



CHAPTER 4

Population structure and diversity of *Ceratocystis polychroma*
prov. nom. on clove in Sulawesi, Indonesia

ABSTRACT

Clove trees in Northern Sulawesi, Indonesia are seriously affected by a decline disease, the cause of which is poorly understood. Clove decline, characterised by wilting, defoliation and tree death is considered to be the most serious problem affecting clove production in this area. An important component of the disease is infestation of trees by the Cerambycid woodborer *Hexamitodera semivelutina*. A newly described and pathogenic *Ceratocystis* sp., *C. polychroma* prov. nom. is also consistently associated with the dying trees. Based on culture morphology, isolates of *C. polychroma* prov. nom. can be separated into three groups, but DNA sequence comparisons for three different gene regions show no differences between them. The aim of this study was to assess the genetic diversity amongst a population of isolates of *C. polychroma* prov. nom. and thus to consider whether phenotypic differences in cultures can be defined genetically. Microsatellite markers developed for the related tree pathogen, *C. fimbriata* were assessed for their usefulness in studying *C. polychroma* prov. nom. Effective markers were then used to infer allele frequencies, which were used in population genetic analysis for 50 isolates of *C. polychroma* prov. nom. Ten of the eleven microsatellite markers developed for *C. fimbriata*, successfully amplified microsatellite regions in *C. polychroma* prov. nom., confirming the close relatedness of these fungi. The fungus had a high gene diversity of $\overline{H} = 0.402$. No unique alleles were observed for any of the three morphological groups. The genotypic diversity was very high, ($G_{ST} = 44.46$) with a maximum of 89.28 %. Linkage disequilibrium analysis revealed that the population reproduces predominantly clonally, but that outcrossing and the generation of new allelic combinations occurs at low frequency. Data from this study suggest that *C. polychroma* prov. nom. is endemic in Sulawesi.

Clove decline is considered to be the most serious problem affecting clove production in Sulawesi. In some plantations, it results in up to 80 % mortality. Dieback affects both seedlings and fully grown trees. The disease is characterised by rapid wilting, defoliation, and twig dieback from the tips that proceeds downwards. Branches and ultimately entire trees die (Liew *et al.* 2003).

Ceratocystis polychroma *prov. nom.* Van Wyk, Liew & Wingfield is a recently described species associated with clove decline in Northern Sulawesi (Chapter 3). The fungus is associated with rapid wilting and death of full-grown trees. A recent investigation has revealed the presence of the trunk borer, *Hexamitodera semivelutina* Hell. (Coleoptera: Cerambycidae) in association with *C. polychroma* *prov. nom.* (Liew *et al.* 2003, Chapter 3). This insect is a well-known pest of *S. aromaticum* L. Merr. & Perry that has invaded many Indonesian islands in the past ten years (Anonymous 2002). Preliminary tests have shown that it is an aggressive pathogen (Wingfield & Liew unpublished). It is, however, not known whether this is a specific association between a fungus and insect or a chance infection of wounds by *C. polychroma* *prov. nom.*

The association of *C. polychroma* *prov. nom.* and its apparent contribution to clove decline in Sulawesi is not surprising. This fungus is closely related to *C. fimbriata* Ellis & Halsted, which is a well-known pathogen of many woody plant species (Kile 1993). Recent studies based on DNA sequence comparisons have shown that *C. fimbriata* represents a species complex (Barnes *et al.* 2001a). Thus new species that have previously been treated under the name *C. fimbriata* have been described. The serious wilt pathogen of *Acacia mearnsii* de Wild. in South Africa, *C. albofundus* De Beer, Wingfield & Morris (Wingfield *et al.* 1996) and the *Eucalyptus* pathogen *C. pirilliformis* (Barnes *et al.* 2003) represent two good examples. *Ceratocystis polychroma* *prov. nom.* appears to be another cryptic species in the *C. fimbriata* species complex, and all of these fungi are evidently pathogenic.

Ceratocystis fimbriata is a homothallic fungus that undergoes unidirectional mating type switching (Olsen 1949, Webster & Butler 1967, Harrington & McNew 1997). Sexual reproduction in ascomycetes is controlled by two opposite mating type genes. In homothallic

species like *C. fimbriata*, both genes (MAT-1 and MAT-2) exist in a single individual, thus self-fertile strains contain both MAT-1 and MAT-2 genes. Single ascospore cultures produced from a selfing event result in approximately half self-sterile strains and half self-fertile strains (Harrington & McNew 1997). In self-fertile strains, the MAT-1 gene has been deleted and this is known as uni-directional mating type switching (Harrington & McNew 1997). These strains cannot self but can cross with self-fertile strains (Harrington & McNew 1997, Witthuhn *et al.* 2000). Although the sexual behaviour of *C. polychroma prov. nom.* has not been studied critically, the fungus appears to be similar to *C. fimbriata*, where single ascospores give rise to either self-sterile or self-fertile isolates.

In earlier studies (Chapter 3), variation in the morphology of *C. polychroma prov. nom.* cultures has been seen. One objective of this study was to use DNA sequence analysis to ascertain if these fungi are the same. The close phylogenetic relationship between *C. polychroma prov. nom.* and *C. fimbriata* (Chapter 3) suggests that tools used to study the latter pathogen might be useful in studying the fungus associated with clove decline. Barnes *et al.* (2001a) developed 11 microsatellite markers for population studies of *C. fimbriata*. These markers have been successfully applied in population studies with *C. albobundus* (Barnes 2002, Nakabonge 2002). Thus, a further aim was to test whether the microsatellite markers developed for *C. fimbriata* could be used in studies of *C. polychroma prov. nom.* and if so, to consider the genetic diversity of a population of *C. polychroma prov. nom.* from dying clove trees in Indonesia. It was hoped that this might resolve questions relating to the different cultural characteristics amongst isolates and to provide some indication of the probable origin of the fungus.

MATERIALS AND METHODS

Fungal isolates and DNA extractions

Fungal isolates were obtained from *Syzygium aromaticum* trees that showed symptoms of decline. Eighteen sample sites in Sulawesi were selected (Table 1 & Fig. 1) for isolation. Isolates were collected either by transferring ascospores from the necks of ascomata in the galleries of the clove borer or by carrot baiting of discoloured vascular tissue as described by Moller & De Vay (1968).

Cultures were purified by transferring masses of ascospores from the tips of ascomatal necks, mycelium or conidial masses from the primary isolation plates onto 2 % (w/v) Malt Extract Agar (MEA) (Biolab, Midrand, South Africa). All cultures used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 2).

Three different groups were identified amongst isolates based solely on cultural characteristics. Cultures were transferred three times by taking single ascospore droplets to ensure that the cultural characteristic remained constant throughout each transfer. The groups were defined based solely on this cultural character; no further studies on the morphology of isolates were considered.

For DNA extraction, isolates (Table 1) were grown on 2 % (w/v) MEA plates for 2 weeks. Mycelium was scraped from the surface of these cultures using a scalpel, and transferred to Eppendorf tubes and lyophilised for 2 days. The lyophilised mycelium was placed in liquid nitrogen, ground to a fine powder using a glass rod, and DNA was extracted using a modified version of the method described by Barnes *et al.* (2001b).

PCR amplification

For the DNA sequence analysis the gene regions chosen were the ITS regions. These gene regions were amplified with the primers ITS1 and ITS4 (White *et al.* 1990). The PCR reactions and annealing temperature were the same as for Chapter 3. Eleven microsatellite primers previously developed for *C. fimbriata* (Barnes *et al.* 2001a) were tested for use in this study. PCR reactions were specific for each primer, as determined by the optimum annealing temperature (Barnes *et al.* 2001a). The PCR reactions consisted of 2 ng genomic DNA, 1 x Expand High Fidelity Buffer containing 1.5 mM MgCl₂ (Roche Molecular Biochemicals), 200 μM of each dNTP, 300 nM of the forward and reverse primer (Barnes *et al.* 2001a) and 0.35 U Expand High Fidelity enzyme. Sterile water was used to adjust the final volume of each reaction to 25 μl. The PCR reaction was initiated with an initial denaturing step of 2 min at 96 °C. This was followed by 10 cycles of 20 s at 94 °C, annealing at 48 s and an extension for 45 s at 72 °C. Another 25 cycles, with a 5 s time increase on the annealing temperature followed. The final elongation cycle was at 72 °C for 10 min. The PCR products were electrophoresed on a 2 % (20 % w/v) agarose gel containing ethidium bromide and visualised

using ultra violet light. A 100 bp molecular weight marker (Roche Diagnostics, Mannheim, Germany) was included to determine the approximate size and concentrations of the amplicons.

DNA Sequencing and analyses

The ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit was used according to the manufacturer's protocols (Applied BioSystems, Foster City, California) to obtain DNA sequences of the PCR amplicons from both directions. The same primer pairs and cleaning techniques were used for the sequence reactions as for the PCR reactions. Sequence reactions were run on an ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California, U.S.A) and sequence electropherograms were analysed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California).

The sequences obtained for the three different cultural groups of *Ceratocystis polychroma* prov. nom. were compared with those of morphologically similar *Ceratocystis* spp. that are available in GenBank (Table 2). Sequences were aligned manually and analysed using PAUP version 4.0b10* [Phylogenetic Analysis Using Parsimony (and other methods)] (Swofford 2002). All data were treated as described in Chapter 3. *Ceratocystis virescens* (Davids.) Moreau was used as the out-group. A partition homogeneity test (Swofford 2002) was used to determine whether the sequence data sets for the three different gene regions could be combined.

The posterior probability of nodes in the phylogenetic tree was determined using the Markov Chain Monte Carlo (MCMC) method (Larget & Simon 1999), with a Bayesian framework. One hundred thousand random trees were generated, sampling every 100th tree and printing every 10th tree. A number of trees generated (4700) had to be discarded as they might have been sampled before the convergence of the Markov chain. For the analysis of the ITS region, gamma rate heterogeneity was set, and no codon specific sites were included. For the β -tubulin and EF1- α sequences, codon specific sites were specified with a site-specific substitution rate and the site partition was treated as a by-codon.

Genescan analyses

Three different sets for genescan analyses were assembled as described by Barnes *et al.* (2001a). Each reaction consisted of 0.2 µl of the different amplicons, 0.3 µl of the loading dye (Perkin Elmer Corporation, California, USA), 1.1 µl formamide as well as 0.6 µl of an internal size standard, GENESCAN-500 TAMRA (Perkin Elmer Corporation, California, USA). PCR amplicons were size fractionated on a 4.25 % PAGE (Polyacrylamide Gel Electrophoresis) gel, on an ABI Prism 377TM DNA sequencer. The sizes of the DNA fragments were determined using a combination of the software programs GeneScan® 2.1 (Perkin Elmer Corporation, California, USA) and Genotyper® 3.0 (Perkin Elmer Corporation, California, USA).

Analyses of data

Gene diversity (\bar{H}) (Nei 1973) was calculated to consider the probability of sampling two different alleles at the same locus within a population. A matrix was assembled where absence "0" or presence "1" of specific alleles was noted. The frequency of each allele in the population was determined and gene diversity was calculated where

$$\bar{H} = 1 - \sum_k x_k^2$$

and x_k^2 is the frequency of the k^{th} genotype (Nei 1973). Genotypic diversity (\hat{G}) and the probability that two individuals taken at random in a population have different genotypes (Nei 1973) were calculated. The number of different genotypes present in the population was calculated by allocating an alphabetic letter to each allelic size at that specific locus. Isolates with the same profile thus had the same genotype. Genotypic diversity was calculated using the formula

$$\hat{G} = \frac{1}{\left[\sum f_x \left(\frac{x}{N} \right)^2 \right]}$$

where N is the sample size, and f is the number of genotypes occurring x times in the sample (Stoddart & Taylor 1988). To determine whether a sufficiently large population of isolates was used in the study, the genotypic diversity was modelled against the number of loci with 1000 resampling repetitions to produce a sigmoidal graph (Stoddart & Taylor 1988).

To determine the level of linkage disequilibrium, the index of association (I_A) was calculated (Taylor, Jacobson & Fisher 1999). The software program Multilocus (Agapow & Burt 2001) was used to randomise the data set (1000 randomisations) in order to model the observed I_A against an optimally outcrossing population of the same genetic background. Therefore, the null hypothesis assumes random mating, and can be rejected with high significance if the observed value does not conform to random mating.

RESULTS

Fungal isolates and DNA extractions

All sites where isolations were made were in the North of Sulawesi. In total, more than 200 isolates of *C. polychroma* prov. nom. were collected from 18 different trees at 18 sites (Table 1 & Fig. 1). Of these isolates, 50 were selected to represent a population of *C. polychroma* prov. nom. to be used for the purpose of this chapter. Isolates were obtained either directly from separate ascomata in the tunnels of the woodborer or from carrot baiting of the discoloured wood.

Three distinct colony morphologies were observed for isolates of *C. polychroma* prov. nom. (Fig. 2). Isolates representing Group I had a flat appearance with little to no aerial mycelium. The white appearance was a result of masses of conidia that formed on the surface of cultures. Isolates in this group were fast growing and represented the largest number obtained from any area. Isolates representing Group II had more aerial mycelium than those in Group I. Group II isolates had a greenish colour and were slower growing than Group I isolates. Isolates residing in Group III had a woolly texture when compared to the other two groups. Group II isolates had a mixture of white and green colour and these were the slowest growing of all three groups of isolates. Isolates in all three different groups produced perithecia. For further study, 20 isolates from Group I, 23 isolates from Group II and seven isolates representing Group III were chosen (Table 1).

PCR amplification

For amplification of the gene regions for DNA sequence analysis, an average amplicon size of 500 bp was obtained for both the ITS regions. PCR products were obtained for ten of the eleven microsatellite marker primers designed for *C. fimbriata* (Barnes *et al.* 2001a). The

primer pair CF 15/16 gave no results even after changing parameters and concentrations of the PCR reaction conditions. All temperatures used for the amplification of the ten *C. polychroma* *prov. nom.* loci were the same as those for *C. fimbriata* (Barnes *et al.* 2001a).

DNA Sequencing and analyses

The data set obtained from the ITS regions produced 538 characters (Appendix), with 75 most parsimonious trees of which one was chosen for presentation (Fig. 3). The tree had a length of 430, with a consistency index (CI) of 0.9628, a homoplasy index of 0.0372, a retention index (RI) of 0.9175 and a rescaled consistency index (RC) of 0.8834. The bootstrap values for the different clades were; 92 % for *C. polychroma* *prov. nom.*, 100 % for *C. pirilliformis* Barnes & Wingfield, 91 % for *C. fimbriata* and 98 % for *C. albofundus* Wingfield, De Beer & Morris. The Bayesian inference programme's posterior probability of the branch nodes supported the bootstrap values. The posterior probability for the branch nodes for the *C. pirilliformis*, *C. fimbriata* and *C. albofundus* clades were 99 %, 80 % and 98 %, respectively. Isolates of *C. polychroma* *prov. nom.* resided in a single discrete clade that grouped separately from all the other clades, with its own posterior probability of 99 % (Fig. 3). No sub-clades or separate clades were evident for the three cultural groups present.

Genescan analyses

The ten microsatellite primers amplified a total of 35 alleles for *C. polychroma* *nom prov.* (Table 3). One of the loci (CF 15/16) produced a monomorphic allele of 155 bp (Table 3). No unique alleles were observed for any of the three groups of isolates based on morphological differences. Thus, isolates representing these three apparent sub-groups were subsequently treated as a single population.

Analyses of data

Loci with two or three observed alleles (AG 1/2, AG 7/8, AG 15/16 & CF 21/22) exhibited a high allele frequency for one of the alleles, while the other allele(s) had a very low frequency. At loci where more than four alleles were observed (CF 5/6, CF 11/12, CF 17/18), with the exception of one locus (CF 13/14), the distribution of the alleles was interspersed. The frequency for each allele (Table 4) was used to determine the gene diversity (Nei 1973). Loci CF 5/6 and CF 17/18 had the highest gene diversity of 0.781 and 0.734, respectively. The

overall gene diversity (\overline{H}) of the population of isolates was $\overline{H} = 0.402$. Locus CF 23/24 was monomorphic and had a gene diversity of 0. This locus was omitted during calculation and did not influence the overall gene diversity of the population. Forty-eight different multilocus genotypes were inferred from the allelic data. Only two genotypes were observed more than once (Table 5). The maximum percentage of genotypic diversity (\hat{G}) was 89.28 %. A plot of genotypic diversity vs. number of loci showed that the data matrix was sufficiently large to allow population genetic inference (Fig. 4). The observed linkage disequilibrium (I_A) was 0.20 and was located within the distribution range for the randomised data sets ($P = 0.20$) (Fig. 5). The null hypothesis that the population is in linkage equilibrium could thus be rejected at a confidence level of $P = 0.20$ (80 % confidence).

DISCUSSION

In this study we have shown that a newly discovered and interesting fungus, *C. polychroma* *prov. nom.* associated with clove decline in Sulawesi, is probably endemic to the area. Isolates of the fungus had a high level of genetic diversity, which suggests that it has been present in Northern Sulawesi for a long time. This is in contrast to what one would expect from a newly introduced pathogen, where the population would most likely be clonal (McDonald 1997).

DNA sequence analysis for the ITS regions showed no evidence of sub-clade formation correlating with the three different groups representing culture morphology. Rather, all the isolates of *C. polychroma* *prov. nom.* grouped together in one clade separate from the other *Ceratocystis* spp. included in this study. This suggests that differences in culture morphology are probably due to a small number of unlinked genes, which control these morphological differences. Microsatellite marker analysis used in this study also showed no correlation to differences in colony morphology.

Based on morphology of cultures, isolates of *C. polychroma* *nom. prov.* could be placed in one of three different groups. However, comparison of these isolates using microsatellite markers showed no clear genetic difference between them. This is consistent with the DNA sequence comparisons using the ITS gene region. There were two isolates that originated from different geographical areas and grouped within different cultural groups but had the same genotype.

This was unexpected, as the genetic differentiation is high within this population. This provides further evidence that there is no basis for grouping *C. polychroma prov. nom.* into three groups based on differences in culture morphology.

This study was facilitated by the fact that most polymorphic markers designed for *C. fimbriata* (Barnes *et al.* 2001a) could be used to study *C. polychroma prov. nom.* These results support DNA based comparisons showing that the two fungi are phylogenetically closely related (Chapter 3). These primers have also been used on the closely related *C. albofundus* (Barnes 2002, Nakabonge 2002), and it is likely that they will be useful for other cryptic species for example *C. pirilliformis* (Barnes *et al.* 2003, Marin 2003) that evidently reside in the *C. fimbriata* species complex.

A total of 35 alleles were identified in the isolates of *C. polychroma prov. nom.*, using ten microsatellite primer pairs. Differences in size of the alleles were observed between isolates of *C. fimbriata*, *C. albofundus* and *C. polychroma prov. nom.* Similar differences were also observed between *C. albofundus* and *C. polychroma prov. nom.* Although microsatellite markers and allele sizes derived from them are not typically appropriate for distinguishing between species, they clearly reflect phylogenetic relationships in the fungi mentioned above. This has also been found in various other fungi such as *Diplodia pinea* (Desm.) Kickx (Burgess, Wingfield & Wingfield 2001) species in the *C. polonica* complex (Marin 2003) and within different phylogenetic groups recognised in *C. fimbriata* (Marin 2003).

At the loci where only two or three alleles were observed, a high allele frequency was observed for one of the alleles, while the other alleles had a very low frequency. This could indicate fixation, where there is a random genetic drift. Genetic drift is the chance that allele frequency can change due to the drawing of the same gametes in the population (Hartl & Clark 1997). This can also be as a result of selection, based on the fact that the one allele is genetically more stable or favourable than the others (Hartl & Clark 1997). Alternatively, the single high allele frequency could indicate an introduction of a selectively advantageous genotype into the population.

Gene diversity for a large collection of *C. polychroma prov. nom.* isolates was high ($\overline{H} = 0.402$) relative to studies on various other fungi (Leung & Williams 1986, Boeger, Chen & McDonald 1993, Goodwin *et al.* 1993, Barnes *et al.* 2001a). The highest gene diversity was obtained for loci CF 5/6 and CF 17/18 indicating a more equal distribution, signifying a high level of variation at this locus. The high level of gene diversity suggests that *C. polychroma prov. nom.* has been present in Sulawesi for a long time. This fungus appears to have a sexual system similar to that of *C. fimbriata*, which is homothallic but with a capacity to outcross (Olson 1949, Webster & Butler 1967, Harrington & McNew 1997). If it had been recently introduced into Sulawesi, we would have expected a much more limited genetic diversity with distinguishable clonal lineages, and this would be similar to the situation with *C. fimbriata* in Uruguay (Barnes 2002). This study included 50 isolates and only two genotypes occurred more than once. The maximum genotypic diversity (89.28 %) was very high; also supporting the view that *C. polychroma prov. nom.* is probably native to Sulawesi.

Linkage disequilibrium for *C. polychroma prov. nom.* showed that the population is predominantly clonal. This is consistent with the fact that the fungus is homothallic and able to produce perithecia from single ascospores. In this regard, it is most like *C. fimbriata* and *Cryphonectria cubensis* (Bruner) Hodges, which is homothallic but where genetic outcrossing can occur (Webster & Butler 1967, Harrington & McNew 1997, Van der Merwe 2000). The test suggests that recombination occurs only occasionally. Self-crossing as well as out-crossing are typically observed in homothallic fungi. Some of the alleles are in equilibrium while some alleles are in disequilibrium. This result is typical of a homothallic fungus. It suggests that the *C. polychroma prov. nom.* population is clonal but recombination as the result of out-crossing does occur at low frequency.

This study has shown that a large group of isolates of *C. polychroma prov. nom.* reflect a high level of genetic diversity. In this regard, they suggest that the fungus is probably native to Sumatra. The fact that the fungus has not been found elsewhere in the world also suggests that some part of Indonesia is most likely its area of origin. This study is limited by the fact that *C. polychroma prov. nom.* is known only from Sumatra and that there are no other populations of the fungus available for comparison.

Clove decline in Sumatra is a relatively new problem and it is clearly associated with damage due to the borer *Hexamitodera semivelutina*. Hell. (Coleoptera: Cerambycidae). This insect is native on cloves in their area of origin in the Molucca islands and was introduced into nearby Sulawesi, where cloves have been introduced. It would be interesting to know whether *C. polychroma* prov. nom. occurs in the Moluccas, or whether this fungus is unique in Sulawesi, capitalising on a niche provided by the borer. The absence of clove decline in the Moluccas where the insect is present suggests that the fungus might be an important component in the overall disease syndrome.

- Agapow, P. M. & Burt, A. (2001) Indices of multilocus disequilibrium. *Molecular Ecology Notes* **1** : 101-102.
- Anonymous (2002) Detection, monitoring and management on invasive plant pests in Indonesia. APEC Symposium on Detection, monitoring and management of invasive plant pests. Chinese Taipei, Sept. 30-Oct. 3.
- Barnes, I. (2002) Taxonomy, phylogeny and population biology of *Ceratocystis* species with particular reference to *Ceratocystis fimbriata*. MSc. Thesis. University of Pretoria, South Africa.
- Barnes, I., Gaur, A., Burgess, T., Roux, J., Wingfield, B. D. & Wingfield, M. J. (2001a) Microsatellite markers reflect intra-specific relationships between isolates of the vascular wilt pathogen, *Ceratocystis fimbriata*. *Molecular Plant Pathology* **2** : 319-325.
- Barnes, I., Roux, J., Coetzee, M. P. A. & Wingfield, M. J. (2001b) Characterization of *Seiridium* spp. associated with cypress canker based on β -tubulin and histone sequences. *Plant Disease* **85** : 317-321.
- Barnes, I., Roux, J., Wingfield, M. J., Old, K. M. & Dudzinski, M. (2003) *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. *Mycologia* **95** : 865-871.
- Burgess, T., Wingfield, M. J. & Wingfield, B. D. (2001) Simple sequence repeat markers distinguish among morphotypes of *Sphaeropsis sapinea*. *Applied and Environmental Microbiology* **67** : 354-362.
- Boeger, J. M., Chen, R. S. & McDonald, B. A. (1993) Gene flow between geographic populations of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. *Phytopathology* **83** : 1148-1154.
- Goodwin, S. B., Saghai-Marooof, M. A., Allard, R. W. & Webster, R. K. (1993) Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. *Mycological Research* **97** : 49-58.
- Harrington, T. C. & McNew, D. L. (1997) Self-fertility and uni-directional mating-type switching in *Ceratocystis coerulescens*, a filamentous ascomycete. *Current Genetics* **32** : 52-59.

- Hartl, D. L. & Clark, A. G. (1997) Principles of population genetics. 3rd edition. 267-314. Sinauer Associates, Inc. Publishers Sunderland, Massachusetts.
- Kile, G. A. (1993) Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In: *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity* (Wingfield, M. J., Seifert, K. A. & Webber, J. F., eds.). 173-183. APS Press, St. Paul, Minnesota.
- Larget, B. & Simon, D. L. (1999) Markov chain monte carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16** : 750-759.
- Leung, H. & Williams, P. H. (1986) Enzyme polymorphism and genetic differentiation among geographic isolates of the rice blast fungus. *Phytopathology* **76** : 778-783.
- Liew, E. C. Y., Wingfield, M. J., Assa, B., Paath, J., Kandowangkossor, D., Sembel, D. T., Summerell, B. A. & Burgess, L. W. (2003) *Ceratocystis fimbriata* associated with clove decline in North Sulawesi. In: 8th International Congress of Plant Pathology, 2-7 February 2003, Christchurch, New Zealand, ICPP 8 Book of Abstracts (ICPP 8 Programme Committee (Falloon, R.E. [chair])), (Ed.): 266, Abstract no. 19.40.
- Marin, M. A. (2003) Phylogenetic and molecular population biology studies on *Ceratocystis* spp. associated with conifer and coffee diseases. PhD. Thesis, University of Pretoria. South Africa.
- McDonald, B. A. (1997) The population genetics of fungi: Tools and Techniques. *Phytopathology* **87** : 448-453.
- Moller, W. & De Vay, J. (1968) Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. *Phytopathology* **58** : 1499-1508.
- Nakabonge, G. (2002) Diseases associated with plantation forestry in Uganda. MSc. Thesis. University of Pretoria. South Africa.
- Nei, M. (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA* **70** : 3321-3323.
- Olsen, E. O. (1949) Genetics of *Ceratostomella*. Strains in *Ceratostomella fimbriata* (Ell. & Hals.) Elliott from sweet potatoes. *Phytopathology* **39** : 548-561.
- Stoddart, J. A. & Taylor, J. F. (1988) Genotypic diversity: estimation and prediction in samples. *Genetics* **118** : 705-711.
- Swofford, D. L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.

- Taylor, J. W., Jacobson, D. J. & Fisher, M. C. (1999) The evolution of asexual fungi: reproduction, speciation and classification. *Annual Review of Phytopathology* **37** : 197-246.
- Van der Merwe, N. A. (2000) Molecular phylogeny and population biology studies on the *Eucalyptus* canker pathogen *Cryphonectria cubensis*. MSc. Thesis. University of Pretoria, South Africa.
- Webster, R. & Butler, E. E. (1967) The origin of self-sterile, cross-fertile strains in *Ceratocystis fimbriata*. *Mycologia* **59** : 212-221.
- White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A sequencing guide to methods and applications* (Innis M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J., eds.): 315-322. Academic Press, San Diego.
- Wingfield, M. J., De Beer, C., Visser, C. & Wingfield, B. D. (1996) A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. *Systematic and Applied Microbiology* **19** : 191-202.
- Witthuhn, R. C., Harrington, T. C. Wingfield, B. D., Steimel, J. P. & Wingfield, M. J. (2000) Deletion of the MAT-2 mating type gene during uni-directional mating type switching in *Ceratocystis*. *Current Genetics* **38** : 48-52.

Table 1. Isolates of *Ceratocystis polychroma* from Sulawesi, used in this study

Isolate number	Region of isolation	Cultural group allocated to
CMW 11416	Toliangoki B	2
CMW 11418	“	1
CMW 11420	“	1
CMW 11423	“	2
CMW 11429	“	2
CMW 11431	Lahendong	2
CMW 11432	“	1
CMW 11438	“	1
CMW 11439	Leilum	1
CMW 11440	“	1
CMW 11441	Kaiwa	1
CMW 11445	“	1
CMW 11446	“	1
CMW 11448	Rumoong	1
CMW 11451	“	1
CMW 11452	“	1
CMW 11456	“	1
CMW 11460	Tumpaap / Pinamorongan	2
CMW 11462	Munte	1
CMW 11463	“	1
CMW 11464	Lalumpe	2
CMW 11466	“	1
CMW 11467	Tulap	2
CMW 11468	Kakas	1
CMW 11469	“	2
CMW 11470	“	3
CMW 11477	“	2
CMW 11480	“	2
CMW 11482	“	2
CMW 11486	Tinoor	2
CMW 11487	“	3
CMW 11488	Kumelembuai	2
CMW 11490	“	1
CMW 11492	“	3
CMW 11493	Motoling A	3
CMW 11495	Motoling B	2
CMW 11497	“	3
CMW 11498	“	2
CMW 11499	Tambelang B	2
CMW 11501	“	2
CMW 11504	“	2
CMW 11507	“	3
CMW 11510	Tambelang C	2
CMW 11511	“	2
CMW 11512	“	2
CMW 11513	“	3
CMW 11517	Koka B	2
CMW 11518	“	2
CMW 11519	“	1
CMW 11520	Kembes	2

Table 2. Isolates of *Ceratocystis* used in this study.

Species	Isolate no. ^a	Alternative numbers	GenBank accession nr.	Date of isolation	Host	Geographical origin	Associated insect	Collector(s)
<i>C. fimbriata</i>	CMW 4835 ^b	None	AF395689 ^c N/A ^d N/A ^e	1998	<i>Gymnocladus dioica</i>	Colombia	None	Wingfield, M. J.
"	CMW 2219 ^b	"	AF395679 ^c N/A ^f AY528975 ^c	1991	<i>Platanus</i>	France	"	Grosclaude, C.
<i>C. albofundus</i>	CMW 2475 ^b	"	AF043605 ^c N/A ^d N/A ^e	1992	<i>Acacia mearnsii</i>	South Africa	"	Mc Leman, S.
"	CMW 2148 ^b	"	AF264910 ^c N/A ^d N/A ^e	N/A	"	"	"	Morris, M. J.
<i>C. pirilliformis</i>	CMW 6569 ^b	"	AF427104 ^c N/A ^d AY528982 ^c	"	<i>Eucalyptus nitens</i>	Australia	"	Wingfield, M. J.
"	CMW 6579 ^b	"	AF427105 ^c N/A ^d AY528983 ^e	"	"	"	"	"
<i>C. polychroma</i> <i>prov. nom</i>	CMW 11418	"	N/A	2002	<i>Syzygium aromaticum</i>	Sulawesi, Indonesia	<i>Hexamitodera semivelutina</i>	Liew, E. C. Y. & Wingfield, M. J.
"	CMW 11420	"	N/A	"	"	"	"	"
"	CMW 11470	"	N/A	"	"	"	"	"
"	CMW 111487	"	N/A	"	"	"	"	"
"	CMW 11497	"	N/A	"	"	"	"	"
"	CMW 11492	"	N/A	"	"	"	"	"
"	CMW 11499	"	N/A	"	"	"	"	"
"	CMW 11501	"	N/A	"	"	"	"	"
"	CMW 11504	"	N/A	"	"	"	"	"
"	CMW 11507	"	N/A	"	"	"	"	"
"	CMW 11513	"	N/A	"	"	"	"	"
<i>C. virescens</i>	CMW 3276 ^b	None	AY528984 ^c AY528990 ^d AY528991 ^e	1963	<i>Quercus</i> sp.	USA.	None	Hinds, T.

^{a, b, c, d, e} Isolates marked with ^a CMW refers to the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, those marked with ^b were sequenced, GenBank accession numbers that are marked with ^c represent the ITS sequences, those marked with ^d represent the β -tubulin sequences and those marked with ^e represent the Elongation Factor sequences.

Table 3. A comparison of alleles obtained from *C. fimbriata*, *C. albofundus* and *C. polychroma* with the microsatellite primers designed for *C. fimbriata*.

Primer pairs	Alleles		
	<i>C. fimbriata</i> ^a	<i>C. albofundus</i> ^a	<i>C. polychroma</i>
AG 1/2	255	None	261
	263		262
	264		
	265		
	266		
	268		
AG 7/8	285	309	276
	287	322	277
	288	323	
	289	325	
	290	326	
	300	327	
	301	331	
	304	332	
	305		
AG 15/16	274	288	277
	276	293	279
AG 17/18	304	310	275
	305	311	276
	307		
	308		
	311		
CF 5/6	365	380	374
	367	387	385
	368		387
	369		398
	370		399
	371		400
	382		
	384		
	385		
CF 11/12	217	None	197
	219		198
	220		199
	222		200
			205
	207		
	210		
CF 13/14	400	None	369
	402		374
	403		376
	405		418
	407		432
	410		446
	413		
	414		
	415		
CF 15/16	477	288	None
	480	293	
	487		
CF 17/18	268	288	271
	271	291	272
	279	292	273
			274
CF 21/22	250	283	232
	255	284	234
	256	285	235
CF 23/24	156	166	155
	160	168	
	168		

^aData obtained from Barnes 2002.

Table 4. Alleles, genotype configuration and allele frequency obtained for isolates of *C. polychroma* *prov. nom.* at each loci.

Locus	Alleles ^a	Genotype configuration ^b	Allele frequency ^c
AG 1/2	261	A	0.68
	262	B	0.32
AG 7/8	276	A	0.70
	277	B	0.30
AG 15/16	277	A	0.94
	279	B	0.06
AG 17/18	275	A	0.20
	276	B	0.80
CF 5/6	374	A	0.26
	385	B	0.06
	387	C	0.02
	398	D	0.16
	399	E	0.26
	400	F	0.24
CF 11/12	197	A	0.06
	198	B	0.38
	199	C	0.08
	200	D	0.04
	205	E	0.08
	207	F	0.14
	210	G	0.02
CF 13/14	369	A	0.66
	374	B	0.04
	376	C	0.06
	418	D	0.06
	432	E	0.04
	446	F	0.02
CF 17/18	271	A	0.22
	272	B	0.28
	273	C	0.18
	274	D	0.32
CF 21/22	232	A	0.04
	234	B	0.90
	235	C	0.06
CF 23/24	155	A	1.00

^a Observed allele size.

^b A final representation of the multilocus genotype, for each genotype that has the same configuration the same character was assigned.

^c Allele frequency is obtained by dividing the total number of occurrence of that allele by the number of isolates in the population.

Table 5. The multilocus genotype for the three *C. polychroma* populations.

Isolate number	Multilocus genotype	Isolate genotype	Isolate number	Multilocus genotype	Isolate genotype
Group I			Group II		
CMW 11418	B B A B F B B B B A	a	CMW 11416	A A A B F F C B C A	u
CMW 11420	B A A A F B C B B A	b	CMW 11423	B B A B E B C B B A	v
CMW 11432	B A A B D B A D B A	c	CMW 11429	A B A B A B B D B A	w
CMW 11438	B A A A A B A D B A	d	CMW 11431	^a A B A B A B A D B A	l
CMW 11439	A A A B F B A D B A	e	CMW 11460	A A A B A B A C B A	x
CMW 11440	A A A B E C A D B A	f	CMW 11464	B A A A E B A D A A	y
CMW 11441	A B A B E B A D B A	g	CMW 11467	A A A B E G A B B A	z
CMW 11445	A B A B E B A D A A	h	CMW 11469	A A A B F A A B B A	aa
CMW 11446	A A A B E B A C B A	i	CMW 11480	A A A B D B A D B A	bb
CMW 11448	A A A A D B A C B A	j	CMW 11482	A B A B A A A B B A	cc
CMW 11451	B A A B F C A C B A	k	CMW 11488	B A A A A C A A B A	dd
CMW 11452	^a A B A B A B A D B A	l	CMW 11495	A B A B A E A A B A	ee
CMW 11456	A A A B A B A D B A	m	CMW 11498	A A A B E E A B B A	ff
CMW 11462	A B A B E B A A B A	n	CMW 11499	A B B B B D F C B A	gg
CMW 11463	B A A A E B A D B A	o	CMW 11501	A B A B F F X A B A	hh
CMW 11466	A A A B E B A A B A	p	CMW 11504	B A A B F E X B B A	ii
CMW 11468	^b A A A A D B A D B A	q	CMW 11510	B B A B E F E A B A	jj
CMW 11477	A A A B A A A A B A	r	CMW 11511	A A A B F F X B B A	kk
CMW 11486	^b A A A A D B A D B A	q	CMW 11512	A A A B A F X B B A	ll
CMW 11490	A A A A F B A A C A	s	CMW 11517	A A A B C F D B B A	mm
CMW 11519	A A A B D B D C B A	t	CMW 11518	A A A B D B D A B A	nn
			CMW 11520	B A A B A B E C B A	oo
			Group III		
			CMW 11470	A A A B A B A B B A	pp
			CMW 11487	B A A B E C A C B A	qq
			CMW 11492	B A A B D B A A B A	rr
			CMW 11493	B A A A F B A D B A	ss
			CMW 11497	A A A B F E A B B A	tt
			CMW 11507	A B B B B D X A B A	uu
			CMW 11513	B B B B B F X C C A	vv

^a Indicates the same genotype

^b Indicates the same genotype



Figure 1. a) Map representing Sulawesi, b) an enlargement of Northern Sulawesi, to indicate (with small dots) the 18 areas where samples were collected.



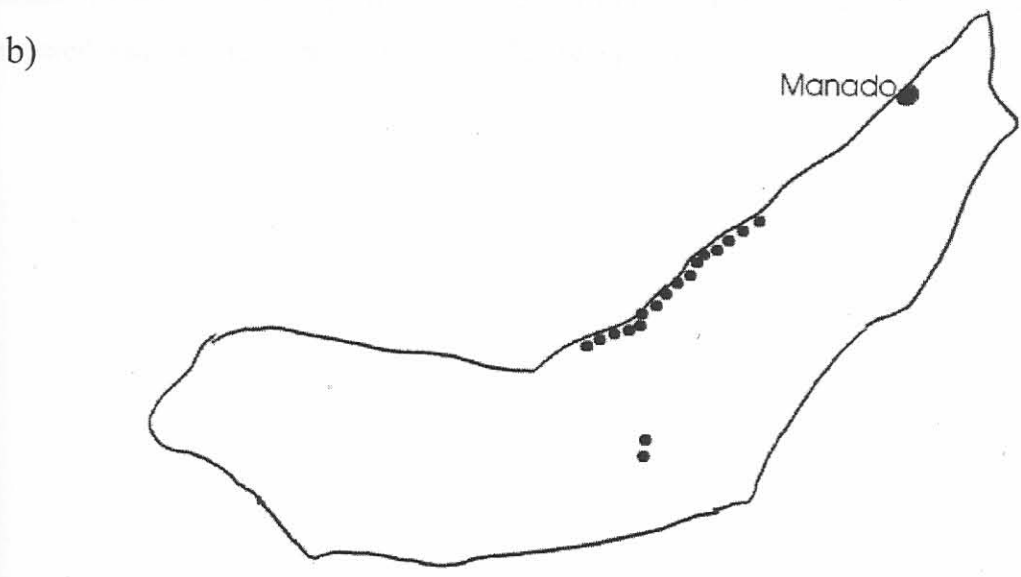
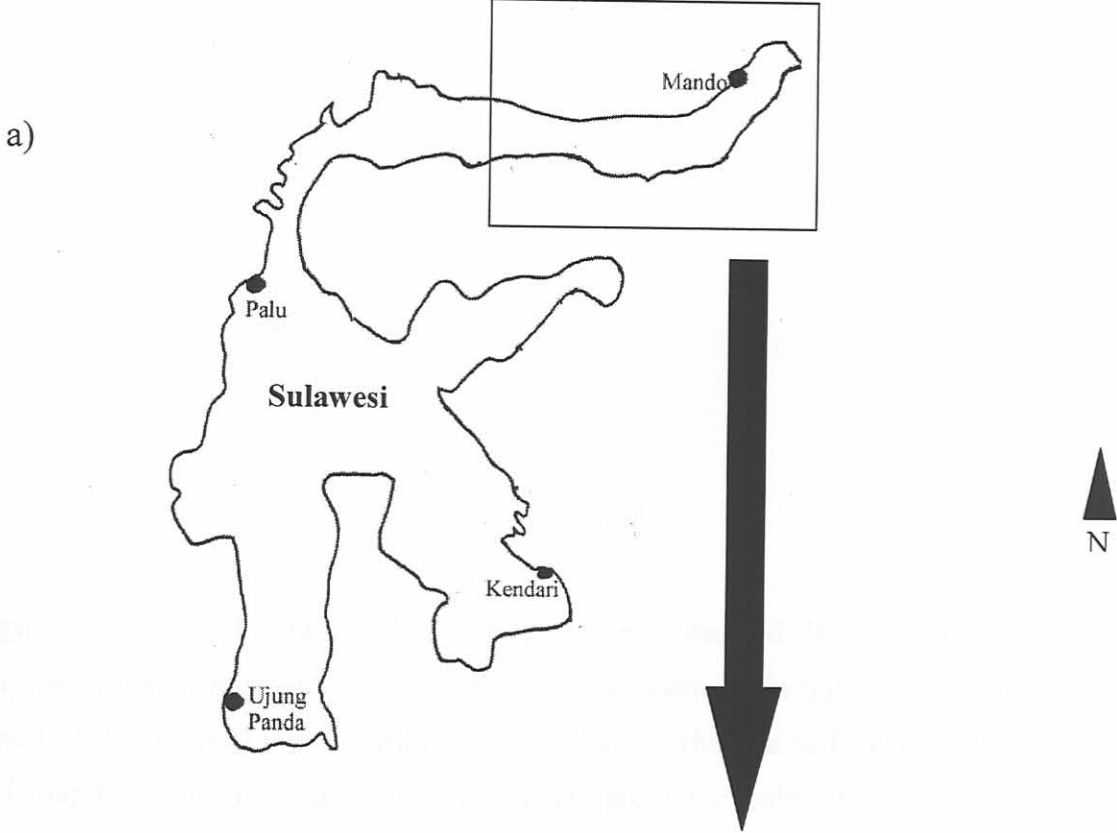
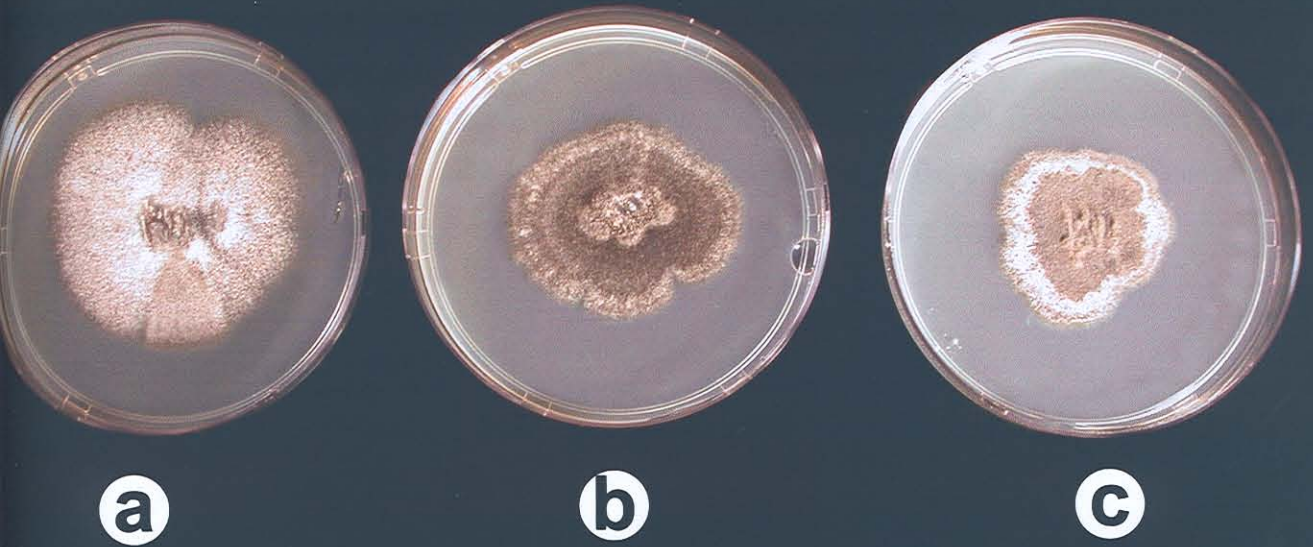
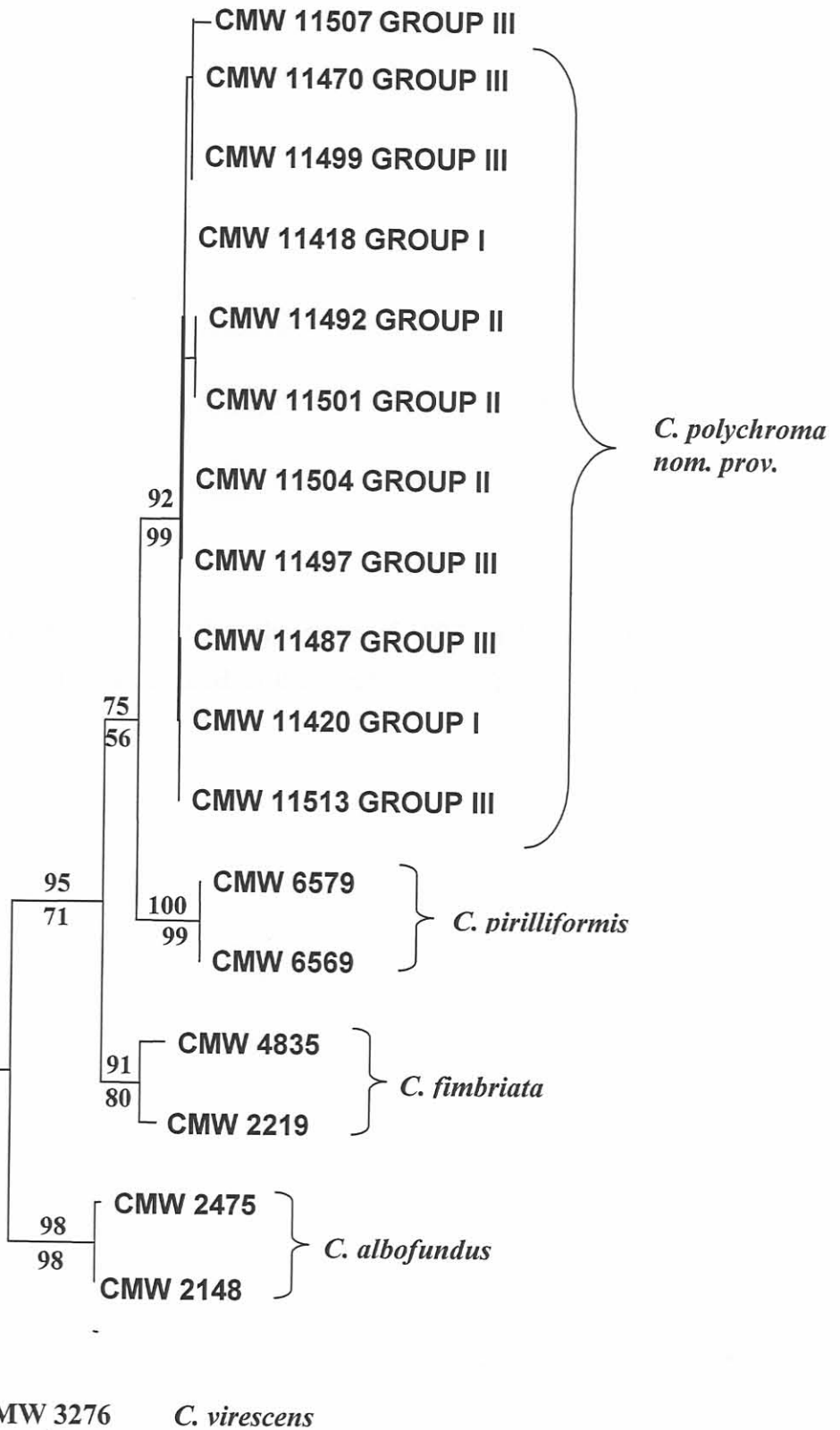


Figure 2. Three different colony morphologies observed in isolates of *Ceratocystis polychroma* prov. nom. isolated from *Syzygium aromaticum* in Sulawesi. a) Group I isolates have little to no aerial mycelia with white conidia covering the surface and a fast growth rate, b) Group II isolates have aerial mycelia and are greener in colour with a slower growth than isolates of Group I, c) Group III isolates have a woolish texture and the slowest growth when compared with isolates representing the other two groups.





changes

Figure 4. A plot of G_{ST} vs. number of loci the mean of 1000 randomisations for each data point indicating that the data matrix used in this study was sufficiently large to allow for population genetic analysis.

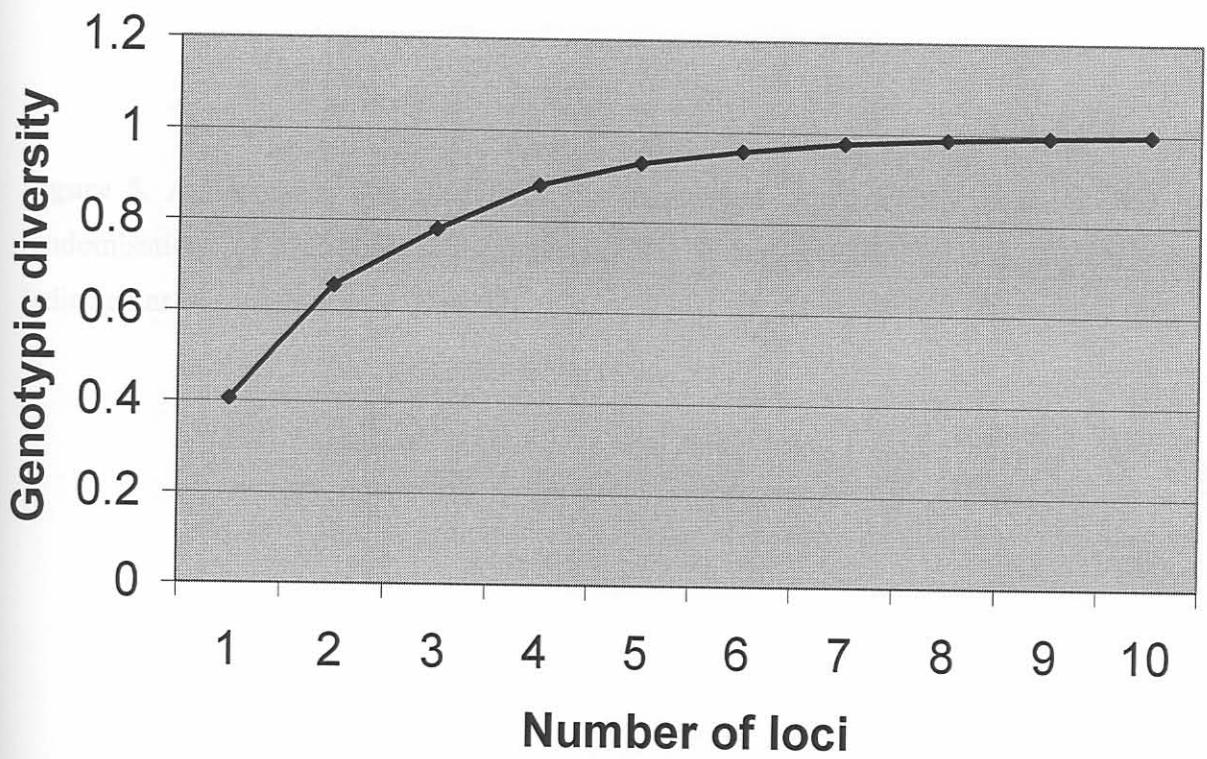
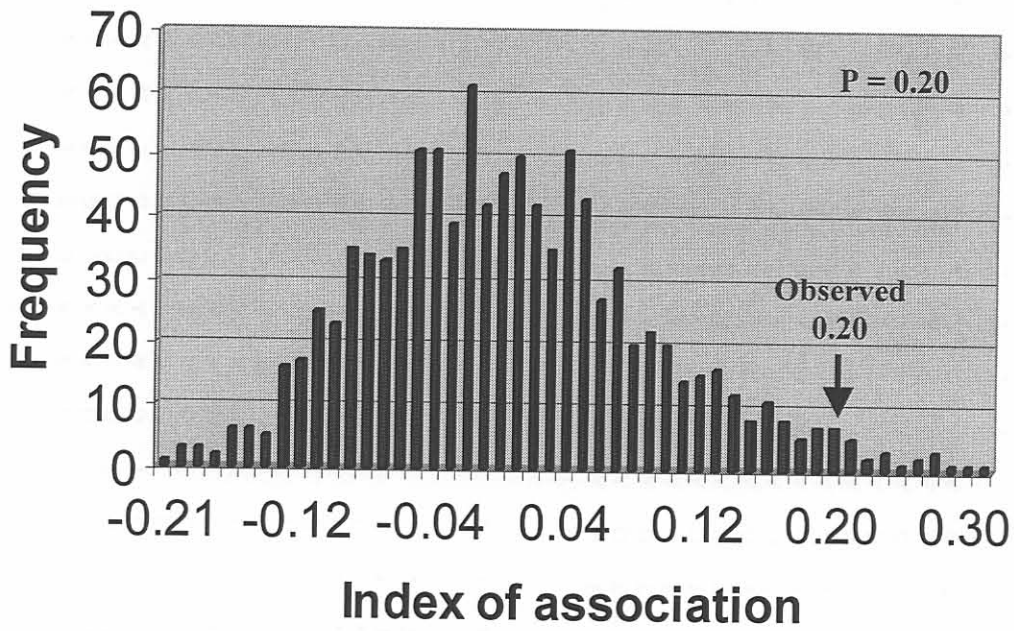


Figure 5. A histogram compiled from the frequencies of associative indices, after 1000 randomisations of the original data matrix. The index obtained for the original data is indicated as an observed value ($P=0.02$).



	1	1	2	0
CMW 11507 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	T	T	A	A	A	C	T	A	T	C	T	T	G	T	G	A	A
CMW 11418 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11470 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11499 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11487 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11420 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11513 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11492 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11504 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11501 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 11497 <i>C. polychroma</i> prov. nom.	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	A	C	T	T	A	T	C	T	T	G	T	G	A	A
CMW 4835 <i>C. fimbriata</i>	C	C	A	T	G	T	G	T	G	A	A	C	A	T	A	C	C	C	T	A	T	C	T	T	G	T	A	A	G
CMW 2219 <i>C. fimbriata</i>	C	C	A	T	G	T	G	T	G	A	A	C	G	T	A	-	C	C	T	A	T	C	T	T	G	T	A	A	G
CMW 6579 <i>C. pirilliformis</i>	C	C	A	T	T	T	G	T	G	A	A	C	G	T	A	A	C	C	T	A	T	C	T	T	G	T	A	A	C
CMW 6569 <i>C. pirilliformis</i>	C	C	A	T	T	T	G	T	G	A	A	C	G	T	A	A	C	C	T	A	T	C	T	T	G	T	A	A	C
CMW 2475 <i>C. albofundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW 2148 <i>C. albofundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW 3276 <i>C. virescens</i>	C	C	A	T	A	T	G	T	G	A	A	C	A	T	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	.	.	3	4	5
CMW 11507 <i>C. polychroma</i> prov. nom.	A	G	A	-	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11418 <i>C. polychroma</i> prov. nom.	A	-	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11470 <i>C. polychroma</i> prov. nom.	A	G	A	-	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11499 <i>C. polychroma</i> prov. nom.	A	G	A	-	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11487 <i>C. polychroma</i> prov. nom.	A	A	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11420 <i>C. polychroma</i> prov. nom.	A	A	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11513 <i>C. polychroma</i> prov. nom.	A	A	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11492 <i>C. polychroma</i> prov. nom.	A	-	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11504 <i>C. polychroma</i> prov. nom.	A	-	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11501 <i>C. polychroma</i> prov. nom.	A	-	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 11497 <i>C. polychroma</i> prov. nom.	A	-	G	G	A	T	G	A	A	C	A	A	A	A	A	-	-	-	-	G	C	T	G	C	T	T	T	T	G
CMW 4835 <i>C. fimbriata</i>	T	G	A	G	A	T	G	A	A	T	-	-	-	-	-	-	-	-	-	G	C	T	G	T	T	T	T	T	G
CMW 2219 <i>C. fimbriata</i>	T	-	G	G	A	T	G	A	A	T	-	-	-	-	-	-	-	-	-	G	C	T	G	T	T	T	T	T	G
CMW 6579 <i>C. pirilliformis</i>	T	-	G	G	A	T	G	A	A	T	A	A	A	C	A	A	T	A	T	G	C	T	G	C	T	T	T	T	G
CMW 6569 <i>C. pirilliformis</i>	-	-	G	G	A	T	G	A	A	T	A	A	A	C	A	A	T	A	T	G	C	T	G	C	T	T	T	T	G
CMW 2475 <i>C. albofundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	C	T	G	C	C	T	T	T	G
CMW 2148 <i>C. albofundus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	C	T	G	C	C	T	T	T	G
CMW 3276 <i>C. virescens</i>	-	-	-	-	-	-	-	-	-	-	-	-	A	C	C	T	A	T	T	A	G	C	G	G	C	T	T	T	T

University of Pretoria etd – Van Wyk, M (2004)

	7															8															9														
	0															0															0														
CMW 11507 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11418 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11470 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11499 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11487 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11420 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11513 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11492 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11504 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11501 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 11497 <i>C. polychroma</i> prov. nom.	C	T	C	-	-	-	-	-	T	T	T	T	A	T	T	T	T	T	G	T	T	A	G	A	A	A	T																		
CMW 4835 <i>C. fimbriata</i>	C	T	C	-	T	T	T	T	T	T	A	T	A	T	T	T	T	C	T	-	-	-	A	G	A	-	-	T																	
CMW 2219 <i>C. fimbriata</i>	C	T	C	-	-	-	-	-	T	T	A	T	A	T	T	T	T	C	G	-	-	-	A	G	A	-	-	T																	
CMW 6579 <i>C. pirilliformis</i>	C	T	C	-	-	-	-	T	T	T	A	A	T	A	T	T	T	T	A	T	-	-	-	A	G	A	A	T																	
CMW 6569 <i>C. pirilliformis</i>	C	T	C	-	-	-	-	T	T	T	A	A	T	A	T	T	T	T	A	T	-	-	-	A	G	A	A	T																	
CMW 2475 <i>C. albofundus</i>	C	T	T	C	-	-	-	-	T	G	T	A	T	A	T	T	T	T	A	-	-	-	A	A	A	T	T	T																	
CMW 2148 <i>C. albofundus</i>	C	T	T	C	-	-	-	-	T	G	T	A	T	A	T	T	T	T	A	-	-	-	A	A	A	T	T	T																	
CMW 3276 <i>C. virescens</i>	T	C	-	T	T	T	T	T	T	T	T	A	T	T	G	T	A	A	-	-	-	A	G	A	A	T	A																		

	2															1															2														
	0															0															0														
CMW 11507 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11418 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11470 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11499 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11487 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11420 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11513 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11492 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11504 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11501 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 11497 <i>C. polychroma</i> prov. nom.	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 4835 <i>C. fimbriata</i>	T	T	-	-	-	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	-																	
CMW 2219 <i>C. fimbriata</i>	T	T	T	-	-	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	A																	
CMW 6579 <i>C. pirilliformis</i>	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	A	A	-	-	A	T	A	A																	
CMW 6569 <i>C. pirilliformis</i>	T	T	T	G	A	T	T	T	C	A	T	T	G	C	T	G	A	G	T	G	A	A	G	C	A	T	A	A																	
CMW 2475 <i>C. albofundus</i>	T	T	T	-	-	A	A	A	A	A	T	T	G	C	T	G	A	G	T	G	G	C	G	C	A	T	A	-																	
CMW 2148 <i>C. albofundus</i>	T	T	T	-	-	A	A	A	A	A	T	T	G	C	T	G	A	G	T	G	G	C	-	-	A	T	A	-																	
CMW 3276 <i>C. virescens</i>	A	T	T	-	-	-	-	C	A	T	T	G	C	T	G	A	G	T	G	-	G	C	A	T	T	A	A	C																	

University of Pretoria etd – Van Wyk, M (2004)

	30															40					50							
CMW 11507 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	-	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11418 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11470 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11499 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11487 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11420 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11513 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11492 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11504 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11501 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 11497 <i>C. polychroma</i> prov. nom.	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 4835 <i>C. fimbriata</i>	A	C	T	A	T	A	A	A	A	A	A	A	A	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 2219 <i>C. fimbriata</i>	-	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 6579 <i>C. pirilliformis</i>	A	A	T	A	A	T	A	A	-	-	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 6569 <i>C. pirilliformis</i>	A	A	T	A	A	T	A	A	-	-	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 2475 <i>C. albofundus</i>	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 2148 <i>C. albofundus</i>	A	C	T	A	T	A	A	A	A	A	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C
CMW 3276 <i>C. virescens</i>	A	T	A	A	T	A	A	-	-	-	-	-	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A

	60															70					80							
CMW 11507 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11418 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11470 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11499 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11487 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11420 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11513 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11492 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11504 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11501 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 11497 <i>C. polychroma</i> prov. nom.	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 4835 <i>C. fimbriata</i>	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 2219 <i>C. fimbriata</i>	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 6579 <i>C. pirilliformis</i>	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 6569 <i>C. pirilliformis</i>	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 2475 <i>C. albofundus</i>	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 2148 <i>C. albofundus</i>	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G
CMW 3276 <i>C. virescens</i>	A	-	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A	G	C	A	T	C	G	A	T	G

	4														5														6													
	0														0														0													
CMW 11507 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11418 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11470 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11499 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11487 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11420 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11513 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11492 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11504 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11501 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 11497 <i>C. polychroma</i> prov. nom.	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 4835 <i>C. fimbriata</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 2219 <i>C. fimbriata</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 6579 <i>C. pirilliformis</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 6569 <i>C. pirilliformis</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 2475 <i>C. albofundus</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 2148 <i>C. albofundus</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														
CMW 3276 <i>C. virescens</i>	A	A	T	C	T	T	T	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A														

	3														3														3													
	7														8														9													
	0														0														0													
CMW 11507 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11418 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11470 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11499 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11487 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11420 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11513 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11492 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11504 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11501 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 11497 <i>C. polychroma</i> prov. nom.	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 4835 <i>C. fimbriata</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 2219 <i>C. fimbriata</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 6579 <i>C. pirilliformis</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 6569 <i>C. pirilliformis</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 2475 <i>C. albofundus</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 2148 <i>C. albofundus</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														
CMW 3276 <i>C. virescens</i>	G	T	A	T	T	C	T	G	C	C	A	G	G	C	A	T	G	C	C	T	G	T	C	C	G	A	G	C														

University of Pretoria etd – Van Wyk, M (2004)

	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
CMW 11507 <i>C. polychroma</i> prov. nom.	-	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	C	G	A	A	A	T	G	T	A	T	C
CMW 11418 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11470 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11499 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11487 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11420 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11513 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11492 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11504 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11501 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 11497 <i>C. polychroma</i> prov. nom.	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 4835 <i>C. fimbriata</i>	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 2219 <i>C. fimbriata</i>	-	C	C	C	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 6579 <i>C. pirilliformis</i>	-	-	-	-	C	T	G	A	G	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 6569 <i>C. pirilliformis</i>	-	-	-	-	C	T	G	A	G	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 2475 <i>C. albofundus</i>	C	C	T	T	C	T	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 2148 <i>C. albofundus</i>	C	C	T	T	C	C	G	A	A	C	A	G	G	C	C	G	C	G	A	A	A	T	G	T	A	T	C
CMW 3276 <i>C. virescens</i>	-	C	C	C	T	C	A	A	C	A	G	G	C	C	A	C	C	G	A	A	A	T	G	T	A	T	C

	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
CMW 11507 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11418 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11470 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11499 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11487 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11420 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11513 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11492 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	T	C	A	A	C	C	T	C
CMW 11504 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11501 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 11497 <i>C. polychroma</i> prov. nom.	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 4835 <i>C. fimbriata</i>	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	C	C	A	A	C	C	T	C
CMW 2219 <i>C. fimbriata</i>	G	G	C	T	G	T	T	A	-	-	-	-	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 6579 <i>C. pirilliformis</i>	G	G	C	T	G	T	T	A	A	-	-	-	-	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 6569 <i>C. pirilliformis</i>	G	G	C	T	G	T	T	A	A	-	-	-	-	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 2475 <i>C. albofundus</i>	G	G	C	T	G	T	T	A	T	T	T	T	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 2148 <i>C. albofundus</i>	G	G	C	T	G	T	T	A	T	T	T	T	T	A	C	T	T	G	C	-	C	A	A	C	C	T	C
CMW 3276 <i>C. virescens</i>	G	G	C	T	G	T	T	-	-	-	-	-	A	C	C	T	T	G	-	A	G	C	T	C	C	T	C

