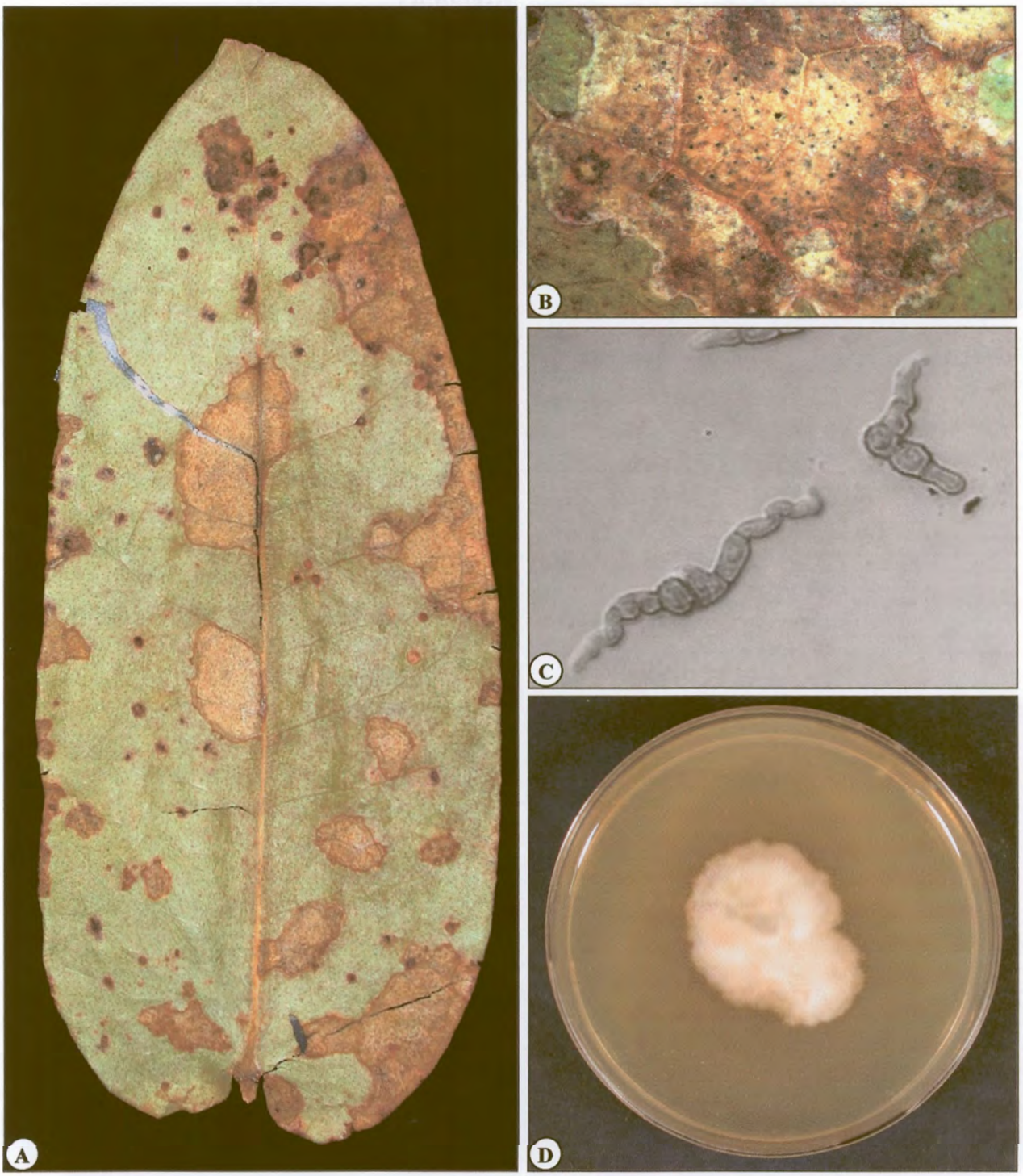

Figure 6. *Mycosphaerella grandis*. (**A**) *E. globulus* leaf with symptoms. (**B**) Lesion. (**C**) Ascospore germination pattern. (**D**) Culture characteristics.

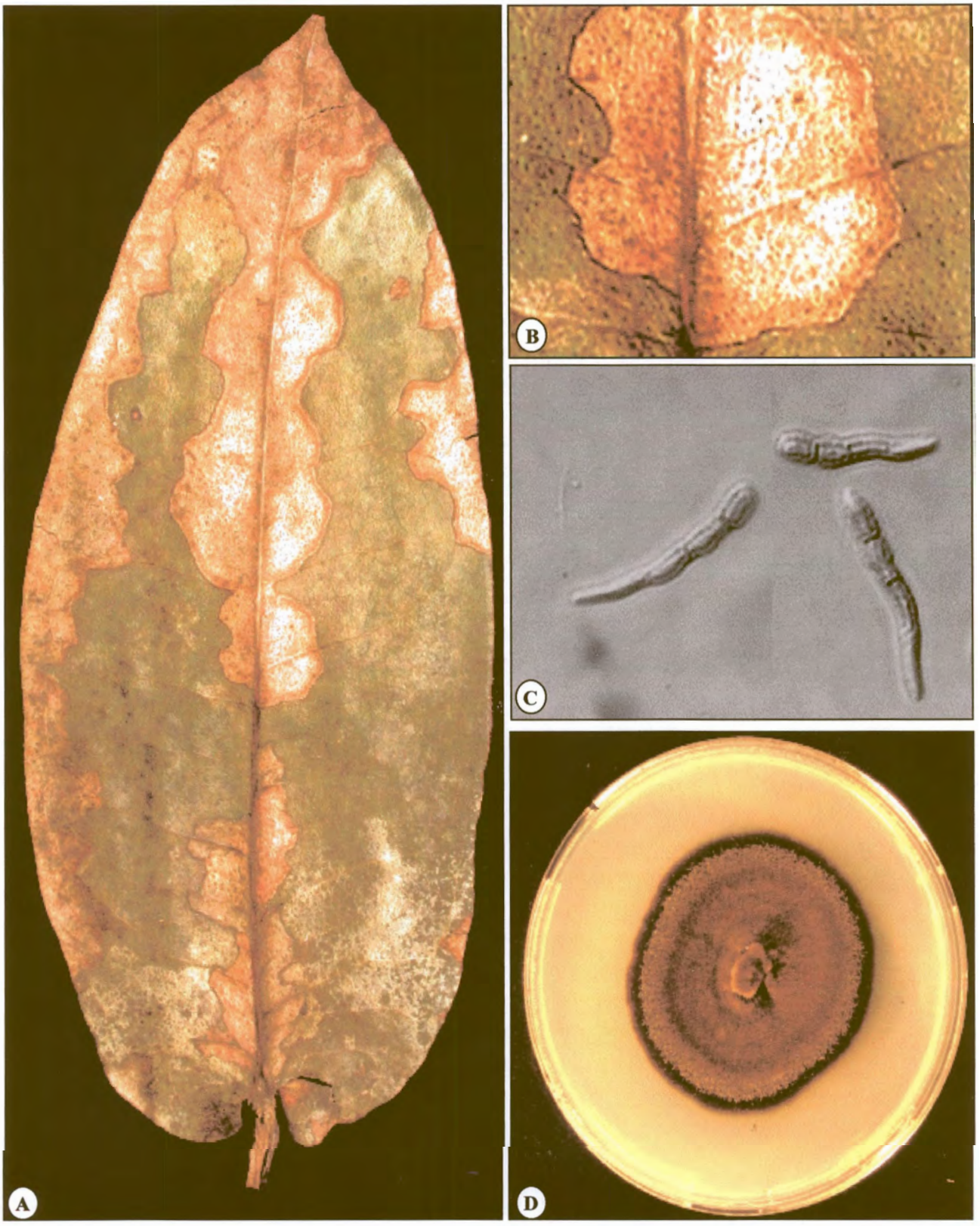

Figure 7. *Mycosphaerella lateralis* **(A)** *E. globulus* leaf with symptoms. **(B)** Lesion. **(C)** Ascospore germination pattern. **(D)** Culture characteristics.

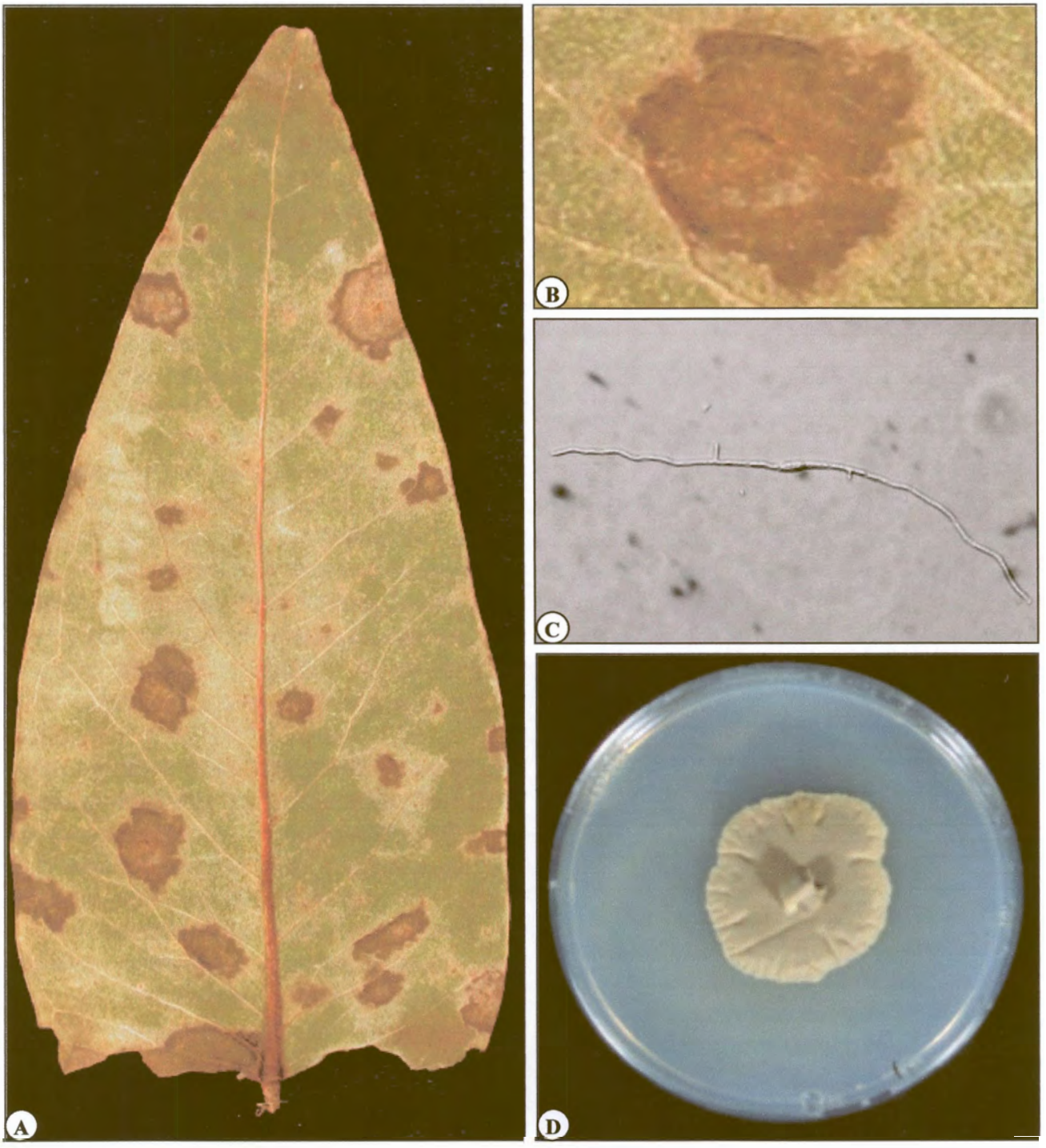
A
B
C
D

Figure 8. *Mycosphaerella molleriana* **(A)** *E. globulus* leaf with symptoms. **(B)** Lesion. **(C)** Ascospore germination pattern. **(D)** Culture characteristics.

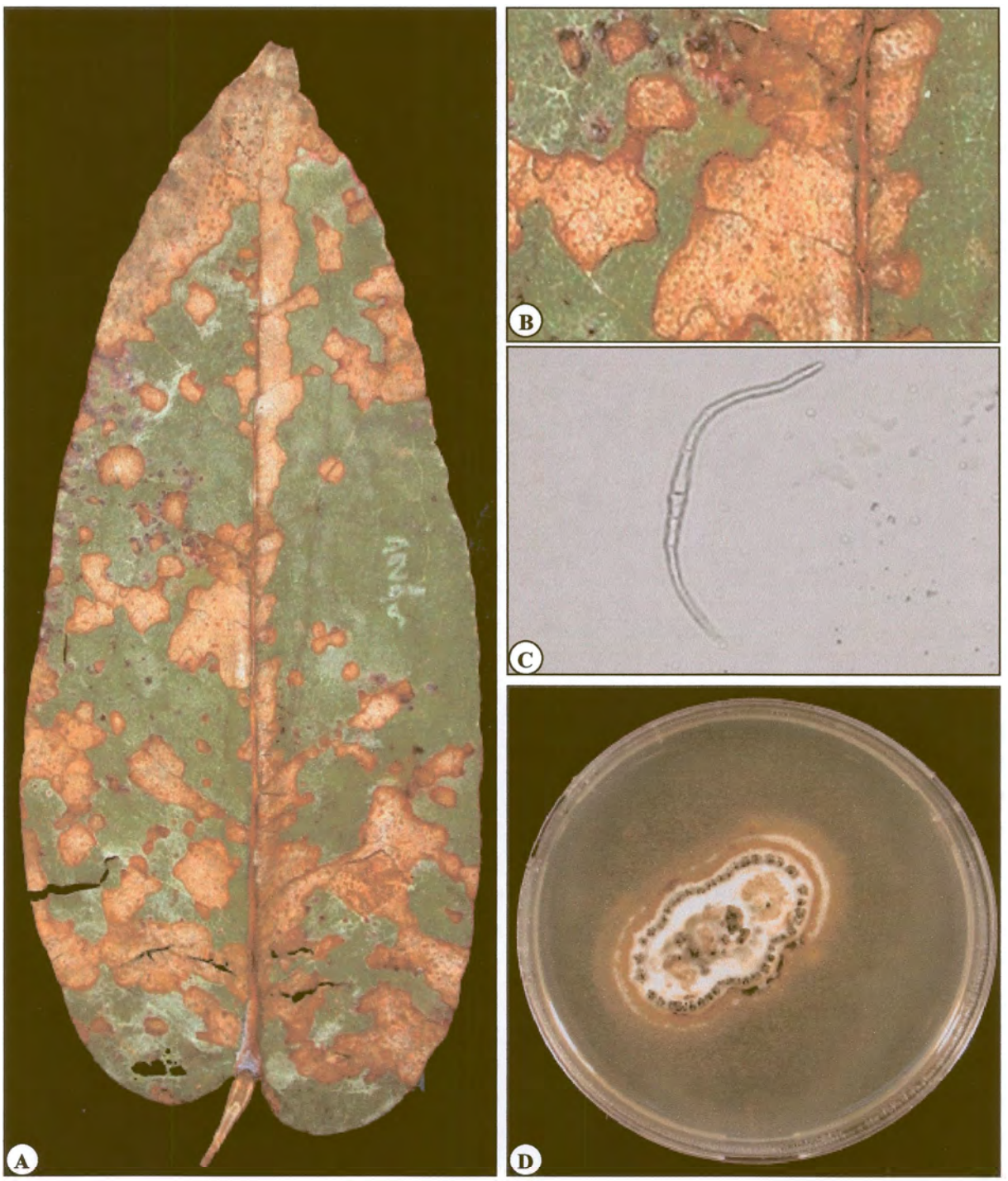
A
B
C
D

Figure 9. *Mycosphaerella parkii* **(A)** *E. globulus* leaf with symptoms. **(B)** Lesion. **(C)** Ascospore germination pattern. **(D)** Culture characteristics.

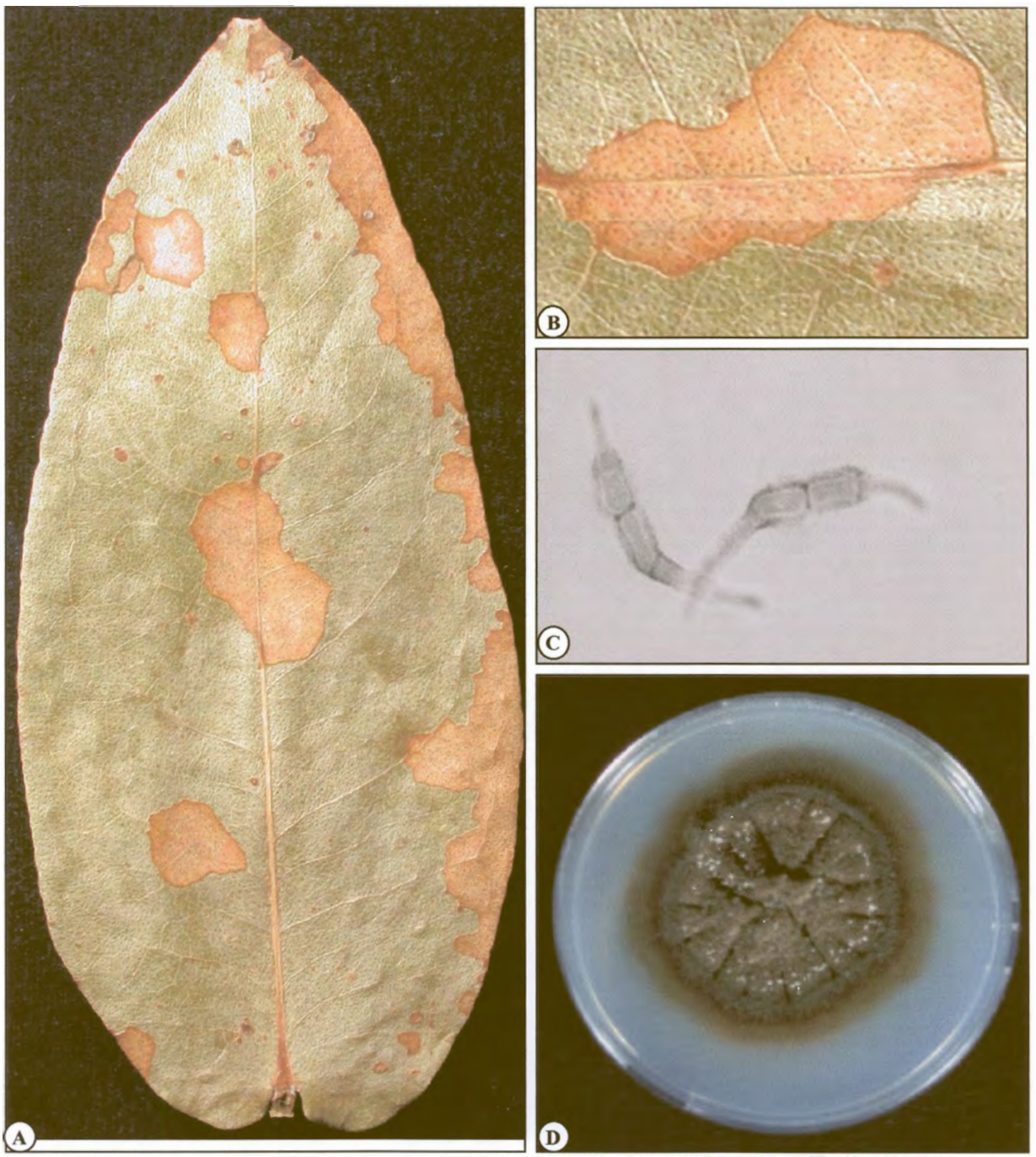
A
B
C
D

Figure 10. *Mycosphaerella walkeri* (**A**) *E. globulus* leaf with symptoms. (**B**) Lesion. (**C**) Ascospore germination pattern. (**D**) Culture characteristics.

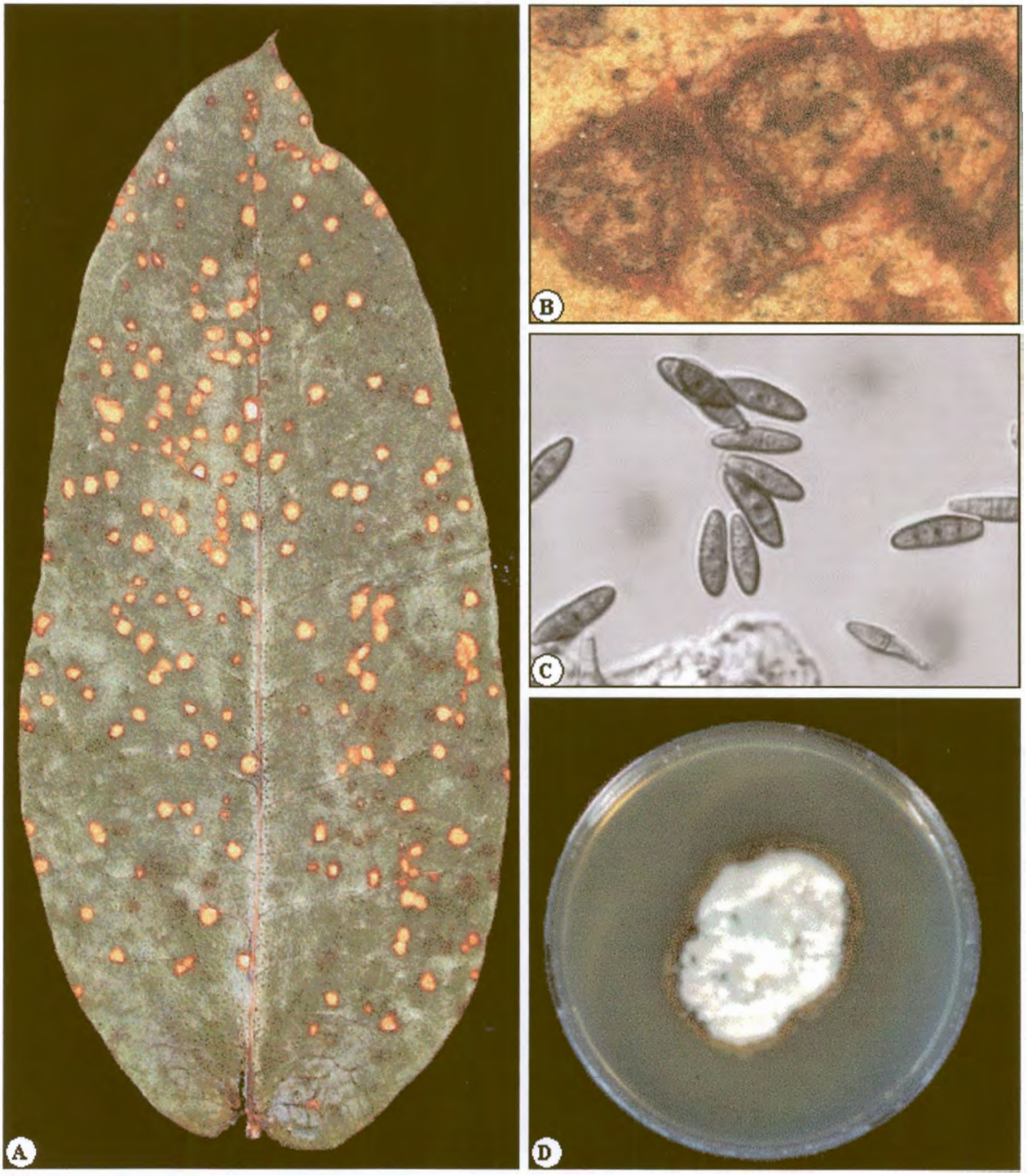

Figure 11: ITS DNA sequences of *Mycosphaerella* species generated using primers ITS1, ITS2 and LR1. Sequence data set contains sequences of *Mycosphaerella* spp. isolated from *Eucalyptus* spp. from Chile.

```
                                10        20        30        40
M.grandis                       TCCGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
CMW 8554                        TCCGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
CMW 8557                        TCCGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
CMW 8570                        TCCGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
M.walkeri STU2768               TCGGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
CMW 10899                       TCCGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
M.africana CMW4945              TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
CMW 8576                        TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
CMW 8574                        TCCGTAGGTG AACCTGCGGA GGGATCATT- CT-GA----T
M.molleriana CMW2734            TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
CMW 8575                        TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
CMW 10898                       TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
CMW 10897                       TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
M.parkii CMW3358                TCCGTAGGTG AACCTGCGGA GGGATCATTA CT-GAG---T
CMW 8573                        TCCGTAGGTG AACCTGCGGA GGGATCATTA CC-GAG---T
CMW 10896                       TCCGTAGGT- AACCTGCGGA GGGATCATTA CC-GAG---T
M.lateralis CMW4935             TCCGTAGGTG AACCTGCGGA GGGATCATTA CCAGA-----
CMW 8581                        TCGAAGGGTG AACCTGCGGA GGGATCATTA CCAGA-----
Ramulispora anguioides          TCCGTAGGTG AACCTGCGGA AGGATCATTA ATAGAGCAAT

                                50        60        70        80
M.grandis                       GAGGGCCT-- -CCGGGCT-C G------ACC TC----CAAC
CMW 8554                        GAGGGCCT-- -CCGGGCT-C G------ACC TC----CAAC
CMW 8557                        GAGGGCCT-- -CCGGGCT-C G------ACC TC----CAAC
CMW 8570                        GAGGGCCT-- -CCGGGCT-C G------ACC TC----CAAC
M.walkeri STU2768               GAGGGCC--- -CCGGCC--C G------ACC TC----CAAC
CMW 10899                       GAGGGCC--- -CCGGCC--C G------ACC TC----CAAC
M.africana CMW4945              GAGGGC-T-C AC-G-CC--C G------ACC TC----CAAC
CMW 8576                        GAGGGC-T-C AC-G-CC--C G------ACC TC----CAAC
CMW 8574                        GAGGGC-T-C AC-G-CC--C G------ACC TC----CAAC
M.molleriana CMW2734            GAGGGC--GC A-AG-CC--C G------ACC TC----CAAC
CMW 8575                        GAGGGC--GC A-AG-CC--C G------ACC TC----CAAC
CMW 10898                       GAGGGC--GC A-AG-CC--C G------ACC TC----CAAC
CMW 10897                       GAGGGC--GC A-AG-CC--C G------ACC TC----CAAC
M.parkii CMW3358                GAGGGTTT-C ACCG--C--C G------ACC TC----CAAC
CMW 8573                        GAGGGCCC-- -CCGGCC--C G------ACC TC----CAAC
CMW 10896                       GAGGGCCC-- -CCGGCC--C G------ACC TC----CAAC
M.lateralis CMW4935             -AG------- ACGCCTCGGC GGAAACGCCG GGG---CCTT
CMW 8581                        -AG------- ACGCTCTGGC GGAAACGCCG GGG---CCTT
Ramulispora anguioides          GAGCGTCAG- -CGCCCCGGG AGCAATCCTG GGGGCCACCC
```

```
                                  90         100        110        120
M.grandis                 CCC------- ---ATT---- GT-----AT- TCC--GAC--

CMW 8554                  CCC------- ---ATT---- GT-----AT- TCC--GAC--
CMW 8557                  CCC------- ---ATT---- GT-----AT- TCC--GAC--
CMW 8570                  CCC------- ---ATT---- GT-----AT- TCC--GAC--
M.walkeri STU2768         CCT------- ----TT---- GT-------- -----GAA--
CMW 10899                 CCT------- ----TT---- GT-------- -----GGA--
M.africana CMW4945        CCT------- ----TT---- GT-------- -----GAA--
CMW 8576                  CCT------- ----TT---- GT-------- -----GAA--
CMW 8574                  CCT------- ----TT---- GT-------- -----GAA--
M.molleriana CMW2734      CCC------- ---AT----- GT------T- TC--------
CMW 8575                  CCC------- ---AT----- GT------T- TC--------
CMW 10898                 CCC------- ---AT----- GT------T- TC--------
CMW 10897                 CCC------- ---AT----- GT------T- TC--------
M.parkii CMW3358          CCT------- ----TT---- GT-------- -----GAA--
CMW 8573                  CCT------- ----TT---- GT-------- -----GAA--
CMW 10896                 CCT------- ----TT---- GT-------- -----GAA--
M.lateralis CMW4935       CGTCCAACCC TTTG-TGAAC GT-----ATC TC--------
CMW 8581                  CGTCCAACCC TTTG-TGAAC GT-----ATC TC--------
Ramulispora anguioides    TCCTCGGAGG GTTTAGAGAC GTCGAGCCTC TCGGAGAAGC

                                  130        140        150        160
M.grandis                 ---------- ---------- -----CTC-- -----TTGTT
CMW 8554                  ---------- ---------- -----CTC-- -----TTGTT
CMW 8557                  ---------- ---------- -----CTC-- -----TTGTT
CMW 8570                  ---------- ---------- -----CTC-- -----TTGTT
M.walkeri STU2768         ---------- --CCCAA--- -----CT--- -----T-GTT
CMW 10899                 ---------- --CCCAA--- -----CT--- -----T-GTT
M.africana CMW4945        ---------- ---CCAA--- -----CTC-- -----T-GTT
CMW 8576                  ---------- ---CCAA--- -----CTC-- -----T-GTT
CMW 8574                  ---------- ---CCAA--- -----CTC-- -----T-GTT
M.molleriana CMW2734      ---------- ----CAAA-C CAC------- -------GTT
CMW 8575                  ---------- ----CAAA-C CAC------- -------GTT
CMW 10898                 ---------- ----CAAA-C CAC------- -------GTT
CMW 10897                 ---------- ----CAAA-C CAC------- -------GTT
M.parkii CMW3358          ---------- ---CCACAA- --CTT----- -------GTT
CMW 8573                  ---------- ----CAC--- ---TTCTC-- -------GTT
CMW 10896                 ---------- ----CAC--- ---TTCTC-- -------GTT
M.lateralis CMW4935       ---------- ---------- ---------- ------TATT
CMW 8581                  ---------- ---------- ---------- ------TATT
Ramulispora anguioides    TCGGTTCAGA CCTCCACCCT TGAATAAAAA ACCTTTGTTG
```

```
                                170         180         190         200

M.grandis                       GCCTCGG--G GGCGACCCG- GCC------T TCGGGCG--T
CMW 8554                        GCCTCGG--G GGCGACCCG- GCC------T TCGGGCG--T
CMW 8557                        GCCTCGG--G GGCGACCCG- GCC------T TCGGGCG--T
CMW 8570                        GCCTCGG--G GGCGACCCG- GCC------T TCGGGCG--T
M.walkeri STU2768               GCTTCGG--G GGCGACCCT- GCCGTC---- TCGGCGGC-G
CMW 10899                       GCTTCGG--G GGCGACCCT- GCCGTC---- TCGGCGGC-G
M.africana CMW4945              GCTTCGG--G GGCGACCCC- GCCGT----T TCGGCGAC--
CMW 8576                        GCTTCGG--G GGCGACCCC- GCCGT----T TCGGCGAC--
CMW 8574                        GCTTCGG--G GGCGACCCC- GCCGT----T TCGGCGAC--
M.molleriana CMW2734            GCCTCGG--G GGCGACCCG- GCCG------ CCGCGCCG--
CMW 8575                        GCCTCGG--G GGCGACCCG- GCCG------ CCGCGCCG--
CMW 10898                       GCCTCGG--G GGCGACCCG- GCCG------ CCGCGCCG--
CMW 10897                       GCCTCGG--G GGCGACCCG- GCCG------ CCGCGCCG--
M.parkii CMW3358                GCTTCGG--G GGCGACCCT- GCCG-----T TCGCGGCATC
CMW 8573                        GCTTCGG--G GGCGACCCT- GCCG------ TCCCGGCGCC
CMW 10896                       GCTTCGG--G GGCGACCCT- GCCG------ TCCCGGCGCC
M.lateralis CMW4935             GCCCCGG--G GG-AACCC-C GCCTGTCAT- ---GGGCGTG
CMW 8581                        GCCCCGG--G GG-AACCC-C GCCTGTCAT- ---GGGCGTG
Ramulispora anguioides          CTTTGGCAGG ACGCCTCGCG CCAGCGGCTT CGGCTGTTGA

                                210         220         230         240

M.grandis                       CGGG-GCCCC CGGTGGACCA -TC---AAAC TCTGCATCT-
CMW 8554                        CGGG-GCCCC CGGTGGACCA -TC---AAAC TCTGCATCT-
CMW 8557                        CGGG-GCCCC CGGTGGACCA -TC---AAAC TCTGCATCT-
CMW 8570                        CGGG-GCCCC CGGTGGACCA -TC---AAAC TCTGCATCT-
M.walkeri STU2768               CGGC-GCCCC CGGAGG-CC- CTC---AAAC ACTGCATCC-
CMW 10899                       CGGC-GCCCC CGGAGG-CC- CTC---AAAC ACTGCATCC-
M.africana CMW4945              -GGCGGCCCC CGGAGGT-CA -TC---AAAC ACTGCATC--
CMW 8576                        -GGC-GCCCC CGGAGGT-CA -TC---AAAC ACTGCATC--
CMW 8574                        -GGC-GCCCC CGGAGGT-CA -TC---AAAC ACTGCATC--
M.molleriana CMW2734            -GG--GCCCC CGGTGGACCC -TC----AAC TCTGCATC--
CMW 8575                        -GG--GCCCC CGGTGGACCC CTC----AAC TCTGCATC--
CMW 10898                       -GG--GCCCC CGGTGGACCC CTC----AAC TCTGCATC--
CMW 10897                       -GG--GCCCC CGGTGGACCC CTC----AAC TCTGCATC--
M.parkii CMW3358                -G-C-GCCCC CGGAGGA--- -T-ACTTAAC CCTGCATCAT
CMW 8573                        GG-C-GCCCC CGGAGGAC-- ---ACCCAAC ACTGCATCCA
CMW 10896                       GG-C-GCCCC CGGAGGAC-- ---ACCCAAC ACTGCATCCA
M.lateralis CMW4935             GG-C----CC CCGGTGGCCA --ACTCAAAC TCTGTTTTTA
CMW 8581                        GG-C----CC CCGGCGGCCA --CTTCAAAC TCTGTTTTTA
Ramulispora anguioides          GTGCCTGCCA GAGG--ACCA -----CAACT CTTGTTTTTA
```

```
                                250        260        270        280

M.grandis                       --TTGACGTC TGA------- -------GTA AAT-A---TT
CMW 8554                        --TTGACGTC TGA------- -------GTA AAT-A---TT
CMW 8557                        --TTGACGTC TGA------- -------GTA AAT-A---TT
CMW 8570                        --TTGACGTC TGA------- -------GTA AAT-A---TT
M.walkeri STU2768               --TCGCGTCG GAGTCTCA-- -------GTA AATGAAA---
CMW 10899                       --TCGCGTCG GAGTCTCA-- -------GTA AATGAAA---
M.africana CMW4945              T-TTGCGTCG GAGTCTTA-- -----AAGTA AATT-AAA--
CMW 8576                        T-TTGCGTCG GAGTCTTA-- -----AAGTA AATTTAAA--
CMW 8574                        T-TTGCGTCG GAGTCTTA-- -----AAGTA AATTTAAA--
M.molleriana CMW2734            T-CTGCGTCT GAGTCACAAA ATC--AA-TC AAT-------
CMW 8575                        T-CTGCGTCT GAGTCACAAA ATC--AA-TC AAT-------
CMW 10898                       T-CTGCGTCT GAGTCACAAA ATC--AA-TC AAT-------
CMW 10897                       T-CTGCGTCT GAGTCACAAA ATC--AA-TC AAT-------
M.parkii CMW3358                TGCGT--CG- --GAGTAATT TT---ATT-- AAT-ACA-T-
CMW 8573                        T--G---CGT CGGAGTGATT TT-GTA---- AATCAAAA--
CMW 10896                       T--G---CGT CGGAGTGATT TT-GTA---- AATCAAAA--
M.lateralis CMW4935             T-TGCCGTCT --GAGTAACA AAC--A---- AATCAAAA--
CMW 8581                        T-TGCCGTCT --GAGTAACA AAC--A---- AATTAAAA--
Ramulispora anguioides          GTG-ATGTCT GAGTACTAT- -----AT--- AAT---AGTT

                                290        300        310        320

M.grandis                       GAATCAATCA AAACTTTTAA CAACGGATCT CTTGGTTCTG
CMW 8554                        GAATCAATCA AAACTTTTAA CAACGGATCT CTTGGTTCTG
CMW 8557                        GAATCAATCA AAACTTTTAA CAACGGATCT CTTGGTTCTG
CMW 8570                        GAATCAATCA AAACTTTTAA CAACGGATCT CTTGGTTCTG
M.walkeri STU2768               --------CA AAACTTTCAA CAACGAATCT CTTGGTTCTG
CMW 10899                       --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
M.africana CMW4945              --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 8576                        --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 8574                        --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
M.molleriana CMW2734            --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 8575                        --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 10898                       --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 10897                       --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
M.parkii CMW3358                ---------A AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 8573                        --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 10896                       --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
M.lateralis CMW4935             --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
CMW 8581                        --------CA AAACTTTCAA CAACGGATCT CTTGGTTCTG
Ramulispora anguioides          ---------A AAACTTTCAA CAACGGATCT CTTGGTTCTG
```

	330	340	350	360
M.grandis	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8554	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8557	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8570	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
M.walkeri STU2768	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 10899	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
M.africana CMW4945	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8576	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8574	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
M.molleriana CMW2734	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8575	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 10898	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 10897	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
M.parkii CMW3358	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8573	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 10896	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
M.lateralis CMW4935	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
CMW 8581	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG
Ramulispora anguioides	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG

	370	380	390	400
M.grandis	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8554	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8557	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8570	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
M.walkeri STU2768	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 10899	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
M.africana CMW4945	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8576	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8574	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
M.molleriana CMW2734	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8575	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 10898	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 10897	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
M.parkii CMW3358	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8573	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 10896	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
M.lateralis CMW4935	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
CMW 8581	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC
Ramulispora anguioides	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC

```
                                410        420        430        440
M.grandis                       ATTGCGCCCC TCGGTATTCC GAGGGGCATG CCTGTTCGAG
CMW 8554                        ATTGCGCCCC TCGGTATTCC GAGGGGCATG CCTGTTCGAG
CMW 8557                        ATTGCGCCCC TCGGTATTCC GAGGGGCATG CCTGTTCGAG
CMW 8570                        ATTGCGCCCC TCGGTATTCC GAGGGGCATG CCTGTTCGAG
M.walkeri STU2768               ATTGCGCCCT CTGGTATTCC GGGGGGCATG CCTGTTCGAG
CMW 10899                       ATTGCGCCCT CTGGTATTCC GGGGGGCATG CCTGTTCGAG
M.africana CMW4945              ATTGCGCCCC GTGGTATTCC GCGGGGCATG CCTGTTCGAG
CMW 8576                        ATTGCGCCCC GTGGTATTCC GCGGGGCATG CCTGTTCGAG
CMW 8574                        ATTGCGCCCC GTGGTATTCC GCGGGGCATG CCTGTTCGAG
M.molleriana CMW2734            ATTGCGCCCT CTGGTATTCC GGAGGGCATG CCTGTTCGAG
CMW 8575                        ATTGCGCCCT CTGGTATTCC GGAGGGCATG CCTGTTCGAG
CMW 10898                       ATTGCGCCCT CTGGTATTCC GGAGGGCATG CCTGTTCGAG
CMW 10897                       ATTGCGCCCT CTGGTATTCC GGAGGGCATG CCTGTTCGAG
M.parkii CMW3358                ATTGCGCCCC GTGGTATTCC GCGGGGCATG CCTGTTCGAG
CMW 8573                        ATTGCGCCCC GTGGTATTCC GCGGGGCATG CCTGTTCGAG
CMW 10896                       ATTGCGCCCC GTGGTATTCC GCGGGGCATG CCTGTTCGAG
M.lateralis CMW4935             ATTGCGCCCC CTGGTATTCC GGGGGGCATG CCTGTTCGAG
CMW 8581                        ATTGCGCCCC CTGGTATTCC GGGGGGCATG CCTGTTCGAG
Ramulispora anguioides          ATTGCGCCCT CTGGTATTCC GGGGGGCATG CCTGTTCGAG

                                450        460        470        480
M.grandis                       CGTCATTTCA CCAC-TCAAG C-CTG-GCTT GGTATTGGGC
CMW 8554                        CGTCATTTCA CCAC-TCAAG C-CTG-GCTT GGTATTGGGC
CMW 8557                        CGTCATTTCA CCAC-TCAAG C-CTG-GCTT GGTATTGGGC
CMW 8570                        CGTCATTTCA CCAC-TCAAG C-CTG-GCTT GGTATTGGGC
M.walkeri STU2768               CGTCATTTCA CCAC-TCAAG C-CTG-GCTT GGTATTGGGC
CMW 10899                       CGTCATTTCA CCAC-TCAAG C-CTG-GCTT GGTATTGGGC
M.africana CMW4945              CGTCATTTCA CCAC-TCAAG C-CTA-GCTT GGTATTGGGC
CMW 8576                        CGTCATTTCA CCAC-TCAAG C-CTA-GCTT GGTATTGGGC
CMW 8574                        CGTCATTTCA CCAC-TCAAG C-CTA-GCTT GGTATTGGGC
M.molleriana CMW2734            CGTCATTACA CCAC-TCCGG C-CTC-GCTG GGTATTGGGC
CMW 8575                        CGTCATTACA CCAC-TCCGG C-CTC-GCTG GGTATTGGGC
CMW 10898                       CGTCATTACA CCAC-TCCGG C-CTC-GCTG GGTATTGGGC
CMW 10897                       CGTCATTACA CCAC-TCCGG C-CTC-GCTG GGTATTGGGC
M.parkii CMW3358                CGTCATTTCA CCAC-TCGAG T-CTGA-CTC GGTATTGGGC
CMW 8573                        CGTCATTTCA CCAC-TCGAG T-CTGA-CTC GGTATTGGGC
CMW 10896                       CGTCATTTCA CCAC-TCGAG T-CTGA-CTC GGTATTGGGC
M.lateralis CMW4935             CGTCATTACA ACCAATCCAG C-CCC-GCTG GGTATTGGGC
CMW 8581                        CGTCATTGCA ACCAATCCAG C-CCC-GCTG GGTATTGGGC
Ramulispora anguioides          CGTCATTATA ACCACTCAAG CTCTC-GCTT GGTATTGGGG
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                                490         500         510         520

M.grandis                       GCCGCGG-TT T---GCCGCG CGCCTCAAAG TCT---CCGG
CMW 8554                        GCCGCGG-TT T---GCCGCG CGCCTCAAAG TCT---CCGG
CMW 8557                        GCCGCGG-TT T---GCCGCG CGCCTCAAAG TCT---CCGG
CMW 8570                        GCCGCGG-TT T---GCCGCG CGCCTCAAAG TCT---CCGG
M.walkeri STU2768               GTCGCGG-T- ----GCCGCG CGCCTCAAAG TCTT--CCGG
CMW 10899                       GTCGCGG-T- ----GCCGCG CGCCTCAAAG TCTT--CCGG
M.africana CMW4945              GTCGCGG-TT -----CCGCG CGCCTTAAAG TCT---CCGG
CMW 8576                        GTCGCGG-TT -----CCGCG CGCCTTAAAG TCT---CCGG
CMW 8574                        GTCGCGG-TT -----CCGCG CGCCTTAAAG TCT---CCGG
M.molleriana CMW2734            GCCGCGGC-- --CT-CCGCG CGCCTCGAAG TCT---CCGG
CMW 8575                        GCCGCGGC-- --CT-CCGCG CGCCTCGAAG TCT---CCGG
CMW 10898                       GCCGCGGC-- --CT-CCGCG CGCCTCGAAG TCT---CCGG
CMW 10897                       GCCGCGGC-- --CT-CCGCG CGCCTCGAAG TCT---CCGG
M.parkii CMW3358                GTCGCGGCTT -CC-GCCGCG CGCCTCAAAG TCT---CCGG
CMW 8573                        GCCGCGCA-- -CC-GCCGCG CGCCTCAAAG TC---CCCGG
CMW 10896                       GCCGCGCA-- -CC-GCCGCG CGCCTCAAAG TC---CCCGG
M.lateralis CMW4935             GTCGCGGC-- --CTGCCGCG CGCCTCAAAG TCTT---CGG
CMW 8581                        GTCGCGGC-- --CTGCCGCG CGCCTCAAAG TCTA---CGG
Ramulispora anguioides          TTCGCGG-TT T-C-GC-G-G --CCTCTAAA -CTCA---GT

                                530         540         550         560

M.grandis                       CTG-AGCCAA CT---GTCTC TAAGCGTTGT GGTTTAA---
CMW 8554                        CTG-AGCCAA CT---GTCTC TAAGCGTTGT GGTTTAA---
CMW 8557                        CTG-AGCCAA CT---GTCTC TAAGCGTTGT GGTTTAA---
CMW 8570                        CTG-AGCCAA CT---GTCTC TAAGCGTTGT GGTTTAA---
M.walkeri STU2768               GTG-AGCTG- --CCCGTCTC CAAGCGTTGT GGCGACT---
CMW 10899                       CTG-AGCTG- --CCCGTCTC CAAGCGTTGT GGCGACT---
M.africana CMW4945              CTG-AGCAGT T---CGTCTC TAAGCGTTGT GGCATAT---
CMW 8576                        CTG-AGCAGT T---CGTCTC TAAGCGTTGT GGCATAT---
CMW 8574                        CTG-AGCAGT T---CGTCTC TAAGCGTTGT GGCATAT---
M.molleriana CMW2734            CCG-AGCCGA C---CGTCTC CAAGCGTTGT GGCACAACTG
CMW 8575                        CCG-AGCCGA C---CGTCTC CAAGCGTTGT GGCACAACTG
CMW 10898                       CCG-AGCCGA C---CGTCTC CAAGCGTTGT GGCACAACTG
CMW 10897                       CCG-AGCCGA C---CGTCTC CAAGCGTTGT GGCACAACTG
M.parkii CMW3358                CTG-GGCAG- C--CCGTCTC CGAGCGTTGT GGCATCACAG
CMW 8573                        CTG-GGCGGG C--CCGTCCC CGAGCGTTGT GATCTCACAG
CMW 10896                       CTG-GGCGGG C--CCGTCCC CGAGCGTTGT GATCTCACAG
M.lateralis CMW4935             CGGAAGCCG- --CCCGTTCC TCTGCGTGAT GACACATCG-
CMW 8581                        CGGAAGCCG- --CCCGTTCC TCTGCGTGAT GACACATCG-
Ramulispora anguioides          GGCGG---TG --CCTGTCGG CTCTACGCGT AGTAATAC--
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                                570        580        590        600

M.grandis                       -TCATC-C-G C-TT-GTGAG AT-CGAA--G GCGA--CGGC
CMW 8554                        -TCATC-C-G C-TT-GTGAG AT-CGAA--G GCGA--CGGC
CMW 8557                        -TCATC-C-G C-TT-GTGAG AT-CGAA--G GCGA--CGGC
CMW 8570                        -TCATC-C-G C-TT-GTGAG AT-CGAA--G GCGA--CGGC
M.walkeri STU2768               -A-TTCGCTT CG----GGG- -CGCGGG-CG GC--CGCGGC
CMW 10899                       -A-TTCGCTT CG----GGG- -CGCGGG-CG GC--CGCGGC
M.africana CMW4945              -ATTTCGCT- -G--AAAGAG TT-CGGGACG GCT-------
CMW 8576                        -ATTTCGCT- -G--AA-GAG TT-CGG-ACG GCT-------
CMW 8574                        -ATTTCGCT- -G--AA-GAG TT-CGG-ACG GCT-------
M.molleriana CMW2734            TT--TCGCTT TC----GGG- AC-CGGTCTG GCGGCGCGCC
CMW 8575                        TT--TCGCTT TC----GGG- AC-CGGTCTG GCGGCGCGCC
CMW 10898                       TT--TCGCTT TC----GGG- AC-CGGTCTG GCGGCGCGCC
CMW 10897                       TT--TCGCTT TC----GGG- AC-CGGTCTG GCGGCGCGCC
M.parkii CMW3358                TTC-TCGCTA ------GGG- AGTCGCGGAC GGCGTCGGCC
CMW 8573                        T-C-TCGCTA ------GGG- AGTCGCGTCC GCCGGCGGCC
CMW 10896                       T-C-TCGCTA ------GGG- AGTCGCGTCC GCCGGCGGCC
M.lateralis CMW4935             ----TCGCTT ------GGGA -CACGGGGGT GAGCGCCCGG
CMW 8581                        ----TCGCTT ------GGGA -CACGGGGGT GAGCGCCCGG
Ramulispora anguioides          TCC-TCGCGA T-----TGAG TCCGGTAGGT TTACTTGCCA

                                610        620        630        640

M.grandis                       C---------G TTAAA-CTTA TTCA---AAG GTTGACCTCG
CMW 8554                        C---------G TTAAA-CTTA TTCA---AAG GTTGACCTCG
CMW 8557                        C---------G TTAAA-CTTA TTCA---AAG GTTGACCTCG
CMW 8570                        C---------G TTAAA-CTTA TTCA---AAG GTTGACCTCG
M.walkeri STU2768               C---------G TTAAATCTTT CAC----AAG GTTGACCTCG
CMW 10899                       C---------G TTAAATCTTT CAC----AAG GTTGACCTCG
M.africana CMW4945              -TTTGGCC-G TTAAATCTTT CTT---AAAG GTTGACCTCG
CMW 8576                        -TTTGGCC-G TTAAATCTTT CTT----AAG GTTGACCTCG
CMW 8574                        -TTTGGCC-G TTAAATCTTT CTT----AAG GTTGACCTCG
M.molleriana CMW2734            ----------G TTAAACCCTT -TCAC-AAAG GTTGACCTCG
CMW 8575                        ----------G TTAAACCCTT -TCACCAAAG GTTGACCTCG
CMW 10898                       ----------G TTAAACCCTT -T-ACCAAAG GTTGACCTCG
CMW 10897                       ----------G TTAAACCCGA -TCACCAAAG GTTGACCTCG
M.parkii CMW3358                ----------G TTAAATACCC --CATCAAAG GTTGACCTCG
CMW 8573                        ----------G TTAAACACCC -CCATCACAG GTTGACCTCG
CMW 10896                       ----------G TTAAACACCC -CCATCACAG GTTGACCTCG
M.lateralis CMW4935             A-AAACATCG GCGGAGACGT CGATTTCAAG GTTGACCTCG
CMW 8581                        A-AAACATCG GCGGAGACGT CGATTTCAAG GTTGACCTCG
Ramulispora anguioides          ACAACC--C- CC--A-A-TT --TTTTACAG GTTGACCTCG
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650 655

M.grandis	GATCAGGTAG GGATA
CMW 8554	GATCAGGTAG GGATA
CMW 8557	GATCAGGTAG GGATA
CMW 8570	GATCAGGTAG GGATA
M.walkeri STU2768	GATCAGGTAG GGATA
CMW 10899	GATCAGGTAG GGATA
M.africana CMW4945	GATCAGGTAG GGATA
CMW 8576	GATCAGGTAG GGATA
CMW 8574	GATCAGGTAG GGATA
M.molleriana CMW2734	GATCAGGTAG GGATA
CMW 8575	GATCAGGTAG GGATA
CMW 10898	GATCAGGTAG GGATA
CMW 10897	GATCAGGTAG GGATA
M.parkii CMW3358	GATCAGGTAG GGATA
CMW 8573	GATCAGGTAG GGATA
CMW 10896	GATCAGGTAG GGATA
M.lateralis CMW4935	GATCAGGTAG GGATA
CMW 8581	GATCAGGTAG GGATA
Ramulispora anguioides	GATCAGGTAG GGATA