

Chapter 2: Fungal diversity associated with maize agricultural soils collected in the Free State and North West provinces of South Africa

Figures

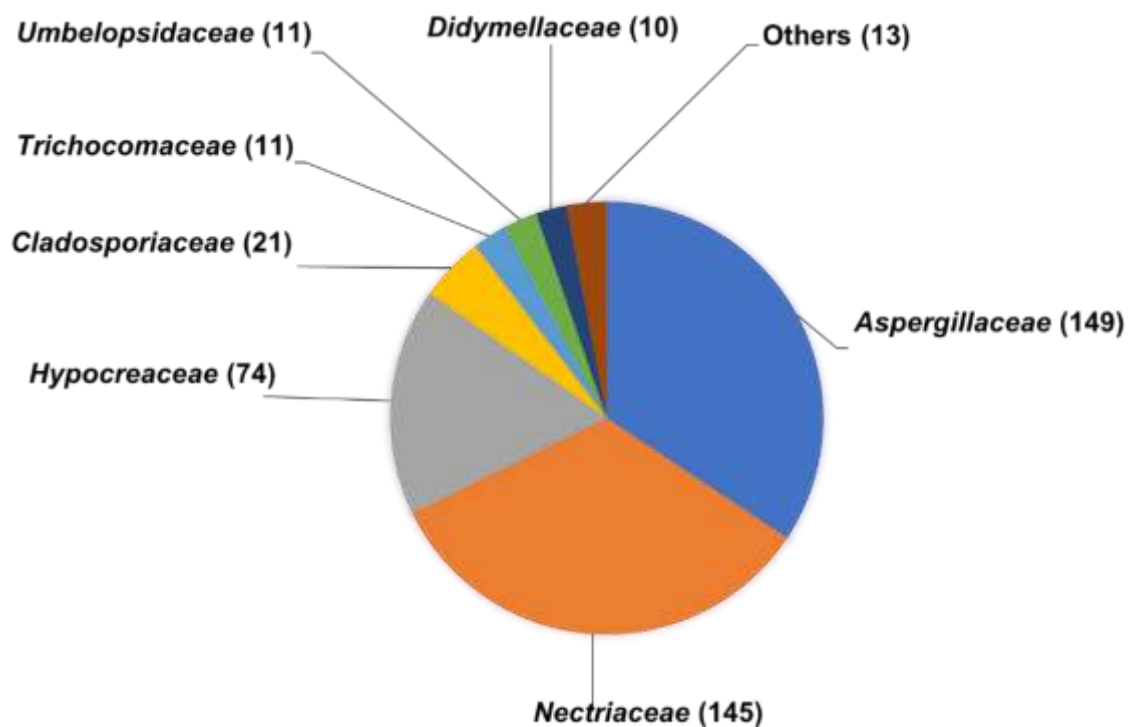


Figure 1: The proportion of fungal strains in maize soils identified to family-level. “Others” include families with less than ten fungal strains (*Aureobasidiaceae*, *Bionectriaceae*, *Chaetomiaceae*, *Clavicipitaceae*, *Cordycipitaceae*, *Cunninghamellaceae*, *Didymosphaeriaceae*, *Mortierellaceae*, *Myrmecridiaceae*, *Ophiocordycipitaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Stachybotryaceae*). Numbers between brackets represent the number of strains obtained for each family.

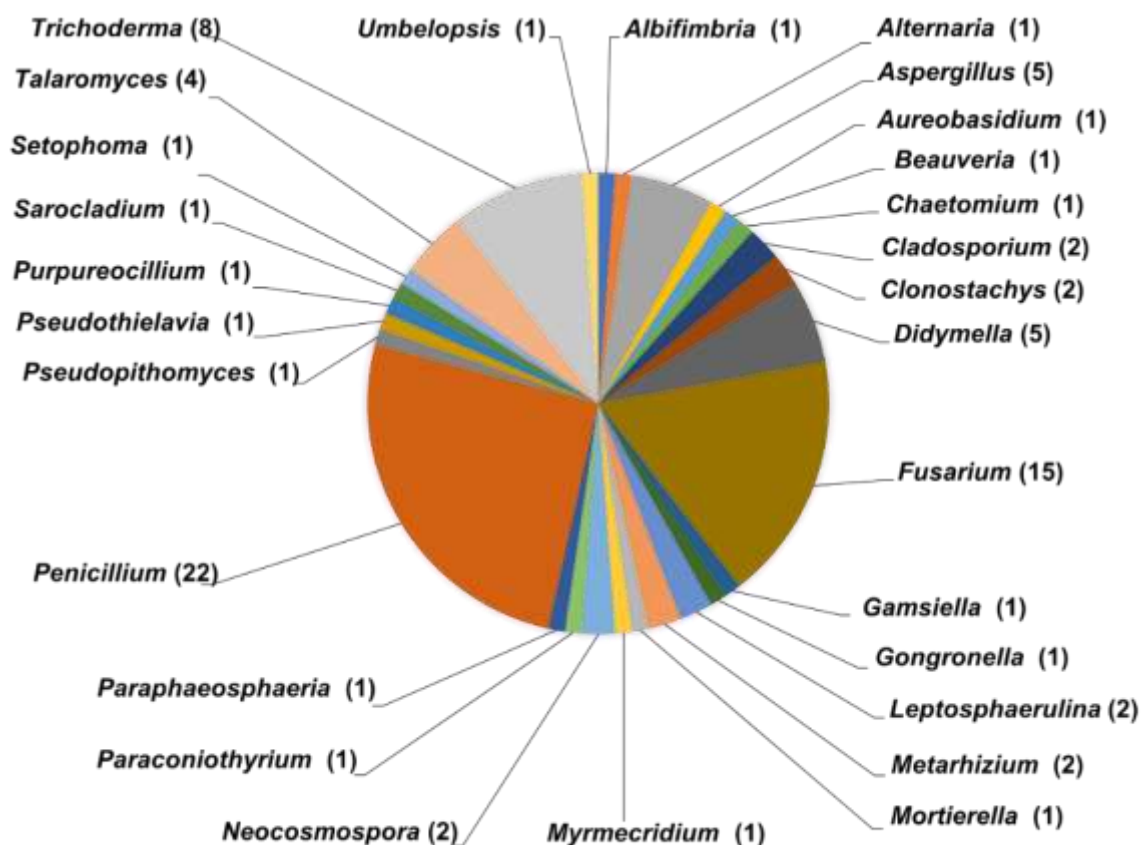


Figure 2: Genera that were found in maize agricultural soils. Numbers between brackets represent the number of species obtained for each genus.

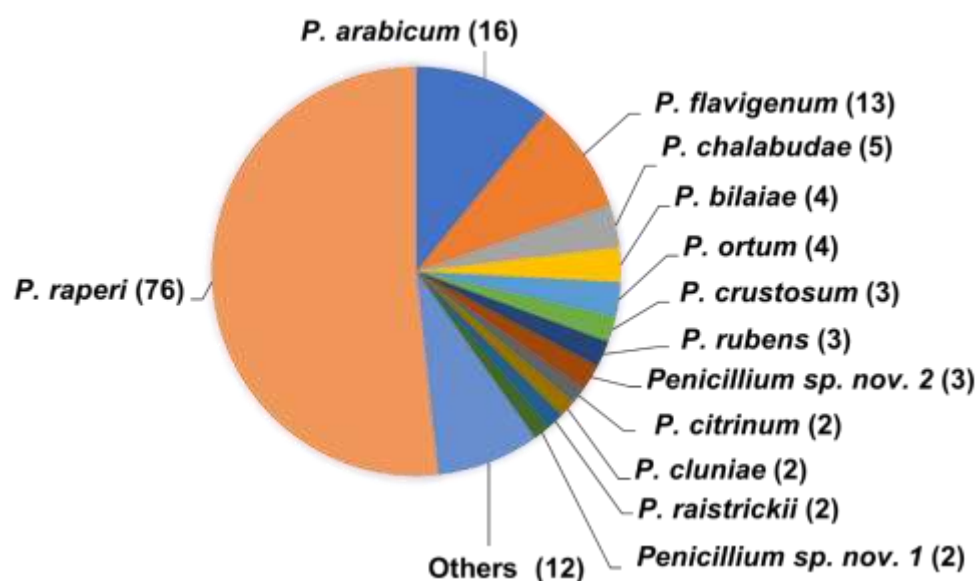


Figure 3: *Penicillium* species isolated from maize agricultural soils. Others = Species that had only one strain isolated (*Penicillium sp. nov. 3*, *P. atrovenetum*, *P. brevicompactum*, *P.*

desertorum, *P. frequentans*, *P. griseofulvum*, *P. murcianum*, *P. olsonii*, *P. sacculum*, *P. sizovae*, *P. striatisporum*, *P. yunnanense*). Numbers between brackets represent the number of strains obtained for each species.

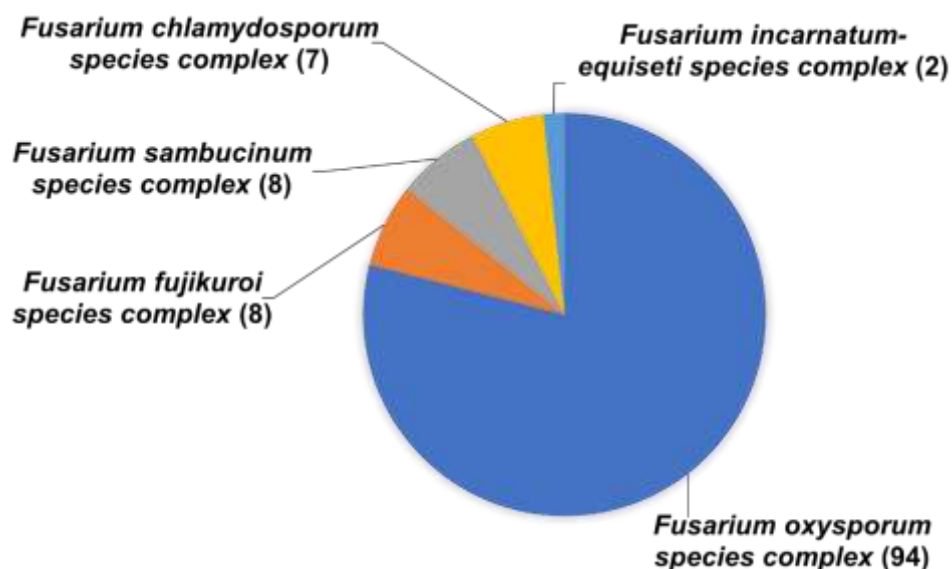


Figure 4: Species complexes isolated in maize agricultural soils. Numbers represent the number of strains obtained for each species complex.

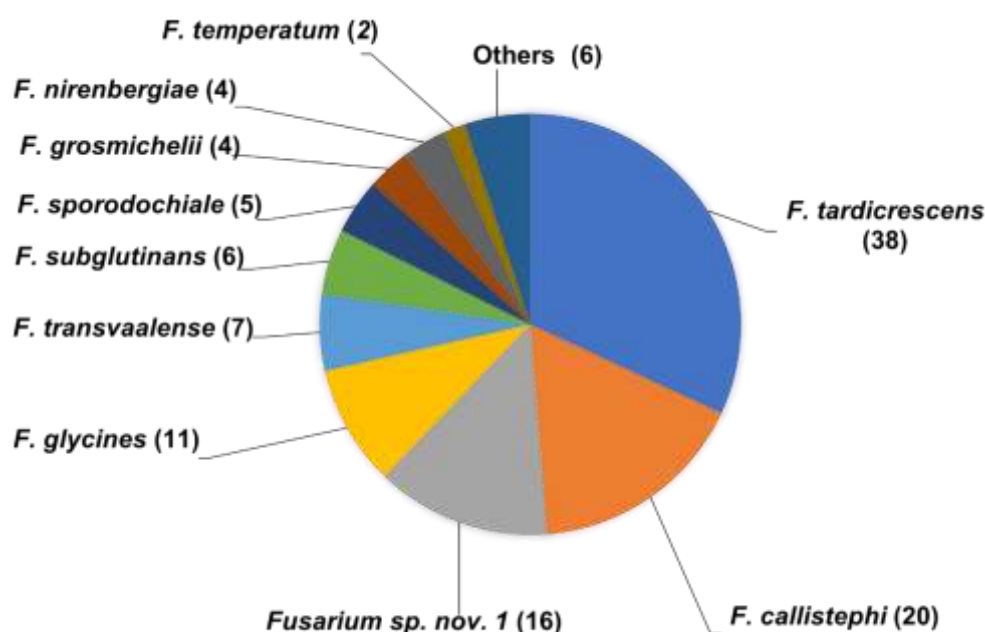


Figure 5: *Fusarium* species isolated in maize agricultural soils. “Other” include species that had only one strain isolated (*F. atrovinosum*, *F. boothii*, *F. chlamydosporum*, *F. clavus*, *F.*

equiseti, and *F. carminascens*). Numbers between brackets represent the number of strains obtained for each species.

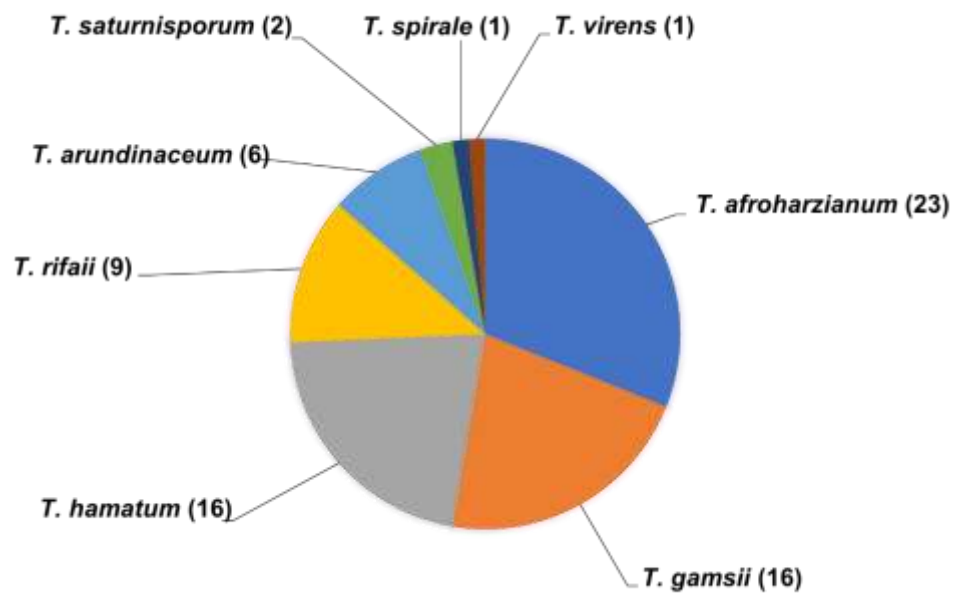


Figure 6: *Trichoderma* species isolated from maize agricultural soils. Numbers between brackets represent the number of strains obtained for each species.

Table 1: Primers used for PCR for different genera

Genus and marker (s)	Primer	Primer (5' to 3')	Reference
<i>Aspergillus</i>	CMD5	CCG AGT ACA AGG ARG CCT TC	(Hong <i>et al.</i> , 2006)
(<i>CaM</i>)	CMD6	CCG ATR GAG GTC ATR ACG TGG	(Hong <i>et al.</i> , 2006)
<i>Cladosporium</i>	EF1-728F	CATCGAGAAGTTCGAGAAGG	(Carbone & Kohn, 1999)
<i>Trichoderma</i>	EF2	GGARGTACCAGTSATCATGTT	(O'Donnell <i>et al.</i> , 1998)
(<i>tef1</i>)			
<i>Fusarium</i>	fusEF1	ATGGGTAAGGARGACAAGAC	(O'Donnell <i>et al.</i> , 1998)
(<i>tef1</i>)	fusEF2	GGARGTACCAGTSATCATGTT	(O'Donnell <i>et al.</i> , 1998)
<i>Penicillium</i>	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	(Glass & Donaldson, 1995)
(<i>tub2/BenA</i>)	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	(Glass & Donaldson, 1995)
Other genera	V9G	TTACGTCCCTGCCCTTTGTA	(De Hoog & Van den Ended, 1998)
(ITS)	LS266	GCATTCCCAAACAACCTCGACTC	(Masclaux <i>et al.</i> , 1995)

Table 2: Thermocycler protocols followed for the amplification of identification markers

DNA region	Primer pairs	PCR thermocycle protocol
<i>CaM</i>	CMD5; CMD6	94 °C 5 min; 35 cycles of 95 °C 45 sec, 55 °C 45 sec, 72 °C 60 s; 72 °C 7 min
<i>tub2/BenA</i>	Bt2a; Bt2b	94 °C 5 min; 35 cycles of 95 °C 45 sec, 55 °C 45 sec, 72 °C 60 sec; 72 °C 7 min
<i>tef1</i> (for <i>Fusarium</i>)	fusEF1; fusEF2	94 °C 5 min; 35 cycles of 94 °C 45 s, 52 °C 45 sec, 72 °C 90 sec; 72 °C 8 min
<i>tef1</i> (for <i>Cladosporium</i> and <i>Trichoderma</i>)	EF1-728F; EF2	94 °C 5 min; 35 cycles of 94 °C 45 sec, 52 °C 45 sec, 72 °C 90 s; 72 °C 8 min
ITS	V9G; LS266	94 °C 5 min; 35 cycles of 95 °C 45 sec, 55 °C 45 sec, 72 °C 60 sec; 72 °C 7 min

Table 3: Genera and fungal species that were identified in 19 maize agricultural farms and CN collection numbers allocated to strains that were preserved.

Species name	Number of strains	Isolation frequency	CN number
<i>Albifimbria</i>	1		
<i>Albifimbria verrucaria</i>	1	1/19	CN030B3
<i>Alternaria</i>	1		
<i>Alternaria alternata</i>	1	1/19	CN042F7
<i>Aspergillus</i>	9		
<i>Aspergillus calidoustus</i>	1	1/19	CN029G4
<i>Aspergillus dimorphicus</i>	1	1/19	CN033A5
<i>Aspergillus pseudodeflectus</i>	4	4/19	CN051D8, CN069G5, CN079D9, CN094H1
<i>Aspergillus terreus</i>	2	1/19	CN079E1, CN069C1
<i>Aspergillus tubingensis</i>	1	1/19	CN069B8
<i>Aureobasidium</i>	1		
<i>Aureobasidium melanogenum</i>	1	1/19	CN029C9
<i>Beauveria</i>	1		
<i>Beauveria bassiana</i>	1	1/19	CN032F8
<i>Chaetomium</i>	1		
<i>Chaetomium madrasense</i>	1	1/19	CN042G2

<i>Cladosporium</i>	21		
<i>Cladosporium cladosporioides</i>	7	5/19	CN029D7, CN033A7, CN033A8, CN033A9, CN041H9, CN070B7, CN094B4
<i>Cladosporium pseudocladosporioides</i>	14	8/19	CN029D6, CN033A6, CN033B1, CN033B4, CN033B6, CN042F6, CN069D1, CN069E8, CN070B5, CN070B8, CN078G7, CN078G8, CN078H1, CN079D2
<i>Clonostachys</i>	3		
<i>Clonostachys rhizophaga</i>	1	1/19	CN082A8
<i>Clonostachys rosea</i>	2	1/19	CN070C3, CN070D3
<i>Didymella</i>	7		
<i>Didymella dimorpha</i>	1	1/19	CN042H3
<i>Didymella glomerata</i>	1	1/19	CN079C9
<i>Didymella pedaeiae</i>	1	1/19	CN029I9
<i>Didymella prosopidis</i>	3	3/19	CN029D4, CN029G5, CN029G6
<i>Didymella subherbarum</i>	1	1/19	CN029C6
<i>Fusarium</i>	119		
<i>Fusarium atrovinosum</i> (FCSC)	1	1/19	CN038I2
<i>Fusarium boothii</i> (FSAMSC)	1	1/19	CN039E2
<i>Fusarium chlamydosporum</i> (FCSC)	1	1/19	CN038I4
<i>Fusarium callispethi</i> (FOSC)	20	8/19	CN038H8, CN038I3, CN039A3, CN039A4, CN039A9, CN039B6, CN039C3, CN039C7, CN039D8, CN051B5, CN051B6, CN051C2, CN051F7, CN081G1, CN081G6, CN081H9, CN081I1, CN081I7, CN082A2, CN082C4

<i>Fusarium carminascens</i> (FOSC)	1	1/19	CN081H8
<i>Fusarium clavus</i> (FIESC)	1	1/19	CN082B3
<i>Fusarium equiseti</i> (FIESC)	1	1/19	CN081G9
<i>Fusarium glycines</i> (FOSC)	11	5/19	CN039B1, CN081H7, CN038I8, CN038I9, CN039A1, CN039A2, CN039A5, CN039A6, CN039B4, CN051E8, CN081I3
<i>Fusarium grosmichellii</i> (FOSC)	4	3/19	CN038I6, CN039B5, CN081G3, CN081I6
<i>Fusarium nirenbergiae</i> (FOSC)	4	2/19	CN038H7, CN051F2, CN051F6, CN082B2
<i>Fusarium</i> sp. nov. 1 (FOSC)	16	6/19	CN038I7, CN039A7, CN039B3, CN039B9, CN039C4, CN039C6, CN039D4, CN039D6, CN039D9, CN039E1, CN051E5, CN081G4, CN081G5, CN081G8, CN081I8, CN082B7
<i>Fusarium sporodochiale</i> (FCSC)	5	2/19	CN039B7, CN039B8, CN051B9, CN051E4, CN051F3
<i>Fusarium subglutinans</i> (FFSC)	6	4/19	CN039E6, CN051C9, CN051D4, CN051D6, CN051F4, CN082B6,
<i>Fusarium tardicrescens</i> (FOSC)	38	11/19	CN038H6, CN038H9, CN038I1, CN039A8, CN039B2, CN039C1, CN039C2, CN039E3, CN039E4, CN051B7, CN051C1, CN051C3, CN051C4, CN051C5, CN051C6, CN051C7, CN051C8, CN051D1, CN051D3, CN051D5, CN051D7, CN051E2, CN051E6, CN051E7, CN051E9, CN081F9, CN081G2, CN081H3, CN081H5, CN081I2 CN081I5, CN082A1, CN082A3, CN082A4, CN082A6, CN082B5, CN082B8, CN082B9
<i>Fusarium temperatum</i> (FFSC)	2	1/19	CN081H4, CN081H6
<i>Fusarium transvaalense</i> (FSAMSC)	7	6/19	CN039C8, CN039C9, CN039D3, CN051B8, CN082A5, CN082B4, CN082C1
Gamsiella	4		
<i>Gamsiella stylospora</i>	4	2/19	CN033E8, CN033F8, CN094F3, CN094G7

Gongronella	1		
<i>Gongronella butleri</i>	1	1/19	CN094F8
Leptosphaerulina	3		
<i>Leptosphaerulina briosiana</i>	2	2/19	CN042H2, CN079A6
<i>Leptosphaerulina chartarum</i>	1	1/19	CN069C3
Metarhizium	3		
<i>Metarhizium anisopliae</i>	2	2/19	CN094G8, CN094H3
<i>Metarhizium pinghaense</i>	1	1/19	CN032G9
Mortierella	1		
<i>Mortierella alpina</i>	1	1/19	CN029I6
Myrmecridium	1		
<i>Myrmecridium schulzeri</i>	1	1/19	CN029G7
Neocosmospora	26		
<i>Neocosmospora falciformis</i>	2	2/19	CN038I5, CN081I4
<i>Neocosmospora solani</i>	24	15/19	CN038H5, CN039C5, CN039D1, CN039D2, CN039D5, CN039D7 CN039E5, CN039E7, CN051B2, CN051B3, CN051B4, CN051D2, CN051D9, CN051E1, CN051E3, CN051F5, CN081G7, CN081H1, CN081H2, CN082A7, CN082B1, CN082C2, CN082C3, CN082C5
Paraconiothyrium	6		
<i>Paraconiothyrium thysanolaenae</i>	6	3/19	CN069F1, CN069F8, CN070C6, CN070C7, CN070D2, CN078H3
Paraphaeosphaeria	1		

<i>Paraphaeosphaeria sporulosa</i>	1	1/19	CN078H5
<i>Penicillium</i>	147		
<i>Penicillium arabicum</i>	16	8/19	CN029C4, CN029F5, CN032G6, CN032H6, CN032H7, CN032H8, CN032I8, CN033A3, CN033A4, CN042B1, CN042E1, CN069G9, CN094C9, CN094E2, CN094E4, CN094E5
<i>Penicillium atrovenetum</i>	1	1/19	CN069H6
<i>Penicillium bilaiae</i>	4	3/19	CN029F7, CN032I3, CN042C6, CN094D9
<i>Penicillium brevicompactum</i>	1	1/19	CN033A2
<i>Penicillium chalabudae</i>	5	3/19	CN029C5, CN078F6, CN078G4, CN078I5, CN078I6
<i>Penicillium citrinum</i>	2	2/19	CN094D2, CN094E3
<i>Penicillium cluniae</i>	2	2/19	CN070C5, CN078H8
<i>Penicillium crustosum</i>	3	2/19	CN032F7, CN032H1, CN042B2
<i>Penicillium desertorum</i>	1	1/19	CN069B6
<i>Penicillium flavigenum</i>	13	6/19	CN029F6, CN029F8, CN029G2, CN029G3, CN042B3, CN042D1, CN042D4, CN042D8, CN042E6, CN042F2, CN069I5, CN070A5, CN078G3
<i>Penicillium frequentans</i>	1	1/19	CN079F3
<i>Penicillium griseofulvum</i>	1	1/19	CN069H7
<i>Penicillium murcianum</i>	1	1/19	CN069B4
<i>Penicillium olsonii</i>	1	1/19	CN029G1
<i>Penicillium ortum</i>	4	2/19	CN070A6, CN079E2, CN079E3, CN079E4

<i>Penicillium raistrickii</i>	2	1/19	CN042B4, CN042B7
<i>Penicillium raperi</i>	76	19/19	CN029C2, CN029C3, CN029C8, CN029H9, CN029I1, CN030A1, CN030A2, CN030A3, CN030A4, CN030A5, CN030A9, CN030B1, CN030B2, CN032F9, CN032G1, CN032G3, CN032G5, CN032G7, CN032G8, CN032H2, CN032H3, CN032H4, CN032H5, CN032H9, CN032I1, CN032I2, CN032I4, CN033A1, CN033E5, CN033F5, CN033G3, CN042C2, CN042F3, CN042G1, CN042G4, CN042G5, CN042G7, CN069A6, CN069B1, CN069F7, CN069F9, CN069G4, CN069H2, CN069H9, CN070D4, CN070D5, CN070D8, CN078G1, CN078I1, CN078I3, CN079A2, CN079A3, CN079A5, CN079B1, CN079B5, CN079B6, CN079B7, CN079F6, CN094C4, CN094C5, CN094C6, CN094C7, CN094C8, CN094D1, CN094D3, CN094D5, CN094D7, CN094D8, CN094E1, CN094E7, CN094E9, CN094F1, CN094F5, CN094F6, CN094H4
<i>Penicillium rubens</i>	3	3/19	CN032G2, CN042F4, CN070A2
<i>Penicillium sacculum</i>	1	1/19	CN070D9
<i>Penicillium sizovae</i>	1	1/19	CN029F9
<i>Penicillium striatisporum</i>	1	1/19	CN078H2
<i>Penicillium yunnanense</i>	1	1/19	CN079F2
<i>Penicillium</i> sp. nov. 1	2	1/19	CN069I8, CN078F7
<i>Penicillium</i> sp. nov. 2	3	1/19	CN070B2, CN079E8, CN079E9
<i>Penicillium</i> sp. nov. 3	1	1/19	CN069A9
<i>Pseudopithomyces</i>	2		
<i>Pseudopithomyces karoo</i>	2	2/19	CN069F2, CN079A8
<i>Pseudothielavia</i>	1		

<i>Pseudothielavia arxii</i>	1	1/19	CN042C9
<i>Purpureocillium</i>	1		
<i>Purpureocillium lilacinum</i>	1	1/19	CN070D7
<i>Sarocladium</i>	1		
<i>Sarocladium zeae</i>	1	1/19	CN030A7
<i>Setophoma</i>	3		
<i>Setophoma terrestris</i>	3	2/19	CN070E1, CN070E2, CN079A9
<i>Talaromyces</i>	11		
<i>Talaromyces pinophilus</i>	1	1/19	CN079B9
<i>Talaromyces purpureogenus</i>	6	5/19	CN032G4, CN032I5, CN032I6, CN079B2, CN094C1, CN094H2
<i>Talaromyces stollii</i>	3	2/19	CN032I7, CN032I9, CN042B5
<i>Talaromyces veerkampii</i>	1	1/19	CN094E6
<i>Trichoderma</i>	73		
<i>Trichoderma afroharzianum</i>	23	13/19	CN029B5, CN029B7, CN029E5, CN029E6, CN029F2, CN033C1, CN033C9, CN041H1, CN041I3, CN069D9, CN069E6, CN078I8, CN079C6, CN093H5, CN093H6, CN093H8, CN093I5, CN093I6, CN093I7, CN093I8, CN094A4, CN094A6, CN094B1
<i>Trichoderma arundinaceum</i>	6	4/19	CN078G5, CN079C2, CN093I9, CN094A1, CN094A2, CN094A3
<i>Trichoderma gamsii</i>	16	8/19	CN029B8, CN029C1, CN029E1, CN029E9, CN033C5, CN033C6, CN033D5, CN033D8, CN041G8, CN041I9, CN070B6, CN079C4, CN093G8, CN094A8, CN094A9, CN094B2

<i>Trichoderma hamatum</i>	16	6/19	CN029A9, CN029B2, CN029E4, CN041G1, CN041G2, CN041H3, CN041H5, CN093G5, CN093G6, CN093G7, CN093H1, CN093H2, CN093H3, CN093I2, CN093I3, CN093I4
<i>Trichoderma rifaii</i>	8	5/19	CN041G9, CN041I1, CN069C8, CN070B9, CN078G6, CN079C8 CN093H7, CN094A7
<i>Trichoderma saturnisporum</i>	2	1/19	CN093H9, CN093I1
<i>Trichoderma spirale</i>	1	1/19	CN029B3
<i>Trichoderma virens</i>	1	1/19	CN094A5
<i>Umbelopsis</i>	11		
<i>Umbelopsis vinacea</i>	11	5/19	CN029D2, CN029D3, CN029I4, CN029I5, CN033E6, CN033E7 CN033E9, CN033G4 CN042G6, CN070D6, CN079E7