The prevalence of *Cladosporium* species in indoor environments

by
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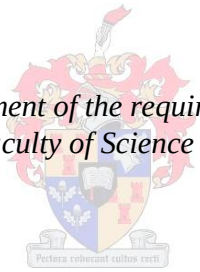

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Supervisor: Prof. Karin Jacobs

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Declaration

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March 2019

Abstract

Cladosporium is among the largest and most diverse genera of hyphomycetes, previously reported to include over 772 names. The olive-green to brown or sometimes black colonies are usually the easiest characteristics used to recognise species of this genus. *Cladosporium* are usually found on both living and dead plant material, and recently in human specimens and indoor environments. Furthermore, *Cladosporium* are associated with allergenic rhinitis, asthma, subcutaneous infections and other infections in both immunocompetent and immunocompromised individuals. Nevertheless, some *Cladosporium* species are useful for the production of antibiotics that work against *Bacillus subtilis* and *Candida albicans*, while some are efficient biological insecticides targeting insects that are resistant to chemical insecticides.

Ambient air pollution has been recognised as the primary source of respiratory illnesses with regards to air pollution. However, in recent years, indoor air has proven to be a potential threat to human health. Indoor environments encompass a lot of gases and microorganisms that may be hazardous to humans. *Cladosporium*, often referred to as outdoor fungi has been commonly isolated in indoor environments over the last few years.

This study investigated the prevalence of a potent allergenic genus, *Cladosporium*, in South African indoor environments. In an attempt to achieve this, air samples collected from different areas of the Western Cape and Gauteng provinces were studied. Following a polyphasic approach used by several authors, isolates were identified and new species described. Species were represented in all species complexes but mostly resolved in the *Cladosporium cladosporioides* species complex, the largest complex of the genus. Here, we present 110 isolates from indoor air samples identified as 18 different species of *Cladosporium*, three of which have been introduced and described as novel.

This study has demonstrated that South African indoor environments contain a large diversity of described and undescribed *Cladosporium* species. Moreover, there is a need for more studies concerning indoor environments with particular regard to this genus.

Opsomming

Cladosporium is van die grootste en mees diverse hyphomycetes genera. Daar is voorheen gerapporteer dat dit meer as 772 name bevat. Die olyfgroen tot bruin of soms swart kolonies is gewoonlik die maklikste eienskap wat gebruik word om die spesies van hierdie genus te herken. *Cladosporium* kom gewoonlik op beide lewende en dooie plantmateriaal voor en is onlangs gekry in menslike monsters en binnenshuise omgewings. Verder word *Cladosporium* ook met allergiese rhinitis, asma, onderhuidse infeksies en ander infeksies in beide immuuntoereikende en immuun gekompromiteerde indiwidue geassosieer. Desnieteenstaande is sommige *Cladosporium*-spesies nuttig vir die vervaardiging van antibiotika teen bakterieë en giste, terwyl sommige doeltreffende insekdoders is teen insekte wat weerstandbiedend teen chemiese insektedoders is.

Omringende lugbesoedeling word erken as die primêre bron van lugwegsiektes a.g.v. lugbesoedeling. In die laaste jare is egter bewys dat binnenshuise lug ‘n potensiële bedreiging vir die mens se gesondheid is. Binnenshuise omgewings sluit baie gasse en mikroörganismes in wat skadelik vir mense kan wees. *Cladosporium,* wat algemeen as ‘n buitenshuise fungus beskou word, is oor die laaste paar jaar dikwels uit binnenshuise omgewings geïsoleer.

Tydens hierdie studie is die voorkoms van ‘n sterk allergeniese genus, *Cladosporium,* in Suid-Afrikaanse binnenshuise omgewings bestudeer. In ‘n poging om dit uit te voer, is lugmonsters wat in verskillende areas in die Wes-Kaap en Gauteng ingesamel is, bestudeer. Met ‘n polifase benadering, wat deur verskeie outeurs gebruik is, is isolate geïdentifiseer en nuwe species beskryf. Species was verteenwoordigend van al die species komplekse, maar het meestal deel van die *Cladosporium cladosporioides* kompleks, die grootste kompleks in die genus, gevorm. Hier lê ons 110 isolate voor, wat vanaf binnenshuise lugmonsters verkry en geïdentifiseer is, as 18 verskillende *Cladosporium* species, waarvan drie voorgehou en beskryf word as nuwe species.

Hierdie studie bewys dat Suid-Afrikaanse binnenshuise omgewings ‘n groot verskeidenheid beskryfde en onbeskryfde *Cladosporium* species bevat. Voorts is daar ‘n behoefte aan meer studies rakende binnenshuise omgewings waarin daar spesifiek op hierdie genus gekonsentreer word.

Dedication

This thesis is dedicated to:

my mother, Lulama Ndlangalavu, and my family-

your words of encouragement kept me going.

I wouldn't have made it without your love and support.

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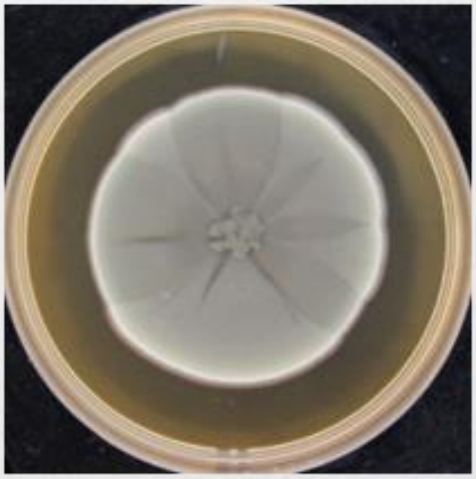
Chapter 1 Introduction



1.1. Background to the research problem

Indoor air pollution (IAP) refers to the contamination of indoor air by human activities, chemicals, and biological pollutants. IAP has been recognised as a public health problem in developing countries (Jafta et al. 2015) while accounting for nearly 4% of diseases worldwide (Kadir et al. 2010). It is responsible for about 2 million deaths annually with over 90% of these occurring in low- and middle-income countries (Kadir et al. 2010). Many people spend most of their time indoors where microorganisms are also inhabitants (Tong et al. 2017). However, these microorganisms can become a source of pollution in indoor air. Frequent exposure to such pollutants can result in adverse health effects (Twaroch et al. 2015).

Microbial growth on building surfaces is linked to dampness and moisture damage (Jayaprakash et al. 2017). Exposure to building material with damp and elevated humidity in places of work and homes has been associated with a variety of adverse health effects such as allergenic rhinitis, asthma, chromoblastomycosis, phaeohyphomycosis, and acute meningitis (Etzel and Rylander 1999, WHO 2000, Gugnani et al. 2006, Jafta et al. 2012, Ogórek et al. 2012, Chen et al. 2013, Zukiewicz-Sobczak et al. 2013, Fukutomi and Taniguchi 2015, Twaroch et al. 2015, Jayaprakash et al. 2017). Known allergen-producing fungi belong to the genera *Alternaria* and *Cladosporium* (Erkara et al. 2009), *Penicillium* and *Aspergillus* (Twaroch et al. 2015), as well as *Mucor* and *Rhizopus* (Zukiewicz-Sobczak et al. 2013).

Cladosporioid hyphomycetes are widespread and cosmopolitan fungi (Crous et al. 2007). The genus, *Cladosporium*, was established in 1816 (Braun et al. 2003) with *Cladosporium herbarum* as the type species. Due to their ubiquitous nature, *Cladosporium* species can be isolated from almost anywhere in the world and from a wide range of substrates including plants (Temperini et al. 2018), soil (Ma et al. 2017), humans (Sandoval-Denis et al. 2015, 2016), air (Bensch et al. 2018), food (Surridge et al. 2003, Razafinarivo et al. 2016), wood, water, building surfaces, textiles, to name a few (Bensch et al. 2012). The genus *Cladosporium* has previously been recorded as having over 772 names (Dugan et al. 2004) and after a recent revision (Bensch et al. 2012) only 170 names of species were recognised in *Cladosporium s. str.* An increasing interest in this genus led to the description of several new species (Crous et al. 2014, Bensch et al. 2015, 2018, Braun et al. 2015, Razafinarivo et al. 2016, Sandoval-Denis et al. 2016, Ma et al. 2017, Marin-Felix et al. 2017). Currently, the genus, *Cladosporium*, comprises 218 recognised species (Bensch et al. 2018).

Species in *Cladosporium* are characterised by the general lack of a sexual stage (AlMatar and Makky 2016) and are typically characterised by their distinct structure of a conidiogenous loci and conidial hila *i.e.*, a raised periclinal rim encircling a central convex dome (Bensch et al. 2018). In addition, *Cladosporium* species have conidia that are easily distributed because of their relatively small size, making them the most common airborne fungi (David 1997, Shelton et al. 2002, Ghiaie et al. 2017). *Cladosporium* species rarely cause human infection, yet they have been associated with human infections in a number of cases (De Hoog et al. 2000, Ogórek et al. 2012, Chen et al. 2013, Grava et al. 2016, Sandoval-Denis et al. 2016, Shi et al. 2016). One of the medically important species is *Cladosporium herbarum*, a species that is known for contaminating medical laboratories and causing lung mycoses (De Hoog et al. 2000, Crous et al. 2007). More clinically important species have been described even though their ability to cause infections is still not understood (Sandoval-Denis et al. 2016). These include *C. cladosporioides*, *C. oxysporum*, *C. sphaerospermum*, and *C. macrocarpum* (Kantarcioglu et al. 2002, Yano et al. 2003, Gugnani et al. 2006, Lalueza et al. 2011, Chen et al. 2013).

Cladosporium cladosporioides is an opportunistic fungus that causes various infections in immunocompetent humans and immunocompromised animals (Kantarcioglu et al. 2002, Zambelli and Griffiths 2015). It is usually the most commonly identified species in clinical isolates, however, a recent study has demonstrated otherwise (Sandoval-Denis et al. 2015). Like *C. cladosporioides*, *C. sphaerospermum* not only affects immune-compromised individuals, cases of *C. sphaerospermum* affecting healthy individuals have also been reported (Yano et al. 2003).

Molecular phylogenetic studies have been implemented in the past to extensively study the genus *Cladosporium* (Crous et al. 2007, Schubert et al. 2007, Zalar et al. 2007, Bensch et al. 2010, 2012, 2015) but it is just recently that an attempt to study this genus in indoor environments has been made (Bensch et al. 2018). Due to the ability of *Cladosporium* species to produce allergens, it is important that *Cladosporium* found in indoor environments be investigated.

1.2. Research question

What is the diversity of *Cladosporium* species in indoor environments in South Africa?

1.3. Research/ problem statement

The genus *Cladosporium* has been studied at length in the last few decades. Researchers have often taken interest in outdoor environmental *Cladosporium* and just recently in clinical *Cladosporium* (Sandoval-Denis et al. 2015, 2016). However, there is scant knowledge about *Cladosporium* in indoor environments (Bensch et al. 2018) and as a result the diversity of indoor *Cladosporium* needs more attention. Moreover, the quality of air in indoor environments plays an important role in human health. Indoor environmental conditions such as humidity enhance the growth of biological contaminants like fungi, which in high concentrations may lead to disease. Fungi such as *Cladosporium* have been associated with human infections (Ogórek et al. 2012, Sandoval-Denis et al. 2016) and have been reported to cause severe allergenic sensitisation in immunocompromised individuals (Ellertsen et al. 2009, Grava et al. 2016).

1.4. Hypothesis and aims

This study was premised on the fact that indoor environments are a source of potential allergenic *Cladosporium*. The study aimed to determine the prevalence of *Cladosporium* species in indoor environments. In order to achieve the aim the following objectives were set:

1.4.1. Identify indoor *Cladosporium* species from South African homes with visible and non-visible fungal contamination.

1.4.2. Describe novel species.

1.5. Significance of research

The quality of air in indoor environments has often been recognised as a health determinant. Tobacco smoke and heating of solid fuels have been identified as major sources of indoor air pollution (IAP) in low- and middle-income countries (Gqaleni 2002, Smith 2003, Tielsch et al. 2009, Kadir et al. 2010, Jafta et al. 2017). Furthermore, increased levels of indoor contaminants may lead to cardiopulmonary diseases ranging from an acute to a chronic state (Fullerton et al. 2011, Gordon et al. 2014, Chafe et al. 2015, Jafta et al. 2017). Some of these cardiopulmonary health effects include lower respiratory tract infections, chronic obstructive lung disease and lung cancer (Gordon et al. 2014, Smith et al. 2014).

A limited number of studies have investigated the association between IAP exposure and TB (Lin et al. 2014, Jafta et al. 2015, 2017), specifically combustion and fuel pollutants, as well as chemical compounds in a gaseous state. While there are a number of international studies

investigating biological pollutants in indoor environments (Kadir et al. 2010, Ogórek et al. 2012, Bensch et al. 2018), there is a limited number of studies in this regard in South Africa. In addition, allergenic sensitisation due to the indoor contaminant, *Cladosporium*, has not yet been investigated. With a rising prevalence of asthma and fungal allergies worldwide, there is a need for a better understanding of the diversity of *Cladosporium* species found in indoor environments and their ability to produce allergens that cause adverse health effects.

1.6. Brief chapter overview

Chapter two of this thesis explains in depth the history of the genus *Cladosporium*, its taxonomy, and its clinical relevance. The third chapter illustrates *Cladosporium* species found in a number of South African homes and provides an overview of their taxonomy and phylogenetic relationships. The description of new species is covered in chapter four while chapter five provides an overall discussion, final conclusions and recommendations for future research.

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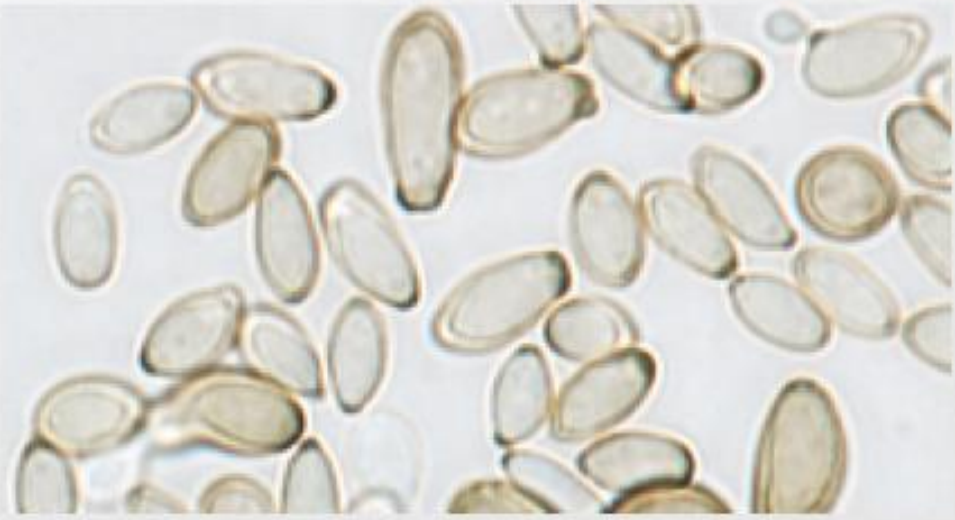
Chapter 2
Literature review



2.1. Indoor air pollution and its sources

The World Health Organization states that everyone has the right to breathe healthy indoor air (WHO 2000b). This is because air quality is one of the major factors that affect the health and well-being of a person. Poor air quality can lead to illness in individuals or even populations and is usually caused by a number of activities such as industrial emissions, smoking and the use of insecticides (Nriagu et al. 1999, Gqaleni 2002). Moreover, indoor environments have become a well-documented source of contaminants that may be harmful to humans. With over fifty percent of the global population relying on solid biomass fuels for domestic use (WHO 2000a, Ezzati and Kammen 2002, Barnes et al. 2009, Kadir et al. 2010), many people are frequently exposed to indoor air pollution (IAP). In low- and middle-income countries where most people are still dependent on burning biomass and fossil fuels such as animal dung, wood, coal, and paraffin, for heat and cooking, IAP has been identified as a major health problem (Smith and Liu 2002, DEA 2011).

IAP is a risk factor for morbidity and mortality, and accounts for about 4% of the diseases globally (Kadir et al. 2010). Just about two million people die annually due to illnesses related to poor indoor air quality (IAQ), with more than 90% of these deaths occurring in low- and middle-income countries (Kadir et al. 2010). While exposure to outdoor air pollution has been associated with adverse health effects, the greatest health risk may be continuous exposure to IAP (DEA 2011). IAP is associated with a range of negative health effects such as tuberculosis (TB), acute lower respiratory tract infections (ALRTIs), chronic obstructive pulmonary disease, lung- and nasopharyngeal cancer, and it increases the risk of otitis media, asthma, low birth weight, still birth, and neonatal mortality (Bruce et al. 2000, Smith et al. 2000, Mishra et al. 2004, Leonardi-Bee et al. 2008, Barnes et al. 2009, Hu and Ran 2009, Kadir et al. 2010, Fullerton et al. 2011, Gordon et al. 2014, Chafe et al. 2015, Jafta et al. 2017). ALRTIs have been reported as the leading causes of death in children below the age of five years (Lopez et al. 2002, Kadir et al. 2010). In South Africa, ALRTIs are among the top four causes of death in children below five years of age (Bradshaw et al. 2003, Barnes et al. 2009).

Exposure to smoke, caused by combustion of biomass fuels, is not the only type of indoor exposure of concern. For instance, second-hand tobacco smoke contributes to poor air quality and is associated with premature death and disease in children and non-smoking adults (Kadir et al. 2010). Consequently, exposure of pregnant women to second-hand smoke has led to low birth weight, and infants exposed to this type of pollution have casually been linked to

sudden infant death syndrome (Mishra et al. 2004, Leonardi-Bee et al. 2008, Kadir et al. 2010). Being exposed to environmental tobacco smoke is also recognised as a major health risk factor for adverse respiratory illnesses (den Boon et al. 2007, Jafta et al. 2015), with those who spend most of their time indoors such as children, the elderly, and the immunocompromised, being more prone to IAP exposure (Jafta et al. 2015). Furthermore, biological pollutants like allergens from fungi in indoor environments are also important, which until just recently, were neglected sources of IAP (Crameri et al. 2014).

Many studies concerning indoor air quality in developing countries have mainly focused on biomass fuels and combustion contaminants, while biological contaminants such as allergens have received very little attention (Jafta et al. 2012). In South Africa, indoor environments have recently been recognised as important contributors to public health. This is supported by a number of studies (Gansan et al. 2002, Gqaleni 2002, Jafta et al. 2012, 2015, 2017) which looked at the quality of indoor environments and how they affect the quality of life. Exposure to indoor contaminants often occurs in homes where people spend most of their time.

There are many household risk factors affecting the IAQ. These are conditions such as dampness of the walls, indoor humidity, poor ventilation, poor building design and construction, poor sanitation, overcrowding or large family size, low-income, to name just a few (Singh 1994, Gansan et al. 2002, Gqaleni 2002, Jafta et al. 2012). These factors create favourable conditions for the growth of different biological contaminants such as fungi (Singh 1994) and bacteria (Wilkins et al. 2016) , which may be detrimental to human health. Thomas et al. (1999) showed that part of the Port Elizabeth population living in low-income houses, experiences health problems such as painful chest and eyes, headaches, as well as skin problems. Gqaleni et al. (1999) found that 60% of the shacks they studied in Port Elizabeth were damp and mouldy, and 20 to 40% children residing in those dwellings showed symptoms of respiratory tract infections. In the 2002 study by Gqaleni on IAQ research in SA, where 200 houses were monitored for dampness and moulds, moulds belonging to the genera *Cladosporium*, *Aspergillus*, and *Penicillium* were predominant in 84 houses. Moreover, 32% of the children tested positive for exposure to fungal allergens. The findings from these studies demonstrate that the less-wealthy households are more prone to fungal illnesses due to their living conditions. In addition, the household risk factors amplify the chances of getting sick from fungal exposure.

Since indoor environments are habitats for microorganisms that can be detrimental to human health, reasonable research interest has been recently give to this aspect (Jafta et al. 2012,

Adams et al. 2015, Tong et al. 2017). Microorganisms in, on, or around our bodies contribute to the biodiversity of microbes in our surroundings (Adams et al. 2013, 2015). Furthermore, the human skin harbours various microbiota including fungi. This would, therefore, mean that the human mycobiome is being shared with the environment (Wilkins et al. 2016). Direct contact with indoor surfaces and shedding of fungi from human clothing, influences the indoor mycobiome. Furthermore, human occupancy affects the quantity of fungi in indoor environments. This is supported by studies that demonstrated that indoor dust, air and surfaces harbour fungi suspected to originate from humans (Adams et al. 2013, 2015, Wilkins et al. 2016). Additionally, indoor microbiota can also be influenced by the interaction between indoor and outdoor microbes. For instance, Adams et al. (2013) have demonstrated that outdoor fungi influence indoor environments. Due to their small size, microorganisms are able to travel long distances with the assistance of air, water and/or animals. Also, outdoor fungi such as *Cladosporium*, which are relatively small and easily dispersed by wind, are predominantly found in indoor environments (Jafta et al. 2012, Bensch et al. 2018).

2.2. Fungal species in association with human health: Allergic reactions

Fungal exposure has been recognised as a source of adverse respiratory symptoms since the 18th century (Wagner 1964, Twaroch et al. 2015). Even though the first fungal allergy was observed over three centuries ago, the relationship between fungal exposure and allergic symptoms has been controversial for years and even today fungi are less recognised as sources of allergens (Simon-Nobbe et al. 2008, Crameri et al. 2014, Twaroch et al. 2015). Fungi are less recognised as allergen sources probably because the exact prevalence of sensitisation resulting from fungal exposure is not known, as there are no set standards for fungal exposure to cause allergies, and because allergic sensitisation differs from person to person. However, there have been a series of papers addressing fungi as allergen sources (D'Amato and Spieksma 1995, Menezes et al. 2004, Ellertsen et al. 2009, Twaroch et al. 2015). The most common allergic reactions associated with fungi are allergic rhinitis and asthma. Allergic rhinitis is defined as an inflammation of the nasal area due to the inhalation of an allergen (Seidman et al. 2015), while asthma is a long term disease caused by the inflammation of the lung airways (Busse and Lemanske 2001), affecting over 300 million individuals world-wide (Rick et al. 2016). Some of the main causes of asthma include air pollution and allergens. These allergies may develop to the extent where they can be lethal.

Of the known and described fungi, about 80 fungal genera are known to cause type I allergies in atopic individuals (Simon-Nobbe et al. 2008, Crameri et al. 2014), but the most important genera are *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus*. These fungi may induce allergic sensitisation if one is exposed to the allergen in clinically relevant concentrations (Twaroch et al. 2015). This means that one may be exposed to fungal spores, but if the threshold is not reached, sensitisation may not be experienced. Sensitisation to allergies differs between the different genera and species. It is believed that for *Alternaria* to cause allergic symptoms, a threshold of 100 conidia per cubic meter must be reached, while for *Cladosporium* the threshold is estimated to be 3000 conidia per cubic meter (D'Amato and Spieksma 1995). *Cladosporium* is one of the most noted genera as an important source of allergens (D'Amato and Spieksma 1995).

A fact sheet produced by the World Health Organization (WHO 2017) reports that there are 235 million people suffering from asthma that mainly affects children. Moreover, the WHO in December 2016 reported an estimate of 383 000 asthma-related deaths for the year, 2015. Asthma affects all classes of countries, however, most death cases that are asthma-related are usually reported from undeveloped- and developing countries (WHO 2017). South Africa (SA) is one of the countries that have the highest asthma recorded deaths for persons between the ages of five and 35, ranking number four in the world (Health24 2016). In South Africa, 58 500 people die from asthma annually, and only two percent of the children receive treatment (Health24 2016). Childhood asthma has become more prevalent in low- and middle income countries over the years (Yakubovich et al. 2016). In Sub-Saharan Africa (SSA), the burden of asthma for children under the age of 15 years has increased. The estimated number of people affected by asthma in the Africa is 50 million, with the majority of those asthmatic people being South Africans (Yakubovich et al. 2016). These statistics suggest a need for better management of asthma. Therefore, it is important to study the causes of asthma and the factors that may induce its prevalence, especially with the ability of *Cladosporium* to trigger asthmatic reactions (Denning et al. 2006, Sharpe et al. 2015).

2.3. A background on *Cladosporium*

2.3.1. A brief history on *Cladosporium*

Cladosporium is a large genus belonging to hyphomycetes which comprised of over 772 names (Dugan et al. 2004) and one of the most common fungi to be isolated from almost anywhere in the world (Schubert et al. 2007). It is known to abundantly occur on dead leaves

of herbaceous woody plants, and has been isolated from air (Bensch et al. 2018), soil (Ma et al. 2017), fruit (Surridge et al. 2003), humans (Sandoval-Denis et al. 2015, 2016), and a number of other substrates. *Cladosporium* species affect humans daily in different ways. Some members of this genus are of medical relevance, for instance *C. herbarum* is known to be a contaminant in clinical laboratories and has been implicated in health effects such as lung mycoses (De Hoog et al. 2000, Schubert et al. 2007). Furthermore, *C. cladosporioides*, a common saprobic species, has been associated with pulmonary and cutaneous infections (De Hoog et al. 2000). However, *C. cladosporioides* has also been reported to produce antibiotics that are effective against *Bacillus subtilis, Escherichia coli* and *Candida albicans,* and are also effective insecticides against insects resistant to chemical insecticides (AlMatar and Makky 2016).

The genus, *Cladosporium*, was first described by Link (1816) with *Cladosporium herbarum* as the type species. Following a long history of *Cladosporium* descriptions and revisions, David (1997) examined the conidiogenous loci and conidial hila of *Cladosporium* using scanning electron microscopy. It was demonstrated that species of this genus were limited to anamorphs of *mycosphaerella*-like ascomycetes consisting of a distinct scar type that he described as coronate, meaning it is composed of a central convex dome surrounded by a raised periclinal rim. A few years later, Dugan et al. (2004) published a checklist of *Cladosporium* names that raised an urgent need for researchers to look into the genus.

Following the approach used by David (1997), more *Cladosporium* species were described and some reallocated to other genera (Braun et al. 2003, Bensch et al. 2005, Schubert and Braun 2005a, 2005b). With the generic affinity of hundreds of *Cladosporium* names being unclear and the number of species belonging to *Cladosporium s. str.* unknown, Schubert and Braun (2005a) initiated monographic examinations of *Cladosporium* (*s. lat.*) species. Some species that were previously excluded from the genus were reassessed and re-described based on type collections. Some species previously assigned to *Cladosporium* were reassigned to the genera *Fusicladium, Parastenella, Passalora, Pseudocercospora,* and *Stenella* as new combinations (Schubert and Braun 2005a). Following their previous paper (Schubert and Braun 2005a), Schubert and Braun (2005b) continued to re-allocate some excluded *Cladosporium* species to *Asperisporium, Dischloridium, Fusicladium, Passalora, Pseudoasperisporium* and *Stenella*.

In the last two decades, *Cladosporium* has been studied at great length based on morphology and molecular studies in order to clarify its generic concept and biodiversity (Schubert and

Braun 2005a, 2005b, Crous et al. 2007, Schubert et al. 2007, Bensch et al. 2010, 2012, 2015, 2018, Sandoval-Denis et al. 2016, Ma et al. 2017). Until recently, *Cladosporium s. lat*. included all dematiaceous hyphomycetes with amero- to phragmosporous conidia formed in acropetal chains. This includes species that essentially did not belong to the genus and this caused a problem to the monograph of the genus it genus encompassed species that were not typical to *Cladosporium*. Therefore, Bensch et al. (2012) presented a monographic revision of the genus, *Cladosporium s. lat*., which included a detailed history of the genus and similar genera, along with details of their phylogeny, systematics and ecology. There were then 170 species recognised as true *Cladosporium* species (Bensch et al. 2012), but due to an ongoing interest on *Cladosporium*, the number has increased from 170 to 218 species (Bensch et al. 2018).

2.3.2. Morphotaxonomy of *Cladosporium*

A comprehensive morphotaxonomy of the genus *Cladosporium* is discussed in Bensch et al. (2012). The following brief taxonomy had been adopted from that paper.

2.3.2.1. Mycelium

Foliicolous and saprobic *Cladosporium* species *in vivo*, often have internal mycelium, the mycelium can sometimes be both internal and external or simply external. The hyphae are septate, often branched, smooth, occasionally sort of rough, and subhyaline, slightly pigmented to dark brown with thin walls, and with aging, thick walls can be observed. *In vitro* most times the mycelium is variable, composed of narrow or wide, subhyaline to pigmented hyphae, with thin walls or thick walls after aging, stromata are usually absent.

2.3.2.2. Conidiophores

In vivo

Cladosporium species possess conidiophores that arise internally or externally from hyphae, from small to large stromatic hyphal aggregation. “They are mostly cylindrical, subcylindrical or filiform, but further differentiation is often due to sympodial proliferations causing geniculations with conidiogenous loci often situated on small lateral shoulders or terminal to intercalary swellings” (Bensch et al. 2012). More than a few species of *Cladosporium* are well-characterised by the presence of mild to distinct geniculate-sinuous conidiophores. Intercalary and apical inflation of conidiophores can be observed at different degrees, ranging from subnodulose to nodulose.

In vitro

The length of conidiophores within different species varies extensively as seen in a number of reports (Crous et al. 2001, 2007, Braun et al. 2003, Schubert and Braun 2005a, 2005b, Schubert et al. 2005, Heuchert et al. 2005, Bensch et al. 2010, 2012, 2015, 2018, Sandoval-Denis et al. 2016, Ma et al. 2017, This study), while the width is much less variable. Conidiophores are almost always formed singly. Several species have branched conidiophores; species whose conidiophores are branched *in vivo* are also branched *in vitro* (Bensch et al. 2012).

2.3.2.3. Conidiogenous cells

Conidiogenous cells are integrated, terminal or intercalary, sometimes reduced conidiophores. The structures of the conidiogenous loci and conidial hila in *Cladosporium* species is more or less the same, with small differences between different species.

2.3.2.4. Conidia

All species of *Cladosporium* have the ability to produce conidia in acropetal chains. One of the useful ways to distinguish particular species is by examining conidial formation, viz conidia formed in chains or solitary. Catenate conidia in *Cladosporium* are acropetal, sympodial and usually branched. The shape of conidia also differs between different species or may be the same for others. Conidial shape can be subglobose, ovoid, ellipsoid, fusiform, limoniform to subcylindrical or cylindrical, with the length, width, and septation of conidia being different too; the width is less variable than the length.

2.4. *Cladosporium* species in association with human infections

Cladosporium species are among the most noted allergenic fungi associated with allergic rhinitis and respiratory arrest in patients with asthma (Black et al. 2000). These species have also been associated with a number of human infections such as chromoblastomycosis, an opportunistic infection described as a chronic skin and subcutaneous tissue fungal infection (Ogórek et al. 2012), and phaeohyphomycosis, a term generally used to describe infections caused by dematiaceous fungi (Revankar 2006). Moreover, *Cladosporium* has been implicated in infections of the central nervous system of immunocompetent individuals (Kantarcioglu et al. 2002, Lalueza et al. 2011, Chen et al. 2013), cervical lymph node (Jayasinghe et al. 2017), legs (Gugnani et al. 2006), and is also known to cause intrabronchial lesions (Yano et al. 2003), onychomycosis (Shi et al. 2016), hypersensitivity pneumonitis (Chiba et al. 2009), and pulmonary infections (Grava et al. 2016). Whilst *Cladosporium* have

been reported to cause such infections, their pathogenicity to humans is not well known, especially given their inability to grow at 37 °C (Sandoval-Denis et al. 2016). *Cladosporium* species of clinical interest have recently been examined by Sandoval-Denis et al. (2015, 2016). Through phylogenetic analysis, new species associated with human and animal infections have been described (Sandoval-Denis et al. 2016), however, their pathogenicity is not yet known.

In attempts to assess the diversity of clinically important *Cladosporium* species associated with human and animal infections, Sandoval-Denis et al. (2015) examined a large set of clinical isolates using phenotypic and DNA sequence data techniques. From this, it was demonstrated that the *C. cladosporioides* complex had the largest species diversity, the highest number of clinically associated species, as well as the largest number of undescribed species. Furthermore, even though *C. cladosporioides* has been extensively cited in literature as being clinically important, it was poorly represented in the isolates studied by Sandoval-Denis et al. (2015). Interestingly, other species belonging to the *C. cladosporioides* complex were reported for the first time in clinical settings. This could be because, as noted by Sandoval-Denis et al. (2015), most of the species that have been associated with clinical settings are actually species complexes encompassing different species. Therefore, there is a possibility that some *C. cladosporioides* infections previously reported, may in fact have been caused by species within the *C. cladosporioides* complex and not by *C. cladosporioides s. str.* Similarly, in the *C. sphaerospermum* complex many isolates were identified as *C. halotolerans*, which has never been associated with human infections.

In retrospect, seemingly some clinically relevant species have been overlooked due to the limited knowledge on *Cladosporium* species of clinical relevance. Moreover, it shows that the studies by Sandoval-Denis et al. (2015, 2016) broaden the species diversity of *Cladosporium* in clinical settings. Sandoval-Denis et al. (2015) identified some undescribed species that were characterised in a follow up study (Sandoval-Denis et al. 2016) by using both molecular and phenotypic criteria, resulting in the description of 10 new species. The human respiratory tract homes some of the newly described species. This comes as no surprise since *Cladosporium* conidia are so small that they can be easily dispersed by wind (Bensch et al. 2012), making them predominant in airborne microbiota. Additionally, *Cladosporium* second to *Alternaria* is an important respiratory allergenic fungus (Twaroch et al. 2015).

2.4.1. Association between *Cladosporium* and TB

The association between *Mycobacterium tuberculosis* and allergies is still unclear (Ellertsen et al. 2009), hence the relationship between TB and allergens remains unclear as well. Nonetheless, Ellertsen et al. (2009) in their study used a questionnaire, TB treatment regimen, and specific and total IgE to determine whether allergenic sensitisation changed after TB patients received treatment. The findings in relation to both specific and total IgE demonstrated a significant decrease in the levels of IgE after a successful TB treatment. Additionally, TB patients after successful treatment had reduced levels of sensitisation to allergens. Those results imply that a weakened immune system, in this case that of a TB patient, may be more susceptible to allergen sensitisation and that there may be some sort of correlation between allergens and TB. Furthermore, the study mentions that healthy individuals are less allergic sensitised to allergens than TB patients whose immune systems have been compromised. This could mean that TB patients are more likely to develop allergies or that allergy patients are more prone to develop TB upon infection with *M. tuberculosis* (Ellertsen et al. 2009), either way one affects the other although the link is not yet clear. *Cladosporium* species are some of the most common allergenic fungi found in indoor environments, and their presence may aggravate the incidence of TB in individuals living with the disease. There is, however, little knowledge about the relationship between *Cladosporium* species and TB incidence.

2.4.2. Chromoblastomycosis

Chromoblastomycosis is a chronic fungal infection of the cutaneous and subcutaneous tissues mostly occurring in individuals in tropical and subtropical regions (Matsumoto et al. 2011, Robles and Ameen 2018). It is a result of an implantation of dark-pigmented fungi that produce thick-walled sclerotic bodies in infected tissues (Robles and Ameen 2018). This type of mycosis is often represented by nodules, plaques, warts or exophytic lesions, mostly affecting lower limbs. Although it is often localised, it can spread to other areas of the body including the central nervous system (Matsumoto et al. 2011). The primary etiological agents for this type of mycosis are *Cladophialophora carrionii*, *Fonsecaea compacta*, *F. monophora, F. pedrosoi, Phialophora verrucosa*, and *Rhinocladiella cerophilum* (Matsumoto et al. 2011). Even though *Cladosporium* is not considered a primary source of chromoblastomycosis it has been in fact reported to cause this type of mycosis (De Hoog et al. 2000, Zambelli and Griffiths 2015).

2.4.3. Phaeohyphomycosis

Phaeohyphomycosis is a type of mycosis known to usually affect immunocompromised individuals such as those suffering from leukaemia, TB, leprosy, diabetes mellitus, HIV/AIDS, and lymphoma (Matsumoto et al. 2011). Unlike chromoblastomycosis which affects lower limbs, phaeohyphomycosis can affect any part of the body. Similar to chromoblastomycosis, *Cladosporium* is not listed among the principal etiological agents for this type of mycosis but has been implicated in some studies (Gugnani et al. 2006).

Cladosporium species are a large component of the environment therefore constant exposure to them is inevitable. Nonetheless, obvious contamination indoors should be removed to avoid the increased risk of sickness. Furthermore, with the literature provided in this chapter it is evident that *Cladosporium* species are indoor contaminants that should be of research interest as they can be detrimental to human health. Moreover, these species should be exploited for their antibiotic-production activity so they may be useful in combating infections caused by other microorganisms.

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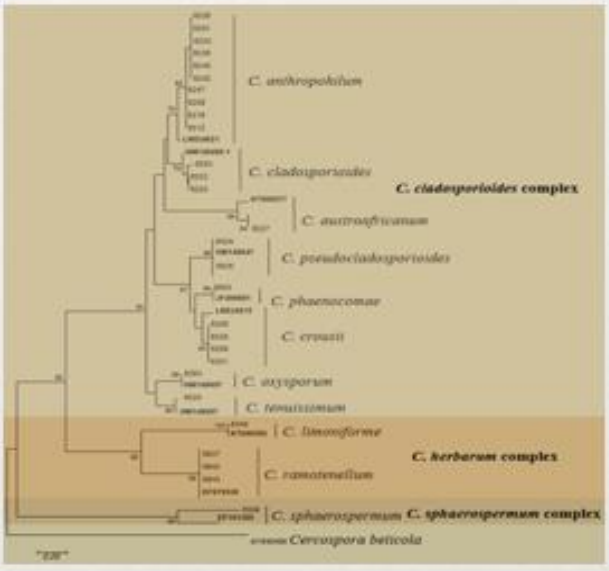
Chapter 3

Cladosporium in indoor environments



Abstract

Indoor air pollution is a major health problem that most developing countries are facing. While the main focus has been on ambient air pollution as the major health issue, indoor air pollution has proven to be a silent killer. With a number of microorganisms such as allergenic fungi, it is important to know which species are found in indoor environments. A survey investigating the prevalence of *Cladosporium* species in indoor environments from the Western Cape and Gauteng was done. The study included 110 isolates from 17 different sites, which through molecular and phylogenetic analysis delimited 18 *Cladosporium* species, three of which were novel. *Cladosporium anthropophilum* was the most common species isolated in indoor environments.

Key words: Indoor air pollution, Indoor environments, *Cladosporium*, New species.

3.1. Introduction

Almost every year in South Africa 20 000 lives are lost to air pollution (Kings 2016). In 2014, a report by WHO demonstrated that air pollution in South Africa was so bad that it exceeded the annual accepted standards measured for the respective driving forces of air pollution (eNCA 2014, Sello 2014, WHO 2014). Areas of heavy industrial developments such as the Vaal Triangle Airshed Priority Area, South Durban Industrial Basin (SDIB) and the Highveld Priority Area (HPA) raised a major concern for air pollution (DEA 2011). Moreover, the two largest and most populated cities in the country, Cape Town and Johannesburg have also raised concerns in the past regarding air pollution. With car emissions and industrialization in these highly populated areas, ambient air pollution is no surprise.

Exposure to outdoor air pollution has been associated with various health issues, however, pollution from indoor environments is the most damaging to health (DEA 2012). According to a report by the South African Institute of Race Relations (Vegter 2016), indoor air pollution is South Africa's most serious air quality problem. Upon closer inspection, mining residues, particulate matter, industrial and car emissions to name a few, are usually at the forefront while there is not enough focus on the most prevalent and difficult problem to solve, indoor air pollution. It is just recently that South African researchers have shown interest in indoor air pollution (Jafta et al. 2015, 2017).

Approximately 20% of South African households are affected by contaminated indoor air (Vegter 2016). Exposure to indoor air pollution is known to play a significant role in chronic pulmonary diseases, different types of cancers including lung and nasopharyngeal cancer, TB, asthma and acute lower respiratory infections in children (Barnes et al. 2009, Jafta et al. 2017). These negative health impacts may be a result of compounds in a gaseous state, particulate matter or biological pollutants. However, biological pollutants are the overlooked sources of indoor air pollution. Indoor environments consist of numerous microorganisms that may or may not be of human benefit. Unknowingly, humans also contribute to indoor air pollution through respiration and shedding of skin flakes and hair, which in both cases release their microbiome in their surrounding environment (Prussin and Marr 2015, Tong et al. 2017). Microorganisms in indoor environments can be isolated from air, dust, and surfaces.

Due to their ubiquitous nature, *Cladosporium* species are the most common airborne fungi to be isolated from the environment. Their spores can be isolated throughout the year but some

European (De Gómez Ana et al. 2006) and American (Ren et al. 2001) studies have shown that in indoor environments they are more prevalent in autumn. Moreover, a study by Ren et al. (2001) showed that within a household, *Cladosporium* species were mostly prevalent in the living area, where families spend most of their time.

Above all, *Cladosporium* species poses a threat to human health. This is suggested by the numerous cases reporting different health issues such as haemorrhagic pneumonia (Grava et al. 2016), cutaneous phaeohyphomycosis (Gugnani et al. 2006) and unilateral cervical lymphadenopathy (Jayasinghe et al. 2017), and intrabronchial lesions (Yano et al. 2003) caused by *C. cladosporioides*, *C. oxysporum*, and *C. sphaerospermum*, respectively. *Cladosporium* has previously been studied in South Africa (Surridge et al. 2003, Zambelli and Griffiths 2015), which is one of the few studies to report *Cladosporium* in indoor environments in this country. The aim was to investigate the occurrence of this genus in indoor environments.

3.2. Methods and materials

Sampling. —Air samples were collected in 13 different houses, with visible and non-visible fungal contamination, located in the Western Cape and Gauteng provinces, using a *MBV MAS-100* Eco-microbial air sampler, which uses 90-100 mm standard Petri dishes. The Petri dishes contained MEA (Product code LAB037 from LAB M, A Neogen Company. 620 Lesher Place, Lansing, MI 48912 USA) or DG18 agar (5g peptone, 10g glucose, 1g KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$, 1.0 ml Dichloran of 0.2% in ethanol, 0.1 chloramphenicol, 15g agar, 1L distilled water) to which the air flow was directed. For each sample 50 litres of air was collected. Samples were collected outside the participants' homes as well as in different rooms inside the houses. The plates were then transported to the lab, incubated at 26 ℃ for 7 days before enumeration. Few isolates from previous studies (Ncube 2016, unpublished), collected in the same manner in five Stellenbosch University residences, were also used as part of this study. Spores from colonies resembling *Cladosporium* were transferred to malt extract agar and pure cultures were obtained through successive transfers.

DNA isolation, amplification and sequence analysis.— The Möller et al. (1992) extraction protocol was used to extract genomic DNA from mycelia of fungal colonies cultivated on MEA. The primers, ITS1 and ITS4 (White et al. 1990), were used for the amplification of a region spanning internal transcribed spacers 1 and 2 and the 5.8S rRNA gene (Bensch et al.

2012). For the resolution to be obtained at species level for *Cladosporium*, it is vital that the ITS region is supplemented with partial gene sequences of the actin gene (ACT) using the primers ACT-512F and ACT-783R (Carbone and Kohn 1999). The PCR amplifications were performed following the PCR conditions described in Bensch et al. (2012). The subsequent fragments were then sequenced using the primers ITS1 or ITS4, and ACT-512F or ACT-783R. The quality of seuences were assessed using Chromas v. 2.5.0 and junk ends were trimmed. The sequences were compared against fungal DNA sequences from previous studies (Bensch et al. 2018) and downloaded from the NCBI's GenBank sequence database using a blast search. Sequences with high similarity, if found, were added to the alignments. The sequences were aligned by ClustalW in Mega v. 7.0.21 and manually checked. Following the phylogenetic analysis of the alignments, phylogenetic trees were constructed using the neighbour-joining options, and branch strength were determined through bootstrap with 1000 replications in Mega v. 7.0.21.

3.3. Results

An illustration of the diversity of the identified species has been presented in a form of a pie chart in Figure 2.1. The distribution of species between indoor and outdoor environments is demonstrated in Figure 2.2 while Figure 2.3 shows regional demonstration. Species have been separated by the environment and area from which they were isolated. Species found in both environments and study areas have also been indicated. Overall summaries detailing the location, environment, isolate and accession numbers are represented in Tables 3.1-3.3.

Phylogenetic analysis was done on the ITS and *act* datasets, representing the *C. cladosporioides, C. herbarum* and *C. sphaerospermum* species complexes. The ITS phylogenetic tree (Figure 2.4) confirmed that ITS lacks resolution to differentiate between many *Cladosporium* species. An overlap between the species complexes is also observed in the ITS tree. Furthermore, the ITS tree consists of all 200 isolates used in the study while the actin tree consists of fewer isolates due to the quality of sequences obtained.

The *C. sphaerospermum* species complex (Figure 3.5) resolved 21 species. Of the known *Cladosporium* species, *C. sloanii* (Clade 1) and *C. dominicanum* (Clade 2) were represented by two of our isolates. About 2% of the isolates were represented by the *C. sphaerospermum* species complex. All isolates represented in this complex were from indoor samples as indicated in Table 3.1.

The *C. herbarum* species complex (Figure 3.6) resolved 39 species in distinct clades. *Cladosporium prolongatum* and *C. paralimoniforme* were found along a long branch, implying they are the same, and *C. soldanellae* and *C. ossifragi* were included in the same clade. The closest relative to *C. paralimoniforme* is *C. prolongatum* and that of *C. soldanellae* is *C. ossifragi*, which could be why this was observed. In both instances, this was a new observation; however, a similar observation is reported in Bensch et al. (2018) where *C. basiinflatum* is always clustered with *C. ramotenellum* in a long branch. Furthermore, about 12% of the isolates in this study were represented in the *C. herbarum* species complex. *Cladosporium ramotenellum* was the only species identified within this species complex, as indicated in Table 3.2.

The *C. cladosporioides* species complex illustrated in Figure 3.7 represented at least 15 species isolated from both indoor and outdoor environments, three of them believed to represent previously undescribed species. The new species were represented by the clades 5, 13 and 15. *Cladosporium anthropophilum* was the most represented species in all isolates, followed by *Cladosporium* sp. (clade 16) and *C. ramotenellum* of the *C. herbarum* species complex. Due to the cryptic nature of species of this complex, only type strains of the species with high similarities were added to the alignments. Some of the isolates did not seem to belong to any clade with the known species, suggesting that they are novel (clade 14 and 16), however, there was no statistical support for this. Therefore, they have not been described and have been referred to as *Cladosporium* sp1 and *Cladosporium* sp.

During the study 200 strains of *Cladosporium* species were isolated. Of these, 110 were the most compatible to be used for identification using a multigene phylogenetic analysis. The majority of isolates were represented by species of the *Cladosporium cladosporioides* complex, with 27 of them representing three novelties, *C. brunneis*, *C. umbraticis*, and *C. civitasaurum*. Moreover, five clinically important species, namely *C. anthropophilum*, *C. cladosporioides*, *C. pseudocladosporioides*, *C. oxysporum*, and *C. phaenocomae* were also identified.

3.4. Discussion

Cladosporium species in the southern hemisphere have mainly been isolated from plant material and soil, and very little is known about those in indoor environments. *Cladosporium* has been reviewed comprehensively to clarify the phylogeny and taxonomy of its species (Crous et al. 2007, Zalar et al. 2007, Bensch et al. 2010, 2012, 2015) and recently molecular

phylogenetic approaches were implemented to study the diversity of *Cladosporium* species in indoor environments (Bensch et al. 2018). It is important to know which fungal species are present in indoor environments, given the fact that some fungi can cause health problems.

While ITS is a suitable locus for identifying isolates belonging to *Cladosporium* and even specific species complexes, additional loci are necessary for species identification (Bensch et al. 2018). As noted by Bensch et al. (2018). An overlap between the species complexes is observed in the ITS tree (Figure 2.4, red coloured boxes), especially in the *C. cladosporioides* and *C. herbarum* species complexes. This is seen in this study where *C. ruguloflabelliforme* and *C. basiinflatum* appear in the *C. cladosporioides* species complex even though they belong to the *C. sphaerospermum* and *C. herbarum* complexes, respectively. With the aid of actin sequences, a better resolution was found and species were identified.

Air samples from different outdoor and indoor environments, mostly houses, were studied, but the interest was mainly in species found in indoor environments. The study had 110 isolates of which 86% of those isolates were represented in the *C. cladosporioides* species complex. A significant number of the isolates did not belong to any known species of this genus, suggesting they are novel. Three potential new species were identified in this study, all from the *C. cladosporioides* species complex. Amongst the species found in this study were *C. oxysporum* and *C. cladosporioides* which have been previously reported to cause human infections (Chen et al. 2013, Jayasinghe et al. 2017). Moreover, a species known from clinical samples (Sandoval-Denis et al. 2016) *C. anthropophilum*, was also identified. Although the clinical relevance and the threshold of this species is not known, its presence in high quantities in indoor environments may be harmful.

In the *C. herbarum* species complex, only *C. ramotenellum* was identified. This species has been previously isolated from indoor environments (Bensch et al. 2018). Furthermore, *C. ramotenellum* has previously been isolated in clinical samples (Sandoval-Denis et al. 2015, 2016) although it is not known to cause human infections. Interestingly, all the samples represented in the *C. herbarum* species complex came from only one of the two study sites, Western Cape; and none were recovered from Gauteng. Gauteng is an inland province in South Africa with subtropical climates while the Western Cape has Mediterranean climates influenced by the oceans surrounding it. It is, therefore, possible that the distribution of *Cladosporium* species, particularly those in this complex, may be geographically influenced.

Based on the studies by Segers et al. (2015) and Bensch et al. (2018), it would be anticipated that species of the *C. sphaerospermum* species complex are the predominant species in indoor environments, an idea that may seem disputed by Horner et al. (2004) who suggested that *C. cladosporioides* is the dominant indoor fungus. However, in this study neither *C. sphaerospermum* nor *C. cladosporioides* were overly represented. As a matter of fact, there were only two species from this complex, *C. sloanii* and *C. dominicanum* representing only two of our isolates, and two isolates identified as *C. cladosporioides*. Interestingly, *C. halotolerans,* which is found abundantly in indoor surfaces (Segers et al. 2015) and house dust (Bensch et al. 2018), was not found at all in this study. This study, however, only used air samples, not swabs from surfaces. This may be the reason why this xerotolerant species was not identified.

Cladosporium species are among the most isolated fungal species in both indoor and outdoor environments (Bensch et al. 2018). The exact number of *Cladosporium* spores required to induce infections and allergic reactions is not definite or known, however, D'Amato and Spieksma (1995) suggested that 3000 spores per cubic meter induce allergic reactions. To the best of my knowledge, there is no known threshold for infections caused by species of this genus. This is problematic because there is no way of knowing if *Cladosporium* species occurring in low numbers in indoor environments can cause infections. Also, there is no evidence yet suggesting that prolonged exposure to low numbers has potential to induce infections.

Additionally, *Cladosporium* species have been implicated in a number of health issues ranging from leg lesions (Gugnani et al. 2006) to infections of the central nervous system (Kantarcioglu et al. 2002, Lalueza et al. 2011, Chen et al. 2013). In this study, some pathogenic species such as *C. cladosporioides,* and *C. oxysporum* were identified. However, these known pathogens did not occur in high quantities, and with no definite threshold, no conclusions could be made on whether the low values of these particular species could actually induce infections. This too applies for every other species identified, more especially the novel ones whose pathogenicity has not yet been studied. This study has shown that indoor environments are home to a number *Cladosporium* species, and that there is a need to understand the pathogenicity of all species belonging to this genus.

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macrocarpum brain abscess after endoscopic ultrasound-guided celiac plexus block. Endoscopy. 43:E9-E10.

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Table 2.1 A representation of species found in the *Cladosporium sphaerospermum* species complex.

Isolate I.D	Species name	Location Western Cape (WC) or Gauteng (G)	Indoor or Outdoor	Clade	Accession numbers	
					ITS	ACT
Clad 155	*C. dominicanum*	G	Indoor	1	MK111439	MK314680
Clad 212	*C. sloanii*	WC	Indoor	2	MK111440	MK314679

Table 2.2 A representation of species found in the *Cladosporium herbarum* species complex.

Isolate I.D	Species name	Location Western Cape (WC) or Gauteng (G)	Indoor or Outdoor	Clade	Accession numbers	
					ITS	ACT
Clad 256	*C. ramotenellum*	WC	Indoor	3	MK111456	MK314681
Clad 264	*C. ramotenellum*	WC	Indoor	3	MK111459	MK306453
Clad 252	*C. ramotenellum*	WC	Indoor	3	MK111455	MK306452
Clad 249	*C. ramotenellum*	WC	Outdoor	3	MK111454	MK306451
Clad 215	*C. ramotenellum*	WC	Indoor	3	MK111452	MK314676
Clad 32	*C. ramotenellum*	WC	Outdoor	3	MK111461	MK314644
Clad 35	*C. ramotenellum*	WC	Outdoor	3	MK111462	MK314645

Clad 37	*C. ramotenellum*	WC	Indoor	3	MK111463	MK329220
Clad 38	*C. ramotenellum*	WC	Outdoor	3	MK111464	MK314646
Clad 29	*C. ramotenellum*	WC	Indoor	3	MK111460	MK314648
Clad 214	*C. ramotenellum*	WC	Indoor	3	MK111451	MK314675
Clad 248	*C. ramotenellum*	WC	Outdoor	3	MK111453	MK306450
Clad 259	*C. ramotenellum*	WC	Indoor	3	MK111457	MK314682

Table 2.3 A representation of species found in the *Cladosporium cladosporioides* species complex.

Isolate I.D	**Species name**	**Location Western Cape (WC) or Gauteng (G)**	**Indoor or Outdoor**	**Clade**	**Accession numbers**	
					ITS	ACT
Clad 67	*C. anthropophilum*	G	Outdoor	4	MK111492	MK314660
Clad 42	*C. anthropophilum*	WC	Indoor	4	MK111489	MK314647
Clad 146	*C. anthropophilum*	G	Outdoor	4	MK111473	MK314636
Clad 123	*C. anthropophilum*	G	Indoor	4	MK111484	MK314662
Clad 108	*C. anthropophilum*	G	Indoor	4	MK111478	MK314639
Clad 120	*C. anthropophilum*	G	Indoor	4	MK111480	MK314661
Clad 128	*C. anthropophilum*	G	Indoor	4	MK111485	MK314663
Clad 74	*C. anthropophilum*	G	Indoor	4	MK111435	MK314659
Clad 117	*C. anthropophilum*	G	Indoor	4	MK111477	MK314665

Clad 110	*C. anthropophilum*	G	Indoor	4	MK111475	MK314664
Clad 72	*C. anthropophilum*	G	Indoor	4	MK111493	MK314656
Clad 125	*C. anthropophilum*	G	Indoor	4	MK111482	MK314666
Clad 84	*C. anthropophilum*	G	Indoor	4	MK111495	MK314651
Clad 80	*C. anthropophilum*	G	Indoor	4	MK111494	MK314652
Clad 94	*C. anthropophilum*	G	Indoor	4	MK111496	MK314642
Clad 95	*C. anthropophilum*	G	Indoor	4	MK111497	MK314643
Clad 277	*C. anthropophilum*	G	Indoor	4	MK111488	MK306449
Clad 280	*C. anthropophilum*	G	Indoor	4	MK111487	MK306448
Clad 121	*C. anthropophilum*	G	Outdoor	4	MK111481	MK314667
Clad 156	*C. anthropophilum*	G	Indoor	4	MK111486	MK314668
Clad 92	*C. brunneis nom prov.*	G	Indoor	5	MK111498	MK314701
Clad 93	*C. brunneis nom prov.*	G	Indoor	5	MK111499	MK314702
Clad 49	*C. brunneis nom prov.*	WC	Outdoor	5	MK111500	MK314697
Clad 65	*C. brunneis nom prov.*	G	Indoor	5	MK111501	MK314698
Clad 141	*C. brunneis nom prov.*	G	Indoor	5	MK111507	MK314704
Clad 119	*C. brunneis nom prov.*	G	Indoor	5	MK111506	MK314703
Clad 83	*C. brunneis nom prov.*	G	Outdoor	5	MK111502	MK314699
Clad 89	*C. brunneis nom prov.*	G	Indoor	5	MK111434	MK314700
Clad 279	*C. brunneis nom prov.*	G	Indoor	5	MK111510	MK314707
Clad 173	*C. brunneis nom prov.*	G	Indoor	5	MK111508	MK314705
Clad 175	*C. brunneis nom prov.*	G	Indoor	5	MK111509	MK314706

Clad 104	*C. austroafricanum*	G	Indoor	19	MK111513	MK314638
Clad 99	*C. austroafricanum*	G	Indoor	19	MK111518	MK306458
Clad 102	*C. austroafricanum*	G	Indoor	19	MK111511	MK314637
Clad 177	*C. xylophilum*	G	Indoor	6	MK111515	MK314673
Clad 98	*C. cladosporioides*	G	Indoor	7	MK111523	MK306457
Clad 97	*C. cladosporioides*	G	Indoor	7	MK111522	MK314641
Clad 165	*C. angustisporum*	G	Indoor	8	MK271392	MK314672
Clad 7	*C. angustisporum*	WC	Indoor	8	MK271397	MK283673
Clad 257	*C. angustisporum*	WC	Indoor	8	MK271394	MK306456
Clad 221	*C. angustisporum*	WC	Indoor	8	MK271393	MK314677
Clad 282	*C. angustisporum*	G	Indoor	8	MK271633	MK314678
Clad 71	*C. angustisporum*	G	Outdoor	8	MK271398	MK314658
Clad 159	*C. angustisporum*	G	Indoor	8	MK271391	MK314671
Clad 73	*C. oxysporum*	G	Outdoor	9	MK111516	MK314657
Clad 86	*C. tenuissimum*	G	Indoor	10	MK111553	MK314649
Clad 87	*C. tenuissimum*	G	Outdoor	10	MK111554	MK314650
Clad 151	*C. asperulatum*	G	Indoor	18	MK111526	MK314640
Clad 64	*C. asperulatum*	G	Indoor	18	MK271396	MK314655
Clad 152	*C. asperulatum*	G	Indoor	18	MK111527	MK314670
Clad 150	*C. myrtacearum*	G	Outdoor	17	MK111541	MK314669
Clad 298	*C. myrtacearum*	G	Outdoor	17	MK111550	MK306455
Clad 12	*C. umbraticis nom prov.*	WC	Indoor	13	MK111530	MK314687

Clad 5	*C. umbraticis nom prov.*	WC	Indoor	13	MK111557	MK283671
Clad 4	*C. umbraticis nom prov.*	WC	Indoor	13	MK111555	MK283670
Clad 48	*C. umbraticis nom prov.*	WC	Indoor	13	MK111556	MK314691
Clad 2	*C. umbraticis nom prov.*	WC	Indoor	13	MK111543	MK283669
Clad 27	*C. umbraticis nom prov.*	WC	Outdoor	13	MK111545	MK314689
Clad 251	*C. umbraticis nom prov.*	WC	Outdoor	13	MK111548	MK314694
Clad 59	*C. umbraticis nom prov.*	G	Indoor	13	MK111561	MK314695
Clad 70	*C. umbraticis nom prov.*	G	Indoor	13	MK111565	MK314696
Clad 13	*C. umbraticis nom prov.*	WC	Indoor	13	MK111539	MK314688
Clad 50	*C. umbraticis nom prov.*	WC	Indoor	13	MK111558	MK314692
Clad 6	*C. umbraticis nom prov.*	WC	Indoor	13	MK111562	MK283672
Clad 56	*C. umbraticis nom prov.*	WC	Outdoor	13	MK111560	MK314693
Clad 33	*C. umbraticis nom prov.*	WC	Indoor	13	MK111610	MK3146960
Clad 1	*Cladosporium* sp. 1	G	Outdoor	16	MK111567	MK321251
Clad 170	*Cladosporium* sp. 1	G	Indoor	16	MK111586	MK321254
Clad 138	*Cladosporium* sp. 1	G	Indoor	16	MK111573	MK321253
Clad 60	*Cladosporium* sp. 1	G	Indoor	16	MK111616	MK321252
Clad 139	*C. civitasaurum nom prov.*	G	Indoor	15	MK111570	MK314708
Clad 140	*C. civitasaurum nom prov.*	G	Indoor	15	MK111571	MK314709
Clad 100	*Cladosporium* sp.	G	Indoor	14	MK111580	MK306459
Clad 103	*Cladosporium* sp.	G	Indoor	14	MK111577	MK306460
Clad 107	*Cladosporium* sp.	G	Indoor	14	MK111579	MK306462

Clad 105	*Cladosporium* sp.	G	Indoor	14	MK111578	MK306461
Clad 113	*Cladosporium* sp.	G	Indoor	14	MK111569	MK306463
Clad 178	*Cladosporium* sp.	G	Outdoor	14	MK111584	MK306464
Clad 226	*Cladosporium* sp.	G	Indoor	14	MK111597	MK306465
Clad 171	*Cladosporium* sp.	G	Indoor	14	MK111589	MK306468
Clad 179	*Cladosporium* sp.	G	Indoor	14	MK111585	MK306469
Clad 222	*Cladosporium* sp.	G	Indoor	14	MK111593	MK306466
Clad 23	*Cladosporium* sp.	WC	Indoor	14	MK111600	MK306470
Clad 77	*Cladosporium* sp.	G	Indoor	14	MK111620	MK306471
Clad 284	*Cladosporium* sp.	G	Indoor	14	MK111606	MK306473
Clad 294	*Cladosporium* sp.	G	Indoor	14	MK111603	MK306454
Clad 300	*Cladosporium* sp.	G	Indoor	14	MK111611	MK306472
Clad 157	*Cladosporium* sp.	G	Indoor	14	MK111583	MK306474
Clad 127	*Cladosporium* sp.	G	Indoor	14	MK111582	MK306475
Clad 69	*Cladosporium* sp.	G	Indoor	14	MK111617	MK306476
Clad 176	*Cladosporium* sp.	G	Indoor	14	MK111587	MK306467
Clad 57	*C. pseudocladosporioides*	G	Indoor	12	MK111532	MK314654
Clad 30	*C. pseudocladosporioides*	WC	Outdoor	12	MK111609	MK314653
Clad 208	*C. pseudocladosporioides*	WC	Indoor	12	MK111601	MK314674
Clad 9	*C. phaenocomae*	WC	Indoor	11	MK111621	MK283674

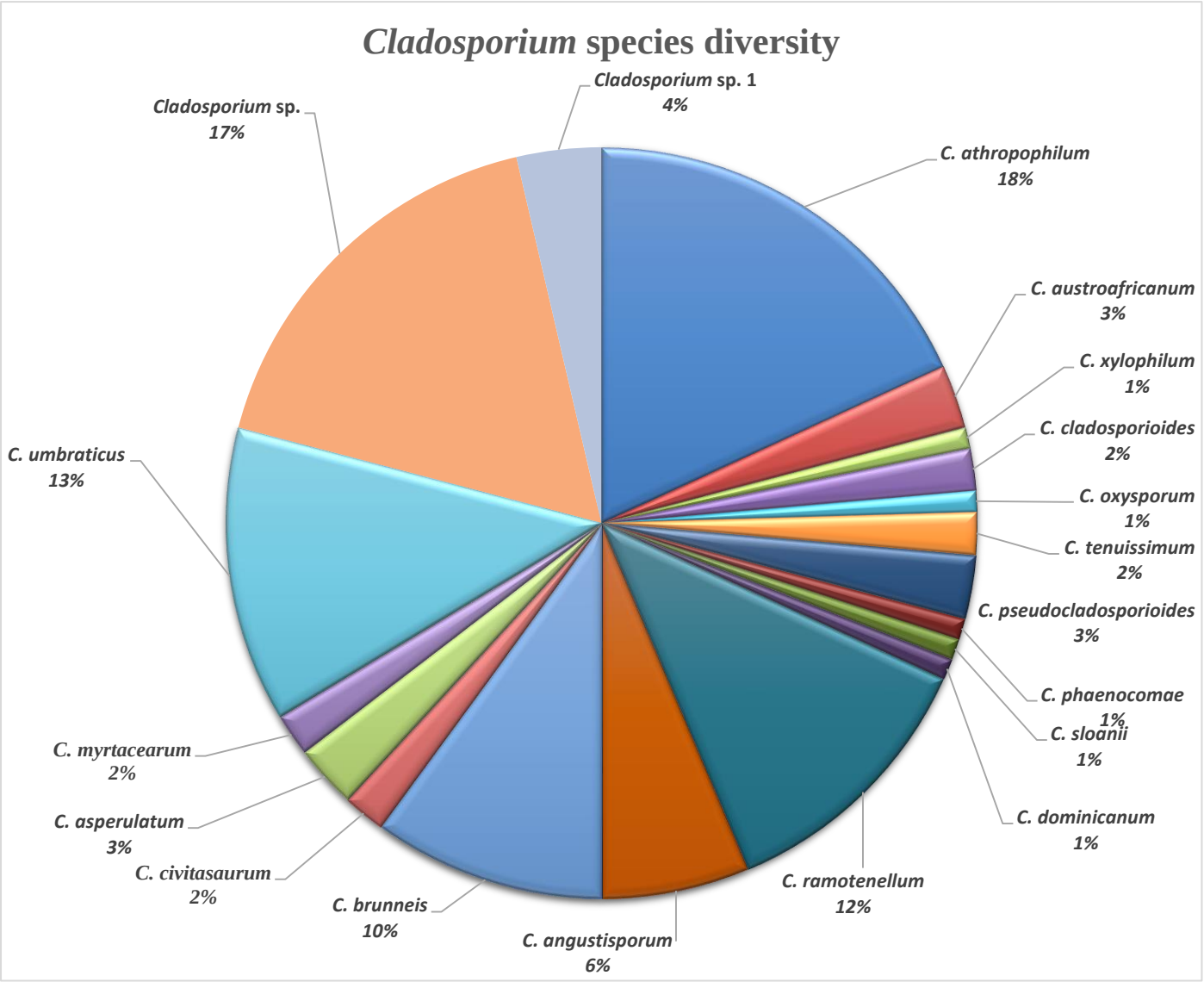


Figure 2.1 A representation of the diversity of *Cladosporium* species isolated from indoor and outdoor areas. The percentages show the prevalence of the identified *Cladosporium* species isolated from both environments.

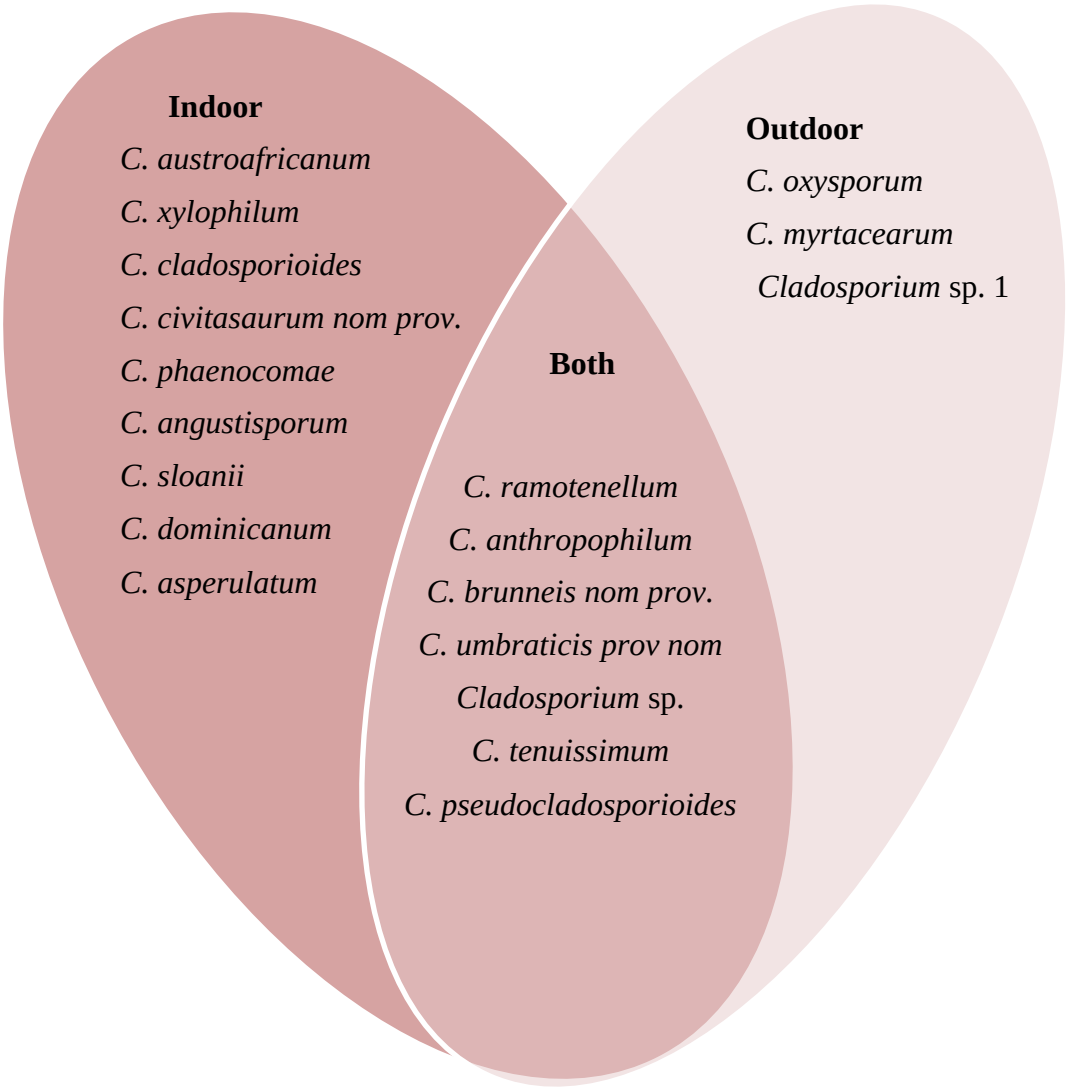


Figure 2.2 *Cladosporium* species distribution between indoor and outdoor environments. Under "Both" are species found in environments.

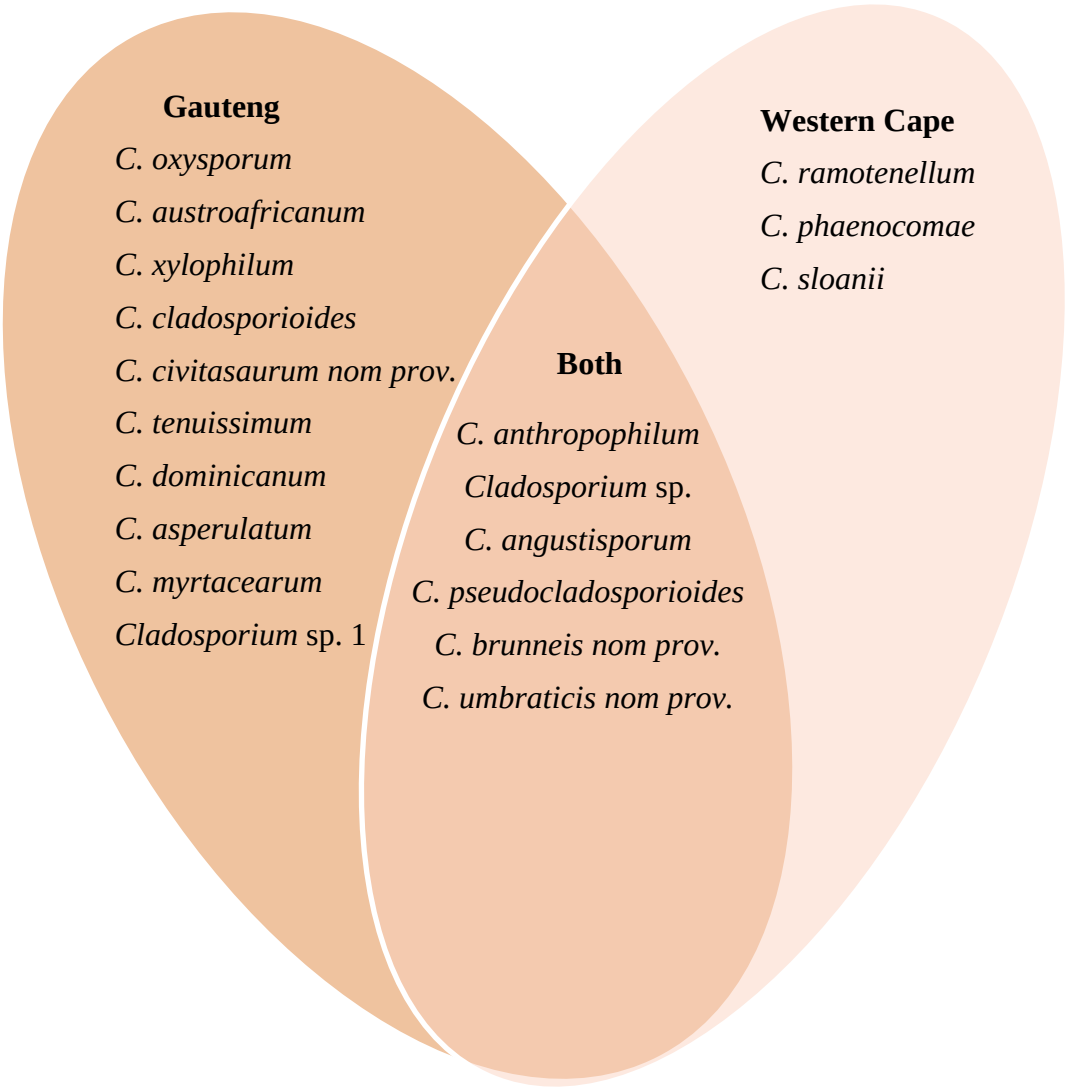


Figure 2.3 *Cladosporium* species distribution between the two studied provinces, Gauteng and Western Cape. Under "Both" are species found in both areas.

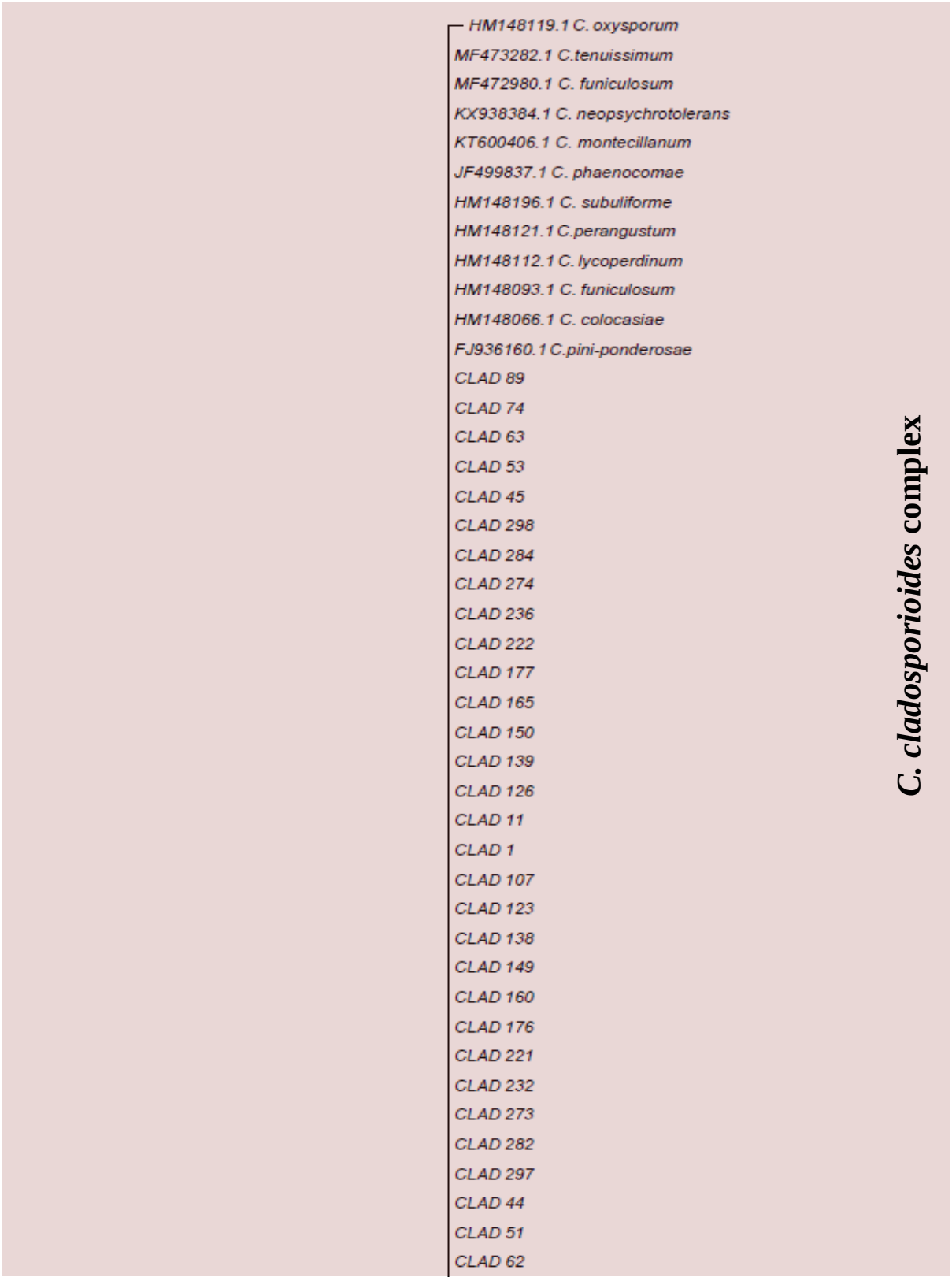
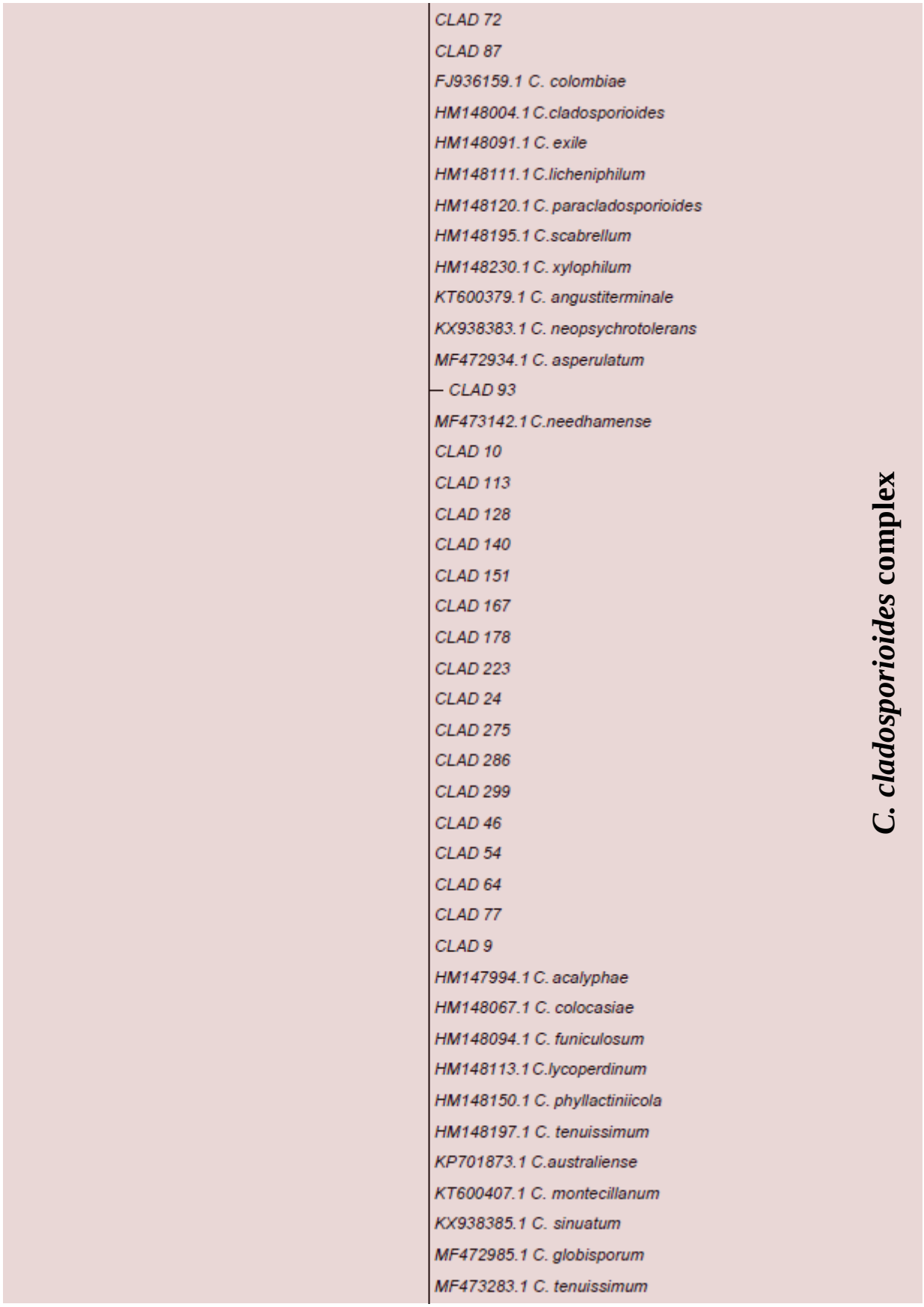


Figure 2.4 A neighbour-joining tree of the *Cladosporium* genus obtained from ITS sequences of 201 strains of *Cladosporium*. The tree is rooted with *Cercospora beticola* CBS 116456. The branch numbers represent bootstrap values of 50% and above. Different coloured blocks distinguish between the species found in this study. Scale bar is 0.02.

Figure 2.4 (continued)



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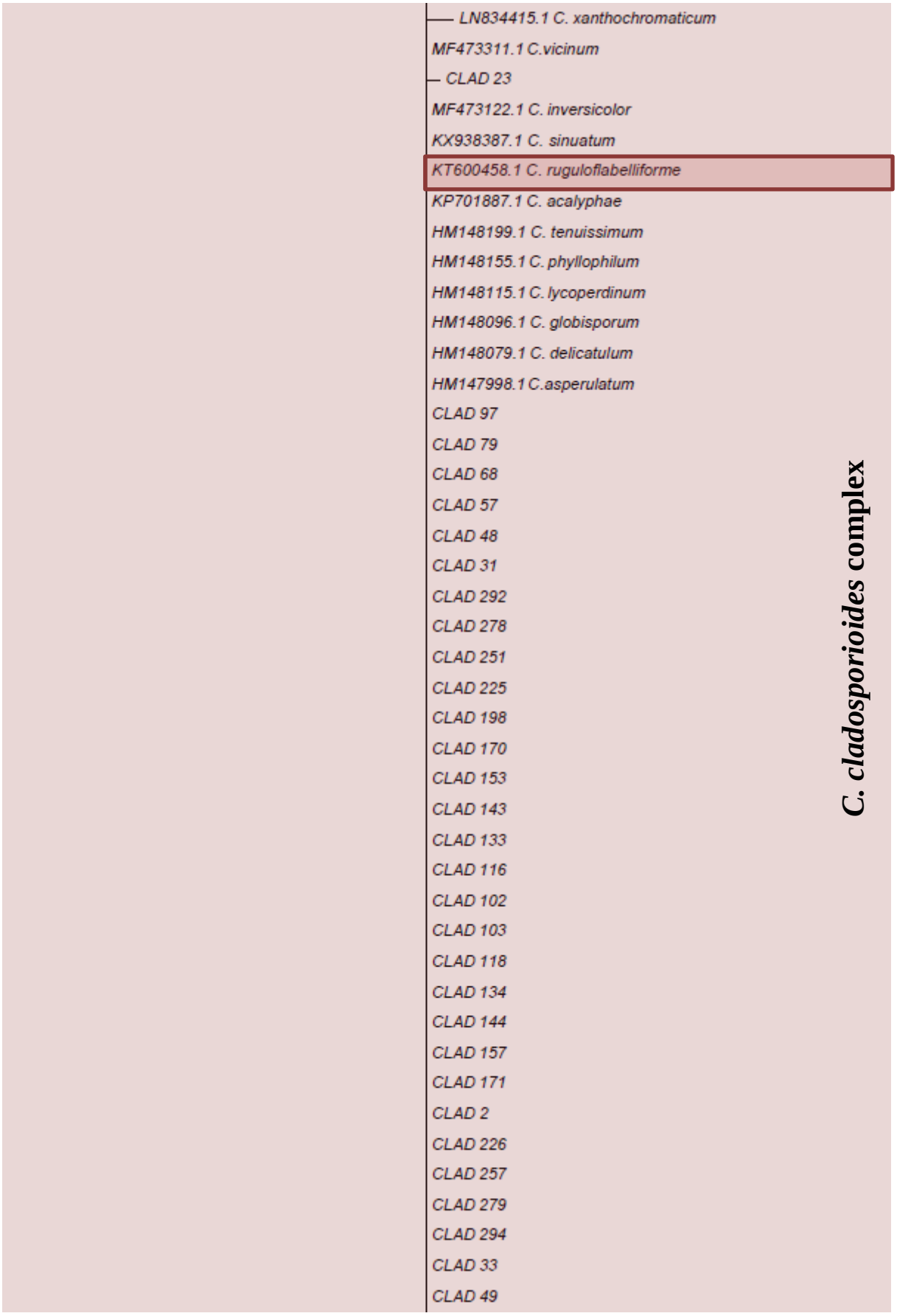

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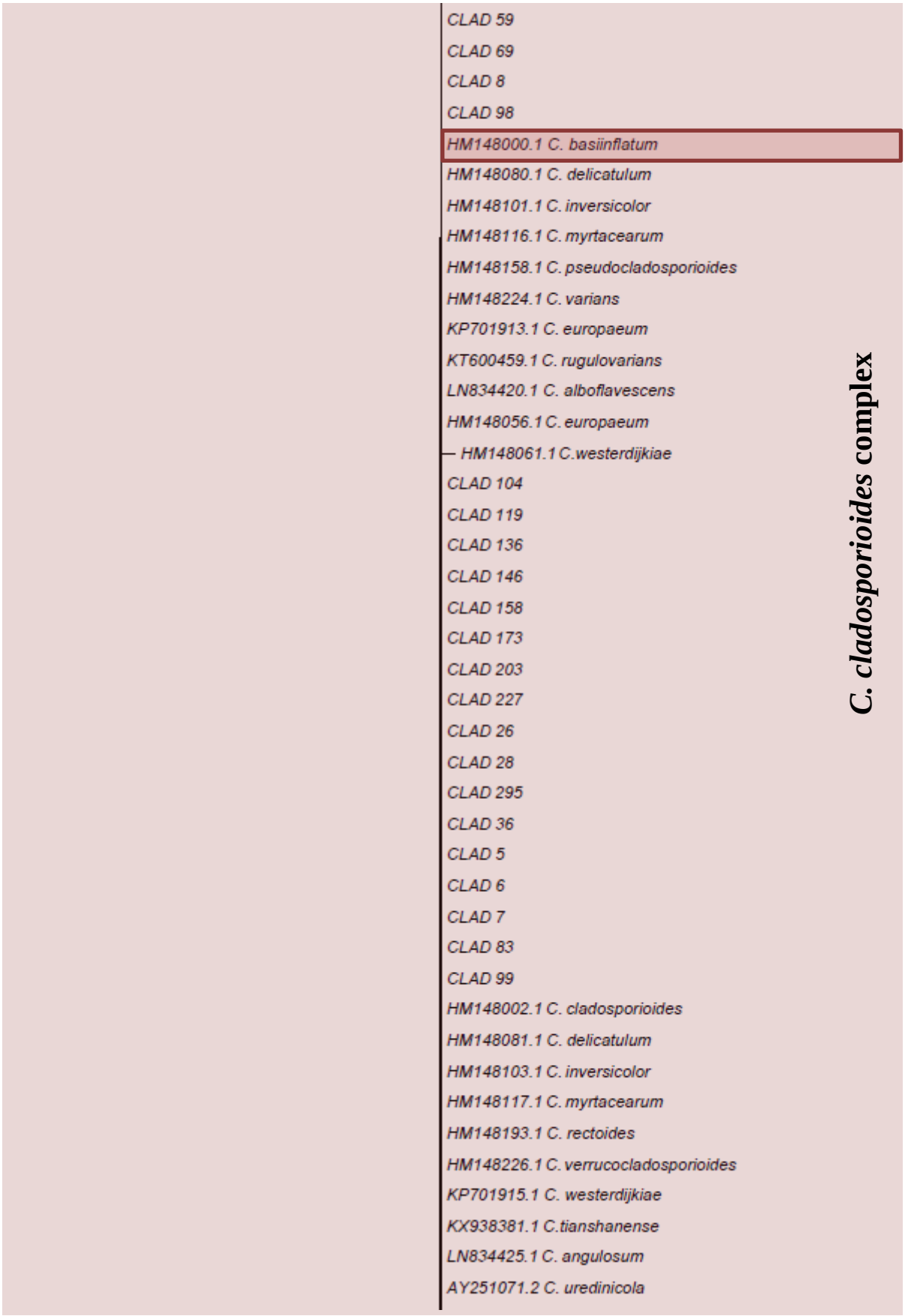

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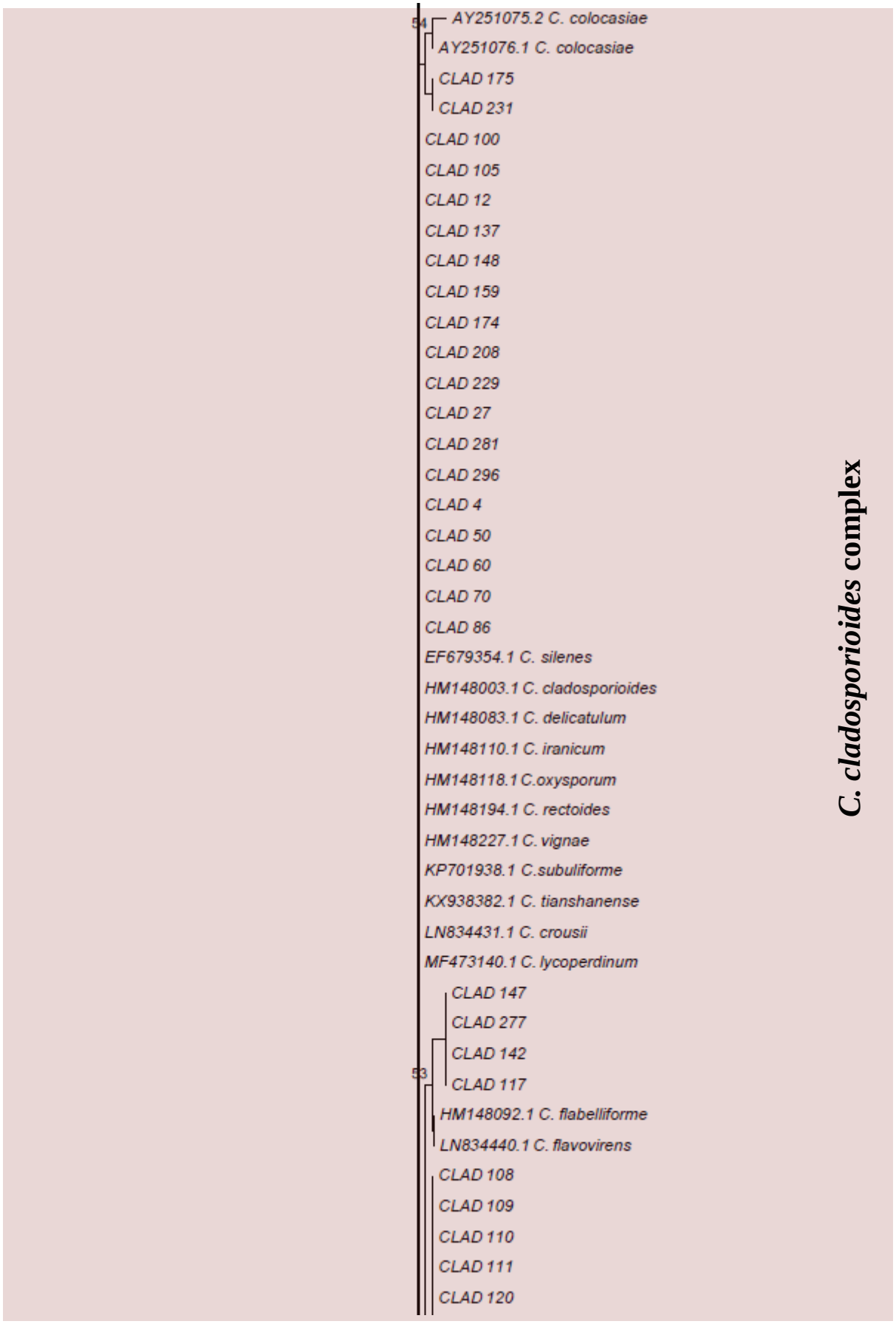

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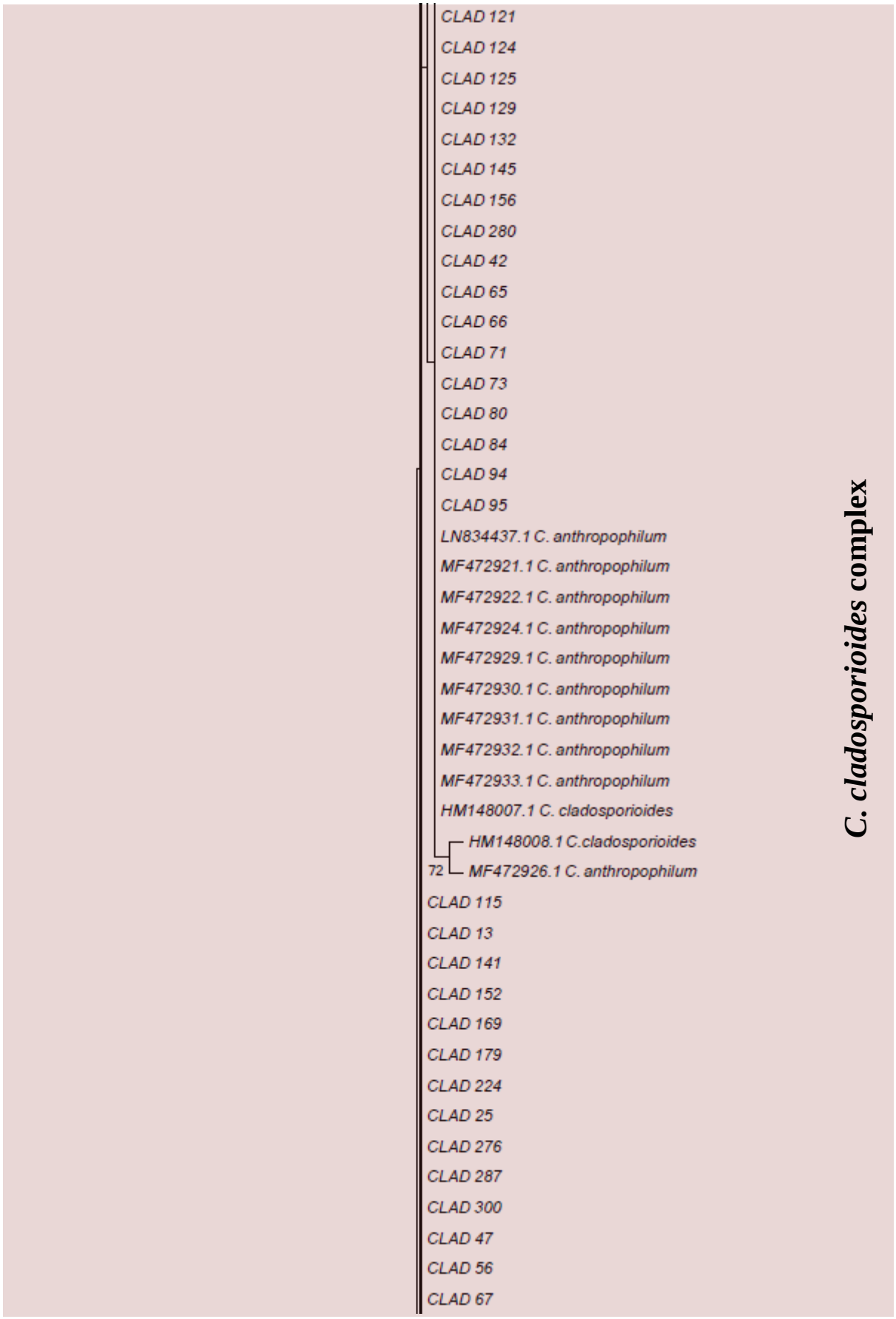

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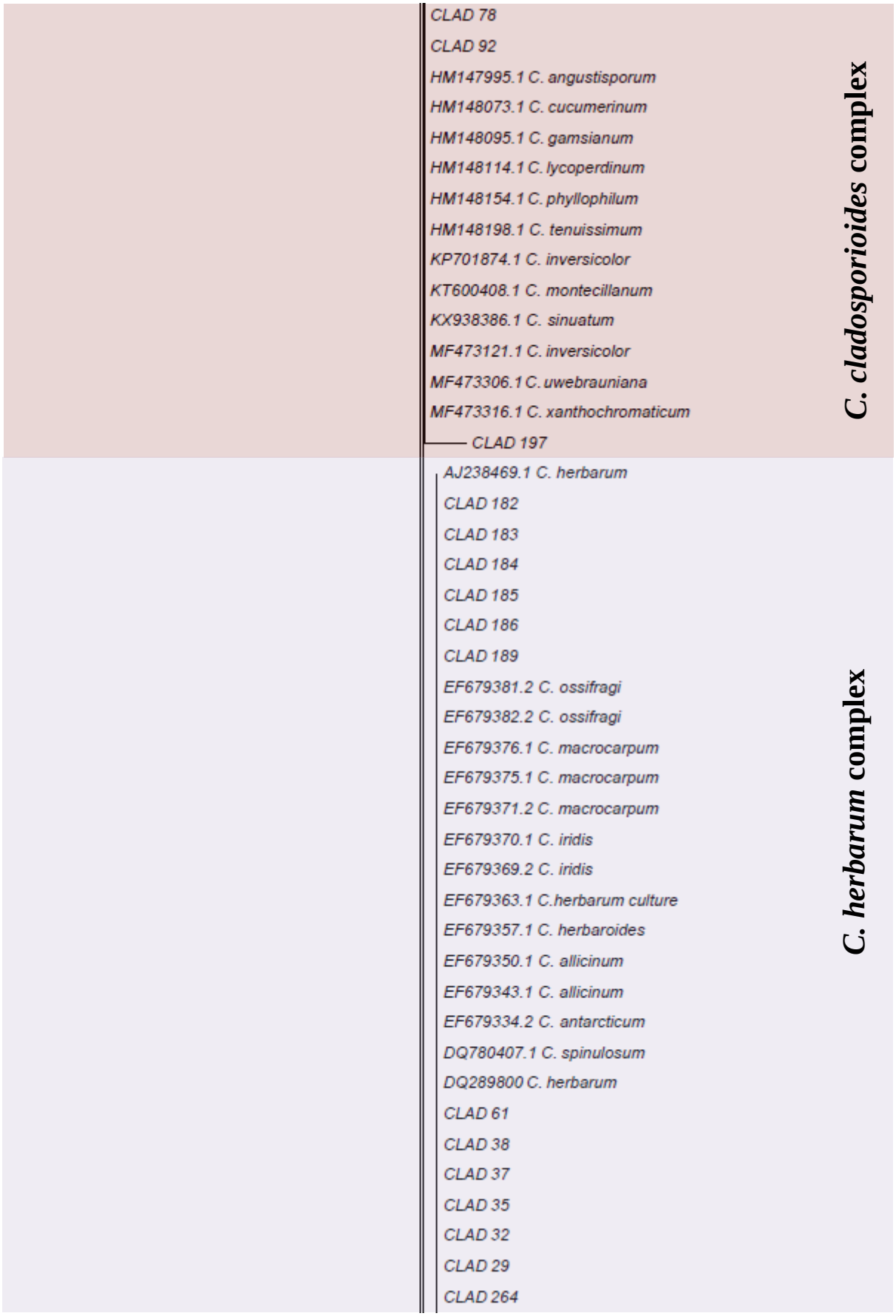

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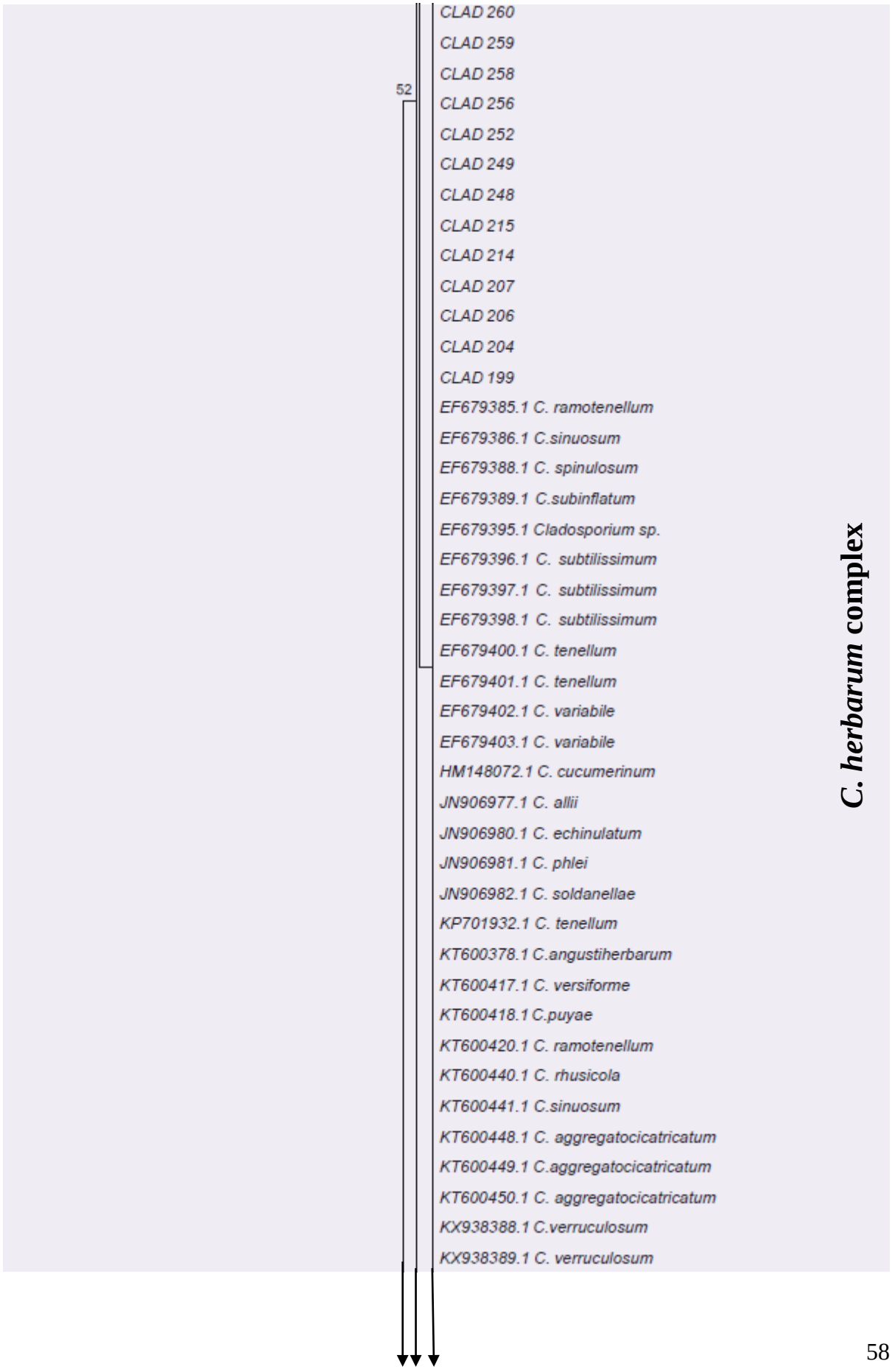

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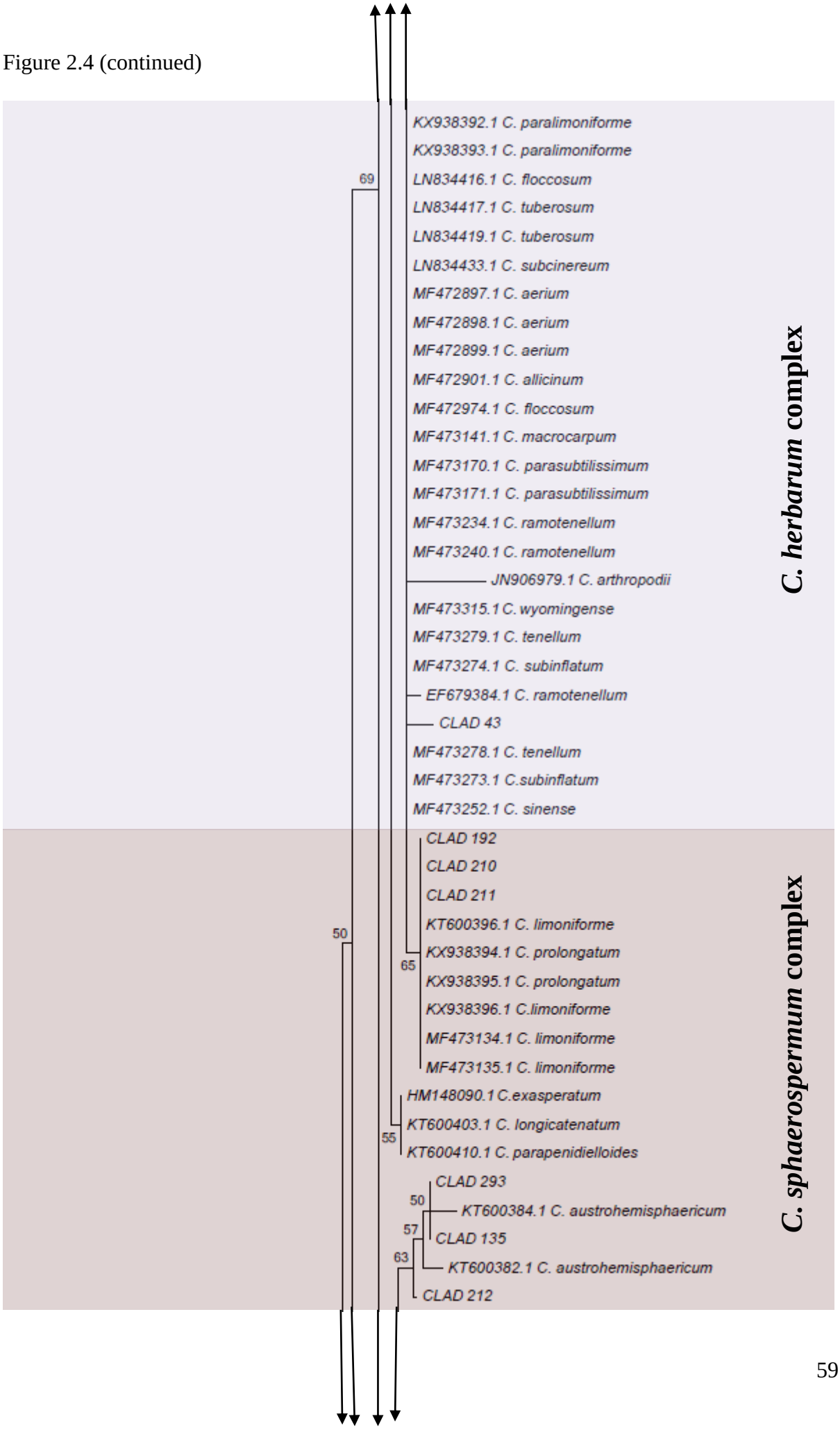

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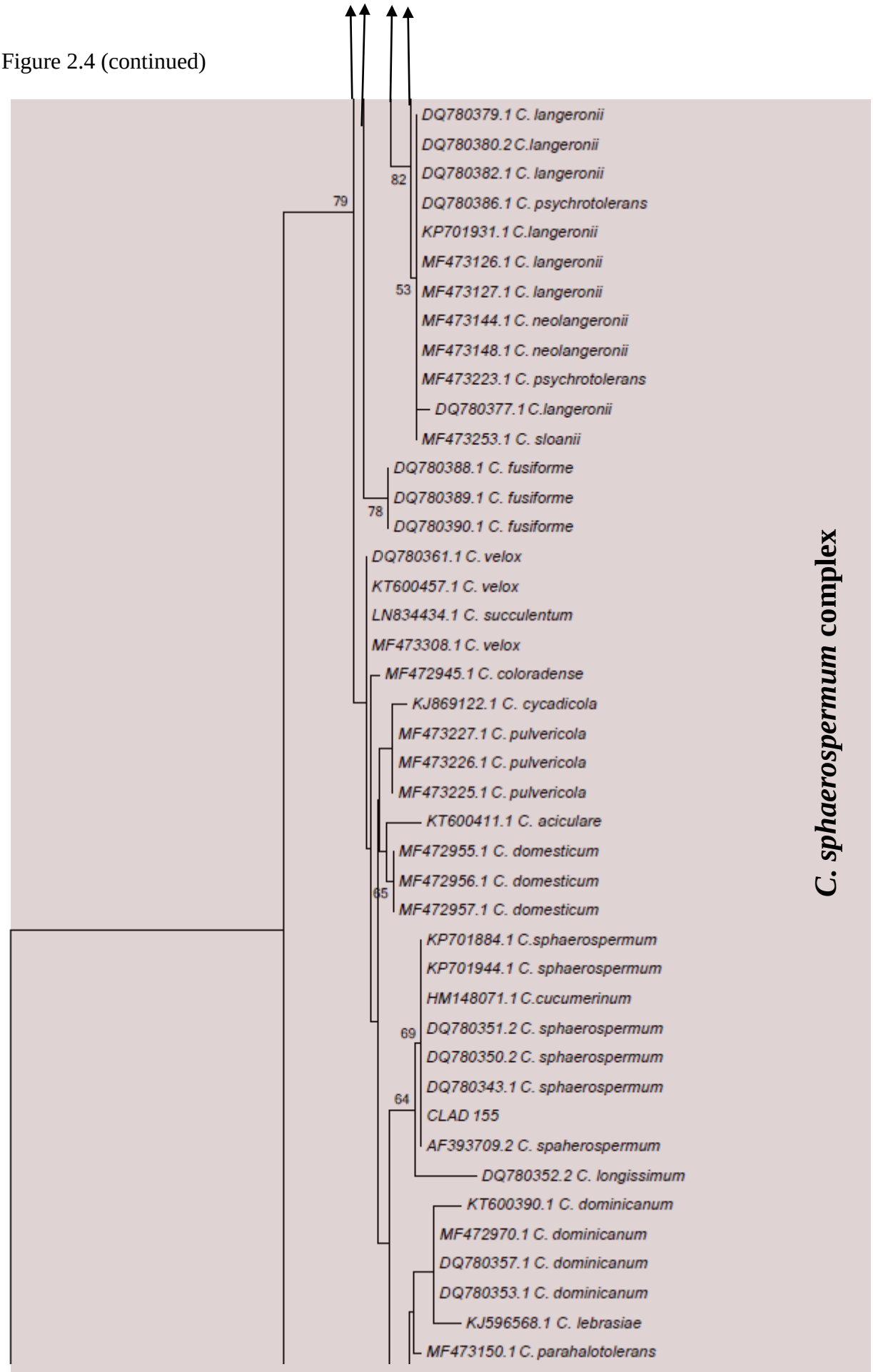

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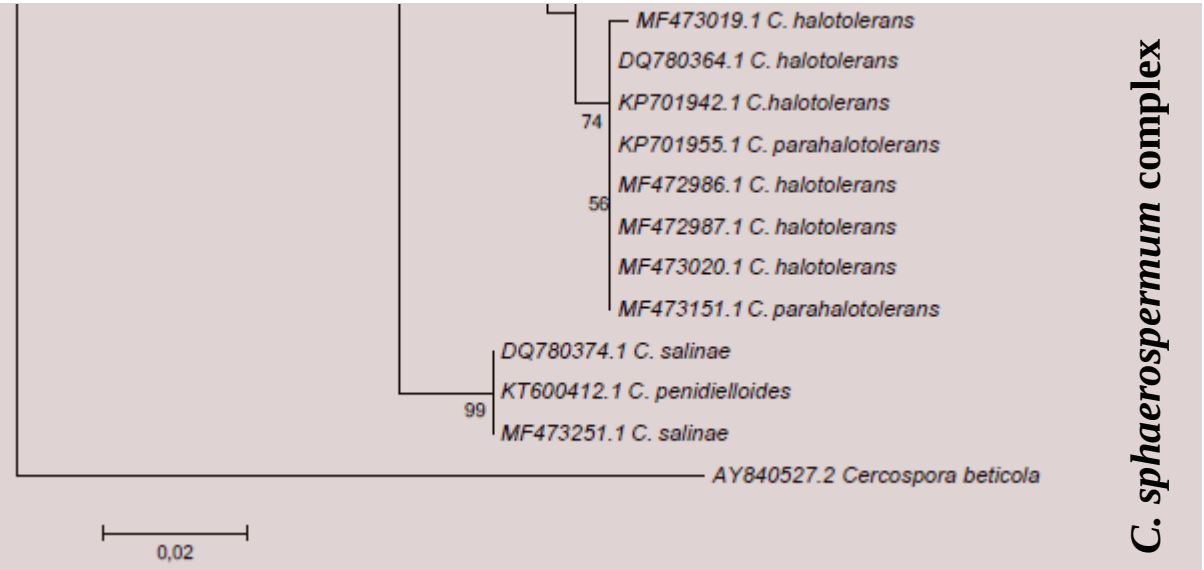


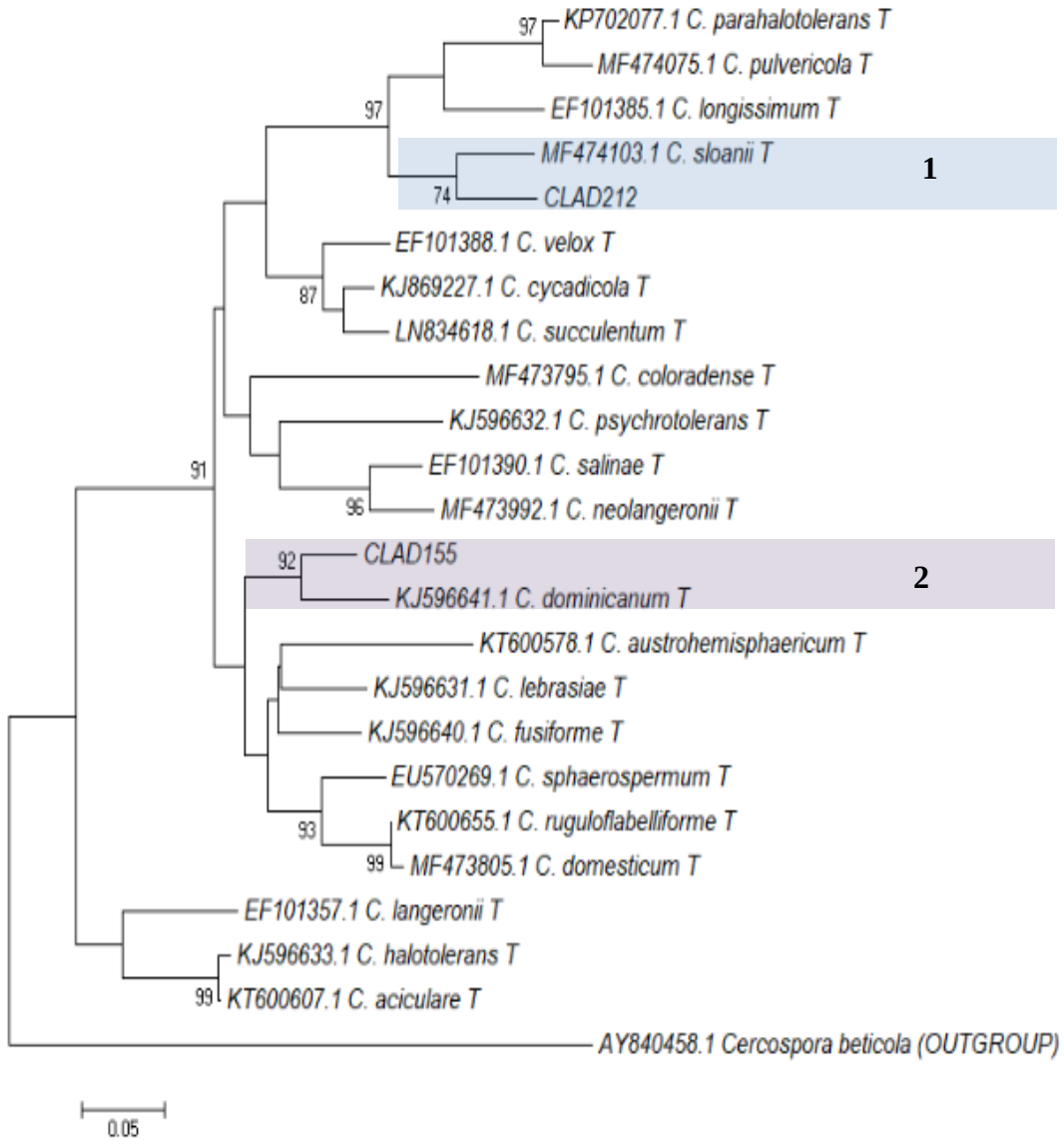


Figure 2.5 A neighbour-joining tree of the members of the *C. sphaerospermum* species complex obtained from actin sequences of 110 strains of *Cladosporium*. The tree is rooted with *Cercospora beticola* CBS 116456. The branch numbers represent bootstrap values of 60% and above. Different coloured blocks distinguish between the species found in this study. The letter "T" refers to type species. Scale bar is 0.05.

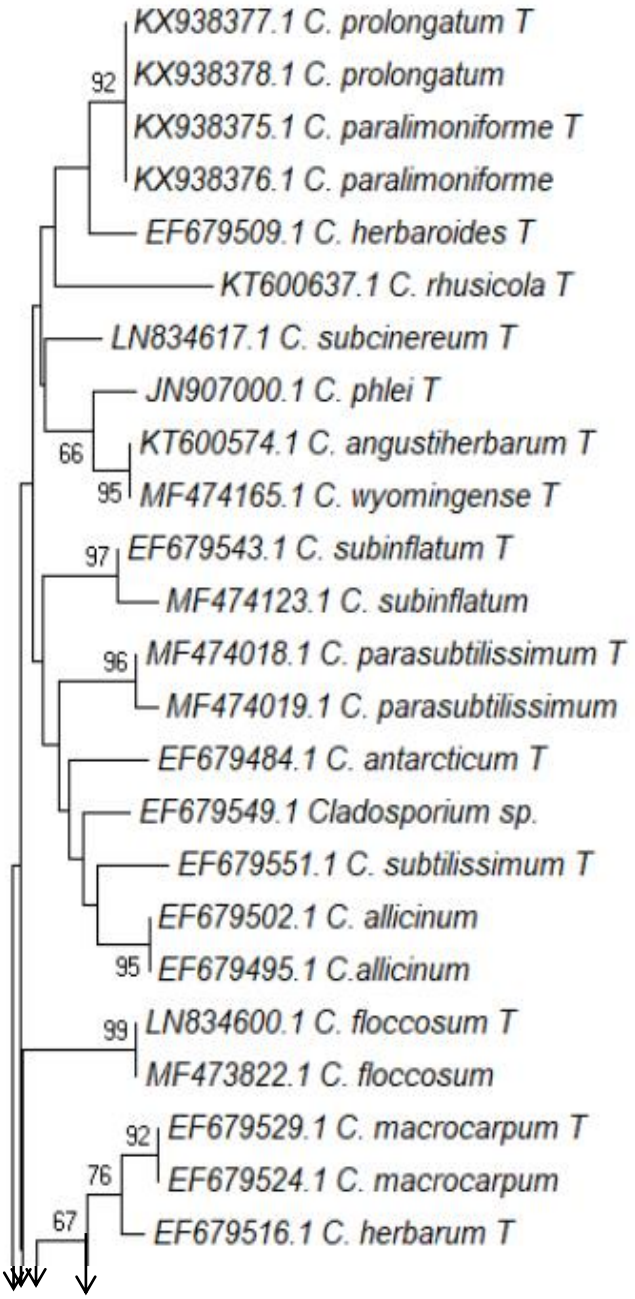
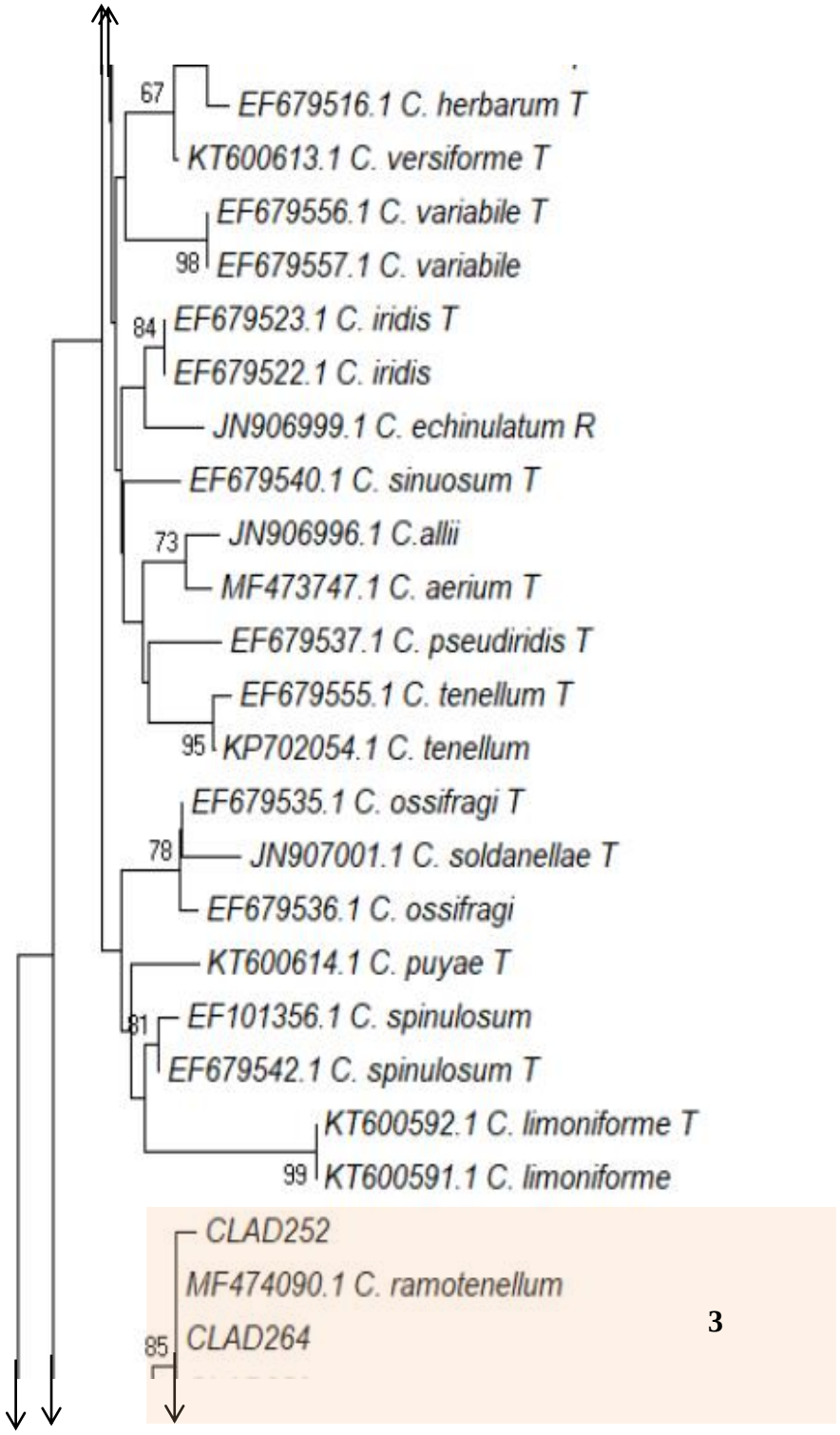


Figure 2.6 A neighbour-joining tree of the members of the *C. herbarum* species complex obtained from actin sequences of 110 strains of *Cladosporium*. The tree is rooted with *Cercospora beticola* CBS 116456. The branch numbers represent bootstrap values of 60% and above. Different coloured blocks distinguish between the species found in this study. The letter "T" refers to type species and "R" to reference strains. The scale bar is 0.05.

(Figure 2.6 continued)



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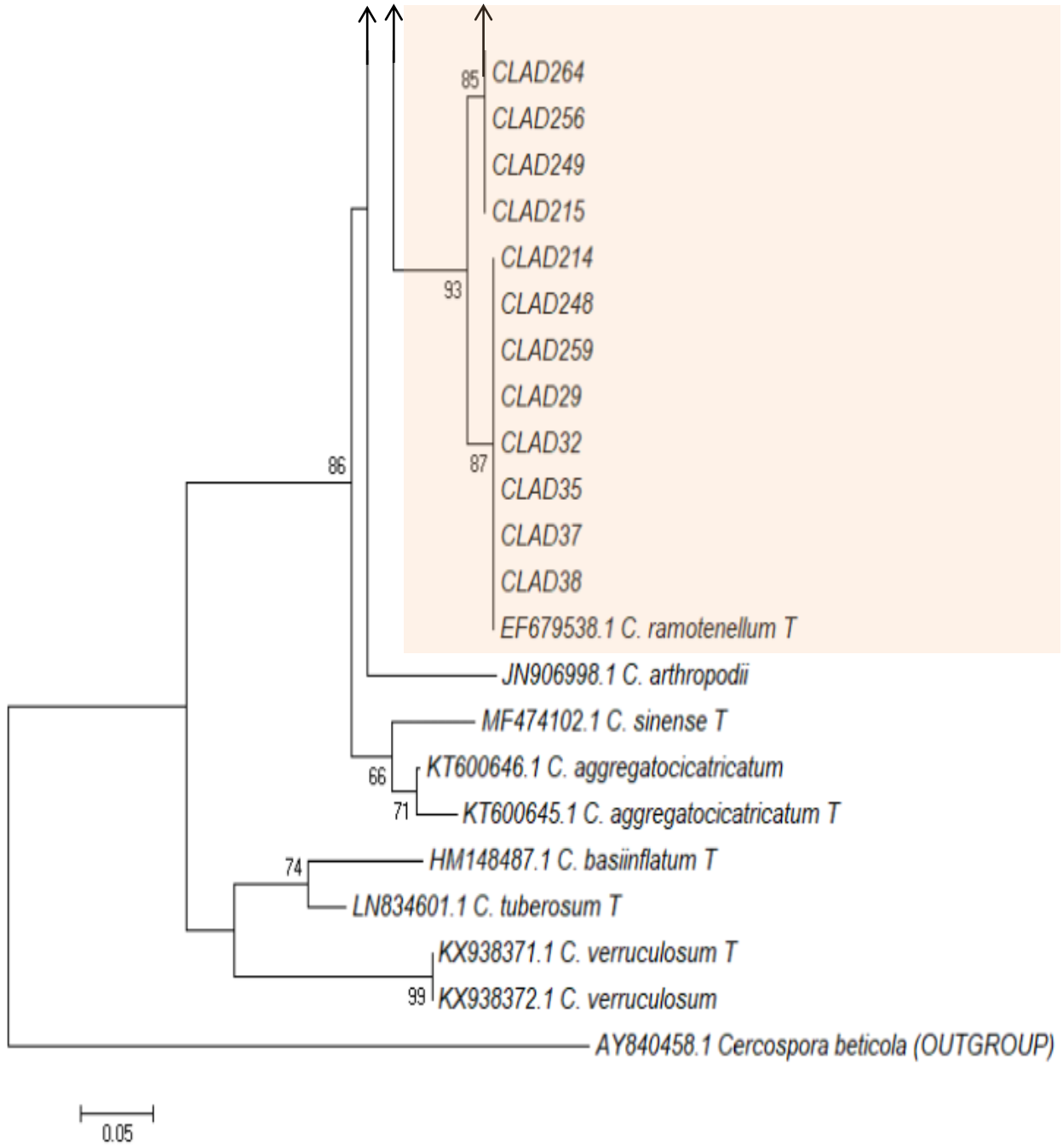

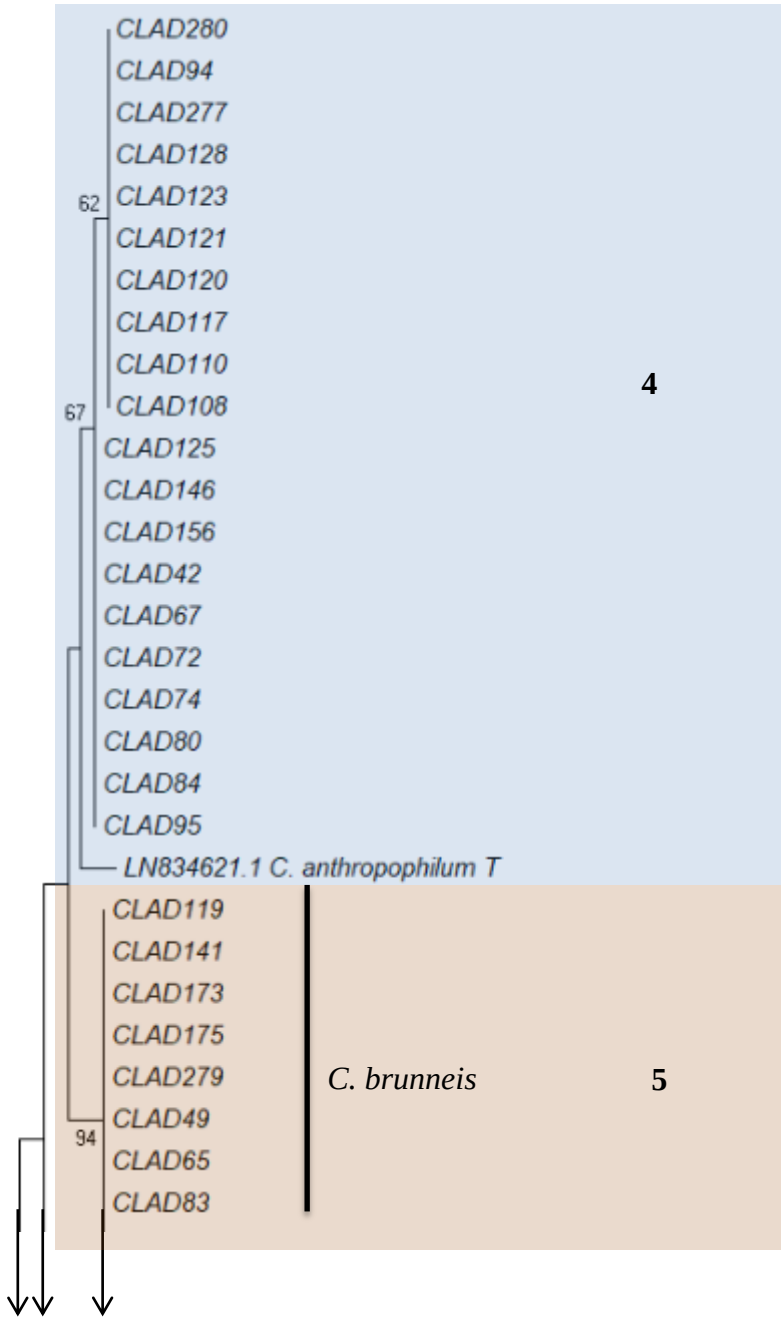

Figure 2.7 A neighbour-joining tree of the members of the *C. cladosporioides* species complex obtained from actin sequences of 110 strains of *Cladosporium*. The tree is rooted with *Cercospora beticola* CBS 116456. The branch numbers represent bootstrap values of 60% and above. Different coloured blocks distinguish between the species found in this study. The letter "T" refers to type species. The scale bar is 0.05.

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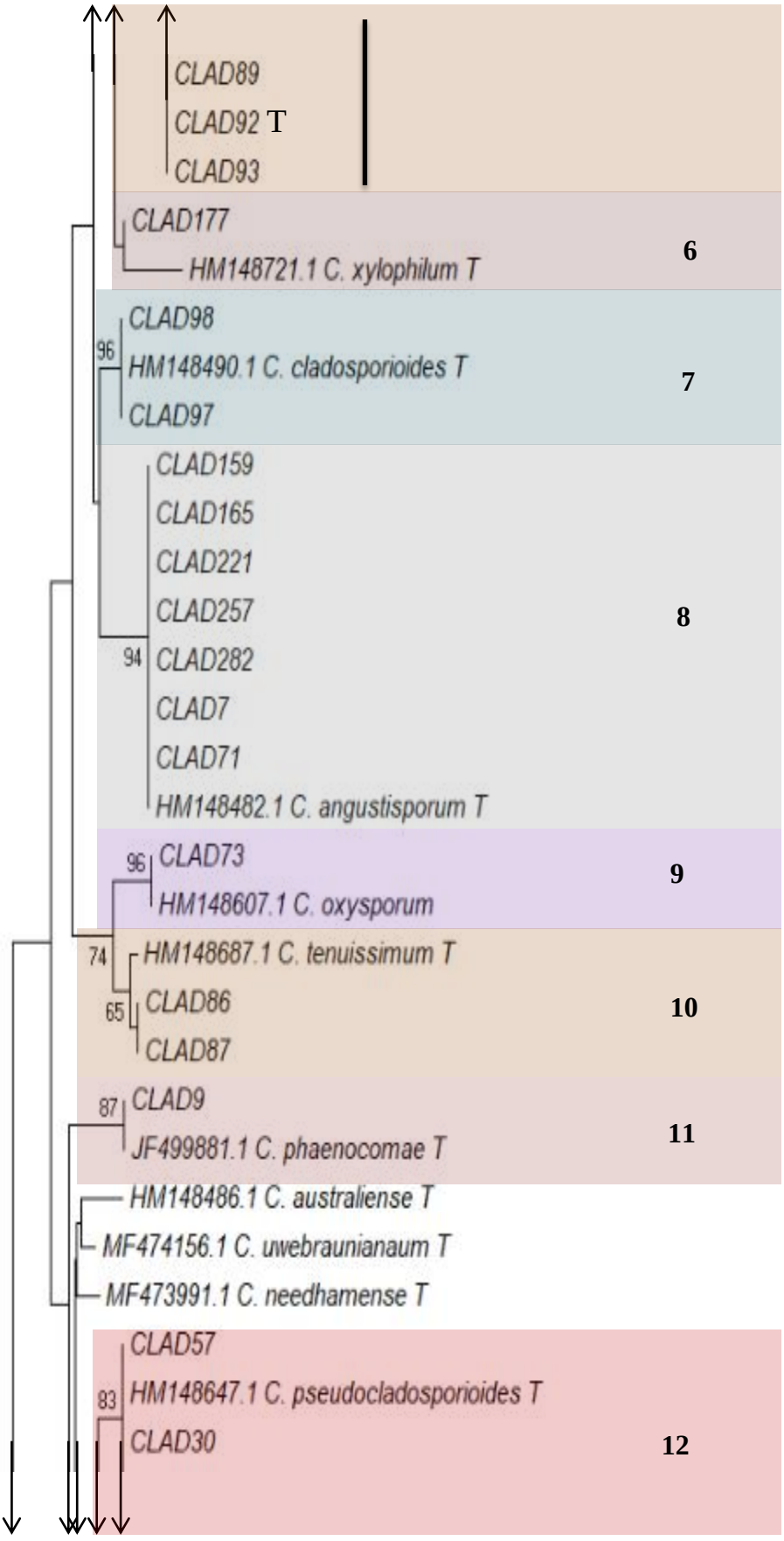

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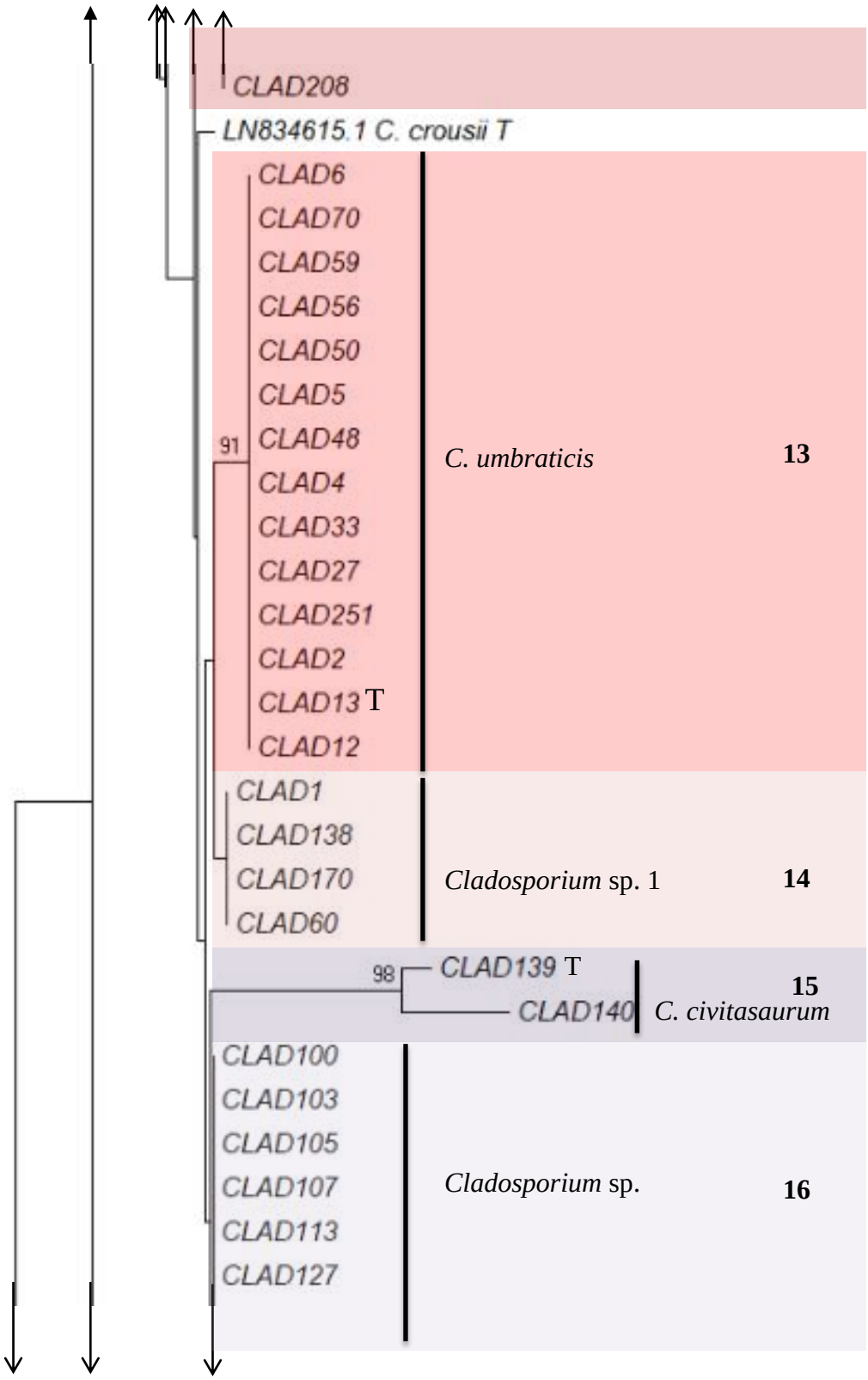

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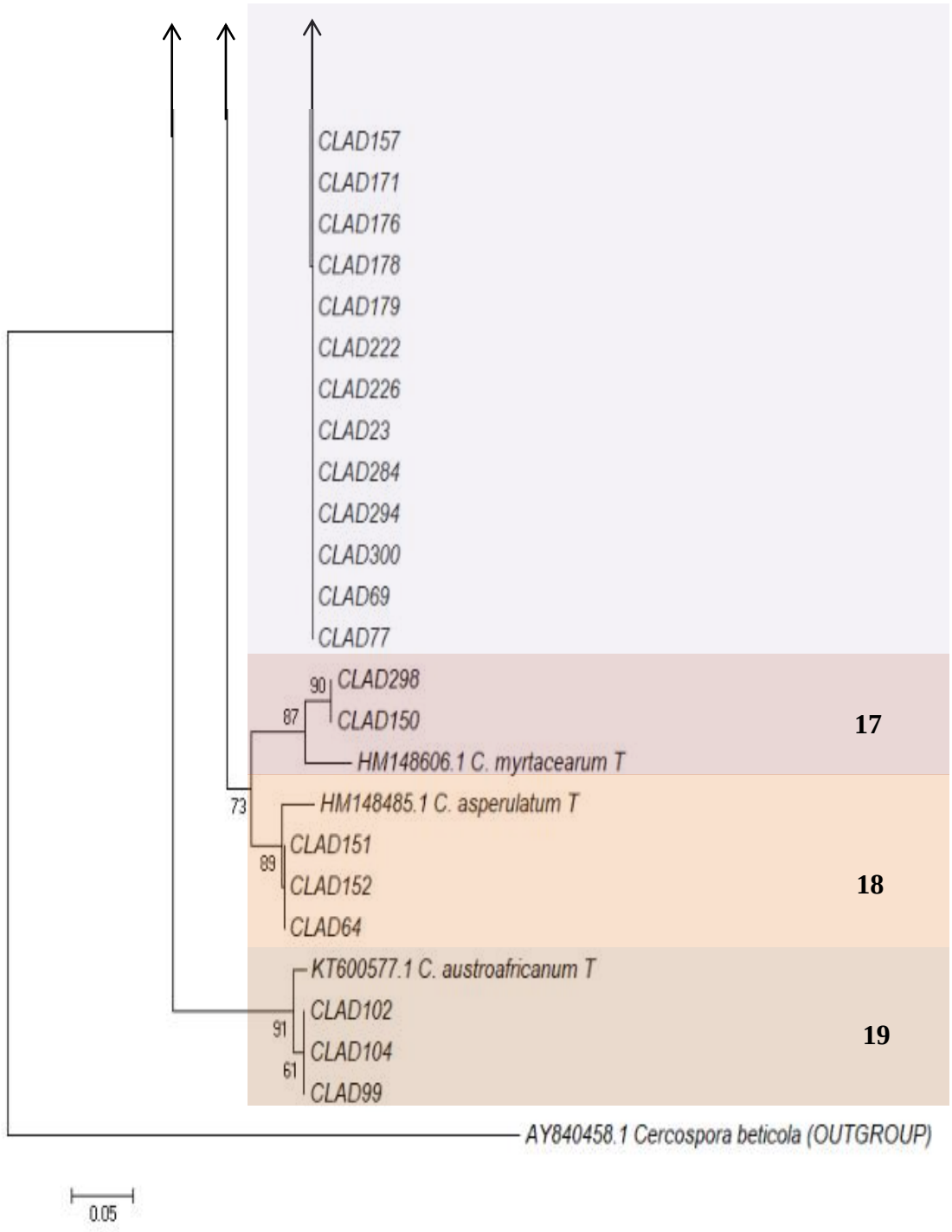
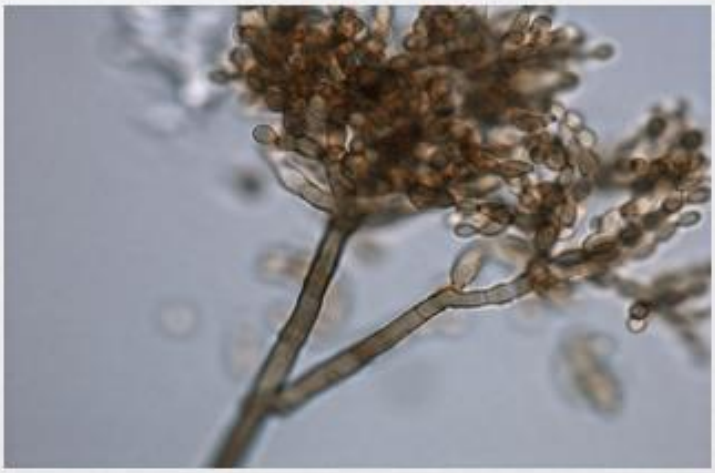


Chapter 4

Three new species of *Cladosporium* from airborne samples



Abstract

Cladosporium species are some of the most heterogeneous and cosmopolitan environmental fungi. In attempts to investigate the prevalence of *Cladosporium* species in indoor environments, a snapshot survey consisting of 110 *Cladosporium* isolates collected in different South African houses, mainly in Johannesburg and Cape Town, was carried out. The study used a polyphasic approach to identify species. Eighteen *Cladosporium* species are treated and three are introduced in this study. All proposed new species belong to the *C. cladosporioides* species complex. Illustrations and descriptions of the new species are provided. The most commonly isolated species indoors is *C. anthropophilum.*

Key words: Taxonomy, New species, Phylogeny, Species complexes, Indoor *Cladosporium*

4.1. Introduction

The genus *Cladosporium*, family *Cladosporiaceae* (*Dothideomycetes*), is defined by its distinct coronate structure of the conidiogenous loci and conidial hila with a raised periclinal rim surrounding the central convex dome (David 1997, Braun et al. 2003). The genus is divided into the *C. sphaerospermum, C. herbarum,* and *C. cladosporioides* species complexes, which are differentiated primarily on morphology (Bensch et al. 2018).

The *Cladosporium cladosporioides* species complex is very complicated, having several cryptic species (Bensch et al. 2010). Species of this complex are very much similar to each other in morphology and are usually distinguished through computer analyses (Bensch et al. 2010) such as phylogenetic analysis, and cultural characteristics and molecular data. Moreover, some species cannot be distinguished by morphology even though they are genetically different. Similar to species of the *C. herbarum* species complex (Schubert et al. 2007), species of the *C. cladosporioides* complex can also be isolated from a broad spectrum of substrates and habitats.

Cladosporium herbarum is the type species of *Cladosporium*, and is one of the most abundant fungi to be isolated in the environment. It is known for its presence on a wide range of substrates including air (Bensch et al. 2018), soil (Ma et al. 2017), and human specimens (De Hoog et al. 2000, Sandoval-Denis et al. 2015, 2016). Species belonging to the *C. herbarum* species complex are characterised by ornamented conidia with the ornamentation alternating between verruculose, verrucose, and spiny (Schubert et al. 2007). In addition, conidia are usually formed in branched or unbranched chains. Conidiophores can both be macro- and micronemetous and are often nodulose, with conidiogenesis restricted at lateral swellings. This feature is an exception in some species of this complex such as *C. subtilissimum,* and *C. limoniforme* (Schubert et al. 2007).

Species of the *Cladosporium sphaerospermum* species complex are characterised by the formation of many globose or subglobose terminal and intercalary conidia (Bensch et al. 2015). All kinds of surface ornamentation observed in the other two species complexes are also observed in this complex because surface ornamentation between species of this complex varies with different species. For instance, surface ornamentation can be nearly smooth (*C. dominicanum*), minutely verruculose (*C. fusiforme, C. langeronii*), verrucose (*C. halotolerans*) or rugose (*C. ruguloflabelliforme*). Additionally, for all species in this complex it can sometimes be challenging to differentiate between conidiophores and hyphae. It has

also been noted that there is more diversity in the species of this complex than the other two species complexes (Bensch et al. 2015).

4.2. Methods and materials

Cultural characteristics were examined from different colonies cultivated for 14 days (Bensch et al. 2010, 2015, 2018) at 26 ℃ on malt extract agar (LAB M). For microscopic examinations, slide preparations were mounted in 85% lactic acid. Important parts of colonies, where conidiophores and conidia were observed under a binocular (Nikon SMZ800) stereomicroscope, were picked using a surgical needle. The slides were then viewed under a light microscope (Nikon Eclipse E800). Micrographs of the isolates were taken as well as measurements of relevant distinguishing features. For conidia, the length and width, of about 50 conidia, were measured. For conidiophores and mycelia, the length, and width were also measured.

4.3. Species description

Cladosporium brunneis Ndlangalavu & Jacobs, *nom prov.*

Etymology: Refers to the brown colour of the conidiophores.

Morphological characteristics: Colonies on MEA reaching 50-55 mm diam after 14 d at 26 ℃, olivaceous green, flat, velvety with whitish vegetative mycelium sparingly scattered at the top, radially furrowed with fimbriated margin, reverse green to black. Mycelium superficial, made up of septate, branched, subhyaline to pigmented, 4.5 µm wide hyphae, slightly but not often rough walls. Conidiophores erect, cylindrical, usually branched and septate, up to 93.5 µm long and 5.0 µm wide, micronemetous conidiophores occasionally formed, 93.5 µm long and 3.5 µm wide. Conidia are solitary, limoniform to fusiform, 4-5(-7) × 2-3.5 (mean±SD: 4.9 ± 0.8 × 2.8 ± 0.7), with a distinctive protruding dark hilum where once attached, microcyclic conidiogenesis not observed.

Specimens examined: *Holotype:* **South Africa, Gauteng,** isolated from indoor air, CLAD92, GenBank MK111498, MK314701.

Substrate & distribution: Species are isolated from both South African provinces and are found both indoors and outdoors.

Notes*:* Closely related to *C. anthropophilum* but different in that morphologically these two species are completely dissimilar. *C. brunneis* has shorter conidiophores, and its conidia are

solitary, limoniform to fusiform while those of *C. anthropophilum* are catenated, oval to ellipsoidal.

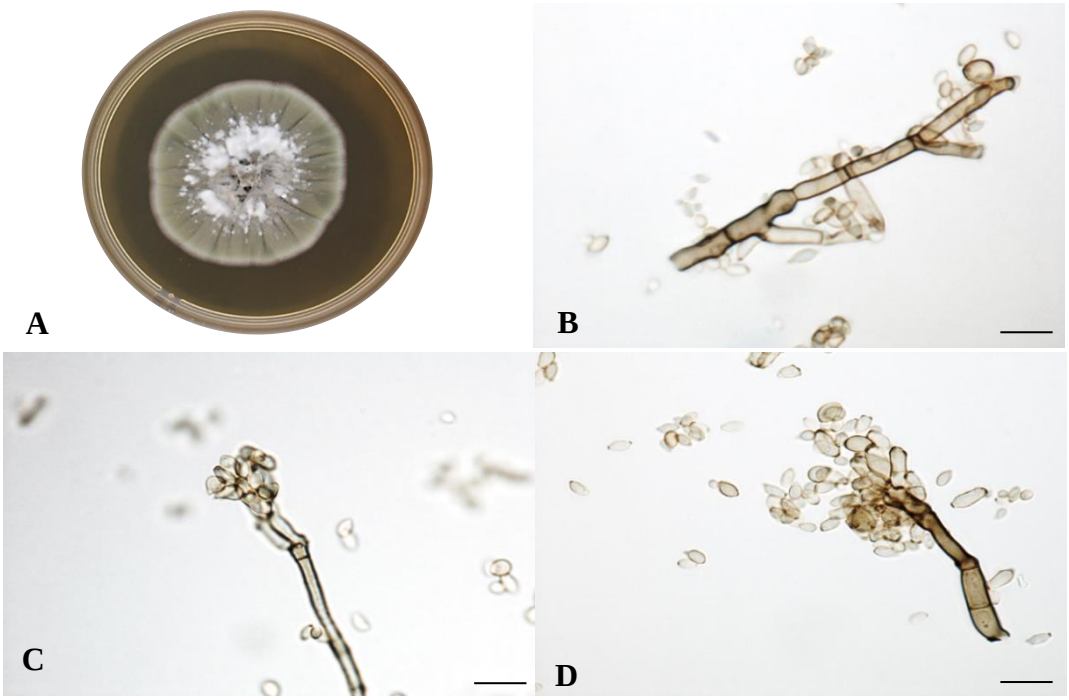


Figure 3.1 ***C. brunneis***. Colony on MEA. **B-D**. Conidiophores and conidia. Scale bars = 10 µm.

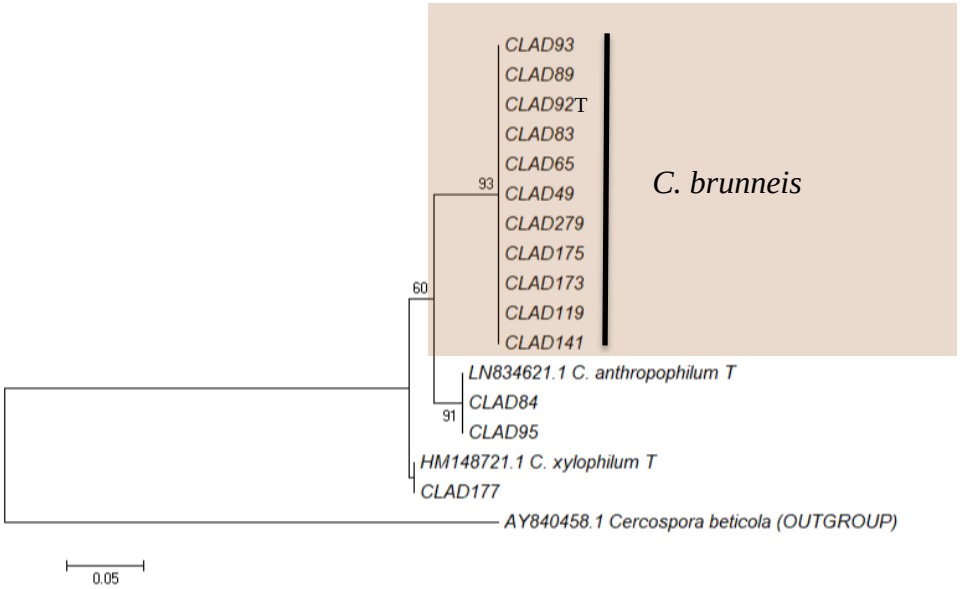


Figure 3.2 A reduced tree showing the placement of the *C. brunneis* in the actin phylogenetic tree.

Cladosporium umbraticis Ndlangalavu & Jacobs, *nom prov*.

Etymology: Name refers to the environment from which the type species was isolated.

Morphological characteristics: Colonies on MEA attaining 55-60 mm diam after 14 d at 26°C, olivaceous green to grey, flat, floccose, black leathery centre as a result of exudates, radially furrowed, whitish entire margin, aerial mycelium abundant, reverse brown to black. Mycelium superficial and submerged, loose, composed of smooth, sometimes rough , branched, septate, 2.5-5 µm wide, dark thick walls, hyaline to pigmented hyphae. Conidiophores erect, cylindrical to oblong, usually branched, arising laterally or terminally from hyphae, septate, pigmented, up to 78.5 µm long and 4.0 µm wide. Conidiogenous cells terminal, cylindrical, subhyaline, 9.5 µm long, 4.0 µm wide and 2.0 µm wide at the base. Conidia occasionally form chains, 2-4(-7) conidia, but mostly solitary, hyaline to subhyaline, unbranched or sparingly branched, obovoid to ellipsoid, (2.5-)3-4.5(-6) × (1.5-)2-3(-3.5) (mean±SD: 3.7 ± 0.9 × 2.2 ± 0.5), distinctive dark hilum where once attached is visible.

Specimens examined: *Holotype:* **South Africa, Western Cape,** isolated from indoor air, CLAD13, GenBank MK111539, MK314688.

Substrate & distribution: Species are isolated from both South African provinces and are found both indoors and outdoors.

Notes: Differs from *C. crousii* in that *C. umbraticis* has shorter conidiogenous cells

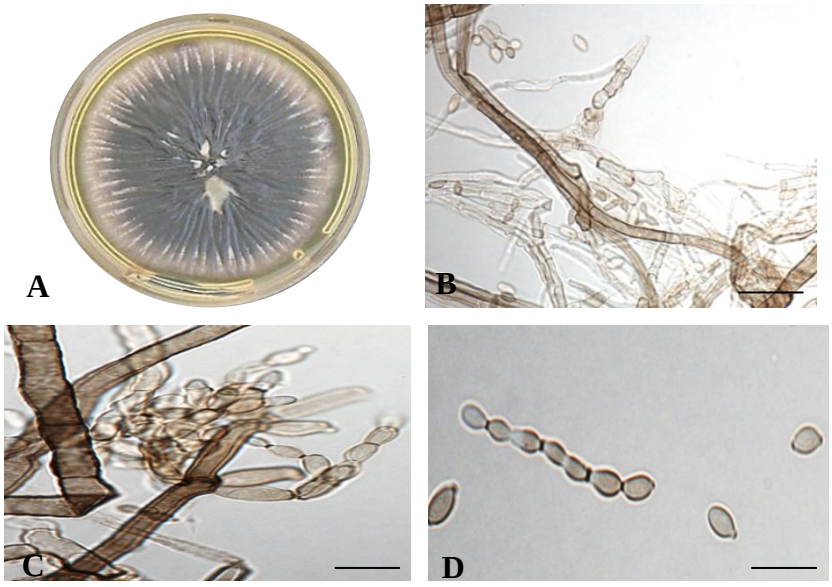


Figure 3.3 ***C. umbraticis.*** **A.** Colony on MEA. **B.** Superficial mycelium. **C-D.** Conidiophores and conidia. Scale bars = 10 µm.

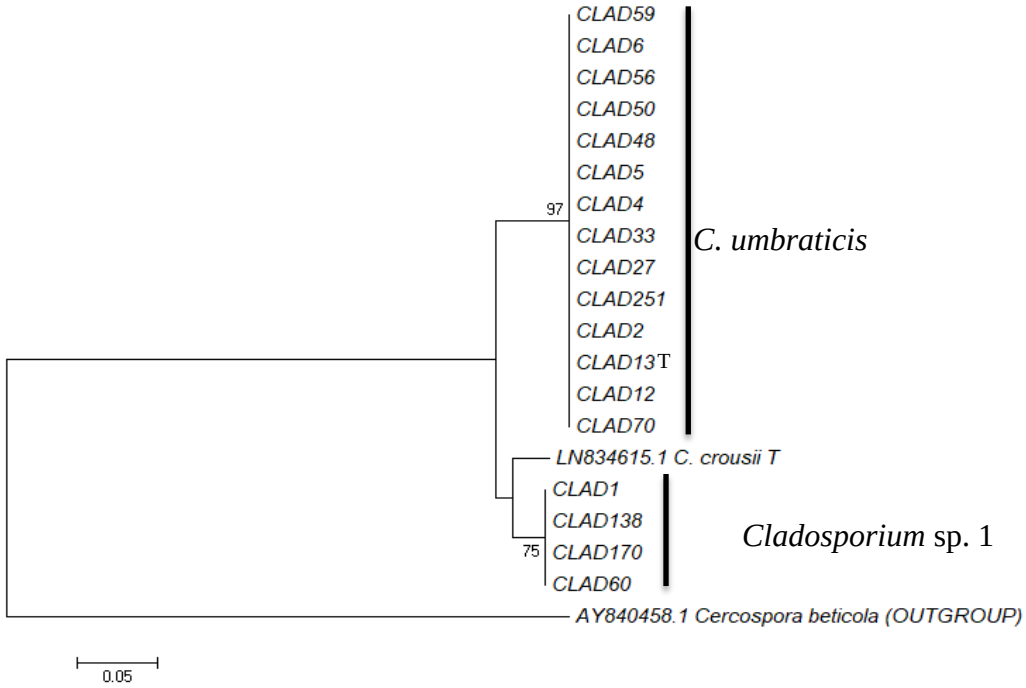


Figure 3.4 A reduced tree showing the placement of the *C. umbraticis* in the actin phylogenetic tree.

Cladosporium civitasaurum Ndlangalavu & Jacobs, *nom prov.*

Etymology: Name refers to the place where it was collected, Johannesburg (Gauteng), known as the "city of gold".

Morphological characteristics: Colonies on MEA attaining 45-50 mm diam after 14 d at 26°C, olivaceous green, flat, velvety, radially furrowed, endulate margin, aerial mycelium abundant, reverse olivaceous green to brownish black. Mycelium superficial, loose, external, composed of smooth, sometimes rough, usually unbranched, septate, 5 µm wide, subhyaline to pigmented hyphae. Conidiophores oblong, slightly curved, usually branched, walls thin or slightly thickened, arising terminally from hyphae, 81-103 µm long and 2.5-4 µm wide. Conidia usually solitary, sometimes form chains of 2-3(-6) conidia, sometimes branched, aseptate, hyaline to subhyaline, ellipsoid, (2-)4-7(-8) × (1-)2-3(-4) (mean±SD: 5.6 ± 1.6 × 2.5 ± 0.6), distinctive dark hilum conspicuous, microcyclic conidiogenesis not observed.

Specimen examined: *Holotype:* **South Africa, Gauteng**, isolated indoor air, CLAD139, GenBank MK111570, MK314708.

Substrate & distribution: Species are isolated from both the Gauteng provinces and are found both indoors and outdoors.

Notes: Differs from *C. crousii* in that *C. civitasaurum* has oblong, slightly curved, and short conidiophores.

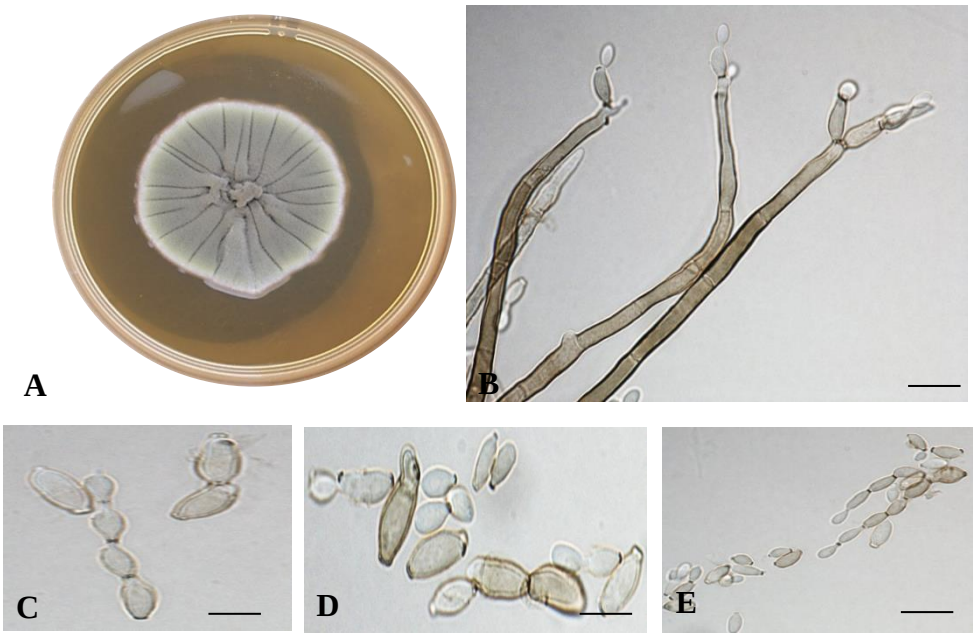


Figure 3.5 ***C. civitasaurum*** **A.** Colony on MEA. **B-E.** Conidiophores and conidia. Scale bars = 10 µm.

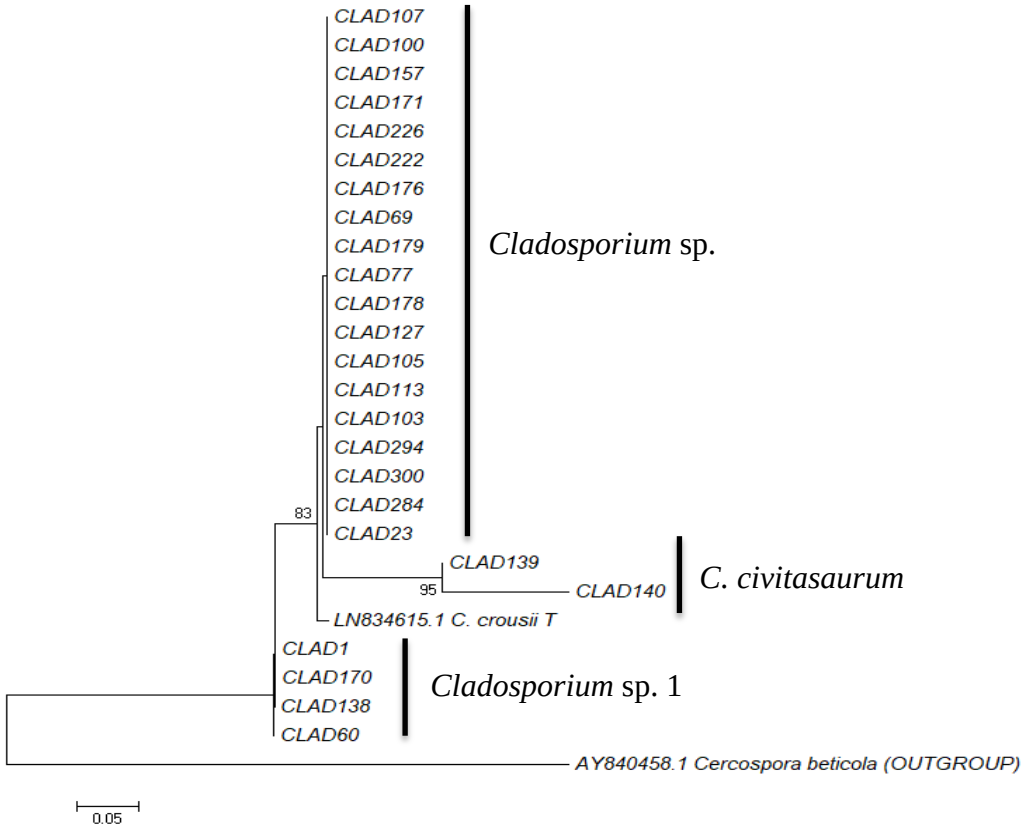


Figure 3.6 A reduced tree showing the placement of the *C. civitasaurum* in the phylogenetic tree.

4.4. Discussion

Cladosporium, previously reported to have more than 772 names (Dugan et al. 2004) has been studied in depth for clarification on its biodiversity and generic concept (Crous et al. 2007, Schubert et al. 2007, Bensch et al. 2010, 2012, 2015, 2018, Ma et al. 2017). Just recently, molecular and phylogenetic studies have been implemented in understanding this genus (Crous et al. 2007, Schubert et al. 2007, Bensch et al. 2012, 2015, 2018). This study contributes to understanding the diversity of this genus using a molecular and phylogenetic approach. With this approach we characterised indoor *Cladosporium* species in a few selected South African indoor environments. Because fungal species are known to possess allergens that cause allergic reactions, it is thus important to know the species housed in our indoor spaces. Sampling from this study was limited to South Africa, in two of the nine provinces in this country and serve as a pilot study. A broader sampling site may give more insight in species distribution across the country.

The new species introduced in this study were collected inside different homes in the Western Cape and Gauteng provinces, and some (very few) were collected just outside the houses where an interaction between indoor and outdoor species is more likely to occur. However, some of the species found indoors were not recovered from outdoor isolates. Nonetheless, in the large number of isolates involved in this study about 25% of the isolates represented the new species described here, with *C. umbraticis* claiming the highest percentage among the species described.

The current study has 5 potential new species, however those not statistically supported to be new (clade 14 and 16) have been excluded, and as a result only 3 new species have been described. Some of the species are morphologically similar to species previously described while some are dissimilar. While *C. civitasaurum* is closely related to *C. crousii*, dissimilarities between the two species were observed. *Cladosporium crousii* has conidiophores that are erect, cylindrical and up to 230 µm long (Sandoval-Denis et al. 2016), while *C. civitasaurum* has oblong, slightly curved conidiophores that can be up to 103 µm long. This shows that the description of species cannot be based on phylogenetic analysis alone, morphological characteristics are also very important. This also demonstrates that species that are genetically similar can have very different morphological features.

One of the most important noticeable observations in the species here is that ramoconidia were not observed. The absence of ramoconidia in all the species can be explained by the fact

that all species do not possess ramoconidia. *Cladosporium anthropophilum*, *C. pseudocladosporioides* and *C. crousii*, which are closely related to each other (Bensch et al. 2018) and related to the new species, have ramoconidia. However, this does not mean that it is a necessity for the newly described species to possess that feature; in fact, this could be a distinguishing feature between the new species and the known *Cladosporium* species they are related to.

Cladosporium anthropophilum is the most isolated species of the known true *Cladosporium* species in this study. *Cladosporium brunneis* is phylogenetically closely related to *C. anthropophilum* but differs in morphology. *Cladosporium anthropophilum* have conidiophores that can be up to 550 µm in length and 2-5 µm in width (Bensch et al. 2018) while those of *C. brunneis* are usually up to 93.5 µm in length and 5 µm wide. However, the conidiophores are branched, septate, erect and cylindrical in both species. *Cladosporium umbraticis* and *C. civitasaurum* are both phylogenetically related to *C. crousii* but are more closely related to each other than *C. crousii*. The difference in their morphology is clear, with formation of conidia, conidial length and conidiophores being different in length and width. Furthermore, in *C. umbraticis* and *C. crousii* conidiogenous cells are observed while they are not in *C. civitasaurum*. The ecology of *C. anthropophilum* and *C. crousii* (Sandoval-Denis et al. 2016), and that of the newly described species, is not yet understood.

The most common traits used to distinguish species from each other here were the length, width, and form of conidiophores, as well as the shape and formation of conidia. Some of the features seem to overlap between accepted *Cladosporium* species and our isolates and this made it difficult to separate them. This study faced a challenge differentiating the newly identified species within the *C. cladosporioides* species complex. This was no surprise as it has been previously noted by Bensch et al. (2010) that species of this complex are very similar in morphology and some species cannot be separated morphologically even though they may be genetically different.

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Chapter 4

Concluding remarks and future research

This study aimed to determine the prevalence of *Cladosporium* species in indoor environments. The results demonstrated that indoor environments, particularly homes, have several described and undescribed fungal species. A large number of isolates represented undescribed *Cladosporium* species, highlighting the potential endemic biodiversity in South Africa. Overall, indoor environments house a great diversity of indoor and outdoor fungi, which potentially pose a threat to human health.

A study by Bensch et al. (2018) found 46 different *Cladosporium* species in indoor environments with 14 of those being in relation with samples derived from human specimens. This study had several isolates representing species from human derived samples as well as species previously reported to cause human infections. This supports the idea that indoor environments are habitats for potential allergenic and pathogenic *Cladosporium* species. Moreover, living conditions such as dampness of the walls, humidity, poor ventilation, and poor sanitation, to name a few create favourable conditions for the growth of different biological contaminants such as *Cladosporium*. It is therefore recommended that homes are kept dry, clean, and dust-free as much as possible. In the case of visible fungi on walls, sinks or bathtubs, proper and thorough cleaning is highly recommended. Additionally, in low-income houses where proper ventilation systems cannot be afforded it is recommended that windows are open daily to help decrease moisture and dampness, and allowing air to enter the house so as to avoid rapid growth of indoor contaminants.

In other studies, *C. halotolerans* was the most common species found in indoor environments, whereas in this study this species was not found. This could be because samples from this study were mainly air samples, and selective media were not used for halotolerant/halophilic organisms. Bensch et al. (2018) identified *C. halotolerans* from South African samples, although all those were collected from house dust. Furthermore, Bensch et al. (2018) suggested that species of the *C. sphaerospermum* species complex can grow at low water activity, which allows them to be persistent on indoor surfaces compared to species from the other two complexes. This could potentially explain why *C. halotolerans* was found

in abundance in their study, where they had dust samples, and not represented in our isolates where only air samples were used.

Cladosporium has not been extensively studied in South Africa, especially in indoor environments. Because of this, and the impact species of this genus have on human health, it is suggested that more studies are done in this country. This study is the first step to understanding indoor *Cladosporium* in South African homes. Further studies on the identification and description of *Cladosporium* species in indoor environments are needed. Comparative studies between high-, middle-, and low-income houses should also be done. Additionally, the relationship between South Africa's leading cause of death, TB, and *Cladosporium* is not yet understood. With studies demonstrating a decrease in allergies after TB treatment, it is safe to say there is a potential link between *Cladosporium* species and TB. Hence, studies investigating the impact *Cladosporium* exposure may have on people living with TB should be explored. Furthermore, the species described in this study should be investigated for their pathogenicity and ability to cause allergies, highlighting the most probable threshold for each species to induce an allergic reaction. Lastly, the prevalence of *Cladosporium* in indoor environments should also be investigated from a seasonal distribution aspect.