

Alice Mthembu

CHAPTER 2

FUNGAL AND OOMYCETE DIVERSITY IN EASTERN CAPE MAIZE FIELDS SOIL.

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 (<i>TEF</i>)	<i>Fusarium</i>	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 <i>et al.</i> , (1998)
			FusEF2	Reverse	GGARGTACCAGTSATCATG	O'Donnell <i>et al.</i> , (2015)
Translation Elongation Factor 1- α (<i>TEF</i>)	<i>Trichoderma Cladosporium</i>	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	CATCGAGAAGTTTCGAGAAGG	Carbone and Kohn, (1999)
			EF2	Reverse	GGARGTACCAGTSATCATGTT	O'Donnell <i>et al.</i> , (1998)
Internal Transcribed spacer 5.8S rDNA (<i>ITS</i>)	Fungi general (unknowns)	Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing 55°C 45sec; 72°C 60sec; 72°C 7 min.	V9G	Forward	TTACGTCCCTGCCCTTTGTA	De Hoog and Gerrits van den Ende (1998)
			LS266	Reverse	GCATTCCCAAACAACCTCGACTC	Masclaux <i>et al.</i> , (1995)

Internal Transcribed Spacer (<i>ITS</i>)	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	GCATTCCAAACAACCTCGACTC	
β -tubulin (<i>BenA</i>)	<i>Penicillium Talaromyces</i>	Denaturation 94°C 5min; 35 cycles 94°C 45sec; annealing at 55°C 45sec; 72°C 60sec; 72°C 7 min.	T10	Forward	GGTAACCAAATCGGTGCTGCTT TC	Glass & Donaldson (1995)
			Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTG GC	Glass & Donaldson (1995)

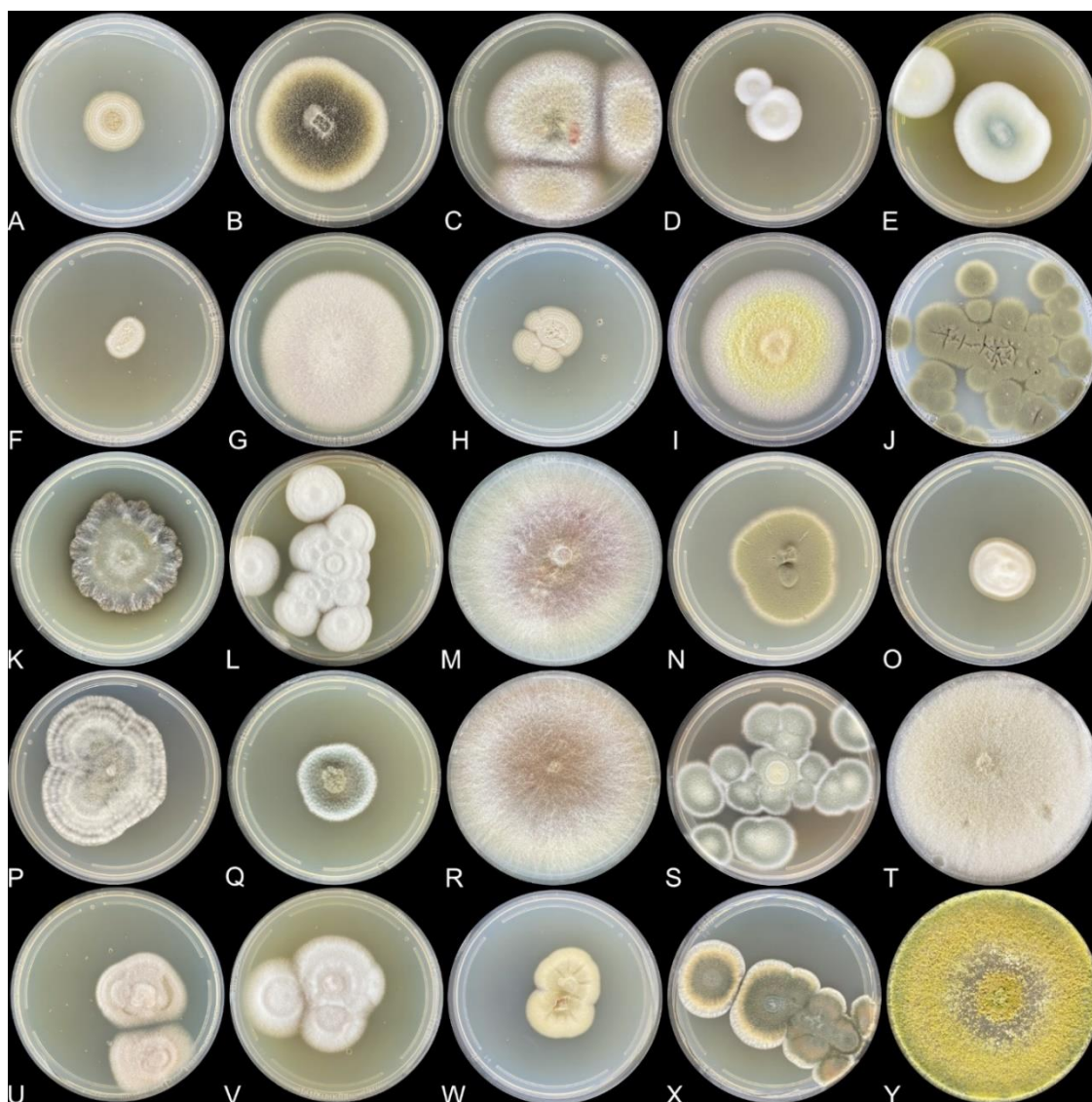


Figure 2.2. A representation of some of the genera isolated in the soil survey. A representation of the genera isolated. **A:** *Albifimbria verrucaria*; **B:** *Alternaria alternata*; **C:** *Arthrinium kogelbergense*; **D:** *Atractium stilbaster*; **E:** *Aspergillus udugawae*; **F:** *Beauveria amorpha*; **G:** *Beauveria 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:** *Microsphaeropsis 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*.

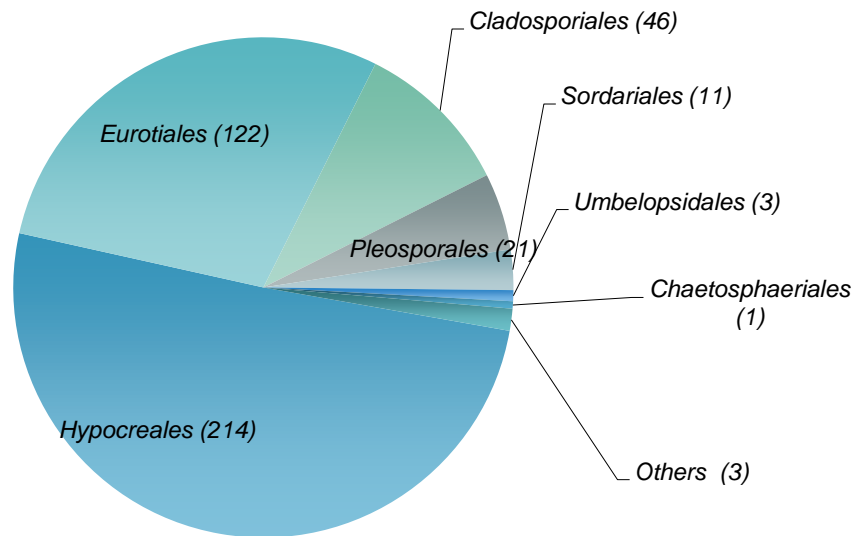


Figure 2.3. The Order that represents the strains obtained in the rhizosphere soil. The numbers in the brackets represent the number of fungal strains in the Order. “Others” include the order with one fungal strain isolated (*Diaporthales*, *Xylariales*, *Mucorales*, *Thelebolales*, *Venturiales*, *Chaetothyriales*).

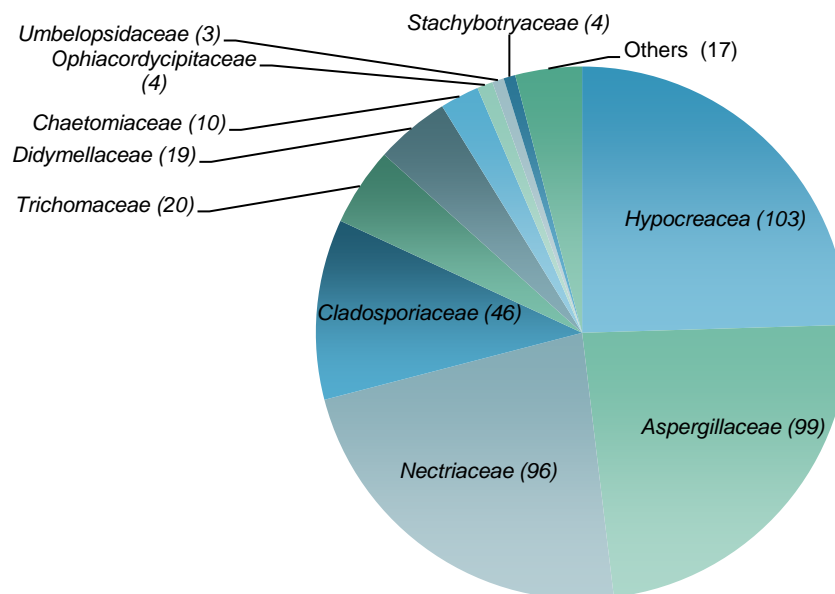


Figure 2.4. The number of strains identified at the family level. The numbers between the brackets show the number of strains in that family. “Others” include the families with only one fungal strain (*Pleiosporaceae*, *Diaporthaceae*, *Apiosporaceae*, *Cunninghamellaceae*, *Pseudeurotiaceae*, *Venturiaceae*,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

Clavicipitaceae, *Cordycipitaceae*, *Vibrissaceae*, *Didymosphaeriaceae*, *Chaetosphaeriaceae*).

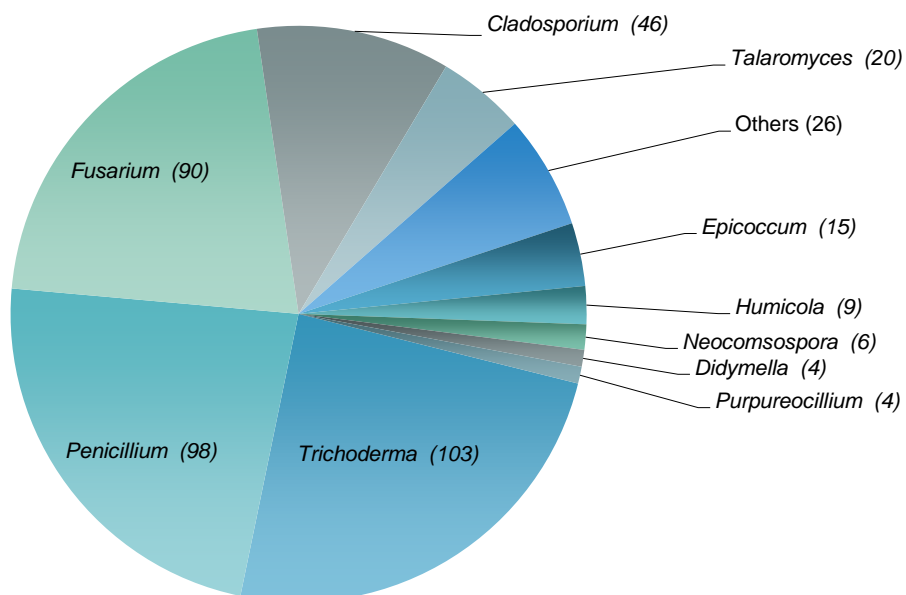


Figure 2.5. Genera found in the maize rhizosphere soils. The numbers in the brackets show the number of strains in the genus. “Others” include genera with less than 5 fungal strains (*Absidia*, *Albifimbria*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Beauveria*, *Chloridium*, *Chrysanthotrachum*, *Exophiala*,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

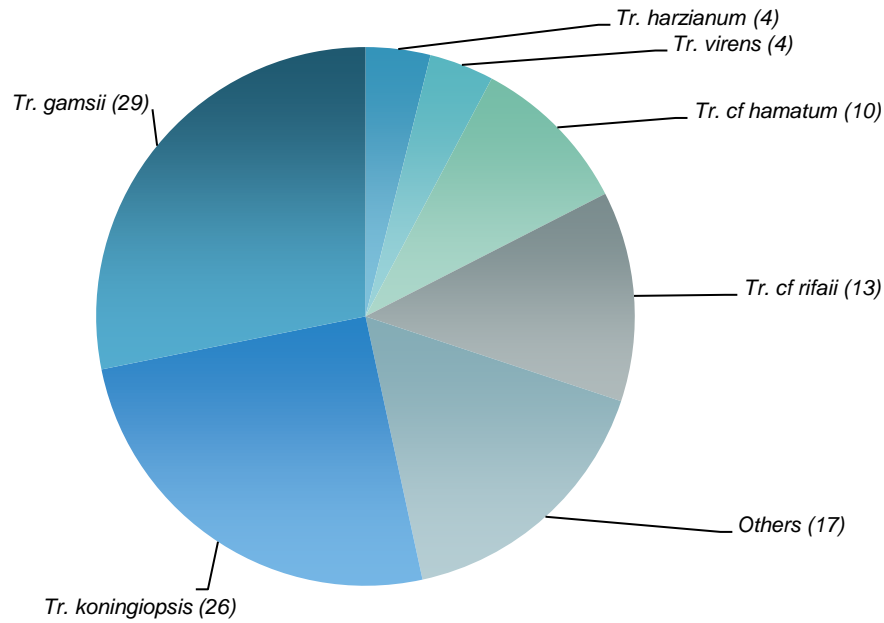


Figure 2.6. *Trichoderma* species identified from the maize rhizosphere soils. The numbers in the brackets show the number of strains obtained. “Others” include species with less than four fungal strains *Trichoderma amoenum*, *Trichoderma afroharzianum*, *Trichoderma arundinaceum*, *Trichoderma subazureum*,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

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*).

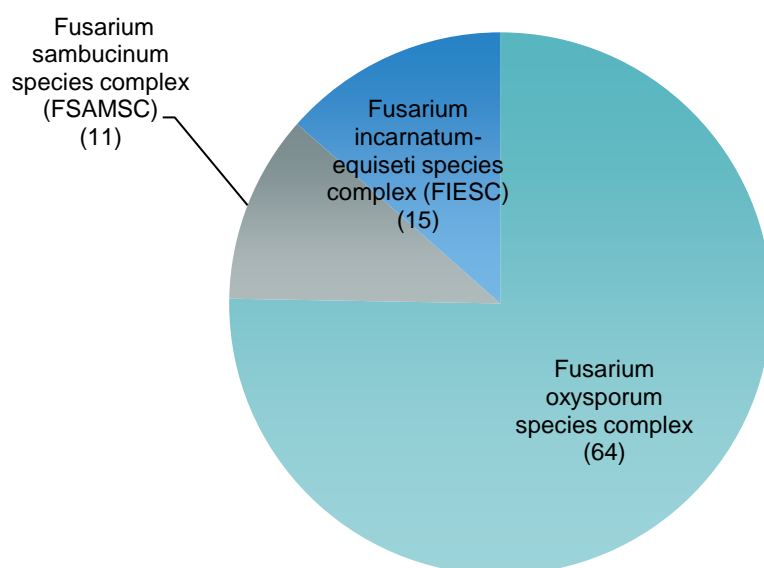


Figure 2.8. *Fusarium* species complexes. The numbers in the brackets show the number of strains in the species complex.

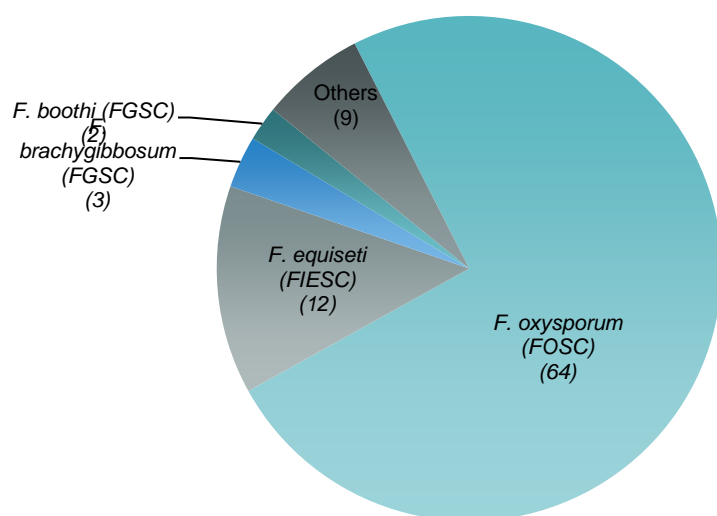


Figure 2.9. *Fusarium* species identified from the maize rhizosphere soils using DNA sequencing. The numbers in the brackets show the number of species obtained. The category others include species such as *F. clavum* (FIESC), *F. transvaalense* (FSAMSC), *F. inflexum* (FOSC), *F. venenatum* (FSAMSC),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

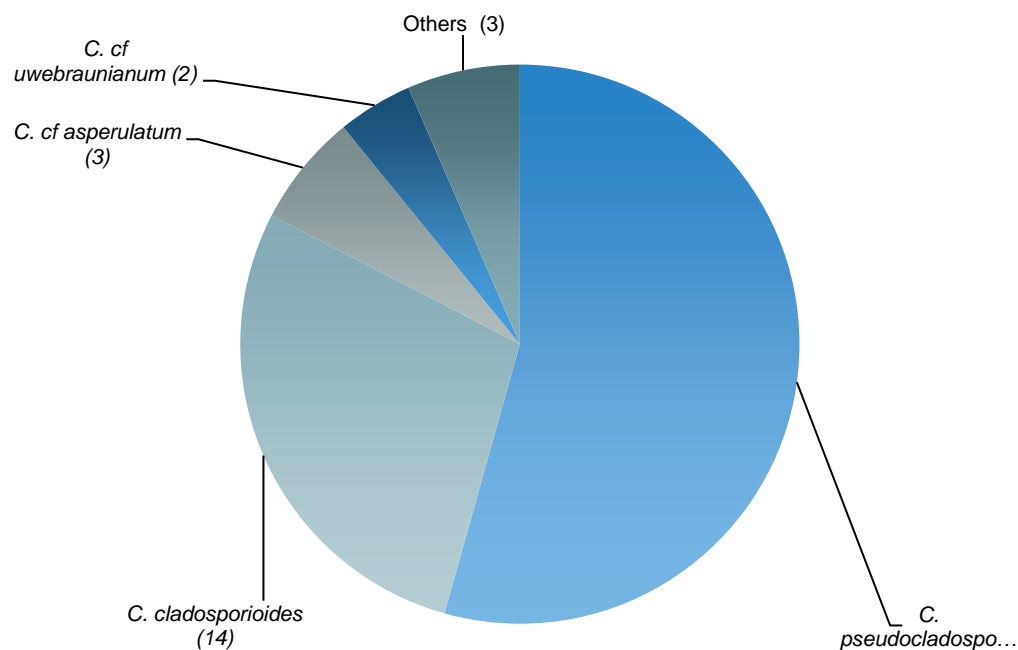


Figure 2.10. *Cladosporium* species identified from the rhizosphere soils. The numbers in brackets show the number of strains. The category “Others” includes *Cladosporium anthropophilum*, *Cladosporium chalastosporoides*, and *Cladosporium cf angustiterminale*.

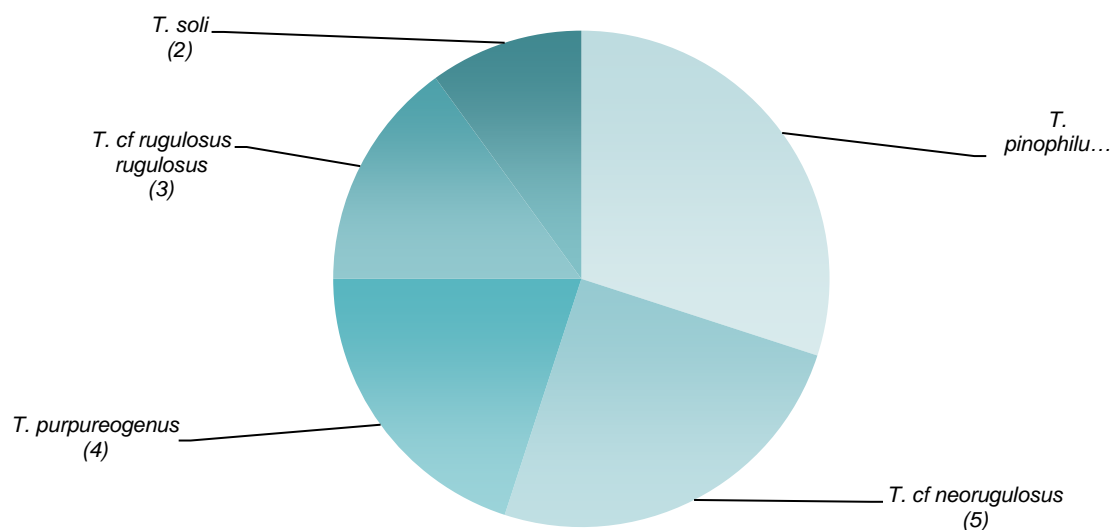


Figure 2.11. *Talaromyces* species identified from the rhizosphere soils. The numbers in brackets show the number of strains.

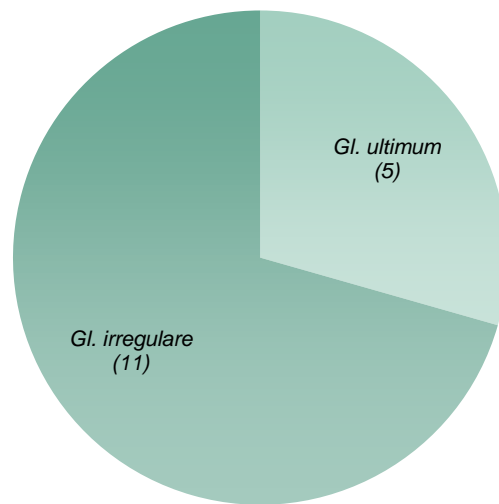


Figure 2.12. A graph representing oomycetes species in the *Globisporangium* genus. The number of strains is represented by the numbers in the brackets.

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
<i>Abisida cf cuneospora</i>	1	1		X ¹		CN113F3 ²
<i>Aspergillus udugawae</i>	1	1			X	CN117E9
<i>Alternaria alternata</i>	1	1		X		CN113C1
<i>Albifimbria verrucaria</i>	3	1		X		CN153A6, CN153A7, CN153B4
<i>Arthrinium kogelbergense</i>	1	1		X		CN113E7
<i>Unknown (Hypocreomycetidae)</i>	1	1		X		CN126D2
<i>Beauveria amorphia</i>	2	2		X		CN126D4, CN131F1
<i>Chloridium aseptatum</i>	2	1		X		CN153A9, CN153B3
<i>Chrysanthotrichum peruvianum</i>	1	1		X		CN121G2

<i>Cladosporium pseudocladosporoides</i>	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
<i>Cladosporium cf uwebraunianum</i>	2	2	X			CN113G7, CN121F5
<i>Cladosporium anthropophilum</i>	1	1	X			CN113G8
<i>Cladosporium cladosporiodes</i>	14	6	X			CN104F1, CN116F8, CN118F9, CN118G3, CN118I6, CN126B8, CN126B9, CN126C3, CN131F9, CN131G1, CN143C3, CN149A5, CN149A6, CN152I2
<i>Cladosporium chalastosporoides</i>	1		X			CN126C6
<i>Cladosporium cf asperulatum</i>	3	2	X			CN121F3, CN148F4, CN148F5,
<i>Cladosporium cf angustiterminale</i>	1	1	X			CN152I3
<i>Didymella prosopidis</i> <i>Didymella spnov</i>	4	2		X		CN113C2, CN 113C5, CN113D5, CN121F6
<i>Epicoccum italicum</i>	1	1		X		CN149B2
<i>Epicoccum cf keratinophilum</i>	1	1		X		CN113F4
<i>Epicoccum ovisporum</i>	1	1		X		CN119B9
<i>Epicoccum thailandicum</i>	1	1		X		CN131F2

<i>Epicoccum viticis</i>	11	2		X		CN113C8, CN113D1, CN113D2, CN113D3, CN113D6, CN113E1, CN119A1, CN119A2, CN119B6 CN119B7, CN119B8
<i>Exophiala pisciphila</i>	1	1		X		CN153B1
<i>Fusarium arcuatisporum</i> (FIESC)	1	1	X			CN104F7
<i>Fusarium brachygibbosum</i> (FSAMSC)	4	1	X			CN154D1, CN145D4, CN154D6, CN154D8
<i>Fusarium boothi</i> (FSAMSC)	2	2	X			CN125D7, CN125E8
<i>Fusarium cerealis</i> (FSAMSC)	1	1	X			CN142C4
<i>Fusarium clavum</i> (FIESC)	1	1	X			CN125C8
<i>Fusarium cf incarnatum</i> (FIESC)	1	1	X			CN125C9
<i>Fusarium equiseti</i> (FIESC)	10	6	X			CN142A9, CN142C9, CN142D1, CN142D4, CN142D5, CN142D8, CN149F8, CN149H1, CN143I3, CN153F1
<i>Fusarium graminearum</i> (FSAMSC)	1	1	X			CN142B2
<i>Fusarium inflexum</i> (FOSC)	2	1	X			CN125D9, CN149F5
<i>Fusarium oxysporum sensu lato</i> (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
<i>Fusarium transvaalense</i> (FSAMSC)	2	1	X			CN154D2, CN154D5
<i>Fusarium venenatum</i> (FSAMSC)	1	1	X			CN142C7
<i>Globisporangium irregulare</i>	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
<i>Globisporangium ultimum</i> var. <i>ultimim</i>	5	6		X		CN-Oom002F1, CN-Oom002F2, CN-Oom002F3, CN-Oom002F7, CN-Oom002C9
<i>Humicola veronae</i>	9			X		CN143H4, CN143H8, CN143I7, CN143I8, CN148F6, CN148F8, CN148F9, CN148G1, CN153E8
<i>Neocosmospora solani</i>	6			X		CN142B5, CN142B6, CN142C2, CN142C6, CN142D6, CN142F4
<i>Metapochonia bulbilosa</i>	1				X	CN121F2
<i>Metarhizium pinghaense</i>	1			X		CN153C3
<i>Paraconiothyrium spnov</i>	1				X	CN143C8, CN148D1

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

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

<i>Talaromyces amestolkiae</i>	1	1			X	CN125G5,
<i>Talaromyces cf neorugulosus</i>	5	1			X	CN125G6, CN125H6, CN126A5, CN126A6, CN125A7
<i>Talaromyces pinophilus</i>	6	1			X	CN125G7, CN125G8, CN125G9, CN125H1, CN125H2, CN125H3
<i>Talaromyces soli</i>	2	1			X	CN131D9, CN131E2
<i>Talaromyces cf rugulosus</i>	1	1		X	X	CN114A2
<i>Talaromyces purpureogenus</i>	1	1			X	CN131D6
<i>Trichoderma afroharzianum</i>	3	1	X			CN153C5, CN153C7, CN153C8,
<i>Trichoderma amoenum</i>	1		X			CN153D1
<i>Trichoderma arundinaceum</i>	1	1	X			CN148G7
<i>Trichoderma gamsii</i>	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
<i>Trichoderma dorotheopsis</i>	2	1	X			CN118I1, CN118I2
<i>Trichoderma hamatum</i>	3	3	X			CN104F5, CN110C3, CN113G2
<i>Trichoderma harzianum</i>	4	3	X			CN117F3, CN131E4, CN131E5, CN143G4

<i>Trichoderma koningiopsis</i>	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
<i>Trichoderma spnov</i>	3			X		CN104F5, CN104F9, CN113F2
<i>Trichoderma cf rifaii</i>	13	8	X			CN121D2, CN121D8, CN131E6, CN131E7, CN131E8, CN131G3, CN143B5, CN143D8, CN143E2, CN143E6, CN143E7, CN143E8, CN148G4
<i>Trichoderma cf hamatum</i>	10	5	X			CN110D1, CN113F5, CN113F7, CN113F8, CN113F9, CN113G1, CN113G3, CN113G5, CN117F5, CN125I3,
<i>Trichoderma cf koningii</i>	2	1	X			CN119B3, CN119B5
<i>Trichoderma subazureum</i>	2	2	X			CN148G8, CN153B9,
<i>Trichoderma virens</i>	4	2	X			CN110C7, CN110C8, CN110C9, CN143A8
<i>Tyrannosorus hystrioides</i>	1	1		X		CN143E9
<i>Umbelopsis vinacea</i>	3	2		X		CN118I5, CN118I7, CN121G5

¹X = DNA sequence generated.

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

³CN-Oom is the collection of oomycetes.

