Cladosporium species in the natural habitat: a source of contamination for indoor environments

by

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Declaration

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Summary

Indoor environments are ideally trusted for the protection of humans from harsh conditions that are normally encountered in the outdoor environment. However, the growth of microorganisms in indoor environments turns these habitats into potential threat of inhabitats safety and wellness. For that reason, this study addresses the issue of indoor air quality particularly fungal contamination in indoor environments. The indoor fungal communities can have a negative impact that compromises human health ranging from allergic diseases to life-threatening conditions. Furthermore, in South Africa there is still a large gap in the research of indoor fungi, thus, it is important that indoor microbiology findings are made available to the public domain. This study focuses on identifying *Cladosporium* species that grow and proliferate in the indoor environment and hopefully contribute to the understanding of their distribution in indoor environments of Southern Africa (SA). This study identified numerous *Cladosporium* species and described novel species using standardised DNA sequencing approaches and morphological diagnosis where necessary. The number of species identified in this study indicated how little we know of fungi from this region (SA) and revealed the obscured richness of *Cladosporium* diversity in SA in indoor environments.

The genus of *Cladosporium* presents species that are of concern in various disciplines including public health and safety. *Cladosporium* species are ubiquitous fungi in the class Dothidiomycetes, order Capnodiales and family of *Cladosporioaceae*. Early attempts to resolve this genus recognised over 720 species in the early 2000s. These species are usually recognised by their dark pigmented colonies (dematiaceous fungi). The recently adopted DNA sequencing approach to identify *Cladosporium* species has resolved the taxonomic ambinguities of the genus and recognised 218 *Cladosporium* species. This emphasised the difficulty in separating *Cladosporium* species only with morphological based approaches. In this study we used both molecular and morphological techniques to identify *Cladosporium* species from indoor samples collected from Western Cape and received from Gauteng and Zimbabwe. Over 350 samples resembled culture characteristics of *Cladosporium* and 194 samples were subjected to molecular analysis, revealing 29 species, of which six are novel.

Opsomming

Binnehuise omgewings is ideaal vir die beskerming van mense teen toestande wat normaalweg in die buitelug voorkom. Dit maak 'n binnenshuise omgewing 'n goeie habitat vir die beskerming van die mens se lewe en welsyn. Weens onbeheerde abiotiese en biotiese faktore kan hierdie habitatte op hul beurt problematies raak vir menslike welstand. Om hierdie rede spreek hierdie studie die kwessie van binnenshuise luggehalte aan, veral swambesmetting in binnenshuise omgewings. Die binnenshuise swamgemeenskappe kan 'n negatiewe impak hê wat die gesondheid van mense in gevaar stel, van allergiese siektes tot lewensgevaarlike toestande. In Suid -Afrika is daar egter steeds 'n groot leemte in die navorsing oor binnenshuise swamme, en daarom is dit belangrik dat binnenshuise mikrobiologiese bevindings aan die publieke beskikbaar gestel word. Hierdie studie fokus op die identifisering van *Cladosporium* -spesies wat in die binnenshuise omgewing groei en vermeerder en hopelik bydra tot die begrip van hul verspreiding in binnenshuise omgewings van Suider -Afrika (SA). Hierdie studie het talle *Cladosporium* -spesies geïdentifiseer en nuwe spesies beskryf met behulp van die gestandaardiseerde DNA -volgordebenaderings en morfologiese diagnose waar nodig. Die aantal spesies wat in hierdie studie geïdentifiseer is, dui aan hoe min ons weet van swamme uit hierdie streek (SA) en het die ongekende rykdom van *Cladosporium* -diversiteit in SA in binnenshuise omgewings onthul.

Die genus *Cladosporium* bevat spesies wat in verskillende dissiplines kommerwekkend is, waaronder openbare gesondheid en veiligheid. *Cladosporium* -spesies is alomteenwoordige swamme in die klas Dothidiomycetes, orde Capnodiales en familie Cladosporioaceae. Soos baie ander swamme, het vroeë pogings om hierdie genus te identifiseer, meer as 720 spesies in die vroeë 2000's uitgeken. Hierdie spesies word gewoonlik erken aan hul donker gepigmenteerde kolonies (dematiaceous fungi) en hul identifikasie was slegs gebaseer op morfologiese karakters. Dit was slegs 'n paar jaar gelede dat spesies van hierdie genus in 'n monografie behandel is deur 'n betroubare polifasiese benadering, wat 218 *Cladosporium* -spesies erken het. Dit beklemtoon die uitdagings om *Cladosporium* -spesies slegs met morfologies gebaseerde benaderings te skei. In hierdie studie het ons morfologiese sowel as molekulêre tegnieke gebruik om *Cladosporium* -spesies te identifiseer uit binnenshuise monsters wat in die Wes -Kaap versamel is, en wat uit Gauteng en Zimbabwe ontvang is. Meer as 350 monsters wat kultuurkenmerke van *Cladosporium* is ondersoek, en 190 monsters is aan 'n molekulêre analise onderwerp, wat 29 spesies onthul het, waarvan 6 nuut is.

Dedication

I would like to dedicate this thesis to my parents (Mrs. M.S. Buthelezi and Mr. B.Z. Buthelezi) and my siblings (Nduduzo, Thembelihle, Thubelihle Buthelezi).

Without your support and love you showed to me it would have been more difficult to accomplish this degree. Many thanks to you.

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1.1 Introduction

For centuries, humans relied on the built environment for safe living conditions and survival. Thus, built environments form a significant and integral part of our life and the majority of people spend most of their time indoors, either in their homes, schools or offices (Adams *et al.*, 2016; Hoisington *et al.*, 2019). This entails that indoor environments are crucial as safe, protective and hospitable spaces (Hoisington *et al.*, 2019; Leung *et al.*, 2019). However, research has revealed a variety of microorganisms that inhabit the unique environmental conditions of indoor environments where their growth is often amplified. This is, however, not desirable as these microorganisms can have a negative impact on inhabitants (Adams *et al.*, 2016). Therefore, research into the dynamics of microbial communities in built environments is important for human health and well-being.

Like other habitats, built environments or indoor environments select for specific microorganisms, or indoor microbiomes (Adams *et al.*, 2016; Ziaee *et al.*, 2018). Fungi form a significant portion of the indoor microbiome and many species have adapted to indoor environmental conditions and nutrient constraints (Segers *et al.*, 2016, 2017). The most prominent indoor fungi include species of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* (Basiloco *et al.*, 2007; Hedayati *et al.*, 2011; Yamamoto *et al.*, 2012; Visagie *et al.*, 2014; Sharpe *et al.*, 2015; Arikoglu *et al.*, 2016; Yan *et al.*, 2016; Bensch *et al.*, 2018). These fungi are referred to as the 'core indoor microbial community' due to their prominent presence in the indoor environment (de Ana *et al.*, 2006). However, the species composition of indoor microbiomes greatly varies and depends on the abiotic factors that support their growth in different types of indoor settings including homes, schools and hospitals (Foladi *et al.*, 2013).

Species of core indoor microbial communities are often identified as disease-causing agents, commonly linked to allergic diseases (Hedayati *et al.*, 2011; Foladi *et al.*, 2013; Fukutomi and Taniguchi, 2015; Ziaee *et al.*, 2018). This is compounded by the fact that individuals are frequently exposed to a large number of spores in confined spaces (Ponce-Caballero *et al.*, 2013). Many studies have shown that indoor fungal contamination often causes allergic reactions that present rhinitis and asthma symptoms, and other respiratory tract infections, sometimes with severe complications depending on the level of exposure and susceptibility (Lugauskasa *et al.*, 2003; Foladi *et al.*, 2013; Zubairi *et al.*, 2014). Recently, there has been a steady increase in reports of people diagnosed with respiratory complications linked to fungal exposure (Hu *et al.*, 2017; Trablesi *et al.*, 2018; Esch *et al.*, 2020).

Studies further suggest that the species composition of indoor microbiomes is often similar to the microbiomes from the immediate or local outdoor environments (Dunn *et al.*, 2013; Adams

et al., 2014; Segers *et al.,* 2016). This forms the basis of the notion that ambient outdoor microbiome emmensly contribute to the indoor microbial community. However, parameters such as residence activities and efficacy of ventilation system often correlates with the microbiome composition (particularly *Cladosporium*) in the indoor environment (Segers *et al.,* 2016; Oh *et al.,* 2019; Guo *et al.,* 2020). Therefore, indoor *Cladosporium* contamination is problematic and compromise indoor air quality and thus requires a thorough investigation of indoor microbiology.

1.2 Air Quality and Fungi in the Indoor Environment

The rapid growth of the global human population, along with urbanisation, require a continuous improvement of building infrastructure to meet increasing demand (Mabahwi *et al.,* 2014; Leung *et al.,* 2019). However, the population growth and urbanisation also coincided with a rise in air pollution (Barberán *et al.,* 2015; Asif *et al.,* 2019; Sylvain *et al.,* 2019). Air pollution includes different components from various origins including chemical, biological, physical, and environmental. These pollutants usually originate from outdoor environments and enters indoor environments through doors and windows. However, pollutants can also originate from the indoor environment due to the activities and behaviour of occupants (Asif *et al.,* 2019; Guo *et al.,* 2020). Studies have shown that many people regularly encounter adverse exposure to pollutants in the indoor environment, which can be more than 100 times higher than the outdoor environment (Wood *et al.,* 2002; Gawrońska and Bakera, 2015; Pitarma *et al.,* 2017). This highlights the importance of maintaining good indoor air quality.

Studies suggest that indoor air pollutants often correlate with immediate outdoor air pollutants. This suggests that outdoor air pollutants migrate into indoor environments through openings such as windows, doors and ventilation systems. The influx of pollutants into the indoor environment implies that the outdoor environment contributes to the rising issue of deteriorating indoor air quality (Saad, 2003; Jafta *et al.,* 2017). In addition, studies suggest that there is a strong correlation of indoor air quality with the state of the occupant's health (Śmiełowska *et al.,* 2017; Wolkoff, 2018). Despite the controversy about the impact of the indoor air (Chang and Gershwin, 2019), many studies agree that some ailments are attributed to problems of the indoor air quality (Badea *et al.,* 2015; Wolkoff, 2018). Poor indoor air quality is currently a global issue and concern. In addition, poor indoor air quality is reported to contribute to global morbidity and mortality of respiratory based infections and other diseases (Mishra, 2003; Gordon *et al.,* 2014; Vanker *et al.,* 2015; Branco *et al.,* 2015).

Ideally, a proper indoor environment will contribute to the wellness and safety of residents (Osborne *et al.*, 2006; WHO, 2015; Vardoulakis *et al.*, 2015; Hoisington *et al.*, 2019). However, it is sometimes not feasible to maintain good indoor air quality, especially in developing countries, including South Africa, as many households still use alternative fuel sources, such as wood and paraffin for cooking and heating (Ferkor and Schraufnagel, 2014; Vanker *et al.*, 2015; Le Roux *et al.*, 2015). This can have a direct or indirect negative impact on indoor air quality because of the production of various gases, including carbon monoxide (CO) and carbon dioxide (CO_2) that are potentially detrimental to human health (Vardoulakis *et al.*, 2015). Currently, very little is known about the indoor air quality in particularly poorer households, and this requires a comprehensive survey and monitoring of indoor air.

There are different indicators used for assessing indoor air quality. The important abiotic markers in the indoor environment include relative humidity (a measure of dampness), temperature (to ensure human thermal comfort) and CO_2 levels (a measure the efficacy of ventilation systems) (Leung and Lee, 2016, Oh *et al.*, 2019). Proper ventilation systems are important to restrict the accumulation of air pollutants in the indoor environment. Higher ventilation rates often change the indoor air quality to be the same as immediate outdoor air due to increased influx of aerosols of outdoor origin (Nazaroff *et al.*, 2013; Kwan *et al.*, 2020). Conversely, a reduced or moderate ventilation rate allows the house to be less influenced by outdoor environmental conditions but still retain the air circulation (Nazaroff *et al.*, 2013).

These ever-changing indoor abiotic factors serve as the selective factor for a specific type of microbiome that can grow in the indoor environment (Kembel *et al.*, 2012, 2014; Leung *et al.*, 2014; Kettleson *et al*., 2015; Leung and Lee, 2016). For example, CO_2 levels above 1000 ppm indicate the risk of poor air quality, usually due to poor ventilation systems or the growth of fungi. Studies suggest that indoor relative humidity should be kept between 40 to 60 % (Arundel *et al.*, 1986; Osborne *et al.*, 2015). Relative humidity above 65 % is known to optimise the growth of sporulating fungi and the production of cell fragments, allergens, mycotoxins, β-glucans as well as volatile organic compounds (Bellotti *et al.*, 2015). The accumulation of these components presents the biohazards, compromises indoor air quality and renders indoor environments a risk to human health (Osborne *et al.*, 2006; Yamamoto *et al.*, 2012).

1.3 Fungal Allergens and Allergy

Fungi were recognised as early as the 18th century as potential cause of adverse respiratory symptoms, long before the term "allergy" was coined by Clemens Von Pirquet (Twaroch *et al.*, 2015). This has been an ongoing concern to the research community and Blackley (1873) found *Penicillium glaucum* to cause bronchial catarrh and chest tightness. Later this was followed by a report that linked fungal spores to asthma (Twaroch *et al.*, 2015). Fungal allergies have been an important concept over the last two decades and various authors confirmed the correlation of fungal exposure with the risk of allergies and other respiratory infections (de Hoog *et al.*, 2000; Osborne *et al.*, 2012; Thornton and Wills, 2015; Twaroch *et al.*, 2015; Mashat, 2015; Crawdford *et al.*, 2015; Levetin *et al.*, 2016; Cingi *et al.*, 2017).

The fungal kingdom consists of eight phyla with only three phyla and 36 genera reported by the WHO/IUIS Allergen Nomenclature Sub-Committee to be the source of 113 recorded allergens (Twaroch *et al.*, 2015; Levetin *et al.*, 2016; Esch *et al.*, 2020). The highest number of fungal allergens have been recorded from fungi in the phylum Ascomycota (88 allergens). Allergens, however, have also been described from the phyla Basidiomycota (23 allergens) and Zygomycota (2 allergens) (Kauffman *et al.*, 2000; Levetin *et al.*, 2016; Wiesner and Klein, 2017, Esch *et al.*, 2020). Allergic fungi are commonly known as being airborne, reproduce asexual (conidia) and have non-motile conidia that often grow on the apex of conidiophores (Esch *et al.*, 2020). One of the unique characters of fungi is their ability to colonise and grow within the host respiratory mucosa, whereas other respiratory allergens such as grass, house dust mite and tree pollen are unable to do so (Twarch *et al.*, 2016; Bartemes and Kita, 2018). The ability of fungi to cause infections is largely determined by the size (diameter) of spores that pass through the tissues to access sensitive organs and also dependent on the host temperature (Esch and Codina, 2017; Esch *et al.*, 2020). Mesophilic fungi with moderate temperature requirements (20 – 30 °C) such as *Cladosporium* and *Alternaria* are less likely to cause pathogenic infections because of the unfavourable human body temperature (Bartemes and Kita, 2018).

The incidence of fungal infections and allergies have increased dramatically over the last few years and many now require expert medical attention (Larenas-Linnemann *et al.*, 2016; Bartemes and Kita, 2018; Abbas *et al.*, 2019). Allergic diseases are defined as inflammatory reactions caused by environmental allergens and are mostly non-pathogenic. It is, however, an excessive or dysregulated immune response rather than the pathogenicity of an antigen that causes an extreme inflammatory response and hay fever is a common symptom (Denning *et al.*, 2014; Osborne *et al.*, 2015). The primary purpose of the immune system is to protect the host

against invasion of microorganisms, but sometimes the responses can be damaging to some tissues (Abbas *et al.*, 2019). Allergic diseases are therefore called 'disorders' caused by immune responses. In general, allergic diseases are species-specific and different fungal species present different degrees of sensitisation (Fukutomi and Taniguchi, 2015). Fungal species linked to allergic diseases and their related allergens that trigger immunoglobuline E (IgE) antibody response are shown in Figure 1.3.1 (Levetin *et al.*, 2016). However, cross-reactivity occurs amongst some of these allergens (Soeria-Atmadja *et al.*, 2007, 2010; Levetin *et al.*, 2016; Esch *et al.*, 2020).

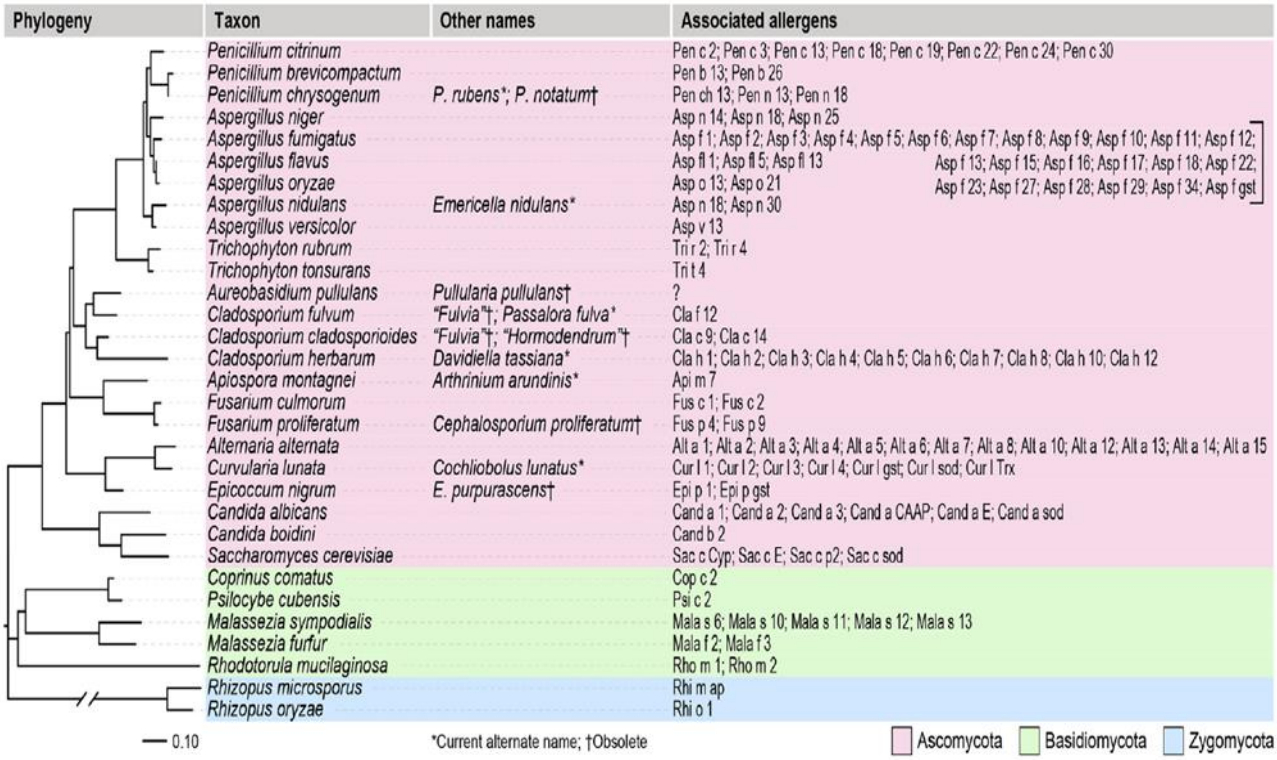

Figure 1.3.1. Phylogeny of common allergenic fungal species from three fungal phyla and relationship of allergenic taxa with associated allergens. From Levetin et al, (2016).

The concept of cross-reactivity refers to the immunogenic determinants (allergens) shared by different fungi. Cross-reactivity of fungal allergens has been explored using different techniques, including immunoblotting, enzyme-linked immunosorbent assay (ELISA) and radioallergosorbent test (RAST) inhibition (Twaroch *et al.*, 2016; Esch *et al.*, 2020). These techniques can identify allergen cross-reactivity and distinguish between independent and parallel sensitisation to multiple fungal allergens (Esch *et al.*, 2020). Allergen crossreacti-vity is often known to be associated with homologous sequences; however, any sizable change in amino acid composition of an antigen can have an impact on IgE reactivity (Esch *et al.*, 2020).

Not all microorganisms can be an antigen, but it is their ability to be recognised by the antibodies and this function is determined by specific proteins that contain epitope properties for T-cells (T_H2) and antibodies (Gómez-Casado and Díaz-Perales, 2016; Abbas *et al.*, 2019). Exposure and sensitisation to immunogenic allergens triggers a classic immune response and manifest in allergic diseases (Osboern *et al.*, 2015; Gómez-Casado and Díaz-Perales, 2016). Allergens can be classified as major (>50 %) or minor (<50 %), and major allergens are often problematic and associated with allergic rhinitis and asthma (Twaroch *et al.*, 2015; Zhang *et al.*, 2017). To date, not enough is known about these proteins or epitopes in order to develop hypoallergenic agents and/or desensitisation agents that can essentially prevent unnecessary immune responses (Twaroch *et al.*, 2015). However, some individuals are atopic, and they have a strong propensity for allergic diseases. These individuals have tend to develop high levels of T_H2 and produce high levels of allergen-specific IgE antibodies in response to allergen/s that may cause less or no immune response to other people (Abbas *et al.*, 2019). To some extent, allergic diseases are considered to have a genetic linkage that accounts for a family history of atopic diseases (Zabriskie, 2009; Abbas *et al.*, 2019; Esch *et al.*, 2020).

An interesting idea is that excessive hygiene during childhood can contribute to the increase of allergic diseases or hypersensitivities later in life (Gómez-Casado and Díaz-Perales, 2016). This is because, at a very early stage of life, the immune system is still underdeveloped and skewed toward producing T_H2-like cytokines. In theory, certain stimuli, including minor infections, can contribute to the immune system development and balance of T-cells responses. Therefore, a child living in the absence of these stimuli i.e., an excessively clean environment, the immature T_H2-like pattern of cytokine production is more likely to persist and contribute to diseases based on dysregulated immune responses (Gómez-Casado and Díaz-Perales, 2016). This is in accordance with the proposed hygiene hypothesis, which suggests that early exposure to infectious agents possibly prevents hypersensitivities later (Lauener *et al* 2012). However, this hypothesis does not promote that young ones must be raised in an environment with inadequate hygiene practices.

1.4 Different Types of Hypersensitivities

Hypersensitivity is classified into four different groups based on the principal underlying mechanisms and clinical manifestations associated with these reactions (Table 1.4.1) (Horner *et al.*, 1995; Cohen, 1998, Crameri *et al.*, 2014) and the common infectious agents for these conditions are outlined on

Table 1.4.**2** (Esch and Codina, 2017). *Type I hypersensitivity*: Commonly known for immediate response post exposure and sensitisation to allergen. This type of hypersensitivity is only for antigens that trigger T_H2 response and react with IgE antibodies attached to mast cells and basophils (Zabriskie, 2009; Abbas *et al.*, 2014, 2019). The antigens that trigger the production of IgE are normally inhaled or ingested. Clinical manifestations for inhaled allergens include abnormal mucus secretion, hay fever, sinusitis and for ingested allergens include contraction of intestinal muscles that lead to increased peristalsis (Crameri *et al.*, 2014; Abbas *et al.*, 2019). *Type II hypersensitivity*: Commonly referred to as cell bound; these reactions are driven by the autoantibodies (IgM and/or IgG) that react or bind to self-antigens on the cell membranes, tissues or circulating due to failure of self-tolerance (Zabriskie, 2009; Abbas *et al.*, 2019). The clinical example of these reactions includes autoimmunity and sometimes haemolytic anaemia (Crameri *et al.*, 2014; Vacher *et al.*, 2015). However, the actual mechanism of autosensitisation and/or autoimmunity is still not clear.

Table 1.4.1. Categories of hypersensitivity stimulated by different allergens. Allergic bronchopulmonary aspergillosis (ABPA) and allergic bronchopulmonary mycosis (ABPM) (from Crameri *et al.*, 2014).

Category	Humoral response	Soluble mediators	Time course	Cellular response	Clinical examples	Fungal diseases
Type I	IgE	Histamine Leukotrienes	Minutes	Smooth muscle constriction and eosiphil infiltration	Rhinitis and allergic asthma	Allergic rhinitis Allergic asthma ABPA ABPM
Type II	IgG, IgM	Complement	1-24 hours	Neutrophil activation Lysis of target cells infiltration and activation of granulocytes	Autoimmunity	unknown
Type III	IgG, IgM	Complement	1-24 hours	Infiltration and activation of granulocytes	Rheumatoid arthritis	Hypersensitivity Pneumonitis Aspergilloma Haemorrhage
Type IV	T cells	Lymphokines	2-3 days	T-cells and macrophage activation	Tuberculosis and contact dermatitis	ABPA Hypersensitivity Pneumonitis

Type III hypersensitivity: Commonly referred to as immune complex because these reactions generally result from antibodies and antigen-antibody complexes, and they are almost similar to type II hypersensitivity (Abbas *et al.*, 2019). This type of hypersensitivity has been most evident in arthus reaction where antibodies (IgM and IgG) attack antigens in heart tissues. The

reaction results in rheumatic fever that sometimes leads to acute heart failure. Typically, these reactions occur after the binding of IgG1 and IgG3 subunits into neutrophils and Fc receptors of macrophages that lead to inflammatory responses. IgM antibodies also drive other similar responses and induce the production of leukocytes (Abbas *et al.*, 2019). This type of reaction has also been seen manifesting as arthritis, where an antigen is subcutaneously administered in an animal that is historically sensitised to the same antigen and has developed an antibody response towards that antigen (Zabriskie, 2009; Crameri *et al.*, 2014). The previously produced antibody moves to the location of the injected antigen and forms immune complexes, attracting a complement activation, neutrophils and macrophages (Zabriskie, 2009; Crameri *et al.*, 2014).

Table 1.4.2. Fungi that are commonly implicated to various hypersensitivities (Esch and Codina, 2017).

Hypersensitivity disease	**Fungi (genera and/or species)**
Allergic rhinitis and asthma	*Agaricus, Alternaria, Aspergillus, Aureobasidium, Bipolaries, Botrytis, Chaetomium, Cladosporium, Curvularia, Drechslera, Epicoccum, Fusarium, Ganoderma, Mucor, Penicillium Phoma, Stemphylium, Trichophyton*
Allergic bronchopulmonary mycoses	*Aspergillus fumigatus, Bipolaris, Candida albicans, Curvularia, Geotrichum*
Allergic fungal sinusitis	*Alternaria, Aspergillus, Bipolaries, Curvalaria, Cladosporium, Drechslera, Fusarium, Penicillium*
Atopic dermatitis	*Candida albicans, Cryptococcus diffluens, Crytococcus liquefaciens, Malassezia (Pityrosporum)*
Hypersensitivity pneumonitis (extrinsic allergic alveolitis)	*Alternaria, Aspergillus, Aureobasidium pullans, Botrytis cinerea, Cryptostroma corticale, Mucor stolinifer, Penicillium, Rhodotorula, Trichosporon, various basidiomycetes* (*Lentinula edodes, Pleurotus pulmonarius*)
Dematophytosis	*Trichophyton, Epidermophyton floccosum, Microsporum canis*

Type IV hypersensitivity: The tissue injury in these reactions is principally caused by macrophages and neutrophils and it is mediated by T-cells cytokines (Zabriskie, 2009; Abbas *et al.*, 2019). T_H1 cytokine produces IFN-γ, the main precursor for macrophages activation, and T_H17 cytokine induces the development and production of neutrophils (Abbas *et al.*, 2014, 2019). These reactions are called delayed hypersensitivity because they manifest one or two days after an individual has encountered the antigen/s (Abbas *et al.*, 2014, 2019). This causes abnormal inflammation which is triggered by the action of macrophages and neutrophils and often results in chronic lesion of necrosis and granulomatosis inflammation (Zabriskie, 2009). The delayed type of hypersensitivity is often used to test if an individual has been exposed and responded to an antigen (Abbas *et al.*, 2019). This hypersensitivity is common in individuals

previously diagnosed with tuberculosis. When these individuals are injected with purified protein derivative (PPD) it results in local swelling that has an accumulation of T-cell and macrophages (Zabriskie, 2009; Abbas *et al.*, 2019).

1.5 Allergic Reaction Mechanism

People depend on innate and adaptive immunity for defence against any infectious agents. The immune system responds systemically and according to the nature of the foreign molecule (antigen). The innate immunity provides an instant first line of protection against various microbial attacks while adaptive immunity follows and progressively develops to provide a characterised and specific host defence (Figure 1.5.1) (Abbas *et al.*, 2014, 2019). These host defenses work together to eradicate the infections. In contrast, type I hypersensitivity results after an antigen has provoked the responses of both innate and adaptive immunity. After the antigen has passed through the epithelial barriers, it gets captured by the dendritic cells (cells of the innate immune system), which then presents it to T-cells to recruit the action of adaptive immunity. The adaptive immune system is divided into humoral and cell-mediated immunity, which is mediated by the B-lymphocytes (producing antibodies) and T-lymphocytes, respectively (Zabriskie, 2009; Abbas *et al.*, 2014, 2019). The production of antibodies is through B-lymphocytes, which is activated by the T-cells and necessary cytokines. Therefore, when B-cells are activated, they proliferate into clonal expansion and differentiate to immunoglobulin producing plasma cells (Abbas *et al.*, 2014).

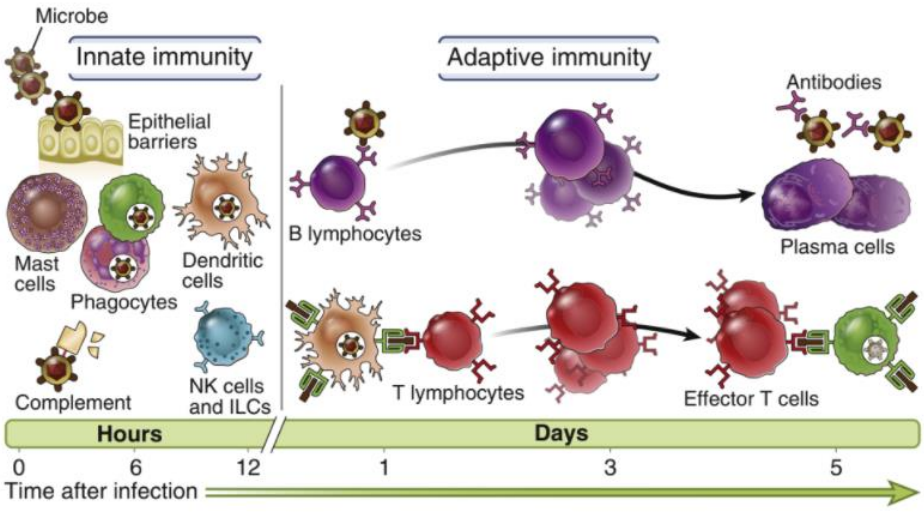

Figure 1.5.1. Two immune systems with their important molecules that provide signals if the host is under attack and further arrange themselves accordingly to remove the attacking agent. After a few hours (0-12 hours) of microbial attack, innate immunity initiates primary defence and subsequently followed by adaptive immunity in the following days (Abbas *et al.*, 2019).

In a case of an individual exposed and sensitised to immunogenic allergens, they experience acute to chronic inflammatory responses (Kurup *et al.*, 2000). The development of allergies is characterised by two phases, namely first exposure and effector phase (Figure 1.5.2). First exposure (Figure 1.5.2.A) is the first phase involving the uptake of allergens by dendritic cells (antigen-presenting cells), which couriers the allergen peptides into differentiated T-cells (T_H2) (Wheatley and Togias, 2015; Gómez-Casado and Díaz-Perales, 2016). The production of T_H2 response is also responsible for activation of cytokine interleukins (IL)-4, IL-5 and IL-13. These interleukins play an important role in the recruitment of the immune complement system for allergic diseases to occur (Ma *et al.*, 2021). The T_H2 convey the response mediated by IL-4 and IL-13 to the B cells to produce allergen-specific IgE antibodies (Renshaw *et al.*, 1994; Kurup *et al.*, 2000; Abbas *et al.*, 2014, 2019). In this event, the function of the IgE antibody is to serve as link between antigen and complement system. Mast cells and basophils provide receptor sites with high affinity (FcεRI) for the binding of IgE antibodies (Wheatley and Togias, 2015). The binding sites FcεRI have three polypeptides chains, one of which binds the Fc portion of IgE antibody (ε heavy chain) very tightly and the other two serve as the signalling chains (Abbas *et al.*, 2014).

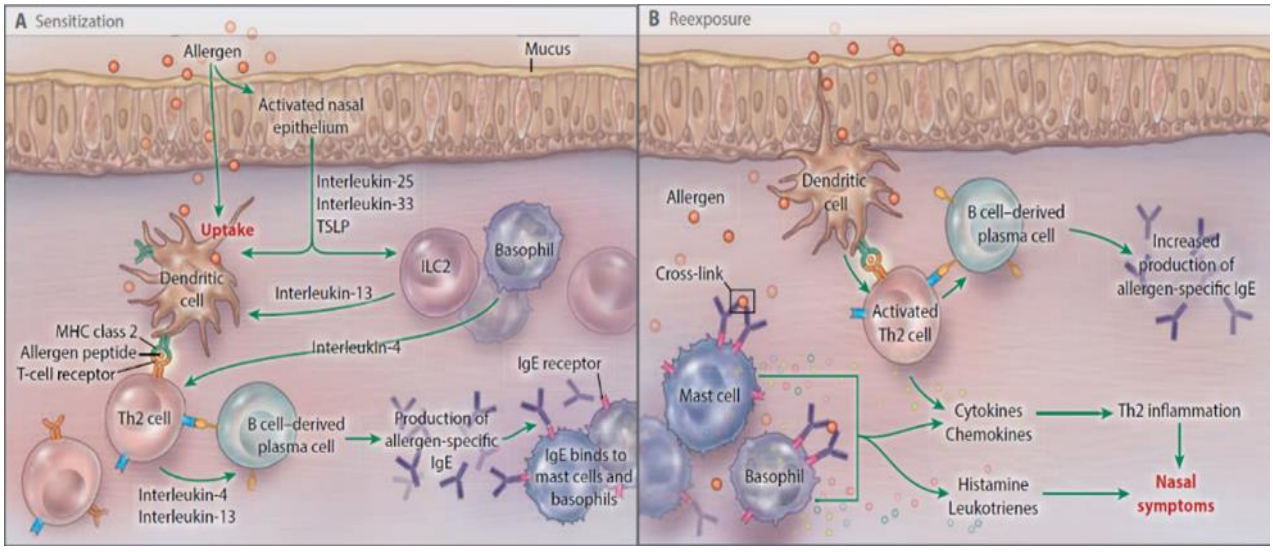

Figure 1.5.2. Classical pathway of immune response toward an allergen (Wheatley and Togias, 2015).

This reaction progresses to the next phase, known as the effector phase (Figure 1.5.2. B), where an individual is experiencing repeated exposure and sensitisation to allergens. The allergens get attached to the allergen-specific IgE antibody on the surface of mast cells and basophils (Kurup *et al.*, 2000; Zabriskie, 2009; Wheatley and Togias, 2015). This attachment leads to the activation of mast cells and basophils. The binding sites (FcεRI) that are carrying the complex

of allergens and allergen-specific IgE antibodies are cross-linked, activating biochemical signals from the signal-transducing polypeptides chains of FcεRI (Abbas *et al.,* 2014). This leads to the degranulation of mast cells and basophils, which produce various volatile mediators, including vasoactive amines, proteases from the granules, products of arachidonic acid and cytokines (Wheatley and Togias, 2015; Abbas *et al.,* 2014, 2019). Histamine being the prominent amine, is responsible for the dilation of small blood vessels, contraction of smooth muscles and exacerbation of vascular permeability.

Proteases are often responsible for injuring local tissues. Metabolites of arachidonic acid, including prostaglandins and leukotrienes, are responsible for vascular dilation and stimulation of smooth muscle contraction (Abbas *et al.,* 2014). Cytokines are responsible for the inflammatory response and late phase reaction through the major involvement of leukocytes (eosinophils, neutrophils and T_H2) that continuously damage tissues (Abbas *et al.,* 2014). This whole response results and clinically manifest as allergic rhinitis, which is commonly characterised as abnormal inflammation, wheezing, runny nose, sneezing and urticaria (hives) (Kurup *et al.,* 2000). South Africa is one of the countries identified as a hot spot of sensitisation to fungi (*Alternaria alternata* and *Cladosporium herbarum*) (Figure 1.5.3) (Twaroch *et al.,* 2015). In South Africa, the prevalence of allergic diseases occurs from toddlers (9 to 38 months) to elders who shows the production of allergen-specific IgE antibodies (Lunjani *et al.,* 2021 Mittermann *et al.,* 2021).

Figure 1.5.3. The world map that shows the geographical distribution of fungal allergy and yellow highlights the countries in which fungal sensitization has been described (Twaroch *et al.,* 2015).

1.6 Control and Diagnosis of Allergies

To date, the control and diagnosis of allergies is still a challenge despite the fact that the mechanisms are well characterised (Wheatley and Togias, 2015; Abbas *et al.,* 2014, 2019).

Allergic diseases remain a challenge to the public, especially in toddlers (2-3 years old), and the symptoms are likely to prevail into the teenage years, and often beyond into adulthood (Salo *et al.*, 2011; Yonekura *et al.*, 2012). In addition, children diagnosed with allergic rhinitis are more likely to have their tonsils and adenoids removed and the myringotomy tube (grommets) replaced (Meltzer *et al.*, 2009). In the United States, allergic rhinitis is diagnosed in approximately 15 % of children, although 30 % of children have self-reported symptoms such as nasal discharge and fever (Salo *et al.*, 2011). These atopic diseases generally progress into severity and in some cases allergic rhinitis progress in severity to asthma.

The diagnostic methods of common allergic diseases are often conducted based on clinical conditions and analyses of presented symptoms (Wheatley and Togias, 2015). The most effective diagnosis relies on proof of sensitisation that can be determined either by the presence of allergen specific-IgE antibodies in the serum or by a positive epicutaneous skin test. The European Academy of Allergy and Clinical Immunology proposed a 'U-shaped' approach that uses both conventional allergy diagnostics along with molecular-based allergy diagnostics, which is called component resolved diagnostics (CRD) (Eiringhaus *et al.*, 2019).

The U-shape approach includes sensitisation patterns and uncovers possible cross-reactivity due to complex polysensitisation to aeroallergens (Fukutomi and Taniguchi, 2015; Eiringhaus *et al.*, 2019). This approach is quite comprehensive compared to conventional techniques that rely on patients' symptoms, clinical history and physical examination. The conventional techniques, however, do not reveal immunological analytes such as T_H2, cytokines (interleukins) and IgE antibody diagnostic accuracy (Lunjani *et al.*, 2021; Eiringhaus *et al.*, 2019). Anti-inflammatory agents, including antihistamines, corticosteroids and long-acting beta2-agonist fused with asthma medication such as montelukast, theophylline and tiotropium, are still useful to reverse the effects of allergic diseases (Novelli *et al.*, 2015; Nixon *et al.*, 2017). Research has also implemented the administration of specific antigens that aim to suppress the excessive secretion of the IgE antibody (desensitisation) to counter the risk of allergic diseases (Abbas *et al.*, 2014; Nixon *et al.*, 2017).

1.7 Genus *Cladosporium*

The Ascomycota presents the largest phylum in the fungal kingdom, and it comprises 15 classes, 68 orders, 327 families, 6 300 genera, and over 65 000 species (Wijayawardene *et al.*, 2017; Esch *et al.*, 2020). The other phyla of the kingdom fungi, Basidiomycota and Zygomycota, have over 30 000 species and 1 000 species, respectively (Esch *et al.*, 2020).

Fungi of Ascomycota are widely distributed and can be found in environmental ecosystems (aquatic and terrestrial), playing key roles in biogeochemical cycles and are largely useful in various industrial applications (Van Wyk *et al.,* 2021). These fungi are also known for adverse health effects and their pathogenic potential range from humans to animals and plants (Sandoval-Denis *et al.,* 2015, 2016; Van Wyk *et al.,* 2021). Link (1816) described the genus *Cladosporium*, with *C. herbarum* as the type species. This is one of the larger genera in the phylum Ascomycota. Furthermore, *Cladosporium* belongs to the class Dothideomycetes, order *Capnodiales* and family *Cladosporiaceae* (Crous *et al.,* 2007; Bensch *et al.,* 2012). Like many other genera, *Cladosporium* has been an interest to many authors with species identification of this fungus relying on morphological characters (David, 1997; Dugan *et al.,* 2004). Dugan *et al.* (2004) assigned 772 names in the genus based on morphological techniques. These species were assigned as dematiaceous fungi that possess branched chains of non-septate and/or septate conidia. Based on the morphological classification, *Cladosporium* became a large and complex, polyphyletic genus (Bensch *et al.,* 2012).

The most prominent feature of *Cladosporium* is the presence of a unique coronate structure of conidiogenous loci and conidial hila, consisting of a central convex dome surrounded by a raised periclinal rim (David, 1997; Braun *et al.,* 2003; Crous *et al.,* 2007; Bensch *et al.,* 2012). In addition, conidiogenous loci and conidial hila of *Cladosporium* and *Heterosporium* were found to be uniform and this suggested that *Heterosporium* was a synonym of *Cladosporium* (David, 1997, Braun *et al.,* 2003; Dugan *et al.,* 2008). David (1997) demonstrated that *Cladosporium* lacks sexual reproduction stages (teleomorphs) but is closely related to *Mycosphaerella*. A phylogenetic study by Braun *et al.* (2003) confirmed that *Cladosporium* is a polyphyletic genus. The closely related *Cladosporium* morphs were assigned to *Mycosphaerella* and were later assigned to a newly introduced genus, *Davidiella* (Crous 2007, Dugan *et al.,* 2008; Bensch *et al.,* 2012). However, there were no observable morphological differences between *Mycosphaerella* and *Davidiella* (Braun *et al.,* 2003), although a phylogenetic study revealed that species of *Mycosphaerella* and *Davidiella* grouped into separate families, *Mycosphaerellaceae* and *Davidiellaceae,* respectively, within the order *Capnodiales* (Schoch *et al.,* 2006).

The separation of *Cladosporium* from *Cladosporium*-like genera was an important step in the taxonomy of this group. However, there was still a significant need to revise the genus with reliable molecular techniques. Part of the studies that contributed to the modern identification of *Cladosporium* include the revision of fungicolous *Cladosporium* (Heuchert *et al.,* 2005) and foliicolous *Cladosporium* (Schubert, 2005). In the continuation of examining *Cladosporium,*

Crous *et al*, (2007) showed that the combination of culture-based techniques and DNA sequencing data (polyphasic approach) presents a better resolution for identification of *Cladosporium* species. The polyphasic approach is currently a standard technique for identification of the *Cladosporium* (Bensch *et al.*, 2012; 2018). The first monographic treatment of *Cladosporium* using a polyphasic approach reduced the number of accepted species names from 772 to 170 (Bensch *et al.*, 2012).

Cladosporium is not just a taxonomically interesting group, but research also continued to explore *Cladosporium* and their impacts in diverse ecosystems. The saprophytic lifestyle of *Cladosporium* species allows them to thrive on various substrates ranging from outdoor to indoor environments (Recio *et al.*, 2012; Bensch *et al.*, 2018). These fungi often contaminate indoor environments, usually damp spaces behind beds or bedroom cupboards, bathroom surfaces, in kitchens under the sink and can grow as visible molds and/ or produce a musty odor of volatile organic compounds (Shams-Ghahfarokhi *et al.*, 2014; Micheluz *et al.*, 2016; Seguel *et al.*, 2017; Sadyś, 2017). In the indoor environment, frequent exposure to *Cladosporium* poses a threat to human health and affect occupants' comfort and wellness. *C. sphaerospermum* is often cited as an opportunistic human pathogen (Zalar 2007). It produces peptidases essential to hydrolyse elastin and laminin in the lungs and disrupt lung tissues (Segers *et al.*, 2016). Recently, *C. sphaerospermum* was isolated from a human brain abscess and showed resistance against numerous antifungal agents (Batra *et al.*, 2019).

Cladosporium is also reported to be associated with various health complications including lung mycoses, phaeohyphomycosis (surface and tissue infection), keratitis, acute meningitis and allergies (de Hoog *et al.*, 2000; Lalueza *et al.*, 2011; Bensch *et al.*, 2012; Chen *et al.*, 2013; Cheng *et al.*, 2015; Levetin *et al.*, 2016, Ma *et al.*, 2021). The most concerning and common effect of *Cladosporium* on human health is their immunogenic allergens. Currently, there are 11 *Cladosporium* allergens (Table 1.7.1**.**) that have been accepted by the WHO/IUS Allergen Nomenclature Sub-Committee, two of them are from *C. cladosporioides* and nine from *C. herbarum* (Esch *et al.*, 2020). *C. herbarum* is known as an outdoor species and is the leading allergenic species in the genus of *Cladosporium* (Fukutomi and Taniguchi, 2015; Levetin *et al.*, 2016; Pomés *et al.*, 2016). Research suggests a spore count as low as 500 spores/m^3 is enough to affect susceptible individuals. Individuals sensitised to *Cladosporium* allergens show symptoms of allergic rhinitis and asthma (Archatz *et al.*, 1995; Fukutomi and Taniguchi, 2015).

Cladosporium allergens also show a great deal of cross-reactivity because it is an aeroallergen and polysensitisation is more likely to occur (Crameri *et al.*, 2014; Fukutomi and Taniguchi, 2015). *Cladosporium* allergen-cross reactivity has been identified from allergen Cla h 8 with

Alt a 8 an *Alternaria* allergen (Crameri *et al.*, 2014). Despite its allergens, *Cladosporium* species are rarely implicated in severe lung infections compared to other fungi such as *Aspergillus* species (Bartemes and Kita, 2018). This is despite the fact that the genes that codes

Table 1.7.1. *Cladosporium* allergens. Adopted and modified from Esch *et al*, (2020).

Species name	Allergen	Biochemical name	MW (kDa)	Accession number
C. cladosporioides	Cla c 14	Transaldolase	36.5	ADK47394
	Cla c 9	Vacuolar serine protease	36	ABQ59329
C. herbarum	Cla h 1	Unknown	13	Unknown
	Cla h 2	Unknown	23	Unknown
	Cla h 5	Acid riosomal protein P2	11	CAA55067
	Cla h 6	Enolase	46	CAA55070
	Cla h 7	YCP4 protein	22	CAA55068
	Cla h 8	Mannitol dehydrogenase	28	AAO91801
	Cla h 9	Vacuola serine protease	45	AAX14379
	Cla h 10	Aldehyde dehydrogenase	53	CAA55072
	Cla h 12	Acid riosomal protein P1	11	CAA59463

for survival in lung tissues have been described in some *Cladosporium* species (Yew *et al.*, 2016; Batra *et al.*, 2019). However, the hostile microenvironment in human lungs, particularly the temperature, remains a restriction for these fungi to cause severe infections. In some cases, *Cladosporium* has been isolated from clinical specimens (Denis-Sendoval *et al.*, 2016) but their clinical impact has not been investigated despite their ability to occur as a opportunistic pathogen.

The impact of *Cladosporium* on human health is concerning and requires more attention from public health officials. However, *Cladosporium* is ubiquitous and often occurs in diverse hosts. In the outdoor environment, *Cladosporium* is known to occur in the soil, dead and/or live plant as phylloplane or endophytes and in air (Bensch *et al.*, 2012). *Cladosporium* on plants are often secondary pathogens on crops or phytopathogens that mostly cause leaf lesions (Barbosa *et al.*, 2001; Kumaresan and Suryanarayanan 2002; Bensch *et al.*, 2012; Marin-Felix *et al.*, 2017). In addition, *C. cladosporioides* have shown fungicidal properties including cladosporin (asperentin), isocladosporin, 5'-hydroxyasperentin, and cladosporin-8-methyl that greatly inhibit the growth of plant pathogens such as *Colletotrichum acutatum*, *Co. fragariae*, and *Co. gloeosporioides* (Wang *et al.*, 2013).

The continuous identification of *Cladosporium* from different substrates has significantly increased the realm of the genus. Today, *Cladosporium* is recognised as a monophyletic genus

with 218 recognised species names (Sandoval-Denis *et al.*, 2015, 2016; Bensch *et al.*, 2015, 2018). The current circumscription of *Cladosporium* is defined as dematiaceous hyphomycetes

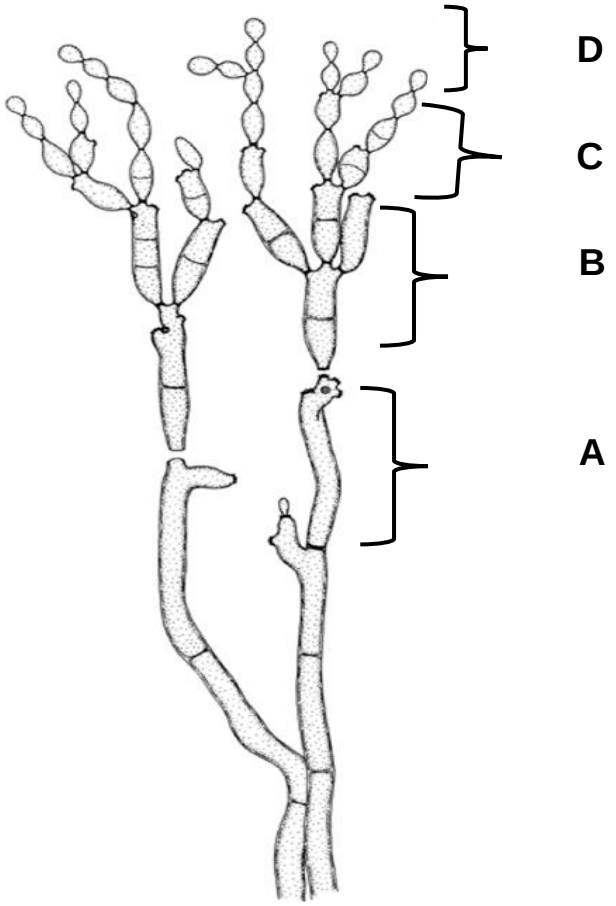

Figure 1.7.1. Overview showing most important features of *Cladosporium*. **A**. Ramoconidium attached to conidiophore. **B**. Secondary ramoconidium. **C**. Intercalary conidia (within the chain). **D**. small, terminal conidia. Adopted and modified from Bensch *et al*, (2012).

that poses solitary to fasciculate conidiophores, often grow sympodially and form unbranched and branched acropetal conidial chains (Figure 1.7.1) (Sandoval-Denis *et al.*, 2016). *Cladosporium* species are identified using multi-locus analysis of the translation elongation factor 1-α gene (EF 1-α), actin gene (ACT) and internal transcribed spacer region (ITS) (Bensch *et al.*, 2012). The topology of *Cladosporium* phylogeny suggests that only EF 1-α and ACT genes have a high resolution to identify *Cladosporium* species and ITS regions are often used for primary identification (Bensch *et al.*, 2018). *Cladosporium* species are grouped into three species complexes, namely *C. cladosporioides*, *C. herbarum* and *C. sphaerospermum* complex (Zalar *et al.*, 2007; Schubert *et al.*, 2007; Bensch *et al.*, 2010). The species complexes of

Cladosporium are primarily based on common morphological features (Bensch *et al.*, 2015; Marin-Felix *et al.*, 2017).

1.8 *Cladosporium* Species Complex

Cladosporium species are genetically distinct but very cryptic making it difficult to separate species with morphological diagnostics (Dugan *et al.*, 2004). However, common key morphological features categorise *Cladosporium* species into three species complexes (Bensch *et al.*, 2012, 2015). The grouping of *Cladosporium* species into complexes is not meant to separate or divide the genus into sub-phylogenetic entities, but it renders a quick and robust species identification (Bensch *et al.*, 2015). These three species complexes usually form their separate clades in the species phylogeny (Bensch *et al.*, 2012, 2018). However, the clustering of *Cladosporium* species into species complexes has not yet been described at a level of functional attributes or perhaps to any common implications. The term species complex used in *Cladosporium* was ruled as not taxonomic fitting by the International Code of Nomenclature but are consistently used in literature (Bensch *et al.*, 2015).

Among the species complexes, Zalar *et al.* (2007) delineated the *C. sphaerospermum* species complex with 23 distinct species (Bensch *et al.*, 2018). Species of *C. sphaerospermum* complex are often found in osmotically stressed environments and at low water activities ($a_w \pm 0.816$) (Zalar *et al.*, 2007, Segers *et al.*, 2015). *C. sphaerospermum* was first described by Penzing (1882) from decaying leaves and branches of citrus. These species are defined by usually having (i) branched septate and dark conidiophores with a length of 150 – 300 µM and width 3.5 – 4 µM, (ii) spherical to ellipsoid, acrogenously formed conidia of 3.4 - 4 µM diameter and (iii) ramoconidia of 6 -14 and 3.5 – 4 µM (Zalar *et al.*, 2007). Most of the commonly recognised features in species of this complex are their numerous globose or sub-globose terminal intercalary conidia with variable surface ornamentation ranging from species to species (Bensch *et al.*, 2015; Marin-Felix *et al.*, 2017). Species of *C. sphaerospermum* complex, in particular *C. halotolerant*, are more prominent indoors compared to the outdoor environment (Bensch *et al.*, 2018). This trend is expected to be at a high peak in winter when the exchange with outdoor air is low (Segars *et al.*, 2015; Bensch *et al.*, 2018).

The *C. cladosporioides* species complex (Bensch *et al.*, 2010) consists of 34 monophyletic species clades. The realm of this species complex has tremendously increased over the years and currently recognise 66 species (Bensch *et al.*, 2018) and form the largest group of the genus. The species of this complex are often characterized by conidiophores that are narrow cylindrical

or cylindrical-oblong, non-nodulose, often non-geniculate. The surface ornamentation of conidia is not consistent among species and ranges from smooth or almost smooth to rough walls (Bensch *et al.*, 2015; Marin-Felix *et al.*, 2017). However, other species of the *C. cladosporioides* complex (*C. oxysporum, C. colocasiae* and partially *C. tenuissimum*) have nodose conidiophores with distinct swellings and are distinct from each other (Bensch *et al.*, 2015). Species of the *C. cladosporioides* complex are widely distributed and have no special ecological requirements as they often grow at moderate temperatures. However, Sandoval-Denis *et al.* (2016) described *Cladosporium* species from clinical specimens and delineated a number of new species under this complex (*C. angulosum, C. flavovirens* and *C. anthropophilum* and some strains of *C. pseudocladosporioides*) with the ability to grow at 35 °C maximum temperatures.

The *C. herbarum* species complex (Schubert *et al.*, 2007) is currently the second largest complex of the genus *Cladosporium* and recognises 37 species (Bensch *et al.*, 2018). This complex is well described morphologically and is phylogenetically monophyletic. However, some species in this complex group are in a single clade and this suggests the occurrence of infraspecific variation. Species infraspecific variation occurred in *C. ramotenellum* and *C. basiinflantum* where *C. basiinflantum* group within the same clade (Bensch *et al.*, 2018). The micro-morphology of these species is well defined by ornamentated conidia that range from verruculose to verrucose, enchinulate or spiny (Bensch *et al.*, 2015; Marin-Felix *et al.*, 2017). Most species of this complex have nodulose conidiophores with conidiogenous cells confined to lateral swellings. This character is not present in all species of this complex (Schubert *et al.*, 2007; Bensch *et al.*, 2015).

1.9 Conclusion

This literature review has covered a wide range of microbial based-problems in our houses. It also serves as a baseline alert to caution the public about the implications of fungal contamination in homes. This poses a commendable challenge to all of us to take care of air quality in our living spaces. However, valuable information about the issue of poor indoor air quality is not available to many societies, especially those affected the most. Studies suggest that fungal growth must be avoided in our homes because they render the house to be a risk. It is, however, an individual responsibility to take necessary hygiene measures to maintain good indoor air quality by taking care of important abiotic factors that normally cultivate the growth and proliferation of fungi indoors, such as ventilation systems and humidity control. The concept of indoor fungal contamination and clinical conditions attributed to fungal exposure is

however, understudied in South Africa. Therefore, with this study we are hoping it will compensate and reduce the gap of this missing information about the genus *Cladosporium* in indoor environments.

Fungi are associated with allergic diseases in humans and their prevalence in respiratory-based allergies account for approximately 20 % - 30 % of atopic individuals and up to 6 % in the general population (Esch *et al.,* 2020). The manifestation of allergic diseases commonly relies on three important factors (i) individual susceptibility (ii) degree of exposure and (iii) how frequent an individual gets exposed and sensitised to fungal propagules that contain allergen/s. However, amongst the fungal propagules such as spores, vegetative cells, or metabolites, the spore size often regulates the sites of infection or deposition of spores between the upper and lower respiratory systems. Normally fungal spores that are less than 5 µm are able to invade the lower airways where the allergy manifest as allergic asthma. The literature is clear about the fact that most of the fungal allergens are heterogeneous and complex. Therefore, when diagnosing fungal allergens, it is important to note that different fungal species partially or entirely share allergenic components. This requires an in-depth understanding of cross-reactivity amongst the fungal allergens when analysing results of skin tests and/or serological tests. This is because there is a serious concern about the immunologic cross-reactivity, particularly between the taxonomic and antigenic related strains, species and even a genera. In general, most fungi have different degrees of allergenicity and prevalence. Therefore, when assessing allergic diseases, it is important to identify the fungal species to keep a record of their allergenicity and for the prescription of specific treatment.

1.10 Problem statement

Fungal growth from built environments has been known for a long time especially in places of residence, schools and hospitals (Guo *et al.*, 2020). The fungi that predominantly grow indoors are referred to as core indoor microbial communities and their resilience in indoor environments is mostly encouraged by their saprophytic lifestyle (Ziaee *et al.,* 2018; Batra *et al.,* 2019). Indoor microbiome is unfortunately not desired since they compromise indoor air quality and impose a continuous threat to human health, safety and general well-being. However, indoor fungi remain grossly understudied, especially in how they interfere with the quality of occupant's health in a short- and long-term period. The fungi that frequently grow in the indoor environment through all seasons include species of *Alternaria*, *Aspergillus, Cladosporium,* and *Penicillium*. These fungi are known for their noxious effect on human health and contain

immunogenic allergens. This study aims to fill the gap in information about the distribution of *Cladosporium* species in Southern African indoor environments.

1.11 Hypothesis and aims

This study was premised on the fact that *Cladosporium* species from immediate outdoor environments are the source of indoor *Cladosporium* contamination. This study aims to investigate the abundance and diversity of *Cladosporium* species from indoor and ambient outdoor environments. In order to achieve the aim, the following objectives were set:

- To identify *Cladosporium* species from indoor and ambient outdoor environments based on multigene species phylogeny.
- To describe novel *Cladosporium* species based on culture character, micromorphology and DNA sequencing data.

1.12 Significance of research

Southern Africa (SA) has an incredibly diverse microbiome which is gradually revealed with research inputs. However, most research focus on the biodiversity of outdoor environments, including soil of different purposes, plants, animals and the human gut microbiome. There has been a less comprehensive attempt to reveal the structure of the microbiome of indoor environments of SA in order to get a sense of their prevalence, impact on human health and factors that support or limit their growth and proliferation. This indicates an urgent need for research input to set at least a baseline database of the indoor microbiome in SA. Therefore, this study establishes a database of *Cladosporium* species that thrive in indoor and outdoor environments. *Cladosporium* species are neglected in SA but are an important part of indoor microbiomes and they are often linked to different indoor-based ailments and other serious life-threatening conditions. Outside the academic sphere, the database of *Cladosporium* species presented in this study is hopefully the most important output since it can be used as a reference dataset to do accurate risk assessments in homes and ultimately inform SA policy-makers on setting safety standards for fungal spores in indoor environments. This study introduces novel *Cladosporium* species that ultimately contribute to revealing the biodiversity richness of SA and increase our knowledge of fungi in SA that can be useful to healthcare practitioners and policymakers.

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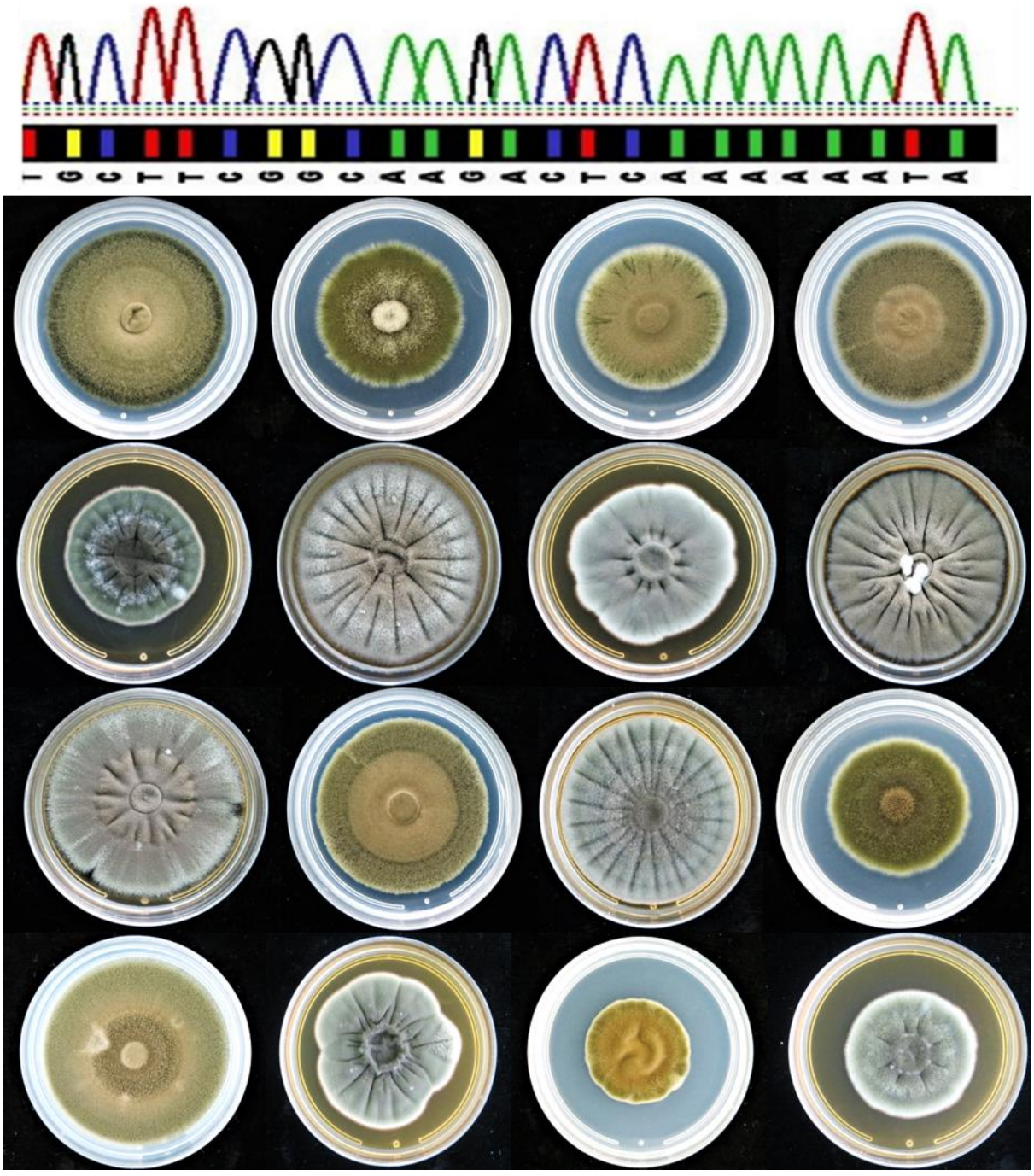
Chapter 2

Investingating *Cladosporium* species from the indoor environment in Southern Africa



Abstract

Species of the genus *Cladosporium* are dematiaceous hyphomycetes in the class Dothideomycetes, and represent ubiquitous fungi with a diverse ecology, ranging from terrestrial to aquatic ecosystems. *Cladosporium* species are dominant in the indoor environment, and it is becoming a concern from a public health view since these fungi are also implicated in human health issues. This study investigated the distribution and occurrence of *Cladosporium* from Southern African indoor environments. Air samples were collected from the Western Cape and other isolates were received from Gauteng and Zimbabwe. DNA sequencing data was generated for two genes, namely the EF 1-α and actin, to identify *Cladosporium* species. Twenty-eight (28) species were recovered from indoor environments in this study. This includes six species that were previously undescribed. This study increases the realm of the genus *Cladosporium* and highlights the need for exploring more ecosystems to recover more of these species, especially from African countries.

Keywords: Indoor environment, human health, *Cladosporium* species, DNA sequencing and species phylogeny

Introduction

Human wellness and their surrounding environments, particularly indoor environments, are connected. In the modern era, one cannot talk about health issues without mentioning the effect of poor indoor air quality. Poor indoor air quality, particularly underpinned by microorganisms has been present for decades, yet it receives little scientific attention. Indoor microbiome is an increasing problem as it affects the livelihood of the occupants of different spaces, including homes, schools, offices, and hospitals (de Ana *et al.*, 2006; Horner *et al.*, 2008; Sharpe *et al.*, 2015; Baurès *et al.*, 2018). These microorganisms cause a number of ailments, subsequently reduce the resident's quality of life and are at the center of sick building syndrome (Morath *et al.*, 2012; Ghaffarian-Hoseini *et al.*, 2018). Amongst the groups of indoor microbiomes, fungi are a dominant group of microorganisms that thrive in indoor spaces where they grow invisible and/ or as visible mycelial and produce musty odours (Nevalainen *et al.*, 2015; Segers *et al.*, 2016).

Some species of the indoor microbial community, i.e., *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium*, are known to be allergenic, opportunistic human pathogens and threaten human health (Ziaee *et al.*, 2018; Bartemes and Kita, 2018; Silva *et al.*, 2019; Batra *et al.*, 2019; Esch *et al.*, 2020). Therefore, fungal growth and proliferation of indoor spaces should receive more attention, especially from a public health view, because of the rising number of immune compromised individuals. This study forms a base to investigate the prevalence and distribution of *Cladosporium* species from Southern African indoor environments. *Cladosporium* species are widely distributed, ranging from indoor to outdoor air, soil, and live and dead plant material (Bensch *et al.*, 2012; Marin-Felix *et al.*, 2017).

Cladosporium species form a large proportion of indoor microbiomes, and these species are linked to various human health conditions and ailments. A recent study by Sandoval-Denis *et al.* (2016) identified *Cladosporium* species from various clinical specimens. Apart from the identification of those species from human organs (including lungs, cerebrospinal fluid and bronchoalveolar lavage fluid), their specific impact is still unknown. Other studies have identified some species of *Cladosporium* as opportunistic pathogens (Vieira, 2001; Tasić and Miladinović-Tasić, 2007), while recent findings implicate some species in human brain abscesses (Batra *et al.*, 2019), express genes that encode for the death of pulmonary epithelial cells (Lo *et al.*, 2020), and cause lung infections (Ma *et al.*, 2021). However, *Cladosporium* species are mostly reported as allergens (Kurup *et al.*, 2002; Crameri *et al.*, 2014; Pomés *et al.*, 2016; Esch *et al.*, 2020). *Cladosporium* species produce immunogenic allergens that are

recorded in the database of WHO/IUIS Allergen Nomenclature, and their allergens cause allergic diseases, including asthma and rhinitis (Ziaee *et al.*, 2018).

The identification of *Cladosporium* species was mainly based on morphological diagnostics, and recognised 772 species (Dugan *et al.*, 2004). However, the continuous isolation and comprehensive revision of *Cladosporium* species using the polyphasic approach resolved the genus to be phylogenetically and morphologically well-defined and separated from other cladosporioid genera (Bensch *et al.*, 2012). The monographic treatment of *Cladosporium* with the adoption of polyphasic analysis recognises 218 names (Bensch *et al.*, 2018). *Cladosporium* forms a monophyletic genus and species of this genus are well circumscribed by a unique coronate structure of the conidiogenous loci and conidial hila, consisting of a central convex dome surrounded by a raised periclinal rim (David, 1997; Crous *et al.*, 2007; Bensch *et al.*, 2012). The genus *Cladosporium* recognises three major species complexes that are morphologically and phylogenetically well described, namely *C. cladosporioides*, *C. herbarum* and *C. sphaerospermum* (Bensch *et al.*, 2015, 2018). In this study, more than 350 putative *Cladosporium* isolates were recovered from indoor environments and over 190 isolates were subjected to polyphasic analyses.

The implication of *Cladosporium* in human health is particularly concerning, considering its prevalence in indoor environments. The source of *Cladosporium* species that occur in the indoor environment is, however, controversial. Studies suggest that these species originate from the outdoor environment and migrate into the indoor environment. However, other scholars suggest that the abundant growth of these fungi in the indoor environment is due to occupant's behaviour and physiochemical parameters of indoor environments. The main research question of this study is, therefore, to investigate the occurrence and distribution patterns of *Cladosporium* species in the indoor and ambient outdoor environments in selected areas in South Africa and Zimbabwe.

Materials and Methods

Sample Collection

Indoor and outdoor air samples for this study were collected and received from various sites in South Africa (Western Cape and Johannesburg) and Zimbabwe following residents' complaints about potential mould problems. All samples were collected using volumetric air sampling with a MAS-100Eco Air Sampler (Merck, Combourg, France). Swab samples were taken from house surfaces where necessary (Figure 2.1). Fifty (50) liters of air were collected onto Petri

dishes containing 2 % malt extract agar (MEA) and in some instances dichloran 18 % glycerol agar (DG18), supplemented with antibiotics (streptomycin and chloramphenicol) to impede bacterial growth and selectively allow fungal growth. Samples were incubated at 26 ºC for a period of 7 days to allow fungal growth. To establish pure cultures of *Cladosporium*, putative dark to brown, velvety colonies with completely dark reverse sides were carefully picked and inoculated onto fresh 2 % MEA, oatmeal agar (OA) and 2 % potato-dextrose agar (PDA) and grown at 26 ºC for 7-14 days (Figure 2.2). All cultures in this study (Table 2.1-2.3) are maintained at Prof K Jacobs's culture collection as spore suspensions at the Department of Microbiology, Stellenbosch University.

Fungal DNA Extraction, Sequencing and Phylogenetic Analysis

Genomic DNA was extracted from *Cladosporium* mycelia using the Quick-DNA fungal/bacterial MiniPrep kit (Zymo Research, USA) according to the manufacturer's protocol. The successful DNA extractions were confirmed on 1% gel electrophoresis as described by Lee *et al.* (2012). DNA amplicons were amplified using PCR as described by Bensch *et al.* (2012). DNA amplification for the partial fragment of the actin gene was performed using primers Act 512F and 783R, respectively (Bensch *et al.*, 2012). The translation elongation factor 1-alpha gene (TEF 1-α) was amplified using primers EF1-728F and EF1-986R, respectively (Bensch *et al.*, 2012). The ITS gene was not included in this phylogenetic analysis since it presents low or poor phylogenetic resolution for *Cladosporium* resulting in a polytomous tree structure (Bensch *et al.*, 2012, 2018). The generated DNA sequences were analysed using FinchTV (Treves, 2010) to remove low quality reading portions of the sequences. High quality sequences were used in a BLAST search to generate a dataset, including ex-type sequences from National center for biotechnology information (NCBI). The multiple alignment of contigs was done using MAFFT version: 1.4.0 (Katoh and Standley, 2013) manually trimmed and concatenated in Geneious Prime version 2020.2.2 (Visagie and Houbraken, 2020). Concatenated sequences were used to construct phylogenetic trees with the Maximum likelihood (ML) and Tamura-Nei model in MEGA-X. The sequences used as reference strains were exclusively selected from sequences previously isolated from the indoor environment and/or clinical specimens where possible (Sandoval-Denis *et al.*, 2016; Bensch *et al.*, 2018).

Results

The species phylogenies were constructed from two concatenated genes (*act* and *tef1*) and are separated into three *Cladosporium* species complexes, *C. cladosporioides* (Figure 2.3), *C. herbarum* (Figure 2.4) and *C. sphaerospermum* (Figure 2.5) according to the classification proposed by Bensch *et al.* (2015, 2018). Sequences obtained from actin and TEF 1-α gene regions have short length ranging from 165 to 190 and 175 to 220 bases, respectively. The species phylogenies (Fig. 2.3-2.5) were constructed with ML. To avoid phylogenetic artefacts, especially from clades with a high number of isolates, we randomly selected certain strains as representatives of those species. The strains that were excluded from participating in the species phylogeny are indicated in bold font (Table 2.1-2.3). In this study, over 190 *Cladosporium* strains were sequenced and analysed. These strains represent 28 species and include six previously undescribed species.

The *C. cladosporioides* species complex (Figure 2.3) contains 37 clades and strains from this study grouped into 21 of these clades. All clades were statistically supported as separate except *C. pseudocladosporioides* and *C. crousii* which were grouping together (clade 17) (Figure 2.3), as was also seen from the study of Bensch *et al.* (2018). It is important to note that clades 11, 15, 19, 23, 27 and 31 contain only sequences generated from this study and suggest to be novel species. However, there are some other isolates that seem to be potential novel species but since they are only available as single strains, they were grouped with the closest known species to avoid assigning single strains as new species.

The phylogeny of C. *herbarum* species complex (Figure 2.4) contains 11 clades and strains from this study clustered into four clades, namely clade 1, 3, 5 and 11. All species clustered in separate, monophyletic clades with high statistical support, except *C. ramotenellum* and *C. basiinflatum*, which grouped together in the same clade (Figure 2.4, Clade 1) as it was also seen from the study of Bensch *et al.* (2018). The phylogeny of *C. sphaerospermum* species complex (Figure 2.5) contains 8 clades and strains from this study clustered in three clades. All species form separate monophyletic clades with high statistical support. Most isolates of the *C. sphaerospermum* species complex were recovered from the swab samples and *C. halotolerant* (Figure 2.5) is the most dominant species with seven strains.

Discussion

Cladosporium species inhabit diverse habitats ranging from aquatic to terrestrial ecosystems. However, very little is known about *Cladosporium*, particularly in African countries (Bensch *et al.*, 2012, 2015, 2018; Sandoval-Denis *et al.*, 2015, 2016). Therefore, this study aimed to fill the gap in the missing information about this genus in Southern Africa, particularly species from indoor environments. Bensch *et al.* (2018) identified 17 species from 52 strains that were isolated from South Africa (SA). Amongst those species, only three were identified from house dust and 14 were from other substrates in the outdoor environment, mostly from herbarium samples. Random isolations for this study, from mould-affected homes in SA over the last few years indicated that *Cladosporium* is the dominant genus isolated from indoor environments and suggested that there are more species that remain undiscovered.

The strains isolated in this study do not represent all *Cladosporium* species known to occur in indoor environments (Bensch *et al.*, 2018). Very few residents were aware of the presence of *Cladosporium* and its role as a major contaminant in the indoor environment, which raises concerns about the health and the safety of individuals who are exposed to these fungi on daily a basis. Especially as there have been a number of reports that implicate *Cladosporium* in various ailments related to indoor environmental conditions (Bush and Portony, 2001; Hu *et al.*, 2017; Ziaee *et al.*, 2018; Bush, 2020).

During this study, some *Cladosporium* species that were previously recovered from clinical specimens in the USA (Sandoval-Denis *et al.*, 2015, 2016) were also recovered from indoor environments in Southern Africa. These include *C. anthropophilum, C. angulosum* and *C. pseudocladosporioides* which were from human cerebrospinal fluid and bronchoalveolar lavage fluid specimens, respectively (Sandoval-Denis *et al.*, 2016). Bensch *et al.* (2018) described a number of new species. Of the current 218 *Cladosporium* species, only two species have officially been reported to produce allergens (*C. cladosporioides* and *C. herbarum*) (Esch *et al.*, 2020) and in someways this make a sense that most *Cladosporium* species are poor investigated for their allergenicity. This highlights a continuous need for intervention of allergists to comprehensively investigate the *Cladosporium* species allergens and/ or patterns of immune system response post-exposure to *Cladosporium* species.

C. herbarum is mostly known as the leading allergen producing species from the genus *Cladosporium,* and it produces nine out of the 11 known *Cladosporium* allergens confirmed by WHO/IUIS Allergen Nomenclature Sub-Committee (Esch *et al.*, 2020). However, some *Cladosporium* allergens have demonstrated a high level of cross-reactivity with other fungi such as *Alternaria* and *Aspergillus* allergens (Crameri *et al.*, 2014). We were not able to isolate

C. herbarum in this study, and this confirm the findings of Bensch *et al.* (2018) that this fungus is not a common indoor fungus but rather an outdoor species, particularly on the phylloplane.

In this study, *C. cladosporioides* was the most dominant species and were found from both indoor and outdoor environments, compared to *C. halotolerans* that was the dominant species in Africa, Asia, North and South America and Europe (Bensch *et al.*, 2018). This prominence of *C. cladospoioides* in indoor and outdoor environments is supported by the early literature (Fradkin *et al.,* 1987; Horner *et al.,* 2004). However, *C. cladosporioides* is an allergenic fungus and contains Cla c 9 and Cla c 14 allergens (Esch *et al.,* 2020). A recent study found that this fungus has the potential to grow in human lungs based on mouse experiments (Ma *et al.,* 2021). However, (Batra *et al.,* 2019) highlighted that the microenvironment of human lungs is hostile to the growth of *Cladosporium* species.

This study only presented a baseline of *Cladosporium* in Southern Africa and suggest that increasing the sampling pool will increase the biodiversity record for Southern Africa. Interestingly, this study generated more strains of the previously single strain species, namely *C. gamsianum, C. phaenocomae* and *C. rugulovarians* (Bensch *et al.,* 2018) and also provided the first report of these species from the indoor environment. *C. gamsianum* and *C. phaenocomae* have previously only been reported from SA, and isolation of these species suggests that they are potentially endemic in SA. This study further reports a number of species for the first time in Southern Africa, including *C. angustisporum, C. uwebraunianum, C. delicatulum, C. subuliforme, C. myrtacearum, C. rugulovarians, C. aggregatocicatricatum, C. asperulatum, C. parahalotolerans, C. limoniforme,* and *C. tenellum*. However, it is worrisome that in past and current literature, the exact functions and/or effects of these species are still unknown.

Phylogenetic analysis of all three species complexes confirmed the findings of Bensch *et al.* (2018), who found that the taxonomy still requires investigation when combining numerous species in one phylogeny. Some of these include *C. ruguloflabelliforme* and *C. basiinflatum,* which can be found within the lineages of *C. cladosporioides* species complex while these species belong to *C. sphaerospermum* and *C. herbarum* species complexes, respectively. Bensch *et al,* (2015) indicated that the grouping of *Cladosporium* species into complexes is mostly driven by common morphological characters rather than phylogenetic relationships. This should be addressed in future monographic and phylogenomics studies.

A second observation was *C. crousii* and *C. pseudocladosporioides* (Figure 2.3) that form a single clade suggesting potential infraspecific variation or conspecificity amongst these species.

C. crousii was described as a single strain species (Sandoval-Denis *et al.,* 2016), and more strains are needed to confirm its phylogenetic position in *Cladosporium*. However, we also confirmed the findings of Bensch *et al.* (2018) that the actin sequence of *C. crousii* is highly similar to sequences of *C. pseudocladosporioides* whereas EF 1-α sequences are completely different from all known *C. pseudocladosporioides*.

Sequences of *C. basiinflatum* and *C. ramotenellum* (Figure 2.4) also form a single clade that suggests these species are conspecific. *C. ramotenellum* was delineated as a novel species even though it clustered within the pre-existing *C. basiinflatum* clade (Bensch *et al.,* 2018), and this was also observed in this study. In this study, sequences of *C. parahalotolerant* (Figure 2.5) grouped in a well-supported clade and may represent a new species. However, these sequences show a continuous phylogenetic pattern with other *C. parahalotolerant* strains (Bensch *et al.,* 2018) and they are morphologically identical to *C. parahalotolerant*.

The phylogenetic analysis from this study supports the general species delineation of previous studies (Sandoval-Denis *et al.,* 2015, 2016; Ma *et al.,* 2017; Bensch *et al.,* 2012, 2015, 2018). In addition, a number of potential new species were identified and some of these will be described in the next chapter. The study broadened our knowledge on the prevalence and diversity of *Cladosporium* in Southern Africa. In addition, it has shown that species of *Cladosporium* dominante the indoor environment, and that many of these species could be endemic to the region. However, it is still unclear what the origin of these species are and will be investigated in future studies. The identification of novel species in this study is in accordance with the notion that Africa has highly diverse fungal communities and significantly contributes to the global understanding of many fungi (Visagie and Houbraken, 2020).

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Figure 2.1. *Cladosporium* contamination from different parts of indoor environments. **A** and **C**. ceiling boards. **B** and **G**. window seals. **D** and **H**. window frames. **E** and **F**. Walls.

Table 2.1. *Cladosporium cladosporioides* species complex isolates and the isolates that are not treated in species phylogeny (Fig. 2.3) are indicated with bold font.

Species	Culture Accession Number	Substrate	Location	GeneBank Accession Numbers	
				tef1	***act***
C. angustisporium	Clad 399	Indoor air	JHB	MZ895041	
	Clad 478	Indoor air	WC		MZ666865
	Clad 350	Indoor air	Zimbabwe	MZ895042	MZ666866
	Clad 333	Indoor air	Zimbabwe	MZ895043	MZ666867
	Clad 351	Indoor air	Zimbabwe	MZ895044	MZ666868
	Clad 345	Indoor air	Zimbabwe	MZ895045	MZ666869
	Clad 362	Indoor air	JHB		MZ666870
	Clad 401	Indoor air	WC	MZ895046	MZ666871
	Clad 396	Indoor air	JHB		MZ666872
	Clad 336	Indoor air	Zimbabwe	MZ895047	MZ666873
	Clad 348	Indoor air	Zimbabwe		MZ666874
C. anthropophilum	Clad 364	Indoor air	JHB	OK086933	MZ694993
	Clad 377	Indoor air	JHB	OK086932	MZ694994
	Clad 379	Indoor air	JHB	OK086931	MZ694995
	Clad 358	Indoor air	Zimbabwe	OK086930	MZ694996
	Clad 380	Indoor air	JHB	OK086929	MZ694997
	Clad 337	Indoor air	Zimbabwe	OK086928	MZ694998
	Clad 381	Indoor air	JHB	OK086927	MZ694999
	Clad 387	Indoor air	JHB	OK086925	MZ695001
C. asperulatum	Clad 357	Indoor air	Zimbabwe	OK086924	MZ695002

Table 2.1. (Continues**)**.

Species	Culture Accession Number	Substrate	Location	GeneBank Accession Numbers	
				tef1	***act***
C. cladoporioides	Clad 397	Indoor air	JHB		MZ695003
	Clad 394	Indoor air	JHB	OK086923	MZ695004
	Clad 390	Indoor air	JHB	OK086922	MZ695005
	Clad 412	Outdoor air	WC	OK086921	MZ695006
	Clad 414	Indoor air	WC		MZ695007
	Clad 500	Outdoor air	WC	OK086920	MZ695008
	Clad 341	Indoor air	Zimbabwe	OK086919	MZ695009
	Clad 398	Indoor air	JHB	OK086918	
	Clad 378	Indoor air	Zimbabwe	OK086917	MZ695010
	Clad 391	Indoor air	Zimbabwe	OK086916	MZ695011
	Clad 483	Indoor air	Zimbabwe	OK086915	MZ695012
	Clad 339	Indoor air	Zimbabwe	OK086914	MZ695013
	Clad 499	Indoor air	WC	OK086913	
	Clad 343	Indoor air	JHB		MZ695014
	Clad 365	Indoor air	JHB	OK086912	MZ694991
	Clad 373	Indoor air	JHB		MZ694992
	Clad 370	Indoor air	JHB	OK086911	MZ695015
	Clad 342	Indoor air	JHB		MZ695016
	Clad 355	Indoor air	Zimbabwe	OK086910	MZ695017
	Clad 359	Indoor air	Zimbabwe	OK086909	MZ695018
	Clad 340	Indoor air	Zimbabwe	OK086908	MZ695019
	Clad 352	Indoor air	Zimbabwe	OK086907	MZ695020
	Clad 494	Indoor air	WC	OK086906	
	Clad 492	Outdoor air	WC		

Table 2.1. (Continues**)**.

Species	Culture accession number	Substrate	Location	GeneBank Accession Numbers	
				tef1	***act***
	Clad 495	Outdoor air	WC	OK086903	
	Clad 498	Outdoor air	WC	OK086905	MZ695021
	Clad 436	Indoor air	WC	OK086904	MZ695022
	Clad 461	Outdoor air	WC	OK086902	
	Clad 353	Outdoor air	JHB	OK086901	MZ695023
	Clad 349	Outdoor air	WC	OK086900	MZ695024
	Clad 491	Outdoor air	WC	OK086899	
C. delicatulum	Clad 476	Indoor air	WC	OK086898	MZ895048
C. gamsianum	Clad 389	Indoor air	WC	OK086897	OK001226
	Clad 368	Indoor air	WC	OK086896	OK001227
C. myrtacearum	Clad 344	Indoor air	Zimbabwe	OK086895	OK001228
C. phaenocomae	Clad 471	Indoor air	WC	OK086894	OK001229
	Clad 318	Indoor air	WC	OK086893	OK001230
	Clad 452	Indoor swab	WC	OK086892	OK001231
	Clad 462	Indoor air	WC	OK086891	OK001232
	Clad 444	Indoor air	WC	OK086890	OK001233
	Clad 409	Indoor air	WC	OK086889	OK001234
	Clad 427	Indoor air	WC	OK086888	MZ895050
	Clad 404	Indoor air	WC	OK086887	OK001236
	Clad 415	Indoor air	WC		OK001235

Table 2.1. (Continued**).**

Species	Culture Accession Number	Isolate Source	Location	GeneBank Accession Numbers	
				tef1	***act***
	Clad 410	Indoor air	WC	OK086886	OK001237
	Clad 417	Indoor air	WC		
	Clad 416	Indoor air	WC	OK086885	OK001238
	Clad 465	Indoor air	WC	OK086884	OK001239
	Clad 453	Indoor air	WC	OK086883	OK001240
	Clad 473	Indoor air	WC		OK001241
	Clad 446	Indoor air	WC	OK086882	OK001242
C. pseudocladosporioides	Clad 451	Indoor air	WC	OK086881	MZ895051
	Clad 454	Outdoor air	WC	OK086880	OK001243
	Clad 334	Indoor air	Zimbabwe		OK001244
	Clad 405	Indoor air	WC	OK086879	OK001245
	Clad 450	Indoor air	WC	OK086878	OK001246
	Clad 411	Indoor air	WC	OK086877	OK001247
	Clad 374	Indoor air	JHB	OK086876	OK001248
	Clad 369	Indoor air	JHB	OK086875	OK018126
	Clad 479	Indoor air	WC		OK018125
	Clad 424	Indoor air	WC	OK086874	OK018124
	Clad 367	Indoor air	JHB	OK086873	OK018123
	Clad 375	Indoor air	JHB	OK086872	OK018122
	Clad 513	Indoor air	WC	OK086871	
	Clad 384	Indoor air	JHB		OK018121
	Clad 363	Indoor air	JHB		OK018120
	Clad 489	Outdoor air	WC	OK086869	OK018118

Table 2.1. (Continued**).**

Species	Culture accession number	Substrate	Location	GeneBank accession numbers	
				tef1	***act***
C. parangustum	Clad 338	Indoor air	Zimbabwe	OK086868	OK018117
C. subuliforme	Clad 361	Indoor air	Zimbabwe		OK018116
C. uwebrauniana	Clad 470	Indoor air	WC	OK086867	OK018115
	Clad 413	Indoor air	WC	OK086866	OK018114
	Clad 460	Outdoor air	WC	OK086865	OK018113
	Clad 466	Indoor air	WC	OK086864	OK018112
	Clad 455	Indoor air	WC	OK086863	OK018111
	Clad 459	Outdoor air	WC	OK086862	OK018110
	Clad 402	Indoor air	WC		
	Clad 448	Outdoor air	WC	OK086861	OK018109
	Clad 467	Outdoor air	WC		OK018108
	Clad 449	Indoor air	WC	OK086860	OK018107
	Clad 435	Outdoor air	WC	OK086859	OK018106
	Clad 475	Indoor air	WC		OK018104
	Clad 441	Indoor air	WC	OK086857	
C. rugulovarians	Clad 497	Outdoor air	WC		OK018103
C. vicinum	Clad 420	Indoor air	WC	OK086856	OK018102
	Clad 472	Indoor air	WC	OK086854	OK018100
	Clad 315	Indoor air	WC	OK086855	OK018101

Table 2.1. (Continued).

Species	Culture Accession Number	Isolate Source	Location	GeneBank Accession Numbers	
				tef1	***act***
C. scabrellum	Clad 347	Indoor air	Zimbabwe	OK086853	MZ895049
C. cibiculi-murrhini sp. nov.	Clad 306	Indoor air	WC	OK135964	
	Clad 312	Indoor air	WC	OK135962	OK086811
	Clad 372	Indoor air	JHB	OK086966	OK086809
	Clad 366	Indoor air	JHB		OK086812
	Clad 332	Indoor environment	Zimbabwe	OK135966	MZ823446
	Clad 376	Indoor environment	JHB	OK135967	OK086814
	Clad 321	Indoor environment	WC		OK086810
	Clad 385	Indoor environment	JHB	OK135965	OK086813
C. kostanzium sp. nov.	Clad 383	Indoor air	JHB		OK086815
	Clad 386	Indoor air	JHB	OK135968	OK086816
C. gracile sp. nov.	Clad 360	Indoor air	Zimbabwe		OK086824
	Clad 346	Indoor air	Zimbabwe		OK086825
C. azaniasporioides sp. nov.	Clad 322	Indoor air	WC	OK135969	OK086817
	Clad 331	Indoor air	WC		OK086818
C. aerem sp. nov.	Clad 323	Indoor air	WC	OK135970	OK086820
	Clad 356	Indoor air	Zimbabwe		OK086819

Table 2.1. (Continued).

Species	Culture Accession Number	Isolate Source	Location	GeneBank Accession Numbers	
				tef1	***act***
C. domusafricanae sp.nov.	Clad 301	Indoor air	WC	OK135972	OK086823
	Clad 428	Indoor air	WC	OK135971	OK086821
	Clad 325	Indoor air	WC		OK086822
	Clad 425	Indoor air	WC		MZ666864

Table 2.2. *Cladosporium herbarums* species complex isolates and the isolates that are not treated in species phylogeny (Fig.2.4) are indicated with bold font.

Species	Culture accession number	Isolation Source	Location	GeneBank accession numbers	
				tef1	***act***
Cladospium limoniforme	Clad 488	Indoor swab	WC	OK086852	MZ895052
	Clad 330	Indoor air	WC	OK086851	OK018099
	Clad 317	Indoor air	WC	OK086850	OK018098
	Clad 328	Indoor air	WC	OK086849	OK018097
	Clad 434	Indoor air	WC	OK086848	OK018096
	Clad 302	Indoor air	WC	OK086847	
	Clad 320	Indoor air	WC	OK086846	OK018095
	Clad 327	Indoor air	WC	OK086845	OK018094
	Clad 305	Indoor air	WC	OK086844	OK018093
	Clad 304	Indoor air	WC	OK086843	OK018092
	Clad 314	Indoor air	WC		OK018091
	Clad 313	Indoor air	WC		OK018090
	Clad 329	Indoor air	WC		OK018089
	Clad 319	Indoor air	WC		OK018088
	Clad 316	Indoor air	WC		OK018087
	Clad 303	Indoor air	WC		OK018086
	Clad 326	Indoor air	WC		OK018085
C. aggregatocicatricatum	Clad 421	Indoor air	WC	OK086934	
C. ramotenellum	Clad 482	Outdoor air	WC		OK018084
	Clad 484	Indoor air	WC	OK086935	OK018083
	Clad 324	Indoor air	WC	OK086936	OK018082

Table 2.2. (Continued).

Species	Culture Accession Number	Substrate	Location	GeneBank accession numbers	
				tef1	***act***
	Clad 307	Outdoor air	WC	OK086937	OK018081
	Clad 311	Indoor air	WC	OK086938	OK018080
	Clad 310	Outdoor air	WC	OK086939	OK018079
	Clad 403	Indoor air	WC	OK086940	
	Clad 486	Indoor air	WC	OK086941	OK018078
	Clad 456	Indoor air	WC	OK086942	OK018077
	Clad 431	Indoor air	WC	OK086943	OK018076
	Clad 422	Indoor air	WC	OK086944	OK018075
	Clad 458	Outdoor air	WC	OK086945	OK018074
	Clad 433	Outdoor air	WC	OK086946	
	Clad 440	Outdoor air	WC	OK086947	
	Clad 443	Indoor air	WC	OK086948	OK018073
	Clad 474	Indoor air	WC		OK018072
	Clad 464	Indoor air	WC		OK018071
	Clad 505	Indoor air	WC	OK086955	OK018068
	Clad 445	Indoor air	WC	OK086858	OK018105
	Clad 406	Indoor air	WC		OK018070
	Clad 437	Indoor air	WC	OK086954	OK018069
C. tenellum	Clad 418	Indoor air	WC	OK086949	OK086806
	Clad 407	Indoor air	WC	OK086950	
	Clad 457	Indoor air	WC	OK086951	OK086807
	Clad 438	Indoor air	WC	OK086952	
	Clad 419	Indoor air	WC	OK086953	OK086808

Table 2.3. *Cladosporium sphaerospermum* species complex isolates and the isolates that are not treated in species phylogeny (Fig. 2.5) are indicated with bold font.

Species	Culture Accession Number	Isolate Source	Location	GeneBank Accession Numbers	
				tef1	***act***
Cladosporim halotolerance	Clad 496	Indoor air	WC	OK086956	OK086826
	Clad 439	Indoor air	WC	OK086957	OK086827
	Clad 487	Indoor air	WC		OK086828
	Clad 382	Indoor air	JHB		OK086829
	Clad 477	Indoor swab	WC	OK086958	OK086830
	Clad 493	Indoor air	WC		OK086831
	Clad 447	Indoor swab	WC	OK086959	OK086832
	Clad 426	Indoor air	WC	OK086960	OK086833
	Clad 463	Indoor air	WC		OK086834
	Clad 480	Indoor swab	WC		OK086835
	Clad 432	Indoor air	WC		MZ666864
			WC		
C. sphaerospermum	Clad 469	Indoor swab	WC	OK086961	OK086836
	Clad 490	Indoor swab	WC		OK086837
	Clad 388	Indoor air	JHB	OK086962	OK086838
	Clad 432	Indoor air	WC		OK086839
	Clad 393	Indoor air	JHB	OK086963	OK086840
C. parahalotolerance	Clad 485	Indoor swab	WC	OK086964	OK086841
	Clad 465	Indoor air	WC	OK086965	
	Clad 442	Indoor air	WC		OK086842

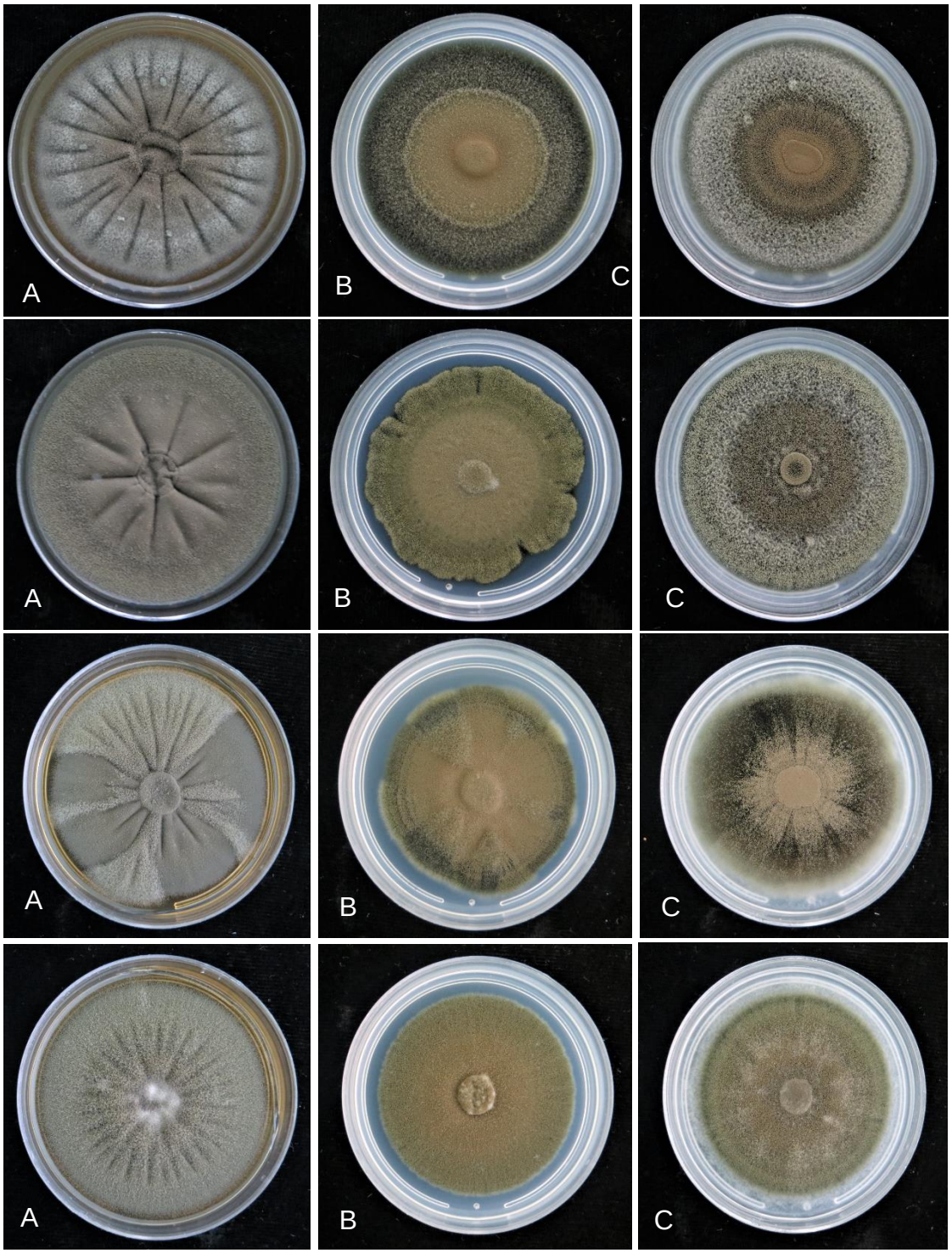


Figure 2.2. A representation of randomly selected isolates to display the morphological cryptic nature of different *Cladosporium* species. Each horizontal column is a culture of different isolates grown on **A**. MEA. **B**. PDA. **C**. OA for 7 to 14 days at 26 °C.

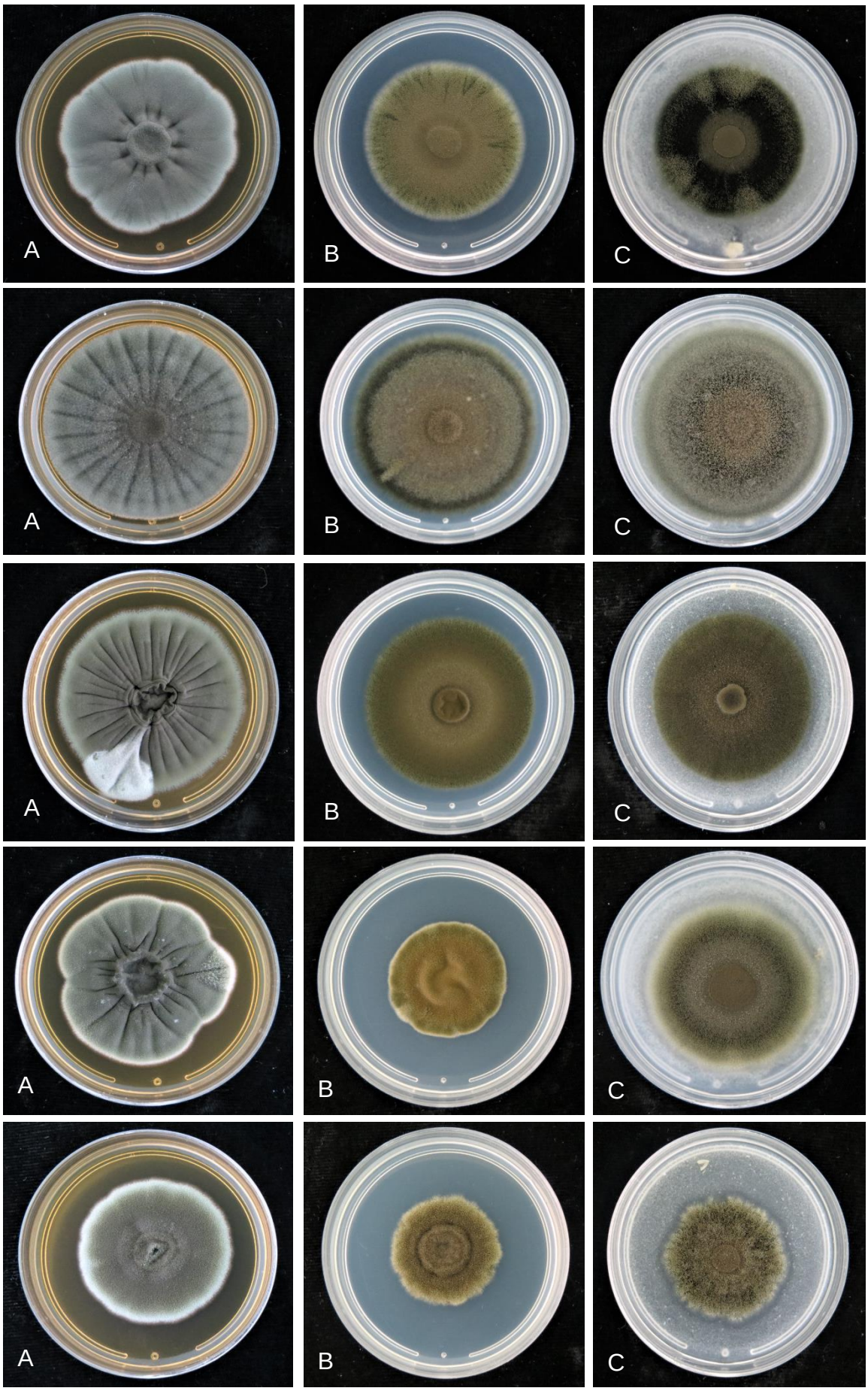


Fig. 2.2. (Continued).

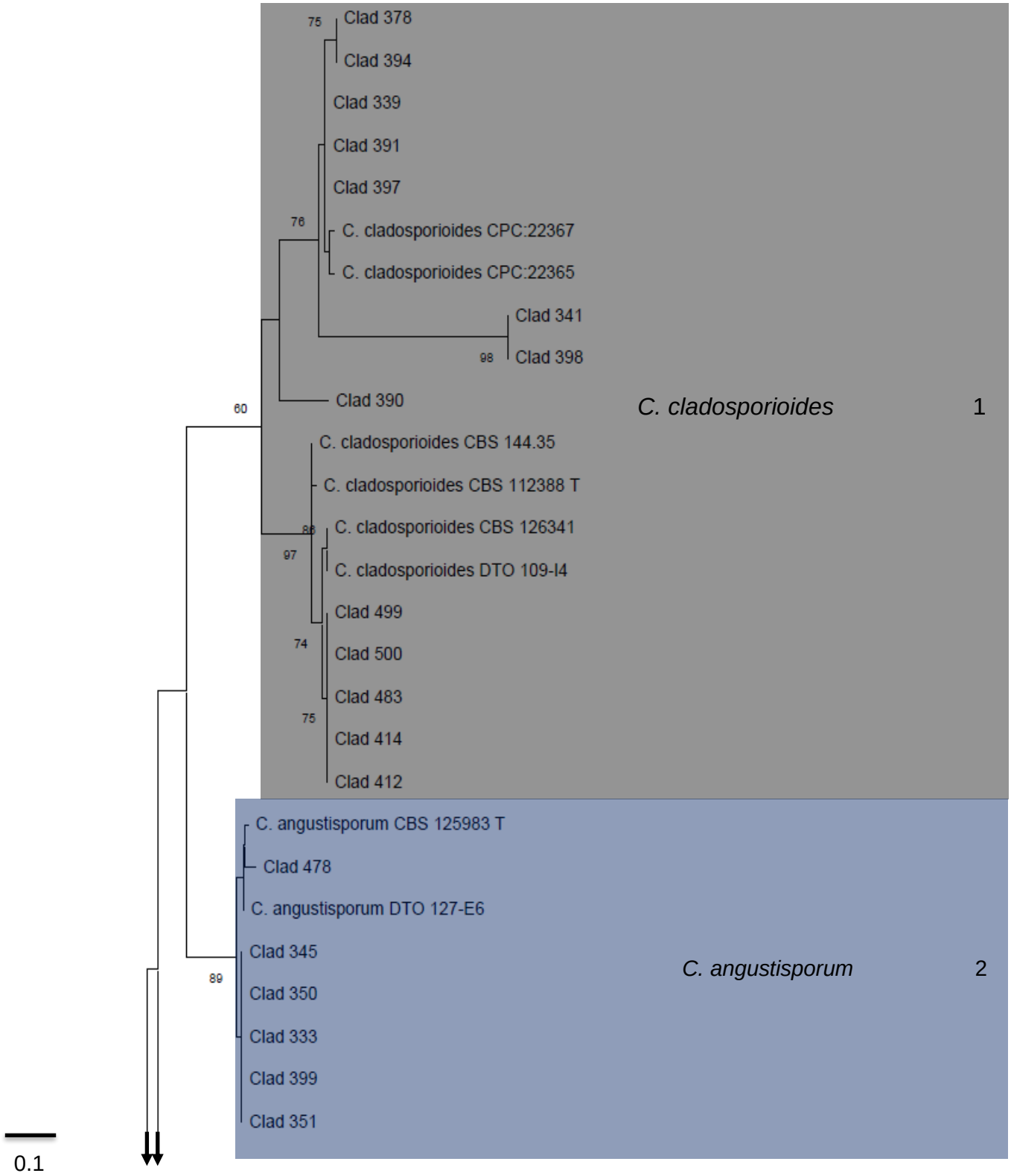


Fig. 2.3. (Continued).

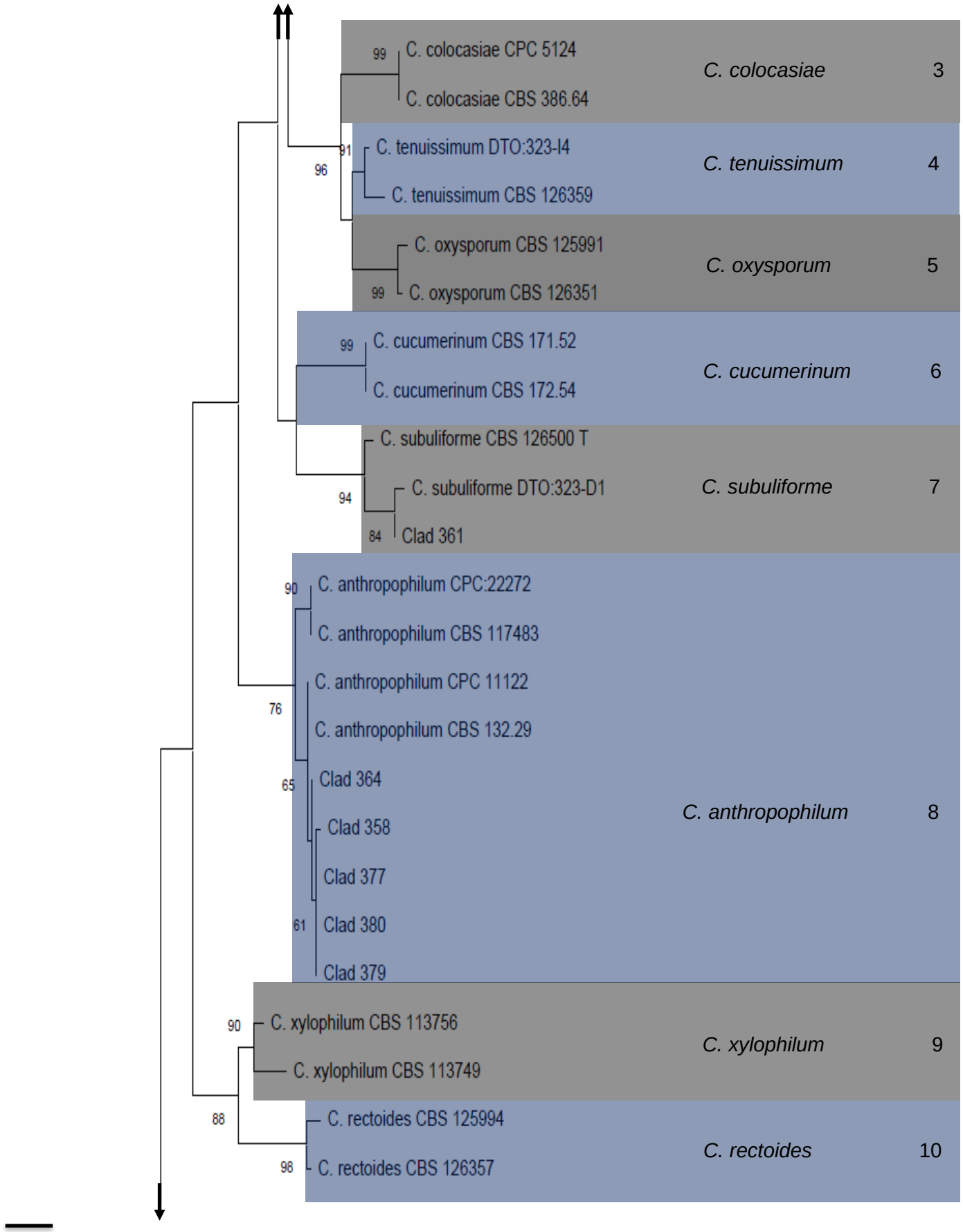


Fig. 2.3. (Continued).

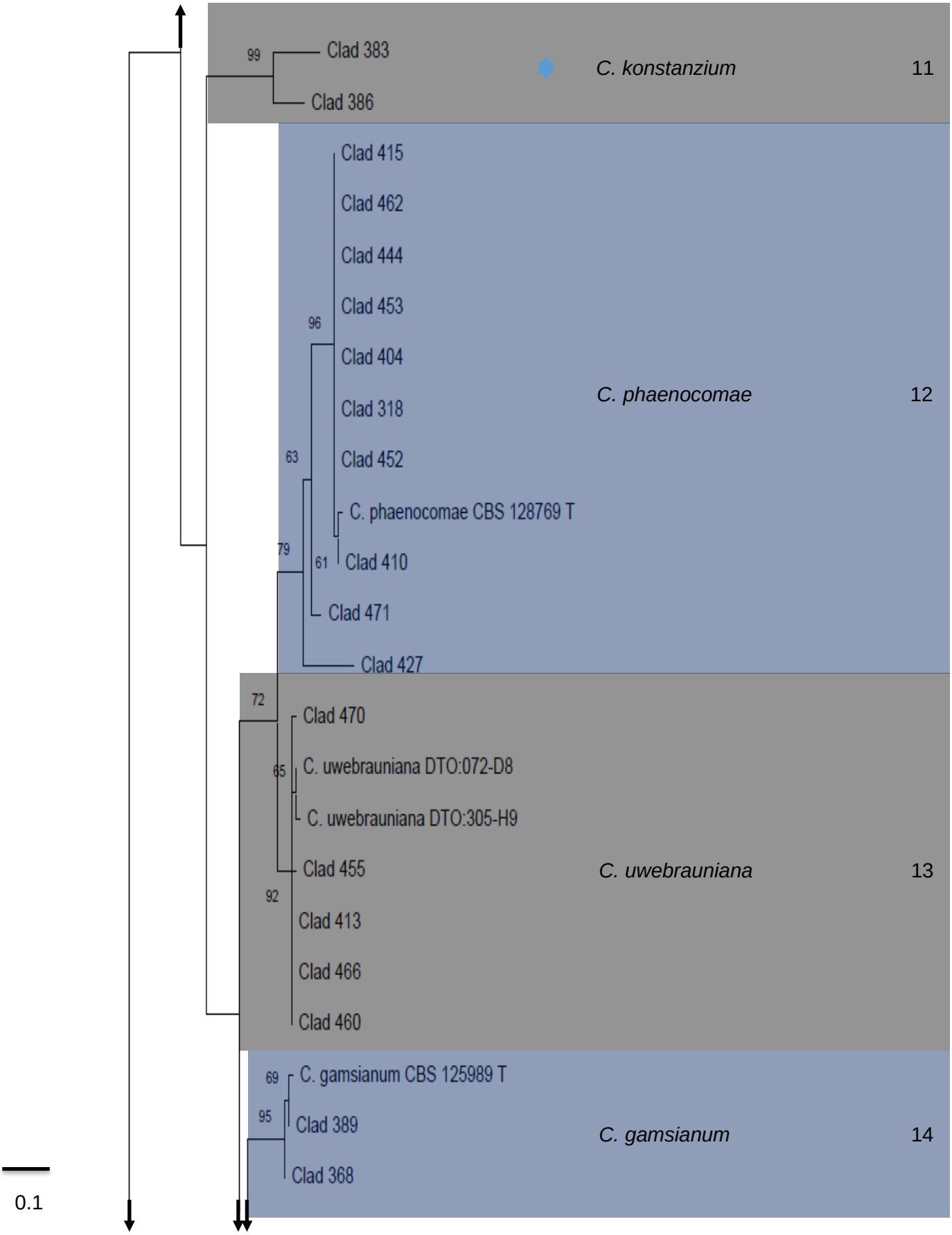
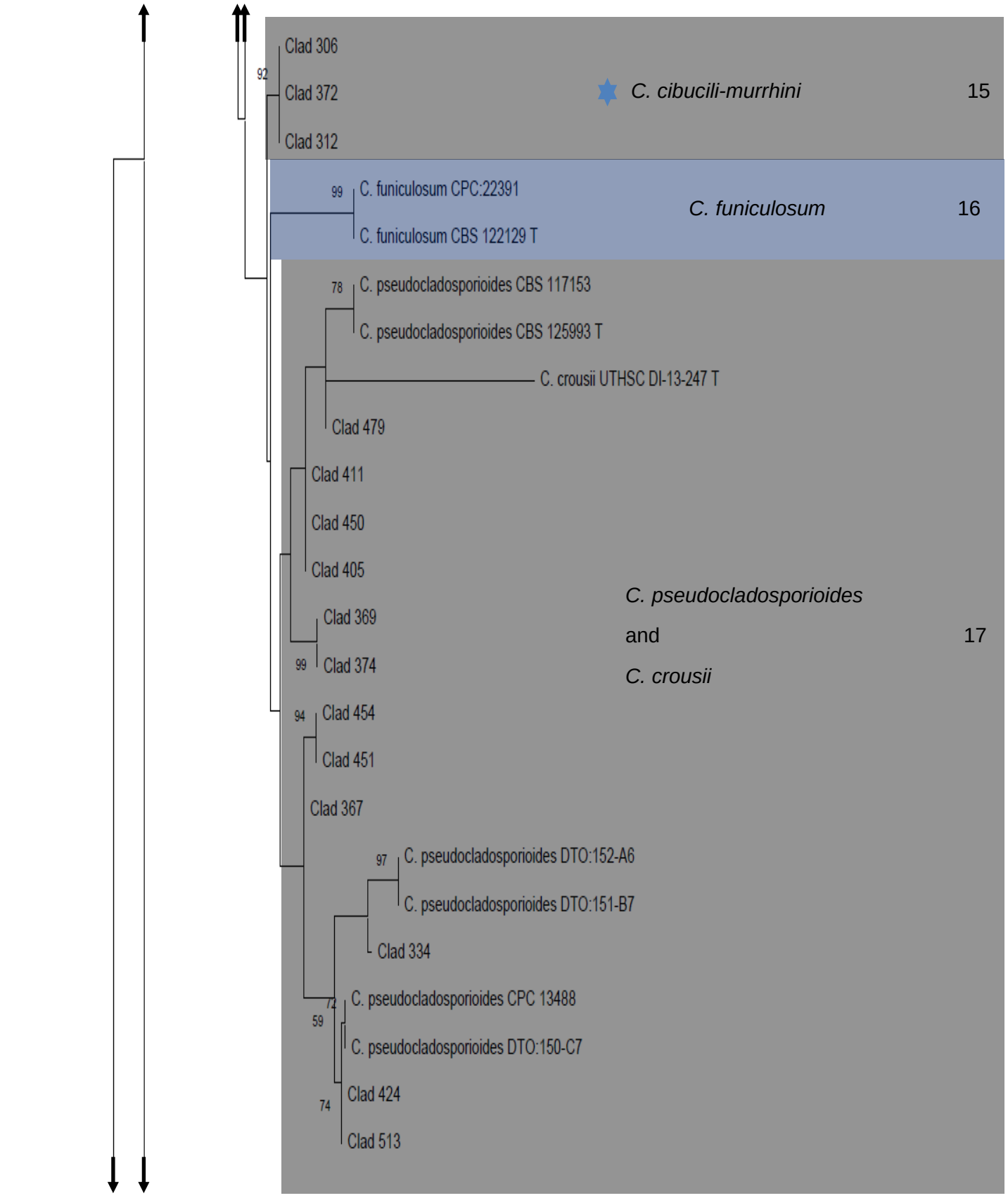


Fig. 2.3. (Continued).



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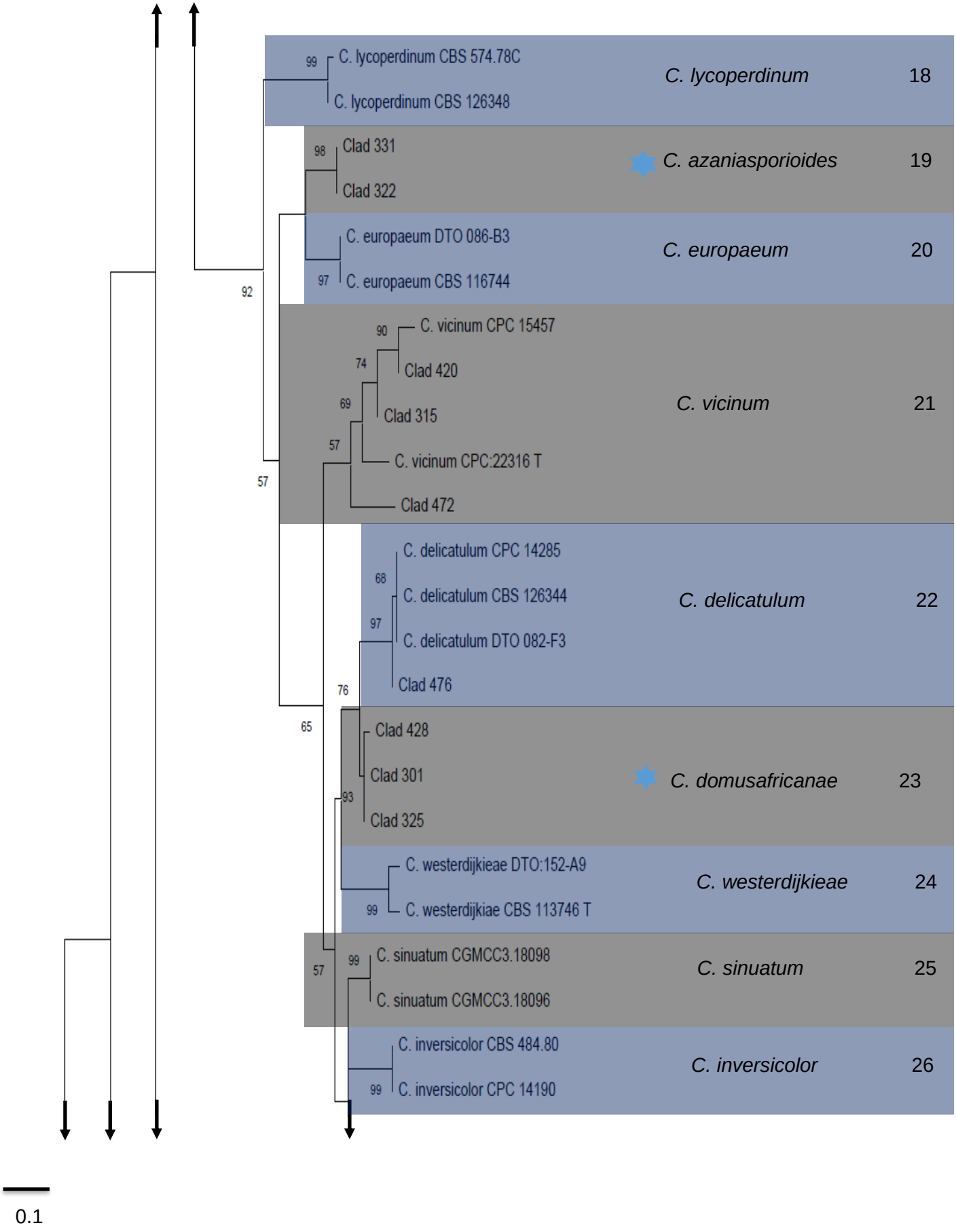


Fig. 2.3. (Continued).

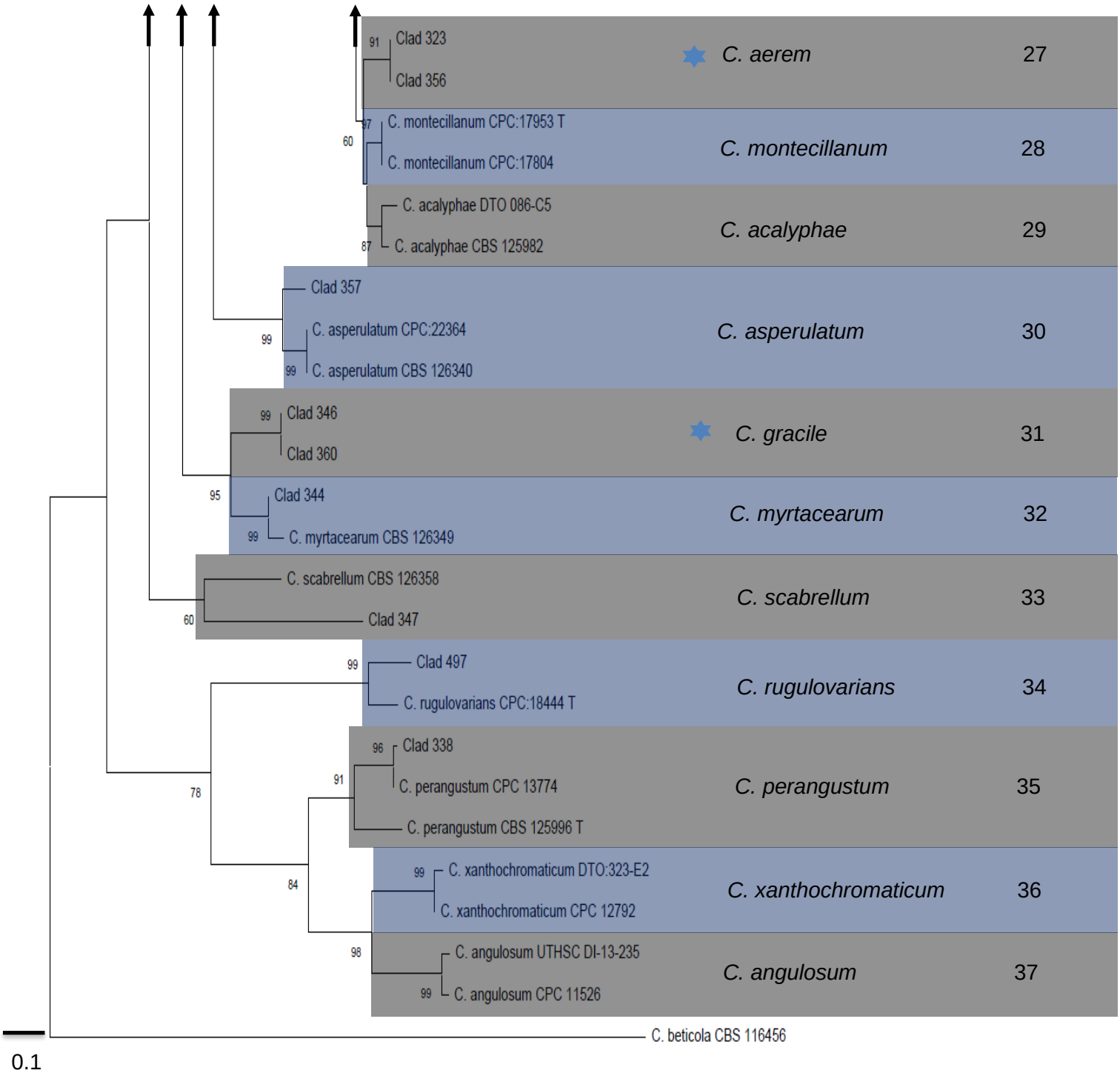


Fig. 2.3.

Figure 2.3. The maximum likelihood tree generated from a heuristic search of *C. cladosporioides* species complex alignments. The support values are on the nodes of each clade and are represented by the bootstrap values of 1000 replications. Bootstrap values lower than 55 were excluded from this analysis. The novel species are indicated with the star before their name. The scale bar is 0.1 and it represents the number of changes. The tree is rooted with *Cercospora beticola* CBS 116456.

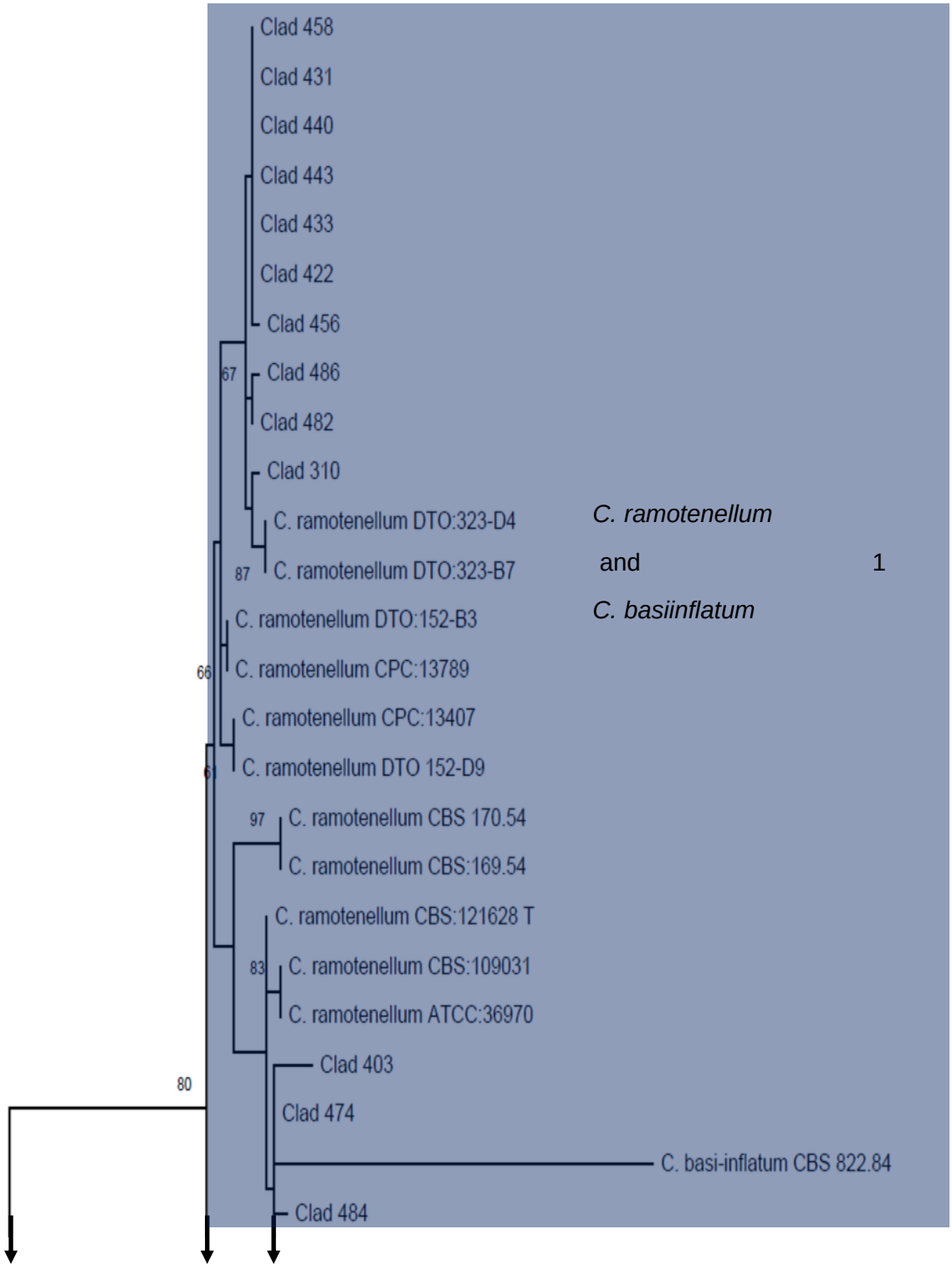


Fig. 2.4. (Continued).

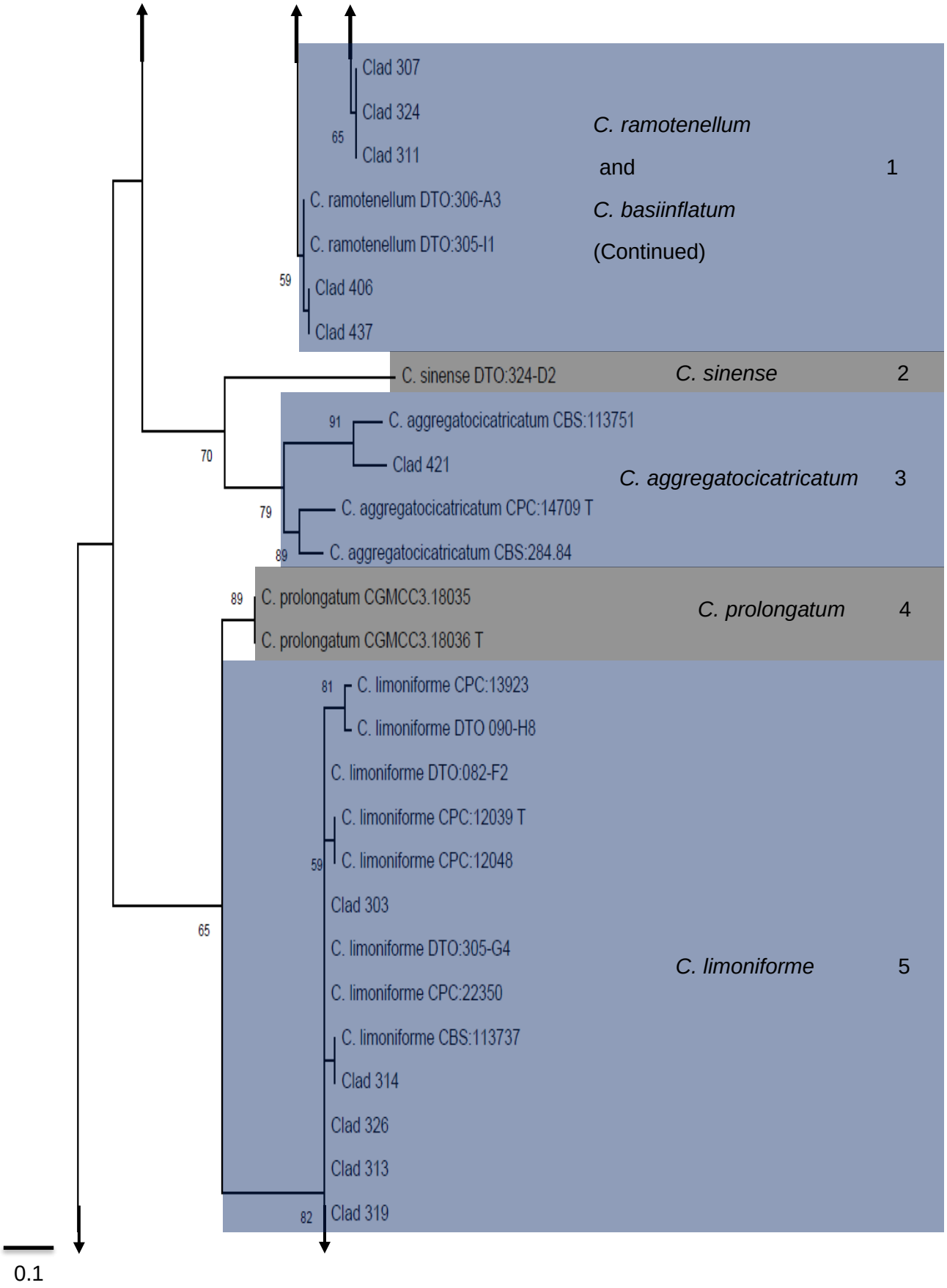


Fig. 2.4. (Continued).

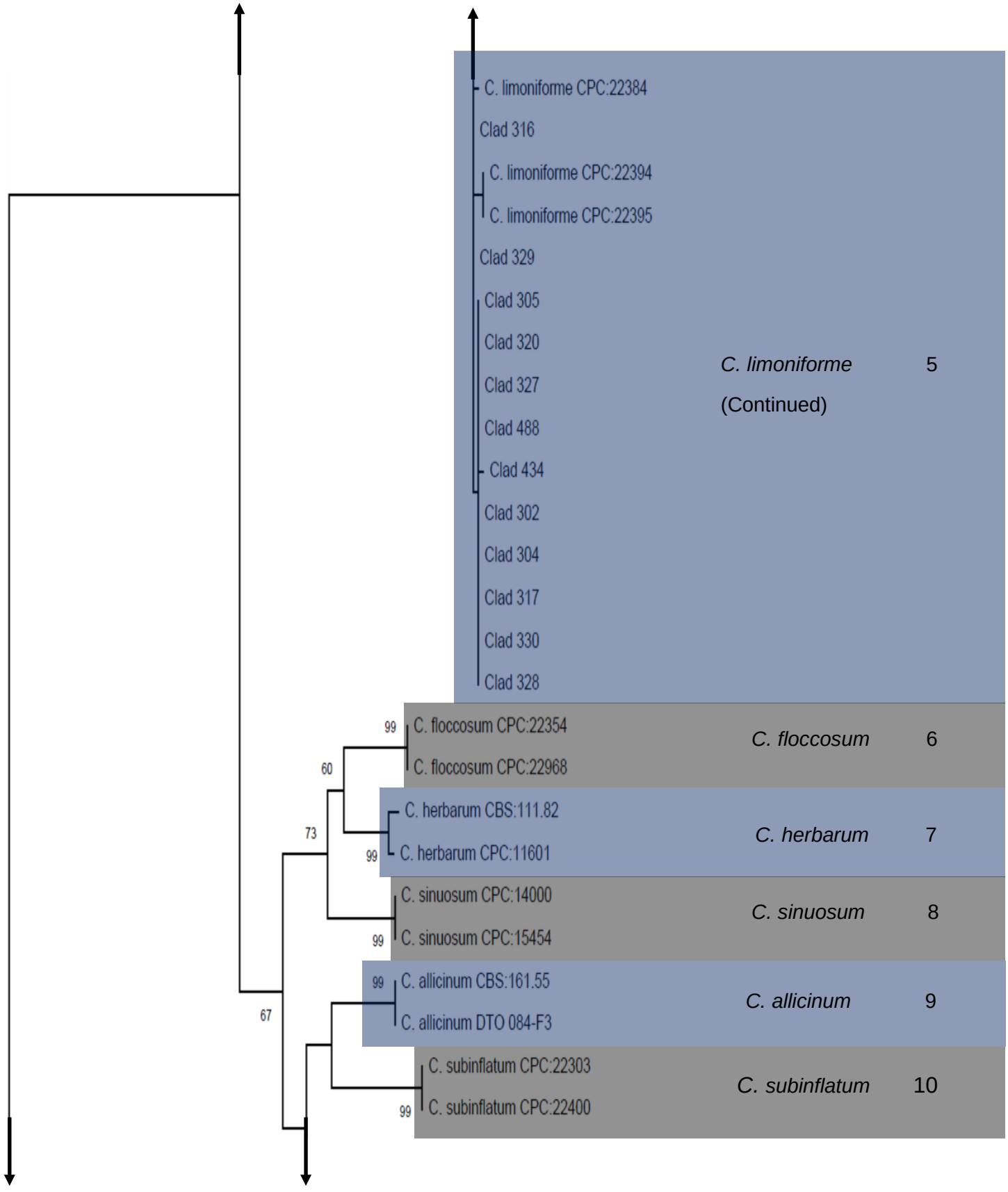


Fig. 2.4. (Continued).

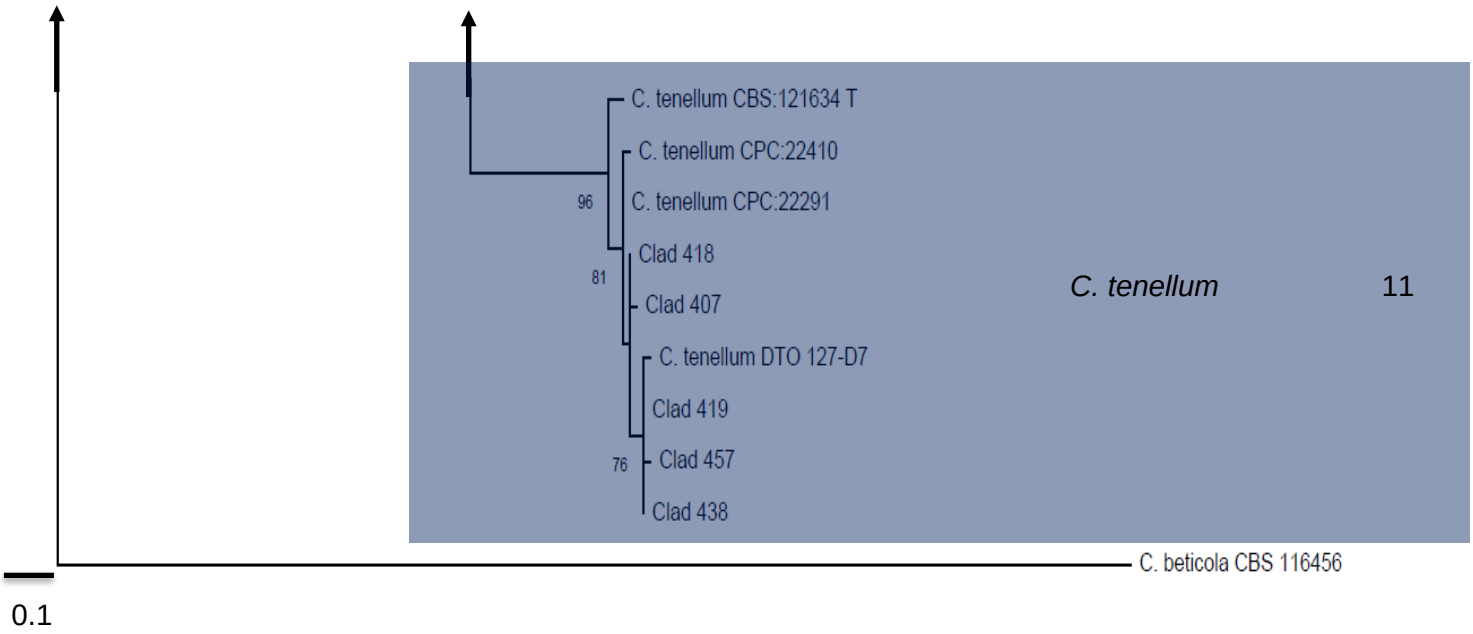


Fig. 2.4.

Figure 2.4. The maximum likelihood phylogeny generated from a heuristic search of *C. herbarum* species complex alignments. The support values are on the nodes of each clade and are represented by the bootstrap values of 1000 replications. Bootstrap values lower than 55 were excluded from this analysis. The novel species are indicated with the star before their name. The scale bar is 0.1 and it represents the number of changes. The tree is rooted with *Cercospora beticola* CBS 116456.

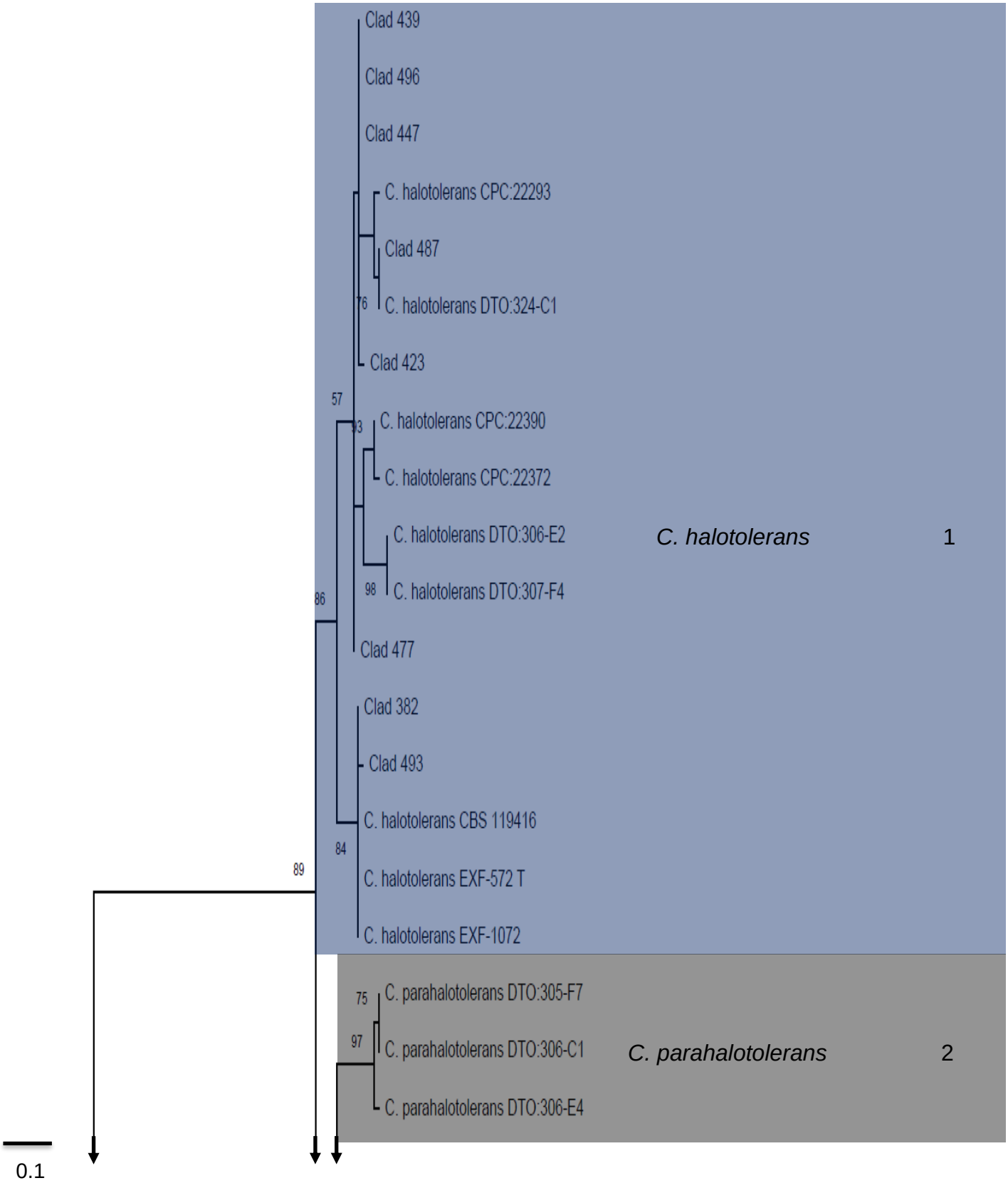


Fig. 2.5. (Continued).

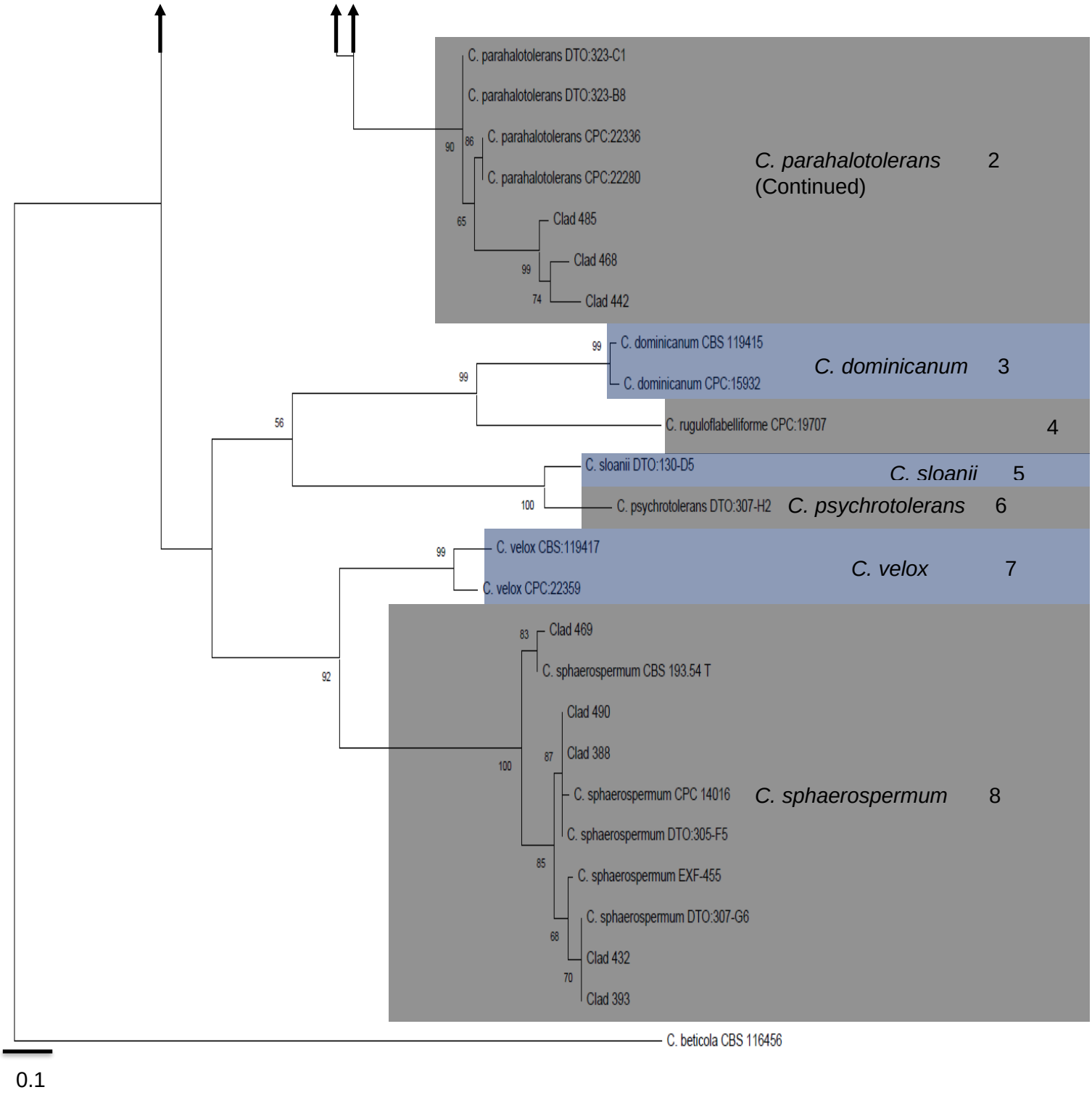
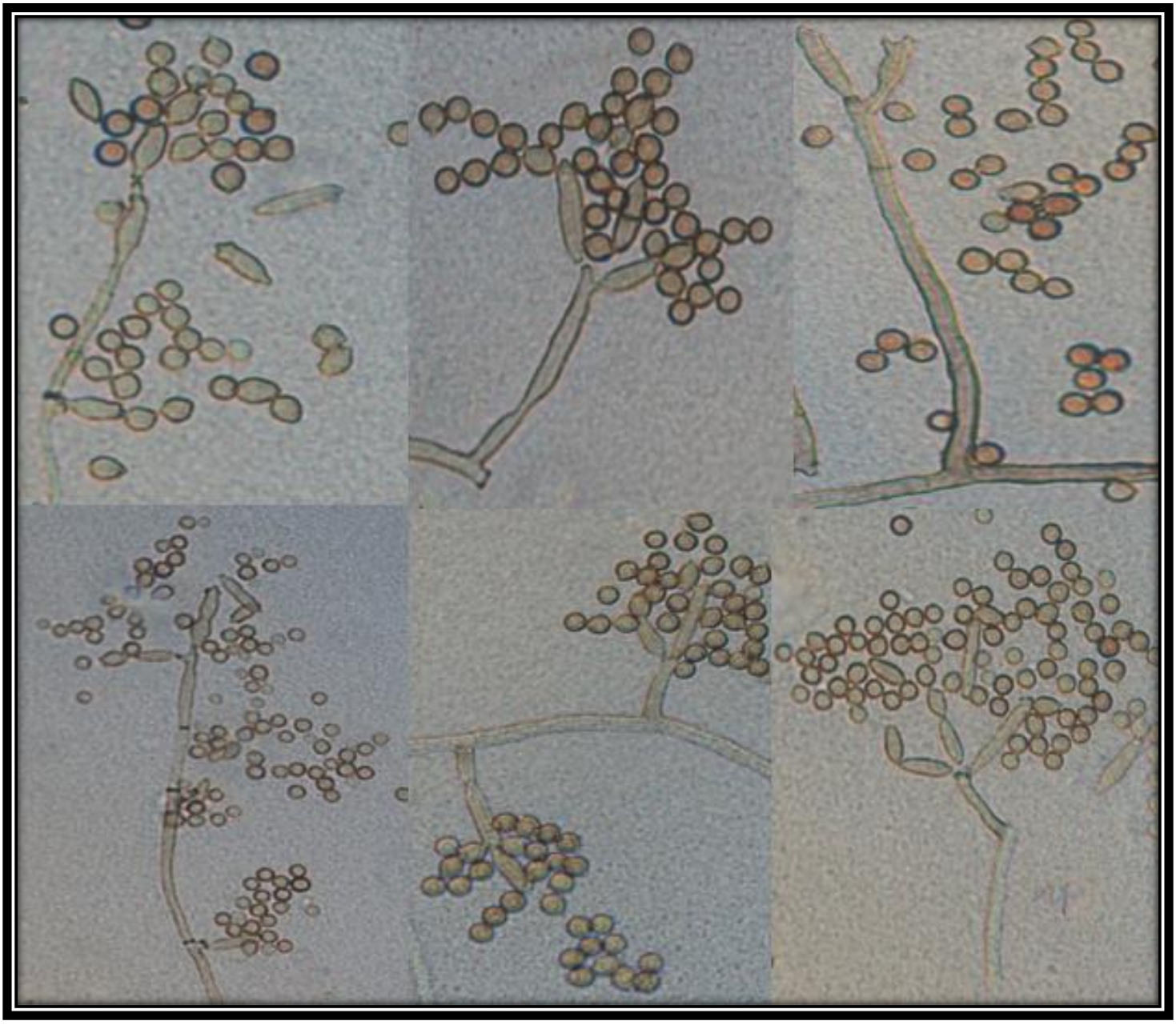


Fig. 2.5.

Figure 2.5. The maximum likelihood phylogeny generated from a heuristic search of *C. sphaerospermum* species complex alignments. The support values are on the nodes of each clade and are represented by the bootstrap values of 1000 replications. Bootstrap values lower than 55 were excluded from this analysis. The novel species are indicated with the star before their name. The scale bar is 0.1 and it represents the number of changes. The tree is rooted with *Cercospora beticola* CBS 11645.

Chapter 3

Six Novel *Cladosporium* Species from the Indoor Environment in Southern Africa



Abstract

The genus of *Cladosporium* resides in class Dothideomycetes, family *Cladosporiaceae* and represents a large and important group of fungi in the order of Capnodiales. *Cladosporium* comprises several species that owe their cosmopolitan distribution to their small size and lightweight spores easily disseminated by the air. In addition, their saprophytic lifestyle enables them to grow and reproduce in different ecosystems. In this study, we described six potential novel species of *Cladosporium* using the standardised approach of DNA sequencing data and diagnostic morphological features. The potential novel species were described as *C. aerem* prov. nom., *C. gracile* prov. nom, *C. azaniasporioides* prov. nom, *C. kostanzium* prov. nom, *C. cibiculi-murrhini* prov. nom and *C. domusafricanae* prov. nom, respectively.

Keywords: Indoor environment, novel species, molecular analysis and morphological characterisation

Introduction

Indoor environments are primary habitats for the safety and survival of the large and growing human population across the globe (Kelley and Gilbert, 2013). However, over the years, this ideal purpose of indoor environments has been continuously disturbed by microbial growth in these environments (Segers *et al.*, 2016). Therefore, personal well-being and healthy livelihoods rely on the comprehensive survey of indoor microbiology (Ghaffarianhoseini *et al.*, 2018; Gilbert and Stephens, 2018). Fungi present an important indoor biohazard and have complicated and compromised the living conditions of many occupants (Segers *et al.*, 2016; Yew *et al.*, 2016; Esch *et al.*, 2020). However, information about this is neither common nor easily accessed by the public and many people are still suffering from fungal related diseases because of a lack of awareness or knowledge (Kalan and Grice, 2018).

Fungal genera that are most frequently reported from the indoor environment include *Alternaria, Aspergillus, Cladosporium* and *Penicillium* (Adams *et al.*, 2013; Hanson *et al.*, 2016; Shan *et al.*, 2019). Some species of these genera are being implicated in various health issues related to the indoor environment. However, identifying these fungi is often not done to species level (Prussin and Marr, 2015; Gilbert and Stephens, 2018; do Nascimento *et al.*, 2019). Few studies adopted the use of next-generation sequencing as an attempt to comprehensively reveal and expand our understanding of the complex taxonomy of species composition that inhabits these spaces (Kelley and Gilbert, 2013; Valeriani *et al.*, 2017, Giampaoli *et al.*, 2020). Most fungal infections are species-specific (Esch *et al.*, 2020) and ideally, the assessment of fungal identification requires conclusive species-level identification. This is because DNA sequencing data often reveals great genetic diversity in species of the same genus (Zimowska *et al.*, 2021), which implies that different species have different characteristics.

Cladosporium species attract attention because it is an important fungus of indoor microbiomes and is able to predominantly grow and proliferate within indoor spaces (Bensch *et al.*, 2018). The early description of *Cladosporium* species identified the genus to be polyphyletic (Braun *et al.*, 2003; Dugan, 2004). Continuous revision with monograph treatments has resolved *Cladosporium* from other *Cladosporium*-like fungi (Bensch *et al.*, 2012). *Cladosporium* species are ubiquitous fungi that exist as saprophytes in different microhabitats of the indoor environment, including drywalls, carpeting, floors, damp surfaces, polymers and also have a wide range of ecological versatility and functional interactions with the natural world (Segars *et al.*, 2017; Alghuthaymi, 2017; Marin-Felix *et al.*, 2017; Bensch *et al.*, 2012, 2018). Correct identification of *Cladosporium* species is mainly based on a proper assessment of the species phylogeny. The dependence on the morphological based approach for the identification of

Cladosporium species is discouraged because there is very little or no morphological differences amongst *Cladosporium* species (Bensch *et al.*, 2012; Zimowska *et al.*, 2021).

Morphological characterisation often only gives partial insight into the identification of the taxa and can be used to place the isolates into larger taxonomic groups. *Cladosporium* currently recognises three major taxonomic groups, namely *C. cladosporioides, C. herbarum* and *C. sphaerospermum* species complexes and these complexes display many species that possess common morphological variations (Bensch *et al.*, 2015; Marin-Felix *et al.*, 2017; Zimowska *et al.*, 2021). This study, describes six novel *Cladosporium* species that are primarily assigned based on the DNA sequencing data generated from actin (ACT) and the elongation factor (EF 1-α) genes. The proposed novel species possess features that are confined within the current circumscription of *Cladosporium* as described by Sandoval-Denis *et al.* (2016) and possess key features of the frequently occurring *Cladosporium* species in the indoor environment (Bensch *et al.*, 2018).

Materials and Methods

Molecular Characteristics

The molecular characteristics were performed as described in the previous chapter (chapter 2). Genomic DNA was extracted from *Cladosporium* mycelia using the Quick-DNA fungal/bacterial MiniPrep kit (Zymo Research, USA) according to manufactures protocol. Partial actin gene was amplified with PCR using Act 512F and 783R primers, respectively (Bensch *et al.*, 2012). Partial translation elongation factor 1-alpha gene (TEF 1-α) was amplified using EF1-728F and EF1-986R primers, respectively (Bensch *et al.*, 2012). PCR amplicons were sequenced as detailed in Bensch *et al.* (2012). The generated DNA sequences were analysed using FinchTV (Treves, 2010) to remove low quality reading portions of the sequences and high quality sequences were used in a BLAST search to generate a dataset, including ex-type sequences from NCBI. The multiple alignments of contigs were done using MAFFT version: 1.4.0 (Katoh and Standley, 2013) manually trimmed and concatenated in Geneious Prime version 2020.2.2 (https://www.geneious.com) (Visagie and Houbraken, 2020). Concatenated sequences were used to construct phylogenetic trees with the Maximum likelihood (ML) and Tamura-Nei model on MEGA-X (Sandoval-Denis *et al.*, 2016).

Macro- and Micro-Morphological Characteristics

Culture characteristics: Spore suspension buffer was prepared using 0.2 % g/ml of agar, 50 % (v/v) of tween and 30 % (v/v) of glycerol and filled up with distilled water. The buffer was

used to make *Cladosporium* spore suspensions which were inoculated onto MEA, PDA, and OA, respectively, and incubated at 25 °C for 14 days (Bensch *et al.*, 2012). Photographs of culture characteristics were taken by Canon camera 12.1 megapixels. *Light microscopy* (LM): Microscopic features were analysed from the cultures cultivated on synthetic nutrient agar SNA (KNO_3 1 g, KCl 0.5 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, sucrose 0.2 g, glucose 0.2 g, agar 14 g and dH_2O 1L) (Samson *et al.*, 2002; Sandoval Denis *et al.*, 2016) for 7-10 days at 26 °C to study the development and branching pattern of conidia, conidiophores, and other important morphological features (Marin-Felix *et al.*, 2017; Bensch *et al.*, 2015, 2018). Usually, *Cladosporium* species have very fragile and complex morphological structures and the use of transparent adhesive tape preparation is advised for their microscope examination (Samson *et al.*, 2002). Therefore, for slide preparations, small squares of adhesive tape were used to lightly pick up the colony and placed on a microscope slide with a drop of Shear's mounting solution and covered with a coverslip (Bensch *et al.*, 2018). A couple of adjustments were made to the pictures by increasing clarity and adjusting the vignette to keep the same background in all pictures. Micro-morphology was progressively examined in the microscope from 10x, 20x and 40x magnification but 50 replicate measurements of diagnostic features were captured and recorded at 40x magnification. The conidiophore and conidial terminology were adopted from Bensch *et al.* (2018). The characterisation of diagnostic morphological features of novel species was compared with the phylogenetically closely related species (Table 3. 1.).

Results

From a larger study (Chapter 2), 20 strains were identified as potentially novel *Cladosporium* species. These strains were collected from different locations in indoor environments, and they belong to the *C. cladosporioides* species complex. In the last decade, studies have agreed on the use of reliable techniques for the identification of *Cladosporium* species and indicated that the use of morphological diagnostics alone is insufficient (Bensch *et al.*, 2012). The isolates were characterised by integrating DNA sequence data of the ACT and EF 1-α genes, culture characteristics and microscopic features (Bensch *et al.*, 2012, 2018). The DNA sequencing data of these isolates were deposited in the GenBank. The strains contain sequences of different lengths ranging from 165-190 bases and 180-220 bases for the ACT and EF 1-α genes, respectively. The heuristic search of *Cladosporium* species phylogeny (Figure 3.1.) showed that the proposed novel species form well delineated clades distinct from closely related species and are statistically well supported by the bootstrap values. The species are described as follows and are compared with closely related species.

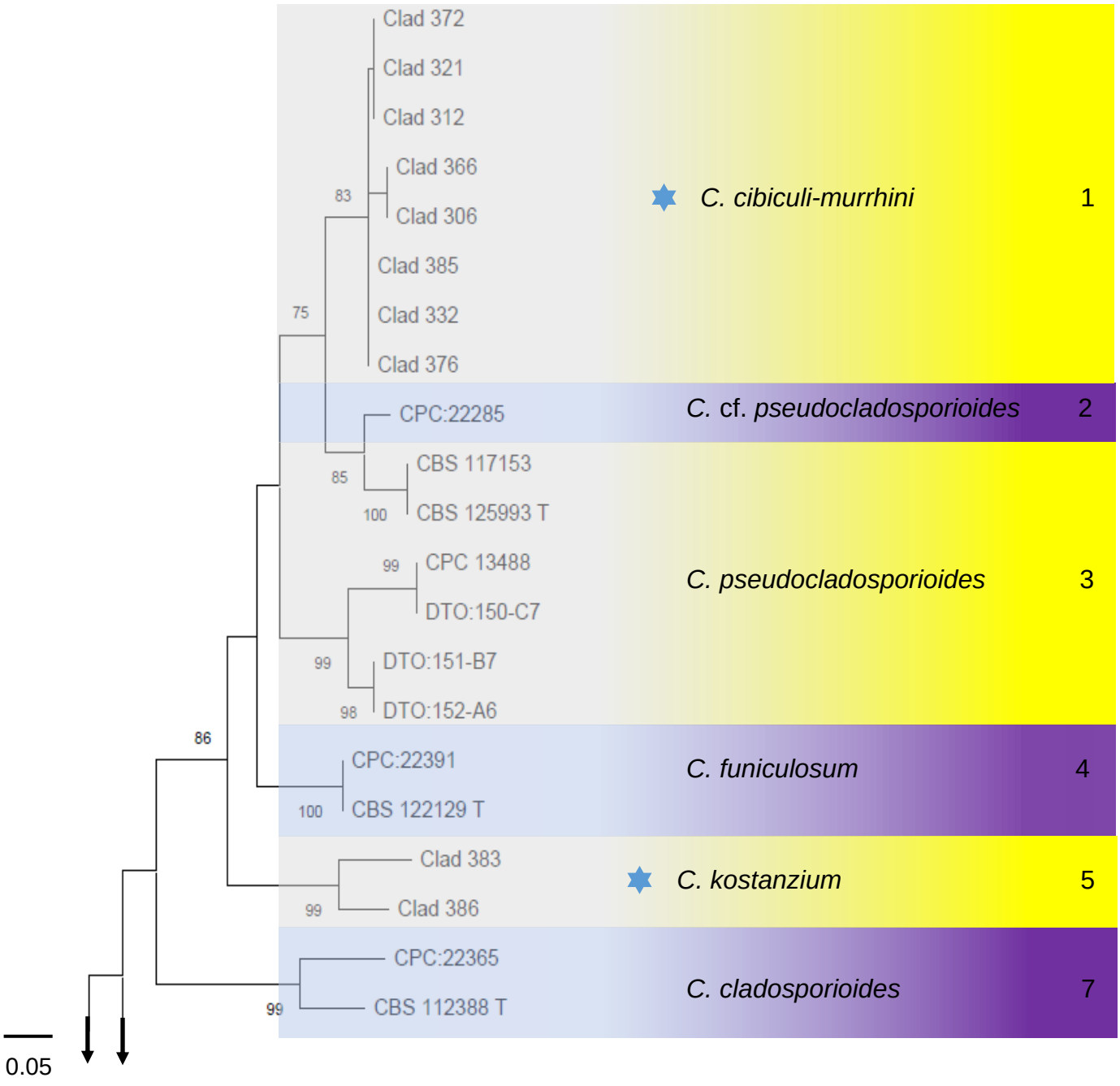


Figure 3.1. The maximum likelihood phylogram was generated from a heuristic search of *ACT + EF1 Cladosporium* sequence alignments. The support values are on the nodes of each clade and are represented by the bootstrap values of 1000 replications. Bootstrap values lower than 75 were excluded from this analysis. The novel species are indicated with the star before their name and numbers next to the species name are for easier referencing of the clade. The scale bar is 0.05 and it represents the number of changes. The tree is rooted with *C. herbarum* CBS:111.82 and CPC:11601.

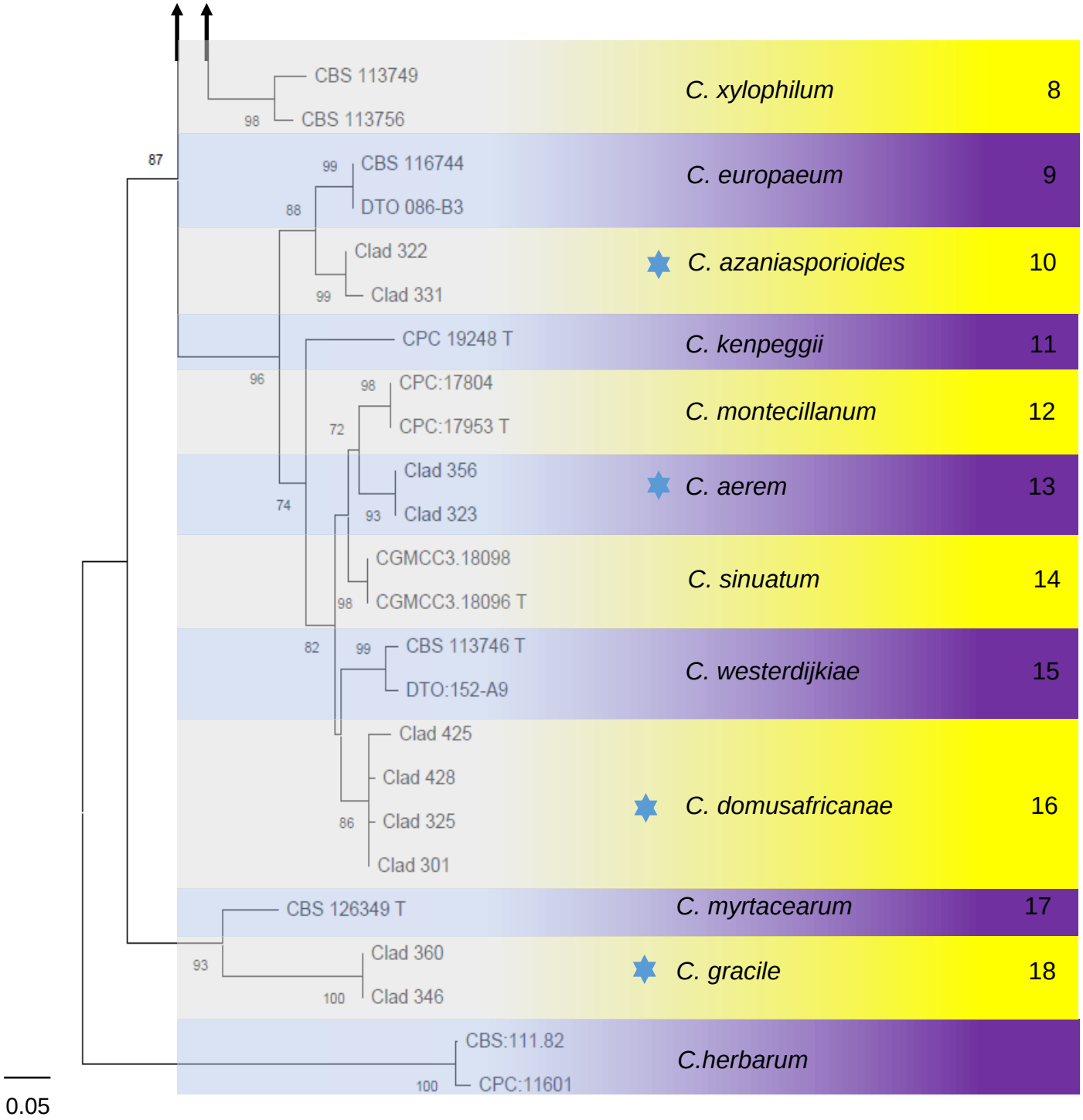


Fig. 3.1. (Continued).

Table 3. 1. Comparisons of macro-and micro-morphological features between novel *Cladosporium* species described in this study and the ex-type species of closely related species (Sandoval-Denis *et al*., 2016; Ma *et al.*, 2017; Bensch *et al.*, 2015, 2018).

***Cladosporium* spp.**	**Conidiophores (µm)**	**Ramoconidia (µm)**	**Conidia (µm)**	**Colony diameter (mm) after 14 days at 25 °C**
C. cibiculi-murrhini **prov. nom**.	(26-)90-172 x (2-)3.5-5	(9-)15-21 x (2.5-)3.5-5.5	(5-)6.5-10.5 x (1.5-)3-3.5(-4.5)	MEA : 60
C. pseudocladosporioides	15-155 x 2-4	19-48 x 3-4	Av: 7 x 3 3-5.5 x (1-)1.5-2.5	PDA : 51; OA : 55 MEA : 52-72
C. cladosporioides	40-300(-350) x (2.5-)3-5	15-50 x (2.5-)3-5	Av: 4.0 x 2.0 3-6(-7) x (1.5-)2-2.5(-3)	PDA : 65-78; OA : 55-73 MEA : 54-72
			Av: 4.7 x 2.4	PDA : 80; OA : 65-70
C. aerem **prov. nom**.	(38-)110-188 x (2-)3.5-4.5	(8-)10-12(-17) x (2-)3-(-4.5)	(4.5-)6-7(-10.5) x (-2)3.5-5.3	MEA : 57
C. sinuatum	9-100(150) x 2-3	5-8.5 x 1.5-4(-5)	Av: 7 x 3.5 1.5-7 x (1-)2-4(-5)	PDA : 56; OA : 52 MEA : 35-42
C. montecillanum	25-130 x 2.5-4	With a truncate, non cladosporioid base	Av: 5 x 3.5 3-5(-6) x (1.5-)2-2.5	PDA : 25-33; OA : 40-45 MEA : 70
			Av: 4.0 x 2.5	PDA : 65-77; OA : 55-70
C. gracile **prov. nom**.	(58-)122-196 x (2.5-)3.5-6.5	(7.5-)15.5-24 x (2-)3-3.5-(-4.5)	(5-)6.5-7(-10) x (2-)2.5-3(-4)	MEA : 48
C. myrtacearum	(35-)55-220(-320) x 3.5-4.5	20-55 x 3.5-4.5	Av: 7 x 3 4.5-8.5 x (2.5-)3-3.5(-4.5) Av: 6.0 x 3.0	PDA : 51; OA : 54

Table 3.1. (Continued).

***Cladosporium* spp.**	**Conidiophores (µm)**	**Ramoconidia (µm)**	**Conidia (µm)**	**Colony diameter (mm)** **after 14 days at 25 °C**
C. azaniasporioides **prov. nom.**	(65-)146-167 x (2-)3-3.5(-4.5)	(8.5-)12-23 x (2-)3-3.5(-4.5)	(3-)4.5-5(-8.5) x (2-)3-4.5	MEA : 58 PDA : 51; OA : 55
C. europaeum	35-150(-290) x (2.5-)3-4.5	18-39 x 3-4	Av: 5 x 3 2.5-4.5(-55) x 2-2.5(-3) Av: 4.0 x 2.5	MEA : 50-76 PDA : 73-82; OA : 55
C. kostanzium **prov. nom.**	(54.5-)95.5-164 x (2.5-)3-4	(9.5-)13-13.5 x (2.5-)3.5-5.5	(4.5-)6.5-8.5 x (1.5-)2.5-4	MEA : 57 PDA : 52; OA : 52
C. funiculosum	10-120 x (2-)2.5-3.5(-4)	Occasionally formed	Av: 6.5 x 1 (2.5-)4-9 x (1.5-)2-2.5(-3) Av: 5.5 x 2.5	MEA : 58-80 PDA : 57-78; OA : 47-67
C. domusafricanae **prov. nom.**	(61.5-)145-203 x (2-)3.5-5.5	(5.5-)9.5-10(-13) x (2.5-)3.5-4.5	(4-)5.5-6(-9) x (2.5-)3-3.5(-4.5)	MEA : 55 PDA : 47; OA : 56
C. westerdijkiae	23-125(-185) x 3-5	22-52 x 3.5-4.5	Av: 6 x 3.5 4-5(-5.5) x 2- 2.5 Av: 4.5 x 2.0	MEA : 46-75 PDA : 61-75; OA : 53-75

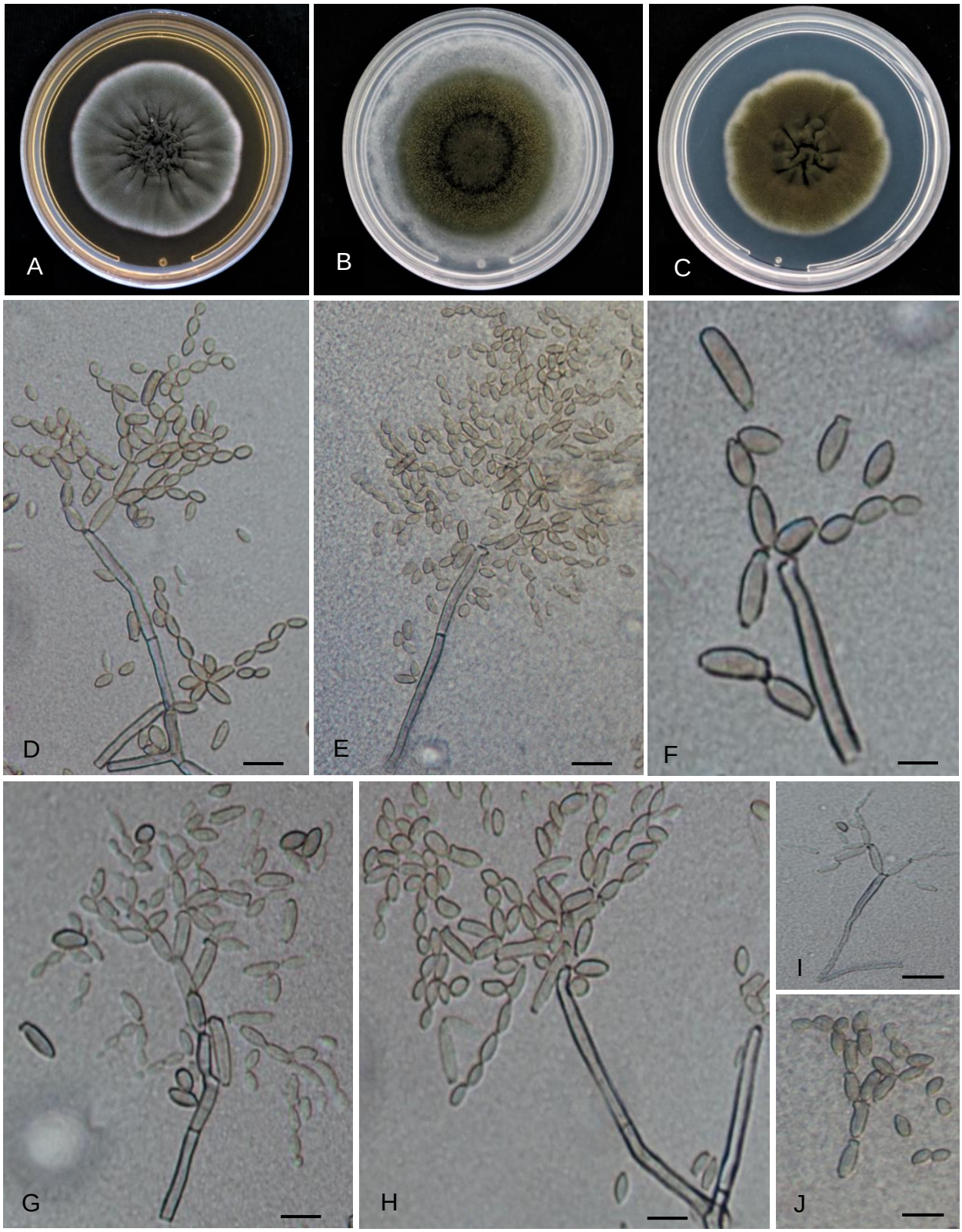


Figure 3.2. *Cladosporium aerem* (Clad 323). **A-C.** Colonies on MEA, OA and PDA. **D-I.** Conidiophores, ramoconidium and conidial chains. **J.** Conidial chains. Scale bar = 10 µm.

Taxonomy

Cladosporium aerem Buthelezi & Jacobs, **prov. nom**, (Figure 3.2.

Etymology: Name refers to *Cladosporium* from air.

Specimens examined: South Africa, Western Cape (Wellington) and Zimbabwe, isolated from the indoor environment. *Holotype*: Clad 323, GenBank: OK135970 & OK086820.

Mycelium immersed and sparingly branched. *Hyphae* sparingly branched and subhyaline. *Conidiophores* macro- and micronematous, erect and straight to somewhat flexuous, arising at the apex or lateral axis of the hyphae, sometimes geniculate, occasionally forming nodules at the apex but non-denticulate, filiforme, subhyaline, cylindrical oblong, (38-)110-188 x (2-)3.5-4(-4.5) µm, 0-1(-2) septate and occasionally with up to 2 septa. *Conidiogenous cells* are intercalary form mostly arising from the apex, cylindrical oblong and seldomly show loci. *Ramoconidia* are formed they are often solitary (8-)12-10 x (2-)3-4.5 µm (av. 12.5 x 3.5). *Conidia* are often catenate and sometimes solitary, obovoid or so, limoniform, rarely spherical, ellipsoid (4.5-)6-7(-10.5) x (2-)3.5-3.5(-5.5) µm (av. ± SD: 7 ± 1.5 x 3.5 ± 0.5), non-septate.

Culture characteristics: Colonies on MEA grow up to 57 mm diameter after 14 days at 25 °C, olivaceous grey with whitish colour in the circumference of the colony, possessing folded and elevated center with radial farrows and have a convex growth pattern. Colonies on OA grow up to 56 mm diameter after 14 days at 25 °C, olivaceous grey to greenish, profusely sporulating with no prominent exudates, have flat or low convex growth pattern. Colonies on PDA grow up to 52 mm diameter after 14 days at 25 °C olivaceous grey, possessing a folded and elevated center, possessing radial farrows that are not maintained throughout the end and have convex growth pattern.

Notes: This species forms a separate clade close to *C. sinuatum* and *C. montecillanum* in a species phylogeny. The relevant morphological diagnostic features for a species rank description confirm that this species is distinct from *C. sinuatum* (Figure 3.1. ; clade 14) (Ma *et al.*, 2017) and *C. montecillanum* (Figure 3.1. ; clade 12) (Bensch *et al.*, 2015). The microscopic features that distinguish *C. aerem* from its ex-type species include its longer conidiophores and conidia that are sometimes globose and longer compared to C. *sinuatum* and *C. montecillanum* (Table 3. 1.).

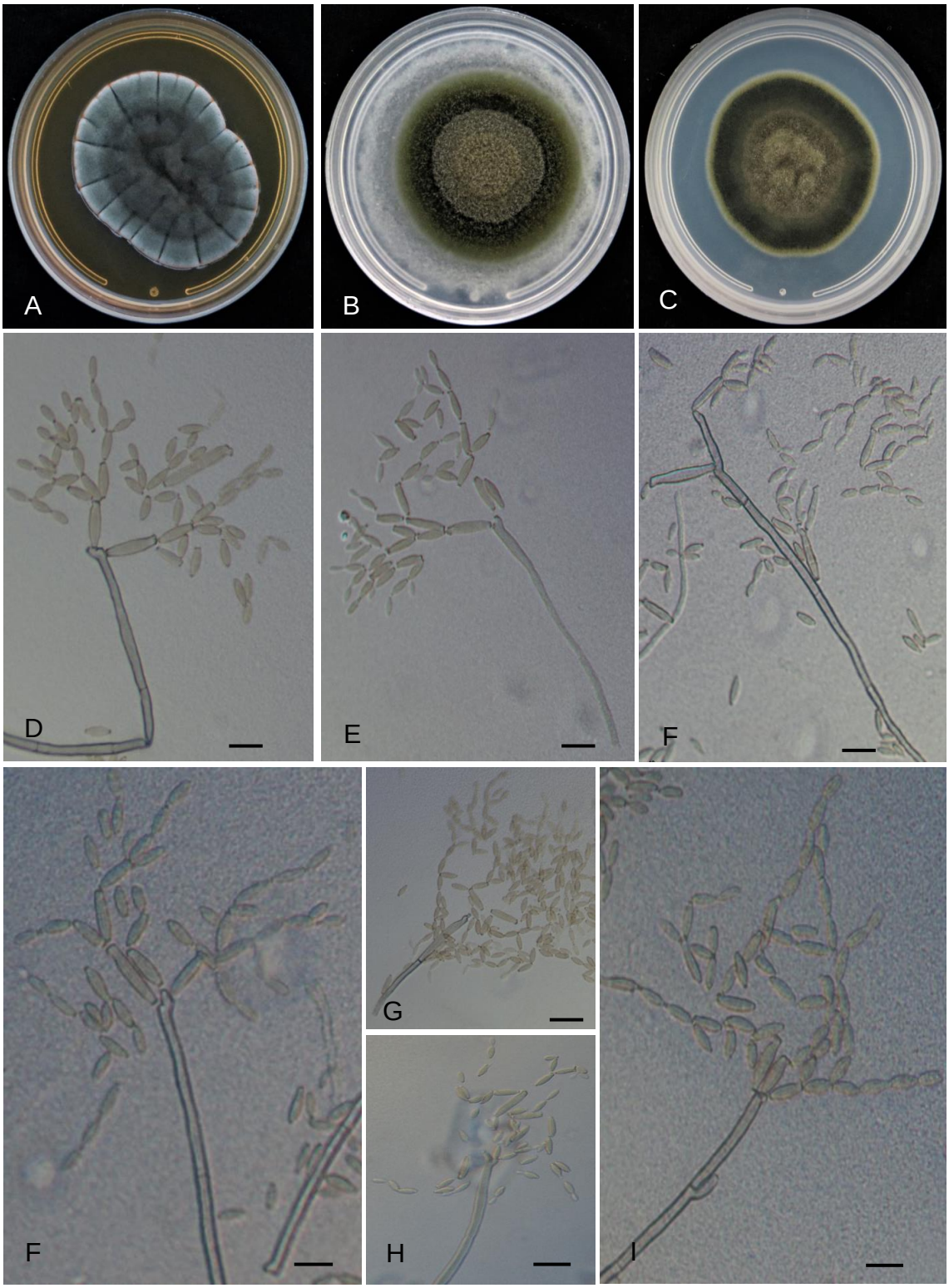


Figure 3.3. *Cladosporium gracile* (Clad 360). **A-C.** Colonies on MEA, OA and PDA. **D-H.** Conidiophores, micronematous conidiophore, ramoconidia and conidial chains. **I.** Conidiophores and conidial chains. Scale bar = 10 µm.

Cladosporium gracile Buthelezi & Jacobs, **prov. nom,** (Figure 3.3).

Etymology: The name refers to thin conidiophores of this fungi.

Specimens examined: Zimbabwe, isolated from the indoor environment. *Holotype*: Clad 360, GenBank: OK086824.

Mycelium immersed. *Conidiophores* macro- but mostly micronematous, erect and straight, often arising from the apex and sometimes at the lateral axis of the growing hyphae, it is plangiotropous, mostly filiforme, sub-cylindrical or cylindrical oblong (58-)122-196x (2.5-) 3.5-3.5(-6.5) µm, 0-1(-3) septate and occasionally showing up to 3 septa, showing dark colour at the ends of the walls, sometimes geniculate but mostly non-denticulate and subhyaline. *Conidiogenous cells* are at the terminal in mostly unbranched or branched chains. *Ramoconidia* are formed, mostly as solitary and often cylindrical oblong (7.5-)15.5-24 x (2-)4-3(-4.5) µm (av. 15.5 x 3.5) and aseptate. *Conidia* often catenate, rarely solitary, mostly obovoid to fusiforme, sometimes limoniform (5-)6.5-7(-10) x (2-)2.5-3(-4) µm (av. ± SD: 7 ± 1 x 3 ± 0.5), aseptate, smooth and non verruculose.

Culture characteristics: Colonies on MEA grow up to 48 mm diameter after 14 days at 25 °C, olivaceous grey and pale grey at the colony margin, the growth pattern is showing radial furrows from the center toward the colony circumference, the center is elevated and folded with a hard texture. Colonies on OA grow up to 54 mm diameter after 14 days at 25 °C, olivaceous grey to greenish toward the end of the colony, showing a low convex growth pattern with the elevated center. Colonies on PDA grow up to 51 mm diameter after 14 days at 25 °C, grey olivaceous mostly at the center and pale green towards colony margin, it is slightly folded at the center with convex growth pattern and profusely sporulating with no prominent exudates.

Notes: All strains of this species were collected from Zimbabwe and the closest related species is *C. myrtacearum* (CBS 126349). *C. gracile* form a highly supported monophyletic clade distinct from *C. myrtacearum* (Figure 3.1. ; Clade 17). The relevant morphological diagnostic features confirm that *C. gracile* is distinct from *C. myrtacearum* (Bensch *et al.*, 2015). *C. gracile* possesses shorter and thicker conidiophores that are less septate and have aseptate, short and occasionally narrow ramoconidia and have no prominent surface ornamentation on conidia, compared to *C. myrtacearum* (Table 3. 1.). In addition, *C. myrtacearum* is not known to occur in the indoor environment and has only been isolated from Australia. In this study, *C. myrtacearum* is identified from indoor environments (*C. myrtacearum* Clad 344, Figure 2.3. Clade 32), suggesting a broader distribution of *C. myrtacearum*.

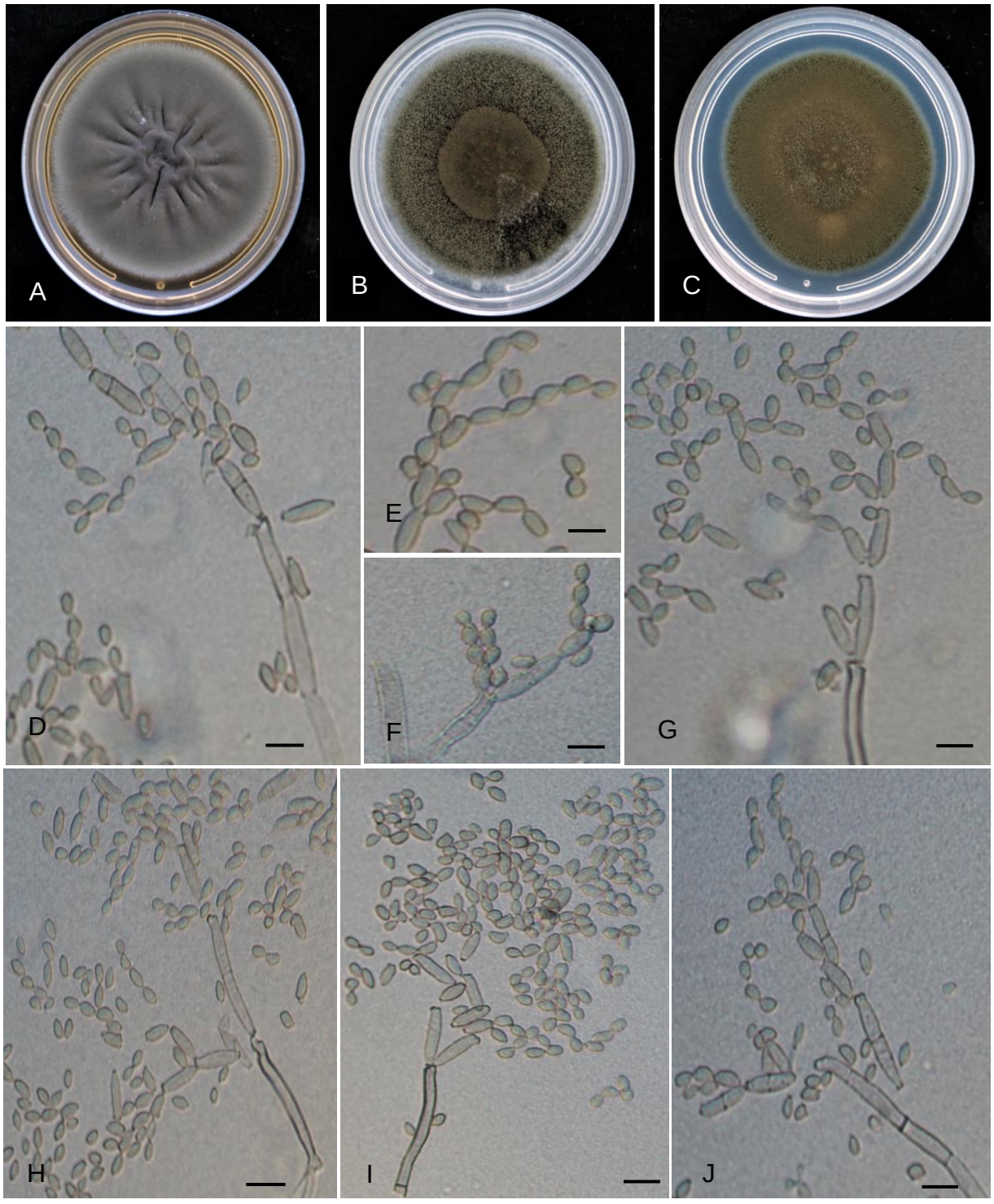


Figure 3.4. *Cladosporium azaniasporioides* (Clad 322). **A-C.** Colonies on MEA, OA and PDA. **D-J.** Unbranched and branched conidiophores, micronematous conidiophore, ramoconidium and conidial chains. Scale bar = 10 µm.

Cladosporium azaniasporioides Buthelezi & Jacobs, **prov. nom**, (Figure 3.4.

Etymology: The name refers to the alternative name for South Africa, Azania where this species was collected from.

Specimens examined: South Africa (Western Cape, Wellington), isolated from the indoor environment. *Holotype*: Clad 322, GenBank: OK135969 & OK086817.

Mycelium immersed. *Conidiophores* macro- and micronematous, erect and straight, somewhat flexible, arising from the apex and lateral axis of the growing hyphae, filiforme, irregular oblong subhyaline (65-)146.5-167 x (2-)3-3.5(-4.5) µm, 0-1(-3) septate, occasionally geniculate and with no projections that can be identified as denticulate or nodulose. *Conidiogenous cells* solitary and in chain form, occasionally denticulate and minutely verruculose or so, mostly terminal, and cylindrical oblong. *Ramoconidia* is formed mostly cylindrical oblong, solitary (8.5-)12-23 x (2-)3-4(-5) µm (av. 12.5 x 3.5) and occasionally septate. *Conidia* numerous often catenate, form branching chains and intercalary conidia observed, obovoid to limoniform, fusiforme, sometimes spherical and non-verruculose (3-)4.5-5(-8) x (2-)3-4.5 µm (av. ± SD: 5 ± 1 x 3. ± 0.5) and showing no septa.

Culture characteristics: Colonies on MEA grow up to 58 mm diameter after 14 days at 25 °C olivaceous grey, profusely sporulating, with no exudates observed, colony is folded and elevated in the center with convex growth pattern and showing radial furrows from the center but not maintained throughout the end of the colony. Colonies on OA grow up to 55 mm diameter after 14 days at 25 °C, olivaceous grey and pale to whitish, colony is flat or having regular growth pattern and have aerial spores not firmly attached to mycelium. Colonies on PDA grow up to 51 mm diameter for 14 days at 25 °C, olivaceous grey, smoke to grey, showing profusely sporulation, minutely elevated center and have convex growth pattern.

Notes: In the phylogeny, *C. azaniasporioides* is closely related to *C. europaeum* (Figure 3.1. , clade 9) which has been recently assigned by Bensch *et al.* (2018) while it was first identified as *C. cladosporioides sensu lato* Lineage 1 because it lacked distinctive morphological features compared to *C. cladosporioides* (Bensch *et al.*, 2010). *C. azaniasporioides* (Figure 3.1. , clade 10) form a strongly supported monophyletic clade that is distinct from *C. europaeum*. *C. azaniasporioides* possess shorter conidiophores and ramoconidia, and longer conidia compared to *C. europaeum* (Table 3.1).

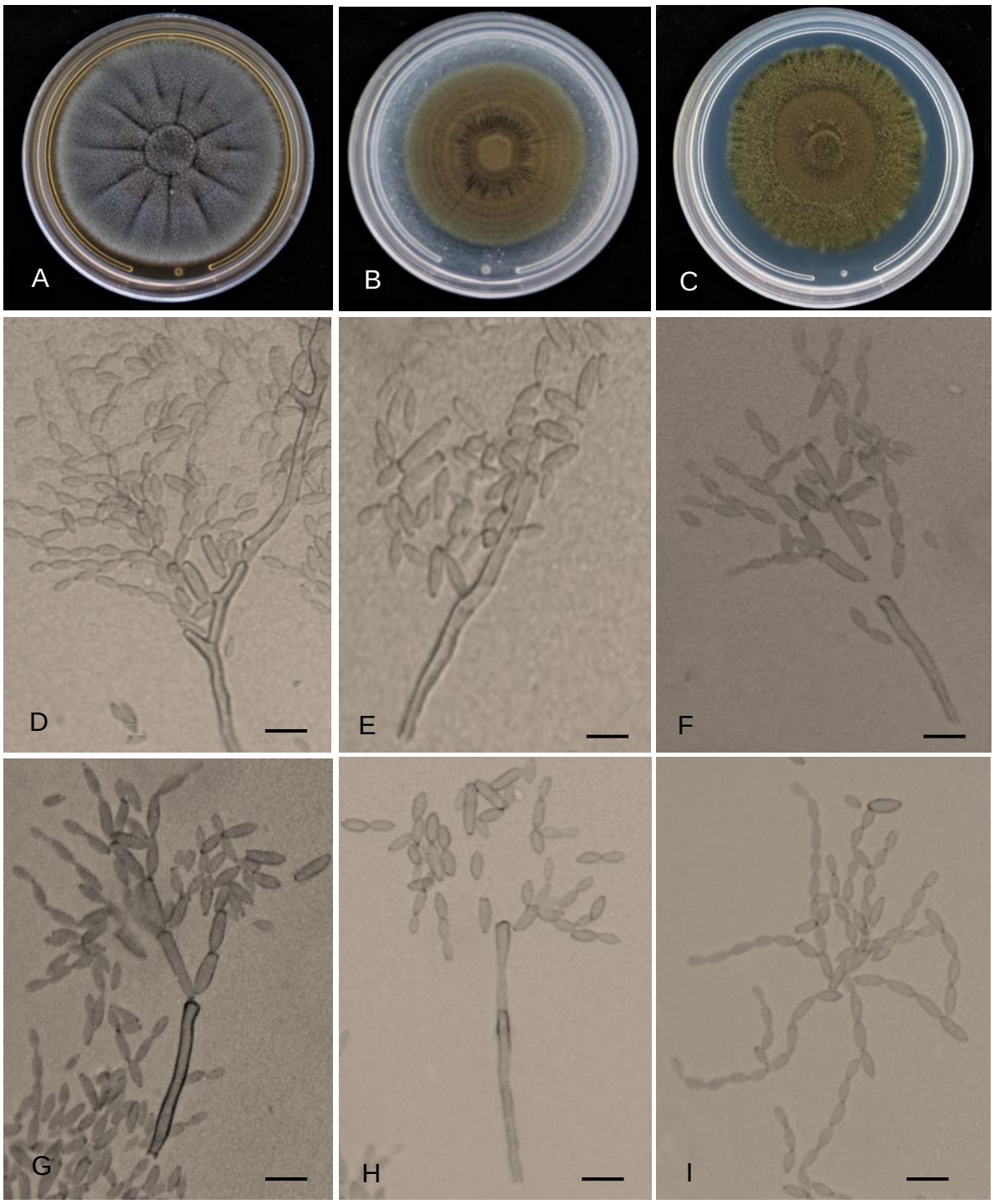


Figure 3.5. *Cladosporium kostanzium* (Clad 383). **A-C.** Colonies on MEA, OA and PDA. **D-G.** Conidiophores and conidial chains. **H.** Micronematous conidiophores and condia. **I.** Conidial chains. Scale bar = 10 µm.

Cladosporium kostanzium Buthelezi and Jacobs, **prov. nom,** (Figure 3.5.

Etymology: With this name we pay tribute to Konstanze Bench for her contribution to the monographic treatment of *Cladosporium*.

Specimens examined: South Africa (Johannesburg), isolated from the indoor environment. *Holotype:* Clad 383, GenBank: OK086815.

Mycelium superficial and immersed. *Conidiophores* macro- and micronematous erect, mostly straight and/or somewhat flexible, often arise plagiotropously at the terminal or lateral axis of the growing hyphae, filiforme, occassionally geniculate and denticulate but non-nodulose, sub-cylindrical or cylindrical oblong, (54.5-)95.5-164 x (2.5-)3-4 µm, 0-1 septa and subhyaline. *Conidiogenous* cells mostly integrated, branching, aseptate and terminal. *Ramoconidia* formed mostly solitary cylindrical oblong (9.5-)13-13.5(-19.5) x (2.5-)3.5-5.5 µm (av. 14 x 3.5) and aseptate. *Conidia* numerous, catenate forming long branched chains and branching to all directions, mostly obovoid to fusiforme, subrostrate, non verruculose, showing smooth surfaces (4.5-)6.5-8.5 x (1,5-)2.5-4 µm (av. ± SD: 6 ± 1 x 3 ± 0.5) and aseptate.

Culture characteristics: Colonies on MEA grows up to 57 mm diameter after 14 days at 25 °C, olivaceous grey to smoke grey, profusely sporulating and have powdery spores, exhibits radial farrows, convex growth pattern and colony elevation at the center. Colonies on OA grows up to 52 mm diameter after 14 days at 25 °C, olivaceous grey to almost greenish towards the colony margin, showing concentric circles from the center to circumference, with convex growth pattern and no prominent exudates observed. Colonies on PDA grow up to 52 mm diameter after 14 days at 25 °C, olivaceous grey to something greenish, profusely sporulating, a bit fluffy towards the colony margin and showing convex growth pattern.

Note: *C. kostanzium* (Figure 3.1. , clade 5) forms a well-supported monophyletic clade distinct from *C. funiculosum* (Figure 3.6. , clade 5). *C. kostanzium* has longer conidiophores and often form ramoconidia, while they are occasionally formed in *C. funiculosum* (Table 3. 1.). In addition, on a plate, *C. funiculosum* grow fluffy, develop white pigment and its conidia often have tapering ends (Bensch *et al.*, 2018) which is lacking in *C. kostanzium*.

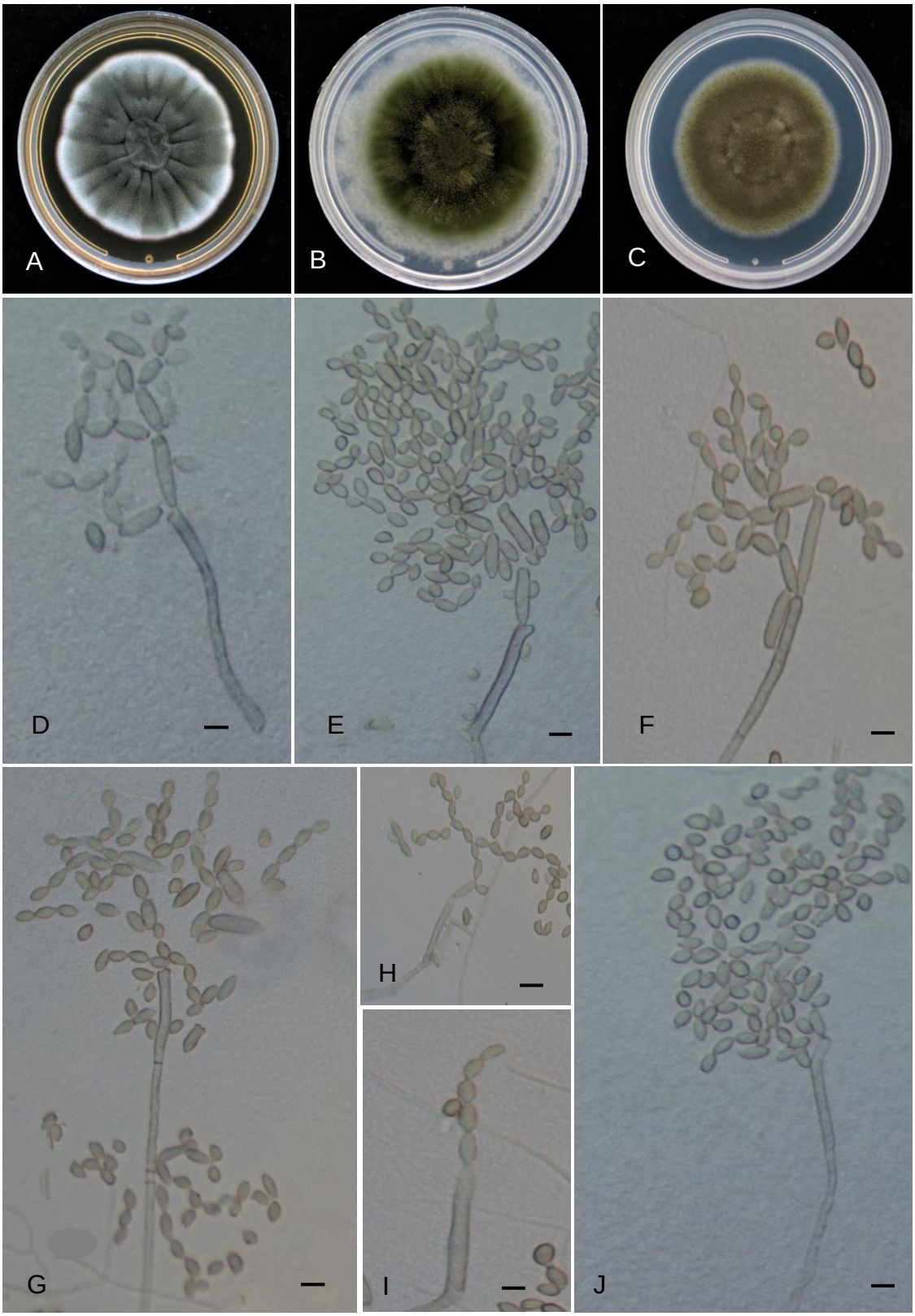


Figure 3.6. *Cladosporium domusafricanae* (Clad 428). **A-C.** Colonies on MEA, OA and PDA. **D-I.** Conidiophores, micronematous conidiophore and conidial chains. **J.** Conidiophores and unbranched conidia. Scale bar = 10 µm.

Cladosporium domusafricanae Buthelezi & Jacobs, **prov. nom**, (Figure 3.6.

Etymology: The name refers to *Cladosporium* from African homes.

Specimens examined: South Africa (Western Cape, Cape Town), isolated from indoor air. *Holotype:* The description and naming of this species is based on strain Clad 428, GenBank: OK135971 & OK086821.

Mycelium immersed and occasionally superficial. *Conidiophores* macro- and micronematous, erect and straight to somewhat flexuous arising at the apex or lateral axis of the hyphae, somewhat cylindrical oblong, sometimes geniculate or so, showing no prominent denticulate nor nodulose, filiforme, (61.5-)145-203 x (2-)3.5-5.5 µm, 0-2 septa and subhyaline. *Conidiogenous cells* formed mostly at the apex and sometimes at the lateral axis, mostly unbranched, sometimes denticulate and cylindrical oblong. *Ramoconidia* mostly solitary possessing subcylindrical or cylindrical oblong shape (5.5-)9.5-10(-13) x (2.5-)3.5-4.5 µm (av. 33 x 9) and non-septate. *Conidia* numerous, catenate, often form branching chains, it arise from terminal but sometimes in lateral axis of the vertical growing conidiophore, possessing obovoid to almost fusiforme shape, occasionally showing spherical shape (4-)5.5-6(-9) x (2.5-)3-4.5 µm (av ± SD: 6 ± 1 x 3 ± 0.5) non verruculose and aseptate.

Culture characteristics: Colonies on MEA grow up to 55 mm diameter after 14 days at 25 °C, olivaceous grey to whitish colour at colony margin, showing folded and elevated centre with radial farrows, convex growth pattern and no prominent exudates observed. Colonies on OA grow up to 56 mm diameter after 14 days at 25 °C, olivaceous grey to dark greenish colour. The colony shows low convex growth pattern with spores firmly attached to mycelium. Colonies on PDA grow up to 47 mm diameter after 14 days at 25 °C, olivaceous grey, showing convex growth pattern, single concentric circle at an elevated center and possesses whitish pigment at the circumference.

Note: *C. domusafricanae* (Figure 3.1. , clade 16) form a well-supported distinct monophyletic clade from *C. westerdijkiae* (Figure 3.1. , clade 15), which is its closest relative. *C. westerdijkiae* was recently reintroduced by Bensch *et al.* (2018) and it was previously described as *C. cladosporioides sensu lato* Lineage 4 due to the lack of morphological distinction compared to *C. cladosporioides* (Bensch *et al.*, 2010). *C. domusafricanae* is phylogenetically and morphologically distinct from *C. westerdijkiae*. *C. cibiculi-murrhini* has longer and thicker conidiophores, shorter ramoconidia and shorter conidia which make it morphological distinguishable from *C. westerdijkiae* (Table 3. 1.).

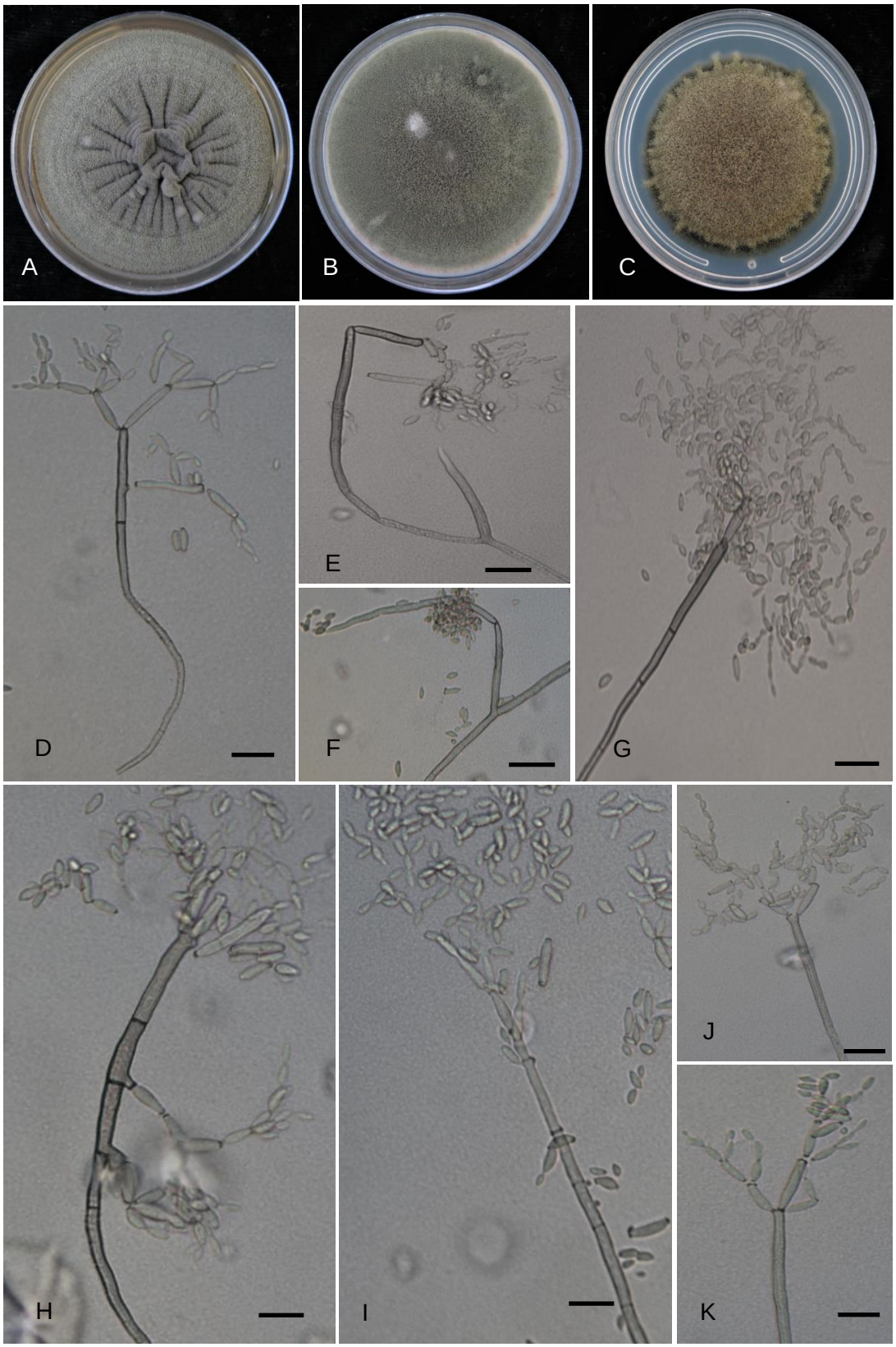


Figure 3.7. *Cladosporium cibiculi-murrhini* (Clad 376). **A-C.** Colonies on MEA, OA and PDA. **D-K.** Conidiophores and micronematous conidiophores, ramoconidia and branched and unbranched conidial chains. Scale bar = 10 µm.

Cladosporium cibiculi-murrhini Buthelezi & Jacobs, **prov. nom,** (Figure 3.7.

Etymology: The name refers to from the mouldy room.

Specimens examined: South Africa (Western Cape, Cape Town) and Johannesburg, isolated from the indoor environment. *Holotype:* Clad 376, GenBank: OK135967 & OK086821.

Mycelium immersed and occasionally superficial. *Conidiophores* macro- and micronematous, erect, straight and flexuous arising at the apex or lateral axis of the hyphae, somewhat cylindrical oblong, sometimes sharply geniculate, occasionally denticulate and or nodulose, filiforme and barely showing protuberant (26-)90-172 x (2-)3.5-5 µm, 0-3(-5) septate and occasionally showing up to 5 septa and subhyaline. *Conidiogenous cells* formed mostly at the apex and sometimes at the lateral axis, sometimes denticulate and cylindrical oblong. *Ramoconidia* mostly solitary possessing, occasionally filiforme, subcylindrical or cylindrical oblong shape (9-)14-15(-21) x (2.5-)3.5-5.5 µm (av. 15 x 3.5) and aseptate. *Conidia* numerous, catenate, often form branching chains, it arise from terminal but sometimes in lateral axis of the vertical growing conidiophore and ramoconidia, possessing obovoid to almost fusiforme shape, occasionally showing globose shape, subrostrate, non-verruculose (5-)6.5-10.5 x (1.5-)3-3.5(-4.5) µm (av. ± SD: 7 ± 1.5 x 3 ± 0.5) and aseptate.

Culture characteristics: Colonies on MEA grow up to 60 mm diameter after 14 days at 25 °C, olivaceous grey colony, showing folded and elevated center with radial furrows that are not maintained throughout to the circumference of the colony, convex growth pattern and no prominent exudates observed. Colonies on OA grow up to 55 mm diameter after 14 days at 25 °C, olivaceous grey to dark greenish colour. The colony shows a low convex growth pattern with spores firmly attached to mycelium. Colonies on PDA grow up to 51 mm diameter after 14 days at 25 °C, olivaceous grey and showing convex growth pattern with a slightly elevated center.

Note: *C. cibiculi-murrhini* (Figure 3.1. , clade 1) form a well-supported monophyletic clade distinct from *C. pseudocladosporioides* (Figure 3.1. , clade 3) and it is phylogenetically close to *C.* cf. *pseudocladosporioides* (Figure 3.1. , clade 2) in this phylogenetic assessment. *C. pseudocladosporioides* (Bensch *et al.,* 2018) is a widely distributed saprophytic species and encompasses many genetic diverse strains which showed a predominance from clinical samples (Sandoval-Denis *et al.,* 2016) and form a large clade with many strains under *C. cladosporioides* species complex (Bensch *et al.*, 2018). Microscopic features of *C. cibiculi-murrhini* show that it has longer conidiophores and shorter ramoconidia than *C.*

pseudocladosporioides (Table 3. 1.). However, the morphological characters of *C. pseudocladosporioides* are rather closely related to *C. cladosporioides* (Bensch *et al.*, 2010).

Discussion

The Consortium for Barcode of Life recommends the ITS gene as the universal barcode for the identification of fungi, partly because it contains regions that show a high success rate of PCR amplification in almost all fungi (Kalan and Grice, 2018). Despite the ease of use, the available fungal database is estimated to represent approximately 1.5 % of the total fungal species diversity on earth and the majority of the fungi are still undiscovered (Kalan and Grice, 2018). Africa is one of the rich treasure troves of biodiversity and many fungi isolated from Africa contributed to the understanding of fungal species in different contexts (Antonelli *et al.*, 2020; Fisher and Garner, 2020). *Cladosporium* is one of the genera where standard protocols and identification methods were explicitly defined in monographs since 2012 (Sandoval-Denis *et al.*, 2016; Ma *et al.*, 2017; Bensch *et al.*, 2012, 2015, 2018). This has made it possible to accurately identify and further assess *Cladosporium* species from different environments using a reliable reference dataset that is easily accessible from MycoBank and GenBank (Bensch *et al.*, 2018).

Cladosporium species are mostly identified based on polyphasic analysis with a combination of secondary marker genes ACT and EF 1-α, for continuous assessment of *Cladosporium* taxonomy (Sandoval-Denis *et al.*, 2016; Bensch *et al.*, 2018). As a result, in the space of six years (2012 to 2018), 48 new *Cladosporium* species has been described using these measures of identification (Crous *et al.*, 2014; Braun *et al.*, 2015; Razafinarivo *et al.*, 2016; Sandoval-Denis *et al.*, 2016; Marin-Felix *et al.*, 2017; Ma *et al.*, 2017; Bensch *et al.*, 2015, 2018). *Cladosporium* species are often morphologically indistinguishable but genetically diverse and the genotype presents the actual species identity, which can only be revealed through the use of DNA sequencing data. The DNA sequencing data of *Cladosporium* species should not be limited to BLAST search results because the intricate identity of a species lies in a good analysis of species phylogeny **(**Zimowska *et al.*, 2021).

In this study, numerous unknown *Cladosporium* isolates collected from indoor environments were subjected to polyphasic analyses, and six novel species were described amongst those isolates. The described novel species all belong to the *C. cladosporioides* species complex (Sandoval-Denis *et al.*, 2016; Ma *et al.* 2017; Bensch *et al.*, 2015, 2018). The identification of numerous indoor *Cladosporium* species as well as novel species is, however, surprising but not unexpected. It is also an indication that indoor environments in Africa, particularly in Southern

Africa, may harbour unique fungal communities which are fairly unexplored and need more investigation to get an insight into their distribution pattern and their biological roles. However, the abundant distribution of *Cladosporium* species from the indoor environment was previously highlighted by Visagie *et al*, (2014), where they identified numerous *Aspergillus, Penicillium* and *Talaromyces* from indoor environments. During their survey, they noticed that *Cladosporium* species constitute a large fraction of the indoor microbiome, which suggested that these species need to be investigated.

Within the indoor environment, different microhabitats become colonised by many fungal species (Kelley and Gilbert, 2013). These habitats present a niche with unique properties such as temperature, humidity and CO_2 compared to the natural world. Fungi in the indoor environment are subjected to an unusual growth and proliferation, which is potentially due to an abnormal switching of gene expressions ranging from metabolising available carbon sources to survival in oligotrophic environments. As a result, this study formulates a partial hypothesis that these genes became selectively fixed in some species and resulted in an overall change of the species genotype clusters. However, in common cases, a new species results from an accumulation of mutations that often results in allele divergence and the development of different genotype clusters (Xu, 2020). In addition, the resulting new species is expected to develop and exhibit different morphological features and ecological requirements. These expected changes are not always guaranteed, and this has had an impact on *Cladosporium* taxonomy (Bensch *et al.*, 2012, 2018), where some phylogenetically novel species were not described as novel species because they had less or no morphological differences from closely related species.

Even though *Cladosporium* species are known as ubiquitous fungi, some species are seemingly strict in specific ecosystem/s and region/s such as *C. herbarum* that is known to only occur outdoors and *C. aerium* only identified in China (Bensch *et al.*, 2018). However, the novel species described in this study are not yet known to occur outside the indoor environment. These new species possess characteristics of *Cladosporium* species that mostly occur in the indoor environment, as described by Bensch *et al.* (2018). Perhaps, with continuous assessment of *Cladosporium* taxonomy from different ecosystems and regions, more strains of these species could be identified. The indoor environment has become an important source of undescribed fungi that preside as opportunistic pathogens and/or irritants, colonisers and contaminants.

Cladosporium species are known for their saprophytic lifestyle and are dark pigmented fungi. However, there has been an evidence that implicates saprobic and dematiaceous fungi with

adverse and life-threatening conditions commonly referred to as melanised fungal infections (Brandt and Warnock, 2003; Batra *et al.*, 2019; Headley *et al.*, 2019). A better understanding of fungal diversity and growth patterns in indoor environments especially in the settings for immune-suppressed individuals (homes, nursing homes and intense care units) will help improve lives and help policymakers to set informed regulations for public health policies.

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Conclusion and Remarks

Indoor environments are important habitats for human safety and wellness. However, there are many factors that play a role to keep indoor environments healthy. Amongst those factors are the prevalence of moulds, particularly *Cladosporium* species, in these ecosystems. The abundance of *Cladosporium* species in indoor environments correlates with the abiotic factors including high CO_2 (≥ 1000 ppm) levels and high relative humidity (≥ 60 %). This suggests that the growth of *Cladosporium* (and other species of indoor microbiomes) in indoor environments can be managed by keeping these parameters at low or moderate levels. The levels of CO_2 and relative humidity mainly depends on the efficacy of ventilation systems. A house with poor ventilation system is characterised by high levels of CO_2 and subsequently increasing levels of humidity that favours the growth and spread the contamination of *Cladosporium* in the air and surfaces of the indoor environment. It is, therefore, important to not only depend on anti-fungal paints and cleaning agents but also an efficient ventilation system because *Cladosporium* grow on those surfaces if there is sufficient moisture. In all air samples of this study, *Cladosporium* colonies were often the dominant group of fungi when the CO_2 and humidity levels were elevated. On the other hand, at relatively low CO_2 and relative humidity levels, the plate count of *Cladosporium* was also low (data not presented).

The study's main objective was to determine the diversity of *Cladosporium* species in indoor environments in Southern Africa. Our study uncovered the previously unknown diversity of *Cladosporium* species in the indoor environment of Southern Africa. The data generated in this study is hopefully a useful component in a continuous investigation of uncovering Southern African biodiversity and highlights the need to continuously explore African biodiversity not only in indoor environments but in a range of different ecosystems. In various regions, *Cladosporium* species are increasingly reported as a major indoor contaminant, where residents also reported respiratory-based complications. *Cladosporium* species are known as allergens, opportunistic pathogens and contaminants. However, the depth of information is lacking in Southern Africa and South Africans are largely affected by allergic diseases in different stages of life (Lunjani *et al.*, 2021). This is often influenced by the socio-economic status and environmental exposure to immunological allergens.

The investigation of allergenic fungi in Africa is important since the recent literature suggest that allergic susceptibility can be genetic and racially linked. Individuals of African origin were found to be highly susceptible and more vulnerable to allergic diseases compared to those of European ancestry (Wegienka *et al.*, 2013; Levin *et al.*, 2014; Lunjani *et al.*, 2021). Allergic diseases are caused by different immunogenic substances ranging from fungi to food and

environmental allergens and this concept needs a multidisciplinary effort to identify these factors in different communities of South Africa. This includes comprehensive indoor microbiology to study the shift of microbiome composition in indoor environments over different seasons. In this study, we observed that *Cladosporium* species composition in the indoor environment was less influenced by outdoor *Cladosporium* species. This is because we often identified species indoors that were not present in the immediate outdoor air. However, it is a limitation of this study that we were not able to further explore other alternatives to investigate the specific type of hypersensitivity these species might cause.

It is surprising and interesting that South African indoor environments are colonised by diverse *Cladosporium* species. The findings of this study suggest that there are more undiscovered taxa, and seemingly more fungi are evolving to thrive in indoor environments. The lack of knowledge about indoor microbiomes put the health and safety of people at risk because they remain unaware of the type of species they are in contact with. This is especially important when one considers that there are more immune compromised people due to different diseases and ageing. Some of these indoor microbiomes can synergise with pre-existing infectious agent/s in immune compromised individuals and worsen the condition. This is a crucial aspect to investigate since, in our findings, we identified species such as *C. cladosporioides, C. halotolerans* and *C. sphaerospermum* that are linked to serious health adversities. However, most of the *Cladosporium* species are taxonomically well described but less investigated into their potential impact and the services they support in the ecosystem. The newly described species in this study still require further investigation in order to understand their potential roles in the indoor environment. Future studies should focus in *in vivo* methods to determine the subsequent responses of immune system components (particularly for type I hypersensitivity) and other respiratory based infections post-exposure in *Cladosporium* species.

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