# Analysis of the mating-type loci of *Chrysoporthe* species

by

**Tshiamo Phakalatsane**

20814412

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In the Faculty of Natural and Agricultural Sciences, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa.

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Supervisor: Prof. Nicolaas A. van der Merwe

Co-supervisor: Dr. P. Markus Wilken

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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DECLARATION

I, undersigned, hereby declare that the dissertation submitted herewith for the degree Magister Scientiae (Genetics) to the University of Pretoria, contains my own work and has not been submitted for any other degree at any other University.

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Tshiamo Phakalatsane

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## Preface

Mating in fungi is controlled by mating-type genes that are localized within mating-type loci. The sexual identity of fungal strains is thus determined by identifying and characterizing these genes. Sexually reproducing fungi can be either homothallic or heterothallic. Homothallic species can complete their life cycle in the absence of a mating partner, while heterothallic species require a genetically compatible mating-type partner to complete their life cycle.

*Chrysoporthe* species are economically important tree pathogens that primarily cause stem and branch canker on *Eucalyptus* spp., *Syzygium* spp., and *Tibouchina* spp. Thus far, the mating systems of only three species from this genus have been characterized. It is of utmost importance to understand the reproductive biology of these pathogenic fungi as sexual reproduction is one way to increase genetic diversity that could lead to increased virulence.

A literature review (Chapter 1) serves as a commentary on the mode of reproduction of *Chrysoporthe* species. This chapter explores different mating systems employed by ascomycetes, highlighting similarities and differences in the genetic composition of the mating-type loci. Further, the review unpacks the current knowledge on the mating strategies and *MAT1* loci from *Chrysoporthe* species.

The genus *Chrysoporthe* accommodates important pathogens that cause diseases on trees in the Myrtales, reducing timber quality and quantity. Thus, it is important to understand how these fungi evolve and adapt to various environmental factors, such as climate and host species. Sequences of fungal genomes provide the opportunity to dissect evolutionary processes, with the goal of reducing or mitigating infections. The main purpose of Chapter 2 was to sequence the genomes of *C. zambiensis* and *C. syzygiicola* using PacBio sequencing. The genomes of these species were assembled using CANU and gene models were predicted using AUGUSTUS. In addition, species identity was confirmed by constructing a phylogenetic tree using single copy orthologs.

Chapter 3 focuses on determining the mating system of *C. zambiensis* and *C. syzygiicola*. The mating type loci were predicted from genome sequences of both species using bioinformatics tools. The mating-type genes of *C. zambiensis* and *C. syzygiicola* were predicted using CLC Genomics Workbench and the mating-type loci were annotated using AUGUSTUS. The identities of the

genes predicted in the mating-type loci were confirmed using BLASTp analysis, and conserved domains analysis of the predicted proteins. The latter was done using InterPro analysis tool. Based on these analyses, there were clear similarities and differences in the mating-type loci of *C. syzygiicola* and *C. zambiensis*. All the genes that are associated with the *MAT1-1* and *MAT1-2* idiomorphs were present in the mating-type loci of both species, thus consisting of homothallic mating system. However, the size of the mating-type locus of *C. zambiensis* was slightly larger than the mating-type locus of *C. syzygiicola*. In addition, the genes that are commonly associated with the flanking regions of the mating-type loci of ascomycetes were not linked to the mating-type locus of *C. zambiensis*.

# Chapter 1

# Literature review: Mating systems of *Chrysoporthe* species

## Introduction

The *Cryphonectriaceae* is a family of fungi in the order Diaporthales that accommodates numerous economically and ecologically important plant pathogens. Some of these pathogens infect tree species in the *Myrtales* (*Melastomataceae* and *Myrtaceae*) across the globe (Granados *et al*., 2020, Mausse-Sitoe *et al*., 2016). This fungal family accommodates 55 species in 41 genera (Jiang *et al*., 2020) that are pathogens of economically important trees in woody ecosystems and forests. The family *Cryphonectriaceae* was initially established based on a type genus, *Cryphonectria parasitica* in the *Cryphonectria-Endothia* (Castlebury *et al*., 2002) species complex, and other type genera such as *Rostraureum*, *Amphilogia* (Gryzenhout *et al*., 2006a), and *Chrysoporthe* (Gryzenhout *et al*., 2004). Fungi in the *Cryphonectriaceae* are distinguished by the formation of orange stromata during their life cycle, and the change of color to purple when subjected to potassium hydroxide (KOH) or yellow when subjected to lactic acid (Gryzenhout *et al*., 2006a, Gryzenhout *et al*., 2004).

In recent years, several new species of *Cryphonectriaceae* have been identified from Africa. For example, *Latruncellus aurorae* was identified as a pathogen of *Galpini transvaalica* in Southern Africa (Vermeulen *et al*., 2011), *Aurifilum marmelostoma* as the causal agent of stem cankers on non-native *Terminalia mantaly* and native *Tibouchina ivorensis* in Cameroon (Begoude *et al.*, 2010) and in South Africa, *Holocryphia eucalypti* was identified as the causal agent of canker and dieback on *Eucalyptus* spp. and *Tibouchina urvilleana* (Gryzenhout *et al*., 2003, Nakabonge *et al*., 2008, Heath *et al*., 2007). Moreover, additional species such as *Celoporthe dispersa* and *Myrtonectria myrtacearum* have been found to cause diseases on *Myrtales* trees in South Africa. These pathogens are the causal agents of canker and die-back on *Heteropyxis canescens, Syzygium cordatum, Tibouchiba granulosa*, and *Heteropyxis natalensis* (Nakabonge *et al.*, 2006a, Ali *et al.*, 2018). Several canker pathogens from the genus *Chrysoporthe* have also been detected in different countries in Africa. Species from the genus *Chrysoporthe* cause stem and branch canker diseases that result in wilting, dieback, and death of infected non-native *Eucalyptus* spp., *Tibouchina* spp.

and native *Syzygium* spp. (Heath *et al*., 2006, Nakabonge *et al.*, 2006b, Chungu *et al*., 2009, Myburg *et al*., 2002). Diseases caused by fungal pathogens from different *Cryphonectriaceae* genera have resulted in significant economic losses in the African forest industry (Wingfield, 2003).

The biogeographic distribution of species from *Cryphonectriaceae* suggests that they have evolved in various parts of the world. For examples, *Holocryphia eucalypti* is widespread in Australia (Heath *et al*., 2007). Although the origin of this pathogen is unknown, it is thought to be introduced to South Africa (Nakabonge *et al.*, 2008). Species from the genus *Celoporthe* have been found infecting trees in South Africa, Zambia, China, and Indonesia (Vermeulen *et al*., 2013b). In addition, species from the genus *Chrysoporthe* have also been detected in tropical and subtropical parts of the world outside of Africa. For instance, *Chrysoporthe cubensis* (Gryzenhout *et al.*, 2004, Hodges Jr *et al*., 1986) is widespread in South America and Central America (Gryzenhout *et al*., 2004, Gryzenhout *et al.*, 2006b, Van der Merwe *et al.*, 2010, Nakabonge *et al*., 2006b), while *Chrysoporthe deuterocubensis*, the closest relative of *C. cubensis* is primarily found in Southeast Asia. Both these species are thought to have been introduced to the African continent through planting non-native *Eucalyptu*s spp. (Gryzenhout *et al*., 2004, Gryzenhout *et al.*, 2006b, Heath *et al.*, 2006, Chen *et al*., 2010). Additionally, the presence of *Chrysoporthe austroafricana* and its close relatives *C. zambiensis* and *C. syzygiicola* on native *Syzygium* trees in Africa, and their absence from other continents, suggests that these species are of African origin (Heath *et al*., 2006, Chungu *et al*., 2010).

The wide geographic distribution of species in the *Cryphonectriaceae* provided them with an opportunity to adapt in different habitats and cause diseases on a wide range of *Myrtales* host. It has been observed that fungi in this family can host-jump between woody plants and ornamental plants. For example, species in *Cryphonectriaceae* can jump between the *Myrtaceae* (*Eucalyptus* spp., *Syzygium* spp., and *Heteropyxis* spp.), *Melastomataceae* (*Tibouchina* spp.), and *Combretaceae* (*Terminalia* spp.), irrespective of the geographic region where the pathogen was detected.

Although the genetic basis for having a wide host range is unknown in species from the *Cryphonectriaceae*, breeding programs have been employed to manage and control diseases that are caused by these phytopathogens (Wingfield *et al*., 2001, Wingfield, 2003). To effectively

eradicate or manage a pathogen it is important to understand factors that play a critical role in generating genetic diversity of species, such as their mode of reproduction, gene flow, mutations, and mating systems (Çelik Oğuz and Karakaya, 2021, Amos and Harwood, 1998). These factors result in the ability of a pathogen to be highly virulent or adapt to various environmental conditions. Therefore, it is important to comprehend the factors that influence the evolution and genetic diversity of pathogens, as this will help with the development of effective control mechanisms. Thus, the main purpose of this review is to understand the reproductive strategies and mating systems that are employed by species from the genus *Chrysoporthe* (Cryphonectriaceae, Diaporthales). It will then further explain the genes that regulate sexual reproduction, and the types of mating systems that different species can employ.

## Mode of reproduction in *Chrysoporthe*

Fungal organisms have evolved diverse modes of reproduction, either sexually or asexually. Sexual reproduction is an ubiquitous process that generates genetic diversity through recombination and crossing-over during meiosis. This provides the organism with the ability to produce progeny that are able to adapt to different environmental conditions, while also eliminating deleterious mutations (Billiard *et al*., 2012, Usher, 2019). Despite having great benefits, sexual reproduction has some disadvantages. It is time consuming and energetically expensive due to the need to find a compatible mating partner of the opposite sex (Nieuwenhuis and James, 2016, Heitman *et al.*, 2013). In contrast, asexual reproduction is a low energy process as only one parent is required for the production of offspring, and the absolute population size can thus rapidly increase under favourable environmental conditions (Heitman *et al*., 2013). There is consequently no genetic variation within an asexual population, which comes with an inherent risk for the persistence of deleterious mutations.

Some filamentous ascomycetes can benefit from both asexual and sexual reproduction by alternating between these modes of reproduction under favourable environmental conditions (Coppin *et al*., 1997). Thus, it is rare to find species that can only reproduce sexually in natural habitats. In addition, sometimes it is difficult to detect the sexual state of a fungal species, making it difficult to determine the mode of reproduction of a fungal species. Furthermore, some filamentous ascomycetes are pleomorphic, meaning that they can produce both anamorph and

teleomorph stages (Nieuwenhuis and James, 2016, Stelzer and Lehtonen, 2016). For example, in *C. puriensis* both asexual (anamorphs) and sexual (teleomorphs) fruiting bodies have been observed in natural habitats (Oliveira *et al*., 2021). This could mean that *C. puriensis* can alternate between sexual and asexual reproduction under conducive environmental factors. When species of *Myrtales* are infected by *C. cubensis* or *C. deuterocubensis,* sexual fruiting bodies are present, irrespective of the host organism (Van Heerden and Wingfield, 2001). In contrast, when *C. austroafricana* causes canker on *Eucalyptus* spp., sexual fruiting bodies are rare, but they occur frequently when *Syzygium* trees are infected (Heath et al., 2006, Nakabonge et al., 2006b). No sexual fruiting bodies have ever been detected when *C. hodgesiana*, *C. syzygiicola*, or *C. zambiensis* infects their host species (Gryzenhout *et al*., 2004, Chungu *et al*., 2010). Although the mode of reproduction in *Chrysoporthe* species is not extensively studied, the presence of sexual and asexual fruiting bodies in these species might be an indicator that some species reproduce sexually while others can reproduce asexually (clonally) under favourable conditions.

It is believed that the process of sexual reproduction has evolved as a mechanism to protect organisms from stressful environmental factors by producing genetically variant offspring and repair damaged DNA via homologous recombination (Wallen and Perlin, 2018). Additionally, sexual reproduction allows a pathogen to evolve along with the host ability to defend itself against the pathogen. Although the ability to reproduce sexually can be beneficial to an organism, not every species in the genus *Chrysoporthe* can produce sexually. For example, *Chrysoporthe hodgesiana* is an asexual species as the sexual state have never been observed (Gryzenhout *et al*., 2004). Therefore, asexual reproduction in some species of *Chrysoporthe* might be favoured because this mode of reproduction has a reduced generation time and reproduction can take place in the absence of a partner.

Sexual reproduction in fungi can be associated with evolutionary costs. For example, a new allele combination generated during recombination (Ni *et al*., 2011) might result in the loss of virulence or reduced pathogenicity (Wallen and Perlin, 2018). Thus, natural selection might favour asexual reproduction in species that have both asexual and sexual modes of reproduction (Otto, 2008). However, the ability of an organism to reproduce sexually will still be maintained due to the selective advantage of adaptation to varying environmental conditions (Heitman, 2006, Billiard *et al*., 2012).

## Mating systems: heterothallism and homothallism

Fungal species have evolved diverse sexual systems which are broadly classified as either heterothallism or homothallism. Heterothallic ascomycetes require a genetically compatible mating-type partner to initiate and complete the sexual cycle. Such a species contains a single mating-type locus in its genome that harbours either a *MAT1-1* or a *MAT1-2* idiomorph (Figure 1) (Kanematsu *et al*., 2007, Kanzi *et al*., 2019, Yin *et al*., 2017). The term idiomorph emphasizes the fact that sequences contained in the mating-type locus are sufficiently dissimilar to not be treated as alleles, and that the *MAT1-1* and *MAT1-2* idiomorphs carry different genes. However, the genes that are encoded by the mating-type idiomorphs in homothallic and heterothallic ascomycetes are functionally similar, in the sense that they are involved in the regulation of sexual reproduction and other sex-related processes (Wilson *et al*., 2019, Rodenburg *et al*., 2018, Kim *et al*., 2015).

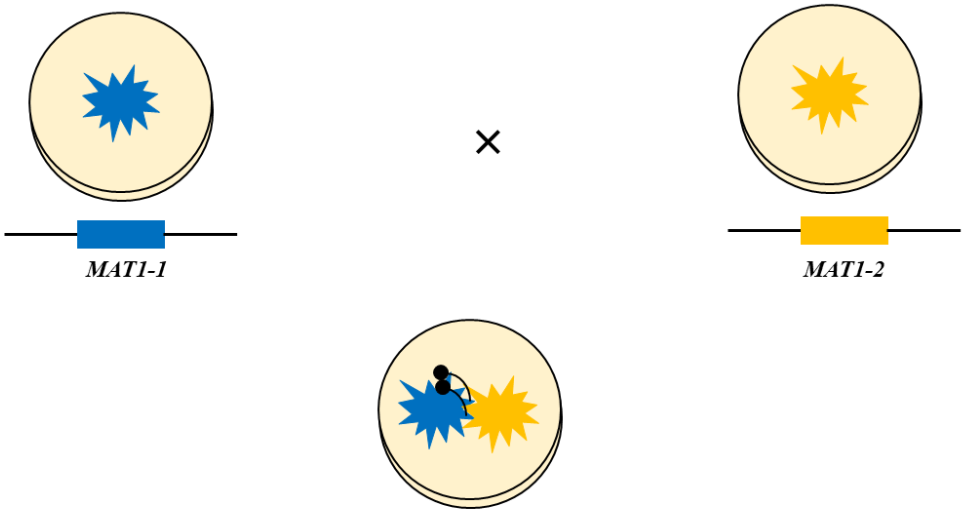
In contrast to heterothallic ascomycetes, homothallic ascomycetes can complete their sexual cycle in the absence of a mating-type partner, and such species are considered as self-fertile (Wilson *et al*., 2015b). A homothallic ascomycete contains all the genes that are necessary for self-fertilization (selfing) to take place. These genes can either be linked in a single locus, or they can be present in different regions in the genome (Dyer *et al*., 2016, Debuchy and Turgeon, 2006) (Figure 1.2). Additionally, fungal species have evolved diverse strategies of producing self-fertile species. Firstly, some species possess primary homothallism. Such species are still self-fertile and the mating-type (*MAT1*) locus contains all of the mating-type genes that would have been encoded by the *MAT1-1* and *MAT1-2* idiomorphs (Paoletti *et al*., 2007, Pöggeler *et al.,* 1997) (Figure 1.2). The genetic mechanism that results in primary homothallism is commonly observed in different orders of Pezizomycotina. Based on genetic analysis, primary homothallism has been confirmed in species of *Diaporthales* (Kanzi *et al*., 2019), *Helotiales* (Robicheau *et al.,* 2017), *Sordariales* (Klix *et al*., 2010), and several other ascomycetes orders.

Secondly, there is secondary homothallism (pseudohomothallism). Fungal species that possess this mating system can reproduce independently from a mating partner (Wilson *et al*., 2015b, Ni *et al*., 2011), as the two opposite mating-types are packaged in a single spore with two nuclei (Arnaise *et al*., 2001) which results in self-fertile progenies (Figure 1.1). Mating-type switching is another example of secondary homothallism. During mating-type switching, an organism mates

independently of a partner by switching from one mating-type to an opposite mating-type, thus resulting in progenies that are self-fertile (Wilson *et al*., 2015b, Ni *et al*., 2011, Haber, 1998). Mating-type switching is commonly observed in *Saccharomyces cerevisiae, Ceratocystis fimbriata,* and *Thielaviopsis cerberus* (Krämer *et al*., 2021, Poggeler, 2001, Wilken *et al*., 2014). Lastly, unisexual mating is a recently discovered mode of homothallism. Such species possess one mating-type idiomorph in the genome and they can reproduce sexually (Wilson *et al*., 2015a, Wilson *et al*., 2021a), thus resulting in self-fertile progeny. Although the genetic mechanisms that result in secondary homothallism and unisexual mating are not well-understood, fungal organisms have evolved different modes of homothallism with the ultimate result of producing self-fertile progeny.

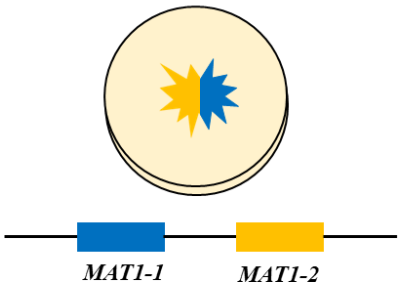
In general, species from the genus *Chrysoporthe* appear to employ homothallic mating system (Kanzi *et al.*, 2019), which might be due to the probability of finding a mate being slim, with the advantages of infrequent outcrossing outweighing the disadvantages of more frequent asexual reproduction. Homothallism imparts unlimited sexual compatibility that is advantageous. Contrarily, heterothallism is more appropriate in environments where mating partners are commonly found, and where the fitness costs is high (Billiard *et al.*, 2012).

## a) Heterothallism




## b) Homothallism

### I. Primary homothallism



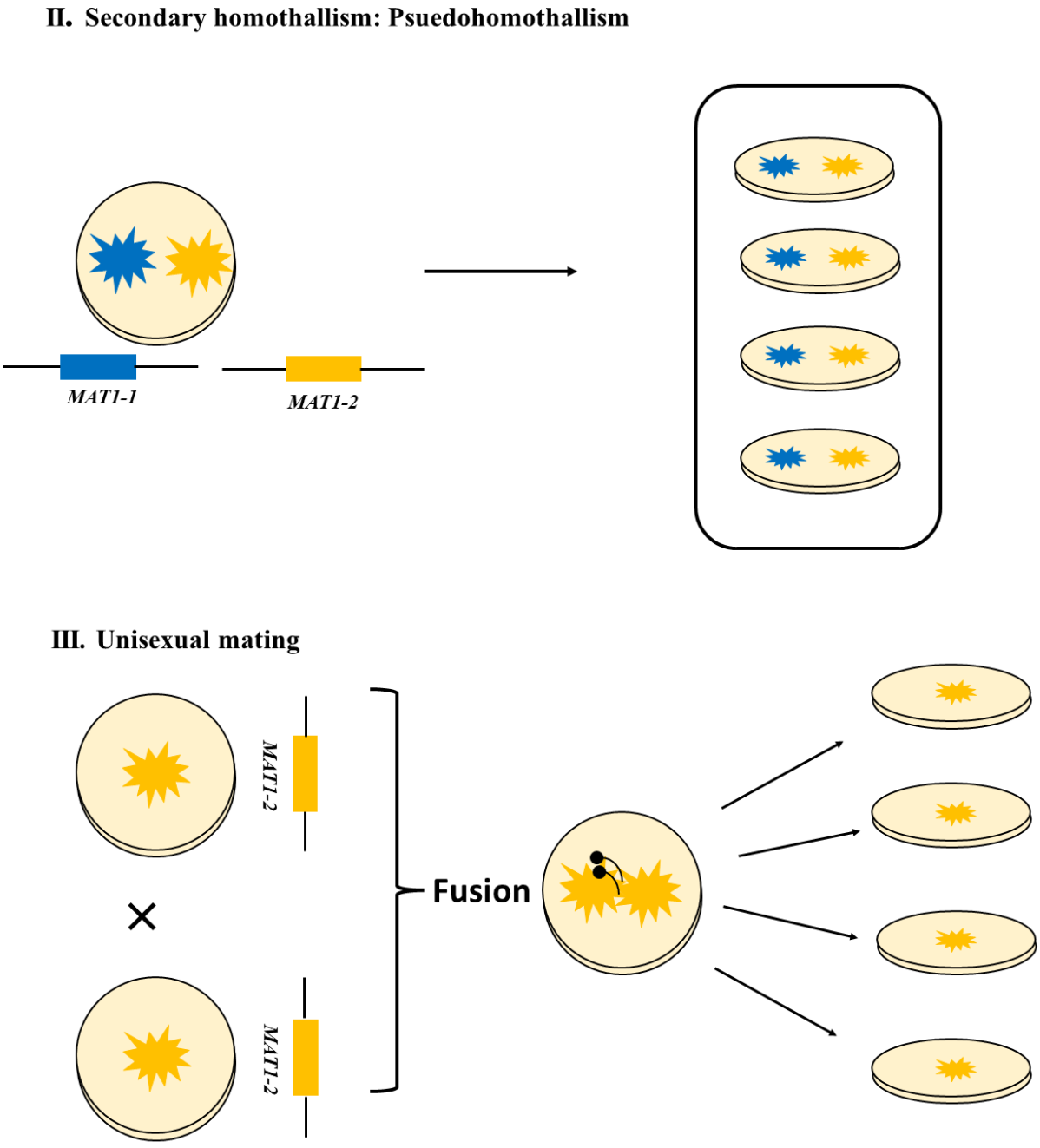



Figure 1.1: Simplified illustration of fungal mating system. (a) Heterothallism, where fungal isolates have either the *MAT1-1* or *MAT1-2* idiomorphs, and both are needed to reproduce. (b)

Homothallism: different modes of homothallism are illustrated. (i) is a representation of primary homothallism, where both mating-type idiomorphs are present inside a single cell, in turn allowing the organisms to produce self-fertile isolates. (ii) Secondary homothallism/pseudohomothallism, where two opposite mating-type are packaged in a single spore with two nuclei thus resulting in self-fertile isolates. (iii) Unisexual mating, when fungal isolates have the same mating-type idiomorph and they can mate or produce sexually even though the opposite mating-type partner is absent.

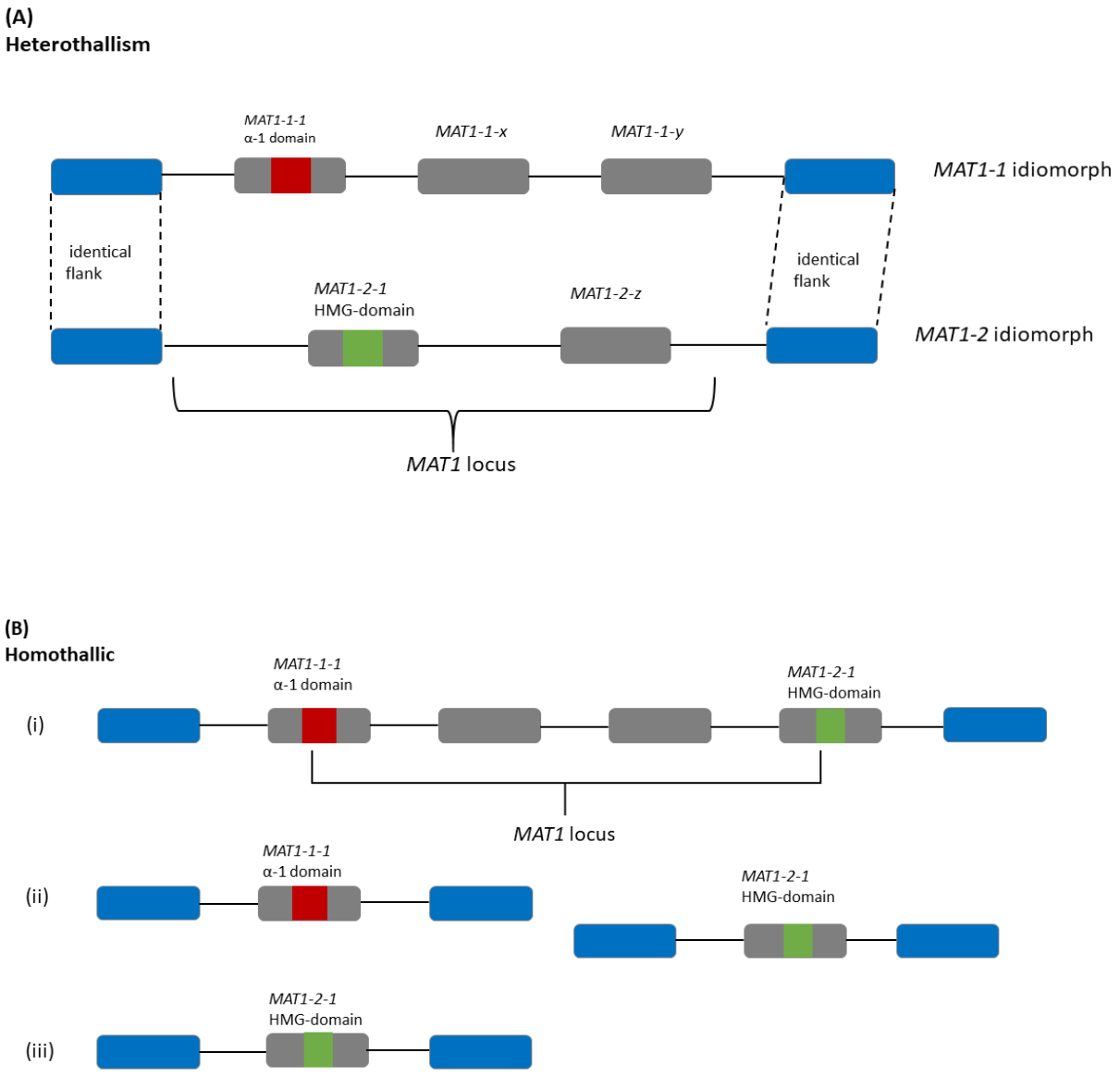



Figure 1.2: Representation of a typical mating-type locus of heterothallic and homothallic filamentous ascomycetes. (A) Heterothallic ascomycetous fungi have either a *MAT1-1* or a *MAT1-2* idiomorph on their mating-type locus. Downstream and upstream of the mating-type locus, there are conserved DNA sequences (blue oval shapes) that are normally associated with the mating-type locus of Pezizomycotina and can be used to confirm the presence of the *MAT1* locus. In the mating-type locus, where either a *MAT1-1* or a *MAT1-2* idiomorph is present, the genes that are encoded by the alternate idiomorphs consists of conserved domains either α-1 domain (red square)

or HMG-box (green square). (B) Schematic representation of the mating-type loci of homothallic ascomycetes. (i) Demonstration of the mating-type locus of homothallic species, genes that are associated with the *MAT1-1* and *MAT1-2* idiomorphs are present in the same locus. (ii) Represents a mating-type locus of some isolates where the mating type locus is unlinked and genes that are associated with opposite idiomorphs are located in different regions in the genome. (iii) A mating-type locus of some homothallic fungi that can reproduce sexually while possessing only one mating-type idiomorph in its genome.

The flanking genes (blue boxes) can either be *APN2, SLA2, COX13,* or *APC5* (Wilken *et al*., 2017). The Figure is not drawn to scale and it was adapted from Dyer *et al*. (2016)

## The content of mating-type loci

Mating in fungal organisms is controlled by the presence of mating-type genes, that are localized within the mating-type locus (*MAT1*) (Debuchy *et al*., 2010, Wilken *et al*., 2018, Turgeon, 1998, Szewczyk and Krappmann, 2010). The mating-type locus consists of mating-type idiomorphs namely the *MAT1-1* and *MAT1-2* that are characterized by the presence of the *MAT1-1-1* and *MAT1-2-1* genes, respectively (Turgeon and Yoder, 2000, Wilken *et al.*, 2017). The *MAT1-1-1* and the *MAT1-2-1* genes that are localized within the *MAT1* locus encodes for proteins with the alpha-1 (α-1) domain and high mobility group (HMG) domain, respectively (Figure 1.2) (Dyer *et al*., 2016, Turgeon and Yoder, 2000, Debuchy and Turgeon, 2006). Mating-type genes are master regulator of sexual reproduction and biological processes that are associated with sexual reproduction, such as by regulating genes involved in meiosis, and determination of mate compatibility (Wilson *et al.*, 2019, Wilson *et al*., 2015a, Nelson, 1996, Mageswari *et al.*, 2016, Wilken *et al*., 2017, Debuchy and Turgeon, 2006).

Additionally, the mating-type genes provide fungal isolates with their sexual identities (Wilson *et al*., 2021b, Wilson *et al*., 2015b). As discussed above, the mating-type locus of heterothallic fungal isolates consists of either one of the two mating-type idiomorphs. For example, the genetic composition the *MAT1-1* idiomorph of a typical heterothallic ascomycetes generally consists of

primary mating-type genes, namely *MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3* (Turgeon and Yoder, 2000, Coppin *et al*., 1997, Duong *et al*., 2013, McGuire *et al*., 2001). In contrast, the *MAT1-2* idiomorph of heterothallic ascomycetes minimally consists of the *MAT1-2-1* gene (Martin *et al*., 2011, McGuire *et al*., 2001). The mating-type locus of homothallic ascomycetes consists of all the core genes of heterothallic species, linked, or unlinked (Figure 1.2) (Poggeler, 2001, Yun *et a*l., 2000, Martin *et al*., 2011, Kück and Böhm, 2013, Kück *et al.*, 2009). Apart from the primary mating-type genes (*MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3*, and *MAT1-2-1*), additional genes may be present in the mating-type idiomorph (Wilken *et al*., 2017, Wilson *et al*., 2021b).

The genetic composition of mating-type loci between species in Pezizomycotina may vary, but the encoded mating-type genes are highly conserved within species (Turgeon, 1998). For instance, the *MAT1-1* idiomorph of heterothallic *Diaporthales* (Sordariomycetes) such as *C. austroafricana*, *Diaporthe W* type, *Diaporthe G* type, *Valsa mali*, and *Cry. parasitica* consists of primary *MAT1* genes. This includes *MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3* genes (McGuire *et al.*, 2001, Kanzi *et al*., 2019, Kanematsu *et al*., 2007, Yin *et al*., 2017). The genetic content of the *MAT1-1* of Diaporthales species is similar to the genetic composition of the *MAT1-1* idiomorph of the model organism *Neurospora crassa* in the order *Sordariales* (Sordariomycetes) (Coppin *et al*., 1997, Poggeler, 2001, Yun *et al*., 2000). The genetic content of the *MAT1-2* idiomorph of *Cry. parasitica* consists of only the *MAT1-2-1* gene (McGuire *et al.*, 2001), and the genetic content of this species is similar to the *MAT1-2* idiomorph of *Gibberella fujikuroi* in the order *Hypocreales* (Sordariomycetes) (Yun *et al*., 2000). However, some variation regarding the genetic content of the *MAT1-2* idiomorph in some species within *Diaporthales* (Sordariomycetes*)* has been observed. For example, the *MAT1-2* idiomorph of *Diaporthe G* type, *Diaporthe W* type, and *Valsa mali* consists of mating-type genes that are homologous to the *MAT1-1* idiomorph (Yin e*t al*., 2017, Kanematsu *et al.*, 2007), whereas the *MAT1-2* idiomorph of *C. austroafricana* contains truncated versions of the *MAT1-1-1* and *MAT1-1-2* gene (Kanzi *et al*., 2019).

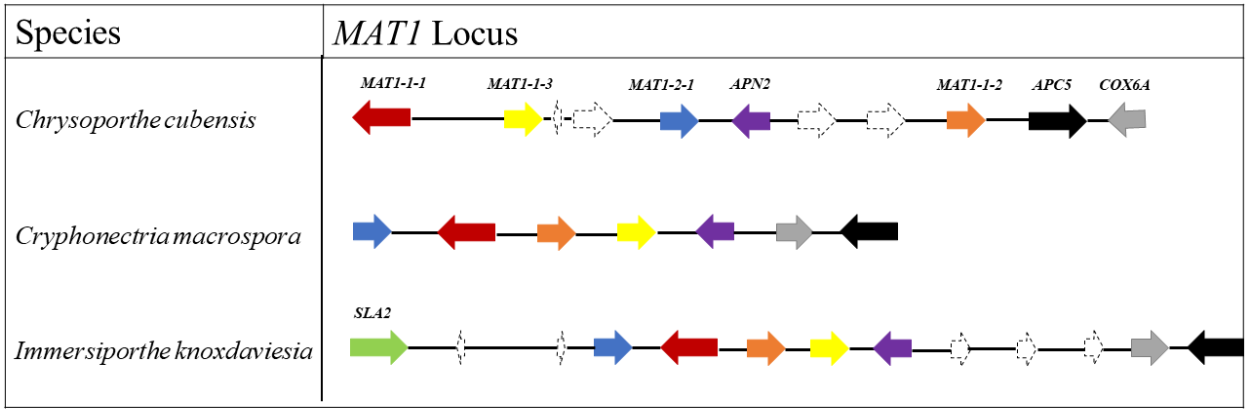
Furthermore, the mating-type locus of *Sordaria macrospora* in the order *Sordariales* represents that of the typical mating-type locus of homothallic species within Sordariomycetes. The mating-type locus of this species consists of *MAT1-1-1, MAT1-1-2, MAT1-1-3,* and *MAT1-2-1* (Pöggeler and Kück, 2000). In addition, species from the order *Diaporthales*, namely *C. cubensis* and *C. deuterocubensis* contains homologs of all four genes, thus constituting of a homothallic mating

system (Kanzi *et al*., 2019). To comprehend the relationship between organisms' mode of reproduction, pathogenicity, and if it will be possible to alter the organisms' reproductive behavior, it is essential to analyze the structural organization of the mating type locus of homothallic and heterothallic species (Yun *et al*., 1999).

Although the genetic composition of some species of *Diaporthales* is similar to that of other species within different orders of Sordariomycetes, the structure of the mating-type locus of some species within *Diaporthales* is quite distinct. In Pezizomycotina, *APN2* (encodes for AP endonuclease), *COX6A* (encodes for Cytochrome C oxidase subunit 6A), and *APC5* (encodes for Anaphase Promoting Complex) genes are located at the end of the *MAT1* locus (Figure 2) (Wilken *et al*., 2017, Nagel *et al*., 2018). However, in the *MAT1* locus of *Chrysoporthe* species, the *APN2* gene is located within the locus. In addition, the *COX13* and *APN2* genes are located within the *MAT1* locus of the apple canker pathogen, *Valsa mali* (Yin *et al*., 2017). The rearrangement of *APN2* in *Chrysoporthe* species and the rearrangement of *COX13* and *APN2* in *Valsa mali* might induce beneficial genetic changes that can be selected for in the mating-type locus of these species (Hartmann *et al*., 2021). Furthermore, the structural organization and genetic content of *MAT1* of Diaporthales might provide insight into the evolution of the mode of reproduction of these species.

Despite extensive investigations to identify and characterize mating-type genes, little is known about the various functions that mating-type genes have during the sexual cycles of different species. Although these investigations have shed some light on the complexity of sexual reproduction in ascomycetes, a detailed understanding of genes that regulate expression of mating-type genes is still required. For example, the mating-type locus of species from *Diaporthales* displays a unique structural organization when compared to other species within *Sordariomycetes*. Thus, the unique structural organization and difference in genetic content might provide insight into the evolution and functions of *MAT1* genes in *Diaporthales*.

a) Homothallism




b) Heterothallism

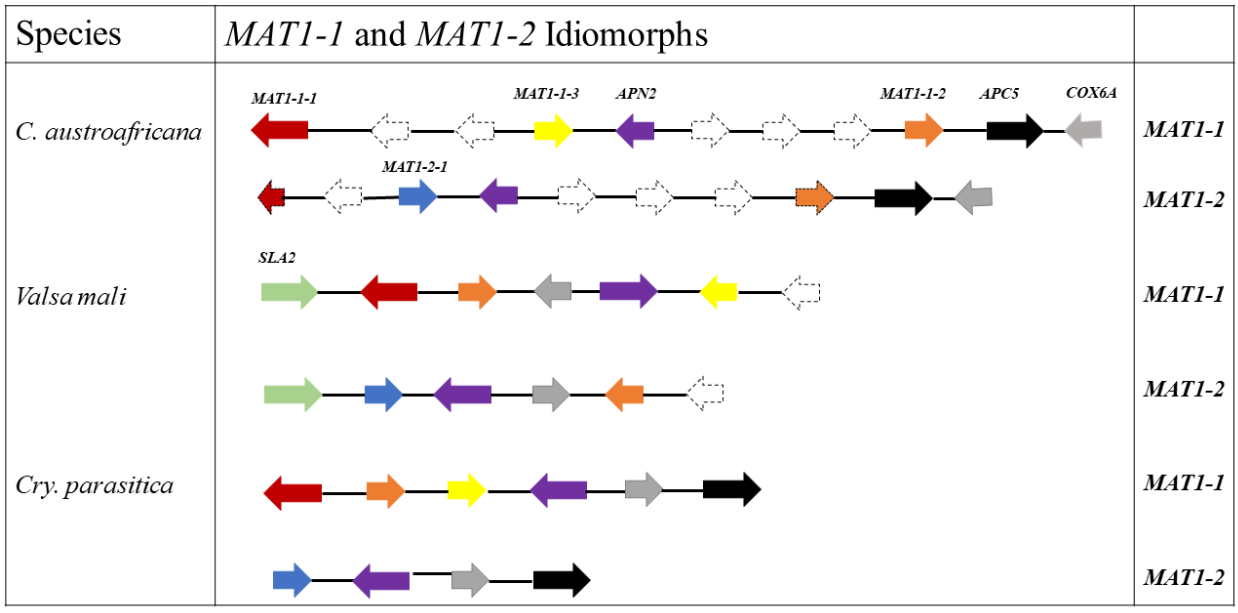



Figure 1.3: Simplified illustration of genetic content of the mating-type loci of homothallic and heterothallic species from different genera in family the *Cryphonectriaceae* and *Valsaceae* in the order *Diaporthales*. All species used in this figure belong to the class Sordariomycetes. The dashed arrows in white are unknown genes or hypothetical proteins. a) An illustration of mating-type loci

of homothallic species in *Cryphonectriaceae, C. cubensis* (Kanzi *et al*., 2019). The mating-type locus of *Cryphonectria macrospora* was constructed using whole genome sequence data (NCBI Accession: GCA_004802535.1), the *MAT1* locus of *Immersiporthe knoxdaviesia* was constructed using whole genome sequence (Wingfield *et al*., 2022). b) An illustration of the mating-type loci of heterothallic species in different orders in *Sordariomycetes*, *C. austroafricana* (Kanzi *et al*., 2019), *Valsa mali* (*Valsaceae*) (Yin *et al*., 2017) and *Cry. parasitica* (McGuire *et al*., 2001), all from the order *Diaporthales*.

## The genus *Chrysoporthe*

*Chrysoporthe* species are canker pathogens of economically important trees from the *Myrtales* (Gryzenhout *et al.*, 2006a). *Chrysoporthe austroafricana* and its closest relatives are important on the African continent because they affect commercial and subsistence plantations of *Eucalyptus* spp., but they also affect native *Syzygium* trees that are culturally important (Wingfield *et al*., 2015b, Wingfield *et al*., 2015a). The devastating nature of canker diseases caused by *Chrysoporthe* species has been a motivational force behind the control of the disease through breeding programs and planting trees that are tolerant to infection (Wingfield, 2003, Guimarães *et al*., 2010).

To date, the genus *Chrysoporthe* consists of nine species, namely *C. austroafricana*, *C. cubensis* (Gryzenhout *et al.*, 2004), *C. deuterocubensis, C. inopina, C. hodgesiana* (Gryzenhout *et al*., 2004*), C. doradensis* (Gryzenhout *et al*., 2005), *C. puriensis* (Oliveira *et al*., 2021), *C. zambiensis*, and *C. syzygiicola* (Chungu *et al.*, 2009). Only five of these species, *C. austroafricana*, *C. zambiensis*, C. *syzygiicola*, *C. deuterocubensis*, and *C, cubensis* cause *Chrysoporthe* canker on *Myrtales* trees in Africa. Species of *Chrysoporthe* have a broad host range that is restricted to species of *Myrtales*, and their ability to infect different host organisms in *Myrtales* makes *Chrysoporthe* canker a high-risk disease. The broad host range of these species could have resulted from planting commercial trees close to where *Chrysoporthe* canker is native on related *Myrtaceae* and/or *Melastomataceae*, or the fungi could have been introduced accidentally by the agricultural and forestry industries (Morris and Moury, 2019).

The availability of whole-genome sequences for some species of *Chrysoporthe* (Wingfield *et al.*, 2015b, Wingfield *et al*., 2015a) provides an opportunity to study sexual reproduction at the sequence level. For example, *MAT1* loci of three *Chrysoporthe* species, namely *C. austroafricana*, *C. cubensis*, and *C. deuterocubensis* has been determined (Kanzi *et al.*, 2019). In addition, sexual fruiting bodies (perithecia) have been observed on hosts of *Chrysoporthe* (Oliveira *et al*., 2021, Heath *et al.*, 2006, Van Heerden and Wingfield, 2001), thus providing evidence of sexual reproduction in natural habitats. These genomic regions and observation of a high level of genetic diversity from population genetic studies provide evidence that sexual reproduction is frequent in species of *Chrysoporthe* (Vermeulen *et al*., 2013a, Oliveira *et al*., 2022).

Additionally, it has been proven that sexual reproduction relies on the regulation of specific genes and signaling pathways (Wilson *et al*., 2019, Debuchy *et al*., 2010). For example, the first step

that initiates sexual reproduction in ascomycetes is mate recognition which is facilitated by a pheromone-receptors signaling pathway called the Mitogen-activated protein (MAP) signal transduction pathway (Wilson *et al.*, 2019, Paoletti *et al*., 2007). Ni *et al.* (2011) concluded that the presence of pheromones and receptors in any genome could imply that mating and mate recognition is possible in any given species, even if the sexual cycle is unknown. For example, in species of *Chrysoporthe* pheromones and receptors have been detected from their genomes (Kanzi *et al*., 2019). Moreover, the presence of mating-type genes in the genomes of species of *Chrysoporthe* provided insight regarding the sexual identities of species (Kanzi *et al*., 2019).

## Future prospects

Plantation forestry is of increasing importance worldwide, due to an increase in demand for forest products. Unfortunately, plantation forests are under threat from diseases caused by fungi (Kanyi *et al*., 2005) that reduce forest product quality and quantity. Therefore, it is important to understand how these fungi evolve and adapt to various environmental factors, such as climate and host species. Sequences of fungal genomes provide the opportunity to dissect evolutionary processes, with the goal of reducing or mitigating infections. Species in the genus *Chrysoporthe* are important on the African continent because they affect both commercial and subsistence plantation trees such as *Eucalyptus,* but they also affect native *Syzygium* trees that are culturally important. Thus, the economic significance of fungi in the genus *Chrysoporthe*, their ecological impact, seemingly wide host ranges in the Myrtales, and their ability to cross infect trees makes them important to plantation industries as well as biodiversity and conservation programs in Africa.

*Chrysoporthe zambiensis* and *C. syzygiicola* are canker pathogens of economically important plantations of *Eucalyptus grandis* and *Syzygium guineense* in Zambia (Chungu *et al*., 2010). Unlike their closest relative *C. austroafricana*, asexual spores (conidia) are the predominant source of inoculum of these species. Thus, one can assume that these species reproduce asexually under favorable environmental conditions. One of the first steps to understand a pathogen is to study its mode of reproduction. This allows researchers to understand the generation time and genetic diversity. Therefore, it is important to determine the mode of reproduction and the mating system of these species by identifying and characterizing the mating-type loci of *C. zambiensis* and *C. syzygiicola*. In addition to mating-type genes it should be considered that other factors play

important roles in the initiation and completion of sexual reproduction. This includes the presence and regulation of signal transduction pathways, and genes that are required for the formation of fruiting bodies, mate recognition (mating), and meiosis (Casselton, 2002, Wilson *et al*., 2019, Arnaise *et al.*, 1997). Currently, the genomes of only five isolates from four species of *Chrysoporthe* are publicly available, of which three species are found in Africa. The primary aim for future research is thus to increase the catalog of genome sequences of *Chrysoporthe* species in Africa, to provide a competitive advantage in combating diseases that are caused by these pathogens. We are particularly interested in sequencing the whole genomes of *C. zambiensis* and *C. syzygiicola* to determine important comparative parameters, including determining the mating systems of both species.

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# Chapter 2: Draft genome sequences of *Chrysoporthe syzygiicola* and *Chrysoporthe zambiensis*

## Abstract

In this study, we report on the genomic sequence information of *Chrysoporthe zambiensis* and *C. syzygiicola* that cause stem cankers on *Eucalyptus* spp. and *Syzygium* spp., respectively. The genomes of these two species slightly vary. The genome size and number of protein-coding genes of *C. zambiensis* were 48 317 394 bp and 15 899, respectively. Moreover, the genome size and number of protein-coding genes of *C. syzygiicola* were 42 500 337 bp and 12 328. This study showed that the genome size and number of gene models of *C. zambiensis* are slightly larger than those of *C. syzygiicola*.

## Introduction

*Chrysoporthe* species are phytopathogens of *Eucalyptus* spp., *Tibouchina* spp., and *Syzygium* trees (Heath *et al*., 2006, Gryzenhout *et al.*, 2004) from different regions of the world. These species are the causal agents *Chrysoporthe* canker, stem canker, branch canker, and dieback which can result in the death of juvenile eucalypts (Wingfield, 2003, Wingfield *et al*., 2001). In Zambia, *C. zambiensis* and *C. syzygiicola* are fungal pathogens of *E. grandis* and *S. guineense*, respectively (Chungu *et al*., 2010). Both species cause stem canker on their respective host organisms. Pathogenicity trials have confirmed that these species are a potential threats of commercially grown *Eucalyptus* trees (Chungu *et al*., 2010) and can result in low quality wood products and reduced yield. Although diseases that are caused by *Chrysoporthe* species have been successfully controlled through breeding hybrid *Eucalyptus* trees, these species remain a threat to the economic stability of the forestry industry (Wingfield, 2003).

Fungal pathogens that reside in the genus *Chrysoporthe* can cause serious damage to forest plantations by threatening the survival of trees. Therefore, it is important to understand the genetic components that contributes to their pathogenicity, broad host range (Van der Merwe *et al*., 2013), and their ability to live in different region of the world (Oliveira *et al*., 2021, Heath et al., 2006, Chen *et al*., 2010). The availability of genome sequence data provides an opportunity to identify pathogenicity factors and genes that are essential in the biosynthesis of secondary metabolites (Aylward *et al*., 2017). To date, whole-genome data of only four species of this genus are publicly

available, and includes *Chrysoporthe austroafricana*, *C. puriensis*, *C. cubensis*, and *C. deuterocubensis* (van der Nest *et al*., 2021, Wingfield *et al*., 2015b, Wingfield *et al.*, 2015a). The primary goal for the current study is to sequence and assemble the genomes of *C. zambiensis* and *C. syzygiicola*, to increase the genome catalog of *Chrysoporthe* species. This data will enable comparative genomics studies that will assist in understanding the biology of *Chrysoporthe* species.

## Sequenced strains

*Chrysoporthe zambiensis*: Zambia, Luapula province, Kapweshi: isolated from *Eucalyptus grandis,* 2008, D. Chungu (CMW29930/CBS124502)

*Chrysoporthe syzygiicola*: Zambia, Luapula province, Samfya: isolated from *Syzygium guineense*, 2008, D. Chungu (CMW29940/CBS124488)

## Materials and Methods

*Chrysoporthe zambiensis* (CMW29930) and *Chrysoporthe syzygiicola* (CMW29940) isolates were obtained from the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa. Using phenol-chloroform, gDNA was isolated from 14-day-old mycelium of these isolates that were grown in 2% (w/v) Malt Extract Broth (Biolab, Merck, South Africa) (Steenkamp *et al*., 1999). High molecular weight gDNA was submitted for long read sequencing using Pacific Bioscience Single-Molecule Real-Time [SMRT] protocol at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. Furthermore, FastQC implemented in the Galaxy platform (https://usegalaxy.eu/root) was used to evaluate the quality the raw reads (Jalili *et al*., 2020). In addition, CANU was used to assembled the genomes (Koren *et al*., 2017), and QUAST (Gurevich *et al*., 2013) was used to determine general genomic statistics such as N50, L50, and GC content for the two isolates. BUSCO (Benchmarking Universal Single-Copy Orthologs) (Simão *et al*., 2015) was used to evaluate the completeness of the draft genomes against the "sordariomycetes" database. Lastly, the AUGUSTUS *de-novo* protein-coding software (Stanke *et al*., 2006, Stanke and Morgenstern, 2005) was used to annotate the draft genomes using gene models from *Neurospora crassa* as reference set.

To confirm the identity of *C. zambiensis* (CMW29930) and *C. syzygiicola* (CMW29940), a phylogenomics approach was used. The draft genomes of CMW29930 and CMW29940, along with previously sequenced genomes of *Chrysoporthe*, were subjected to BUSCO analyses. A Python 3.8 command line script was used to parse the BUSCO output files and identify the genes that were complete and shared between all species in the analysis. The translated amino acid sequences were aligned using MUSCLE v. 5 (Edgar, 2021), followed by automatic *in silico* trimming of each alignment using TrimAl v. 1.2 (McGowan *et al.*, 2020). Amino acid alignments were concatenated to form a supermatrix, which was subsequently subjected to maximum-likelihood analysis using IQ-TREE v. 1.6.12 (Nguyen *et al*., 2015). Confidence values in nodes were assessed using 1000 bootstrap replicates.

## Results

Phylogenetic analysis of *C. zambiensis* (CMW29930) and *C. syzygiicola* (CMW29940) using single copy orthologs obtained from BUSCO analyses confirmed the identity of the isolates with 100% bootstrap values (Figure 2.1). A summary of the genome sizes and general genome statistics of *C. zambiensis* and *C. syzygiicola* are provided in Table 2.1. The *Chrysoporthe zambiensis* genome was estimated to be 48 317 394 bp (48.3 Mb), consisting of 211 contigs. The L50 and N50 of the assembled genome were 19 and 691 378 bp, respectively. Moreover, the predicted gene models for *C. zambiensis* were 15 899 and BUSCO predicted 96.2% completeness for this genome. For *C. syzygiicola*, the estimated genome size was 42 500 337 bp (42.5 Mb), comprising of 233 contigs. The general statistics, L50 and N50 of the *C. syzygiicola* draft genome were 21 and 617 420 bp, respectively. The predicted gene models from AUGUSTUS were 12 328, and BUSCO predicted a 95% genome completeness.

When compared to other species of *Chrysoporthe*, the genome size (48.3 Mb) and the predicted gene models (15 899) of *C. zambiensis* were slightly larger than genomes of other *Chrysoporthe* spp. For example, the *C. austroafricana* genome is predicted to encode 13 484 protein coding genes in a genome of 44.6Mb (Wingfield *et al.*, 2015a). The assembled genome has 6 414 contigs with a N50 of 33.52Mb and an L50 of 5 (https://www.ncbi.nlm.nih.gov/). The estimated size of *C. deuterocubensis* genome is 43.9 Mb and the predicted gene models were 13 772. The genome of

*C. deuterocubensis* consists of 6 500 contigs and an N50 value of 4.14Mb and a L50 value of 5 (https://www.ncbi.nlm.nih.gov/) (Wingfield *et al*., 2015b). While the genome size of *C. cubensis* is 42.6 Mb and with 13 121 gene models (Wingfield *et al*., 2015b). This genome consists of 3 326 contigs, and N50 value of 3.38Mb and an L50 value of 5 (https://www.ncbi.nlm.nih.gov/). In addition, the genome size of *C. puriensis* is 44.66Mb and consists of 13 166 gene models. Furthermore the genome of this species has 14 contigs, L50 value of 5 and an N50 value of 4.78Mb (van der Nest *et al*., 2021). Lastly, the estimated genome size, and gene models of *C. syzygiicola* were slightly smaller than that of other *Chrysoporthe* spp. The genome of this species is 42.5 Mb in size and consists of 12 328 gene models with 233 contigs and the N50 of 691.38Kb and L50 of 19. In addition, when the genomes of *Chrysoporthe deuterocubensis*, *Chrysoporthe puriensis*, *Chrysoporthe cubensis*, and *Chrysoporthe austroafricana* were compared, it appears that the completeness of these genomes were 95%, 98%, 94%, and 94% complete, respectively (van der Nest *et al*., 2021, Wingfield *et al*., 2015a, Wingfield *et al*., 2015b).

## Discussion

The availability of fungal genomic data plays important in understanding how organisms live and survive in various habitats. Moreover, genomic data can also be used to target genomic regions that are important in the production of natural products such as secondary metabolites. Furthermore, in order to understand the mode of infection and pathogenicity, genome sequences can be used to provide a glimpse of potential effectors and toxins that pathogens (Mangwanda *et al.*, 2016). There is increasing data on the availability of genomic data. Therefore, variation in genome size and gene content has been observed in distantly and closely related species within different phyla of fungi (Mohanta and Bae, 2015, Aylward *et al*., 2017). Although there is variation in genome size and gene content in the genome of fungal species, there is a link between the gene size, gene content, and pathogenicity of fungal species (Spanu *et al*., 2010, Duplessis *et al.*, 2011).

Based on publicly available fungal genome sequences, the genome size of fungal species generally ranges from 30-40 Mb, while the average genome size in Ascomycota is 36.91 Mb (Mohanta and Bae, 2015, Aylward et al., 2017). However, some fungal pathogens are much larger in size (Mohanta and Bae, 2015). In the current study, the genome size of *Chrysoporthe* species differs slightly. Comparative analysis of the genome size for different genera in the Ascomycota are

diverse and even species within the same genus show some variation at genomic level (Mohanta and Bae, 2015). The variability in genome size of Ascomycota implies that species from different genera and within the same genus are dynamic in nature. The variability in the genome size of fungi is the result of noncoding and repetitive DNA, gene duplication and indels (Mohanta and Bae, 2015, Taylor *et al*., 2017). The importance of the differences in the genome sizes of species of *Chrysoporthe* is unknown. However, the geographic distribution and host preferences of these species might have a significant contribution to the evolution of their genome sizes. The draft genomes of *C. zambiensis* and *C. syzygiicola* that were generated in this study can be used to understand the biology of these pathogens. In addition, these draft genomes can also be used to determine comparative parameters such as mating-type status and their abilities to produce secondary metabolites.

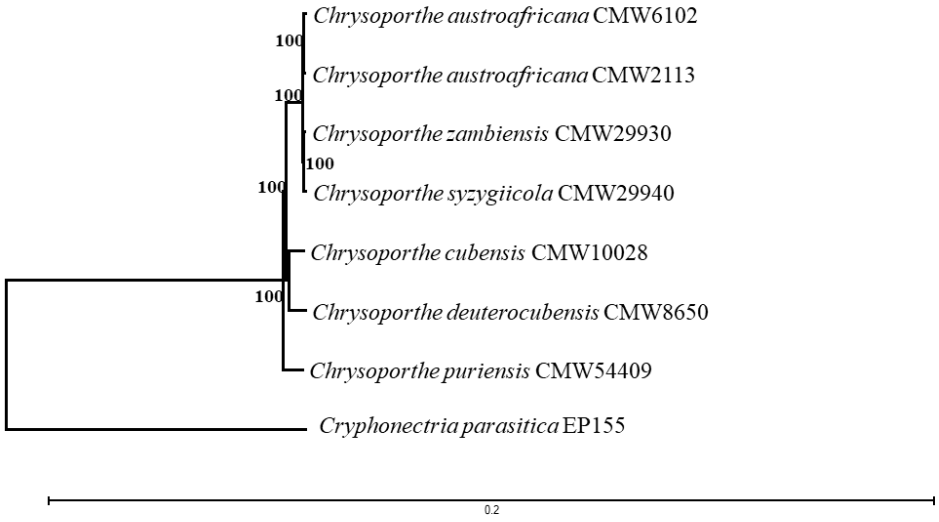

Figure 2.1: A maximum-likelihood tree generated based on the combined BUSCO protein sequences of 3490 shared complete BUSCO genes from genomes of *Chrysoporthe* spp. Percentages at nodes denote bootstrap values (1000 replicates), while *Cryphonectria parasitica* EP155 was used as an outgroup.

Table 2.1: Genomic statistics for *C. zambiensis* and *C. syzygiicola*

| **Assembly metrics** | *C. zambiensis* | *C. syzygiicola* |
|---|---|---|
| Genome size bp | 48 317 394 | 42 500 337 |
| Number of contigs | 211 | 233 |
| GC content % | 56.57 | 55.43 |
| N50 | 691 378 | 617 420 |
| L50 | 19 | 21 |
| Number of Ns per 100 Kbp | 0.00 | 0.00 |
| **BUSCO statistics** | | |
| Overall completeness % | 96.2 | 95 |
| BUSCO complete (C) | 3672 | 3631 |
| Single copy orthologs | 3664 | 3625 |
| Duplicated orthologs | 8 | 6 |
| Fragmented orthologs | 34 | 39 |
| Missing BUSCO orthologs | 111 | 147 |

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genome sequences of *Ceratocystis eucalypticola*, *Chrysoporthe cubensis*, *C. deuterocubensis*, *Davidsoniella virescens*, *Fusarium temperatum*, *Graphilbum fragrans*, *Penicillium nordicum*, and *Thielaviopsis musarum. IMA fungus,* 6**,** 493.

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# Chapter 3

# Homothallism in two species of *Chrysoporthe* from Zambia

## Abstract

*Chrysoporthe syzygiicola* and *C. zambiensis* were first described in Zambia, where cause stem canker on *Syzygium guineense* and *Eucalyptus grandis*, respectively. These diseases are typical of *Chrysoporthe* species which includes many important phytopathogens. The taxonomic descriptions of C. *zambiensis* and *C. syzygiicola* were based on their anamorphic states, as no sexual state is known. The main purpose of this work was to use whole genome sequences to identify and define the mating type (*MAT1*) locus of these two Zambian *Chrysoporthe* species. Unique *MAT1* loci for *Chrysoporthe zambiensis* and *Chrysoporthe syzygiicola* were described, and consists of the *MAT1-1-1*, *MAT1-1-2*, and *MAT1-2-1* gene, but no *MAT1-1-3* gene was present. Both *MAT1-1* (*MAT1-1* and *MAT1-2*) and *MAT1-2* (*MAT1-2-1*) genes were present at the single locus, which suggests that *C. zambiensis* and *C. syzygiicola* have homothallic mating system.

## Introduction

Filamentous ascomycetes can show diverse mating strategies that include sexual and asexual reproduction. Sexual mating strategies are generally categorized as either homothallic or heterothallic. Heterothallic individuals require a genetically suitable partner to complete their sexual cycle (Debuchy and Turgeon, 2006, Dyer *et al*., 2016), while homothallic fungi are regarded as self-fertile and do not require a partner for sexual reproduction (Wilson *et al*., 2015, Coppin *et al*., 1997). Both mating strategies are beneficial to fungal organisms. Homothallism allows fungi to produce self-fertile offspring that can quickly colonize a new niche. Heterothallism and outcrossing are beneficial in circumstances where mating-type partners are plentiful and fitness costs for selfing are considerable (Billiard *et al*., 2012), for example when genetic diversity in a population is selectively advantageous.

In ascomycetes, mating is governed by mating-type genes that are localized within the *MAT1* locus (Wilson *et al*., 2019, Wilson *et al*., 2021). The *MAT1* genes are primary regulator of reproduction, and determine mating compatibility of filamentous ascomycetes (Wilson *et al*., 2019, Casselton, 2002). In heterothallic fungi, the *MAT1* locus consists of either a *MAT1-1* or a *MAT1-2* idiomorph (Coppin *et al*., 1997). The *MAT1-1* idiomorph is defined by the *MAT1-1-1* gene encoding a protein with an alpha-l box, whereas the *MAT1-2* idiomorph is defined by the *MAT1-2-1* gene that encodes a protein with a high-mobility-group (HMG) box domain (Turgeon and Yoder, 2000, Wilken *et*

*al.*, 2017, Dyer *et al*., 2016). In comparison, the *MAT1* locus of homothallic species harbors homologous genes that are associated with both *MAT1-1* and *MAT1-2* in the same genome, and that can either be linked in a single locus or unlinked (Wilson *et al*., 2015, Dyer *et al*., 2016).

The genus *Chrysoporthe* consists of fungal pathogens that cause *Chrysoporthe* canker on *Myrtales* trees, notably economically important forest trees as well as ornamental trees (Nakabonge *et al*., 2006, Myburg *et al*., 2002). Most species of *Chrysoporthe* commonly display sexual fruiting bodies (perithecia) in natural habits (Nakabonge *et al*., 2006, Heath *et al*., 2006, Chen *et al*., 2010, Van Heerden and Wingfield, 2001, Oliveira *et al*., 2021), although sexual reproduction is not frequently observed under laboratory conditions. For other *Chrysoporthe* species such as *C. hodgesiana*, *C. zambiensis*, and *C. syzygiicola*, sexual fruiting bodies are rarely observed even under natural conditions (Gryzenhout *et al*., 2004, Chungu *et al*., 2010). In the absence of observable perithecia the mating-types can provide evidence for the possibility of sexual reproduction in these species.

Mating behavior of *C. austroafricana*, *C. cubensis*, and *C. deuterocubensis* have been characterized. For example, *C. austroafricana* has a heterothallic mating system consists of a *MAT1-1* or a *MAT1-2* idiomorph (Kanzi *et al*., 2019). The *MAT1-1* idiomorph of *C. austroafricana* contains the *MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3* genes. The genetic composition of the *MAT1-1* idiomorph of *C. austroafricana* is similar to that of other heterothallic species in Sordariomycetes (Pöggeler and Kück, 2000, McGuire *et al*., 2001, Duong *et al*., 2013). The *MAT1-2* idiomorph of *C. austroafricana* contains the *MAT1-2-1* gene, but some irregularities were observed. The *MAT1-2* idiomorph of this species consists of truncated *MAT1-1-1* and *MAT1-1-2* genes that are usually associated with the *MAT1-1* idiomorph (Kanzi *et al*., 2019). Additionally, the *MAT1* loci of *C. cubensis* and *C. deuterocubensis* were typical for homothallism (Kanzi *et al*., 2019).

The study conducted by Kanzi *et al*. (2019) was important in determining the mating system of three *Chrysoporthe* species that occur in Africa. However, there is no genetic information regarding sexual reproduction of other *Chrysoporthe* species. Population studies have attempted to infer mating systems in some *Chrysoporthe* by considering genetic diversity. An example is *Chrysoporthe puriensis*, for which microsatellite markers were used to reveal high level of genetic diversity in the Brazilian population of this species (Oliveira *et al*., 2022). Such high levels of

diversity might be an indicator of recombination during heterothallic mating, indicating the presence of cryptic sex.

The mode of reproduction and the genetic basis of sexual reproduction in *C. zambiensis* and *C. syzygiicola*, both of which are African species, are unknown. Therefore, the aim of this study was to identify and characterize the mating-type genes and infer the mating systems of these species using whole-genome sequences. Phylogenies were also constructed to investigate any conflicts that might exist between the *MAT1* genes and the species phylogeny. The structure of the *MAT1* loci of *C. zambiensis* and *C. syzygiicola* were also compared with other *Chrysoporthe* species for which genome sequences were previously published.

## Materials and Methods

### Mating-type genes and structure of the mating type loci of *C. syzygiicola* (CMW29940) and *C. zambiensis* (CMW299300)

Draft genomes of *C. syzygiicola* and *C. zambiensis* that were sequences in Chapter 2 were used to characterize and determine their mating type loci of these two species. The publicly available gene models for a well-defined mating-type locus of *Cryphonectria* (*Cryphonectriaceae*) was used to identify the *MAT1* locus of *Chrysoporthe* species. The protein sequences for the *MAT1* gene models of *Cry. parasitica*, namely *MAT1-1-1* (AAK83346.1), *MAT1-1-2* (AAK83345.1), *MAT1-1-3* (AAK83344.1), and *MAT1-2-1* (AAK83343.1) were retrieved from the National Center of Biotechnology Information (NCBI) GenBank database using their accession numbers. These sequences were used as query sequences against contigs of the draft genomes of CMW29930 and CMW29940. tBLASTn searches (Gertz *et al*., 2006, Altschul *et al*., 1990) were performed using CLC Main Workbench v.20.0 (CLC Bio, Aarhus, Denmark) to search for homologs of the *Cry. parasitica* mating-type genes in the *Chrysoporthe* genomes. In addition, tBLASTn searches were also used to identify genes normally associated with the fungal *MAT1* locus (Wilken *et al*., 2017), which includes the *APN2* (NCBI Accession Number: VM1G_08163), *COX6A* (NCBI Accession Number: VM1G_08162), and *APC5* genes (Yin *et al*., 2017). Only sequences with at least 50% query coverage and contigs that produced matches with an E-value $\leq 0.01$ were considered as

possible homologs of the *MAT1* genes or genes that are associated with the flanking regions of the *MAT1* locus.

To annotate the contigs that putatively contain *MAT1* genes and its flanking genes, contigs were subjected to *de novo* gene prediction using the web-based AUGUSTUS Gene Prediction Software (Stanke and Morgenstern, 2005, Stanke *et al.*, 2006). The gene models from *Neurospora crassa* were used as references for AUGUSTUS Gene Prediction. The GFF output file produced was used to annotate the contigs that contained these genes using the "annotate with GFF/GTF/GVF file" tool that is implemented in CLC Main Workbench v.20.0. BLASTp with default parameters were used to functionally characterize the predicted protein sequences of the putative *MAT1* genes, as well as genes associated with the *MAT1* locus at the NCBI GenBank database. In addition, the conserved domains that are associated with the mating-type genes were confirmed using the protein database InterPro (https://www.ebi.ac.uk/interpro/search/sequence/) (Jones *et al*., 2014, Finn *et al.*, 2017) and Pfam (https://pfam.xfam.org/) (El-Gebali *et al*., 2019).

To understand the structural differences and similarities of the *MAT1* loci of *Chrysoporthe*, the structure of the *MAT1* loci of previously published species *Chrysoporthe* (Kanzi *et al*., 2019) were reconstructed from available complete genome sequences these species and mapped onto the tree.

## Phylogenetic analysis of mating-type genes

A maximum-likelihood phylogeny of species of *Chrysoporthe* was constructed from protein sequences of the mating-type genes using the IQ-TREE webserver v.1.6.12 (Nguyen *et al*., 2015), using the built-in selection of the best evolutionary model. These analyses included mating-type gene sequences of the pathogenic filamentous ascomycetes *C. puriensis* (Oliveira *et al.*, 2021, van der Nest *et al*., 2021), *C. austroafricana* (Wingfield *et al*., 2015a), *C. cubensis*, and *C. deuterocubensis* (Wingfield *et al*., 2015b), with *Cry. parasitica* (McGuire *et al*., 2001) as an outgroup taxon. MAFFT v7.1 (Katoh and Standley, 2013) was used to perform multiple sequence alignments of mating-type genes and the combined dataset was visualized in CLC Main Workbench v20.0. Poorly aligned regions from multiple sequence alignment were trimmed. Furthermore, a species tree was constructed for comparison of tree topologies. The genome sequences of previously sequenced species of *Chrysoporthe*, including *C. syzygiicola* and *C.*

*zambiensis* were subjected to BUSCO analyses with the "sordariomycetes" gene models. A Python 3.8 command line script was used to parse the BUSCO output files and identify the complete single-copy orthologs shared between species. The translated amino acid sequences that are encoded by the identified single-copy orthologs were aligned using MUSCLE v. 5 (Edgar, 2021), followed by automatic *in silico* trimming of each alignment using TrimAl v. 1.2 (McGowan *et al*., 2020). The individual amino acid alignments were concatenated to form a supermatrix, which was subsequently subjected to maximum-likelihood analysis using IQ-TREE v. 1.6.12 (Nguyen *et al.*, 2015). Confidence values in nodes were assessed using 1000 bootstrap replicates.

To determine whether the observed mating-type genes from the genome of *C. syzygiicola* and *C. zambiensis* were conserved, the inferred amino acids sequences of *C. syzygiicola* and *C. zambiensis*, including those of *C. puriensis*, *C. cubensis*, *C. deuterocubensis*, and *Cry. parasitica* were aligned using MAFFT and compared with each other. To compare the corresponding amino acid sequence between these isolates the "create pairwise comparison" tool that is implemented in CLC Genomics Workbench v.20.0 was used to determine sequence similarities.

## Results

### Mating-type genes and structure of the mating-type loci of *C. syzygiicola* and *C. zambiensis*

The tBLASTn search against the whole-genome assembly of *C. syzygiicola* revealed the presence of *MAT1* genes and genes that are associated with the flanking regions of the *MAT1* locus on the same contig. The *MAT1-1-1*, *MAT1-1-2*, *MAT1-2-1*, DNA Lyase (*APN2),* Anaphase Promoting Complex (*APC5),* and Cytochrome C Oxidase subunit 6A (*COX6A*) genes were identified on contig ctg-000040F of the draft genome. AUGUSTUS predicted an additional five genes positioned between the *MAT1* genes, placing these within the mating-type locus of *C. syzygiicola* (Figure 1). These genes located within the *MAT1* locus did not show any sequence similarities to any of the proteins present in the NCBI GenBank database and no domains were detected from protein databases such as InterPro and Pfam.

tBLASTn searches against the draft genome assembly of *C. zambiensis* revealed the presence of the *MAT1-1-1*, *MAT1-2-1,* and *APN2* genes on a single contig (ctg000166F), while the *MAT1-1-2*

gene was identified on a separate contig (ctg000072F). The genes associated with the flanking regions of the mating-type loci of fungal species (Wilken *et al.*, 2017, Li *et al*., 2013) were not linked to the mating-type locus of *C. zambiensis*. In addition to the *MAT1* genes, seven genes with no known functions were present within the *MAT1* locus of *C. zambiensis* and none of these genes showed any amino acid sequence similarities with proteins from the GenBank database and no domains were detected from the protein database such as InterPro and Pfam (Figure 3.1).

In the mating type locus of *C. syzygiicola,* the putative *MAT1-1-1* gene is 1 098 bp long (CDS 1 096), and codes for 387 amino acids with no intron. In addition, this *MAT1-1-1* gene encodes for a protein with the alpha_box domain (IPR006856) (Turgeon and Yoder, 2000, Wilken *et al*., 2017). The putative *MAT1-1-2* genes is 1 279 bp long (CDS is 1 119 bp) in size and it contained two introns (90bp and 67 bp). The predicted *MAT1-1-2* gene encoded 373 amino acids, and no conserved motifs were detected against the Interpro and Pfam protein domain databases. The putative *MAT1-2-1* gene is 1 008 bp long (CDS 879 bp), coding for 293 amino acids, and it contained two introns (60bp and 69bp). The expected HMG box domain (IPR009071) that characterize the *MAT1-2-1* gene was detected in both the Pfam and InterPro protein databases. The size of the mating-type locus is 25.25 kb. Moreover, based on the observed genetic composition of the mating-type locus of *C. syzygiicola*, this species has a homothallic mating system. Therefore, it is self-fertile and can complete its life cycle in the absence of a mating-type partner.

In the mating-type locus of *C. zambiensis*, the predicted *MAT1-1-1* gene is 1 161 bp long (CDS 1 161bp) and codes for 387 amino acids that harbor the characterizing domain, namely the MATalpha_HMG box domain (IPR006856), and with introns detected in this gene. The putative *MAT1-1-2* gene was 1 280bp long, with a CDS of 1 119 bp long and two introns of 71 bp and 90 bp. The predicted *MAT1-1-2* gene codes for 373 amino acids, and no conserved motifs were detected against Interpro and Pfam protein domain databases. The predicted *MAT1-2-1* gene was 1 008 bp long with a CDS of 879 bp and two introns (60 bp and 69 bp). The *MAT1-2-1* gene codes 293 amino acids and the expected HMG box domain (IPR009071) that characterizes this gene was detected in both Pfam and InterPro protein databases. The size of the *MAT1* locus of *C. zambiensis* was 28.97 kb. Based on the genetic content of the mating-type locus of this species, *C. zambiensis* employs homothallic mating system.

The structure of the mating-type loci of *C. syzygiicola* and *C. zambiensis* were compared with the structure of the mating-type loci of other *Chrysoporthe* species using a species tree (Figure 3.2). Based on the structural comparison of the *MAT1* loci of these species, the genetic content of the *MAT1* loci of *C. syzygiicola* and *C. zambiensis* differed slightly from the genetic content of other *Chrysoporthe* spp. For example, the *MAT1* loci of homothallic *Chrysoporthe* species consist of genes that are associated with both the *MAT1-1* and *MAT1-2* idiomorphs, including *MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3*, and *MAT1-2-1* genes. The *MAT1* loci of *C. zambiensis* and *C. syzygiicola* contain gene sequences for *MAT1-1-1*, *MAT1-1-2*, and *MAT1-2-1* that are homologous to *MAT1-1* and *MAT1-2* idiomorphs, but the *MAT1-1-3* gene is absent in the mating-type loci of both species. In addition, genes that are associated with the flanking regions of the mating-type locus of Pezizomycotina, such as *COX6A* and *APC5* were absent from the mating-type locus of *C. zambiensis*. The structure of the mating-type loci of *Chrysoporthe* species is thus unique to the *MAT1* loci of filamentous ascomycetes.

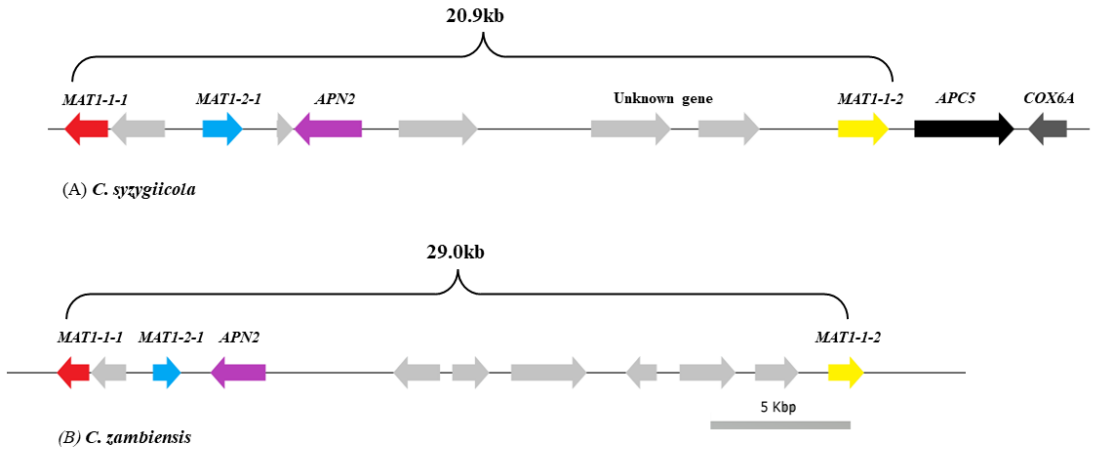



Figure 3.1: An illustration of the mating-type loci of (A) *C. syzygiicola* and (B) *C. zambiensis*. The mating-type loci of these species are drawn to scale. The mating-type genes are color-coded and the genes that are colored in light gray are genes with unknown function or no known sequence similarities when compared with genes from NCBI.

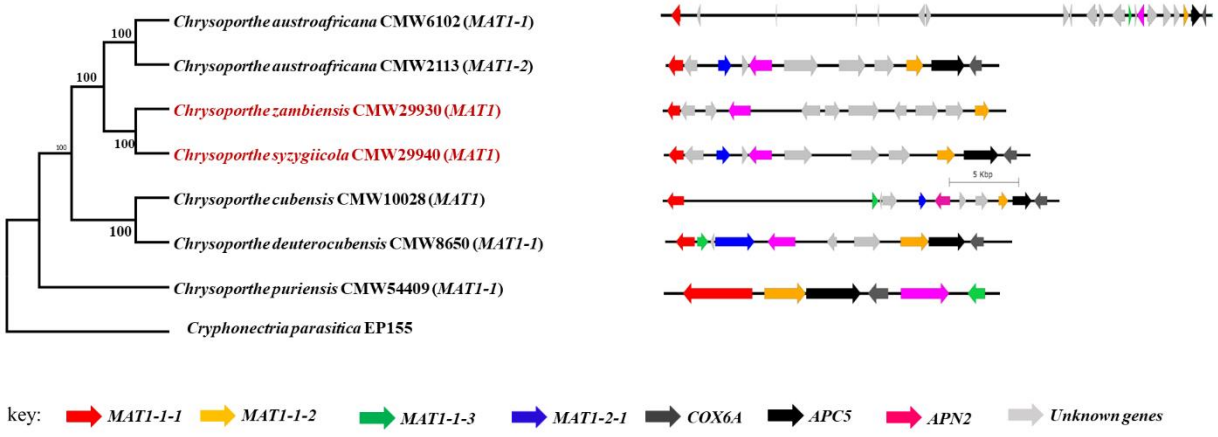



Figure 3.2: Structural comparison of the *MAT1* loci of *Chrysoporthe* spp. *Cryphonectria parasitica* was used as an outgroup taxon. Species that are highlighted in red are those that are under

investigation. *MAT1-1* and *MAT1-2* represent the idiomorphs and *MAT1* represents homothallic the *MAT1* locus.

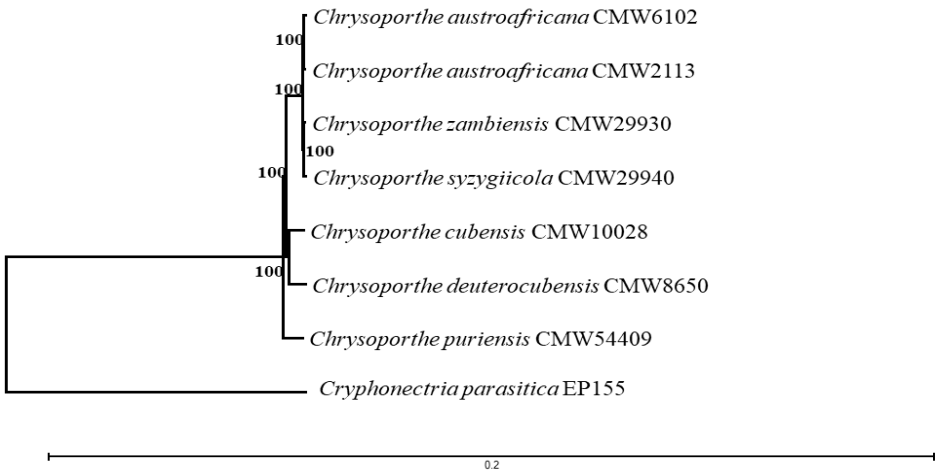
## Phylogenetic analysis of the mating-type genes

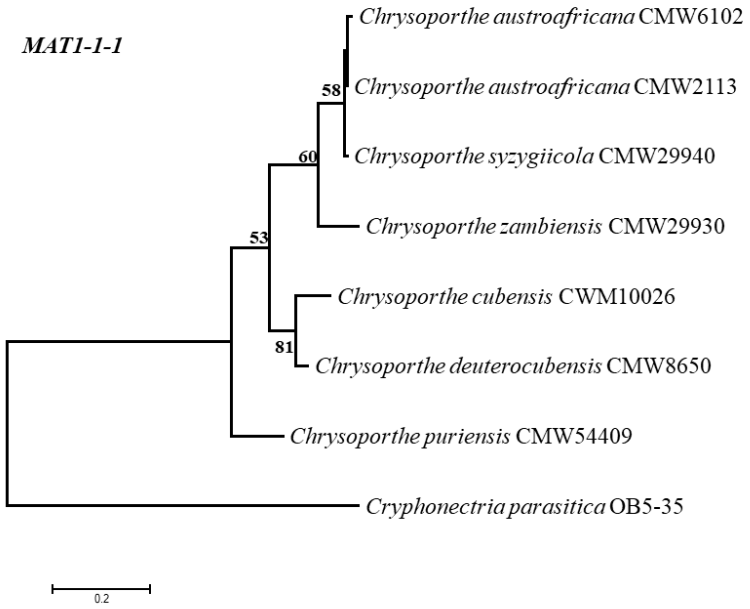
Based on pairwise sequence comparison, the amino acid identity of the predicted *C. syzygiicola MAT1-1-1* gene was 91.29%, 97.51%, 98.76, 84.65%, 87.55 %, and 84.23 % when compared with the *MAT1-1-1* proteins of *C. zambiensis*, *C. austroafricana* (CMW6102), *C. austroafricana* (CMW2113), *C. cubensis*, *C. deuterocubensis* and *C. puriensis*, respectively (supplementary table 3.1). The *MAT1-1-2* protein of *C. syzygiicola* shared 100% sequence identity with *MAT1-1-2* protein sequence of both isolates of *C. austroafricana* and 98.11%, 97.57%, 96.76%, and 95.41% identity with *MAT1-1-2* protein sequence of *C. zambiensis*, *C. cubensis*, *C. deuterocubensis*, and *C. puriensis*, respectively. The predicted *MAT1-2-1* protein of *C. syzygiicola* shared sequence identity with species of *Chrysoporthe* that ranged from 96% to 99.62% (Supplementary table 3.1).

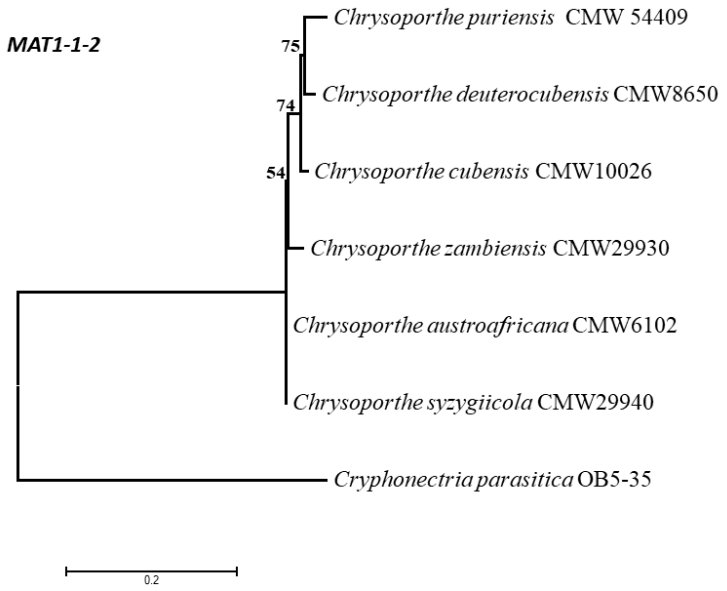
Pairwise comparison of the predicted mating-type genes of *C. zambiensis* were done against those of *Chrysoporthe* species. The *MAT1-1-1* protein of *C. zambiensis* shared sequence identity ranging from 83% to 91.29% (Supplementary Table 3.2). The predicted *MAT1-2-1* protein sequence of *C. zambiensis* shared 98.11% identity with the *MAT1-1-2* protein of both isolates of *C. austroafricana* (CMW6102 and CMW2113) and *C. syzygiicola*. While the predicted *MAT1-1-2* protein sequence of *C. zambiensis* shared 96.22%, 95.41% and 94.05% identity with *C. cubensis*, *C. deuterocubensis*, and *C. puriensis* respectively. For comparison of the *MAT1-2-1* protein sequence, *C. austroafricana* (CMW2113) and *C. cubensis* shared a percentage identity of 97.48% with *C. zambiensis*, while a 94.6 % and 97.12% identity were shared with *C. deuterocubensis* and *C. syzygiicola*, respectively. When protein sequence of *MAT1* genes of *C. zambiensis* and *C. syzygiicola* were compared with *Cry. parasitica* the sequence identity was lower (Supplementary Tables 3.1 and 3.2).

The generated maximum likelihood phylogeny for the putative *MAT1* genes *MAT1-1-1*, *MAT1-1-2*, and *MAT1-2-1* grouped *C. syzygiicola* and *C. zambiensis* under different clades. In the maximum-likelihood phylogeny for the *MAT1-1-1* gene tree, *C. zambiensis* and *C. syzygiicola* are grouped under the same clade as *C. austroafricana*. For the *MAT1-2-1* phylogeny, *C. zambiensis*

grouped under the same clade as *C. cubensis* and *C. deuterocubensis.* Lastly, for the *MAT1-2-1* phylogeny *C. zambiensis* grouped in the same clade as *C. cubensis* and *C. deuterocubensis.* In the *MAT1-1-1* gene phylogeny, *C. zambiensis* and *C. syzygiicola* grouped in the same clade as *C. austroafricana* which was moderately supported by a 60% bootstrap value. In addition, the grouping of *C. zambiensis* in the same clade as *C. deuterocubensis* and *C. cubensis* in the *MAT1-2-1* gene tree had a strong bootstrap support of 76% (Figure 3.3). Overall *Chrysoporthe* species grouped under the same clade, thus indicating that the *MAT1* genes evolved from a common ancestor. However, in the constructed phylogenetic trees (Figure 3.3), the species of interest, namely *C. zambiensis* and *C. syzygiicola* did not group as predicted by the reference phylogenetic tree. Thus, the exact evolutionary relationship of species of *Chrysoporthe* based on the mating-type genes could not be determined. In addition, the constructed species tree (reference tree) was incongruent with the gene trees. Moreover, species that are closely related to each other i.e., *C. austroafricana, C. syzygiicola*, and *C. zambiensis* consist of different mating systems.


Chrysoporthe austroafricana CMW6102
Chrysoporthe austroafricana CMW2113
Chrysoporthe zambiensis CMW29930
Chrysoporthe syzygiicola CMW29940
Chrysoporthe cubensis CMW10028
Chrysoporthe deuterocubensis CMW8650
Chrysoporthe puriensis CMW54409
Cryphonectria parasitica EP155
100
100
100
100
100
0.2


MAT1-1-1
Chrysoporthe austroafricana CMW6102
Chrysoporthe austroafricana CMW2113
Chrysoporthe syzygiicola CMW29940
Chrysoporthe zambiensis CMW29930
Chrysoporthe cubensis CWM10026
Chrysoporthe deuterocubensis CMW8650
Chrysoporthe puriensis CMW54409
Cryphonectria parasitica OB5-35
58
60
53
81
0.2


MAT1-1-2
75
74
54
Chrysoporthe puriensis CMW 54409
Chrysoporthe deuterocubensis CMW8650
Chrysoporthe cubensis CMW10026
Chrysoporthe zambiensis CMW29930
Chrysoporthe austroafricana CMW6102
Chrysoporthe syzygiicola CMW29940
Cryphonectria parasitica OB5-35
0.2

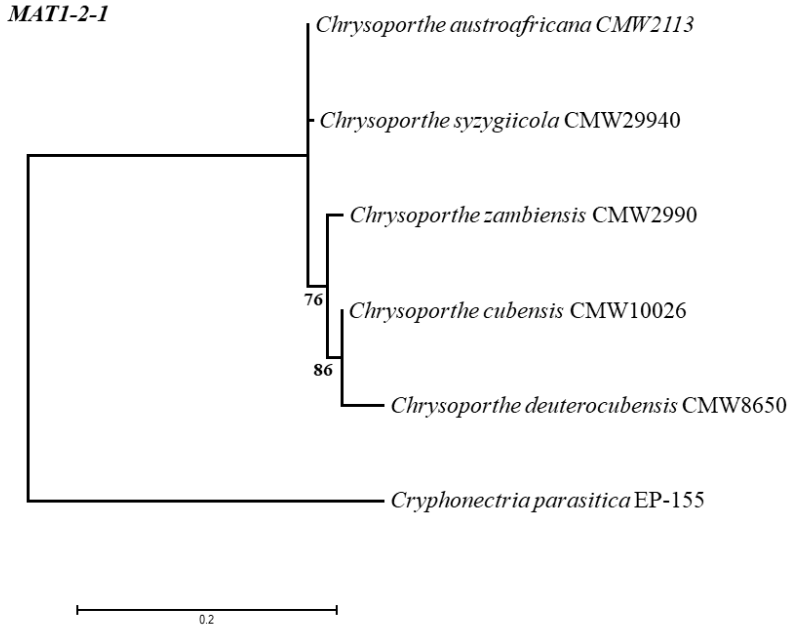

MAT1-2-1
Chrysoporthe austroafricana CMW2113
Chrysoporthe syzygiicola CMW29940
Chrysoporthe zambiensis CMW2990
76
Chrysoporthe cubensis CMW10026
86
Chrysoporthe deuterocubensis CMW8650
Cryphonectria parasitica EP-155
0.2

Figure 3.3: Maximum-likelihood phylogenetic trees of species of *Chrysoporthe* using mating-type genes and *Cryphonectria parasitica* was used as an outgroup taxon. The CDS for *MAT1-1-1* had 241 characters, *MAT1-1-2* had 370 characters, and *MAT1-2-1* had 278 characters. For comparison of tree topologies, a Maximum-likelihood phylogeny was generated using the combined BUSCO amino acid sequences of 3490 shared complete BUSCO genes from genomes of *Chrysoporthe* spp. Percentages at nodes denote bootstrap vales (1000 replicates), while *Cryphonectria parasitica* EP155 was used as an outgroup. IQ tree webserver (Nguyen *et al*., 2015) was used to draw the phylogenies using the built-in selection of the best evolutionary model.

## Discussion

The availability of genome sequences for *C. syzygiicola* and *C. zambiensis* has allowed for the identification and characterization of the mating-type loci of these species. The availability of this data in turn provided insight on the mating system that is employed by these species. This study indicated that the *MAT1* loci of *C. zambiensis* and *C. syzygiicola* comprised of genes that are characteristic of homothallic mating systems, where *MAT1-1* and *MAT1-2* genes co-occur in the same genome (Wilson *et al*., 2015, Dyer *et al*., 2016). The *MAT1* loci of both species harbored the *MAT1-1-1*, *MAT1-1-2*, and *MAT1-2-1* genes. Apart from the mating-type genes, the *MAT1* loci of filamentous ascomycetes are usually associated with genes such as *APN2, COX6A,* and *APC5* that are occur in the flanking regions (Wilken *et al.*, 2017, Bihon *et al*., 2014, Nagel *et al.*, 2018). These genes were associated with the *MAT1* locus of *C. syzygiicola*, but *COX6A* and *APC5* were not associated with the *MAT1* locus of *C. zambiensis*. The genetic contents of the *MAT1* loci of *C. zambiensis* and *C. syzygiicola* slightly differ from the *MAT1* loci of other *Chrysoporthe* species.

Generally, the mating-type locus of homothallic and heterothallic Sordariomycetes harbor the *MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3*, and *MAT1-2-1* genes (Debuchy and Turgeon, 2006, Kück and Böhm, 2013, Kück *et al*., 2009). However, the *MAT1-1-3* gene was absent in the *MAT1* locus of *C. syzygiicola* and *C. zambiensis*. Although the latter gene is frequently found in the *MAT1* locus of *Diaporthales* species and other fungi (Debuchy and Turgeon, 2006, Kanematsu *et al*., 2007, Kanzi *et al*., 2019, Martin *et al.*, 2011, McGuire *et al*., 2001), the *MAT1-1-3* gene can also be absent in some heterothallic (Yokoyama *et al.*, 2003, Yokoyama *et al*., 2005) and homothallic species (Wilken *et al*., 2014, Li *et al*., 2020). To date, the significance of the presence or absence

of the *MAT1-1-3* gene in the *MAT1* loci of species of *Chrysoporthe* is not known. However, in other fungi, specifically *Villosiclava virens*, the *MAT1-1-3* gene is essential for pathogenicity, sexual development, and asexual reproduction (Yong *et al*., 2020).

The predicted mating-type genes in the *MAT1* loci of *C. zambiensis* and *C. syzygiicola* have high sequence identity when compared with the *MAT1* genes of other *Chrysoporthe* species (Supplementary Tables 3.1 and 3.2). The sizes of the putative *MAT1-1-1* genes of *Chrysoporthe* species from Zambia were similar and the alpha-1 domain that characterizes the *MAT1-1-1* gene was also present. However, no intron was observed in the *MAT1-1-1* gene of *C. syzygiicola* and *C. zambiensis*, a trade that seem unique among the *Chrysoporthe* species as well as *Diaporthales* in general. The size of the putative *MAT1-1-1* and *MAT1-1-2* genes of *C. syzygiicola* and *C. zambiensis* were slightly smaller in size, in comparison to the size of the *MAT1-1-1* and *MAT1-1-2* genes of *C. austroafricana*, *C. cubensis*, and *C. deuterocubensis* (Kanzi *et al*., 2019). In addition, no conserved domain was observed in the *MAT1-1-2* gene of *C. syzygiicola* and *C. zambiensis*, a trade that seem to be unique in these species. The gene sizes and intron sizes of the *MAT1-2-1* genes of *C. syzygiicola* and *C. zambiensis* were conserved and like that of other *Chrysoporthe* species (Kanzi *et al*., 2019). In addition, the presence of unknown genes within the mating-type loci of *C. zambiensis* and *C. syzygiicola* seems to be a common feature that occurs in the *MAT1* loci of *Chrysoporthe* species (Figure 3.3)(Kanzi *et al*., 2019).

The mating-type loci of *Chrysoporthe* species are very distinct. For example, the size of the mating-type locus of *C. zambiensis* (29.0 kb) is larger than the size of the *MAT1* locus of *C. syzygiicola* (20.9 kb) and *C. deuterocubensis* (18.2 kb). Similarly, the size of the *MAT1* locus of *C. cubensis* (45.0 kb) and the *MAT1-1* (133.8 kb) and *MAT1-2* (19.4 kb) idiomorphs of *C. austroafricana* were slightly larger when compared to other *Chrysoporthe* spp. The mating-type loci of *Chrysoporthe* species are consistently larger than the expected *MAT1* loci size of most filamentous ascomycetes that have been studied. The size variations of the *MAT1* loci of *Chrysoporthe* spp. might be attributable to the presence of varying numbers of genes of unknown functions and the presence of transposable elements, observed in other *Chrysoporthe* spp. (Kanzi *et al*., 2019). The presence of transposable elements in the *MAT1* loci of fungal species is associated with the expansion of the *MAT1* locus, introducing genetic variation, and suppressing

recombination in this region if sexual reproduction is possible (Li *et al.*, 2013, Hartmann *et al.*, 2021)

Compared to a typical Sordariomycetes *MAT1* locus, the structure of the *MAT1* locus of *Chrysoporthe* species is unique (Wilken *et al*., 2017, Debuchy and Turgeon, 2006, Dyer *et al*., 2016). For example, a gene that is associated with the flanking regions of *MAT1* loci such as *APN2* (AP endonuclease) (Fraser *et al*., 2007, Bihon *et al*., 2014, Li *et al*., 2013, Nagel *et al*., 2018) is present within the *MAT1* loci of all *Chrysoporthe* spp. studied thus far. Gene organization in the *MAT1* loci of *C. zambiensis* and *C. syzygiicola* is similar to what has been observed in other Chrysoporthe species (Kanzi *et al*., 2019). However, *COX6A* and *APC5* genes are in the *MAT1* locus of *C. zambiensis*, instead of flanking it, when compared to the *MAT1* loci of other *Chrysoporthe* species (Figure 3.2). Therefore, the structural configurations of the *MAT1* loci in the genus *Chrysoporthe* differ from each other and from that of other ascomycetes.

In many filamentous ascomycetes, the structural configuration of the *MAT1* locus is *SLA2-MAT1-APN2/COX3A/APC5* (Wilken *et al.*, 2017) and the presence of these genes adjacent to the *MAT1* locus plays a crucial role in the characterization of the *MAT1* locus. However, in some species of Diaporthales, the structural configuration of the *MAT1* locus is distinct from other filamentous ascomycetes. For example, in the *MAT1* locus of *Valsa mali*, *APN2* and *COX13* genes are located within the locus (Yin *et al.*, 2017). Additionally, the *APN2* gene is located within the *MAT1* loci of *Chrysoporthe* species (Figure 3.1). The significance of the rearrangements of the *MAT1* loci of these species are unknown. However, rearrangement of these genes in the *MAT1* locus might induce beneficial genetic changes that can be selected for (Hartmann *et al*., 2021), and thus might play a crucial role in the evolution of the *MAT1* locus of Diaporthales.

In this study, the mating systems of *C. syzygiicola* and *C. zambiensis* were determined as homothallic, making each individual self-fertile and capable of completing the life cycle in the absence of a mating partner (Wilson *et al*., 2015). Based on the current analyses, there was no evidence of another mode of homothallism such as mating-type switching, pseudohomothallism, or unidirectional mating in *Chrysoporthe* species. The characterization of the mating-type genes in the two species from Zambia indicate that they can reproduce sexually. However, the absence of perithecia in natural habitats could indicate that cryptic sex is taking place. In some fungal pathogens, the process of sexual reproduction and the presence of mating-type genes in an

organism is associated with virulence (Heitman *et al*., 2014, Yong *et al*., 2020). It is unknown whether *C. syzygiicola* and *C. zambiensis* reproduce sexually under natural habitats because no sexual state has been observed. Therefore, functional studies for the mating-type genes of *Chrysoporthe* species might be useful to understand the role these genes in virulence, as well as the significance of the absent *MAT1-1-3* gene in the genomes of *C. zambiensis* and *C. syzygiicola*.

## Conclusion

Mating-type loci have been structurally characterized in a number of filamentous fungi. However only few investigations have been conducted to determine the function of the genes that are encoded in the *MAT1*. In most cases, *MAT1* genes have been knocked out to determine functionality of the mating-type genes in fungi (Yong *et al*., 2020). While in other species, mutants of mating-type genes had no effect on the sexual cycle or pathogenicity of the species (Wilson *et al.*, 2021, Kück and Böhm, 2013, Klix *et al*., 2010). In this study, the structure of the mating-type loci of two asexual species of *Chrysoporthe* was characterized, thus providing these species with their sexual identities. For future research it will be essential to determine the function of mating-type genes in *Chrysoporthe* by using homologous recombination to substitute the *MAT1-1* locus by *MAT1-2* in heterothallic strains of *Chrysoporthe*. Therefore, functional genomics will be necessary to discover essential gene function or identify suppressor or activators of gene expression.

Supplementary Table 3.1: Sequence comparison of the coding sequences of the mating-type genes of *C. syzygiicola*, other species of *Chrysoporthe* and *Cry. parasitica*

| Species/ Isolates number and NCBI accession number | Genes | Percentage identity (%) |
|---|---|---|
| | *MAT1-1-1* | |
| | | |
| *C. zambiensis* CMW29930 | | 91.29 |
| *C. austroafricana* CMW6102 | | 97.51 |
| *C. austroafricana* CMW2113 | | 98.76 |
| *C. cubensis* CMW10028 | | 84.65 |
| *C. deuterocubensis* CMW8650 | | 87.55 |
| *C. puriensis* CMW54409 | | 84.23 |
| *Cry. parasitica* AAK83346.1 | | 46.47 |
| | *MAT1-1-2* | |
| | | |
| *C. zambiensis* CMW29930 | | 98.11 |
| *C. austroafricana* CMW6102 | | 100 |
| *C. austroafricana* CMW2113 | | 100 |
| *C. cubensis* CMW10028 | | 97.57 |
| *C. deuterocubensis* CMW8650 | | 96.76 |
| *C. puriensis* CMW54409 | | 95.41 |
| *Cry. parasitica* AAK83345.1 | | 52.15 |
| | *MAT1-2-1* | |
| | | |
| *C. zambiensis* CMW29930 | | 97.12 |
| *C. austroafricana* CMW6102 | | Absent |
| *C. austroafricana* CMW2113 | | 99.64 |
| *C. cubensis* CMW10028 | | 97.12 |
| *C. deuterocubensis* CMW8650 | | 94.24 |
| *C. puriensis* CMW54409 | | Absent |
| *Cry. parasitica* AAK83343.1 | | 62.23 |

Supplementary Table 3.2: Sequence comparison of the coding sequences of the mating-type genes of *C. zambiensis*, other species of *Chrysoporthe* and *Cry. parasitica*

| Species/ Isolate number and/ NCBI accession number | **Genes** | **Percentage identity (%)** |
|---|---|---|
| | *MAT1-1-1* | |
| | | |
| *C. syzygiicola* CMW29940 | | 91.29 |
| *C. austroafricana* CMW6012 | | 92.57 |
| *C. austroafricana* CMW2113 | | 84.57 |
| *C. cubensis* CMW10028 | | 87.55 |
| *C. deuterocubensis* CMW8650 | | 83.55 |
| *C. puriensis* CMW54409 | | 83.82 |
| *Cry. parasitica* AAK83346.1 | | 45.23 |
| | *MAT1-1-2* | |
| | | |
| *C. syzygiicola* CMW29940 | | 98.11 |
| *C. austroafricana* CMW6012 | | 98.11 |
| *C. austroafricana* CMW2113 | | 98.11 |
| *C. cubensis* CMW10028 | | 96.22 |
| *C. deuterocubensis* CMW8650 | | 95.41 |
| *C. puriensis* CMW54409 | | 94.05 |
| *Cry. parasitica* AAK83346.1 | | 51.61 |
| | *MAT1-2-1* | |
| | | |
| *C. syzygiicola* CMW29940 | | 97.12 |
| *C. austroafricana* CMW6012 | | Absent |
| *C. austroafricana* CMW2113 | | 97.48 |
| *C. cubensis* CMW10028 | | 97.48 |
| *C. deuterocubensis* CMW8650 | | 94.6 |
| *C. puriensis* CMW54409 | | Absent |
| *Cry. parasitica* AAK83346.1 | | 61.87 |

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# Summary

The genus *Chrysoporthe* resides in the family Cryphonectriaceae in the order Diaporthales. Currently, the genus *Chrysoporthe* accommodates nine species that have been described thus far. Species that reside in this genus are pathogens of forest plantations and ornamental trees in the order Myrtales. These species are primary pathogens of commercially grown trees such as *Tibouchina* spp., *Syzygium* spp., and *Eucalyptus* spp. The wide host distribution of these species makes them high-risk pathogens that reduce yield and quality of forest products. The diseases that are caused by these species are generally known as *Chrysoporthe* canker, stem canker, and die-back which results in swollen bark, cracking of the bark, and in some cases results in the death of young *Eucalyptus* spp. Several previous studies focused on studying the reproductive biology and genes that control reproduction of these pathogens, in order to understand their ability to adapt to various environmental factors. It is well-known that sexual reproduction is one of the common factors that play a role in generating genetic variation. Sexual reproduction is controlled by mating-type genes that are located within the mating-type locus. The main aim of this research project was to characterize the mating-type genes of two *Chrysoporthe* species from Zambia, namely *C. zambiensis* and *C. syzygiicola*.

To characterize these genes, the draft genome sequences of both species were produced, and the respective genes were identified and characterized. Based on the analysis of this study, mating-type genes such as *MAT1-1-1*, *MAT1-1-2*, and *MAT1-2-1* were identified in the mating-type locus of both species. However, the *MAT1-1-3* gene that is commonly present in the mating-type loci of *Chrysoporthe* species is absent. In addition, the presence of these genes in the genomes of both species implies that both species of *Chrysoporthe* from Zambia have homothallic mating systems. Availability of genome sequences of *Chrysoporthe* species played a crucial role in determining the mating system of these species. In addition, based on the presented data the genome size *C. zambiensis* and *C. syzygiicola* slightly differs from genome size of other publicly available *Chrysoporthe* species. Many pathogenic fungi have genes encoding sexual machinery in their genomes, but no known sexual cycle has been observed in natural habitats or under laboratory. Thus far, no sexual has been observed in *C. syzygiicola* and *C. zambiensis*. In order to understand the role of the identified mating-type genes in *Chrysoporthe* species, functional analysis studies

will be essential. In addition, functional analysis studies will provide a clear indication of the role of *MAT1* genes and how are they linked with virulence thus providing biologist with an opportunity to control diseases caused by these species.