## 

## Chapter 3

# Mycosphaerella species causing leaf disease in South African Eucalyptus plantations 




#### Abstract

Commercial Eucalyptus plantations provide an important source of hardwood for several industries worldwide. Several species of Mycosphaerella are associated with a destructive Eucalyptus leaf disease known as Mycosphaerella leaf disease (MLD). During 2000, a survey was undertaken in several commercial Eucalyptus growing areas of South Africa to determine the identity of the Mycosphaerella spp. contributing to outbreaks of MLD. Symptomatic leaf samples were collected from three major Eucalyptus growing areas and the Mycosphaerella spp. were isolated. Isolates were identified using ascospore germination patterns and sequence data from the ribosomal DNA operon. Six species, namely; M. ellipsoidea, M. irregulariramosa, M. juvenis, M. lateralis, M. marksii, M. nubilosa as well as an undescribed species of Mycosphaerella were identified. Mycosphaerella nubilosa was most commonly isolated, particularly on E. nitens and appears to be a dominant species contributing to MLD. Data obtained in this study show that MLD is caused by a complex of species contributing to disease outbreaks in South Africa.


## INTRODUCTION

Eucalyptus L'Heritier is a large genus in the Myrtaceae, that includes approximately 700 species (Potts \& Pederick 2000). Most of these species are native to Australia and Papua New Guinea, where they occur in large natural forests (Poynton 1979). Eucalyptus spp. are grown commercially in intensively managed plantations in both the Northern and Southern Hemispheres. In South Africa, Eucalyptus spp. comprise 47 percent of the total 1.5 million ha of commercial plantations (Edwards 2000). These trees are now amongst a small number of favoured forestry species throughout the world. As such, they rival Pinus spp. in their use as a commercial forestry resource. Eucalyptus spp. are, however, susceptible to many pathogens that threaten plantations (Wingfield et al. 1995, Wingfield 1999). Species of Mycosphaerella Johanson are regarded to be some of the most important Eucalyptus leaf pathogens.

The genus Mycosphaerella is large and includes approximately 2000 species (Corlett 1991, 1995, Aptroot et al. 1999). Species of Mycosphaerella include both saprophytes and parasites that infect woody and herbaceous hosts and generally cause leaf diseases (Arx 1983). Several species of Mycosphaerella are associated with Mycosphaerella leaf disease (MLD) of Eucalyptus spp. in many countries where these trees are grown as a commercial hardwood resource (Crous 1998, Carnegie 2000).

Symptoms associated with MLD are variable and can differ depending on the Mycosphaerella spp. involved. The primary symptoms are leaf spots that reduce the photosynthetic capacity of leaves and result in defoliation (Beresford 1978, Ganapathi 1979). In cases of severe infection, the disease can spread to young shoots and branches, where cankers are formed, resulting in gum exudation and eventual twig die-back (Dick 1982, Dick \& Gadgil 1983). This symptom leads to the formation of multi-stemmed trees, which are unsuitable for milling (Beresford 1978). Trees often become physiologically stressed and stunted, resulting in reduced growth, infection by secondary pathogens, and increased silvicultural costs.

The taxonomy and identification of Mycosphaerella spp. is complicated and beset with problems. Traditionally, host affiliations have been the primary factor considered for identification. This approach has, however, been recognized as
unreliable (Corlett 1991, Aptroot \& Lucking 2001). Recently, cultural characteristics and anamorph-teleomorph connections have received attention in the classification of Mycosphaerella spp., and today 23 anamorph genera, within the coelomycetes and hyphomycetes, are accepted as Mycosphaerella anamorphs (Arx 1983, Crous 1998, Crous et al. 2000). Ascospore germination patterns have also provided useful taxonomic characters (Park \& Keane 1982a, Crous 1998). More recently DNA sequence data have contributed substantially to the identification of Mycosphaerella spp. occurring on Eucalyptus spp and other hosts (Crous et al. 1999, 2001).

Several Mycosphaerella spp. are readily isolated from diseased Eucalyptus leaves in South Africa (Crous 1998). During initial studies of MLD in this country, the disease was attributed to M. molleriana (Thüm.) Lindau (Doidge 1950). Subsequent studies, particularly on E. nitens (Deane et Maiden) Maiden, suggested that M. nubilosa was the only Mycosphaerella spp. causing MLD in South Africa (Purnell \& Lundquist 1986, Crous et al. 1989). More recently, several new species of Mycosphaerella were identified on Eucalyptus spp., including M. africana Crous \& M.J. Wingf., M. crystallina Crous \& M.J. Wingf., M. ellipsoidea Crous \& M.J. Wingf and M. juvenis Crous \& M.J. Wingf. (Crous \& Wingfield 1996, Crous 1998). The impact, relative occurrence and importance of these Mycosphaerella spp. on commercial forestry in South Africa, however, remains unclear.

The aim of this study was to consider the occurrence of Mycosphaerella spp. on Eucalyptus spp., specifically in commercial plantations experiencing outbreaks of MLD. To achieve this goal, surveys were conducted and samples collected from plantations in three major Eucalyptus growing areas of South Africa. Mycosphaerella spp. were recovered from diseased leaves and identified based on ascospore germination patterns, leaf symptoms, cultural characteristics and sequence data from the Internal Transcribed Spacer (ITS) region of the rDNA operon.

## MATERIALS AND METHODS

## Sample collection

Samples of diseased Eucalyptus leaves were collected from various plantations in three major Eucalyptus growing areas of South Africa. The plantations were selected based on advice received from foresters and occurred in the KwaZulu-Natal Midlands, Tzaneen in the Northern Province and Umtata in the Eastern Cape Province. From KwaZulu-Natal, diseased leaves were collected from two plantations. These were Clairemont (latitude $29^{\circ} 40^{\prime \prime}$, longitude $29^{\circ} 45^{\prime \prime}$ ) near Bulwer and the Enon plantation (latitude $29^{\circ} 49^{\prime \prime}$, longitude $30^{\circ} 13^{\prime \prime}$ ) near Richmond. A total of 126 trees were sampled ( 65 trees from Clairemont, 61 trees from Enon) from these two plantations with ten leaves collected from each diseased tree. These samples were collected from several different Eucalyptus spp. including E. nitens, E. bicostata Maiden, Blakely et Simmonds, E. macarthurii Deane et Maiden, E. smithii R. T. Baker, E. dunnii Maiden, E. grandis Hill ex Maiden and one clone of E. grandis $\times E$. nitens.

Samples from Tzaneen were collected from a plantation belonging to Northern Timbers (latitude $23^{\circ} 40^{\prime \prime}-23^{\circ} 58^{\prime \prime}$, longitude $30^{\circ}-30^{\circ} 15^{\prime \prime}$ ). A total of 33 trees were sampled and ten leaves were collected from each diseased tree. The Eucalyptus spp. sampled included E. grandis and E. grandis $\times$ camaldulensis. Samples were also collected from 10 trees in Umtata (Eastern Cape Province), these were all from naturally regenerated $E$. grandis trees, where ten leaves were collected from each tree. In all cases, diseased leaves were placed in brown paper bags after collection and transported to the laboratory, where Mycosphaerella isolations were conducted within three days.

## Isolation procedures and isolates examined

Leaves displaying a wide array of different lesions were chosen for isolations. The number of leaves used from each sample varied from two to five, depending on the number and types of lesions present on leaves. An effort was made to isolate from as
many different lesion types as possible, even though there was no particular symptom that was obviously dominant. One to three lesions per leaf were excised and used for isolations. Excised lesions were placed in water for approximately two hours after which they were placed on double sided adhesive tape and adhered to the insides of Petri dish lids over 2\% malt extract agar (MEA) (wt/v) (Biolab, South Africa), with the pseudothecia facing the agar surface (Crous 1998). Petri dishes were incubated in the dark for 24 hours to allow for ascospore release and germination on MEA. Following incubation, individual germinating ascospores were subcultured onto $2 \%$ MEA and incubated at $25^{\circ} \mathrm{C}$ in the dark.

Once colonies had formed, they were incubated at $25^{\circ} \mathrm{C}$ under continuous cool white light in an incubator. Germinating ascospores were also sub-cultured onto Carnation Leaf Agar (CLA) [ $1 \%$ water agar (wt/v) (Biolab, South Africa) with sterilized carnation leaves placed onto medium] and incubated at $25^{\circ} \mathrm{C}$ under continuous near-ultra-violet light (nuv, 250 nm ) to promote the production of any asexual states. All cultures retained from this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

## Symptoms and morphology of Mycosphaerella spp.

All Mycosphaerella isolates obtained from Eucalyptus spp. in this study were considered in terms of lesion characteristics, ascospore germination patterns and cultural characteristics. Lesions were characterized based on colour, shape, and ascomatal position.

After ascospore discharge from pseudothecia onto MEA, germinating ascospores were transferred to microscope slides and mounted in lactophenol for microscopic examination. Ascospores were evaluated based on characteristics known to be taxonomically relevant for Mycosphaerella spp. (Park \& Keane 1982a, Crous 1998). From these criteria, the ascospore germination patterns were grouped as outlined by Crous (1998). Characteristics were compared with those for other Mycosphaerella spp. described from Eucalyptus spp. (Crous 1998).

Following isolation, cultures of Mycosphaerella spp. were grouped according to cultural colour (Rayner 1970) and morphology. Mycosphaerella cultures were also cultured onto CLA in an attempt to induce anamorph formation (Crous 1998). Two to four representative isolates grouped according to morphology, culture characteristics and ascospore germination patterns, were chosen for DNA sequencing (Table 1). The sequences of these isolates were compared to those from other Mycosphaerella spp. known to cause MLD on Eucalyptus spp. (Crous et al. 2001) (Table 1).

## DNA Isolation

Following growth of axenic cultures, mycelium was scraped directly from agar plates and used for DNA isolation. Harvested mycelium was dried under vacuum and lypholized with liquid nitrogen. DNA was isolated using the method of Raeder \& Broda (1985) with minor variations. The $1: 1$ phenol:chloroform purification step was repeated until the interphase between the two aqueous phases was clean of any cellular debris. Nucleic acids were precipitated by the addition of $10 \% 3 \mathrm{M} \mathrm{NaAc}$ and 2 volumes of absolute ethanol and incubated at $-20^{\circ} \mathrm{C}$ for 2 hours. DNA was further purified by washing with $70 \%$ ethanol and dried under vacuum, after which the resulting DNA pellet was resuspended in $50 \mu \mathrm{l}$ SABAX water. RnaseA $(10.0 \mu \mathrm{~g} / \mu \mathrm{l})$ was added to the DNA samples, and incubated at $37^{\circ} \mathrm{C}$ for three to four hours to digest any residual protein or RNA. DNA was visualized on a $1 \%$ agarose gel (wt/v) (Boehringer Mannheim, Germany) stained with ethidium bromide and viewed under an ultra-violet light. DNA was quantified for all samples with a Beckman DU Series 60 Spectrophotometer (Beckman, Germany).

## PCR Amplification and Purification

Isolated DNA ( $50-90 \mathrm{ng}$ ) was used as a template for the Polymerase Chain Reaction (PCR). The Internal Transcribed Spacer (ITS) region of the rDNA operon, was targeted for amplification using primers ITS 1 ( $5^{\prime}$ - TCC GTA GGT GAA CCT GCG G $-3^{\prime}$ ) and LR1 ( $5^{\prime}$ - GGT TGG TTT CTT TTC CT $-3^{\prime}$ ) (White et al. 1990). The ITS 1 and ITS 2 regions including the 5.8 S gene were amplified. DNA was amplified in a
$50 \mu 1$ reaction volume containing PCR buffer ( 10 mM Tris- $\mathrm{HCL}, 1.5 \mathrm{mM} \mathrm{MgCl} 2,50$ $\mathrm{mM} \mathrm{KCl}, \mathrm{pH} 8.3)$ (Roche Diagnostics, South Africa), 2.5 mM of each dNTP (dATP, dTTP, dCTP and dGTP) (Roche Diagnostics, South Africa), $0.2 \mu \mathrm{M}$ of primers ITS1 and LR1 (MWG Biotech, Germany) and 2.5 U Taq DNA polymerase (Roche Diagnostics, South Africa). SABAX water was used to achieve the total volume of 50 $\mu \mathrm{l}$.

PCR reactions were carried out using an Eppendorf Mastercycler gradient PCR machine (Eppendorf Scientific, Germany). PCR reaction conditions included an initial denaturation temperature of $96^{\circ} \mathrm{C}$ for 2 min . Following initial denaturation, 40 cycles of template denaturation for 30 s at $94^{\circ} \mathrm{C}$, primer annealing for 30 s at $53^{\circ} \mathrm{C}$ and chain elongation for 2 min at $75^{\circ} \mathrm{C}$ were carried out with a final elongation at $75^{\circ} \mathrm{C}$ for 7 min . A negative control using water and no template DNA and a positive control containing DNA of a Mycosphaerella sp., was used for each reaction. PCR products were visualized in $2 \%$ agarose gels stained with ethidium bromide and viewed under ultra-violet light. Sizes of PCR products were determined against a 100 bp molecular weight marker XIV (Roche Diagnostics, South Africa). PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics, South Africa). After PCR purification, concentrations of purified PCR products were determined by running products on a $2 \%$ agarose gel stained with ethidium bromide, together with a 100 bp molecular weight marker XIV and viewed under ultra-violet light.

## DNA sequencing and data analysis

Purified PCR products were used as template DNA for sequencing reactions on an ABI PRISM ${ }^{\text {TM }} 377$ Automated DNA sequencer (Perkin Elmer, Norwalk, CON). The ABI Prism Big Dye Terminator Cycle sequencing reaction kit (Perkin Elmer Biosystems, USA) was used for the sequencing reactions. Sequencing reactions were conducted with the same primers used for the PCR reaction, however, two internal primers ITS3 ( $5^{\prime}$ - GCA TCG ATG AAG AAC GCA GC -3 ') and ITS2 ( $5^{\prime}$ - GCT GCG TTC TTC ATC GAT GC $-3^{\prime}$ ) (White et al. 1990) were used as well to completely sequence both DNA strands of the ITS region.

Sequences were analyzed using Sequence Navigator version 1.0.1 (Perkin-Elmer, Applied Biosystems, Foster City, CA). Sequence alignments were done using the Clustal function of Sequence Navigator and gaps were inserted manually where necessary. Phylogenetic analysis of aligned sequences was conducted using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0bl (Swofford 1998). The Heuristic search function was used to generate the most parsimonious trees. Starting trees for the analyses were obtained by stepwise addition, with the MULPAR function effective. Tree Bisection Reconnection (TBR) was used as the swapping algorithm with maximum parsimony as an optimal criterion. All characters in the analysis were of equal weight. Branch support was evaluated by performing a Bootstrap search of 1000 replicates on the aligned sequences. Published sequences of Mycosphaerella spp. from Eucalyptus spp. were obtained from Genebank and compared to those sequences obtained for the Mycosphaerella spp. isolated in this study. Following the analysis all resulting trees were rooted to an outgroup. The taxon chosen for this purpose was Ramulispora anguoides (Nirenberg) Crous, which resides in the genus Tapesia and has been shown to be an appropriate outgroup for Mycosphaerella spp.(Crous et al. 2001).

## RESULTS

Isolation procedures and isolates examined

Mycosphaerella ascospores were obtained from most, but not all lesions. Isolates of Mycosphaerella were, however, recovered from a wide variety of Eucalyptus spp. and all of the three areas sampled. A total of 382 isolates of Mycosphaerella were recovered, namely 257 from the Clairemont and Enon plantations (KwaZulu-Natal Midlands), 71 from Tzaneen (Northern Province) and 54 from Umtata (Eastern Cape Province). These isolates could be separated into 7 groups based on lesion type, cultural morphology and ascospore germination patterns. Based on symptoms, cultural characteristics and germination patterns, five species of Mycosphaerella were identified from the KwaZulu-Natal Midlands. These included M. ellipsoidea Crous \& M. J. Wingf., M. juvenis, M. lateralis Crous \& M.J. Wingf., M. marksii and M. nubilosa. Four species were identified from Tzaneen, namely M. irregulariramosa

Crous \& M.J. Wingf., M. lateralis and M. marksii and an apparently undescribed Mycosphaerella species. Two species, M. nubilosa and M. juvenis, emerged from samples originating in Umtata in the Eastern Cape.

## Symptoms and Morphology

## Mycosphaerella ellipsoidea

Mycosphaerella ellipsoidea was isolated only from the KwaZulu-Natal Midlands. In total, six cultures of M. ellipsoidea were collected from two E. nitens trees (Enon plantation). Lesions were present on both leaf surfaces (amphigenous) and lesions were circular to sub-circular in shape with raised borders and a medium brown to dark brown colour (Figure 1B, Figure 2F). Pseudothecia were black, amphigenous and single. Ascospores showed a typical Type I ascospore germination pattern (Figure 3F) (Crous 1998). Ascospores germinated from both poles, producing parallel germ tubes with lateral projections (Crous 1998). Colonies of M. ellipsoidea had even to uneven margins. Colonies did not sector but did exhibit folding into the medium. Aerial mycelium was moderate to profuse with white fluffy patches. Colonies were olivaceous black 27 '"'m (reverse) (Figure 4B). Incubation of this species on CLA resulted in the production of an asexual state that was identified as Uwebraunia ellipsoidea Crous \& M. J. Wingf.

## Mycosphaerella irregulariramosa

Eight isolates of M. irregulariramosa were collected from juvenile leaves on six $E$. grandis trees from Tzaneen (Northern Timbers plantation). Lesions of this species were amphigenous, light brown, with a dark brown border (Figure 1F). Lesions were circular to irregular in shape, and varied in size from $2-25 \mathrm{~mm}$ (Figure 2B). Pseudothecia were black, amphigenous, single, and evenly dispersed. Ascospores showed a Type I germination pattern and germinated from both poles producing parallel germ tubes (Figure 3B) (Crous 1998). Lateral, secondary germ tubes were also produced from the original ascospore. Colonies had regular to irregular margins, but were predominantly irregular. Although colonies were unsectored, they did fold
into the agar medium after prolonged incubation. Aerial mycelium was sparse. Colonies were iron grey 23 ',"',i (surface) and olivaceous black $27{ }^{\prime}, ' m$ to greenish black $33^{\prime,}, \gg \mathrm{k}$ (reverse) (Figure 4F). Pseudocercospora irregulariramosa Crous \& M. J. Wingf., the anamorph of M. irregulariramosa was readily produced on CLA medium.

## Mycosphaerella juvenis

Mycosphaerella juvenis was isolated from KwaZulu-Natal and from Umtata. A total of twelve cultures of $M$. juvenis were collected from KwaZulu-Natal on hosts such as, E. nitens (Clairemont plantation), E. grandis (Enon plantation), E. smithii (Enon plantation), E. dunni (Enon plantation) and E. grandis $\times$ E. nitens (Enon plantation). Four cultures of $M$. juvenis were collected from three naturally regenerated E. grandis trees near Umtata.

Lesions of M. juvenis were amphigenous, varying in shape from circular to irregular, with some lesions coalescing to form larger spreading lesions (Figure 1C). Lesions were light brown on the abaxial surface and a darker brown on the adaxial surface. Lesions were often surrounded by raised dark borders, particularly on the adaxial leaf surface (Figure 2C). Pseudothecia of M. juvenis occurred only on the abaxial leaf surface (hypophyllous). Pseudothecia were single, black and numerous. Ascospore germination showed a typical Type F germination pattern (Crous 1998). Ascospores germinated from both poles and produced germ tubes that grew parallel to the long axis of the spores. Upon germination, the ascospores became constricted at the median septum and the spores became prominently swollen (Figure 3C). Colonies of M. juvenis had even margins and produced erect hyphal tufts. Aerial mycelium was moderate to profuse. Colonies were olivaceous black 29 '"' m to olivaceous grey
 (Figure 4C). Uwebraunia juvenis Crous \& M. J. Wingf., the anamorph of M. juvenis was produced in culture on CLA.

## Mycosphaerella lateralis

Mycosphaerella lateralis was collected from KwaZulu-Natal and Tzaneen. From KwaZulu-Natal, two isolates of M. lateralis were obtained from one $E$. nitens tree (Clairemont plantation). Thirteen isolates of $M$. lateralis were collected from thirteen E. grandis trees in Tzaneen (Northern timbers plantation).

Leaf spots were amphigenous, grey to dark brown in colour with raised borders (Figure 1D). Lesions were the same colour on the reverse side (abaxial) of the leaf and were circular to sub-circular in shape (Figure 2D). Smaller lesions often coalesced to form larger lesions of a more irregular shape. Pseudothecia were black, predominantly epiphyllous, and sparse. Ascospores of M. lateralis germinated from both poles and produced germination tubes that grew roughly parallel to the long axis of the spore (Type I) (Figure 3D). Ascospores showed a constriction at the septum upon germination. After a longer period of ascospore germination, lateral branches were produced from the primary germ tube (Crous 1998). Cultures of M. lateralis had irregular margins and sparse to medium aerial mycelium. Colonies were not sectored but did show some folding into the agar medium. Colonies were olivaceous 21 '"m to 23 '"'" $i$ (surface) and grey olivaceous 23 '"'i (reverse) (Figure 4D). The anamorph of M. lateralis, Uwebraunia lateralis Crous \& M. J. Wingf., was readily produced on CLA after one to two weeks of incubation under near-ultra-violet light.

## Mycosphaerella marksii

Mycosphaerella marksii was collected from the KwaZulu-Natal midlands and Tzaneen areas. A total of twelve isolates of M. marksii were isolated from one tree each of $E$. grandis, E. bicostata and E. smithii (Enon plantation). Five isolates were obtained from Tzaneen from five E. grandis trees (Northern timbers plantation).

Leaf spots extended through the leaf lamina and were visible on both the abaxial and adaxial leaf surfaces (Figure 1E). Lesions were light to dark brown in colour with a raised brown border. A faint red margin could be observed around the majority of lesions (Figure 2E). Pseudothecia of M. marksii were located predominantly on the adaxial leaf surface (epiphyllous). Ascospores showed a typical Type B germination
pattern, germinating from both poles with long germ tubes growing parallel to the long axis of the spores. Ascospores did not constrict or swell upon germination (Figure 3E) (Crous 1998). This species was characterized by colonies with smooth or uneven edges and folding into the agar medium to form sectors with sparse aerial mycelium. Colonies were raised above the agar with a roughly concentric colony morphology. Colonies were pale olivaceous grey 23 ',",' f to olivaceous grey $22^{\prime,}{ }^{\prime}, \mathrm{\prime} \mathrm{i}$ (surface) and olivaceous black 27 '"'m (reverse) (Figure 4E). Incubation of isolates of M. marksii on CLA did not result in the formation of any anamorph. However, ascomata were readily produced in culture.

## Mycosphaerella nubilosa

Mycosphaerella nubilosa was collected from KwaZulu-Natal and Umtata. M. nubilosa was the dominant fungus in both these regions. From KwaZulu-Natal, a total of 232 isolates were obtained from four Eucalyptus species, 47 E. nitens trees (Clairemont plantation), two E. bicostata trees (Enon plantation), two E. dunnii trees (Enon plantation) and five trees representing a E. grandis $\times$ nitens clone (Enon plantation). From Umtata, a total of 50 M . nubilosa cultures were collected from 10 naturally regenerated $E$. grandis trees.

Lesions of M. nubilosa varied in size from small spots to large blotches across the leaf surface (Figure 1A). Lesions were round to irregular in shape and frequently coalesced with other lesions to form larger blotches. Lesions were amphigenous, light to pale brown in colour and had a raised brown to light brown margins (Figure 2A). Pseudothecia of M. nubilosa were amphigenous, but predominantly hypophyllous. Ascospores of M. nubilosa showed typical Type C ascospore germination patterns (Crous 1998). Ascospores germinated from both poles and produced germ tubes that grew roughly parallel to the long axis of the spore. No lateral branches were produced and the ascospores showed a slight constriction at the median septum (Figure 3A). Cultures of M. nubilosa had regular to irregular margins with little aerial mycelium. Cultures were initially pale olivaceous grey 23 '","'f becoming dark olivaceous grey $23{ }^{\prime}{ }^{\prime}, \gg 1$ (surface) (Figure 4A) (Crous 1998). No anamorph was produced in culture.

## Mycosphaerella sp.

An apparently unidentified species of Mycosphaerella was collected from 43 E. grandis trees in Tzaneen (Northern timbers plantation). This species was dominant in Tzaneen and was represented by a total of 45 isolates. This fungus was not found in other forestry areas surveyed. Based on all key morphological characteristics used to identify Mycosphaerella spp. from Eucalyptus, it was concluded that this fungus represented a new taxon. It is thus described as follows:

The description of this fungal species does not represent a formal mycological description and is only described here for thesis purposes. This description should not be cited. A formal mycological description of this fungus will be published at a later date in a recognized mycological journal.

## Species description

Mycosphaerella fori sp. nov. Fig. 6 A-E, 7A-E.

Anamorph. Pseudocercospora fori sp. nov.

Leaf spots amphigenous, subcircular to irregular, $2-30 \mathrm{~mm}$ diam., becoming confluent along leaf margins, grey, surrounded by a dark brown outer zone, and a thin red-purple border, confluent with the leaf surface; abaxial surface medium brown with a dark brown outer zone. Pseudothecia predominantly epiphyllous, single, black, immersed becoming erumpent, globose, $50-100 \mu \mathrm{~m}$ diam.; apical ostiole $5-10 \mu \mathrm{~m}$ diam.; wall of 2-3 layers of medium brown textura angularis. Asci aparaphysate, fasciculate, bitunicate, subsessile, subcylindrical to narrowly obclavate, straight or slightly incurved, 8 -spored, $35-55 \times 6-10 \mu \mathrm{~m}$. Ascospores 3 - to multiseriate, overlapping, hyaline, guttulate, thick-walled, straight to slightly curved, narrowly ellipsoid with subobtuse apices, medianly 1 -septate, widest at unconstricted septum, tapering equally toward both ends, (12-)14-16(-20) $\times 3(-3.5) \mu \mathrm{m}$ in vivo, $11-14 \mathrm{x}$ $2.5-3.5 \mu \mathrm{~m}$ in vitro. Mycelium immersed and superficial, of smooth, branched, septate, pale brown hyphae, $3-4 \mu \mathrm{~m}$ diam. Caespituli fasiculate, predominantly epiphyllous, medium brown on leaves, up to $150 \mu \mathrm{~m}$ wide and $90 \mu \mathrm{~m}$ high.

Conidiophores occurring singly on secondary mycelium as lateral projections, or arranged in fascicles; fascicles arising from the upper cells of a medium brown stroma up to $60 \mu \mathrm{~m}$ wide and $50 \mu \mathrm{~m}$ high; conidiophores smooth, unbranched or rarely branched below, 1-3-septate, subcylindrical, straight to geniculate-sinuous, pale brown, $20-60 \times 2.5-4 \mu \mathrm{~m}$. Conidiogenous cells terminal, subcylindrical, straight or with several geniculations, pale brown, monoblastic or polyblastic, sympodial, or proliferating $1-3$ times percurrently near the apex, $15-30 \times 2.5-3.5 \mu \mathrm{~m}$, with truncate apices; conidial scars unthickened. Conidia solitary, subcylindrical, pale brown, smooth, variously curved, apex subobtuse, base truncate, (50-)70-90(-100) x 2-3(3.5) $\mu \mathrm{m}$, indistinctly $1-3$-septate; hilum unthickened, $1.5-2 \mu \mathrm{~m}$ wide.

HOLOTYPE. SOUTH AFRICA. NORTHERN PROVINCE: Tzaneen, on leaves of E. grandis 2000, G.C. Hunter (PREM 57305, teleomorph; PREM 57306, anamorph), cultures ex type CMW 9095.

Etymology. Raper (1987), states that Tzaneen is a North Sotho term indicating "where the people used to meet" or "in a basket". The epithet, is therefore to be translated " of the forum", or by extension "of Tzaneen".

Ascospore germination on MEA after 24 h . Type C. Ascospores do not darken on MEA, and germinate from both ends, with germ tubes parallel to long axis of spore, and with no visible distortion thereof. Some constriction occurs at the original ascospore septum, with ascospores becoming 3-4 $\mu \mathrm{m}$ diam.

Cultures. Colonies $35-39 \mathrm{~mm}$ diam on MEA after 1 month at $25^{\circ} \mathrm{C}$. Colonies olivaceous, $21^{\prime \prime} \mathrm{k}$ (reverse) and smoke grey, $21^{\prime \prime}{ }^{\prime}$ 'f to grey olivaceous, $21^{\prime}{ }^{\prime}{ }^{\prime} \mathrm{i}$ (surface) (Rayner 1970). Colony centre smoke grey, while colony border is grey olivaceous. Aerial mycelium profuse. Margins regular and smooth. Colony surface not sectored and no folding occurs. Only the anamorph, $P$. fori is observed in culture.

Cardinal Temperatures. Min. above $5^{\circ} \mathrm{C}$, opt. $20-25^{\circ} \mathrm{C}$, max. below $35^{\circ} \mathrm{C}$.

Distribution. Tzaneen, Northern Province, South Africa.

Notes: Mycosphaerella fori is most similar to M. gracilis Crous \& Alfenas, but can be distinguished by its ascospore germination pattern, where ascospores remain unconstricted in M. gracilis (type B). Furthermore, conidia of Pseudocercospora fori taper towards their apices, whereas conidia of $P$. gracilis Crous \& Alfenas are cylindrical.

## PCR amplification and sequence data analysis

Amplification of the ITS region of the rDNA operon resulted in amplification products of approximately 600 bp for all isolates. Sequence and parsimony analysis of representative isolates combined with published sequences of Mycosphaerella spp. on Eucalyptus spp. produced four most parsimonious trees with a length of 1562 steps $(\mathrm{CI}=0.5038, \mathrm{RI}=0.7879, \mathrm{HI}=0.4962)$ using the heuristic search option. Of the 657 characters that were analyzed; 257 of these were constant, 106 were parsimonyuninformative and 295 were parsimony-informative. A bootstrap search of 1000 replicates produced a tree of the same topology as the most parsimonious tree (Figure 5). The total data set could be resolved into two major clades. The first clade comprised a larger monophyletic Mycosphaerella clade and the second clade was represented by species producing a Dissoconium de Hoog, van Oorschot \& Hijwegen anamorph. Within the larger Mycosphaerella clade, several anamorph genera, associated with those Mycosphaerella spp. occurring on Eucalyptus were represented. These included Stenella Syd., Pseudocercospora Speg., Cercostigmina U. Braun, Sonderhenia H. Swart \& J. Walker, Uwebraunia Crous \& M. J Wingf., Phaeophleospora Rangel, Mycovellosiella Rangel and Colletogloeopsis Crous \& M. J. Wingf.

Based on isolations made from South African Eucalyptus leaves, six previously recognized species and one new species of Mycosphaerella could be identified from the sequence data. These taxa correlated with those identified based on morphology. Thus, sequence data clearly separated $M$. marksii, M. irregulariramosa, M. ellipsoidea, M. juvenis, M. nubilosa, M. lateralis and M. fori. Four of the species
isolated during this survey, M. marksii, M. irregulariramosa, M. ellipsoidea and M. lateralis were supported with a $100 \%$ bootstrap support, and showed no base differences when compared with their respective reference isolates. Mycosphaerella nubilosa isolates were also supported with a strong bootstrap support and had four base differences when compared with the reference isolate of M. nubilosa (CMW 3282) originally collected from Australia. Isolates of M. juvenis grouped within the Uwebraunia sub-clade, together with two reference isolates of $M$. juvenis (CMW 4936 \& CMW4937) from South Africa, and showed eight base pair differences when compared with the reference strains. Isolates of M. lateralis grouped outside of the larger Mycosphaerella clade within a smaller, well supported second clade, together with reference isolates from South Africa (CMW 5164) and Zambia (CMW 4935). This smaller clade is represented by species with a Dissoconium anamorph, of which M. lateralis is the only Mycosphaerella species associated with this anamorph genus (Crous et al. 1999).

Isolates CMW 9094 \& CMW 9095, representing M. fori from Tzaneen, grouped in a clade comprising Pseudocercospora species from Eucalyptus and Syzygium. These isolates grouped close to Pseudocercospora eucalyptorum Crous, M. J. Wingf., Marasas \& B. Sutton isolates, which is known to occur on Eucalyptus leaves in South Africa. They were, however, distinct from this species, grouping within their own clade with high bootstrap support ( $99 \%$ ).

## DISCUSSION

Results from this study have provided substantial clarification to our understanding of the occurrence of Mycosphaerella spp. in commercial Eucalyptus plantations in South Africa. While a relatively large number of species of Mycosphaerella have previously been reported from South Africa (Crous \& Wingfield 1996, Crous 1998), there has been very little information available regarding their relative importance and distribution. The focus of the present study was to include the most important Eucalyptus spp. in South Africa and to concentrate collections in plantations where leaf spot problems were being experienced. As a result, we were able to detect 6 of the total number of species previously reported on Eucalyptus in South Africa, and a previously undescribed new species was also collected.

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It is clear from this study that one species, M. nubilosa, is dominant in disease outbreaks throughout the sampled areas, particularly KwaZulu-Natal and Umtata. Mycosphaerella nubilosa is one of the main pathogens responsible for MLD in Australia and New Zealand (Park \& Keane 1982a, b, Dick \& Gadgil 1983). It is now clear that it is also an important pathogen in South Africa, where it causes severe leaf spotting and defoliation of young Eucalyptus trees, especially E. nitens. This species was, however, also isolated from other Eucalyptus spp. including E. bicostata, E. dunni and E. grandis. Among these species, E. grandis and E. dunni are particularly important in South Africa, but M. nubilosa does not appear to cause severe damage on these species.

Mycosphaerella marksii was first identified from Victoria in Australia where it was infecting both adult and juvenile leaves of several Eucalyptus spp. (Carnegie \& Keane 1994). Previous surveys of Eucalyptus spp. in South Africa showed that M. marksii was present in the Western Cape, KwaZulu-Natal and Gauteng Provinces (Crous \& Wingfield 1996). Results of the present study have expanded our knowledge of the geographic distribution of this fungus to now include the Northern Province. The known host range of $M$. marksii includes $E$. botryoides Smith, $E$. fraxinoides Deane et Maiden, E. globulus Labill., E. grandis, E. nitens, E. quadrangulata Deane et Maiden and E. saligna Smith (Crous 1998). During this study, M. marksii was also found on E. bicostata and E. smithii, the latter of which is an important plantation species. However, the low number of isolates of M. marksii collected suggests that this fungus is not a major contributor to MLD outbreaks.

In this study, M. lateralis was found on diseased leaves of E. nitens and E. grandis leaves from KwaZulu-Natal and Tzaneen. This identification is not surprising as the fungus was previously known to occur in these areas (Crous \& Wingfield 1996). However, our results have expanded the known host range of this fungus to include $E$. grandis. Other than in South Africa, M. lateralis is known to occur in Zambia, and has recently been found in Queensland, Australia (Crous 1998, Maxwell et al. 1999).

Mycosphaerella juvenis was previously considered as the most important species contributing to outbreaks of MLB in South Africa (Crous \& Wingfield 1996, Crous
1998). It was thus surprising that this fungus was encountered relatively infrequently in the present study. The reason for the apparent change in status of this fungus is not clear. It is possible that species of Mycosphaerella causing leaf disease differ in their distributions and occurrence in different years and this matter deserves further study. From the samples studied in the present study it is clear that M. nubilosa is now dominant. This finding has emerged from a study that included considerably more material from a wider geographical area than has ever previously been sampled. Unlike M. nubilosa, M. juvenis has never been found in Australia, and it is thus difficult to assess its relative importance. However, due to its perceived importance in South Africa, M. juvenis is considered an important quarantine pathogen in Australia.

Mycosphaerella ellipsoidea is a relatively newly described Mycosphaerella species that has previously been known only from the Western Cape Province of South Africa (Crous \& Wingfield 1996). In this study, M. ellipsoidea was collected from KwaZulu-Natal from leaves of $E$. nitens, although its incidence was low. Its appearance, for the first time in a commercially important forestry area deserves consideration. In this study, M. irregulariramosa was isolated from leaves of $E$. grandis from Tzaneen. Although this fungus has previously been reported from Tzaneen, this is the first report of its occurrence on E. grandis. Only 8 isolates of this species were recovered in this study, and relative to other species, this was the least common in the area.

The identification of a new species of Mycosphaerella was surprising. This is because extensive collections have been made in the past, resulting in the unusual situation that the largest number of Mycosphaerella spp. from Eucalyptus are known in South Africa. Mycosphaerella fori appears to be the most important species responsible for leaf spot in the Tzaneen area. It was found on the most important Eucalyptus sp. grown in South Africa, and there is concern that it might spread to other areas in the future.

A large number of Mycosphaerella spp. have been reported on Eucalyptus spp. from South Africa. These include, M. africana, M. ellipsoidea, M. endophytica, M. irregulariramosa, M. juvenis, M. lateralis, M. marksii, M. crystallina and M. nubilosa (Crous 1998). Six of these species were collected during the present study. The three
species not collected were, M. africana, M. endophytica and M. crystallina. M. africana and M. endophytica were originally identified from Stellenbosch in the Western Cape Province (Crous 1998), which is outside the commercial Eucalyptus growing area. These fungi have either been geographically isolated, or they are marginally important. The other species not collected was $M$. crystallina, that is known to occur in the KwaZulu-Natal province on leaves of E. bicostata and E. grandis $\times$ camaldulensis (Crous \& Wingfield 1996). The absence of this species in this study suggests that it is probably not an important pathogen in commercial plantations.

Determining the relative importance of Mycosphaerella spp. causing MLD on Eucalyptus is difficult. This is due to the fact that many species are associated with the disease and that it is extremely difficult to identify them. Although results of this study have shown that it is possible to define species based on morphology, these characters are variable. Generally identifications made in the absence of DNA sequence data are viewed with some circumscription. Clearly, it is impossible to obtain DNA sequences for the large number of isolates that emerge from extensive surveys. Rapid DNA-based procedures for the identification of Mycosphaerella spp. on Eucalyptus are thus needed. These would not only facilitate efforts to improve disease management but they would be extremely valuable in the application of more stringent and meaningful quarantine.

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Table 1. Isolates included in sequence analysis of Mycosphaerella species.

| Isolate No. | Teleomorph (Mycosphaerella) | Anamorph | GENBANK Accession No. | Origin |
| :---: | :---: | :---: | :---: | :---: |
| CMW 4945 ${ }^{1}$ | M. africana | Unknown | AF309602 | Eucalyptus, South Africa |
| $\text { CMW } 4944^{1}$ | M. colombiensis | Pseudocercospora colombiensis | AF309612 | Eucalyptus, Colombia |
| CMW 2732 | M. cryptica | Colletogloeopsis nubilosum | AF309622 | Eucalyptus, Chile |
| CMW 3279 ${ }^{1}$ | M. cryptica | Colletogloeopsis nubilosum | AF309623 | Eucalyptus, Australia |
| $\text { CMW } 3042^{1}$ | M. crystallina | Pseudocercospora crystallina | AF309611 | Eucalyptus, South Africa |
| CMW 4934, 5166 | M. ellipsoidea | Uwebraunia ellipsoidea | AF309592, AF309593 | Eucalyptus, South Africa |
| CMW 9098* | M. ellipsoidea | Uwebraunia ellipsoidea | AF468874 | Eucalyptus, KwaZulu-Natal, RSA |
| CMW 9099* | M. ellipsoidea | Uwebraunia ellipsoidea | AF468875 | Eucalyptus, KwaZulu-Natal, RSA |
| CMW 9100* | M. ellipsoidea | Uwebraunia ellipsoidea | AF468876 | Eucalyptus, KwaZulu-Natal, RSA |
| $\text { CMW } 5224^{1}$ | M. flexuosa | Unknown | AF309603 | Eucalyptus, Colombia |
| CMW 9094* | M. fori | Pseudocercospora fori | AF468868 | Eucalyptus, Tzaneen, RSA |
| CMW 9095* | M. fori | Pseudocercospora fori | AF468869 | Eucalyptus, Tzaneen, RSA |
| $\text { CMW } 4942^{1}$ | M. heimii | Pseudocercospora heimii | AF309606 | Eucalyptus, Madagascar |
| CMW 3046 ${ }^{1}$ | M. heimioides | Pseudocercospora heimioides | AF309609 | Eucalyptus, Indonesia |
| $\begin{aligned} & \text { CMW } 4943^{1}, \\ & 5149^{1} \end{aligned}$ | M. irregulariramosa | Pseudocercospora irregulariramosa | AF309607, AF309608 | Eucalyptus, South Africa |


| CMW 9097* | M. irregulariramosa | Pseudocercospora irregulariramosa | AF468877 | Eucalyptus, Tzaneen, RSA |
| :---: | :---: | :---: | :---: | :---: |
| CMW 5825* | M. irregulariramosa | Pseudocercospora irregulariramosa | AF468878 | Eucalyptus, Tzaneen, RSA |
| CMW 4937, 4936 | M. juvenis | Uwebraunia juvenis | AF309604, AF309605 | Eucalyptus, South Africa |
| CMW 9101* | M. juvenis | Uwebraunia juvenis | AF468879 | Eucalyptus, Umtata, RSA |
| CMW 9102* | M. juvenis | Uwebraunia juvenis | AF468880 | Eucalyptus, KwaZulu-Natal, RSA |
| CMW 9103* | M. juvenis | Uwebraunia juvenis | AF468881 | Eucalyptus, KwaZulu-Natal, RSA |
| STE-U $825^{1}$ | M. lateralis | Dissoconium dekkeri | AF309624 | Eucalyptus, South Africa |
| CMW 4935 | M. lateralis | Dissoconium dekkeri | AF309625 | Eucalyptus, Zambia |
| CMW 9106* | M. lateralis | Dissoconium dekkeri | AF468882 | Eucalyptus, Tzaneen, RSA |
| CMW 9107* | M. lateralis | Dissoconium dekkeri | AF468883 | Eucalyptus, KwaZulu-Natal, RSA |
| STE-U 348 | M. marasasii | Stenella marasasii | AF309591 | Syzygium, South Africa |
| CMW 5150, 3278 | M. marksii | Unknown | AF309588, AF309598 | Eucalyptus, Australia |
| CMW 9090* | M. marksii | Unknown | AF468870 | Eucalyptus, KwaZulu-Natal, RSA |
| CMW 9091* | M. marksii | Unknown | AF 468871 | Eucalyptus, KwaZulu-Natal, RSA |
| CMW 9092* | M. marksii | Unknown | AF468872 | Eucalyptus, Tzaneen, RSA |
| CMW 9093* | M. marksii | Unknown | AF468873 | Eucalyptus, Tzaneen, RSA |
| CMW 2734 | M. molleriana | Colletogloeopsis molleriana | AF309619 | Eucalyptus, U.S.A |


| CMW 4940 | M. molleriana | Colletogloeopsis molleriana | AF 309620 | Eucalyptus, Portugal |
| :--- | :--- | :--- | :--- | :--- |
| CMW 3282 | M. nubilosa | Unknown | $\mathrm{AF309618}$ | Eucalyptus, Australia |
| CMW 9104* | M. nubilosa | Unknown | AF 449096 | Eucalyptus, KwaZulu-Natal, <br> CMW 9105* |
| M. nubilosa | Unknown | Eucalyptus, Umtata, RSA |  |  |
| CMW 3358 |  |  |  |  |


| CMW 5349 | Mycosphaerella state <br> unknown | Pseudocercospora syzgiicola AF309600 |
| :--- | :--- | :--- |

Figure 1: Adaxial view of leaf symptoms associated with Mycosphaerella species isolated from South Africa. (A) M. nubilosa, (B) M. ellipsoidea, (C) M. juvenis, (D) M. lateralis, (E) M. marksii and (F) M. irregulariramosa


Figure 2: Lesions showing pseudothecial arrangement of Mycosphaerella species isolated from South Africa. (A) M. nubilosa, (B) M. irregulariramosa, (C) M. juvenis, (D) M. lateralis, (E) M. marksii, (F) M. ellipsoidea.


Figure 3: Ascospore germination patterns of Mycosphaerella species isolated from South Africa. (A) M. nubilosa Type C, (B) M. irregulariramosa Type I, (C) M. juvenis Type F, (D) M.lateralis Type I, (E) M. marksii Type B, (F) M. ellipsoidea Type I. See Crous (1998) for definition of germination type. Scale bar $=10 \mu \mathrm{~m}$.


Figure 4: Cultural morphology of Mycosphaerella species isolated from Pietermaritzburg, Tzaneen and Umtata. (A) M. nubilosa, (B) M. ellipsoidea, (C) M. juvenis, (D) M. lateralis (E) M. marksii, (F) M. irregulariramosa


Figure 5: Cladogram of Mycosphaerella species on Myrtaceae and Mycosphaerella species isolated from various Eucalyptus spp. One of 4 most parsimonious trees (length $=$ $1562, \mathrm{CI}=0.5038, \mathrm{RI}=0.7879, \mathrm{HI}=0.4962$ ) inferred from heuristic searches using PAUP version 4.0 b 1 . Bootstrap support values of 1000 replicates is listed above branches

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Figure 6: Teleomorph and anamorph structures of $M$. fori sp. nov., identified from leaves of E. grandis in Tzaneen, South Africa. (A) Bitunicate 8 -spored asci. (B) Fascicle of conidiophores arising from a brown stroma. (C) Type C ascospore germination pattern, with spores germinating from both spore plows and producing parallel germ tubes with no visible constriction. (D) (E) Solitary, subcylindrical conidia showing a subobtuse apex and a truncate base. Scale bar $=10 \mu \mathrm{~m}$.


Figure 7: Line drawing of $M$. fori sp. nov and $P$. fori sp . nov. (A) Subcylindrical to narrowly obclavate, straight or incurved asci. (B) Thick-walled ellipsoidal ascospores with subobtuse apices. (C) Type C ascospore germination patterns. (D) Pale brown variously curved solitary conidia. (E) Conidiophores and terminal pale brown conidiogenous cells showing several geniculations. Scale bar $=10 \mu \mathrm{~m}$.


Figure 8: ITS DNA sequences of Mycosphaerella species from Myrtaceae generated using primers ITS 1, ITS 2, ITS 3 and LR 1. Sequence data set contains sequences of Mycosphaerella spp. isolated from various Eucalyptus spp. during this study and published sequences of Mycosphaerella spp. and their anamorphs from other Myrtaceous hosts. (Table 1). Gaps inserted during sequence alignments are indicated by a dash (-).
M.marksiiCMW5150
M. marksiiCMW3278

CMW9090
CMW9091
CMW9092
CMW9093
M. parkiiCMW3358
M. marasasiiSTEU348
P. natalensisCMW4948
P. robustaCMW5151
P. syzygiicolaCMW5349

CMW9094
CMW9095
P. eucalyptorumCMW5228
P. eucalyptorum5229
P. basiramiferaCMW5227
P. paraguayensisCMW5146
C. syzygiiCMW5348
M. colombiensisCMW4944
M. _irregulariramosaCMW4943TCCGTAGGTG AACC----TG
M. _irregulariramosaCMW5149TCCGTAGGTG AACC----TG CMW̄9097
CMW5825
M. heimiiCMW4942
M._crystallinaCMW3032
M._heimioidesCMW3046
M. _walkeriSTEU2768
M. _walkeriSTEU2769
M._africanaCMW4945
M._-ellpsoideaCMW4943
M._ellpsoideaCMW5166

CMW9098
CMW9099
CMW9100
P._eugeniaeCMW5351
P._destructansSTEU1366
M. tasmaniensisCMW5005
M. flexuosaCMW5224
M. _juvenisCMW4937
M._juvenisCMW4936

CMW9101
CMW9102
CMW9103
M. nubilosaCMW3282

CMW̄9104
CMW9105
M. mollerianaCMW2734
M._mollerianaCMW4940
M._crypticaCMW2732
M._crypticaCMW3279
M._suttoniaeSTEU1346
M. -lateralisCMW4935
M._lateralisSTEU825

CMW9106
CMW9107
Ramulispora_anguioides

102030
40
TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG ATCC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGAAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG ATCC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG CGGAGGGATC ATTACT-GAG CGGAGGGATC ATTACT-GAG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCGGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCGGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG AGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG AGGAGGGATC ATTACT-GAG TCCCGTAGTG AACC----TG AGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG AGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG AGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCGGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACT-GAG TCCGTAGGTG AACC----TG CGTAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACC-GAG TCCGTAGGTG AACC----TG CGGAGGGATC ATTACCAGATCCGTAGGTG AACC----TG CGGAGGGATC ATTACCAGATCCGTAGGTG AACC----TG CGGAGGGATC ATTACCAGATCCGTAGGTG AACC----TG CGGAGGGATC ATTACCAGATCCGTAGGTG AACC----TG CGGAAGGATC ATTAATAGAG
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CMW9091
CMW9092
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M. _walkeriSTEU2769
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M._ellpsoideaCMW4943
M._-ellpsoideaCMW5166

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M. -flexuosaCMW5224
M. _juvenisCMW4937
M. _juvenisCMW4936

CMW9101
CMW9102
CMW9103
M._nubilosaCMW3282

CMW9104

## CMW9105

M. mollerianaCMW2734
M._mollerianaCMW4940
M._crypticaCMW2732
M._crypticaCMW3279
M._suttoniaeSTEU1346
M. _lateralisCMW4935
M. ${ }^{\text {lateralisSTEU825 }}$

CMW9106
CMW9107

| 50 | 60 | 70 | 80 |
| :---: | :---: | :---: | :---: |
| TGAGGGT | TT-CGGC-CC | A | CCTC----CA |
| TGAGGGT | TT-CGGC-CC |  | CCTC----CA |
| TGAGGGT | TT-CGGC-CC |  | ССTC----CA |
| -TGAGGGT | TT-CGGC-CC | G--------A | CCTC----CA |
| -TGAGGGT | TT-CGGC-CC |  | CCTC----CA |
| -TGAGGGT | TT-CGGC-CC |  | CCTC----CA |
| -TGAGGGT | TTCACC-GCC |  | CCTC----CA |
| -TGAGGGC | CTCCAGT-CC |  | CCTC----CA |
| TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| -TGAGGGC | -TCACG-CCC |  | ССтС----CA |
| TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| -TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| -TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| TGAGGGT | -TCACG-CCC |  | CCTC----CA |
| TGAGGGC | CTCCG-GTCC |  | CCTC----CA |
| TGAGGGC | TTC--GGTCC |  | CCTC----CA |
| TGAGGGC | TTC--GGTCC | A | CCTC----CA |
| TGA | TTC--GGTCC |  | CCTC----CA |
| -TGAGGGC | TTC--GGTCC |  | CCTC----CA |
| -TGAGGGC | TA---GGTCC |  | CCTC----CA |
| TGAGGGT | TCGG---TCC |  | CCTC----CA |
| -TGAGGGC | TTC--GGTCC |  | ССTC----CA |
| TGAGGGC | C--CCGGCCC |  | CCTC----CA |
| TGAGGGC | C-CCGG-CCC |  | CCTC----CA |
| -TGAGGGC | -TCACG-CCC |  | CCTC----CA |
| TGAGGGC | -TCACG-CCC |  | TCT------A |
| -TGAGGGC | -TCACG-CCC | A--------T | TCT------A |
| -TGAGGGC | -TCACG-CCC |  | TCT |
| TGAGGGC | -TCACG-CCC |  | A |
| -TGAGGGC | -TCACG-CCC |  | TC |
| -TGAGGGC | CTTCGGTC-C |  | CCTC----CA |
| -TGAGGGC | CTTCCGCC-G |  | ССТС----CA |
| -TGAGGGC | CTTCGGGCTC |  | ССтС----CA |
| -TGAGGG- | CTCCGG-CCC |  | ССтС----CA |
| -TGAGGG- | CTCCGG-CCC |  | ССтС----CA |
| TGAGGG- | CTCCGG-CCC |  | ССтС----CA |
| -TGAGGG- | CTCCGG-CCC |  | CCTC----CA |
| -TGAGGG- | CTCCGG-CCC |  | CCTC----CA |
| -TGAGGG- | CTCCGG-CCC | - | ССтС----CA |
| -TGCGGGC | GCCAGCCCG- |  | ССтС----CA |
| TGCGGGC | GCCAGCCCG- |  | CCTC----CA |
| -TGCGGGC | GCCAGCCCG- |  | CCTC----CA |
| -TGAGGGC | -GCAAGCC-C |  | ССTC----CA |
| -TGAGGGC | -GCAAGCC-C |  | ССTC----CA |
| -TGAGGGC | CTCCGGGTCC |  | ССтС----СА |
| -TGAGGGC | GCCCG--CCC |  | CCTC----CA |
| -CGAGGGC | GTCAGGCC-C |  | CCTC----CG |
| Ag | -ACGCCTC | GGC--GGAAA | CGCCG |
| AG | ---ACGCCTC | GGC--GGAAA | CGCCGGGG-- |
| -AG--- | --ACGCCTC | GGC--GGAAA | CGCCGGGG |
|  | GC |  |  |

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---TGAGGGT TT-CGGC-CC G---------A CCTC----CA
---TGAGGGT TT-CGGC-CC G---------A CCTC----CA
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---TGAGGGC -TCACG-CCC G---------A CCTC----CA
---TGAGGGC -TCACG-CCC G---------C CCTC----CA
---TGAGGGC -TCACG-CCC G---------A CCTC----CA
rgaggge -TCACG-CCC G--
---TGAGGGC -TCACG-CCC G--------A CCTC----CA
---TGAGGGC -TCACG-CCC G--------A CCTC----CA
---TGAGGGC -TCACG-CCC G---------A CCTC----CA
---TGAGGGC -TCACG-CCC G---------C CCTC----CA
---TGAGGGT -TCACG-CCC G---------A CCTC----CA
---TGAGGGC CTCCG-GTCC G---------A CCTC----CA
HGAGGGC THC--GGICC G--------A CCIC----CA
---TGAGGGC TTC--GGTCC G--------A CCTC----CA
---TGAGGGC TTC--GGTCC G--------A CCTC----CA
---TGAGGGC TA---GGTCC G--------A CCTC----CA
---TGAGGGT TCGG---TCC G---------A CCTC----CA
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---TGAGGGC -TCACG-CCC G--------A CCTC----CA
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---TGAGGGC CTTCCGCC-G G---------A CCTC----CA
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---TGAGGG- CTCCGG-CCC G--------A ССТС---CA
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---TGAGGG- CTCCGG-CCC G--------A CCTC----CA
---TGCGGGC GCCAGCCCG- ----------A CCTC----CA
---TGCGGGC GCCAGCCCG- ----------A CCTC----CA
---TGAGGGC -GCAAGCC-C G---------A CCTC----CA
---TGAGGGC -GCAAGCC-C G--------A CCTC----CA
---TGAGGGC CTCCGGGTCC G---------A CCTC----CA
---TGAGGGC GCCCG--CCC G---------A CCTC----CA
---CGAGGGC GTCAGGCC-C G---------A CCTC----CG
-----AG--- ---ACGCCTC GGC--GGAAA CGCCGGGG--
-----AG--- ---ACGCCTC GGC--GGAAA CGCCGGGG--
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-----AG--- ---ACGCCTC GGC--GGAAA CGCCGGGG--
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M. _marksiiCMW3278

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P._robustaCMW5151
P._syzygiicolaCMW5349

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P. _basiramiferaCMW5227
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C. _syzygiiCMW5348
M. colombiensisCMW4944
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M._irregulariramosaCMW5149

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M. _heimioidesCMW3046
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M. tasmaniensisCMW5005
M. flexuosaCMW5224
M. juvenisCMW4937
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CMW9103
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CMW9106
CMW9107
Ramulispora_anguioides

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CMW9105
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Ramulispora_anguioides

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Ramulispora_anguioides

170
-GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCG -GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCGG -GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCGG -GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCGG -GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCGG -GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCGG -GTTGCTTCG G--GGGCGAC CCT-GCCG-- ---TTCGCGG -GTTGCTTCG G--GGGCGAC CCT-GCCT-- ---TTC-GGG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC -ACTTCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC -ACTTCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC -ACTTCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC C-CTGCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC C-CTGCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC -ACTTCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC -ACTTCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGAC GACTTGGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGAC GACTCCGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGGC A-CTTTGTCG TGTTGCTTCG G--GGGCGAC CCT-GCCGC- --TTCGGCGG TGTTGCTTCG G--GGGCGAC CCT-GCCG-- --CTTCGGCG TGTTGCTTCG G--GGGCGAC CCT-GCCG-- --CTTCGGCG TGTTGCTTCG G--GGGCGAC CCT-GCCG-- --CTTCGGCG TGTTGCTTCG G--GGGCGAC CCT-GCCG-- --CTTCGGCG TGTTGCTTCG G--GGGCGAC CCT-GCCG-- --CTTGGGCG TGTTGCTTCG G--GGGCGAC CCT-GCCGC- ---TTTGGCG TGTTGCTTCG G--GGGCGAC CCT-GCCG-- --CTTCGGCG -GTTGCTTCG G--GGGCGAC CCT-GCCGTC ----TCGGCG
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TGTTGCTTCG G--GGGCGAC CCG-GCCGTC ---CGGGCCG
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-GTTGCCTCG G--GGGCGAC CCG-GCCCTC ---TGGGT-G
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-GTTGCCTCG G--GGGCGAC CCG-GCCCCC G--------CG
-GTTGCCTCG G--GGGCGAC CCG-GCCCCC G--------CG
-GTTGCCTCG G--GGGCGAC CCG-GCCG-- ---C-CGCGC
-GTTGCCTCG G--GGGCGAC CCG-GCCG-- ---C-CGCGC -GTTGCGTCG G--GGCCGAC CCT-GCCGCC ------GT-G TGTTGCCTCG G--GGGCGAC CCG-GCCG-- ------CCGTG -GTTGCCTCG G--GGGCGAC CCG-GCC--- ----GCCGCG TATTGCCCCG G---GGGAAC CC-CGCCTGT CAT----GGG TATTGCCCCG G---GGGAAC CC-CGCCTGT CAT----GGG TATTGCCCCG G---GGGAAC CC-CGCCTGT CAT----GGG TATTGCCCCG G---GGGAAC CC-CGCCTGT CAT----GGG TGTTGCTTTG GCAGGACGCC TCGCGCCAGC GGCTTCGGCT
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CMW9092
CMW9093
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C. _syzygiicMW5348
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CMW9106
CMW9107
Ramulispora_anguioides

210
220
230
240
CG-CGGC--G CCCCCGGGGG AAA--TCA-- AACACTGCGT
CG-CGGC--G CCCCCGGGGG AAA--TCA-- AACACTGCGT
CG-CGGC--G CCCCCGGGGG AAA--TCA-- AACACTGCGT
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CG-CGGC--G CCCCCGGGGG AAA--TCA-- AACACTGCGT
CATCGC---G CCCCCGGAGG A----TACTT AACCCTGCAT
CAG-TGC--G CCCCCGGAGG ATATCAA--A A-CGCTGCAT
CC-GGGC--G CCCCCGAAGG TC--TCCA-- AACACTGCAT
CC-GGGC--G CCCCCGAAGG TC--TCCA-- AACACTGCAT
CC-GGGC--G CCCCCGAAGG TC--TCCA-- AACACTGCAT
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CC-GGGC--G CCCCCGAAGG TC--TCCA-- AACACTGCAT
CC-GGGC--G CCCCCGGAGG TC--TCCA-- AACACTGCAT
CC-GGGC--G CCCCCGGAGG TC--TCCA-- AACACTGCAT
CC-GGGC--G CCCCCGGAGG TC--TTCT-A AACACTGCAT CC-GGGC--G CCCCCGGAGG TC--TTCT-A AACACTGCAT CC--GGGC-G CCCCCGGAGG TCCATC---- ---TCTGCGT TG-CGGC--G CCCCCGGAGG CCATCA---- AACACTGCAT GT-GCGGC-G CCCCCGGAGG CCATTA---- AACACTGCAT GT-GCGGC-G CCCCCGGAGG CCATTA---- AACACTGCAT GT-GCGGC-G CCCCCGGAGG CCATTA---- AACACTGCAT GT-GCGGC-G CCCCCGGAGG CCATTA---- AACACTGCAT GT-GCGGC-G CCCCCGGAGG CCATTA---- AACACTGCAT GT-GCGGC-G CCCCCGGAGG -CCA---TTA AACACTGCAT GT-GCGGC-G CCCCCGGAGG CCAT-----A AACACTGCAT GC-GCGGC-G CCCCCGGAGG -CCC-TC--A AACACTGCAT GC-GCGGC-G CCCCCGGAGG C--CCTC--A AACACTGCAT AC-G-GCG-G CCCCCGGAGG TCATCA---A A-CACTGCAT CGGTGGC--G CTCCCGGTGG CCAATTATTA AACTCTGCAT CGGTGGC--G CTCCCGGTGG CCAATTATTA AACTCTGCAT CGGTGGC--G CTCCCGGTGG CCAATTATTA AACTCTGCAT CGGTGGC--G CTCCCGGTGG CCAATTATTA AACTCTGCAT CGGTGGC--G CTCCCGGTGG CCAATTATTA AACTCTGCAT G--GAGGT-G CCCCCGGTGG CCCCATC--A AACTCTGCAT A---GG-T-G CCCCCGGTGG CCCCA-TCA- AACTCTGCAT CC-GC----- CCCCCGGTGG ACCCCCTCTC AACTCT-CGC CC-GGG---G CCCCCGGCGG ACACC-TCA- A-CTCTGCAT CC-GGG---G CCCCCGGCGG ACCAC-TCA- A-CTCTGCAT CC-GGG---G CCCCCGGCGG ACCAC-TCA- A-CTCTGCAT CC-GGG---G CCCCCGGCGG ACCAC-TCA- A-CTCTGCAT CC-GGG---G CCCCCGGCGG ACCAC-TCA- A-CTCTGCAT CC-GGG---G CCCCCGGCGG ACCAC-TCA- A-CTCTGCAT CC---GGG-G CCCTCGCAGG ACCCCTC--A ACG-CTGCAT CC---GGG-G CCCTCGCAGG ACCCCTC--A ACG-CTGCAT CC---GGG-G CCCTCGCAGG ACCCCTC--A ACG-CTGCAT CG--G-G--G CCCCCGGTGG ACCC-TC--A A-CTCTGCAT CG-GG-GC-- -CCCCGGTGG ACCCT-C--A A-CTCTGCAT CC-GGG---G CCCCCGGCGG ACCCCT---C AACTCTGCAT CC-G-G-G-G CCCCCGGCGG ACCCCTC--A A-CTCTGCAT TC-GGG-C-- CCCCCTGAGG ---ACCCTCT AACCCTGCGT CGTG-GGC-- CCCC-GGTGG CCA--ACTCA AACTC-TGTT CGTG-GGC-- CCCC-GGTGG CCA--ACTCA AACTC-TGTT CGTG-GGC-- CCCC-GGTGG CCA--ACTCA AACTC-TGTT CGTG-GGC-- CCCC-GGTGG CCA--ACTCA AACTC-TGTT GTTGAGTG-C CTGCCAGAGG ACCA----CA ACTCTTGTTT
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CMW9106
CMW9107
Ramulispora_anguioides

| 250 | 260 | 270 | 280 |
| :---: | :---: | :---: | :---: |
| CAATTTG-TG | TCGGAGTACT |  |  |
| CAATTTG-TG | TCGGAGTACT |  | A |
| CAATTTG-TG | TCGGAGTACT |  | -GTTAAT--A |
| CAATTTG-TG | TCGGAGTACT |  | -GTTAAT--A |
| CAATTTG-TG | TCGGAGTACT |  | -GTtAAT--A |
| CAATTTG-TG | TCGGAGTACT |  | GTtAAT--A |
| CA-TT--GCG | TCGGAGTAAT | T------T-T | A-TTAATACA |
| C--TTT-GCG | TCGGAGTATC | -A-----AT- | CAAATT-G |
| C--TTTG-CG | TCGGAGT--T |  | -CAAATT-A |
| C--TCTG-CG | TCGGAGT--T | TA | -CAAATT-A |
| C--TTTG-CG | TCGGAGT--T | TA | -CAAATT-A |
| C--TTTG-CG | TCGGAGT--T |  | -CAAATT-A |
| C--TTTG-CG | TCGGAGT | TA | -CAAATT-A |
| C--TTTG-CG | TCGGAGT--T | TA | -CAAATT-A |
| C--TTTG-CG | TCGGAGT--T |  | -CAAATT-A |
| C--TTTG-CG | TCGGAGT--T | TA | -CAAATT-A |
| C--TTTG-CG | TCGGAGT--T | TA-----A | A-CAAATT-A |
| CGGAGTTTAA | GTCAATTA | A-GCAAGTTT | AAGTCAATTA |
| C--ATTG-CG | TCGGAG---T | AA | AGtadatG-A |
| CA--TTG-CG | TCGGAG---T |  | GTAAAT-TA |
| CA--TTG-CG | TCGGAG---T | TA | GTAAAT-TA |
| CA--TTG-CG | TCGGAG---T | TA | GTAAAT-TA |
| CA--TTG-CG | TCGGAG---T | T | AAAT-TA |
| CA--TTG-CG | TCGGAGTAA- | -------A-- | AGTAAAT-TA |
| -CA-TT-GCG | TCGGAGTTAA | AG-----TAA | ATtAAAC--A |
| CA--TTG-CG | TCG | -AA | AGTAAAT-TA |
| CC---TCGCG | TCGGAGTCTC |  | -Gtadatgan |
| CC--TCG-CG | TCGGAGTCTC |  | GTAAATG-A |
| C--TTTG-CG | TCGGAGTCTT | A | GTAAAT-TA |
| C--TCTTGCG | TCGGAGTCTT | -A-----A-- | -GAAATTTA |
| C--TCTTGCG | TCGGAGTCTT | -A-----A-- | A |
| C--TCTTGCG | TCGGAGTCTT | -A-----A-- | -GAAATTTA |
| C--TCTTGCG | TCGGAGTCTT | -A | A-GAAATTTA |
| C--TCTTGCG | TCGGAGTCTT | -A-----A-- | -GAAATTTA |
| CTCTT-G-CG | TCGGAGTCTT | CA-----AAA | G---AATTCA |
| CT-CTTGC-G | TCGGAGTCTT | C | -AAGAATTCA |
| GT-CCCGCCG | TCT-AGTCTT | TG-----ATT | ATTGAATTGA |
| CT--TTGC-G | TCTGAGTATG | A | -TTGAATCAA |
| CT--GTGC-G | TCTGAGTA-A | AT- | -TTGAATCAA |
| CT--GTGC-G | tCTGAGTA-A | AT-----AT- | -TTGAATCAA |
| CT--GTGC-G | TCTGAGTA-A | -AT- | -TTGAATCAA |
| CT--GTGC-G | TCTGAGTA-A | AT- | -TTGAATCAA |
| CT--GTGC-G | TCTGAGTA-A | AT-----AT | -TTGAATCAA |
| C--TGTG-CG | TCGGAGTAAT | AC-----AA- | -CCAATC-A |
| C--TGTG-CG | TCGGAGTAAT | AC-----AA- | --CCAATC-A |
| C--TGTG-CG | TCGGAGTAAT | AC-----AA- | --CCAATC-A |
| C--TCTG-CG | TCTGAGTCAC | AA-----AA- | --TCAATC-A |
| C--TCTG-CG | TCTGAGTCAC | AA-----AA- | --TCAATC-A |
| C--TTTG-CG | TCTGAGTGAT | - | CGAAAAT-CA |
| C--TTTG-CG | TCTGAGTGAT |  | CGAAAAT-CA |
| CC-TCTTGCG | TCTGAGTCGT | -G-----AGT | A-GAAATTGA |
| TTTATTGCCG | TCTGAGTAAC | AA------A- | --CAAATCAA |
| TTTATTGCCG | TCTGAGTAAC | AA------A | --CAAATCAA |
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| TTAGTG-ATG | TCTGAGTACT |  | AT |

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M. marksiiCMW3278 CMW9090
CMW9091
CMW9092
CMW9093
M. _parkiiCMW3358
M. _marasasiiSTEU348
P._natalensisCMW4948
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CMW9095
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CMW̄9106
CMW9107
Ramulispora_anguioides

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M. -marksiiCMW3278

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Ramulispora_anguioides

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Ramulispora_anguioides

AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGC GAATCATCCA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCCGT GAATAATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG
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M. marksiiCMW3278

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Ramulispora_anguioides

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Ramulispora_anguioides

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Ramulispora_anguioides

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| GC | TAGG---GAG | C-CGCGGA-G |  |
| TCGC | TAGG---GAG | C-CGCGGA-G |  |
| TCGC | TAGG---GAG | - |  |
| GC | TAGG---GAG | C-CGCGGA-G |  |
| CAGTTCTCGC | TAGG---GAG | T-CGCGGAC- |  |
| -T-C--GCGC | TAG | A |  |
| CA-A-TTCGC | TTCG---GAG | TGCGGG-T-G | GC |
| CA-A-TTCGC | TTTG---GAG | CGCGGG-T-G |  |
| CA-A-TTCGC | A | TG |  |
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| AACTATTCGC | GAG |  |  |
| CT-A-TTCGC | TTCG---GAG | G-C-CGGG-C |  |
| CGC | AG | G | GC |
| TCGC | A | G |  |
| -TCGC | TTCG---GAG | G-CCGGGT-G | GC |
| TC | GAG | - |  |
| -TCGC | TTCG---GAG | G-T-CGGG-T | G |
| TATTCGC | TTCG---GAG | GTCGG----- | GTGG |
| CGC | TTTG---GAG | T |  |
| A-TTCGC | TTCG---GGG | CGCGGG-C | G |
| ------TCGC | TT-C--GGGG | C--GCGGG-C |  |
| -CGC | TG--AAAGAG | TT--CGGGAC |  |
| -TCGC | TAGG---GAT | GACA-GGTCT | GT |
| -TCGC | TAGG---GAT | GACA-GGTCT |  |
| -A-T--TCGC | TAGG---GAT | GACA-GGTCT | GTC |
| -A-T--TCGC | TAGG---GAT | GACA-GGTCT |  |
| -A-T--TCGC | TAGG---GAT | GACA-GGTCT | GTC |
| TTCAC | TG-G--GGAG | G-ACGGGT-C |  |
| C | TGGG---GAG | CACG-GGTC- |  |
| GT--TCGC | GACG---GAG | C-CG-GCCC- |  |
| TGGA--CCGC | TTGT---GA | TAT |  |
| TGGA--CCGC | GC---GAG | TACG-GGAC- |  |
| TGGA--CCGC | TTGC---GAG | TA |  |
| TGGA--CCGC | TTGC---GAG | TACG-GGAC- |  |
| TGGA--CCGC | TTGC---GAG | TACG-GGAC- |  |
| TGGA--CCGC | TTGC---GA | TACG-GGAC |  |
| GT---TTCGC | T--GACGGGG | ACC--GGTCT |  |
| GT---TTCGC | T--GACGGGG | ACC--GGTCT |  |
| GT---TTCGC | T--GACGGGG | ACC--GGTCT |  |
| GT-T--TCGC | TTTC---GGG | -AC-CGGTCT | GG |
| GT-T--TCGC | TTTC---GGG | -AC-CGGTCT |  |
| GT---TTCGC | TTCC---GGG | A-CCGGTC-T | GG |
| GT---TTCGC | TT--CCGG-G | AC--CGGTCT |  |
| CTGTGC-CGC | TTCC---GGG | AC-C-GGTCT |  |
| -TCGC | TTGG---GA- | CACGGGGGTG |  |
| TCGC | TTGG---GA | ACGGGG |  |
| --TCGC | TTGG---GA- | CACGGGGGTG |  |
| -TCGC | GA | CACGGGGGTG |  |
|  |  |  |  |

600
C-----TCGC TAGG---GAG C-CGCGGA-G GG--CGTTGG
C-----TCGC TAGG---GAG C-CGCGGA-G GG--CGTTGG
C-----TCGC TAGG---GAG C-CGCGGA-G GG--CGTTGG
C-----TCGC TAGG---GAG C-CGCGGA-G GG--CGTTGG
C-----TCGC TAGG---GAG C-CGCGGA-G GG--CGTTGG
CAGTTCTCGC TAGG---GAG T-CGCGGAC- GGC--GTCGG
-T-C--GCGC TAGG---GAG T-CGCGGGCA CAC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTTG---GAG CGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGG-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGA-T-G GCC---GCGG
CA-A-TTCGC TTCG---GAG TGCGGA-T-G GCC---GCGG
AACTATTCGC TTCG---GAG -----GGTGG GTGGCCGCGG
CT-A-TTCGC TTCG---GAG G-C-CGGG-C GG--CCGCGG
------TCGC TTCG---GAG G-CCGGGT-G GCC--GC-GG
------TCGC TTCG---GAG G-CCGGGT-G GCC--GC-GG
------TCGC TTCG---GAG G-CCGGGT-G GCC--GC-GG
--CTATTCGC TTCG---GAG GTCGG----- GTGGCCGCGG
C------CGC TTTG---GAG ---GCGGG-T GGC----CGG
---A-TTCGC TTCG---GGG --CGCGGG-C GGC--CGCGG
------TCGC TT-C--GGGG C--GCGGG-C GGC-CGC-GG
TT-----CGC TG--AAAGAG TT--CGGGAC GGCT-TTTGG
-A-T--TCGC TAGG---GAT GACA-GGTCT GTC---GCGG
-A-T--TCGC TAGG---GAT GACA-GGTCT GTC---GCGG
-A-T--TCGC TAGG---GAT GACA-GGTCT GTC---GCGG
-A-T--TCGC TAGG---GAT GACA-GGTCT GTC---GCGG
-A-T--TCGC TAGG---GAT GACA-GGTCT GTC---GCGG
-----TTCAC TG-G--GGAG G-ACGGGT-C TGC-CGC-GG
------TCGC TGGG---GAG CACG-GGTC- TGCCGCG-GC
--GT--TCGC GACG---GAG C-CG-GCCC- GGCGTGG-GC
TGGA--CCGC TTGC---GAG TACG-GGAC- GTCCTCG-GC
TGGA--CCGC TTGC---GAG TACG-GGAC- GTCCTCG-GC
TGGA--CCGC TTGC---GAG TACG-GGAC- GTCCTCG-GC
TGGA--CCGC TTGC---GAG TACG-GGAC- GTCCTCG-GC
GT---TTCGC T--GACGGGG ACC--GGTCT GGCGGCGCGC
GT---TTCGC T--GACGGGG ACC--GGTCT GGCGGCGCGC
GT---TTCGC T--GACGGGG ACC--GGTCT GGCGGCGCGC
GT-T--TCGC TTTC---GGG -AC-CGGTCT GGCGGCGCGC
GT-T--TCGC TTTC---GGG -AC-CGGTCT GGCGGCGCGC
GT---TTCGC TT--CCGG-G AC--CGGTCT GGCG-TCGCG
CTGTGC-CGC TTCC---GGG AC-C-GGTCT GGCGGTGTGC
------TCGC TTGG---GA- CACGGGGGTG AGCGCCCGGA
------TCGC TTGG---GA- CACGGGGGTG AGCGCCCGGA
------TCGC TTGG---GA- CACGGGGGTG AGCGCCCGGA
------TCGC GAT---TGAG TCCGGTA--- GGTTTACTTG
M._marksiiCMW5150
M. marksiiCMW3278 CMW59090
CMW9091
CMW9092
CMW9093
M. parkiiCMW3358
M. -marasasiiSTEU348
P._natalensisCMW4948
P._robustaCMW5151
P._syzygiicolaCMW5349

CMW9094
CMW9095
P._eucalyptorumCMW5228
P. eucalyptorum5229
P. -basiramiferacMW5227
P. -paraguayensisCMW5146
C. _syzygiicMW5348
M. colombiensisCMW4944
M. _irregulariramosaCMW4943
M._irregulariramosaCMW5149

CMW9097
CMW5825
M. _heimiiCMW4942
M._crystallinaCMW3032
M. heimioidesCMW3046
M. _walkeriSTEU2768
M._walkeriSTEU2769
M._africanaCMW4945
M._ellpsoideaCMW4943
M._ellpsoideaCMW5166

CMW9098
CMW9099
CMW9100
P._eugeniaeCMW5351
P._destructansSTEU1366
M. tasmaniensisCMW5005
M. -flexuosaCMW5224
M. _juvenisCMW4937
M._juvenisCMW4936

CMW9101
CMW9102
CMW9103
M. nubilosaCMW3282

CMW 9104
CMW9105
M. _mollerianaCMW2734
M. mollerianaCMW4940
M. -crypticaCMW2732
M._crypticaCMW3279
M._suttoniaeSTEU1346
M. lateralisCMW4935
M. lateralisSTEU825

CMW9106
CMW9107
Ramulispora_anguioides

| 610 | 62 | 630 | 640 |
| :---: | :---: | :---: | :---: |
|  | GTtAAACACC | CAAA | G |
|  | GTtAAACACC | CCAT--CAAA | GGTTGAC-CT |
|  | GTtaAACACC | CCAT--CAAA | GG |
|  | GTTAAACAC | CCAT--CAAA | GG |
|  | GTtAAACACC | CCAT--CAAA | GGT |
|  | GTtAAACACC | CCAT--CAAA | GG |
|  | GTtAAATACC | CCAT--CAAA | GG |
|  | TTAAATACC | CCAT--CAAA | GGTTGAC-C |
|  | AT | TATT--CAAA | GGI |
|  | TTAAAT | TATT--GAAA | GG |
|  | TTAAATCTT | TATT--CAAA | GGTTGAC-C |
|  | T | TATT--CAAA | GGI |
|  | TTAAAT | TATT--CAAA | GG |
|  | ITAAATCTT | TATT--CAAA | GGI |
|  | AAATCTT | TATT--CAAA | GG |
|  | TTAAATCTI | TATT--CAAA | GG |
|  | ITAAATCTT | TATT--CAAA | GGI |
|  | ITAAATCTT | TATTCA-AA- | GG |
|  | GTTAAATCTT | TCA---CAA- | GGTTGAC-C |
|  | ITAAATCTT | T---CACAA- | GGTTGAC-C |
|  | ITAAATCTT | T---CACAA- | GG |
|  | TTAAATCTT | T---CACAA- | G |
|  | TAAAT | T---CACAA- |  |
| C | ITAAATCTT | T---CACAA- | GGI |
|  | TAAAT | T---CACAA- | GGTTGAC-C |
|  | TTAAATCTT | T---CACAA- |  |
|  | GTTAAATCTT | TCAC----AA | GGTT |
|  | TTAAATCTT | TCAC----AA | GGTTGAC-C |
|  | GTTAAATCTT | TCTT---AAA | GGTTGA |
|  | TtAAAT | TATA-ACA | GGT |
|  | TAAATCTT | TATA- |  |
|  | ITAAATCTT | tata | GGTTGAC-C |
|  | TAAA | tata-ACA |  |
|  | TTAAATCTT | TATA-ACA | GGTTGA |
|  | TtAAAT | T-AT--CAAA | GG |
|  | TtAAAT-CC | T-TATCA-AA | GG |
|  | CCAACGACC | C-CATCTTCA | GG |
|  | TAAA-CTT | Attacacas- | GGTTGAC-C |
|  | TTAAACCCT | TTTAT-CAAA | GGT |
|  | ItaAACCCT | TTTAT-CAAA | GGT |
|  | ITAAACCCT | tTtat-CAAA | GGI |
|  | TtAAACCCT | TtTAT-CAAA | GGI |
|  | TTAAACCCT | tTTAT-CAAA | G |
|  | ITAAACCCT | T-TCACCAAA | GGTTG |
|  | ITAAACCCT | T-TCACCAAA | GG |
|  | TTAAACCCT | T-TCACCAAA | GGTT |
|  | TTAAACCCT | T-TCAC-AAA | GG |
|  | GTTAAACCCT | T-TCA-CAAA | GG |
|  | TCAACCCCC | TCTC-TCACA | GGTTG |
|  | GTCAACCCCC | TCTC-TCACA | GGTTG |
|  | GTCAAACCCC | T-TCATCAAA | GGI |
| --AAACATCG | GCGGAGACGT | CGATTTC-AA | GGTTG |
| --AAACATCG | GCGGAGACGT | CGATTTC-AA | GGTTG |
| -AAACATCG | GCGGAGACGT | CGATTTC-AA | GGTTG |
| AAACATCG | GCGGAGAC | CGATtTC-AA |  |
|  |  |  |  |

M. marksiiCMW5150
M. marksiiCMW3278

CMW̄9090
CMW9091
CMW9092
CMW9093
M. parkiiCMW3358
M. marasasiiSTEU348
P. natalensisCMW4948
P._robustaCMW5151
P. syzygiicolacMW5349

CMW̄9094
CMW9095
P._eucalyptorumCMW5228
P. eucalyptorum5229
P. -basiramiferacMW5227
P._paraguayensisCMW5146
C._syzygiiCMW5348
M. colombiensisCMW4944
M. irregulariramosaCMW4943
M. _irregulariramosaCMW5149

CMW̄9097
CMW5825
M. heimiiCMW4942
M._crystallinaCMW3032
M. heimioidesCMW3046
M._walkeriSTEU2768
M._walkeriSTEU2769
M._africanaCMW4945
M._-ellpsoideaCMW4943
M._ellpsoideaCMW5166

CMW9098
CMW9099
CMW9100
P. _eugeniaeCMW5351
P._destructansSTEU1366
M. tasmaniensisCMW5005
M. flexuosaCMW5224
M. _juvenisCMW4937
M._juvenisCMW4936

CMW9101
CMW9102
CMW9103
M. nubilosaCMW3282

CMW̄9104
CMW9105
M. _mollerianaCMW2734
M. mollerianaCMW4940
M._crypticaCMW2732
M._crypticaCMW3279
M._suttoniaeSTEU1346
M. ${ }^{-1 a t e r a l i s C M W 4935}$
M._lateralisSTEU825

CMW9106
CMW9107
Ramulispora_anguioides

640
650
CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAAGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGG AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGC AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA CGGATCAGGT AGGGATA

