Survey of soil fungal and oomycete diversity from maize field soils in the Eastern Cape, South Africa

by

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Declaration of originality

I, Alice Mthembu declare that the dissertation, which I hereby submit for the degree Master of Science at the University of Pretoria, is my work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: 

DATE: 25 March 2024

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“Every good and perfect gift is from above, coming down from the Father of lights, with whom there is no variation or shadow due to change”.

PREFACE

Maize is the major food and feed grain in South Africa and is the third most produced grain crop worldwide. Recently, the Eastern Cape province was identified as a region with the potential to be developed into a larger maize-producing region. For this goal to be a reality, research and development efforts are needed. Maize production in South Africa is threatened by fungal diseases which may have a negative impact on crop quality and yield. One problem for the Eastern Cape is that little is known about the fungal and oomycete diversity, or potential pathogens, that are associated with maize rhizosphere soils in the province. This study thus aims to provide baseline knowledge on what fungal species are present in Eastern Cape maize farm soils and provide a better idea of whether these soils contain potential maize pathogens. This information is important to help develop relevant management strategies to deal with these diseases in the future. Through the support of emerging farmers by the national government, the local economy can be sustained, and people's livelihoods can be improved by ensuring food security and job creation.

The first chapter provides a literature review that discusses soilborne diseases that are known to affect maize, especially in South Africa. It also highlights knowledge on their distribution, symptoms, and common management strategies. Furthermore, we discuss maize production in the Eastern Cape and the problems faced by the emerging farmers sector in the region.

Chapter two is focused on a survey of fungal and oomycete diversity in Eastern Cape maize farm rhizosphere soils. We aimed to isolate and culture fungi and oomycetes from soils and then identify isolates using both morphology and DNA sequence data. The isolates were accessioned into the working CN collection (working collection for the Applied Mycology group) at FABI (Forestry and Agricultural Biotechnology Institute) making them available for future research projects. DNA was extracted from strains, polymerase chain reactions (PCRs) were performed, and Sanger sequences were generated for gene regions most appropriate for species identifications in respective genera. Strains were then identified to species level by comparing their newly generated sequences using BLAST with the reference database available on NCBI GenBank. For genera such as *Fusarium, Penicillium,* and *Trichoderma,* sequences were compared to locally curated databases created from recent taxonomic revisions. This study identified 437 strains to 87 species representing 30

genera. Several strains obtained in this study belonged to species previously reported to cause maize diseases such as Fusarium stalk rot, Diplodia stalk rot, and Pythium root and stalk rot. No disease symptoms were observed during the fieldwork.

In chapter three we propose and describe a new *Penicillium* species classified in the section *Canescentia* series *Atrovenata*. The species is described using a polyphasic species concept that incorporates morphology and phylogeny.

SUMMARY

Maize plays a crucial role as a staple food and feed grain in South Africa and globally. Fungal diseases pose a significant threat to maize yields in South Africa, particularly in the Eastern Cape where fungal and oomycete diversity in maize rhizosphere soils is limited. The first chapter presents a comprehensive literature review focused on soilborne diseases affecting maize, with a particular emphasis on those prevalent in South Africa. It covers their distribution, symptoms, and commonly employed management strategies. Additionally, the chapter delves into maize production in the Eastern Cape, addressing the challenges encountered by the emerging farmers in this region. Chapter 2 (first research chapter) focuses on a survey of fungal and oomycete diversity in rhizosphere soils from maize farms in the Eastern Cape. Our goal was to isolate and culture fungi and oomycetes from these soils and identify the isolates using both morphological characteristics and DNA sequence data. Isolation resulted in 421 fungal and 16 oomycete strains The most dominant fungal genera from the soil collected were *Penicillium* (n=98)*, Fusarium* (n=90), *Cladosporium* (n=46), and *Trichoderma* (n=103), with *Fusarium oxysporum sensu lato* (n=64), *Trichoderma gamsii* (n=29), and *Penicillium cremeogriseum* (n=18) among the most common species. Several pathogenic fungal species like *A. alternata, F. graminearum, Beauveria amorpha, S. maydis, G. irregulare and G. ultimum*, were isolated from this study and have been reported to cause root and stalk rot in maize. There is a large variation in the distribution of fungal and oomycete species across all farms. The fungal and oomycete communities that were dominant in the soils belonged to the genera *Fusarium, Penicillium, Trichoderma,* and *Globisporangium.* None of the isolated *Penicillium* and *Trichoderma* species have been reported to cause diseases in maize in South Africa. Notably, *Globisporangium irregulare* was the predominant oomycete species identified. Several strains belonged to species known to cause maize diseases, such as Fusarium stalk rot, Diplodia stalk rot, and Pythium root and stalk rot. Chapter 3 (second research chapter) of this study provided the description of a newly proposed *Penicillium* species in the section *Canescentia* series Atrovenata. We described the species based on its unique DNA sequences and provide morphological evidence for its formal description. Overall this study shows that the fungal communities detected in the maize rhizosphere soils are relatively diverse and some have been reported to cause important maize diseases.

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SOILBORNE DISEASES AFFECTING MAIZE PRODUCTION IN SOUTH AFRICA.

ABSTRACT

Maize is one of South Africa's most important crops and a source of food for humans and animals. Various soilborne diseases caused by fungal and oomycete phytopathogens can greatly affect maize production and cause significant losses. The most important soilborne diseases affecting maize include charcoal root rot (*Macrophomina phaseolina*), Diplodia stalk rot (*Stenocarpella maydis*), Fusarium stalk rot (*Fusarium verticillioides*), Gibberella stalk rot (*Fusarium graminearum*), and Pythium root and stalk rot (*Pythium aphinidermatum)*. Farmers use various control measures to manage these pathogens. This review is focused on the soilborne diseases that affect maize production, their prevalence, symptoms, economic importance, and common management strategies to control them. It also discusses the problems faced by the subsistence farmers in the Eastern Cape.

MAIZE PRODUCTION IN SOUTH AFRICA

Maize (*Zea mays*) is the most widely cultivated grain crop in the world after wheat and rice (Erenstein *et al*., 2022). It was first introduced to tropical Africa by the Portuguese in the 17th century to supply their trading ports (Sihlobo, 2018). Later, African farmers adopted the crop due to its high energy yield and short growing season (Cherniwchan and Moreno-Cruz, 2019). Maize was introduced to South Africa in 1655 after the arrival of the Dutch colonialists and has become a staple food for most South Africans while it is also an important source of feed grain for animals (Sihlobo, 2018). Maize is grown throughout South Africa, in tropical, subtropical, and temperate regions (Hadisu-Bello, 2020), with the Free State (34%), Mpumalanga (24%) and North-West (18%) provinces being the largest producers, accounting for about three-quarters of total maize production. Production depends on a stable water supply at specific planting and growing times. In South Africa, 80% of maize is grown on dry land and 20% on irrigated land (du Plessis, 2003).

Much of the South African maize is produced by commercial farmers, but small-scale and subsistence farmers also make an important contribution.

The maize produced in South Africa accounts for about 45% of the gross domestic product in the agricultural sector (Adisa *et al.,* 2019). Many smallholder and subsistence farmers play an important role in ensuring better food security in their home countries, where they grow maize for their consumption and sell it to members of the local community (Hlophe-Ginindza and Mpandeli, 2020). In Southern Africa, there is a high rate of urbanization combined with high population growth (Johan and May, 2020). As a result, food insecurity and malnutrition are increasing in urban and rural areas (Johan and May, 2020). In Africa, 23% of people are undernourished with 45% of deaths linked to hunger (World Food Programme, 2019). The situation in South Africa is also of great concern, as many households are food insecure. The population is growing rapidly and there is still much that needs to be done to ensure food security, putting the United Nations Sustainable Development Goal 2 of zero hunger (SDG2) at risk.

Maize is traditionally a significant part of a staple diet and an imported raw material for animal feed in South Africa (Adisa *et al*., 2019). There is therefore an urgent need for the continuous production of maize to ensure food security (Hlophe-Ginindza and Mpandeli, 2020). Another issue is climate change, which is placing a lot of pressure on crop farming (Wilson *et al.,* 2022). Extreme weather changes such as heavy rainfall and extremely high temperatures lead to yield losses, which can lead to hunger and malnutrition (Zhang *et al*., 2022). It will therefore be difficult to manage crops as the weather cannot be predicted. Mangani *et al.* (2019) predicted a yield reduction of 1% associated with each degree day above 30°C under optimal rainy weather conditions for the major maize-growing regions in South Africa. Mangani *et al.* (2019) also reported a 24.3% reduction in maize yields in the 2014/15 planting season due to drought and heat waves. In the 2017/18 season, maize production decreased by a further 21.4% compared to previous seasons (Pahlane *et al.,* 2018). Researchers have projected a 10% decline in maize yields by 2050 using data from HadCM2 climate scenarios for Africa (Jones and Thornton, 2003). Further, Ruane *et al.,* (2018) projected a 20% decrease in maize yield driven by an increase of 1.5 and 2°C in air temperature in sub-Saharan Africa for the mid-century (2046-2065) (Ruane *et al.,* 2018). These problems suggest that effective strategies are needed to combat the negative effects of climate change. These include educating farmers on climate change adaptation and behaviour (Moore and Lobell, 2014), improving irrigation

facilities, breeding more resistant maize (Aragon *et al*., 2021), and providing advice and financial support to farmers where it is most needed.

South Africa produces about 60% white maize and 40% yellow maize (Mokone, 2017). White maize is typically used for human consumption (mostly as maize meal) and yellow maize is mainly used as animal feed (Esterhuizen and Kreamer, 2011). In the 2021/22 season, about 15.3 million metric tons of maize were produced. This represents a 10% decline from previous years, as shown in Figure 1.1 below (Galal, 2022). Exports of maize to other countries totalled 3.2 million tonnes in 2021/22, contributing significantly to the GDP of South Africa (Galal, 2022). Exports to Africa alone contributed about R25.5 billion (South African Grain Information Service, 2021).

Maize can be affected by various fungal diseases that infect cobs (or ears), leaves, stems, or roots. In the wet climatic conditions of KwaZulu-Natal and the Eastern Cape provinces, foliar diseases caused by fungi are a persistent challenge to maize production (Nsibo *et al.,* 2019). The three main foliar pathogens that affect maize production in South Africa include Grey Leaf Spot (GLS), Northern Corn Leaf Blight (NCLB) and common rust (CR) (Muller *et al.,* 2016; Haasbroek *et al.,* 2014). These diseases usually lead to lower-yielding crops and thus cause economic losses on both local and global scales. Common maize diseases affecting production include ear and stalk rot, Gray Leaf spot, Northern corn leaf blight and common rust (GrainSA, 2016).

Maize is also prone to mycotoxin contamination, which poses a health risk to consumers and results in economic losses (Misihairabgwi *et al*., 2017). The effects of mycotoxins on humans range from acute poisoning to long-term effects such as immune response deficiency and cancer (World Health Organization, 2018). Species belonging to the fungal genera *Aspergillus, Fusarium,* and *Penicillium* are known to produce most of the regulated mycotoxins (Greeff-Laubscher *et al.,* 2020). Fumonisins (FUMs), zearalenone (ZEA), aflatoxins (AFs) and deoxynivalenol (DON) are the most common mycotoxins associated with maize (Munkvold, *et al.,* 2021). In South Africa, the most common mycotoxins detected during routine testing include Aflatoxin B1, Fumonisin B1 (FUMB1), ZEA, and DON (Shephard *et al.,* 2010; Mwalwayo and Thole, 2016).

MAIZE PRODUCTION IN THE EASTERN CAPE

The Eastern Cape province contributes 1% of South Africa's total maize production, with 60% of maize produced by subsistence farmers and 40% by commercial farmers (Grain SA, 2015). Emerging farmers from the province typically report that their production is affected by pests and diseases, mostly of unknown nature. This leads to stunted growth, crop damage, and poor crop yields. These losses cause an increase in food prices and lead to hunger in the homelands. Since maize is a staple food in South Africa, it has a significant impact on smallholder farmers' livelihoods and food security (Zuma *et al*. 2018). Although maize production in the Eastern Cape contributes only a small portion of the country's economy, it ensures food security, the Eastern Cape has been identified for increased future production. Therefore, extension programs are needed to support farmers in the region and identify which pests and diseases are limiting maize production. This will allow farmers to better manage their crops and adopt more effective pest and disease control strategies.

MAIZE FARMING SECTORS IN SOUTH AFRICA AND THE CHALLENGES FACED BY FARMERS IN SOUTH AFRICA, EASTERN CAPE

There are three main types of farming practices in South African agriculture, commercial, emerging and subsistence farming (Zantsi *et al.,* 2019). South Africa has approximately 30 000 commercial farmers (AECI Plant Health, 2021). Maize is produced by about 9 000 commercial farmers who employ about 128 000 South Africans, while the rest is produced by thousands of subsistence farmers. (National Department of Agriculture, 2018). In South Africa, 98% of maize comes from commercial farming, while 2% comes from emerging farmers (Department of Agriculture Forestry and Fisheries, 2014).

a) Commercial farming is when crops are produced by a farmer to sell on the market for profit gain (Baipheti and Jacobs, 2009). In commercial farming, the crops are grown in large quantities. Commercial farmers mostly produce maize in millions of tonnes; Therefore, they need a lot of land, the latest technologies and expertise to achieve the optimised production targets (Gouse *et al.,* 2016). It is capital-intensive and thus requires a large amount of money for maintenance (Meintjies, 2017). Commercial farming requires the use of chemical fertilizers, pesticides, insecticides and weed killers (Gouse *et al*., 2016). In South Africa, the total land used for commercial agriculture is 46.4

million hectares, which is about 37.9% of the country's total land area (122.5 million hectares) (Stats SA, 2022). Commercial agriculture land comprises mainly grazing land and arable land which is used for crop production, and this accounts for 7.6 million hectares (Stats SA, 2022). The average annual commercial maize production in South Africa is 10–12 million tonnes on more than 2.5 million hectares of land (GrainSA, 2015).

b) Emerging farmers produce crops for the consumption of their family but are more market-orientated crop sellers who aspire to sell and commercialise their crops (Nieuwoudt, 2000). Emerging farmers can be regarded as farmers who have started small in terms of the crop production business while commercial farmers have been producing crops for a while and can produce large quantities (Matsimela, 2020).
c) Subsistence farming/Small-scale farming was better defined by Barnett *et al.,* (1997) as "agriculture and its associated activities that form a living strategy where the main products are used or consumed directly by the farmers, with little or no purchased inputs, and a small percentage of output marketed." Unlike other types of farming, subsistence farmers are more concerned with survival. They produce maize on a smaller scale than commercial farmers. Smallholder farming plays a very important role in the reduction of food-insecure households and has the potential to eliminate poverty in both rural and urban areas. This type of farming improves people's livelihoods, however, farmers in these communities face several challenges such as impacts of climate change, poor agricultural extension services, low levels of education, lack of knowledge on innovative methods to increase productivity and sustainability, as well as low levels of financial support from the government (Aliber & Hart, 2009). There is therefore a significant need for increasing smallholder/subsistence agriculture which will ensure long-term food security in the country.

FACTORS AFFECTING DISEASE EMERGENCE AND DEVELOPMENT IN MAIZE

It was previously established that the occurrence of plant diseases depends on three main factors namely the host plant, the pathogen, and the environment, which is

known as the disease triangle (Velásquez *et al.,* 2018) (Figure 1.2). These factors influence the occurrence and severity of plant diseases, the susceptibility of the host plant, the presence of a disease-causing pathogen, and favourable environmental conditions (Tjosvold, 2018). If one of the three factors is not present, no disease occurs. For example, a resistant maize hybrid prevents the disease from occurring because one side of the triangle (susceptible host) is broken (Tjosvold, 2018). The disease pyramid or tetrahedron was later introduced by some pathologists and it allows for the addition of one or more factors (Agrios, 2005). Proposed factors include human factors, vectors, and time. A three-dimensional disease pyramid or tetrahedron has been commonly used after the addition of these parameters as shown in Figure 1.3 (Agrios, 2005).

Humans contribute to the disease triangle through human activities, by employing various agricultural practices (Francl, 2001). Cultivation practices by humans affect a pathogen's life cycle. For example, genetic manipulation of plant hosts through breeding and genetic engineering, planting large expanses of genetically similar crops. Irrigation and the use of greenhouses can also affect the occurrence and severity of certain diseases (Francl, 2001).

Maize production can be hampered by both soil-borne and foliar diseases; however, this study focuses more on soil-borne diseases. Soilborne diseases that affect maize include root, crown, and stalk rots. Maize diseases require certain environmental conditions to develop. When conditions are favourable and the pathogen/inoculum is present in the host plant, the diseases begin to develop and infect the plant (Grain SA, 2016). These diseases are not fully understood since they are caused by more than one pathogen, and this is known as the disease complex. Root rot and stalk rots are generally associated with each other. Whitney and Mortimore (1957) reported that maize roots may be complete diseases with no stalk rot, but stalk rots will always occur when there is root rot. Certain conditions affect the progression of the disease complex, and these include, environmental conditions, soil conditions, cultivation practices, and maize plant resistance (Shekhar and Singh, 2021).

The environment is the most complex of the four factors, but it can be controlled to reduce the likelihood of disease occurrence. Some factors to consider include planting dates (time) and the region where the maize is to be planted. For this reason, maize

is grown in specific regions at specific times of the year when the general environmental conditions are known. This is where the issue of climate change comes in because it affects the annual conditions at certain times of the year. The largest maize producers in South Africa; Mpumalanga, Free State, and North-West plant their maize around late October to mid-December (Cruex *et al.,* 2022). Maize is predominately grown on dry soils, so maize production is dependent on the rainy season (Cruex *et al.,* 2022). To counter the problem of climate change, plant breeders have developed resistant maize cultivars that can withstand these specific harsh environmental conditions.

SOIL HEALTH: HEALTHY SOIL FOR GROWING MAIZE

Soil is known to be one of the most important reservoirs of biodiversity (Jeffrey *et al.,* 2011). Bacteria, fungi, insects, protozoa nematodes and many other microorganisms live and interact in the soil (Rønn *et al.,* 2012). They all play different roles in soil health by helping to fix nitrogen, regulating the flux of nutrients to plants, decomposition of organic matter, and degrading metabolic by-products and agrochemicals (Kayiva *et al.,* 2019; Frąc *et al.,* 2018). For example, nitrogen fixation is one of the most important processes for maize. As an essential nutrient for maize and an important determinant of grain yield, nitrogen plays an important role in photosynthesis as well as the absorption of water and minerals, vacuole storage, and xylem transport (Asibi *et al.,* 2019). There are several groups of nitrogen-fixing bacteria associated with maize roots, including *Enterobacter* species and *Azospirilum* species (Zeffa *et al.,* 2019; Van Dommelen *et al.,* 2009). By infecting the root system of the host plant with nitrogen-fixing bacteria, they cause it to form nodules in which the bacteria can grow. In the root nodules, the bacteria convert gas from the atmosphere into fixed nitrogen, such as ammonia, which the plants can use.

Rhizosphere fungal communities are hotspots and are essential for the healthy growth of maize. Fungal populations influence the diversity and composition of maize plant communities, affecting plant growth through mutualistic relationships, pathogenicity, nutrient availability, and cycling (Wardle, 2002; Wagg *et al.,* 2014; Hannula *et al.,* 2017). Moreover, fungi produce hormones, control root pathogens, and protect plants from drought (Baum *et al.,* 2015; El-Komy *et al.,* 2015; Jayne and Quigley, 2014). Thus, understanding microbial communities in maize rhizosphere soils is essential for understanding their ecological roles and relationships with crop plants.

According to Wang *et al.* (2017), healthy soils have more beneficial microbes and greater microbial diversity. Fungal species are important for the growth of plants such as maize because they suppress plant root diseases and fungi promote healthier plants by attacking plant pathogens with fungal enzymes (Hoorman, 2016). When fungal diversity is high, fungi compete for nutrients, which means that plant survival is high due to the suppression of fungal diseases (Gupta and Neate, 1999; Barnett *et al.,* 2006). An example of a beneficial fungus is mycorrhizal fungi. Maize forms a symbiotic relationship with mycorrhiza (Bona *et al.,* 2016). The mycorrhizal fungus attaches itself to the root system of maize (Bonfante and Genre, 2010; Javot *et al.,* 2007). The fungus obtains sugars from the maize through the photosynthetic process of maize. The mycorrhizal fungi then enhance the uptake of water and nutrients into the plant itself. More specifically mycorrhizal fungi help plants with the uptake of phosphorus, one of the most important nutrients that plants need. It has been reported that when mycorrhizal fungi are present in the soil, the plants are less susceptible to water stress (O'Callaghan *et al*., 2022). At least three benefits are attributed to the mycorrhizal relationship: nutrients are taken up by the plant more efficiently, water usage is improved, and plants are protected from diseases (Begum *et al.,* 2019).

COMMON FUNGAL AND OOMYCETE SOILBORNE DISEASES OF MAIZE AND THEIR MANAGEMENT

Maize is susceptible to a wide range of phytopathogens including fungi and oomycetes. Soil-borne diseases can typically enter plants via roots and eventually typically cause root rot or stem rot (Koike *et al.,* 2003). The most common soil-borne fungal pathogens affecting maize include *Fusarium oxysporum* (root, crown, and stalk rot), *Pythium* species (seedling blight), *Phytophthora species, Rhizoctonia solani* (damping off and seedling blight) and *Macrophomina phaseolina* (charcoal rot) (Grain SA, 2016).

Among the groups of phytopathogens that cause many serious maize diseases are the oomycetes, traditionally classified as Phycomycetes or "lower fungi", which are further divided into *Pythium* and *Phytophthora* (Roosman and Palm, 2006) and are also known as water molds; resemble fungi but are phylogenetically related to brown algae (Gunderson *et al.,* 1987). It was previously reported by Uzuhashi *et al.,* (2010) that *Pythium* genus is highly divergent and divided into five monophyletic clades that are well or moderately supported. Clades are distinguished based on their sporangial

morphology, for example ovoid, globose, filamentous, or elongated. According to the relationship between morphology and phylogeny, the taxonomy of the genus *Pythium* was revised, and several new taxonomic revisions have been proposed (Uzuhashi *et al.,* 2010). *Pythium* is composed of four new genera based on phylogeny and morphology: *Ovatisporangium, Globisporangium, Elongisporangium,* and *Pilasporangium,* which are separated from *Pythium sensu. lato*. (Uzuhashi *et al.,* 2010). *Pythium* and *Globisporangium* are two sister genera of plant pathogenic oomycetes with ubiquitous distribution and are responsible for infecting diverse crops including maize (Kirk *et al.,* 2001). *Gobisporangium irregulare* (formerly known as *Pythium irregulare*) along with *Pythium aphanidermatum* are the two most common and important pathogens of maize (Matsumoto *et al.,* 2000; Daughtrey and Benson, 2005).

Table 1.1 presents a summary of the most common soilborne fungal and oomycete diseases affecting maize. Among the commonly reported stalk rots in South Africa are Charcoal, Diplodia, Gibberella, and Fusarium stalk rots (Flett and van Rensburg, 2021). Additionally, certain *Pythium* species have been reported as causal agents of stalk rots (Song *et al.,* 2015).

FUSARIUM STALK ROT

Causal species: ***Fusarium verticillioides*** (Sacc.) Nirenberg, Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 169: 26 (1976) [MB#314223]

=*Fusarium moniliforme* J. Sheld., Annual Report of the Nebraska Agricultural Experimental Station 17: 23 (1904) [MB#142842]

=*Oospora cephalosporioides* Luchetti & Favilli, Annali della Facoltà di agraria della R. Università di Pisa 1: 399 (1938) [MB#492231]

=*Gibberella moniliformis* Wineland, Journal of Agricultural Research 28: 909 (1924) [MB#271553]

Fusarium stalk rot is the most widely reported stalk rot disease and is caused by several *Fusarium* species (Roncero *et al.,* 2003), including *F. verticillioides, F. annulatum* (previously identified as *F. proliferatum*), *F. subglutinans* and *F. temperatum* which all belong to the *Fusarium fujikuroi* species complex (FFSC). The

main causal agent for Fusarium ear rot is *F. verticillioides* which is widely distributed in maize-producing farms in South Africa (Ncube, 2012). Other *Fusarium* species causing Fusarium ear rot in the Eastern Cape includes *F. oxysporum, F. scirpi* and *F. sporodochiale* (Price *et al.,* 2024).

Economic importance

F. verticillioides is widely distributed in maize-growing areas of South Africa and can produce fumonisins as a secondary metabolite (Rensburg *et al.,* 2014). *F. verticillioides* grows from the roots and upwards towards the stalk and into the ears, which then leads to stalk rots. This pathogen causes most of the damage to maize as stalk and ear rot in maize. It is also known to cause rots in sugarcane, rice, and asparagus (Flett, 2015). Fusarium stalk rot typically reduces maize productivity by 10%. In severely affected areas, it can reduce production by 30–50% (Gai *et al.,* 2018).

Disease symptoms

Maize plants infected by Fusarium stalk rot have a shredded pith that may be whitish pink to salmon-coloured, and they may die prematurely (Figure 1.4) (Jeschke, 2018). Brown streaks may be seen on the lower internodes (Figure 1.5) (Crop Protection Network, 2022). Maize plants infected by *F. verticilioides* turn from a healthy green colour to a dull green and the lower turn to a yellowish colour at the base. As the roots develop after infection, they take on a brownish colour and turn dark black when the roots are completely rotten (Jeschke, 2018). Decay of the internal stalk pith tissue of the stalk may occur and rotting may also occur (Jeschke, 2018).

Fusarium stalk rot disease cycle and epidemiology

F. verticillioides overwinters in mycelial form on leaf debris, soil maize or other dead plant debris (Young and Kucharek, 1977). It infects the plant directly through the roots and causes root rot and decay on the lower stalk. The fungal pathogen grows on healthy stalks and requires certain conditions to cause stalk rot. The spores produced can be spread by wind or splash water and spread on the leaves (Crop Protection Network, 2016). The spores can be washed onto the leaves by rainwater. Infection occurs at the roots, wounds in the stalk or on the leaves (Crop Protection Network, 2016).

Management strategies

Several strategies are used to manage Fusarium stalk, for example, planting in well-drained soils to avoid excessive soil moisture (Okello *et al.,* 2019), rotation of crops and reduction of stress where possible (Pioneer Seeds, 2021). Other control measures that can be implemented include ploughing crop residues under and balanced fertility. There are currently no registered fungicides for Fusarium stalk rot (Grain SA, 2016). Cross stress can never be entirely removed, but it can be reduced by using good crops as well as by managing soil and water. Stress levels can be reduced by avoiding compaction and maintaining soil quality. Management of irrigation is critical to minimize crop stress in dry regions (Pioneer Seeds, 2023). Controlling rootworms and other insects causes injury to the roots of maize plants (Okello *et al*., 2019).

GIBBERELLA STALK ROT

Causal species: ***Fusarium graminearum*** Schwabe, Flora Anhaltina 2: 285 (1839) [MB#200256]

=*Fusarium stictoides* Durieu & Mont., Exploration scientifique de l'Algérie 1 (9): 334 (1848) [MB#240445]

=*Fusarium caricis* Oudem., Verslagen en Mededelingen van de Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 7: 325 (1890) [MB#197359]

=*Fusarium graminearum var. caricis* (Oudem.) Wollenw., Zeitschrift für Parasitenkunde 3: 365 (1931) [MB#266352]

=*Gibberella saubinetii var. mate* Speg., Anales del Museo Nacional de Historia Natural Buenos Aires 17: 129 (1908) [MB#137060]

=*Sphaeria zeae* Schwein., Schriften der Naturforschenden Gesellschaft zu Leipzig 1: 48 (1822) [MB#142679]

=*Gibberella zeae* (Schwein.) Petch, Annales Mycologici 34 (3): 260 (1936) [MB#255496]

=*Dothidea zeae* (Schwein.) Schwein., Transactions of the American Philosophical Society 4 (2): 230 (1832) [MB#474955]

=*Hendersoniopsis zeae* (Schwein.) Woron. (1922) [MB#565011]

=*Fusisporium insidiosum* Berk., Gardeners' Chronicle 1860: 480 (1860) [MB#199407]

=*Fusarium insidiosum* (Berk.) Sacc., Sylloge Fungorum 4: 707 (1886) [MB#231227]

=*Fusarium funicola Tassi, Bollettino del Laboratorio de Orto Botanico Reale Universita Siena 3:* 131 (1900) [MB#201368]

Fusarium rostratum Appel & Wollenw., Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft 8: 30 (1910) [MB#205201]

=*Gibberella saubinetii* var. *flacca* Wollenw., Zeitschrift für Parasitenkunde 3: 433 (1931) [MB#277518]

=*Gibberella saubinetii* var. *tetraspora* Feltgen, Vorstudien zu einer Pilzflora des Grossherzogthums Luxemburg. Nachträge III: 302 (1903) [MB#137066]

=*Gibberella saubinetii* var. *calami* Henn., Beiblatt, Hedwigia 42: (79) (1903) [MB#137245]

=*Fusarium mollerianum* Thüm., Instituto de Coimbra 28: 263 (1881) [MB#143167]

=*Selenosporium bufonicola* Speg., Anales del Museo Nacional de Historia Natural Buenos Aires ser. 3, 13: 458 (1911) [MB#187970]

=*Fusarium bufonicola* (Speg.) Sacc. & Trotter, Sylloge Fungorum 22: 1486 (1913) [MB#223866]

=*Fusarium rhoicola* Fautrey, Revue Mycologique Toulouse 17: 171 (1895) [MB#214867]

=*Sphaeria saubinetii* Durieu & Mont., Exploration scientifique de l'Algérie 1 (12): 479 (1849) [MB#509484]

=*Gibbera saubinetii* (Durieu & Mont.) Mont., Sylloge generum specierumque plantarum cryptogamarum: 252 (1856) [MB#206606]

=*Gibberella pulicaris* subsp. saubinetii (Durieu & Mont.) Sacc., Michelia 1 (3): 317 (1878) [MB#509408]

=*Gibberella saubinetii* subsp. pachyspora Sacc., Michelia 2 (6): 74 (1880) [MB#562912]

=*Gibberella saubinetii var. pachyspora* (Sacc.) Sacc., Sylloge Fungorum 2: 555 (1883) [MB#137050]

=*Gibberella saubinetii f. acuum* Feltgen, Vorstudien zu einer Pilzflora des Grossherzogthums Luxemburg. Nachträge III: 303 (1903) [MB#588635]

=*Gibberella saubinetii var. acuum* (Feltgen) Sacc. & D. Sacc., Sylloge Fungorum 17: 813 (1903) [MB#137247]

Gibberella stalk rot is caused by *Fusarium* species classified in the *Fusarium sambucinum* species complex (FSamSC), but the main causal agent is *Fusarium graminearum*. Other hosts affected by this fungus are oats, barley, sorghum, and wheat (Flett and Rensburg, 2022). *F. graminearum* is generally not a soil-borne pathogen but causes diseases such as ear and stalk rot in maize (Grain SA, 2021).

Economic importance

Gibberella stalk rot is a common problem in maize-growing areas around the world as well as in South Africa. This fungus can cause major economic losses due to the premature death of maize plants. The fungal disease interferes with the translocation of water and nutrients during the process of grain fill resulting in weakened stems (Freije and Wise, 2016). Grain filling is reduced because of fungal growth and disruptions in water and nutrient uptake by the xylem and phloem (GrainSA, 2021).

Symptoms

The symptoms of Gibberella stalk rot are like other stalk rot such as Fusarium stalk rot. A pinkish-reddish discolouration is the main symptom seen on the stalk (Figure 1.6). Plants affected by this fungus wilt; the leaves turn colour from light green to dull green. Another important symptom is the disintegration of the pith, which leads to the death of the stem and eventually to the rotting of the root system (Freije and Wise, 2016). When the stem tissue disintegrates, this causes lodging and rotting of the root system, which in turn leads to lodging of the roots (van Rensburg and Flett, 2021).

Gibberella stalk rot epidemiology and disease cycle

The fungal pathogen overwinters on maize debris and sometimes on seed. When conditions are cool and moist, spores are carried by air currents to maize ears and stalks and infection occurs (Pannar Seed, 2011). After pollination, infection of the stalk

occurs on the leaves and around the roots. The fungal pathogen can also enter the plant through the roots, grow upwards and infect the stem.

Management strategies

Management strategies for Gibberella stalk root include crop rotation and the use of tillage systems that break down and incorporate plant residues (PioneerSeeds, 2021). Growing resistant hybrids is also recommended (van Rensburg and Flett, 2012). Gibberella stalk rot may also be resistant to hybrids that are resistant to other stalk rots such as Diplodia. There are no fungicides available to control Gibberella stalk rot. However, the use of fungicides for the control of foliar diseases can reduce stress on the plant and thus reduce the severity of stalk rot and lodging.

CHARCOAL ROT

Causal species: ***Macrophomina phaseolina*** (Tassi) Goid., Annali della Sperimentazione Agaria 1 (3): 457 (1947) [MB#300023]

=*Rhizoctonia lamellifera* W. Small, Trans. Br. mycol. Soc.: 152 (1924) [MB#268900]

=*Sclerotium bataticola* Taubenh., Phytopathologia: 164 (1913) [MB#235618]

=*Rhizoctonia bataticola* (Taubenh.) E.J. Butler, Bull. Minist. Agric. Egypt 49, Bot. Sect.: 65 (1925) [MB#250965]

=*Macrophoma faseoli* Maubl. (?) [MB#492017]

=*Macrophomina philippinensis* Petr., Annales Mycologici 21 (3-4): 314 (1923) [MB#274223]

=*Dothiorella philippinensis* (Petr.) Petr., Repertorium Specierum Novarum Regni Vegetabilis Beihefte 42: 248 (1927) [MB#274218]

=*Macrophoma corchori* Sawada, Taiwan Nöjiho (Formosan Agric. Review): 868-871 (1916) [MB#189465]

=*Macrophoma 14esame* Sawada: 118 (1922) [MB#277988]

=*Macrophoma phaseoli* Maubl., Bulletin Trimestriel de la Société Mycologique de France 21 (1): 90 (1905) [MB#235970]

=*Dothiorella phaseoli* (Maubl.) Petr., Repertorium Specierum Novarum Regni Vegetabilis Beihefte 42: 241 (1927) [MB#274187]

=*Macrophomina phaseoli* (Maubl.) S.F. Ashby, Transactions of the British Mycological Society 12 (2-3): 141 (1927) [MB#274190]

=*Botryodiplodia phaseoli* (Maubl.) Thirum., Phytopathology 43 (11): 610 (1953) [MB#293796]

=*Tiarosporella phaseoli* (Maubl.) Aa, Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen: 4 (1977) [MB#324612]

Charcoal rot is caused by the fungus *Macrophomina phaseolina*. The fungus is a common soilborne fungus. It causes diseases such as stalk rot, root rot and charcoal rot, as well as seedling blight in maize (Marquez *et al*., 2021). *Macrophomina phaseolina* infects a wide range of species and has a wide geographical distribution. It can be found in areas where maize, sorghum and wheat are grown (Zitter *et al.,* 1996). It is a seed-borne fungus that inhibits root growth and produces black sclerotia (Khan, 2017).

Symptoms of Charcoal stalk rot

The first symptoms appear after flowering with drying of the upper leaf tissue, stalk lodging and premature death (Khokhar *et al.,* 2014). The pith of the infested maize plant appears grey over time due to the developing microsclerotia (Figure 1.7) (Grain SA, 2012). The rot later colonizes the vascular tissue, which disturbs the water translocation of the plants, causing water deficiency symptoms. The leaves turn yellow and dry out (Khokhar *et al.,* 2012).

Charcoal rot disease cycle and epidemiology

The disease cycle of charcoal rot is caused by *Macrophomina phaseolina* (Ghosh *et al.,* 2018*)*. The fungus overwinters in sclerotia form in crop residues and the soil. It then causes infection through the roots (Mukherjee *et al.,* 1983). The primary source of inoculum is the microsclerotia present in the soil (Gupta *et al*., 2012). The microsclerotia germinate (between 30 and 35 degrees Celsius) and a germ tube is formed (Shekhar and Kumar, 2012). An appressorium develops and penetrates through the epidermis of the host. Upon infection, the fungus affects the vascular system of the host plant, disrupting water and nutrient transport to the upper parts of the host (Marquez *et al.,* 2021). This then causes the plant to wilt and the stem to appear greyish due to the microsclerotia. In extreme infestations and favourable conditions, premature death often occurs (Smith and Carvil, 1997). A new cycle begins

when the microsclerotia in the roots and stem debris return to the soil or they can survive in the soil for up to 15 years (Gupta *et al.,* 2012)

Management strategies

Crop rotation to nonhost crops, such as small grains, can help reduce disease potential by growing resistant hybrids. These are the most widely used strategies. Plant residues can also be removed as another strategy. During the flowering stage, good water management must be maintained to avoid stressing the plant (Davis, 206).

DIPLODIA STALK ROT

Causal species: ***Stenocarpella maydis*** (Berk.) B. Sutton, The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata: 432 (1980) [MB#116258]

=*Diplodia zeae* Lév., Annales des Sciences Naturelles Botanique 9: 258 (1848) [MB#244611]

=*Macrodiplodia zeae* (Lév.) Petr. & Syd., Annales Mycologici 21 (3-4): 189 (1923) [MB#282284]

=*Hendersonia zeae* (Lév.) Hazsl., Math. termiesz. Közlem. Magg. Tudom. Akad.: 48 (1872) [MB#160014]

=*Phaeostagonosporopsis zeae* (Lév.) Woron., Défense des Plantes Leningrad 2: 333 (1925) [MB#282287]

=*Sphaeria zeae* Schwein., Transactions of the American Philosophical Society 4 (2): 207 (1832) [MB#474954]

=*Diplodia maydicola* Speg., Anales del Museo Nacional de Historia Natural Buenos Aires ser. 3, 13: 370 [no. 933] (1911) [MB#241451]

=*Diplodia zeae-maydis* Mekht., Botanicheskie Materialy: 156 (1962) [MB#330079]

Diplodia stalk rot is a soil-borne disease caused by the fungus *Sternocarpellla maydis* previously known as *Diplodia maydis*. The primary host for this pathogen is maize and sweet maize. This maize disease often occurs during early rainfall and in very hot seasons with late drought.

Symptoms

Symptoms of this disease include wilting and greyish-green leaves. Internodes on the lower stalk become spongy and pycnidia cluster near the nodes of the rind covered with white mycelia. Diplodia stalk rot causes a straw-brown discolouration of the lower nodes and internal decay of the pith, leaving only the vascular tissue intact (Figure 1.8). As the stalk is weakened, this can cause the maize plant to lodge during strong winds and rain before harvest (Figure 7B) (Flett and van Rensburg, 2021).

Economic importance

This fungal pathogen is of major economic importance, causing root and stalk rot in maize (Wicklow *et al.,* 2011). Diplodia stalk rot occurs extensively in all maize-growing areas of South Africa. The disease shows itself in a season with early rainfall and hot, as well as late droughts. It causes drastic damage and results in lodging and poor grain filling (Flett and van Rensburg). This disease reduces maize yields by decreasing the nutrient and moisture uptake in the maize ears. Sugars and nutrients are extracted from the stalks by the ears, which promotes fungal growth and further reduces nutrient uptake. Lodging during drying can result in further losses; 5% to 20% of losses may be due to Diplodia stalk rot and lodging (Flett and van Rensburg, 2021).

Diplodia stalk rot life cycle and epidemiology

The fungal pathogen overwinters in mycelium form on the soil surface. When conditions are warm and moist, pycnidia develop and release spores that are spread by wind and rain. Other plants are infected through the crown and roots, less frequently via the nodes between the crown and ear. Infection occurs within two to three weeks after silking under favourable conditions (Flett and van Rensburg, 2022).

Management strategies

Management strategies include crop rotation that reduces inoculum by removing maize stubble before the next maize planting. In a crop rotation programme, soybeans are a good alternative, followed by groundnuts, wheat, and dry beans. Removing crop residue by ploughing, grazing, or burning reduces inoculum in the field. Stress reduction is another way to manage the disease. Stress can be reduced by adjusting planting dates to avoid drought. There are currently no registered fungicides against the disease in South Africa. However, a good full protection spray programme against foliar diseases has been reported to improve the standability (Flett and van Rensburg,

2021). Maize is the only host for the fungus that causes Diplodia ear and stalk rots; therefore, rotating to crops other than maize for 1-2 years is very effective at reducing the inoculum in the field (Grain SA, 2021).

PYTHIUM ROOT ROT

Pythium root rot is caused by multiple species that belong to the genus *Pythium* including *Pythium aphanidermatum, Globiposporangium irregulare (previously known as Pythium irregulare, Globipsporangium ultimum* (Harvey *et al.*, 2008). *Pythium* species are known to cause root rot, seed rot, damping off, and seedling blight in maize crops (White, 2000). The fungus survives in the soil and maize residues. When the soil is cool (10 to 15°C), the fungus germinates. The most favourable conditions are cold, moist soils. The main favourable conditions are very wet and moist soil conditions (Grain SA, 2012).

Economic importance

P. aphanidermatum can result in economic losses by stunting and slowing the growth, due to the weakening of the root system of the plants (Packer and Clay,2000). Root rots and damping off are extreme diseases caused by *Pythium* species, they result in poor germination and thin seedlings in maize crops, ornamentals, vegetables, and forest trees in nurseries (Bickel and Koehler, 2021).

Symptoms

Maize plants infected with Pythium root rot have dark areas extending to the stem. The infected areas may become translucent, soft, and watery. Other symptoms include stunted, slow-growing plants, to severely infected, dead plants. The roots become brown rotten and mesocotyls (Figure 1.9) (Agrios, 2005).

Pythium stalk rot disease cycle and epidemiology

Pythium species overwinter in the soil as oospores, hyphae or sporangia. *Pythium* develops and colonizes the maize plant through the production of hyphae, threadlike, filamentous cells that absorb nutrients from the host (Martin and Loper, 1999). Thick-walled oospores are produced when the hyphae from opposite mating types meet. The oospores are the overwintering structures during this process. When the environmental conditions are favourable, oospores produce a germ tube and infect the plant directly (that is if enough amount of water is present), an oospore may produce

sporangia, which might then produce motile, biflagellate zoospores, which then encyst and germinate on the host plant (Moorman *et al.,* 2002).

Management strategies

In the effective control of *Pythium* diseases, more focus is placed on ensuring a good drainage system and water management. Sanitation and prevention are important and play a major role in reducing disease development and pathogen spread. To control *Pythium* species infections, excessive irrigation and accumulation of standing water should be avoided (Goldberg *et al.,* 1992; Rao *et al.,* 1978). Fungicides such as metalaxyl in combination with other fungicides protect seedlings from *Pythium* species infections and can help control *Pythium* (Rao *et al.,* 1978; Martin and Loper 1999). Fungicides need to be rotated to prevent the build-up of resistant strains (Moorman *et al.,* 2002) Minimizing stress helps to reduce Pythium stalk root rot.

PYTHIUM STALK ROT

Pythium stalk rot is an oomycete disease caused by several *Pythium species* (Gai *et al.,* 2018), including *P. aphanidermatum*, *G. irregulare* (previously known as *Pythium irregulare*) and *Globisporangium ultimum* (Harvey *et al.,* 2008). It always occurs during humid and wet seasonal conditions. *Pythium* species are known for their role as pathogens causing seed rot, damping-off and seedling blight and root rot (White, 2000). During the seedling stage of maize in its life cycle, *Pythium* infections cause pre- and post-emergence damping off, and seeds and seedlings decay before and after the crop emerges from the soil (Arora *et al.,* 2021).

Economic importance

Most *Pythium* species that affect maize are mostly associated with maize seedlings as well as old roots of maize. *Pythium* species have been reported to cause severe losses in soils with poor drainage systems, in monocultures and in areas with conservation tillage is done (Rao *et al.,* 1987). These oomycetes are known to be major root pathogens in maize-producing countries such as the USA and Europe, but their economic importance has been determined in Southern Africa (Grain SA, 2016).

Symptoms

P. aphanidermatum causes the rotting of the internodes above the soil (Figure 1.10). It causes the rind and pith to become soft, brownish and to be water-soaked. The stalk

eventually twists and falls over (Figure 1.11). The vascular tissue of the plant is not affected and so the plant can retain its green colour (Harvey *et al*., 2008).

Pythium stalk rot disease cycle and epidemiology

The oospores of *Pythium* species overwinter in plant and soil residue (Martin and Loper, 1999). The oospores are the primary survival structures during overwintering. They are very resistant to extreme drought and survive in soils for long periods without organic matter or suitable conditions (Martin and Loper, 1999). The spores germinate when conditions are favourable and they produce mycelium or sporangia that also release oospores (Hendrix and Campel, 1983). These oomycete pathogens infect roots and are more active during rainy seasons with moist soils (Hendrix and Campbel, 1983).

Management strategies

Management strategies related to Pythium stalk rot include eliminating low areas in the field where maize is planted as well as improving drainage in the soils, avoiding overwatering and applying fungicides (Davis, 2016; Tuf and Landscape Syngenta, 2022).

CONCLUSION

This review described the soilborne pathogens affecting maize crops, their distribution, potential economic impact as well as management strategies. It also provided insight into the challenges faced by emerging farmers in the Eastern Cape in relation to maize pathogen infestation and the lack of support from agricultural extension services. There is a lack of knowledge about what fungal species occur in maize soils in the province, including potential soil-borne pathogens. Furthermore, little information on how to manage these diseases is available. To fill this gap, surveys are needed to create the baseline knowledge needed on what fungi (or oomycetes) and what diseases are prevalent and in which areas they occur. Such surveys will contribute to future research into applying disease control strategies. This research will contribute to the long-term goal of ensuring food security in South Africa in the face of an ever-growing population. One way to ensure food security is to empower and support emerging farmers. Education and training can be improved through agricultural extension services and technology, as well as using applications to communicate agricultural advice and climate information. Improved crop varieties adapted to

different environments should be used by farmers to ensure higher and more sustainable crop production. Sustainable disease management strategies help farmers avoid crop damage, leading to an increase in local food security.

FIGURES AND TABLES

Table 1.1 List of common soil-borne fungal and oomycete pathogens associated with maize diseases.

Fungal species	**Disease-associated**	**Reference**
Colletoytichum graminicola (Ces.) G.W. Wils	Seedling blight, Anthracnose disease, red stalk rot, foliar leaf blight	(Bergstrom and Nicholson, 1990)
Curvularia eragostidis (Hennings) J.A. Meyer	Curvularia leaf spot Stalk and root rot (secondary stalk invader)	(Shurtleff *et al.,* 1993) (White, 1999)
Exserohilum pedicellatum (Henry) K.J. and E.G. Suggs	Seedling blight, Root rot seedling blight Cob-rot, wilting, stunting	(Shurtleff *et al.,* 1993) (Gilbert, 2003)
Fusarium chlamydosporum (Wollenw. and Reiking)	Root, crown, and stalk rot Ear rot	(Morales-Rodriguez *et al*., 2007)
Fusarium equiseti (Corda) (Sacc.)	Fusarium head blight Minor root rot, stalk rot, crown rot	(Nicolaisen *et al.,* 2009) (Shurtleff et al., 1993)
Fusarium graminearum (Schwabe)	*Gibberella* root, crown, and stalk rot Seedling blight *Fusarium* head blight	(Logrieco *et al.,* 2003)
Fusarium oxysporum (Schlectend)	Minor root rots and wilts Minor stalk rots *Fusarium* head blight Seedling blight and root rot	(Shurtleff *et al.,* 1993)
Fusarium verticilloides (Sacc.)	*Fusarium* ear, cob, and stalk rot *Fusarium* kernel, root and stalk rot, seed rot and seedling blight *Fusarium* head blight	**(**Shurtleff *et al.,* 1993) (Christensen *et al.,* 2014) (Logrieco *et al.,* 2002)

Globisporangium irregulare (Syn=Pythium irregulare)	Root and stalk rot	(Mao *et al*., 1998)
Macrophomina phaseolina (Tassi) Goid	Charcoal rot, Seedling rot-seedling blight, Root and stem rot	(Nicolaisen *et al.,* 2009) (White, 1999) (Francl, 1998)
Pythium aphanidermatum (Edson) Fitzp	Pythium stalk and root rot Seedling blight	(White, 1999)
Pythium graminicola (Subramanian)	Pythium root and stalk rot	(Shurtleff *et al*., 1993)
Rhizoctonia solani (Kühn)	Seedling blight, damping off Rhizoctonia root, crown, and stalk rot	(Shurtleff *et al.,* 1993) (White, 1999)
Stenocarpella maydis (Berk.) Sutton	Diplodia stalk rot	(Lamprecht *et al.,* 2011)

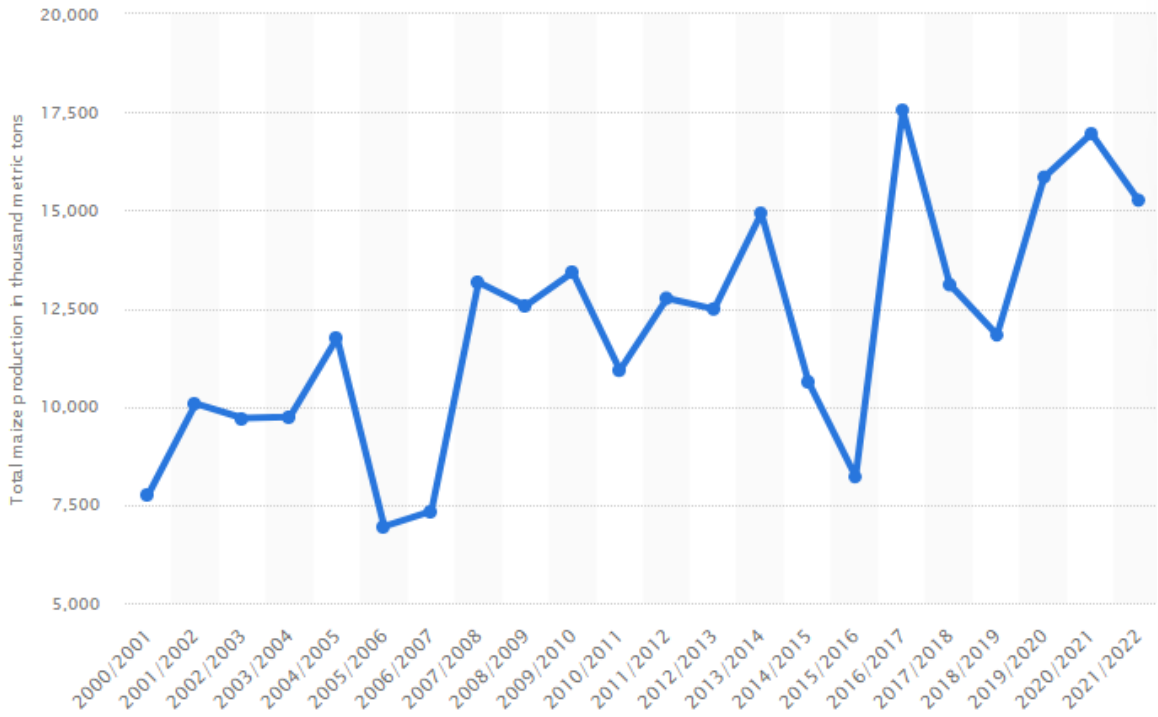

Figure 1.1. South Africa's maize production from 2000 to 2022 (Galal (Statista; https://www.statista.com/statistics/1134833/production-of-maize-in-south-africa/), 2022) © Statista 2024.

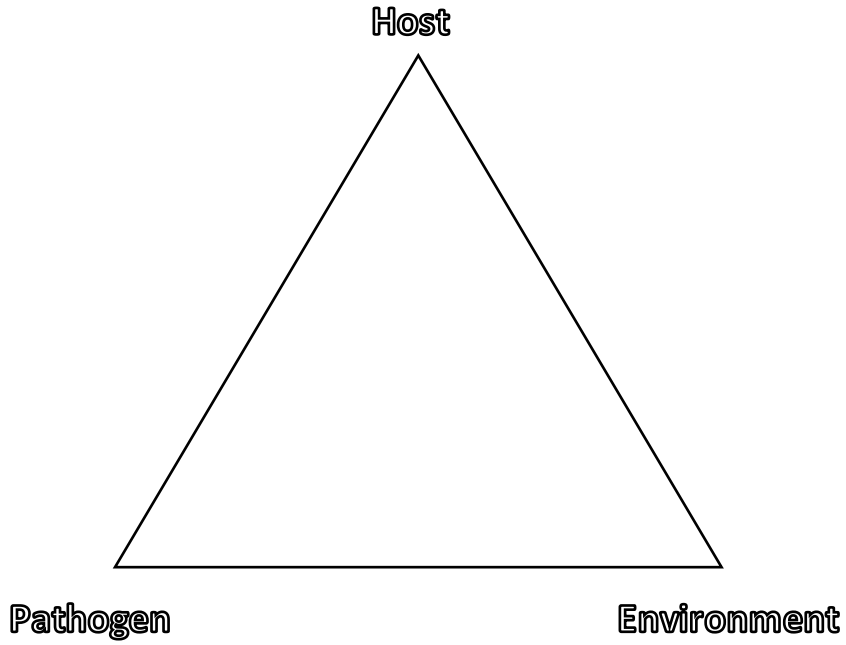

Figure 1.2. The disease pyramid shows the factors that need to coexist for a disease to occur.

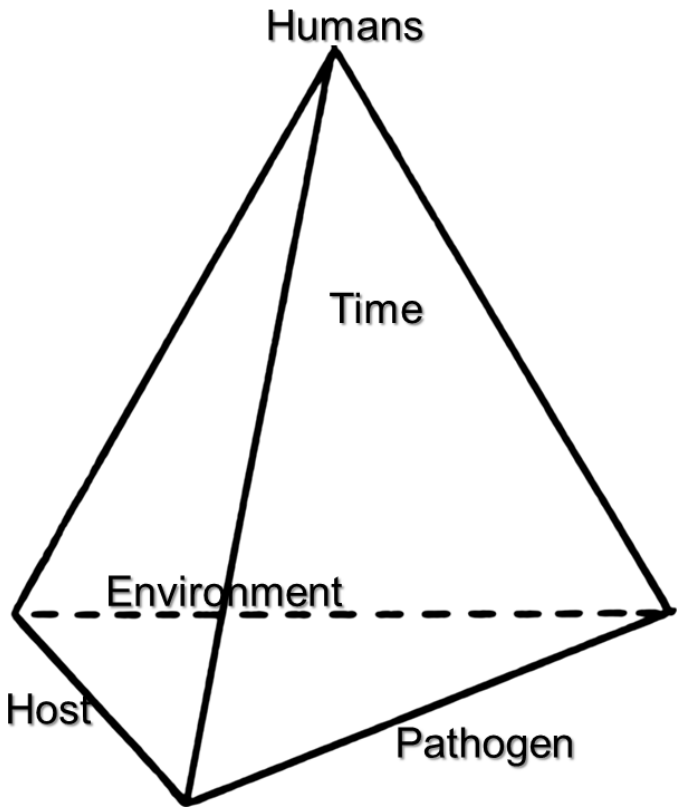

Figure 1.3. The Disease Pyramid. (Agrios, 2005).

Figure 1.4. Stalk rot caused by *Fusarium* may cause pink or salmon-coloured shredded pith. (Alison Robertson, 2022; Crop Protection Network) https://cropprotectionnetwork.org/encyclopedia/fusarium-stalk-rot-of-corn ©2024 by the Crop Protection Network.

Figure 1.5. Brown streaks appear on the lower internodes because of Fusarium stalk rot (Alison Robertson, 2022; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/fusarium-stalk-rot-of-corn) ©2024 by the Crop Protection Network.

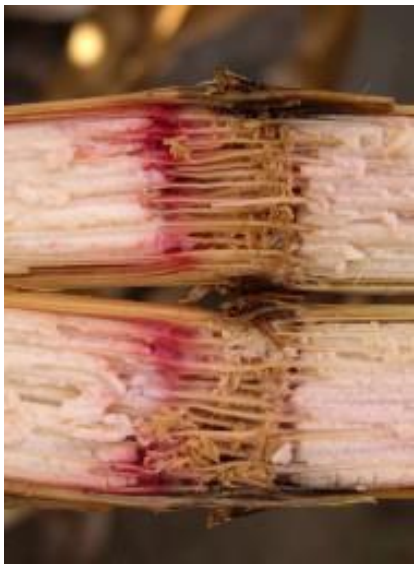

Figure 1.6. Gibberella stalk rot results in internal discolouration and pith shredding (Gary Munkvold, 2021; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/gibberella-crown-rot-and-stalk-rot-of-corn) ©2024 by the Crop Protection Network.

Figure 1.7. Charcoal-like, grey discolouration may appear on the stalk from numerous microsclerotia characteristic of charcoal rot (Mueller, 2020; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/charcoal-rot-of-corn) ©2024 by the Crop Protection Network.

Figure 1.8. Diplodia stalk rot can result in small black pycnidia on the lower internodes. (Gary Munkvold, 2021; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/diplodia-stalk-rot-of-corn) ©2024 by the Crop Protection Network.

Figure 1.9. Stalk lodging from Diplodia stalk rot. (Gary Munkvold, 2021; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/diplodia-stalk-rot-of-corn) ©2024 by the Crop Protection Network.

Figure 1.10. Pythium stalk rot can cause the stalk to twist and fall over. (Alison Robertson, 2021; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/pythium-stalk-rot-of-corn) ©2024 by the Crop Protection Network.

Figure 1.11. Pythium stalk rot causes decay of the first internode above the soil. (Alison Robertson, 2021; Crop Protection Network; https://cropprotectionnetwork.org/encyclopedia/pythium-stalk-rot-of-corn) ©2024 by the Crop Protection Network.

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CHAPTER 2

FUNGAL AND OOMYCETE DIVERSITY IN EASTERN CAPE MAIZE FIELDS SOIL.

ABSTRACT

Maize is a staple food and one of the most important grain crops grown in South Africa. Currently, knowledge of fungi and oomycetes present in Eastern Cape soils is limited, especially on maize farms. The aims of this study were therefore to conduct a survey using modern taxonomic approaches while establishing important baseline information for the province. Bulk rhizosphere soil samples were collected from 22 farms across four districts of the Eastern Cape. Fungal isolations were made by preparing serial dilutions and plating these onto potato dextrose agar (PDA), water agar (WA) and Fusarium Selective Media (FSM). Oomycetes, on the other hand, were isolated using the soil bait technique using rose petals and leaves. Isolation plates were incubated at ±21°C for seven days. The colonies of interest were then transferred to clean ¼ PDA plates and pure cultures were made. A total of 421 fungal and 16 oomycete strains were isolated. These were identified to genus level based on morphology where possible, after which DNA sequencing was performed targeting gene regions informative to the genera of interest. Gene regions included β-tubulin (*BenA*) for *Penicillium*, translation elongation factor 1-α (*TEF*) for *Cladosporium, Fusarium,* and *Trichoderma*; the internal transcribed spacer (ITS) region for strains that could not be morphologically identified to a genus; and ITS for oomycetes. *Penicillium* (n=98), *Fusarium* (n=90), *Cladosporium* (n=46), and *Trichoderma* (n=103) were the most frequently isolated, but *Epicoccum* (n=15), *Neocosmospora* (n = 6), *Phoma* (n = 2), and *Talaromyces* (n=20) were also identified. The most frequently isolated fungal species were *Fusarium oxysporum sensu lato* (n =64), *Trichoderma gamsii* (n = 29), and *Penicillium cremeogriseum* (n = 18). *Globisporangium irregulare* (n=11) was the most frequently isolated oomycete, but *Globisporangium ultimum* (n=5) was also identified. Several strains belonged to species previously reported to cause maize diseases such as Fusarium stalk rot, Diplodia stalk rot and Pythium root and stalk rot. However, no disease symptoms were observed during the fieldwork. This study is the first report on the fungal and oomycete species present in Eastern Cape maize soils identified using DNA sequence data. It provides much-needed baseline

knowledge on the fungi and oomycetes and potential soilborne fungal pathogens present on maize farms in the Eastern Cape.

INTRODUCTION

Maize (*Zea mays*) is the world's third most important cereal crop after wheat and rice (Bawa, 2021), but in South Africa, it is the most important grain and a staple food for most of the country. Maize contains about 72% starch, 10% protein and 4% fat, making it a very useful source of energy for humans and animals (Maluleke, 2020). It is also a versatile crop that serves several purposes. Industrial uses include the production of starch, sweeteners, oils, beverages, adhesives like glue, and industrial alcohols (Maluleke, 2020), while maize can be used to produce a range of animal feeds, textiles, paper, and pharmaceuticals (Maluleke, 2020).

Maize plays a significant role in the diet of more than 60% of the South African population. Per capita consumption of maize and maize-based foods is particularly high in South Africa, Lesotho, Malawi and Zambia, averaging 100 kg per capita per year (FAOStat, 2021; Erenstein *et al.,* 2022). As a C4 crop, it has excellent photosynthetic efficiency and can grow well in a variety of environments, including tropical, subtropical, and temperate regions (Erenstein *et al.,* 2022). In South Africa, maize is mostly produced in areas with summer rainfall (Hadisu-Bello *et al.,* 2020). Our main production areas are in the Free State (43%), Mpumalanga (24%) and North-West (16%), while KwaZulu-Natal (5%), Gauteng (5%), Northern Cape (4%), and Limpopo (2%), while the Eastern Cape (1%) and Western Cape (1%) supply only a small percentage of maize in the country (Maluleke, 2020).

Both white and yellow maize are grown in South Africa. About 60% of the maize produced is white and 40% yellow (SA National Department of Agriculture, 2020). White maize is primarily used for human consumption in the form of maize meal, while yellow maize is mostly used in the animal feed industry (Adama, 2023). Around 10–12 million tonnes are produced annually on around 2.5 million hectares (Gravelet-Blondin, 2021). In the 2020/2021 season, South Africa recorded up to 14.6 million tonnes of maize with the Free State producing the largest amount (Galal, 2021). Currently, commercial agriculture accounts for only 40% of Eastern Cape production, with subsistence and emerging agriculture the main agricultural practices among the

particularly poor households in the province, while producing most maize (Kibirige, 2016; Kibirige and Obi, 2015; GrainSA, 2015).

Soil microbial communities play a vital role in the effort to produce healthy mature ears. The major fungal groups previously reported to be commonly associated with maize soils are *Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium* and *Trichoderma* (Bhattacharya and Raha, 2002). The main function of these filamentous fungi in soil is to decompose dead organic matter and support soil aggregation. However, some fungal and oomycete species can cause devastating diseases, including stalk, root and seed rots as well as downy mildew, damping-off and seedling blights. Some of the most common soilborne diseases that affect maize are charcoal root rot (*Macrophomina phaseolina*), Diplodia stalk rot (*Stenocarpella maydis*), Fusarium stalk rot (*Fusarium verticillioides*), Pythium root and stalk rot (*Pythium aphinidermatum*), and Gibberella stalk rot (*Fusarium graminearum*) (Flett and van Rensburg, 2021), which occur mainly during periods of drought. These microbes usually invade the roots and establish themselves as endophytes where some species may cause disease while others like some *Trichoderma* species promote plant growth, (Tseng *et al.,* 2020). Understanding the complex interactions between soil microbial communities, plants and various other factors is an important part of better understanding what constitutes a healthy soil structure and provides valuable information for sustainable farming practices to control diseases in agriculture (Suman *et al.,* 2022).

Maize seedlings are also vulnerable to soilborne oomycete pathogens, especially from the genera *Phytophthora, Globisporangium* and *Pythium* (Rojas *et al.,* 2019; Schmidt *et al.,* 2020). *Pythium* recently underwent several taxonomic revisions and, in the process, was split into five genera, namely *Elongisporangium, Globisporangium, Ovatisporangium, Pilasporangium* and *Pythium*. These newly introduced genera follow their sporangial morphology (Uzuhashi *et al.,* 2010). This resulted in *Pythium irregulare*, *P. sylvaticum, P. ultimum* var*. sporangiiferum,* and *P. ultimum* var*. ultimum* that were renamed as *Globisporangium irregulare, G. sylvaticum, G. sporangiiferum* and *G. ultimum*, respectively (Eggertson *et al*., 2023). *Globisporangium sylvaticum* is thought to be the most damaging oomycete pathogen of seeds and seedlings in both corn and soybean (Dorrance *et al.,* 2004, Jiang *et al*., 2012).

The South African government has collaborated with many development partners to raise public knowledge and awareness on managing maize pests and diseases at the emerging maize farms in the Eastern Cape. However, these farmers have traditionally been neglected in terms of research and development, meaning the knowledge on what diseases impact production in the province is lacking. There is also very little data on the fungal and oomycete communities associated with maize soils. Past fungal diversity survey-based species identifications on morphological observations, which may have resulted in species being misidentified. From the late 1990s, advancements in molecular biology technologies made it possible for taxonomists to generate DNA sequences for fungi and as a result led to more accurate sequence-based identifications. In light of this, our study aimed to document the rhizosphere bulk soil fungi and oomycete communities of Eastern Cape maize farms using these more modern taxonomic approaches to identify species.

MATERIALS AND METHODS

Soil sampling

A total of 110 maize rhizosphere bulk soils were collected into sterile plastic bags from 22 Eastern Cape farms situated across the OR Tambo (31.4632° S, 29.2321°E), Chris Hani (31. 8743° S, 26. 7968° E), Joe Gqabi (30.9850° S, 26.9852° E), and Alfred Nzo Districts (30. 5483° S, 28.8597° E) from 21–26 February 2021 (Figure 2.1). At each field, plants were randomly selected, and soil was collected around roots at a depth of 5–10 cm using a small sterile shovel. The shovel was sterilized by spraying it with 70% ethanol and wiping it with paper towel between each soil sample. Each bag of soil was labelled appropriately by indicating the farm name, date, and place of collection. Soil samples were stored at 4°C until processed. The 110 soil samples that were collected from each field were pooled to represent one sample for each of the 22 fields.

Fungal isolations

Fungal isolations were made using two approaches. Firstly, a dilution series was prepared by suspending 10 g of soil in 90 mL of sterile distilled water and serially diluting the suspension down to 1×10^{-4}. For each dilution, 1 ml suspension was plated in duplicate onto Difco™ Potato Dextrose Agar (PDA) (Becton, Dickson and Company, Sparks, USA) and Water Agar (WA) (WA: 20 g/L agar). Secondly, the soil was plated directly onto Fusarium Selective Media (FSM) which selects for *Fusarium* (15 g

peptone powder, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$ and 20 g Difco Agar in 1 L, and 1 g PCNB dissolved in acetone and 2 mL of chloramphenicol working solution). All media was supplemented with 2 mL chloramphenicol (50 ppm) and 3 mL streptomycin (100 ppm) to suppress bacterial growth. Plates were incubated at ± 21°C for 7 d and examined daily for fungal growth using a stereo microscope.

After incubation, colonies of interest were sub-cultured and purified onto ¼ PDA. These were incubated for another 7 d at ± 21°C. Single spore isolations were made for *Fusarium* by suspending spores in 100 µl of sterile distilled water in Eppendorf tubes. The suspension was mixed well and plated onto ¼ PDA with the plates facing down overnight. Single germinating spores were then cut out, transferred onto fresh ¼ PDA, and incubated for another 7 d at ± 21°C. Fungal isolates were grouped and identified into genus based on their morphological features before preserving them. All isolates were preserved as agar blocks or spore suspensions in cryovials containing 10% glycerol, stored at -80°C and accessioned into the CN working culture collection of the Applied Mycology group housed at FABI (Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa).

Oomycete isolations

For oomycetes, we used the baiting method described by Burgess *et al.,* (2020). Approximately 200 g of soil was added to a 1 L plastic container containing 100 ml of distilled water. The soil particles were allowed to settle for 24 h. After 24 h, the water surface was cleaned with sterile paper towels to remove floating debris. Oomycetes were then baited using *Rosa alba* petals and *Hedera canariensis* leaves. The baits were checked daily for 3–4 d for infection symptoms/lesions.

The lesions were excised with a sterile scalpel and plated onto Phytophthora and Pythium selective medium. The media (per L) used included NARPH (17 g cornmeal agar amended with 1mL nystatin, 100mg ampicillin, 10mg rifampicin, 100mg pentachloronitrobenzene (PCNB), and 50mg hymexazol) (Huberli *et al*., 2000) and NAR (17g cornmeal agar amended with 1mL nystatin, 100mg ampicillin, and 100mg rifampicin) (Simamora *et al*., 2017). All cultures were incubated for 10 d at ± 21°C and checked daily for hyphal growth. Hyphal tips were transferred onto carrot agar and incubated for another 10 d (Coffey and Coffey, 2015). The obtained isolates were then accessioned into the Applied Mycology Group (CN-Oom) working culture collection

housed at FABI and preserved in double distilled water in cryotubes kept at room temperature (± 21°C).

DNA Extraction, Amplification and Sequencing

DNA for highly melanized fungi, like *Penicillium, Cladosporium,* or *Trichoderma* was extracted from 7 d old ¼ PDA colonies using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, CA, USA) according to the manufacturer's instructions. For oomycetes and hyaline fungi like *Fusarium*, we used the Prepman™ Ultra (Thermo Fischer Scientific, Warrington, UK) kit according to the manufacturer's instructions. The DNA extracts were stored at -20°C and accessioned into the CN-DNA collection housed at FABI.

Amplification and Sanger Sequencing

Polymerase Chain Reactions (PCR) were performed in 25 µl volume reactions. The primer pairs and PCR amplification protocols are listed in Table 2.1. Reaction mixtures consisted of 5 µl Bioline 5X MyTaq Reaction buffer (Bioline, England, UK), 17.5 µl MilliQ water, 0.15 µl BioLine MyTaq DNA polymerase (Bioline Ltd, USA Inc.), 0.5 µl 10 µM reverse and forward primers and 1 µl DNA template. Gene regions amplified were dependent on strain identities at genus level (see Table 2.1). These regions were translation elongation factor-1-alpha (*TEF*), β-tubulin (*BenA*), and the internal transcribed spacer rDNA region (ITS).

The resulting PCR products were separated on a 1% agarose gel electrophoresis, stained with GelRed (Thermo Fischer Scientific, Massachusetts, USA), and eletrocrophosed alongside a 0.5µg/L GeneRuler 100bp DNA ladder (Thermo Fisher Scientific) and examined under UV light using a Bio-Rad Gel Doc™ EZ Imager (Bio-Rad Laboratories, Inc., USA). A clean-up step using ExoSap-IT PCR product cleanup reagent (Thermo Fischer Scientific, Massachusetts, USA) was performed to purify the PCR products and remove primer dimers and excess nucleotides. A reaction containing 4 µl PCR product and 7.5 µl of Exosap-IT reagent was prepared in strip tubes and subjected to a thermocycler at 37°C for 15 minutes and 85°C for 15 min.

The resulting pure PCR products were sequenced in both directions using the BigDye Terminator Sequencing kit v. 3.1 (Applied Biosystems, Foster City, California, USA) with the same primers used for PCR amplification. The thermal cycle profile had an initial denaturation temperature of 94°C for 5 min, followed by 40 cycles at 96°C for 30

sec, 50°C for 10 sec, and a final elongation step at 60°C for 4 min. Sequence reactions were run on the ABI PRISM 3500xL auto-sequencer (Applied Biosystems, Foster City, California) at the University of Pretoria sequencing facility. Contig sequences were assembled using Geneious Prime v. 2021.0.3 (BioMatters Ltd., Auckland, New Zealand). Newly generated DNA sequences were compared against sequences on NCBI (National Centre for Biotechnology Information) through BLASTn (Basic Local Alignment Search Tool) to obtain initial identifications to species level.

RESULTS

Fungal identifications

Isolations from the 22 soil samples resulted in 421 fungal strains. Figure 2.2 shows the representation of some of the genera isolated in this survey. Based on morphology and DNA sequence data, strains were identified into 14 orders (Figure 2.3), 22 families (Figure 2.4) and 29 genera (Figure 2.5). At order level, *Hypocreales* (n=210) and *Eurotiales* (n=119) were most frequently isolated, followed by *Capnodiales* (n=46), *Pleosporales* (n=22), *Sordariales* (n=11), *Umbelopsidales* (n=3), *Chaetosphaeriales* (n=2), *Chaetothyriales* (n=1), *Diaporthales* (n=1), *Hetotiales* (n=1), *Mucorales* (n=1), *Thelebolales* (n=1), *Venturiales* (n=1) and *Xylariales* (n=1). At family level, *Hypocreaceae* (n=103), *Aspergillaceae* (n=99) and *Nectriaceae* (n=96) were most frequent, followed by *Cladosporiaceae* (n=43), *Trichocomaceae* (n=20), *Didymellaceae* (n=19), *Chaetomiaceae* (n=10), *Chaetosphaeriaceae* (n=1), *Didymosphaeriaceae* (n=1), *Ophiocordycipitaceae* (n=4), *Umbelopsidaceae* (n=3), *Stachybotryaceae* (n=3), *Apiosporaceae* (n=1), *Clavicipitaceae* (n=1), *Cordycipitaceae* (n=1), *Cunninghamellaceae* (n=1), *Diaporthaceae* (n=1), *Herpotrichiellaceae* (n=1), *Pleosporaceae* (n=1), *Pseudeurotiaceae* (n=1), *Venturiaceae* (n=1) and *Vibrisseaceae* (n=1).

Genera and species identified included (Figure 2.5, Table 2.2) *Absidia* (n=1), *Alfbifimbria* (n=3), *Alternaria* (n=1), *Arthrinium* (n=1), *Aspergillus* (n=1), *Beauveria* (n=2), *Chrysanthotrichum* (n=1), *Chloridium* (n=2), *Cladosporium* (n=46), *Didymella (n=4), Epicoccum (n=15), Exophiala* (n=1), *Fusarium (n*=90*), Humicola* (n=9), *Metapochonia* (n=1), *Metarhizium* (n=1), *Neocosmospora* (n=6), *Paraconiothyrum* (n=1), *Penicillium* (n=98*), Phialocephala* (n=2), *Pseudogymnoascus* (n=1), *Purpureocillium* (n=4), *Staphylotrichum* (n=1), *Sternocarpella* (n=1), *Talaromyces*

(n=20), *Trichoderma* (n=103), *Tyrannosaurus* (n=1) and *Umbelopsis* (n=3). All species identified are shown in Table 2.2.

Trichoderma is classified as *Hypocreaceae*. The 103 strains were identified to 12 species, namely *T. afroharzianum* (n=3), *T. amoenum* (n=1), *T. arundinaceum* (n=1), *T. cf koningii* (n=2), *T. cf rifaii* (n=13), *T. dorothopsis* (n=2), *T. gamsii* (n=29), *T. hamatum* (n=10), *T. harzianum* (n=4), *T. koningiopsis* (n=26), *T. subazureum* (n=2), *T. virens* (n=4) (Figure 2.6). *Trichoderma gamsii* was the most commonly isolated species followed by *T. koningiopsis, T. cf rifaii* and *T. hamatum. T. gamsii* was recovered from all 22 farms, while *T. koningiopsis, T. rifaii* and *T. hamatum* were recovered from 9/22, 4/22 and 5/22 farms respectively.

Aspergillaceae was also commonly isolated with 99 fungal strains represented by 18 *Penicillium* species and a single isolate of *Aspergillus udugawae*. Species from these genera are typically not associated with maize diseases in South Africa. *Penicillium* strains (Figure 2.7) were identified as *P. adametzii* (n=10), *P. annulatum* (n=1), *P. allsoppiae* (n=1), *P. brevicompactum* (n=1), *P. cf camponotum* (n=1), *P. cf restrictum* (n=3), *P. cremeogriseum* (n=18), *P. cf ortum* (n=3), *P. cf pole-evansii* (n=4), *P. melini* (n=1), *P. onobense* (n=8), *P. ortum* (n=15), *P. pulvillorum* (n=9), *P. raperi* (n=13). *P. rubens* (n=4), *P. skrjabinii* (n=4), *P. subrubescens* (n=1), and *P. virgatum* (n=1). *Penicillium cremeogriseum* was the most commonly isolated species and was recovered from 7/22 farms, followed by *P.ortum, P. raperi* and *P. pulvillorum*, respectively recovered from 4/22, 6/22 and 3/22 farms.

Fusarium (n=90) and *Neocosmospora* (n=6) belonging to *Nectriaceae* contain several important grain pathogens. The 90 *Fusarium* strains represented 12 species in three species complexes (Figure 2.8), namely the *Fusarium oxysporum* species complex (FOSC), *Fusarium sambucinum* species complex (FSAMSC), and the *Fusarium incarnatum-equiseti* species complex (FIESC). Species identified included *F. arcuatisporum* (FIESC; n=1), *F. boothi* (FSAMSC; n=2), *F, brachygibbosum* (FSAMSC; n=3), *F. cerealis* (FSAMSC; n=1), *F. clavum* (FIESC; n=1), *F. cf incarnatum* (FIESC; n=1), *F. equiseti* (FIESC; n=13), *F. graminearum* (FSAMSC; n=1), *F. inflexum* (FOSC; n=2), *F. oxysporum sensu lato* (FOSC; n=64), *F. transvaalense* (FSAMSC; n=2) and *F. venenatum* (FSAMSC; n=1) (Figure 2.9). *Fusarium oxysporum sensu lato* was the most isolated species and was recovered from 15/22 farms. *Fusarium* species

in the FOSC were the most common species to be isolated in this survey and they are known to be associated with maize seeds and roots. Other *Fusarium* species that occurred often across samples included *F. equiseti* (n=10) followed by *F. brachygibbosum* (n=4). *Neocosmospora* (previously the *Fusarium solani* species complex) species identified included *N. solani* with six strains being recovered from 3/22 farms.

Several other species were commonly isolated in this survey including *Cladosporium pseudocladosporoides* (n=24; Figure 2.10), *Talaromyces pinophilus* (n=6; Figure 2.11), *Humicola veronae* (n=9) and *Epicoccum viticis* (n=11). These are typically non-pathogenic soil inhabiting fungi. Some species of economic importance were also isolated including *Alternaria alternata* (n=1) and *Sternocarpella maydis* (n=1). These two species are both known to be important pathogens of maize but were isolated infrequently. For example, *A. alternata* is known to be a leaf pathogen to maize and causes leaf blight and can result in major economic losses. *S. maydis* is a fungal pathogen that is associated with maize seeds and is known to cause Diplodia ear and stalk rot in maize.

Oomycete identifications

Oomycete isolations from the 22 soil samples resulted in 16 strains. Strains were identified as *Globisporangium irregulare* (n=11) and *Globisporangium ultimum* (n=5) (Table 2.2; Figure 2.12). Both these species are known to be pathogenic to maize and they cause seedling blight, root and stem rots in maize.

DISCUSSION

Maize is one of South Africa's most important crops, but its production can be greatly impacted by fungal and oomycete diseases. The Eastern Cape province was identified by the South African government as a region with great production potential. Baseline knowledge on the pests and diseases that impact maize production is however lacking. Progress has been made to monitor and document pests and foliar pathogens across the province, with the current study documenting fungal and oomycete diversity associated with rhizosphere soils.

No research has been conducted in the Eastern Cape that looks at the fungal and oomycete diversity in the maize field soils, because traditionally, no money was spent on research in the region. This study is the first of its kind in the Eastern Cape province

to investigate the fungal and oomycete diversity occurring in the maize rhizosphere soils. Many emerging farmers in the Eastern Cape province are struggling with disease infestation in their maize fields and have reported that diseases are one of the factors limiting production. Several farmer development programs implemented by the government support emerging farmers in the Eastern Cape, as the province's maize industry wants to become one of the main producers and a healthy percentage of food producers in the country.

Fungal communities were remarkably similar to a study that completed a similar survey at commercial farms in the Free State and North-West provinces by Qikani, 2023. Qikani (2023) reported 28 genera and 80 species, with mostly *Fusarium, Neocosmospora, Penicillium,* and *Trichoderma* being found to dominate the maize rhizosphere soils of both provinces. The fungal species that dominated the soil were *Fusarium tardicrescens, Neocosmospora solani, Penicillium raperi,* and *Trichoderma afroharzianum*. In our study, it was observed that *Trichoderma* was more common than *Fusarium* compared to the previous study. The present study, however, found that the fungal species that dominated the rhizosphere soil belong to the genera *Trichoderma* (n=103)*, Penicillium* (n=98)*, Fusarium* (n=90) *and Cladosporium* (n=46) respectively with *Trichoderma koningiopsis*, *Penicillium cremeogriseum*, *Fusarium oxysporum sensu lato* being the most dominant fungal species.

In a study conducted by Viviers (2014) over 101 localities through the commercial maize-producing regions of South Africa, seventy different fungal species were isolated from maize seedling roots and the species isolated included species from the genera *Aspergillus, Clonostachys, Fusarium, Trichoderma* and *Penicillium.* The most isolated species were *Aspergillus niger, Neocosmospora solani, Fusarium verticillioides* and *Fusarium oxysporum* which partially correlates with the results of this study. A wide range of *Fusarium* species were commonly isolated from maize seedling rots. The *Fusarium* isolates were confirmed based on DNA sequence data as part of the polyphasic approach. The workhouse gene region was the ITS region in conjunction with *TEF* for the identification of *Fusarium* species.

Beyers (2019), used qPCR to identify and quantify the twelve most commonly occurring root and crown rot soilborne pathogens of maize in South Africa. From the study conducted by Beyers (2019), the most prominent fungi were found to be *Phoma*

spp., Fusarium chlamydosporum., Pythium spp., Fusarium oxysporum and *Fusarium graminearum*. The results obtained in the above study correlate with the results obtained in our study except for *Fusarium chlaymadosporum and Phoma spp*. The communities not differing much between the two studies could be attributed to the fact that both the Free State and Eastern Cape's climates are characterized by hot dry summers and cold moist winters. The fungal diversity of soil is known to be influenced by the local environmental conditions (Tardy *et al.,* 2015) as well as the physical and chemical properties of the soil. This determines the composition of extant fungal communities (Requena *et al.,* 2001). The fungal species identified in the previous study are well-known pathogens of maize including *Fusarium graminearum, Fusarium chlamydosporum* and *Fusarium oxysporum*.

Trichoderma was the most frequently isolated genus in this study with 103 strains identified to 29 species. *Trichoderma gamsii* was especially common and was isolated from all samples collected. *Trichoderma koningiopsis* was also commonly isolated and occurred in 9 samples. *Trichoderma* is a ubiquitous filamentous fungus associated with numerous substrates, such as rhizosphere soil, foliar environments, and decaying plant matter. *Trichoderma* species are widely used as a biological control agent effective against many phytopathogens, where they suppress soilborne diseases and enhance resistance. Furthermore, agricultural applications of *Trichoderma* strains have been shown to promote nutrient uptake and growth of plants, as well as the control of soilborne pathogens such as *Fusarium, Phytophthora, and Rhizoctonia.* This is possible through direct and indirect mechanisms of processes such as mycoparasitism, enabling host resistance, and competition for space and nutrients. (Kubicek *et al.,* 2008; Jaklitsch and Voglmayr, 2015; Samuels and Hebbar, 2015; Ghazanfar *et al.,* 2018).

Trichoderma species are among the most studied fungi and the most commonly used biological control agents in agriculture. There are several biological control products that are available and registered worldwide for the control of *Fusarium* diseases on various crops. However, only one biological control product has been registered in South Africa for the control of *Fusarium* species. The product is known as Tri-Cure (*Trichoderma harzianum* isolate MIT04). There is a vast potential for controlling *Fusarium* in maize using biological agent formulations as well as in other crops like wheat, barley, oats and legumes (Hasan, 2010).

Many *Trichoderma* species are registered as biological control agents across the world. The most typically used species are *T.afroharzianum, T harzianum, T asperellum, T atroviridae, T viridae, T virens, T longibrachiatum T. polysporum*, and *T. asperellum* (Di Marco *et al.,* 2022). *Trichoderma gamsii* has been proven to be a good biological control agent of Fusarium Head Blight (FHB) of wheat. A study by Risoli *et al.,* 2023 proved (*Trichoderma gamsii* T06085) to be an effective a biological control agent of Fusarium Head Blight (FHB) and regulates biocontrol-relevant defence genes expression in wheat (Alukumbura *et al.,* 2022). Several studies have also been conducted to investigate the ability of *Trichoderma gamsii* T6085 to control Fusarium Head Blight symptoms as well as the build-up of mycotoxins together with mycoparasitic, antagonistic and competitive activities against *F. graminearum,* which is known to be one of the main causal agents of FHB on wheat has been extensively studied (Matarese *et al.,* 2012; Sarrocco *et al.,* 2013).

A recent report in 2018 however, reported on the severe infestation of *Trichoderma* observed on maize cobs for the first time in several experimental fields in the southern parts of Germany (Pfordt *et al.,* 2020). It was however surprising that the causal agent was found to be *T. afroharzianum*. It was the first report in Europe on *Trichoderma* species as a pathogen causing severe yield losses in maize. *Trichoderma afroharzianum* causing ear rot in maize was also detected in a couple of cases in Italy on maize kernels showing cob rot symptoms (Sanna *et al*., 2022).

Penicillium was the second most frequently isolated genus with 98 strains identified to 18 species. *Penicillium cremeogriseum* was the most commonly isolated but was isolated from only 15/22 samples. *Penicillium* was thus diverse but individual species did not form part of the core community. This was different from commercial farms of the North-West and Free State in a survey conducted by Qikani 2022 where they found the same *Penicillium* across the board. *Penicillium* is taxonomically diverse with 483 accepted species (Visagie *et al.,* 2014, Houbraken *et al.,* 2020).

Several reports have shown that *Penicillium* species interact with the roots of plants to enhance plant growth through nutrient absorption, secretion of plant hormones, and tolerance to abiotic stress (Hyakumachi, 1994; Shivanna *et al.,* 1994; Khan *et al.,* 2008), *e.g P. chrysogenum* has been proven to promote plant growth and improving early stages of maize development under saline conditions (Galeano *et al.,* 2023).

Some *Penicillium* species such as *P. citrinum* and P. *roqueforti* (Khan *et al.,* 2008; Ikram *et al.,* 2018) secrete plant hormones like indole-3-acetic acid (IAA) and gibberellin (GA) and facilitate phosphate solubilization, which may contribute to plant growth (Khan *et al*., 2008; Kim *et al*., 2011; Radhakrishnan, Shim *et al*., 2013). This survey found *P. raperi*, a potential biological control agent that produces multiple metabolites and is antagonistic to phytopathogens and insects postharvest.

Fusarium was the third most frequently isolated genus with 90 strains identified to nine species. *Fusarium* species isolated include *Fusarium oxysporum sensu lato, Fusarium equiseti* as well and *F. graminearum* which have been reported to cause ear, crown root and stalk rot in maize. Most of these species belong to the *Fusarium oxysporum species complex* with *Fusarium oxysporum sensu lato* identified from 15 samples. *Fusarium oxysporum sensu lato* is a complex of *Fusarium* species that are the causal agents of various important diseases on a wide variety of plants including maize. The most important and common diseases caused by *Fusarium oxysporum sensu lato* are seed rot and root rot on maize. *F. oxysporum* is widely distributed and overwinters and can survive for many years in the soil as spores and on crop residues. This serves as an important source of fungal infection, when weather conditions are favourable a disease outbreak occurs (Paugh *et al.,* 2021). Stalk rot infections and outbreaks are more common in hot and rainy summer conditions (Shi *et al.,* 2017). The fungus is involved in seedling diseases which affects the germination of seeds and emergence, and this affects the development of seeds in maize (Varela *et al.,* 2013). These are economically important diseases affecting maize in South Africa. Sixty-four (*TEF*) sequences were generated for this species complex and additional work is needed to determine the relationship between the species.

Fusarium equiseti (FIESC) and *Fusarium boothi* (FSAMSC) were also identified and are considered important pathogens of maize. *Fusarium equiseti* is known to be a weak pathogen on cereal crops and is occasionally associated with Fusarium Head Blight infected kernels (Rubella *et al.,* 2004). Furthermore, members of the *Fusarium incarnatum-equiseti* species complex (FIESC) are rarely considered major pathogens of disease outbreaks. However, they have been identified as co-occurring fungal pathogens during infections (Vilani *et al.,* 2016). *Fusarium equiseti* has been recently reported to cause post-flowering stalk rot of maize in India (Swamy *et al.,* 2020). It has also been reported to cause wilt on *Capsium chinense* in Mexico as well as chilli wilt

in Kashmir along with other species such as *F. oxysporum* and *N. solani* (Mejia-Bautista *et al.,* 2016; Hami *et al.,* 2021). *Fusarium oxysporum sensu lato* is pathogenic to maize and is known to cause wilting, root, and crown rot as well as necrosis in maize.

Fusarium boothii is a member of the *Fusarium-sambucinum* species complex. It is an important pathogen known to cause Gibberella ear rot of maize as well as Fusarium Head Blight of wheat. A previous report shows the occurrence of the cereal pathogen *F. boothii* from trees as hosts for the first time. In a study conducted by Gryzenhout and Landman (2016), it was shown that *F boothii* infected the common native tree *Vachellia erioloba* and a non-native, unrelated pecan tree. *Fusarium boothii* is known to only infect barley, maize and wheat in countries in the Europe, South America, the USA and South Africa. No reports show it to infect a native plant. Two other different hosts that were infected included soybean and tomato. Other *Fusarium* pathogens are *F. equiseti, F. brachygibbosum* and *F graminearum* which cause root, stalk, and ear rot in maize.

Oomycetes were not common in soils, but 16 isolates representing two *Globisporangium* species were obtained. Five oomycete strains were identified as *Globisporangium ultimum* (formerly *Pythium ultimum*) and 11 were identified as *Globisporangium irregulare*. Both *G. irregulare* and *G. ultimum* are well-known pathogens of maize and are known to cause seedling blights, damping off and root rots in maize (Koch *et al.,* 2022).

Globisporangium species primarily attacks monocotyledonous plants causing root rot, while *Phytophthora* primarily attacks dicotyledonous such as beans, tomatoes and eggplants causing damping off and root rot (Ho, 2018). The genus *Globisporangium*, was separated from *Pythium* and was invented to group species with globular sporangia (Uzuhashi *et al.,* 2010). This makes sense as to why we did not recover any *Phytophthora* even though NARPH (cornmeal agar amended with nystatin, ampicillin, rifampicin, pentachloronitrobenzene (PCNB), and hymexazol) was used as a selective media. Researchers have previously found that maize roots do not attract *Phytophthora capsica* zoospores, inhibiting both the swimming of zoospores and the formation of cystospores. This results in peppers having more resistance to *Phytophthora* blight (Yang *et al.,* 2014). Another reason could be that *Pythium* is

generally fast-growing compared to *Phytophthora* (Ho, 2018). Semi-selective media such as V8-RPBH may be used in future studies to increase *Phytophthora* species isolated (Rojas *et al.*, 2019).

In the study conducted by Qikani (2023), the oomycete species that were identified in the maize rhizosphere soils of Free State and North-West provinces are *G. irregulare, G. ultimum* and *P. tolurosum*. These results correlate with the results of our study except for *P. tolurosum,* Qikani (2023), also did not recover *Phytophthora* species in their study.

Pythium species are the major pathogens reported with limited data on *Phytophthora* infestation on maize. Several studies have been conducted in South Africa that document fungal and oomycete diseases and their effects on agricultural farming and forestry (Linde *et al*., 1994; Maseko *et al.*, 2002; Spies *et al*., 2011). However, there is little knowledge about their diversity in maize farms of South Africa including the Eastern Cape. Controlling these diseases is possible through various types of cultivation practices, such as using disease-resistant cultivars, pathogen-free seeds, crop rotation, appropriate planting dates, and chemical and biological methods (Gqozo *et al*., 2020).

In a study conducted by Rojas *et al.,* 2019 in Michigan, eighty-four oomycete species were identified from soybean seedlings using the ITS region of the rDNA. *Pythium* was predominantly isolated across the samples followed *by Phytophthora, Phytopythium* and *Aphanomyces* species. The most commonly isolated species were *P. heterothallicum* (12.2%), *Phytophthora sojae* (9.3%), *P. ultimum* (6.1%), *P. perplexum* (6.0%), *P. irregulare* (5.8%), *P. oopapillum* (3.2%), *P. inflatum* (2.9%), *P. attrantheridium* (2.7%), *P. intermedium* (2.7%), *P. rostratifingens* (2.6%) and *P. ultimum var ultimum* (2.5%).

In nine provinces of El-Minia Governorate, Egypt, 374 *Pythium* isolates were recovered and identified from the rhizosphere soils of maize plants grown in 100 different fields. Five *Pythium* species were isolated, and they were *P. deliense, P. graminicola, P. irregulare, P. oligandrum* and *P. splendens.* The present study only isolated and identified *P. irregulare* (now known as *G. irregulare*) in low occurrences except for the other four isolates. A recent report showed that *G. irregulare* and *G.*

ultimum var. *sporangiiferum* were associated with the damping-off of soybean in Brazil (Molin *et al.,* 2021).

In Southwestern Uganda, root rots were discovered on maize, sorghum, peas and potato. These were obtained from farmer's fields where they were intercropped with beans that had been previously infected with root rot (Gichiru *et al.,* 2016). Identifications were done using the ITS region and twenty-one *Pythium* species were found to be associated with root rot in crops that were commonly intercropped with beans. The most predominantly found *Pythium* species was *P. ultimum* (now known as *G. ultimum*). Other *Pythium* species found include *P. torulosum, P. folliculosum, P. acanthicum, P. spinosum* and *P. olidandrum.*

Globisporangium species as well as *Pyhtium* species cause huge economic losses in agricultural and forestry industries. For example, in Kenya and Rwanda, it has been reported that *Pythium* root rot caused up to 70% yield losses in bean cultivars grown locally. Moreover, 35-54% and 12-17% are lost under temperatures that range from 18°C and 28°C with lettuce that is grown through hydroponics (Stanghellini and Kronland, 1986; Nzungize *et a*l., 2012). *Pythium* root rot has also been reported to reduce ginger production by 50-90% in areas that are severely affected (Rai *et al.,* 2018).

None of the obtained fungal and oomycete species were found to cause diseases in the fields, but their continuous monitoring and screening of diseases will have to be done because factors such as climate change and cultivation practices may alter the diversity of the soil and thereby result in disease in the maize fields. In this regard, future studies will have to investigate the environmental and climatic conditions that may alter these strains to be pathogenic to maize. It is expected that by 2050, the world's population will increase by more than a third, or 2.3 billion people. By 2050, global agriculture will need to increase by 60-70% from current levels to meet the increased demand for food. As a result, it is imperative to invest in research aimed at identifying the pests and diseases that lead to losses for farmers. By doing so, it is possible to prevent and control pests and diseases. The results of this study provide the baseline knowledge needed for the fungal and oomycete communities present in these soils and the potential soil-borne pathogens that may be present in these soils.

CONCLUSION

Fungal and oomycete diversity in The Eastern Cape was expolored for the first time in this study and provides a baseline knowledge needed on what fungal and oomycete species are present in the region. In this study, relatively diverse fungal communities were detected in maize rhizosphere soils, and some have been reported to be important pathogens of maize. Some species in the genera *Penicillium* and *Trichoderma* are known to be beneficial for the healthy growth of plants through their interaction with maize roots. Diverse fungal communities in maize soils have a significant impact on maize soils whereby they improve soil fertility, nutrient cycling, diseases suppression as well as plant growth promotion. This directly has a positive impact to the farmer in that there will be improved crop yields, enhanced soil health and longevity as soils with diverse fungal communities are resilient to drought, diseases and other environmental stresses. A new species of *Penicillium* belonging to the section *Canescentia* has been found and is described in chapter 3 of this dissertation. The findings of this study provide a baseline knowledge required on what fungal and oomycete species are present in Eastern Cape maize farm soils.

FIGURES AND TABLES

Figure 2.1. Map showing the four districts visited to sample rhizosphere soil samples in the Eastern Cape province.

Table 2.1. Table showing primers used for amplification and sequencing.

Locus	Genus	PCR amplification profile	Primer pairs	Direction	Primer sequence (5'-3')	Reference
Translation Elongation Factor 1-α *(TEF)*	*Fusarium*	Denaturation 94°C 5min; 30 cycles of 94°C 45sec; annealing at 52°C 60sec; 72°C 60sec; 72 °C 7min.	FusEF1	Forward	ATGGGTAAGGARGACAAGAC	O'Donnell *et al*., (1998)
			FusEF2	Reverse	GGARGTACCAGTSATCATG	O'Donnell *et al.,* (2015)
Translation Elongation Factor 1-α *(TEF)*	*Trichoderma* *Cladosporium*	Denaturation 94 °C 5 min; 35 cycles of 94 °C 45 sec, annealing 52 °C 45 sec, 72 °C 90 s; 72 °C 8 min.	EF1-728F	Forward	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn, (1999)
			EF2	Reverse	GGARGTACCAGTSATCATGTT	O'Donnell *et al.,* (1998)
Internal Transcribed spacer 5.8S rDNA *(ITS)*	Fungi general (unknowns)	Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing 55°C	V9G	Forward	TTACGTCCCTGCCCTTTGTA	De Hoog and Gerrits van den Ende (1998)

		45sec; 72°C 60sec; 72°C 7 min.	LS266	Reverse	GCATTCCCAAACAACTCGACTC	Masclaux *et al.,* (1995)
Internal Transcribed Spacer *(ITS)*	Oomycetes	Denaturation 94 °C 3 min; 35 cycles 94°C 60 sec; annealing at 55°C for 60 sec; 72°C for 60 sec; 72°C for 10 min.	ITS6	Forward	TTACGTCCCTGCCCTTTGTA	De Hoog and Van den Ended, (1998)
			ITS4	Reverse	GCATTCCAAACAACTCGACTC	
β-tubulin (*BenA*)	*Penicillium* *Talaromyces*	Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing at 55°C 45sec; 72°C 60sec; 72°C 7 min.	T10	Forward	GGTAACCAAATCGGTGCTGCTTTC	Glass & Donaldson (1995)
			Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	Glass & Donaldson (1995)

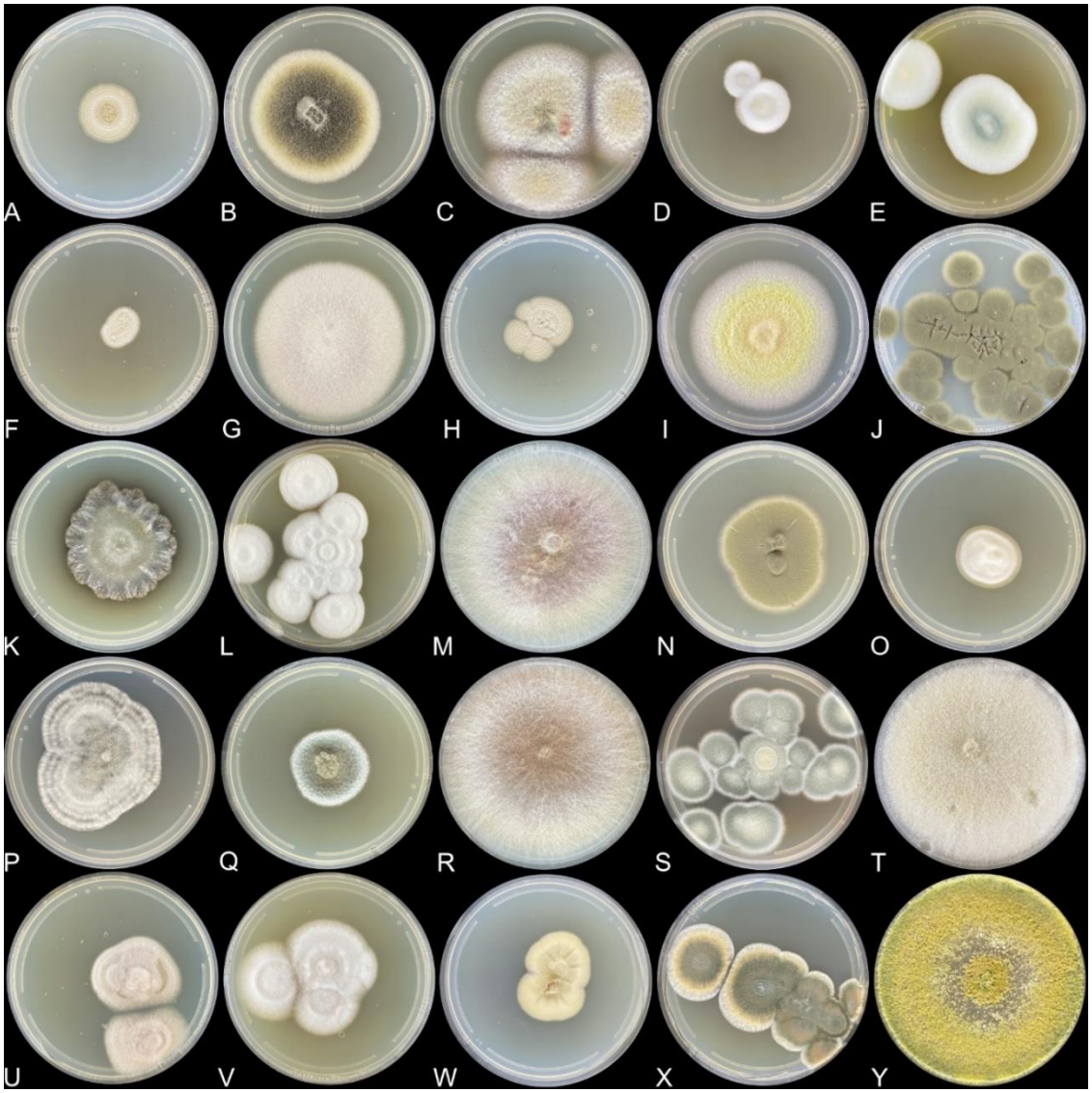

Figure 2.2. A representation of some of the genera isolated in the soil survey. A representation of the genera isolated. **A:** *Albifimbria verrucaria;* **B:** *Alternaria alternata;* **C:** *Arthrinium kogelbergense;* **D:** *Atractium stilbaster;* **E** *Aspergillus udugawae;* **F:** *Beavaria amorpha;* **G:** *Beavaria amorpha;* **H:** *Chaetosphaeria;* **I:** *Chloridium fuscum:* **J:** *Cladosporium pseudocladosporoides:* **K:** *Chrysanthotrichum peruvianum;* **L:** *Exophiala pisciphila;* **M:** *Fusarium inflexum;* **N:** *Humicola veronae;* **O:** *Metapochonia bulbilosa* **P** *Metarhizium pinghaense* **Q** *Microspharopsis arundis* **R** *Neocosmospora solani;* **S:** *Penicillium pulvillorum;* ***T:*** *Phialocephala sp.;* **U:** *Purpureocillium lilacinum;* **V:** *Staphylotrichum cf coccosporum;* **W:** *Stenocarpella maydis;* **X:** *Talaromyces purpureogenus;* **Y:** *Trichoderma harzianum*.

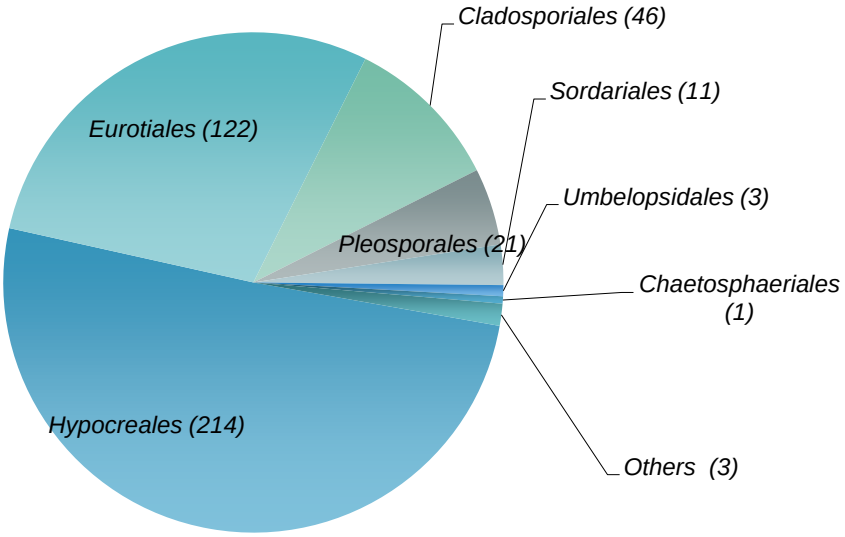

Figure 2.3. The Order that represents the strains obtained in the rhizosphere soil. The numbers in the brackets represent the number of fungal strains in the Order. “Others” include the order with one fungal strain isolated (*Diaporthales, Xylariales, Mucorales, Thelebolales, Venturiales, Chaetothyriales*).

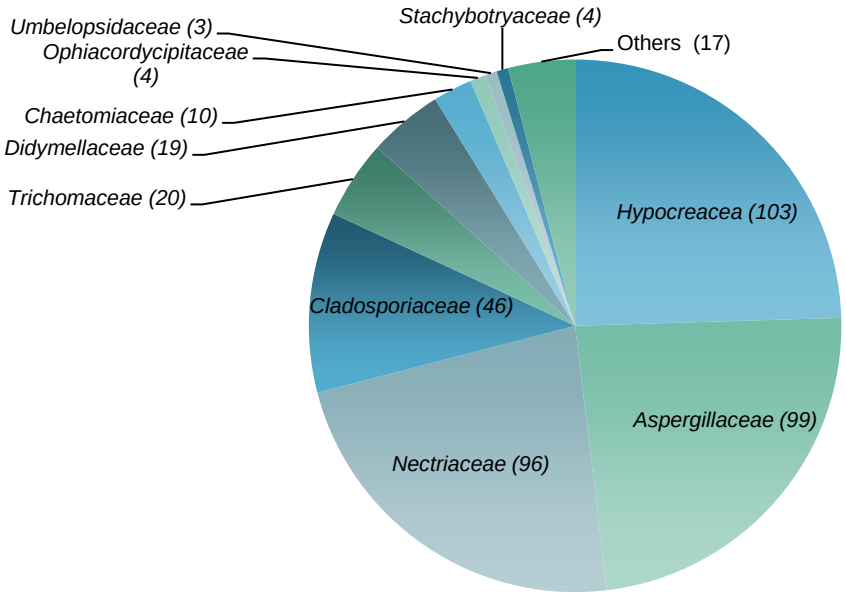

Figure 2.4. The number of strains identified at the family level. The numbers between the brackets show the number of strains in that family. “Others” include the families with only one fungal strain (*Pleosporaceae, Diaporthaceae, Apiosporaceae, Cunninghamellaceae, Pseudeurotiaceae, Venturiaceae, Herpotrichiellaceae*

Clavicipitaceae, Cordycipitaceae, Vibrisseaceae, Didymosphaeriaceae, Chaetosphariaceae).

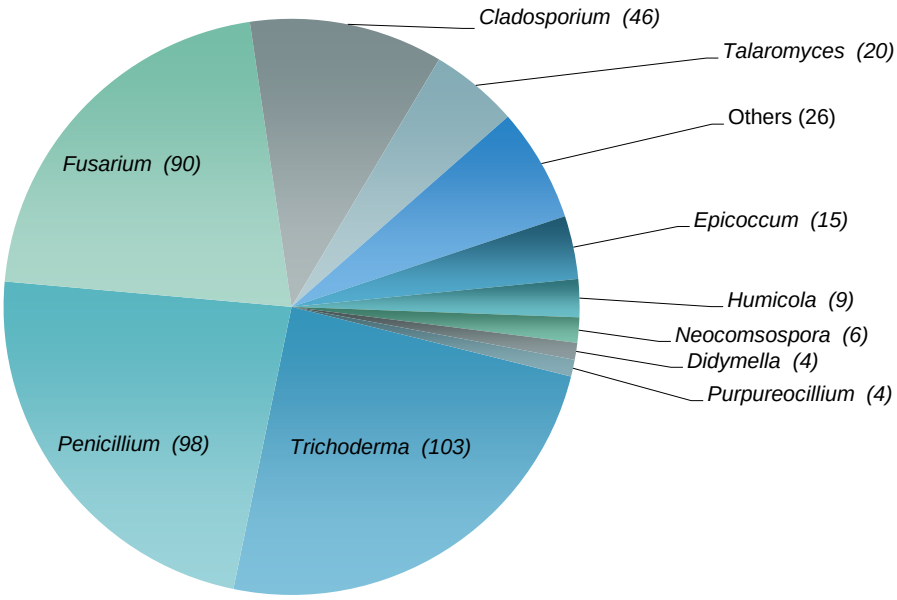

Figure 2.5. Genera found in the maize rhizosphere soils. The numbers in the brackets show the number of strains in the genus. “Others” include genera with less than 5 fungal strains (*Absidia, Albifimbria, Alternaria, Arthrinium, Aspergillus, Beauveria, Chloridium, Chrysanthotrichum, Exophiala, Metapochonia, Metarhizium, Paraconiothyrium, Pseudogymnoascus, Staphylotrichum, Stenocarpella Tyrannosorus, Umbelopsis, Chloridium, and Phialocephala*).

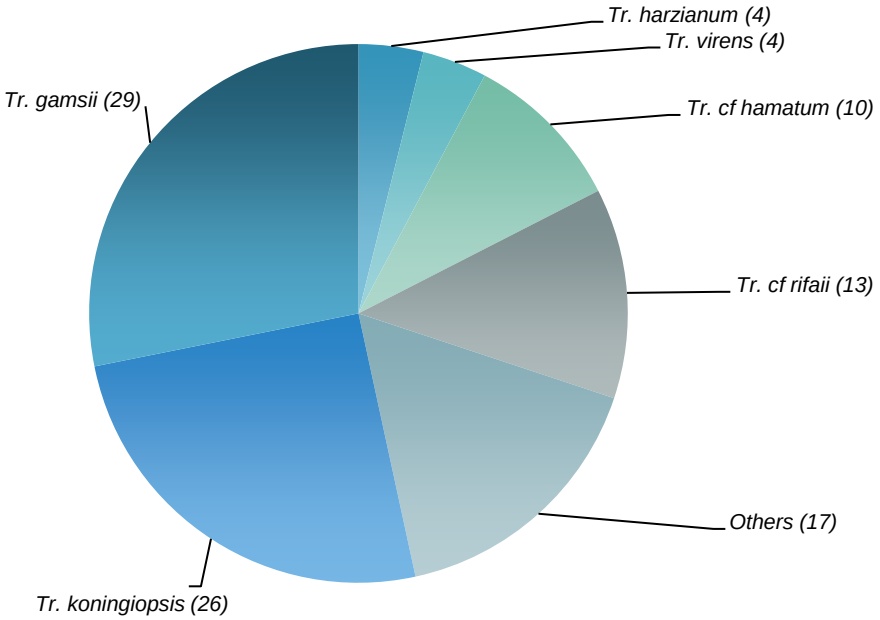

Figure 2.6. *Trichoderma* species identified from the maize rhizosphere soils. The numbers in the brackets show the number of strains obtained. “Others” include species with less than four fungal strains *Trichoderma amoenum, Trichoderma afroharzianum, Trichoderma arundinaceum*, *Trichoderma subazureum, Trichoderma cf koningii, Trichoderma dorothopsis.*

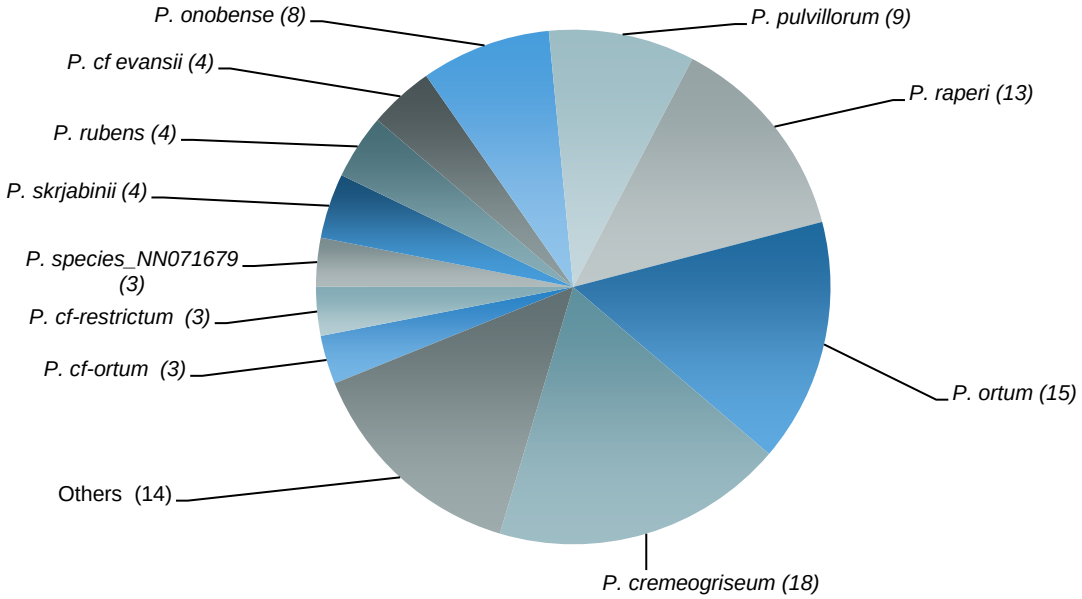

Figure 2.7. *Penicillium* species identified from the rhizosphere soils. The numbers in the brackets show the number of species. “Others” include species that only had one fungal strain (*Penicillium adametzii*, *Penicillium allsoppiae*, *Penicillium melini,*

Penicillium brevicompactum, Penicillium annulatum, Penicillium subrubescens, Penicillium cf camponotum, P. cf pulvillorum and *Penicillium virgatum).*

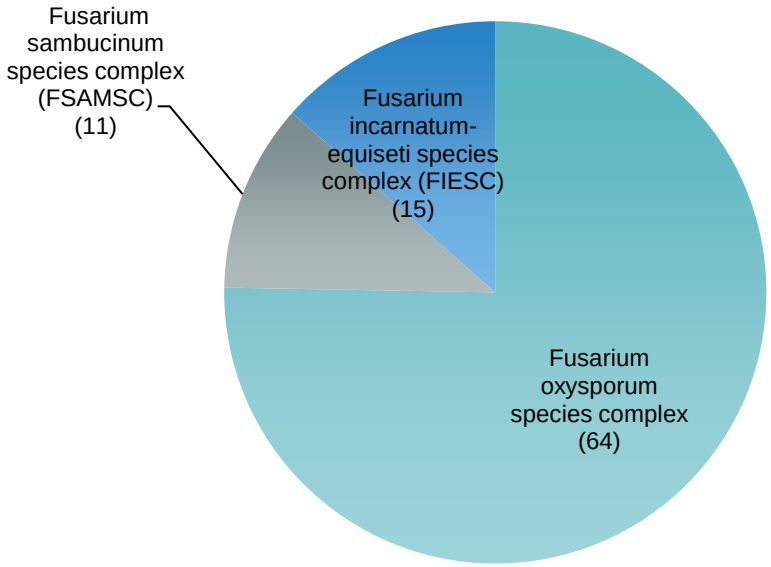

Figure 2.8. Fusarium species complexes. The numbers in the brackets show the number of strains in the species complex.

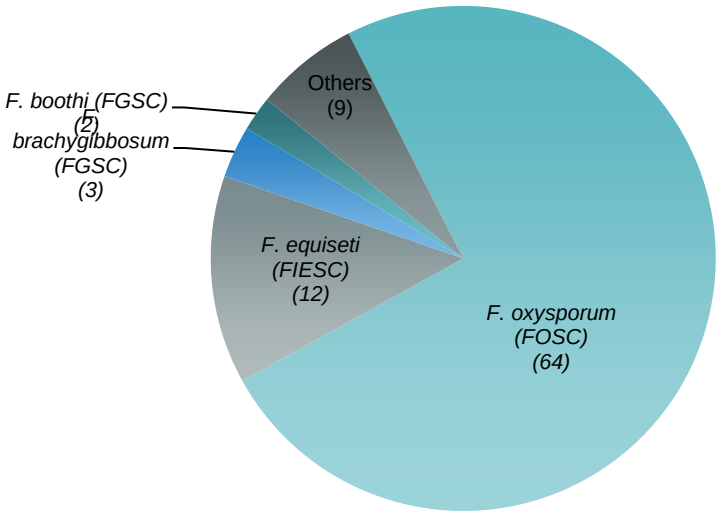

Figure 2.9. *Fusarium* species identified from the maize rhizosphere soils using DNA sequencing. The numbers in the brackets show the number of species obtained. The category others include species such as *F clavum* (FIESC*), F. transvaalense* (FSAMSC), *F. inflexum* (FOSC), *F. venenatum* (FSAMSC), *F. cerealis* (FSAMSC), *F. graminearum* (FSAMSC), *F. arcuatisporum* (FIESC).

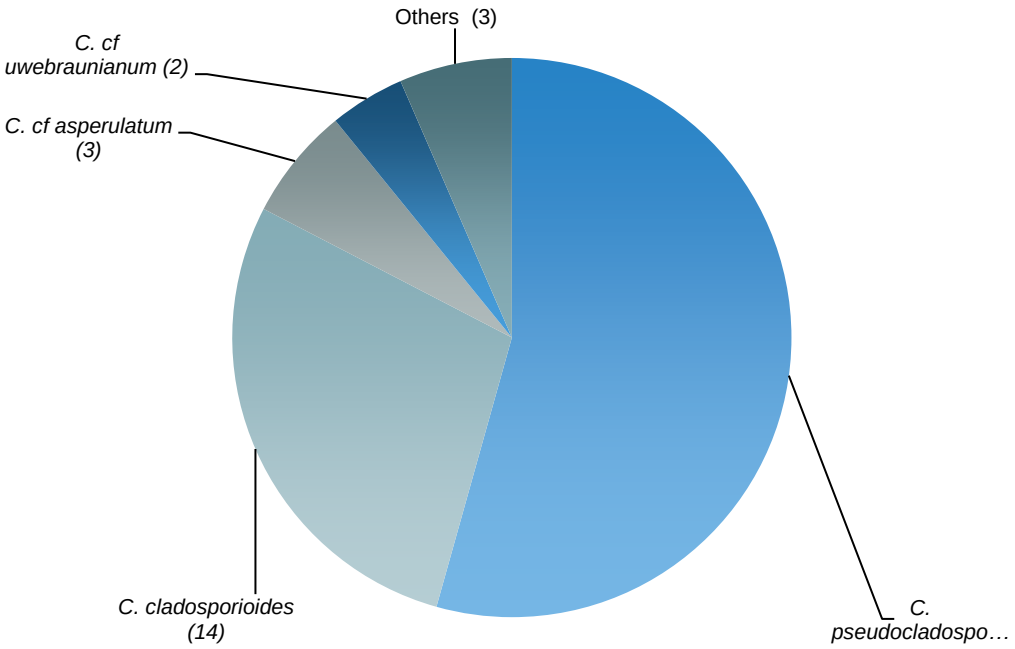

Figure 2.10. *Cladosporium* species identified from the rhizosphere soils. The numbers in brackets show the number of strains. The category “Others” includes *Cladosporium anthropophilum, Cladosporium chalastosporoides, and Cladosporium cf angustiterminale.*

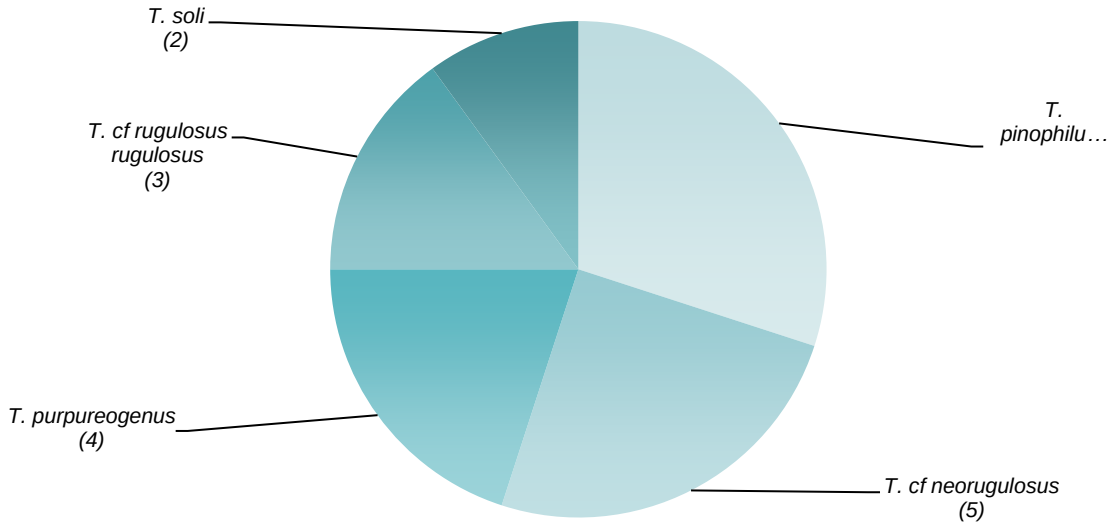

Figure 2.11. *Talaromyces* species identified from the rhizosphere soils. The numbers in brackets show the number of strains.

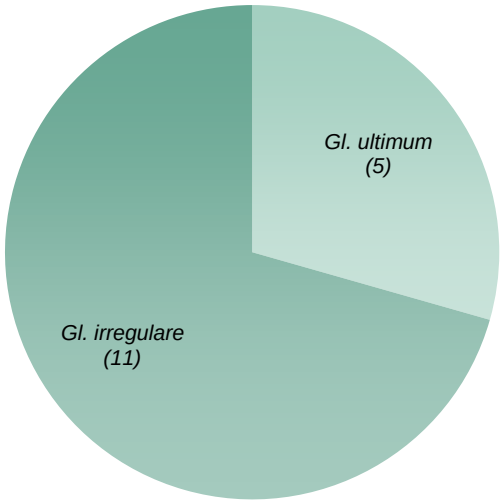

Figure 2.12. A graph representing oomycetes species in the *Globisporangium* genus. The number of strains is represented by the numbers in the brackets.

Table 2.2. Fungal and oomycete species identifications through DNA sequencing using gene regions of interest. The table also shows the isolation frequency of the species as well as the CN collection numbers allocated to them.

Species name	**Number of strains**	**Isolation frequency (22 farms)**	***TEF***	***ITS***	***BenA***	**CN Number**
Abisida cf cuneospora	1	1		X[1]		CN113F3[2]
Aspergillus udugawae	1	1			X	CN117E9
Alternaria alternata	1	1		X		CN113C1
Albifimbria verrucaria	3	1		X		CN153A6, CN153A7, CN153B4
Arthrinium kogelbergense	1	1		X		CN113E7
Unknown (Hypocreomycetidae)	1	1		X		CN126D2
Beauveria amorpha	2	2		X		CN126D4, CN131F1
Chloridium aseptatum	2	1		X		CN153A9, CN153B3
Chrysanthotrichum peruvianum	1	1		X		CN121G2

Cladosporium pseudocladosporoides	24	5	X			CN113G9, CN113H1, CN113H2, CN113H3, CN113H4, CN117G1, CN118F6, CN118F7, CN118F9, CN118G1, CN118G2, CN118G3, CN121F4, CN121F5, CN126B8, CN126B9, CN126C1, CN126C2, CN131F4, CN131F5, CN131F6, CN131F7, CN131F8, CN149A4
Cladosporium cf uwebraunianum	2	2	X			CN113G7, CN121F5
Cladosporium anthropophilum	1	1	X			CN113G8
Cladosporium cladosporiodes	14	6	X			CN104F1, CN116F8, CN118F9, CN118G3, CN118I6, CN126B8, CN126B9, CN126C3, CN131F9, CN131G1, CN143C3, CN149A5, CN149A6, CN152I2
Cladosporium chalastosporoides	1		X			CN126C6
Cladosporium cf asperulatum	3	2	X			CN121F3, CN148F4, CN148F5,
Cladosporium cf angustiterminale	1	1	X			CN152I3
Didymella prosopidis Didymella spnov	4	2		X		CN113C2, CN 113C5, CN113D5, CN121F6
Epicoccum italicum	1	1		X		CN149B2
Epicoccum cf keratinophilum	1	1		X		CN113F4
Epicoccum ovisporum	1	1		X		CN119B9
Epicoccum thailandicum	1	1		X		CN131F2

Epicoccum viticis	11	2		X		CN113C8, CN113D1, CN113D2, CN113D3, CN113D6, CN113E1, CN119A1, CN119A2, CN119B6 CN119B7, CN119B8
Exophiala pisciphila	1	1		X		CN153B1
Fusarium arcuatisporum (FIESC)	1	1	X			CN104F7
Fusarium brachygibbosum (FSAMSC)	4	1	X			CN154D1, CN145D4, CN154D6, CN154D8
Fusarium boothi (FSAMSC)	2	2	X			CN125D7, CN125E8
Fusarium cerealis (FSAMSC)	1	1	X			CN142C4
Fusarium clavum (FIESC)	1	1	X			CN125C8
Fusarium cf incarnatum (FIESC)	1	1	X			CN125C9
Fusarium equiseti (FIESC)	10	6	X			CN142A9, CN142C9, CN142D1, CN142D4, CN142D5, CN142D8, CN149F8, CN149H1, CN143I3, CN153F1
Fusarium graminearum (FSAMSC)	1	1	X			CN142B2
Fusarium inflexum (FOSC)	2	1	X			CN125D9, CN149F5
Fusarium oxysporum sensu lato (FOSC)	64	22	X			CN113B4, CN125C4, CN125C5, CN125D2, CN125D8, CN125E2, CN125E4, CN125E7, CN125E9, CN125F1, CN126B1, CN142B1, CN142B3, CN142B4, CN142B7, CN142B8, CN142B9, CN142C3, CN142C5, CN142D7, CN142E4, CN142E5, CN142E7, CN142E8, CN142F1, CN142F2, CN142F3, CN142F5, CN142F7, CN142F9, CN142H4, CN149F4, CN149F6, CN149F7, CN149G1, CN149G2, CN149G3, CN149G4, CN149G5, CN149G6,

						CN149G8, CN149G9, CN149H2, CN149H4, CN149H6, CN149H7, CN149H8, CN153E3, CN153F2, CN153F3, CN153F4, CN153F5, CN153F8, CN153F9, CN153G1, CN153G2, CN153G3, CN153G4, CN153H3, CN153H4, CN154C3, CN154C8, CN154C9, CN154D3
Fusarium transvaalense (FSAMSC)	2	1	X			CN154D2, CN154D5
Fusarium venenatum (FSAMSC)	1	1	X			CN142C7
Globisporangium irregulare	11	11		X		CN-Oom002C1[3], CN-Oom002D5, CN-Oom002D6, CN-Oom002D8, CN-Oom002D9, CN-Oom002E1, CN-Oom002E3, CN-Oom002E5, CN-Oom002E6, CN-Oom002E7, CN-Oom002F5
Globisporangium ultimum var. ultimim	5	6		X		CN-Oom002F1, CN-Oom002F2, CN-Oom002F3, CN-Oom002F7, CN-Oom002C9
Humicola veronae	9			X		CN143H4, CN143H8, CN143I7, CN143I8, CN148F6, CN148F8, CN148F9, CN148G1, CN153E8
Neocosmospora solani	6			X		CN142B5, CN142B6, CN142C2, CN142C6, CN142D6, CN142F4
Metapochonia bulbilosa	1				X	CN121F2
Metarhizium pinghaense	1			X		CN153C3
Paraconiothyrium spnov	1				X	CN143C8, CN148D1

Paraconiothyrium estuarinum	1			X		
Penicillium allsoppiae	1	1			X	CN148E4
Penicillium adametzii	2	1			X	CN121E1, CN121E3
Penicillium annulatum	1	1			X	CN119A5
Penicillium brevicompactum	1	1			X	CN117E1
Penicillium cf camponotum	1	1			X	CN143C2
Penicillium cremeogriseum	18	5			X	CN113I5, CN113I6, CN113I8, CN114B1, CN114B2, CN114B3, CN114B4, CN114B5, CN114B6, CN117E4, CN119A8, CN119A9, CN121E2, CN121E4, CN121E5, CN121E6, CN148E5, CN148H1
Penicillium cf pole-evansii	4	4			X	CN143D7, CN143F7, CN152I5, CN153E3
Penicillium melinii	1	1			X	CN113I7
Penicillium pulvillorum	9	4			X	CN113H6, CN113H8, CN113H9, CN113I3, CN113I9, CN114A1, CN114A8, CN117E8, CN153D3
Penicillium cf pulvillorum	3	2			X	CN113I2, CN113I3, CN117E2
Penicillium raperi	13	4			X	CN113E9, CN113H5, CN113I4 CN114A7, CN121E7, CN121E8, CN121E9 CN131D5, CN131D8, CN131E1, CN143F9, CN143G1, CN143G2
Penicillium rubens	4	2			X	CN125H4, CN125H5, CN126A8, CN126A9

Penicillium subrubescens	2	2			X	CN119A6, CN143D2
Penicillium skrjabinii	4	3			X	CN118H2, CN118H3, CN143C1, CN152I6,
Penicillium onobense	8	4	X		X	CN104F6, CN104F8, CN113H7, CN117E6, CN119A4 CN143D3, CN143D4, CN152I8,
Penicillium ortum	15	4			X	CN113I1, CN114A6, CN114A9, CN117D5, CN117D6 CN117D7, CN117D8, CN117D9, CN117E3 CN117E7, CN148G7, CN148G9, CN148I7, CN148I8, CN148I9
Penicillium cf ortum	3	2			X	CN118H5, CN118H6, CN126B2
Penicillium cf restrictum	3	2			X	CN131E3, CN131D7, CN148F8,
Penicillium virgatum	1	1			X	CN143F6
Pennicillium spnov NN071679	4	2			X	CN118H4, CN118H7, CN143D4, CN143D5
Phialocephala sp	2	1		X		CN143C6, CN143C7
Pseudogymnoascus roseus	1	1		X		CN121G3
Purpureocillium lilacinum	4	2			X	CN110C6, CN113E3, CN113C4, CN143B6,
Staphylotrichum cf coccosporum	1	1		X		CN113C7
Stenocarpella maydis	1	1		X		CN113E6
Talaromyces purpureogenus	4	1			X	CN131D6, CN148E2, CN148E3, CN148E6

Talaromyces amestolkiae	1	1			X	CN125G5,
Talaromyces cf neorugulosus	5	1			X	CN125G6, CN125H6, CN126A5, CN126A6, CN125A7
Talaromyces pinophilus	6	1			X	CN125G7, CN125G8, CN125G9, CN125H1, CN125H2, CN125H3
Talaromyces soli	2	1			X	CN131D9, CN131E2
Talaromyces cf rugulosus	1	1		X	X	CN114A2
Talaromyces purpureogenus	1	1			X	CN131D6
Trichoderma afroharzianum	3	1	X			CN153C5, CN153C7, CN153C8,
Trichoderma amoenum	1		X			CN153D1
Trichoderma arundinaceum	1	1	X			CN148G7
Trichoderma gamsii	29	6	X			CN113G6, CN117F1, CN117F2, CN117F4, CN118I3, CN118I4, CN119B4, CN121D7, CN125H7, CN125H8, CN125H9, CN125I2, CN131E9, CN131G4, CN143D9, CN143E1, CN143G6, CN143G8, CN143G9, CN143H1, CN148E8, CN148E9, CN148F1, CN148F2, CN153B5, CN153B6, CN153B7, CN153B8, CN153C1
Trichoderma dorothopsis	2	1	X			CN118I1, CN118I2
Trichoderma hamatum	3	3	X			CN104F5, CN110C3, CN113G2
Trichoderma harzianum	4	3	X			CN117F3, CN131E4, CN131E5, CN143G4

Trichoderma koningiopsis	26	12	X			CN113F6, CN113G4, CN121D6, CN125I1, CN125I4, CN126B3, CN126B4, CN126B5, CN126B6, CN126B7, CN143A5, CN143A7, CN143B1, CN143B2, CN143B3, CN143B4, CN143E3, CN143E4, CN143E5, CN148G3, CN148G5, CN152I9, CN153A1, CN153A2, CN153A3, CN153C2
Trichoderma spnov	3			X		CN104F5, CN104F9, CN113F2
Trichoderma cf rifaii	13	8	X			CN121D2, CN121D8, CN131E6, CN131E7, CN131E8, CN131G3, CN143B5, CN143D8, CN143E2, CN143E6, CN143E7, CN143E8, CN148G4
Trichoderma cf hamatum	10	5	X			CN110D1, CN113F5, CN113F7, CN113F8, CN113F9, CN113G1, CN113G3, CN113G5, CN117F5, CN125I3,
Trichoderma cf koningii	2	1	X			CN119B3, CN119B5
Trichoderma subazureum	2	2	X			CN148G8, CN153B9,
Trichoderma virens	4	2	X			CN110C7, CN110C8, CN110C9, CN143A8
Tyrannosorus hystrioides	1	1		X		CN143E9
Umbelopsis vinacea	3	2		X		CN118I5, CN118I7, CN121G5

[1]X = DNA sequence generated.

[2]CN is the fungal working culture collection housed at FABI (Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

[3]CN-Oom is the collection of oomycetes.

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CHAPTER 3

PENICILLIUM VIRIDIGENUM PROV. NOM., A NEW SPECIES ISOLATED FROM MAIZE FARMS OF THE EASTERN CAPE OF SOUTH AFRICA.

ABSTRACT

The genus *Penicillium* is diverse and occurs worldwide with species that have a large economic impact on humans. During a survey studying fungal and oomycete diversity in soils collected at Eastern Cape maize farms, a new *Penicillium* species was found. *Penicillium viridigenum prov. nom*. is described using morphological characters and sequence data of the Internal Transcribed Spacer 5.8S rDNA region (ITS), β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit *(RPB2)* gene regions. The phylogenies placed the new species as a close relative of *P. pole-evansii* in the section *Canescentia* series *Atroveneta*. Colonies of the two species were found to be similar on Czapek yeast autolysate agar (CYA) where they produce large amounts of yellow exudates and soluble pigments. On Malt Extract Agar (MEA) the colonies were found to have floccose texture, dense sporulation and greyish-green conidia.

Keywords: DNA sequences, *Eurotiales*, multigene phylogeny, *Penicillium janczewskii,* polyphasic species concept.

INTRODUCTION

The genus *Penicillium* (*Eurotiomycetes, Eurotiales, Aspergillaceae*) is a well-known genus and one of the most common fungal genera that is often found in substrates like air, soil, indoor environments and food products (Visagie *et al.,* 2014). Many of its species are widely distributed around the world and have an important impact on human life in both industry and in the field of medicine. In addition to causing food spoilage as post-harvest pathogens, they also produce mycotoxins (Houbraken and Samson, 2022). *Penicillium* plays important ecological roles in nature, such as the recycling of nutrients and the decomposition of organic matter. Some species play an important role in the food industry where they are used for the manufacturing of cheese. For example, *Penicillium roqueforti* is used to make blue-veined cheese (Lund *et al.,* 1995). Some species are opportunistic pathogens, but others are used in the biotechnology industry to produce important chemicals (Houbraken and Samson, 2020). *Penicillium nalgiovense* and *Penicillium salamii* are both used as starter

cultures for sausage fermentation (Wang *et al.,* 2017). Some species have been reported as useful producers of enzymes for biotechnology and pharmaceutical purposes, also to produce antibiotics like penicillin and cholesterol-lowering agent lovastatin (Brian *et al.,* 1946; Laich *et al*., 2002; Shiono *et al.,* 2008; Houbraken and Samson, 2011). *Penicillium rubens* (former name*: Penicillium chrysogenum*) and *Penicillium nalgiovense are* important species well known to produce penicillin (Houbraken *et al.,* 2011; Laich *et al.,* 2002). Penicillin has made a huge impact in the field of medicine for the treatment of bacterial diseases (Thom, 1945). Furthermore, some *Penicillium* species are well known to produce organic acids and various enzymes that degrade different types of complex biomolecules (Geiser *et al.,* 2006, Pitt and Hocking 2009, Samson *et al.,* 2010). Most species in the section *Canescentia* can produce secondary metabolites which can play an important role in the soil for healthy development of plants (Visagie *et al*., 2021). As more and more genomes become available for groups like section *Canescentia*, it will be interesting to understand the potential metabolites that these species may produce (Grijseels *et al.,* 2016; Visagie *et al.,* 2023). With the wide range of impacts the genus can have on our lives, it is important to be able to accurately identify strains to species level.

Many soil fungal surveys around the world show that *Penicillium* is one of the most dominant fungal genera (Christensen *et al.,* 2000). A survey was conducted from soil in the fynbos region of South Africa, and it was discovered that the region is a hotspot for *Penicillium* diversity with 29 new species being described among 61 that were identified (Visagie *et al.,* 2021).

Members of *Penicillium* section *Canescentia* are found worldwide and are mostly found in soil and forest litter (Domsch *et al.,* 1980). The conidiophores of these species are terminally biverticillate, with subterminal branching and short, swollen phialides (Pitt, 1979; Houbraken and Samson, 2011). The section *Canescentia* is based mainly on *P. canescens* and P. *janczewskii* and includes species such as *P. antarcticum*, *P. atrovenetum* and *P. novae-zeelandiae* (Visagie *et al.,* 2021).

Recent studies on *Penicillium* have focused on building a strong and robust taxonomy (Visagie *et al.,* 2014, Houbraken *et al.,* 2020, Visagie, 2024). *Penicillium* falls under the phylum Ascomycota, Order *Eurotiales* and belongs phylogenetically to the family *Aspergilaceae*, currently containing 483 accepted species (Houbraken *et al.,* 2020).

Classification of *Penicillium* based on morphology was shown to be difficult due to various complexities and resulted in many misidentifications (Visagie *et al.,* 2021). As such, the phylogenetic species concept has become standard in *Penicillium* (Houbraken *et al*., 2020; Visagie *et al.,* 2024). South Africa is thought to be a biodiversity hotspot for *Penicillium* and many new species of *Penicillium* await descriptions from it (Visagie *et al.,* 2021). During a survey exploring the Eastern Cape maize soils, 18 species belonging to *Penicillium* were identified. One of these could not be identified at the time and we propose a new species below. We support this using multi-gene phylogenies and morphological comparisons with closely related species.

MATERIALS AND METHODS

Isolates

The strains used for this description were isolated from 22 maize-related soils sampling sites in the Eastern Cape province of South Africa. Other strains come from Free State, Mpumalanga and Western Cape provinces. Strains were deposited into the CN working collection of the Applied Mycology group at FABI and preserved as spore suspension in 10% glycerol at -80°C. Strains were also deposited into the CMW (the culture collection of the Forestry and Agricultural Biotechnology Institute) and CMW-IA (the culture collection of Innovation Africa), housed at FABI.

Morphological identifications

Macromorphological characters were assessed by inoculating strains in a three-point fashion onto Czapek yeast autolysate agar (CYA), malt extract agar (MEA: Oxoid CM0059), yeast extract sucrose agar (YES), Czapek yeast autolysate agar with 5% NaCl (CYAS), dichloran-18%-glycerol agar (DG18: Oxoid CM0729) creatine sucrose agar (CREA) and Oatmeal Agar (OA). Plates were prepared in 90 mm Petri dishes and incubated at 25°C for 7 d in the dark. Additional CYA plates were incubated at 10, 15, 20, 30 and 37°C for 7d. This follows the methods recommended by Visagie *et al.,* (2014b). After incubation, the plates were observed, and morphological characters were recorded. The colour codes and names used in the species description follow the Methuen Handbook of Colour (Kornerup and Wanscher, 1967). The colonies were captured with a Sony a6400 camera. Microscopic observations were made using the

Zeiss AXIO Imager. Affinity Photo v. 1.7.3 (Serif (Europe) Ltd, Nottingham, UK) was used to arrange the photos and create a photo plate.

DNA extraction, sequencing and phylogenetic analysis

DNA was extracted from 7d old colonies grown on MEA using the Fungal/Bacterial DNA kit (ZymoResearch, California, USA). The DNA was accessioned into the CN-DNA collection and stored at -20°C. The gene regions chosen for amplification were beta-tubulin (*BenA*), calmodulin (*CaM*)*,* Internal Transcribed Spacer rDNA region (ITS) and RNA polymerase II second largest subunit (*RPB2*). Polymerase Chain Reactions were performed for *ITS*, *BenA, CaM* and *RPB2* using primer pairs and amplification protocols as shown in Table 3.1 (Visagie *et al.,* 2014; Houbraken *et al.,* 2020). Each PCR consisted of 17.5 µl MilliQ water, 5.0 µl Bioline 5X MyTaq Reaction buffer (Bioline, England, UK), 0.15 µl BioLine MyTaq DNA polymerase (Bioline Ltd, USA Inc.), 0.5 µl 10 µM reverse and forward primers and 1.0 µl of the DNA template.

After PCR, the resulting products were assessed on a 1% agarose gel electrophoresis and examined under UV light. The products were then purified using ExoSap-IT PCR product clean-up reagent (Thermo Fischer Scientific, Massachusetts, USA) by setting up reactions containing 4 µl of PCR product and 7.5 µl of Exosap-IT reagent in strip tubes and subjecting them to a thermocycler at 37°C for 15 min and 85°C for 15 min. Afterwards, products were sequenced in both directions using the BigDye Terminator Sequencing kit v.3.1 (Applied Biosystems, Foster City, California, USA) with the same primers used for PCR amplification. The cycling conditions were as follows; initial denaturation temperature of 94°C for 5 min, followed by 40 cycles at 96°C for 30 s, 50°C for 10 s, and a final elongation step at 60°C for 4 min. Sequencing reactions were analysed at the University of Pretoria sequencing facility on an ABI PRISM 3500xL Auto-sequencer (Applied Biosystems, Foster City California, USA). Contigs were assembled and edited using Geneious Prime v. 2.1 2019. (BioMatter, Auckland, New Zealand).

A reference sequence dataset largely based on Houbraken *et al.,* 2020; Visagie *et al.,* 2021; Visagie *et al.,* 2023 was assembled having both ex-type and reference sequences of all accepted species belonging to *Penicillium* section *Canescentia* series *Atroveneta* (See table 3.2). The datasets were aligned in MAFT V. 7.490 (Katoh and Standley, 2013) using the G-INS-I option after which the sequences were manually

trimmed and adjusted. *P. janczewskii* was used as the outgroup. Single gene maximum likelihood (ML) trees were constructed in IQ-Tree v 1.6.12 (Nguyen *et al.,* 2015) and support in nodes were calculated using bootstrap analysis with 1000 replicates. After this, a multigene tree was generated with alignments first concatenated in FasconCAT-G and a concatenated tree constructed as described above. The most appropriate substitution model for each partition was selected using the integrated PartitionFinder v. 2.1.1 (Lanfer *et al.,* 2017). Trees were visualized using FigTree v1.4.4 (Rambaut, 2018) and edited on Affinity Publisher v1.10.4 (Serif, Europe) Ltd, Nottingham, UK).

RESULTS

Phylogenetic analysis

The alignments for *BenA, CaM,* ITS and *RPB2* datasets were respectively 360bp, 345bp, 464bp and 580bp long. ITS had a poor performance in identifying the new species. This is because ITS has insufficient variation to delineate several species of section *Canescentia* as well as in other groups, this has led to (Visagie *et al.,* 2014b) proposing that *BenA* be used as a secondary marker for identification for the genus *Penicillium*. *BenA, CaM* and *RPB2* performed well and were able to distinguish the new species from other known species having at least 2, 3, and 4 bp differences respectively. Single gene phylogenetic trees are shown in (Figure 3.1–3.5). The Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept was applied here, and it is the most used concept to delineate species in *Penicillium* taxonomic studies (Visagie *et al.,* 2016). The different gene regions resolved the strains in consistent clades concordant with each otherwhile they had a significant number of base pair differences. The new species shares the same ITS sequence with *P. nucicola*. Phylogenies consistently resolved *P. viridigenum* as a close relative of *P. pole-evansii* in series *Atroveneta.*

TAXONOMY

Penicillium viridigenum Mthembu, Van Vuuren, Yilmaz & Visagie, *prov. nom.*

Subgeneric classification: subgenus: *Penicillium*, section *Canescentia*, series *Atroveneta.*

Etymology. Latin*, viridigenum,* '*viridis*' meaning green and '*genum*' meaning production, named after the striking green pigment, produced by this species on oatmeal agar especially.

Typus: **South Africa**, Free State, Vrede, from soil, 2020, *N. Qikani,* CMW-IA 003483 (**holotype**, preserved as metabolically inactive culture, culture ex-type CMW-IA 003483 = CMW 59679 = CN069H6).

Material examined: **South Africa**, Eastern Cape, Humansdorp (-34.003599, 24.74712), from pasture mulch, May 2020, *C. Dewing, collected by A. Davis,* CMW-IA 002940 = CMW 61154 = CN049F8, CMW-IA 003488 = CMW 61454 = CN071D4. **South Africa**, Free State, Vrede, from soil, 2020, *N. Qikani,* CMW-IA 003484 = CMW 60007 = CN069I8. **South Africa**, Eastern Cape, Aberdeen (-32.387997, 24.217217), from soil, 21 April 2021, *C.M. Visagie*, CMW-IA 005958 = CMW 61977 = CN165B3, CMW-IA 005959 = CMW 61978 = CN165B4. **South Africa**, Eastern Cape, Middelburg (-31.837003, 24.860625), from soil, 21 April 2021, *C.M. Visagie*, CMW-IA 005960 = CMW 61979 = CN165B5, CMW-IA 005961 = CMW 61980 = CN165B6, CMW-IA 005962 = CMW 61981 = CN165B7, CMW-IA 005963 = CMW 61982 = CN165B8.

Colony diameters 25°C, 7d (in mm): CYA 24–33; CYA 30°C 20–27; CYA 37°C no growth; MEA 25–30; YES 33–38; DG18 24–33; CYAS 26–34; CREA 8–10.

Colony characters — CYA 25°C, 7 d: Colonies are moderately deep, radially sulcate, moderately crateriform; margins low to moderate, narrow, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–26D3); soluble pigments yellow; exudates yellow; reverse olive yellow to oak brown (3D6–5D6). MEA 25°C, 7d: Colonies moderately deep, lightly sulcate, raised at the centre; margins low to moderate, narrow, entire; margins entire; mycelia white; texture floccose; sporulation moderately dense; *conidia en masse* dull to greyish green (27D4–27D5, 25D4); soluble pigments absent; exudates yellow; reverse light brown to oak brown (5D6). YES 25°C, 7d: Colonies deep, radially sulcate, crateriform; margins deep, wide, entire; mycelia white; texture floccose; sporulation dense; *conidia en masse* dull to greyish green (25C); soluble pigments absent; exudates absent; reverse greyish to golden yellow (4B6–4C6). DG18 25°C, 7 d: Colonies low to moderately deep, slightly sulcate, slightly raised at the centre; margins low, narrow, entire; mycelia white; texture floccose; sporulation dense, *conidia en masse* dull green

(26D); soluble pigments absent; exudates absent; reverse yellow greyish to yellow (3B5–4B7). OA 25°C, 7 d: Colonies low, plane; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderately dense, *conidia en masse* greenish grey (26D2); soluble pigments green; exudates yellow. Strikingly green pigment is produced on oatmeal agar which is a unique character. CREA 25°C, 7 d: Acid not produced.

Micromorphology: Conidiophores biverticillate, minor proportion terverticillate, stipes rough, 80–142 x 2.5–4.5 µm; branches 20–65 x 2.5–3.5 µm; metulae divergent, 2–3 per stipe/branch, 6.5–12 x 2.5–4 µm; phialides ampulliform, 5.5–12 x 2.5–3.5 µm; average length phialide/ metulae 0.65; conidia rough, globose, 2–4 x 2–4 µm (1.6±0.5 x 1.6±0.5 µm), average width/length = 0.87, n=50.

Distinguishing characters: *Penicillium viridigenum* is morphologically similar and phylogenetically closely related to *P. pole-evansii* (Visagie *et al*., 2021). Colonies of the two species are similar on most media, producing large amounts of yellow exudates and soluble pigments on CYA, while they produce green pigments and green-yellow reverses on OA. Microscopically, these species are also identical. *Penicillium viridigenum* is thus introduced based on its unique DNA sequences.

DISCUSSION

Penicillium is a ubiquitous fungal genus that can easily be isolated from air and soil. Many reports show a high frequency and diversity of *Penicillium* in the soils from various climatic conditions and geographical regions of the world (Liang, 2020). Visagie *et al.,* 2014 hinted that many *Penicillium* still await discovery across South Africa.

Here we introduce *Penicillium viridigenum*, a species isolated from various substrates and regions of South Africa, including maize rhizosphere soils, pasture grass and pasture millet, in the Eastern Cape, Free State, Mpumalanga and Western Cape provinces. The species was found commonly across many substrates that were examined. The described species was isolated from maize rhizosphere soil from the Free State province. It will be interesting to see in the future what this species does and why it is so common.

Even though the new species is morphologically identical to *P. pole-evansii*, strains were resolved in distinct consistent clades, while they had a significant number of base pair differences (*BenA* = 2, *CaM* = 3 and *RPB2* = 4). *Penicillium* taxonomy has broadly adopted a phylogenetic species concept with GCPSR (Genealogical Concordance Phylogenetic Species Concept) (Visagie *et al.,* 2016) the most widely applied. With this approach, new species are being described at a rapid pace (Visagie *et al.* 2024) but due diligence is needed. In our case, we have collected many specimens from many locations and substrates that consistently had distinct sequences from its closest relative. However, comparisons are complicated by the fact that *P. pole-evansii* was described from a single isolate.

CONCLUSION

South Africa is very diverse with many new *Penicillium* waiting to be discovered. Here we described one of these based on its unique DNA sequences. The description of this new species through multi-gene sequence analysis contributes to the current sequence database available for *Penicillium* as a genus. The findings of the present study enhances to our understsnding of fungal diversity in maize soils of the Eastern Cape.

FIGURES AND TABLES

Table 3.1. Primers for sequence amplification used in the PCR reaction and sequencing.

Locus	PCR amplification profile	Primer pairs	Direction	Primer sequence (5'-3')	Reference
RNA polymerase II second largest subunit *(RPB2)*	Denaturation 94°C 5min; 30 cycles of 94°C 45sec; annealing at 55°C 45sec; 72°C 60sec; 72 °C 7min.	5F	Forward	GAY GAY MGW GAT CAY TTY GG	Liu *et al.,* 1999
		7CR	Reverse	CCC ATR GCT TGY TTR CCC AT	Liu *et al.,* 1999
Calmodulin *(CaM)*	Denaturation 94°C 5 min; 35 cycles of 94 °C 45 sec, annealing 55 °C 45 sec, 72°C 60sec; 72 °C 7 min.	CMD5	Forward	CCG AGT ACA AGG ARG CCT TC	Hong *et al.,* 2006
		CMD6	Reverse	CCG ATR GAG GTC ATR ACG TGG	Hong *et al.,* 2006
Internal Transcribed spacer 5.8S rDNA *(ITS)*	Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing 55°C 45sec; 72°C 60sec; 72°C 7 min.	V9G	Forward	TTA CGT CCC TGC CCT TTG TA	De Hoog and Gerrits van den Ende (1998)
		LS266	Reverse	GCA TTC CCA AAC AAC TCG ACT C	Masclaux *et al.,* (1995)

Internal Transcribed Spacer *(ITS)*	Denaturation 94°C 3 min; 35 cycles 94°C 60 sec; annealing at 55°C for 60 sec; 72°C for 60 sec; 72°C for 7 min.	ITS1	Forward	TCC GTA GGT GAA CCT GCG G	White *et al.,* 1990
		ITS4	Reverse	TCC TCC GCT TAT TGA TAT GC	White *et al.,* 1990
β-tubulin (*BenA*)	Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing at 55°C 45sec; 72°C 60sec; 72°C 7 min.	Bt2a	Forward	GGT AAC CAA ATC GGT GCT GCT TTC	Glass & Donaldson (1995)
		Bt2b	Reverse	ACC CTC AGT GTA GTG ACC CTT GGC	Glass & Donaldson (1995)

Table 3.2. NCBI GenBank accession numbers for *Penicillium* species in the section *Canescentia* series *Atroveneta* used for the phylogenetic analyses. [T]: ex-type strains.

Species name	Strains	GenBank:B*enA*	GenBank:*CaM*	GenBank:*ITS*	GenBank: *RPB2*
P. antarcticum	CBS100492[T]	MN969371	MN969236	KJ34503	JN406653
P. antarcticum	CBS116938	KP016925	KP016827	KP016845	KP016848
P. antarcticum	CBS116939	KP016921	JX157255	KP016829	KP016849
P. atrovenetum	CBS241.56[T]	JX140944	KJ867004	AF033492	JN121467
P. atrovenetum	CBS243.56	JX140945	MN969241	KP016835	JN121467
P. atrovenetum	NRRL2571	KJ775171	KJ775405	KJ775678	MN969116
P. janczewskii	CBS221.28[T]	KJ866967	KJ866998	KC411682	KP016853
P. coralligerum	CBS114.69	KJ866970	KJ866991	KP016836	KP016847
P. coralligerum	CBS123.65[T]	MN969378	MN969248	JN617667	JN406632
P. novae-zeelandiae	CBS137.41[T]	MN969390	MN969279	JN617688	JN406628
P. novae-zeelandiae	CV0042	JX140956	JX157352	JX140853	KP016864
P. nucicola	KAS2101	KT887807	KT887768	KT887846	OR146006
P. nucicola	KAS2203[T]	KT887821	KT887782	KT887860	MN969171
P. nucicola	CBS140987 [T]	KT887821	KT887782	KT887860	MN969171
P. pole-evansii	CBS138946[T]	JX141005	JX157412	JX140831	KP016911
Penicillium viridigenum	CN069H6=CMW59679=CMWIA003483 [T]	n.a.	n.a	n.a	n.a

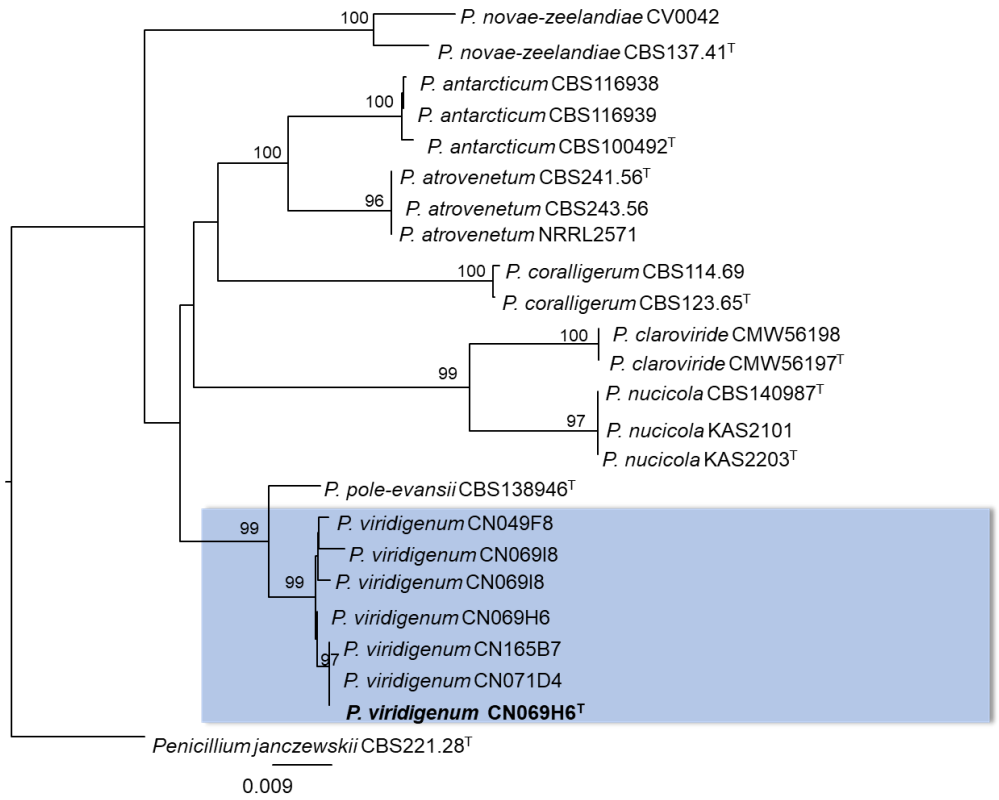

Figure 3.1. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on a concatenated dataset of *BenA, CaM,* ITS *and RPB2*. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^{T}= ex-type).

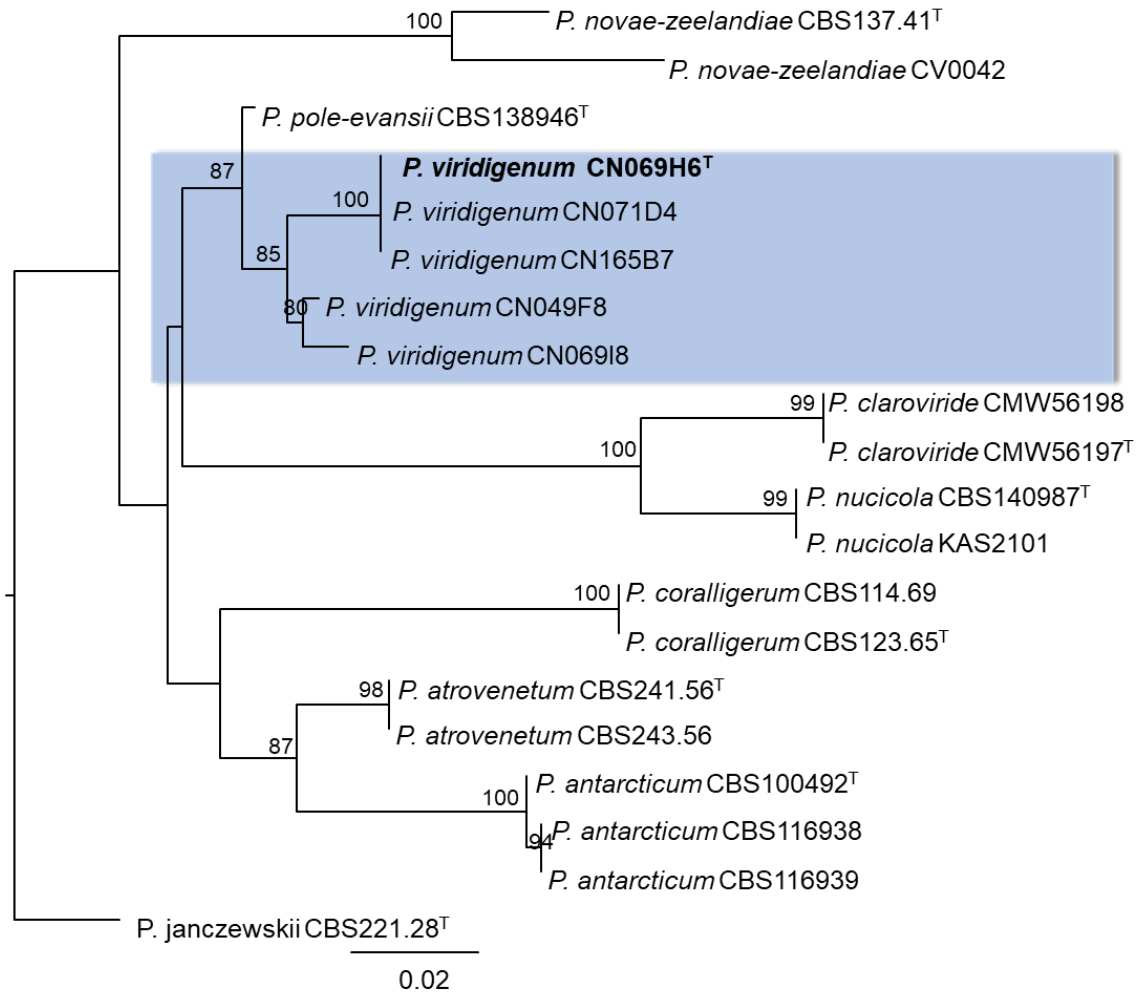

Figure 3.2. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on dataset of *BenA locus*. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^{T}= ex-type).

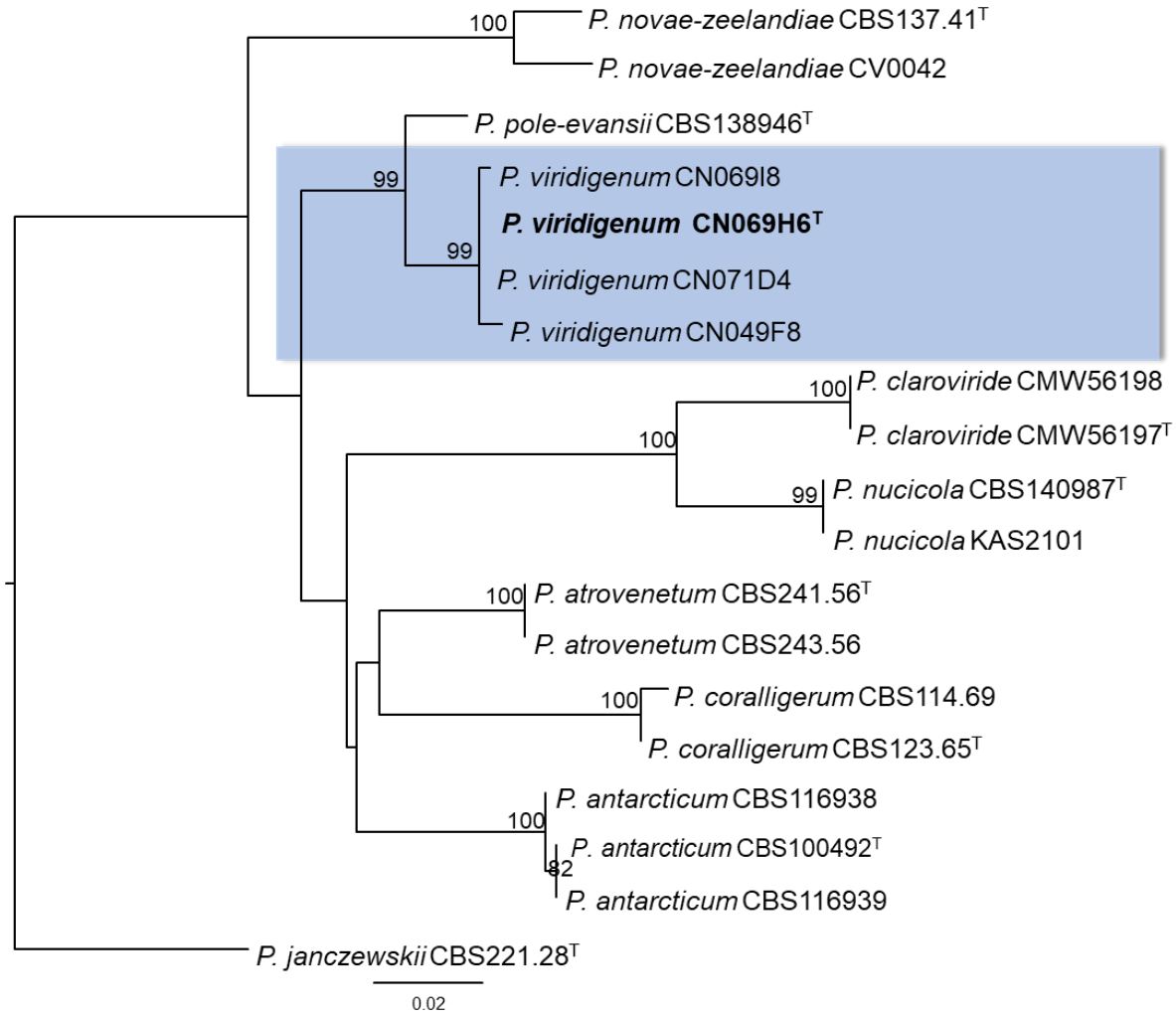

Figure 3.3. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on dataset of *CaM* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^{T}= ex-type).

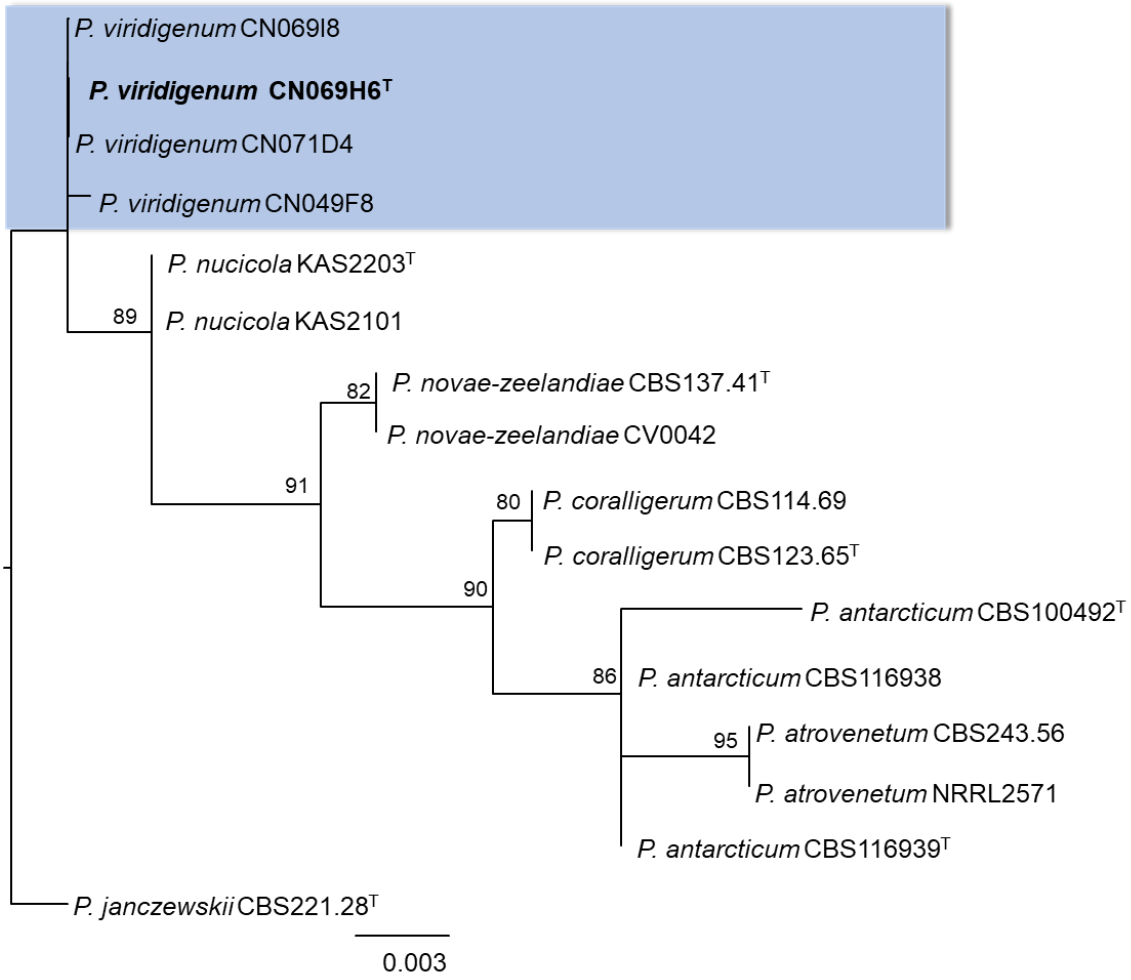

Figure 3.4. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on a dataset of *ITS* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^{T}= ex-type).

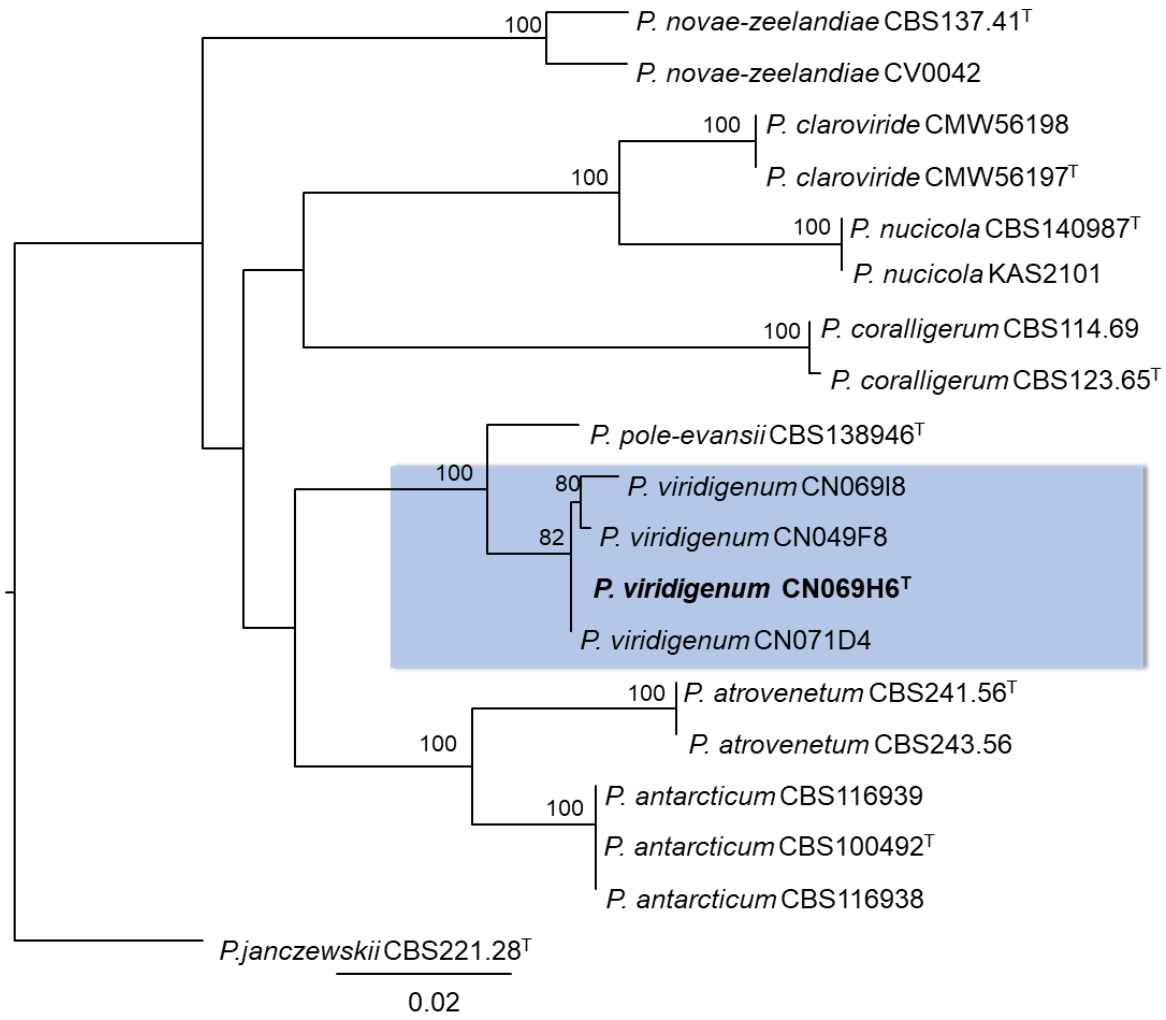

Figure 3.5. Maximum Likelihood Phylogenetic tree of *Penicillium* section *Canescentia* series *Atroveneta* based on a dataset *RPB2* locus. *Penicillium janczewskii* was chosen as the outgroup. The species name highlighted in blue represents the new species that was named *Penicillium viridigenum*. Bootstrap values higher than 80% are indicated on the branch nodes. (^{T}= ex-type).

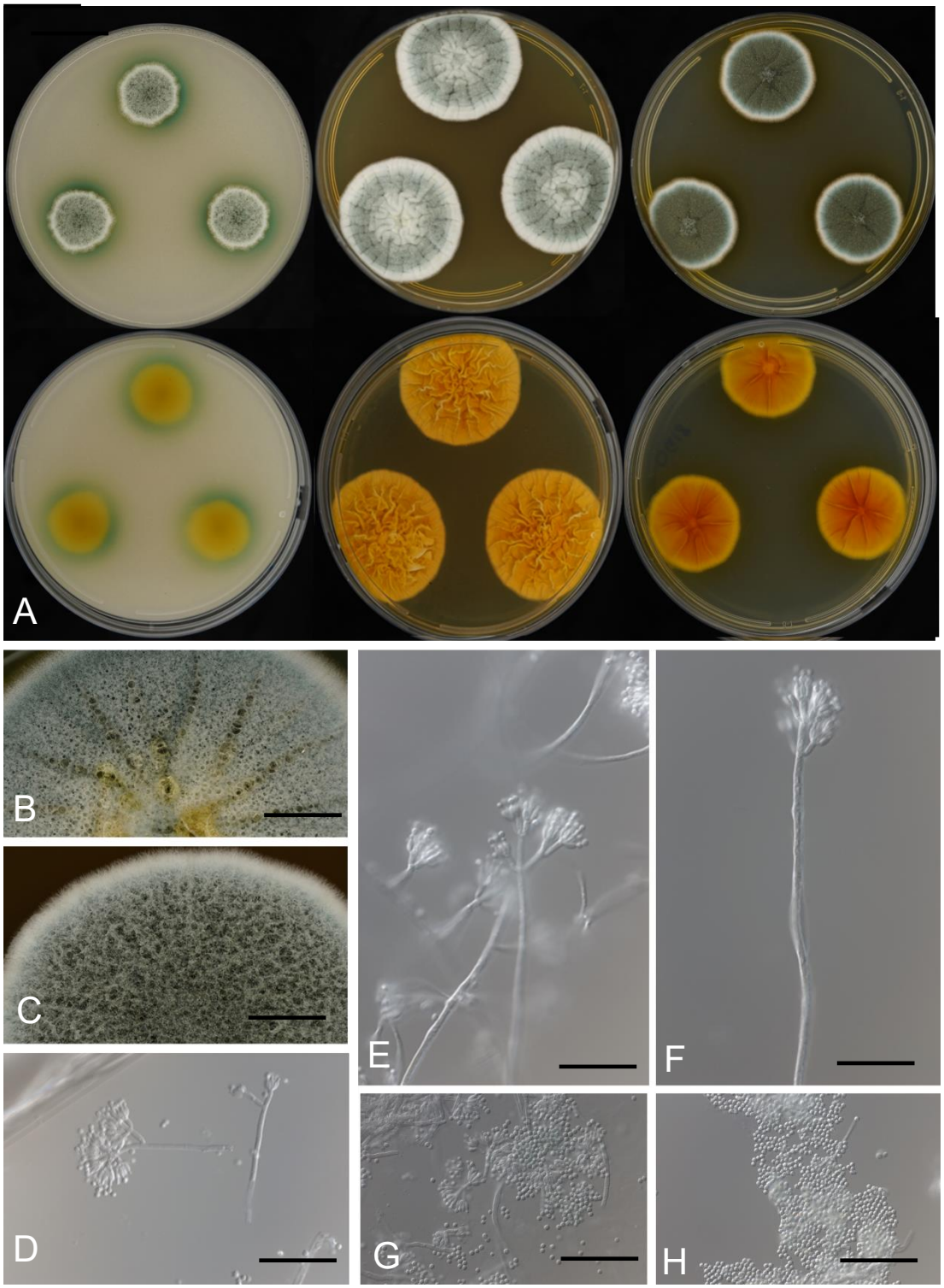

Figure 3.6. Morphological characters of *Penicillium viridigenum*; A. Colonies (top row, left to right: OA, MEA, CYA; bottom row, left to right: OA reverse, MEA reverse, CYA reverse); B, C. colony texture on MEA and CYA. D-F. conidiophores; G-H. conidia. Scale bars B-H = 10µm.

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