Diversity of *Fusarium* species associated with healthy and malformed *Syzygium cordatum* inflorescences in South Africa

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Abstract

Syzygium cordatum (Myrtacease) is one of the most common encountered and widely distributed tree species indigenous to South Africa. This tree is often affected by a malformation disease characterized by grossly misshapen inflorescences that do not bear fruit. Because such symptoms have previously been attributed to *Fusarium* species in other plants, the aim of this study was to determine the diversity of *Fusarium* species associated with *S. cordatum* inflorescences. Healthy and malformed *S. cordatum* inflorescences were collected from Gauteng, Mpumalanga, Western Cape, and KwaZulu-Natal. A total of 118 *Fusarium* isolates were obtained from healthy (19) and malformed (99) inflorescences and identified using the translation elongation factor 1 alpha (*TEF1*α) gene region. The results revealed that 39 isolates belonged to the *Fusarium fujikuroi* species complex (FFSC), 45 isolates to the *Fusarium oxysporum* species complex (FOSC), 33 to the *Fusarium incarnatum-equiseti* species complex (FIESC) and one isolate resided in the *Fusarium chlamydosporum* species complex (FCSC). Phylogenetic analysis separated these isolates into 15 species, of which five (two in the FFSC, three in the FIESC) are new to science. No obvious patterns were found with respect to species recovered in different geographic areas sampled. However, FFSC species, were all recovered only from malformed inflorescences.

Keywords

Fusarium, *Syzygium cordatum*, malformation, species diversity

Declarations

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Conflicts of interest/Competing interests

To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, upon reasonable request. The sequence data generated was submitted to GenBank

Code availability

Not applicable

Authors' contributions

All authors conceived research.

R.I.M. conducted experiments.

All authors contributed material.

R.I.M., N.Y. and G.F. analyzed data and conducted statistical analyses.

R.I.M. wrote the manuscript.

E.T.S. secured funding.

All authors read, edited, and approved the manuscript.

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Introduction

Syzygium cordatum (family Myrtaceae) is a tree species indigenous to South Africa that commonly occurs along watercourses on forest margins and in swampy areas. The species is widely planted as an ornamental and used in rural areas for firewood and timber (Dlamini, 1981). In these areas, the fruits are also a source of food, while the bark is used in traditional medicines (Palgrave, 1977; Pooley, 1993; Van Wyk, 1997; 2011). Throughout its native range, as well as in urban areas, *S. cordatum* inflorescences are commonly malformed. These malformed structures are associated with the abnormal development of vegetative shoots and floral panicles that are excessively branched and characterized by unusually shortened and thickened rachises. These flowers are typically sterile, are unable to bear fruits or abort prematurely (Rani et al., 2013).

Various biotic and abiotic factors can cause flower malformation, often as a result of hormonal imbalances elicited by these factors (Kaur and Kaur, 2018). Some of the most important abiotic conditions that trigger malformation include exposure to temperature outside the plant's normal temperature range, drought stress, and nutrient deficiencies (Sim et al., 2004; Engin and Gokbayrak, 2010). Biotic factors resulting in malformation include damage caused by gall-forming insects (e.g., aphids, scale insects, and psyllids) or infection by nematodes and phytophagous mites (Sim et al., 2004). Additionally, infections by various fungi, bacteria, and viruses are also known to cause flower malformation, either as a result of infection or as an adaptive defense mechanism by the host plant (Shykoff and Kaltz, 1998).

Symptoms observed on *S. cordatum*, are similar to those associated with the so-called mango malformation disease (MMD) on *Mangifera indica* (Summanwar et al., 1966; Schlosser, 1971; Marasas et al., 2006). By making use of infection studies, a range of species in the fungal genus *Fusarium* has been identified as causal agents of MMD in various countries of Asia, Africa, the Americas, Europe, and the Middle East. These species include *F. mangiferae* (previously reported as *F. subglutinans*) (Freeman et al., 1999; Steenkamp et al., 2000; Britz et al., 2002; Ploetz et al.,

2002; Kvas et al., 2008; Liew et al., 2016), *F. sterilihyphosum* (Steenkamp et al., 2000; Britz et al., 2002), *F. mexicanum* (Otero-Colina et al., 2010), *F. tupiense* (Lima et al., 2012), *F. proliferatum* (Zhan et al., 2010; Liew et al., 2016) and *F. pseudocircinatum* (Freeman et al., 2014; Liew et al., 2016).

Fusarium represents a ubiquitous group of filamentous fungi within the class Ascomycetes. The genus is of significant importance, with estimates suggesting that at least 80% of economically important food crops are affected by pathogens in this group (Leslie and Summerell, 2006a). *Fusarium* species can infect plants at every stage of growth and can cause diseases such as seed, root, stalk, ear and kernel rot, seedling and head blight, as well as, cankers, wilts, and leaf diseases (Leslie and Summerell, 2006a; Summerell and Leslie, 2011). Some *Fusarium* species are also regarded as endophytes or saprotrophs (Summerell et al., 2010). Additionally, some species in this genus pose serious health risks to both humans and animals due to the role they play in producing a variety of toxic secondary metabolites, which can cause acute or chronic diseases (Desjardin, 2006).

The aim of this study was to determine the diversity of *Fusarium* species obtained from *S. cordatum* inflorescences. We hypothesized that several *Fusarium* species would be isolated from this indigenous plant species. We sampled and compared healthy and malformed *S. cordatum* inflorescences. This was done in order to understand which of these *Fusarium* species might contribute to the malformation disease, especially due to the similarity of malformed *S. cordatum* inflorescences to MMD.

Materials and Methods

Sample collection and fungal isolations

In 2008, malformed inflorescences were collected from *S. cordatum* trees at various locations across South Africa, including Gauteng, Mpumalanga, Western Cape, and KwaZulu-Natal (Table 1). Thereafter, a second and more comprehensive collection was made in 2016 from trees growing in KwaZulu-Natal (Table 1). During the 2016 survey, both healthy and malformed inflorescences were collected, with five malformed and one healthy inflorescence sampled per tree. In total, 15

Table 1 Origin and putative species of the isolates from *Syzygium cordatum* used in this study

From: Diversity of *Fusarium* species associated with healthy and malformed *Syzygium cordatum* inflorescences in South Africa

CMW isolate number	Collection date	Province	Location	Tissue[a]	FID Based ID[b]	%	Phylogeny Based ID	Species complex[c]	GenBank Accession Numbers (TEF1α)
CMW 55303	2016	KwaZulu Natal	Mzingazi	MI	*F. fujikuroi*	100	*F. fujikuroi*	FFSC	MZ966199
CMWF 1156	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	100	*F. fujikuroi*	FFSC	MZ966200
CMWF 1171	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	97,95	*F. fujikuroi*	FFSC	MZ966205
CMWF 1173	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	95,68	*F. fujikuroi*	FFSC	MZ966210
CMWF 894	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	99,69	*F. fujikuroi*	FFSC	MZ966209
CMWF 898	2008	Gauteng	Pretoria	MI	*F. fujikuroi*	100	*F. fujikuroi*	FFSC	MZ966206
CMWF 900	2008	KwaZulu Natal	Penington	MI	*F. fujikuroi*	100	*F. fujikuroi*	FFSC	MZ966204
CMW 55766	2016	KwaZulu Natal	Nyalazi	MI	*F.proliferatum*	100	*F. annulatum*	FFSC	MZ966202
CMWF 954	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	94,87	*F. fujikuroi*	FFSC	MZ966207
CMWF 902	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	100	*F. fujikuroi*	FFSC	MZ966203
CMWF 936	2008	KwaZulu Natal	Penington	MI	*F. fujikuroi*	99,7	*F. fujikuroi*	FFSC	MZ966208
CMWF 949	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	95,73	*F. fujikuroi*	FFSC	MZ966201
CMW 55298	2016	KwaZulu Natal	Mtubatuba	MI	*F. proliferatum*	99,39	*F. annulatum*	FFSC	MZ966183
CMW 55301	2016	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,04	*F. annulatum*	FFSC	MZ966193
CMW 55302	2016	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	95,3	*F. annulatum*	FFSC	MZ966196
CMW 55776	2016	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,04	*F. fujikuroi*	FFSC	MZ966184

CMWF 1000	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,39	*F. annulatum*	FFSC	MZ966191
CMWF 1009	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	98,61	*F. annulatum*	FFSC	MZ966189
CMWF 1132	2008	Mpumalanga	Buffelskloof	MI	*F. proliferatum*	96,12	*F. annulatum*	FFSC	MZ966192
CMWF 1133	2008	KwaZulu Natal	Richards bay	MI	*F. proliferatum*	99,09	*F. annulatum*	FFSC	MZ966197
CMWF 951	2008	Western Cape	Belville	MI	*F. proliferatum*	96,12	*F. annulatum*	FFSC	MZ966194
CMWF 965	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,72	*F. annulatum*	FFSC	MZ966198
CMWF 966	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	97,35	*F. annulatum*	FFSC	MZ966195
CMWF 976	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	98,95	*F. annulatum*	FFSC	MZ966190
CMWF 980	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,85	*F. annulatum*	FFSC	MZ966187
CMWF 990	2008	Gauteng	Pretoria	MI	*F. proliferatum*	97,5	*F. annulatum*	FFSC	MZ966186
CMWF 994	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,98	*F. annulatum*	FFSC	MZ966185
CMWF 999	2008	KwaZulu Natal	Kwambonambi	MI	*F. proliferatum*	99,53	*F. annulatum*	FFSC	MZ966188
CMWF 1029	2008	KwaZulu Natal	Kwambonambi	MI	*F. sacchari*	99,69	*F. sacchari*	FFSC	MZ966211
CMWF 1139	2008	KwaZulu Natal	Kwambonambi	MI	*F. verticillioides*	97,56	*F. verticillioides*	FFSC	MZ966218
CMW 55299	2016	KwaZulu Natal	Mtubatuba	MI	*F. verticillioides*	99,21	*Fusarium sp. nov.* 2	FFSC	MZ966212
CMW 55753	2016	KwaZulu Natal	Kwambonambi	MI	*F. verticillioides*	98,7	*Fusarium sp. nov.* 2	FFSC	MZ966213
CMW 55769	2016	KwaZulu Natal	Mzingazi	MI	*F. verticillioides*	97,8	*Fusarium sp. nov.* 2	FFSC	MZ966215
CMWF 1151	2008	Gauteng	Pretoria	MI	*F. verticillioides*	98,6	*Fusarium sp. nov.* 2	FFSC	MZ966214
CMWF 956	2008	KwaZulu Natal	Kwambonambi	MI	*F. verticillioides*	97	*Fusarium sp. nov.* 2	FFSC	MZ966217
CMWF 973	2008	KwaZulu Natal	Kwambonambi	MI	*F. verticillioides*	97,51	*Fusarium sp. nov.* 2	FFSC	MZ966216

CMW 55304	2016	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	97,1	*Fusarium sp. nov*. 1	FFSC	MZ966179
CMW 55305	2016	KwaZulu Natal	Dukuduku	MI	*F. fujikuroi*	96	*Fusarium sp. nov*. 1	FFSC	MZ966182
CMWF 924	2008	KwaZulu Natal	Kwambonambi	MI	*F. fujikuroi*	98,21	*Fusarium sp. nov*. 1	FFSC	MZ966181
CMW 55321	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	95,94	*F. oxysporum*	FOSC	MZ966263
CMWF 1048	2008	Western Cape	Belville	MI	*F. oxysporum*	99,33	*F. oxysporum*	FOSC	MZ966247
CMW 55765	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	99,84	*F. oxysporum*	FOSC	MZ966254
CMW 55767	2016	KwaZulu Natal	Dukuduku	HI	*F. oxysporum*	98,48	*F. oxysporum*	FOSC	MZ966261
CMWF 1055	2008	Western Cape	Belville	MI	*F. oxysporum*	92,5	*F. oxysporum*	FOSC	MZ966252
CMWF 938	2008	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	98,03	*F. oxysporum*	FOSC	MZ966257
CMWF 979	2008	Western Cape	Belville	MI	*F. oxysporum*	97,86	*F. oxysporum*	FOSC	MZ966258
CMWF 1036	2008	Western Cape	Belville	MI	*F. oxysporum*	99,19	*F. oxysporum*	FOSC	MZ966251
CMWF 1043	2008	Western Cape	Belville	MI	*F. oxysporum*	97,95	*F. oxysporum*	FOSC	MZ966259
CMWF 955	2008	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	99,7	*F. oxysporum*	FOSC	MZ966255
CMW 55316	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	98,54	*F. oxysporum*	FOSC	MZ966253
CMWF 985	2008	Western Cape	Belville	MI	*F. oxysporum*	95,55	*F. oxysporum*	FOSC	MZ966262
CMW 55313	2016	KwaZulu Natal	Mzingazi	HI	*F. oxysporum*	99,83	*F. oxysporum*	FOSC	MZ966260
CMW 55314	2016	KwaZulu Natal	Mzingazi	HI	*F. oxysporum*	99,55	*F. oxysporum*	FOSC	MZ966249
CMW 55759	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	95,39	*F. oxysporum*	FOSC	MZ966256
CMW 55311	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	99,81	*F. oxysporum*	FOSC	MZ966248
CMW 55319	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	94,67	*F. oxysporum*	FOSC	MZ966250
CMWF 1058	2008	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	98,96	*F. oxysporum*	FOSC	MZ966232
CMW 55312	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	98,27	*F. oxysporum*	FOSC	MZ966231

CMW 55773	2016	KwaZulu Natal	Dukuduku	HI	*F. oxysporum*	94,31	*F. oxysporum*	FOSC	MZ966243
CMW 55318	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	97,88	*F. oxysporum*	FOSC	MZ966233
CMW 55324	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	98,63	*F. oxysporum*	FOSC	MZ966244
CMW 55778	2016	KwaZulu Natal	Mzingazi	HI	*F. oxysporum*	99,53	*F. oxysporum*	FOSC	MZ966222
CMW 55317	2016	KwaZulu Natal	Nyalazi	MI	*F. oxysporum*	97,46	*F. oxysporum*	FOSC	MZ966228
CMW 55758	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	98,43	*F. oxysporum*	FOSC	MZ966227
CMW 55756	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	98,25	*F. oxysporum*	FOSC	MZ966221
CMW 55323	2016	KwaZulu Natal	Dukuduku	MI	*F. oxysporum*	98,48	*F. oxysporum*	FOSC	MZ966245
CMW 55763	2016	KwaZulu Natal	Mtubatuba	MI	*F. oxysporum*	97,22	*F. oxysporum*	FOSC	MZ966229
CMW 55760	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	96,03	*F. oxysporum*	FOSC	MZ966236
CMW 55757	2016	KwaZulu Natal	Mtubatuba	MI	*F. oxysporum*	99,7	*F. oxysporum*	FOSC	MZ966226
CMW 55315	2016	KwaZulu Natal	Dukuduku	HI	*F. oxysporum*	99,7	*F. oxysporum*	FOSC	MZ966220
CMW 55307	2016	KwaZulu Natal	Mtubatuba	MI	*F. oxysporum*	99,7	*F. oxysporum*	FOSC	MZ966239
CMW 55322	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	98,05	*F. oxysporum*	FOSC	MZ966224
CMW 55764	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	98,48	*F. oxysporum*	FOSC	MZ966240
CMWF 1019	2008	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	98,72	*F. oxysporum*	FOSC	MZ966223
CMWF 1057	2008	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	97,73	*F. oxysporum*	FOSC	MZ966230
CMWF 1046	2008	Western Cape	Belville	MI	*F. oxysporum*	98,67	*F. oxysporum*	FOSC	MZ966225
CMW 55768	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	92,57	*F. oxysporum*	FOSC	MZ966241

CMW 55777	2016	KwaZulu Natal	Nyalazi	MI	*F. oxysporum*	99,85	*F. oxysporum*	FOSC	MZ966237
CMW 55320	2016	KwaZulu Natal	Mzingazi	MI	*F. oxysporum*	93,47	*F. oxysporum*	FOSC	MZ966238
CMW 55771	2016	KwaZulu Natal	Mtubatuba	MI	*F. oxysporum*	97,33	*F. oxysporum*	FOSC	MZ966246
CMW 55309	2016	KwaZulu Natal	Mzingazi	HI	*F. oxysporum*	99,4	*F. oxysporum*	FOSC	MZ966234
CMW 55774	2016	KwaZulu Natal	Mtubatuba	MI	*F. oxysporum*	93,04	*F. oxysporum*	FOSC	MZ966242
CMW 55310	2016	KwaZulu Natal	Kwambonambi	MI	*F. oxysporum*	93,2	*F. oxysporum*	FOSC	MZ966235
CMW 55306	2016	KwaZulu Natal	Mzingazi	HI	*F. oxysporum*	93,67	*F. oxysporum*	FOSC	MZ966219
CMW 55738	2016	KwaZulu Natal	Kwambonambi	MI	*F. equiseti*	93,8	*Fusarium sp. nov.* 3	FIESC	MZ966178
CMW 55737	2016	KwaZulu Natal	Kwambonambi	MI	*F. scirpi*	88	*Fusarium sp. nov.* 3	FIESC	MZ966177
CMW 55736	2016	KwaZulu Natal	Kwambonambi	MI	*F. camptocera*	92,67	*Fusarium sp. nov.* 3	FIESC	MZ966174
CMW 55742	2016	KwaZulu Natal	Kwambonambi	HI	*F. equiseti*	92,22	*Fusarium sp. nov.* 3	FIESC	MZ966175
CMW 55743	2016	KwaZulu Natal	Kwambonambi	HI	*F. caatingaense*	90,8	*Fusarium sp. nov.* 3	FIESC	MZ966176
CMW 55735	2016	KwaZulu Natal	Kwambonambi	MI	*F. caatingaense*	96,502	*Fusarium sp. nov.* 4	FIESC	MZ966155
CMW 55739	2016	KwaZulu Natal	Kwambonambi	MI	*F. caatingaense*	96,05	*Fusarium sp. nov.* 4	FIESC	MZ966156
CMWF 1056	2008	Western Cape	Belville	MI	*F. guilinense*	95,95	*Fusarium sp. nov.* 5	FIESC	MZ966153
CMW 55747	2016	KwaZulu Natal	Kwambonambi	MI	*F. guilinense*	96,75	*Fusarium sp. nov.* 5	FIESC	MZ966152
CMWF 1031	2008	Western Cape	Hartenbos	MI	*F. guilinense*	98,98	*Fusarium sp. nov.* 5	FIESC	MZ966150
CMWF 1017	2008	KwaZulu Natal	Kwambonambi	MI	*F. guilinense*	98,67	*Fusarium sp. nov.* 5	FIESC	MZ966151
CMW 55745	2016	KwaZulu Natal	Mtubatuba	HI	*F. guilinense*	91,49	*Fusarium sp. nov.* 5	FIESC	MZ966149

CMW 55329	2016	KwaZulu Natal	Kwambonambi	HI	*F. caatingaense*	97,01	*F. caatingaense*	FIESC	MZ966148
CMW 55328	2016	KwaZulu Natal	Kwambonambi	MI	*F. caatingaense*	98,88	*F. caatingaense*	FIESC	MZ966146
CMWF 1007	2008	KwaZulu Natal	Kwambonambi	MI	*F. caatingaense*	97,64	*F. caatingaense*	FIESC	MZ966154
CMWF 1028	2008	Western Cape	Hartenbos	MI	*F. caatingaense*	99,2	*F. caatingaense*	FIESC	MZ966147
CMWF 1182	2008	Western Cape	Belville	MI	*F. scirpi*	98,32	*F. scirpi*	FIESC	MZ966173
CMW 55730	2016	KwaZulu Natal	Nyalazi	MI	*F. lacertarum*	95,66	*F. lacertarum*	FIESC	MZ966159
CMW 55744	2016	KwaZulu Natal	Mtubatuba	HI	*F. lacertarum*	94,94	*F. lacertarum*	FIESC	MZ966158
CMW 55734	2016	KwaZulu Natal	Kwambonambi	MI	*F. lacertarum*	97,24	*F. lacertarum*	FIESC	MZ966157
CMW 55330	2016	KwaZulu Natal	Kwambonambi	MI	*F. clavum*	97,4	*F. clavum*	FIESC	MZ966170
CMW 55761	2016	KwaZulu Natal	Kwambonambi	HI	*F. clavum*	95,61	*F. clavum*	FIESC	MZ966163
CMW 55327	2016	KwaZulu Natal	Mtubatuba	HI	*F. clavum*	97,43	*F. clavum*	FIESC	MZ966166
CMW 55333	2016	KwaZulu Natal	Mzingazi	MI	*F. clavum*	97,92	*F. clavum*	FIESC	MZ966169
CMW 55750	2016	KwaZulu Natal	Nyalazi	MI	*F. clavum*	98,56	*F. clavum*	FIESC	MZ966168
CMW 55775	2016	KwaZulu Natal	Kwambonambi	HI	*F. clavum*	97,83	*F. clavum*	FIESC	MZ966165
CMW 55752	2016	KwaZulu Natal	Mzingazi	MI	*F. clavum*	97,75	*F. clavum*	FIESC	MZ966162
CMW 55754	2016	KwaZulu Natal	Kwambonambi	HI	*F. clavum*	97,98	*F. clavum*	FIESC	MZ966167
CMW 55326	2016	KwaZulu Natal	Mzingazi	MI	*F. clavum*	95,15	*F. clavum*	FIESC	MZ966161
CMW 55334	2016	KwaZulu Natal	Mtubatuba	MI	*F. clavum*	93,63	*F. clavum*	FIESC	MZ966164
CMW 55325	2016	KwaZulu Natal	Kwambonambi	HI	*F. clavum*	98,44	*F. clavum*	FIESC	MZ966160
CMW 55332	2016	KwaZulu Natal	Mzingazi	MI	*F. clavum*	84,11	*F. clavum*	FIESC	MZ966172

CMW 55331	2016	KwaZulu Natal	Mzingazi	MI	*F. clavum*	97,64	*F. clavum*	FIESC	MZ966171
CMWF 1051	2008	KwaZulu Natal	Kwambonambi	HI	*F. chlamydosporum*	998,3	*F. sporodochiale*	FCSC	MZ966264

[a]Tissue indicates whether the inflorescence was malformed or healthy (MI= malformed inflorescence, HI = healthy inflorescence)

[b] FID Based ID represent the top BLAST hit for the *TEF1*α sequence searches against the *Fusarium* ID database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Geiser et al. 2004).

[c] FFSC denotes the *F. fujikuroi* species complex, FOSC denotes the *F. oxysporum* species complex, FIESC denotes the *F. incarnatum-equiseti* species complex and FCSC denotes the *F. chylamydosporum* species complex.

S. cordatum trees were sampled from different locations, covering the areas of Dukuduku, Mtubatuba, Nyalazi and Mzingazi in addition to Kwambonambi, in the KwaZulu-Natal province. Prior to fungal isolations, the plant material was surface disinfested by submerging small pieces of an inflorescence in 1% (v/v) sodium hypochlorite solution and then 70% (v/v) ethanol, for 1 min each. Samples were then rinsed in sterile distilled water and plated directly onto Potato Dextrose Agar (PDA; 20g/L PDA, 5g/L Agar; Biolab Diagnostics, Wadeville South Africa) medium and onto *Fusarium* selective medium (FSM) (Nash and Snyder, 1962). Following incubation at 25 ºC for 7 days, isolates resembling *Fusarium* were transferred to fresh PDA plates and incubated at 25 ºC for 7 days. For each isolate, a spore suspension was then prepared by flooding the plate with sterile water and used to inoculate water agar medium (WA; 20g/L Agar; Biolab Diagnostics). Single germinating conidia were then used to establish pure cultures for the respective isolates, which were subsequently stored in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

DNA extraction, PCR amplification and sequencing

DNA was extracted from 7-day-old cultures using a modified CTAB (hexadecyltrimethylammonium bromide) method (Steenkamp et al., 1999). Briefly, fungal tissue was scraped from the growth medium and homogenized in 500µl DNA extraction buffer containing 100mM Tris-HCl (pH 8.0), 10mM ethylenediaminetetra-acetic acid di-sodium salt (EDTA; pH 8.0), 2% (w/v) sodium dodecyl sulphate and 0.2µg/µl proteinaise K (Sigma-Aldrich, St Loius, Missouri, USA). This was followed by freezing for 10 min at -70 ºC, defrosting and incubation at 60 ºC for 5 minutes, after which the sample was subjected to a standard phenol-chloroform extraction procedure (Maniatis et al.,1982; Sambrook et al., 1989). Extracted nucleic acids were precipitated using absolute ethanol and incubation at -20 ºC overnight, and then harvested by centrifugation at 14,000 rpm for 30 min at 4 ºC. Precipitates were washed in 500µl 70% ethanol, centrifuged at 14, 000 rpm for 10 min at 4 ºC, air dried and resuspended in 50µl of sterile distilled water (Sambrook et al., 1989). Extracted DNA was visualized and quantified using agarose gel electrophoresis and a nanodrop spectrophotometer (nanodrop, Wilmington, USA), respectively. PCR of the gene encoding the translation elongation factor 1-alpha region (*TEF1*α)

was carried out using the primer pairs and amplification protocols as described in Yilmaz et al. (2021). Each 25µl reaction mixture contained 20 ng of DNA, 200µM dNTPs, (Fermentas, Nunningen, Germany), 10µM of each primer and 2.5 mM $MgCl_2$, as well as 0.1 µM *Taq* polymerase and reaction buffer (Roche, Molecular Biochemicals, Manheim, Germany).

PCR products were purified using ExoSAP-IT (Affymetrix Inc., Santa Clara, California, USA) and electrophoresed to assess product integrity and to estimate the DNA concentration. Cleaned products were sequenced in both directions with the original primers using the ABI 377 automated sequencer and the BigDye Terminator v 3.1 sequencer cycle sequencing kit (Applied Biosystems, Foster City, CA). Electropherograms were visualized and consensus sequences were generated from forward and reverse sequences using BioEdit version 7.0.9 (Hall, 1999). Newly generated sequences were submitted to GenBank, and accession numbers are provided in Table 1.

Sequence analysis

All *TEF1*α sequences generated in this study were compared, using BLAST searches, with those in the Fusarium-ID database (http://isolate.fusariumdb.org/ index.php) maintatined by Geiser et al. (2004). This was done to obtain putative identifications for the isolates examined. These comparisons also revealed the species complexes to which the respective isolates belonged. For the different species complexes, *TEF1*α sequences generated in this study were added to reference sequence datasets built around the ex-type sequences published by O'Donnell et al. (2000, 2009), Sandoval-Denis et al. (2018a; 2018b), Maryani et al. (2019), Lombard et al. (2019a; 2019b) and Yilmaz et al. (2021).

All sequence data sets were aligned with MAFFT v. 1.764b (Katoh and Standley; 2013), using the G-INS-i option, and manually trimmed in Geneious Primer (11.0.5) where needed. The respective datasets were then subjected to Maximum Likelihood (ML) analysis with IQtree v. 1.6.8 (Nguyen et al., 2015) using the most suitable substitute model parameters, as indicated by Modelfinder (Kalyaanamoorthy et al., 2017). Branch support was estimated in IQtree using the same model parameters and 1000 pseudoreplicates using of UFBoot (Minh et al., 2013). Trees were visualized

in Figtree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree) and edited for presentation in Affinity Designer v 1.6.1 (Serif (Europe) Ltd, Nottingham, UK).

Results

Fungal Isolates

A total of 118 *Fusarium* isolates were obtained from *S. cordatum* inflorescences collected in this study (Table 1; Figure 1). In 2008, 47 *Fusarium* isolates were obtained from malformed *S. cordatum* in Gauteng (3 isolates), Mpumalanga (1 isolate), Western Cape (12 isolates) and KwaZulu-Natal (31 isolates). The higher number of samples obtained from the KwaZulu-Natal province, prompted the subsequent collection in 2016 where 15 *S. cordatum* trees were sampled from different locations, covering a larger area including Dukuduku, Mtubutaba, Nyalazi and Mzingazi in addition to Kwambonambi. During this latter collection, healthy inflorescences were also examined. From the second collection, a total of 71 *Fusarium* isolates were obtained, 53 of which were isolated from malformed inflorescences and 18 from healthy inflorescences.

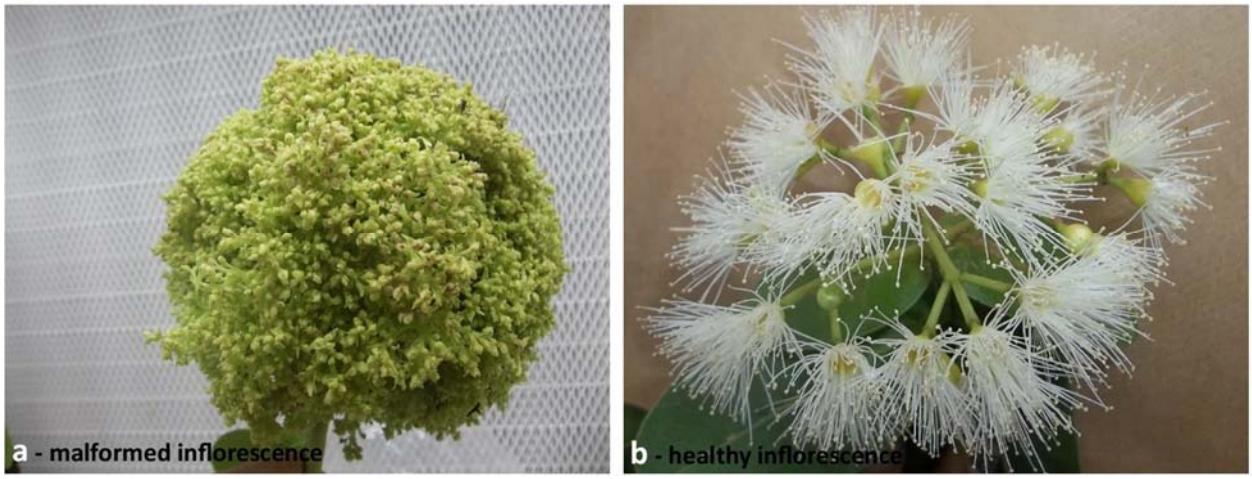


Fig. 1. Malformed (a) and Healthy (b) inflorescences of *Syzygium cordatum* trees in South Africa.

Identification of Fusarium *species*

BLAST searches of the *TEF1*α sequences determined in this study against those in the Fusarium-ID database revealed that the 118 isolates examined represented four different species complexes

in the genus *Fusarium* (Table 1). These were as follows: *F. fujikuroi* species complex (FFSC; 39 isolates), *F. oxysporum* species complex (FOSC; 45 isolates), *F. incarnatum-equiseti* species complex (FIESC; 33 isolates) and *F. chylamydosporum* species complex (FCSC; 1 isolate). The majority of the isolates (38%) were therefore members of FOSC, followed by FFSC (33%) and the FIESC (28%).

In order to assign the isolates to species, phylogenetic analyses of *TEF1*α sequences were performed. Dataset characteristics and substitution models applied during the analysis are summarized in Table 2. In all cases, datasets were made up of sequences generated in this study and those for known species and reference strains in the particular species complexes.

The *TEF1*α based phylogeny separated the 39 FFSC isolates examined into six well-supported and exclusive groups representing existing or new species residing in the so-called African, Asian, and American clades of the complex (O'Donnell, et al., 1998) (Figure 2). Of the 39 isolates, 33 isolates formed part of the Asian clade where they resided in clades representing *F. annulatum* (16 isolates), *F. fujikuroi* (12 isolates), and *F. sacchari* (1 isolate). *Fusarium annulatum* was re-introduced by Yilmaz et al. (2021) as the name for some isolates previously identified as "*F. proliferatum*". Previous studies (including whole genome sequence analysis) showed that the ex-type of *F. annulatum* (CBS 258.54^{T}) resolved in the same clade with isolates named "*F. proliferatum*" (O'Donnell et al., 1998; Brankovics et al., 2020). The ex-type specimen for *F. proliferatum* was however never preserved. Yilmaz et al. (2021) therefore used CBS 480.96 as an epitype of *F. proliferatum*, which was isolated from the same substrate (soil) and location (Papua New Guinea) and resembled the original drawing of *F. proliferatum*. The 16 isolates examined in this study clustered together with the ex-type of *F. annulatum,* although our BLAST results matched those of *F. proliferatum*. Of the seven African clade isolates, one isolate grouped with *F. verticillioides*. The remaining six isolates formed a unique cluster, which was designated as *Fusarium* sp. nov. 2. The three American clade isolates also formed a unique cluster including NRRL 25807 and they are designated as *Fusarium* sp. nov. 1.

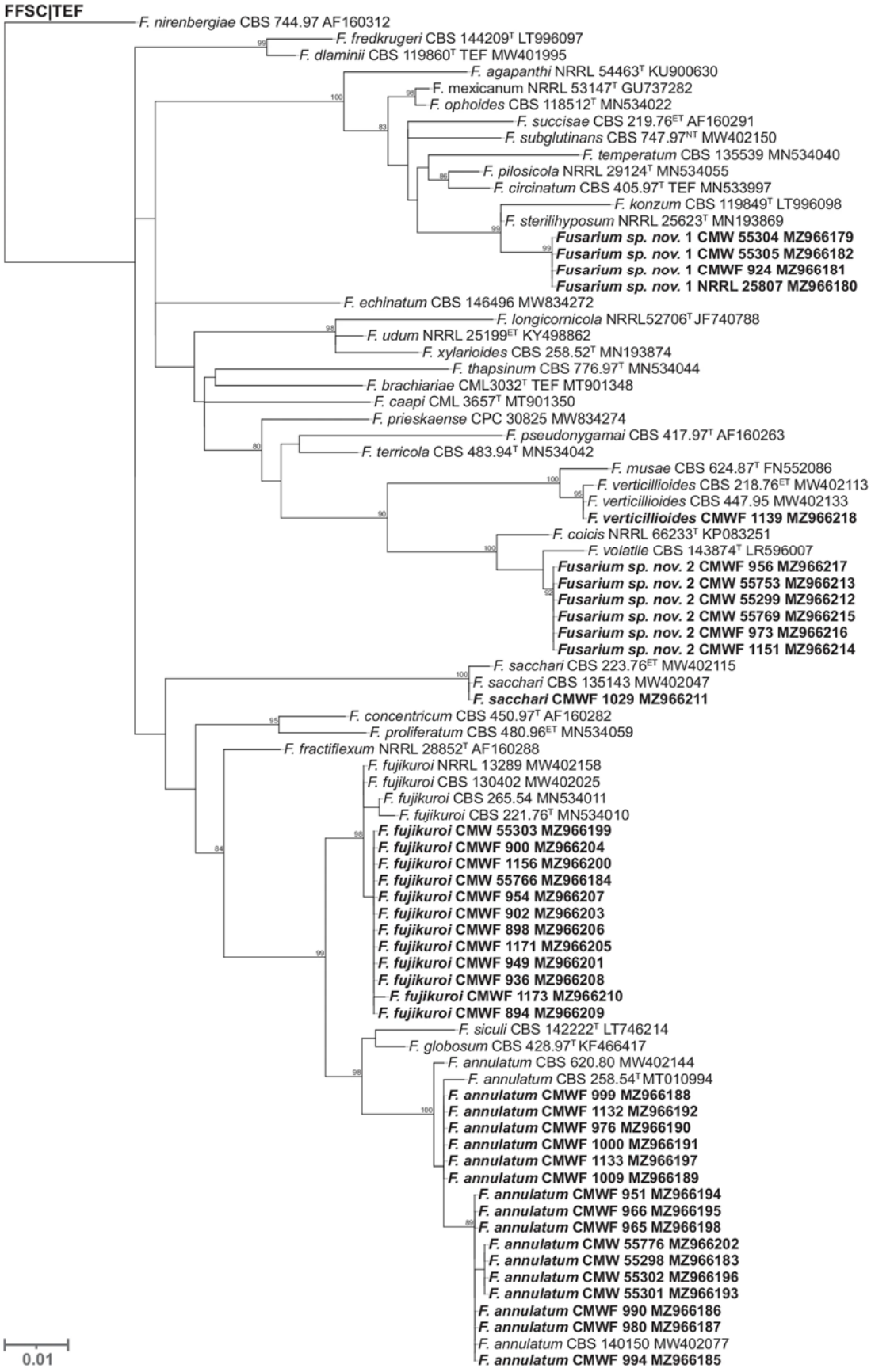
Table 2 Model parameters for the maximum likelihood phylogenetic analyses performed for the respective datasets analyzed in this study

From: Diversity of *Fusarium* species associated with healthy and malformed *Syzygium cordatum* inflorescences in South Africa

Dataset [a]	Number of taxa	Alignment length (nucleotides)	Model parameters [b]	References
FFSC				
*TEF1*α	82	668	TNe+G4 Unequal transition/transversion rates with unequal purine/pyrimidine rates and equal base frequencies (TNe) and 4 discrete gamma (G) rate categories to account for rate heterogeneity across sites	Tamura and Nei, 1993
FOSC				
*TEF1*α	107	574	TNe+G4	Tamura and Nei, 1993
FIESC				
*TEF1*α	79	622	TNe+G4	Tamura and Nei, 1993
FCSC				
*TEF1*α	12	696	TIM2e+G4 Transition model with equal base frequencies (TIM2e) and 4 discrete gamma (G) rate categories to account for rate heterogeneity across sites	Yang, 1994

[a] Individual datasets were constructed for *TEF1*α gene region of the respective species complexes of *Fusarium*, where FFSC denotes the *F. fujikuroi* species complex, FOSC denotes the *F. oxysporum* species complex, FIESC denotes the *F. incarnatum-equiseti* species complex and FCSC denotes the *F. chylamydosporum* species complex.

[b] Model parameters implemented in IQtree v. 1.6.8 (Nguyen et al. 2015) and determined for each dataset using Modelfinder (Kalyaanamoorthy et al. 2017).


FFSC|TEF
F. nirenbergiae CBS 744.97 AF160312
F. fredkrugeri CBS 144209T LT996097
F. dlaminii CBS 119860T TEF MW401995
F. agapanthi NRRL 54463T KU900630
F. mexicanum NRRL 53147T GU737282
F. ophoides CBS 118512T MN534022
F. succisae CBS 219.76ET AF160291
F. subglutinans CBS 747.97NT MW402150
F. temperatum CBS 135539 MN534040
F. pilosicola NRRL 29124T MN534055
F. circinatum CBS 405.97T TEF MN533997
F. konzum CBS 119849T LT996098
F. sterilihyposum NRRL 25623T MN193869
Fusarium sp. nov. 1 CMW 55304 MZ966179
Fusarium sp. nov. 1 CMW 55305 MZ966182
Fusarium sp. nov. 1 CMWF 924 MZ966181
Fusarium sp. nov. 1 NRRL 25807 MZ966180
F. echinatum CBS 146496 MW834272
F. longicornicola NRRL52706T JF740788
F. udum NRRL 25199ET KY498862
F. xylarioides CBS 258.52T MN193874
F. thapsinum CBS 776.97T MN534044
F. brachiariae CML3032T TEF MT901348
F. caapi CML 3657T MT901350
F. prieskaense CPC 30825 MW834274
F. pseudonygamai CBS 417.97T AF160263
F. terricola CBS 483.94T MN534042
F. musae CBS 624.87T FN552086
F. verticillioides CBS 218.76ET MW402113
F. verticillioides CBS 447.95 MW402133
F. verticillioides CMWF 1139 MZ966218
F. coicis NRRL 66233T KP083251
F. volatile CBS 143874T LR596007
Fusarium sp. nov. 2 CMWF 956 MZ966217
Fusarium sp. nov. 2 CMW 55753 MZ966213
Fusarium sp. nov. 2 CMW 55299 MZ966212
Fusarium sp. nov. 2 CMW 55769 MZ966215
Fusarium sp. nov. 2 CMWF 973 MZ966216
Fusarium sp. nov. 2 CMWF 1151 MZ966214
F. sacchari CBS 223.76ET MW402115
F. sacchari CBS 135143 MW402047
F. sacchari CMWF 1029 MZ966211
F. concentricum CBS 450.97T AF160282
F. proliferatum CBS 480.96ET MN534059
F. fractiflexum NRRL 28852T AF160288
F. fujikuroi NRRL 13289 MW402158
F. fujikuroi CBS 130402 MW402025
F. fujikuroi CBS 265.54 MN534011
F. fujikuroi CBS 221.76T MN534010
F. fujikuroi CMW 55303 MZ966199
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F. fujikuroi CMWF 1156 MZ966200
F. fujikuroi CMW 55766 MZ966184
F. fujikuroi CMWF 954 MZ966207
F. fujikuroi CMWF 902 MZ966203
F. fujikuroi CMWF 898 MZ966206
F. fujikuroi CMWF 1171 MZ966205
F. fujikuroi CMWF 949 MZ966201
F. fujikuroi CMWF 936 MZ966208
F. fujikuroi CMWF 1173 MZ966210
F. fujikuroi CMWF 894 MZ966209
F. siculi CBS 142222T LT746214
F. globosum CBS 428.97T KF466417
F. annulatum CBS 620.80 MW402144
F. annulatum CBS 258.54T MT010994
F. annulatum CMWF 999 MZ966188
F. annulatum CMWF 1132 MZ966192
F. annulatum CMWF 976 MZ966190
F. annulatum CMWF 1000 MZ966191
F. annulatum CMWF 1133 MZ966197
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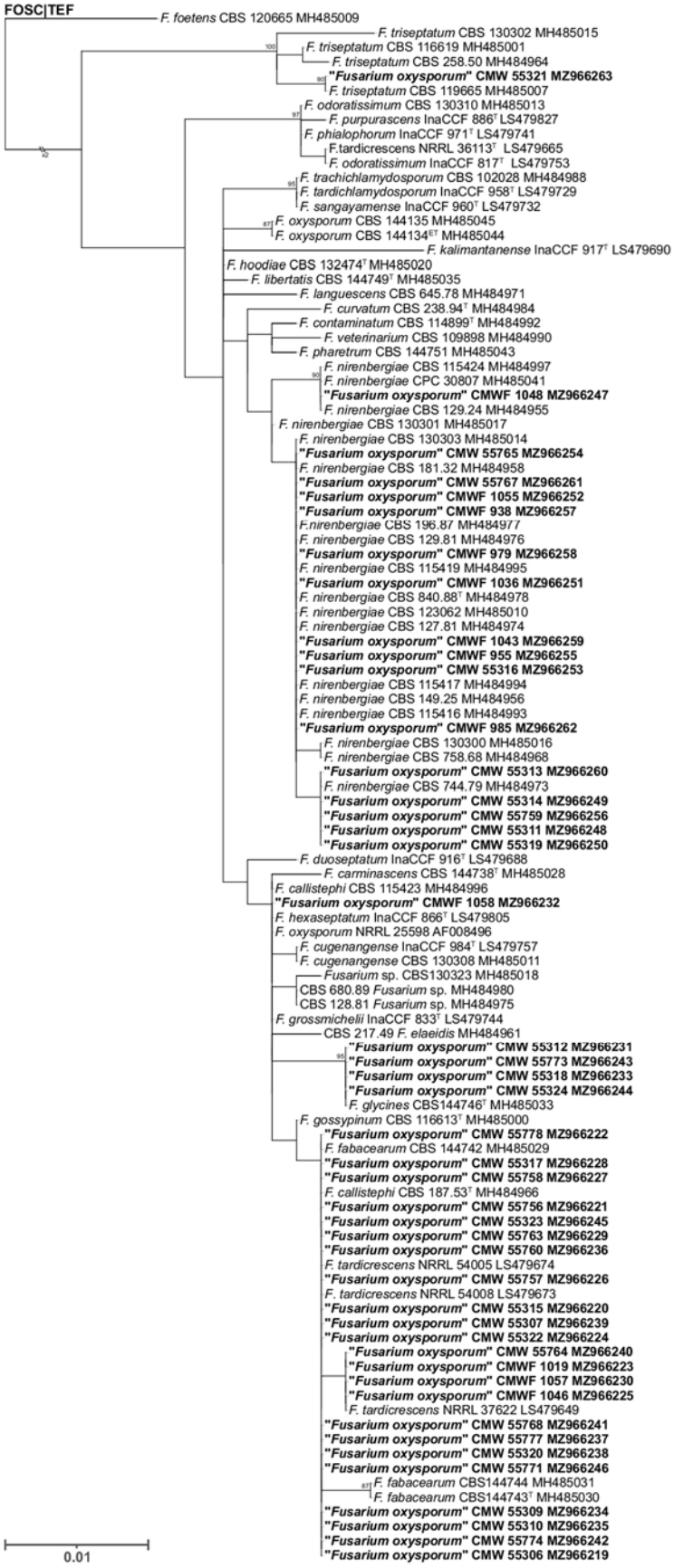
F. annulatum CMWF 951 MZ966194
F. annulatum CMWF 966 MZ966195
F. annulatum CMWF 965 MZ966198
F. annulatum CMW 55776 MZ966202
F. annulatum CMW 55298 MZ966183
F. annulatum CMW 55302 MZ966196
F. annulatum CMW 55301 MZ966193
F. annulatum CMWF 990 MZ966186
F. annulatum CMWF 980 MZ966187
F. annulatum CBS 140150 MW402077
F. annulatum CMWF 994 MZ966185
0.01

Fig 2. ML tree based on *TEF1α* showing identities and diversity of *Fusarium* species in FFSC associated with *Syzgium cordatum*. Bootstrap values ≥80% are shown above branches. Sequences obtained from ex-type cultures are indicated by ^{T}, epi-type cultures indicated by ^{ET}, neo-type cultures indicated by ^{NT}. GenBank accession numbers are shown next to the culture number with the accession numbers. The sequences created during this study are indicated by bold text. The tree was rooted to *F. nirenbergiae* (CBS 744.97).


FOSC|TEF
F. foetens CBS 120665 MH485009
F. triseptatum CBS 130302 MH485015
F. triseptatum CBS 116619 MH485001
F. triseptatum CBS 258.50 MH484964
"Fusarium oxysporum" CMW 55321 MZ966263
F. triseptatum CBS 119665 MH485007
F. odoratissimum CBS 130310 MH485013
F. purpurascens InaCCF 886T LS479827
F. phialophorum InaCCF 971T LS479741
F.tardicrescens NRRL 36113T LS479665
F. odoratissimum InaCCF 817T LS479753
F. trachichlamydosporum CBS 102028 MH484988
F. tardichlamydosporum InaCCF 958T LS479729
F. sangayamense InaCCF 960T LS479732
F. oxysporum CBS 144135 MH485045
F. oxysporum CBS 144134ET MH485044
F. kalimantanense InaCCF 917T LS479690
F. hoodiae CBS 132474T MH485020
F. libertatis CBS 144749T MH485035
F. languescens CBS 645.78 MH484971
F. curvatum CBS 238.94T MH484984
F. contaminatum CBS 114899T MH484992
F. veterinarium CBS 109898 MH484990
F. pharetrum CBS 144751 MH485043
F. nirenbergiae CBS 115424 MH484997
F. nirenbergiae CPC 30807 MH485041
"Fusarium oxysporum" CMWF 1048 MZ966247
F. nirenbergiae CBS 129.24 MH484955
F. nirenbergiae CBS 130301 MH485017
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F. nirenbergiae CBS 181.32 MH484958
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"Fusarium oxysporum" CMWF 1055 MZ966252
"Fusarium oxysporum" CMWF 938 MZ966257
F.nirenbergiae CBS 196.87 MH484977
F. nirenbergiae CBS 129.81 MH484976
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F. nirenbergiae CBS 115419 MH484995
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F. nirenbergiae CBS 840.88T MH484978
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"Fusarium oxysporum" CMW 55319 MZ966250
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F. carminascens CBS 144738T MH485028
F. callistephi CBS 115423 MH484996
"Fusarium oxysporum" CMWF 1058 MZ966232
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F. oxysporum NRRL 25598 AF008496
F. cugenangense InaCCF 984T LS479757
F. cugenangense CBS 130308 MH485011
Fusarium sp. CBS130323 MH485018
CBS 680.89 Fusarium sp. MH484980
CBS 128.81 Fusarium sp. MH484975
F. grossmichelii InaCCF 833T LS479744
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F. gossypinum CBS 116613T MH485000
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F. fabacearum CBS 144742 MH485029
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F. callistephi CBS 187.53T MH484966
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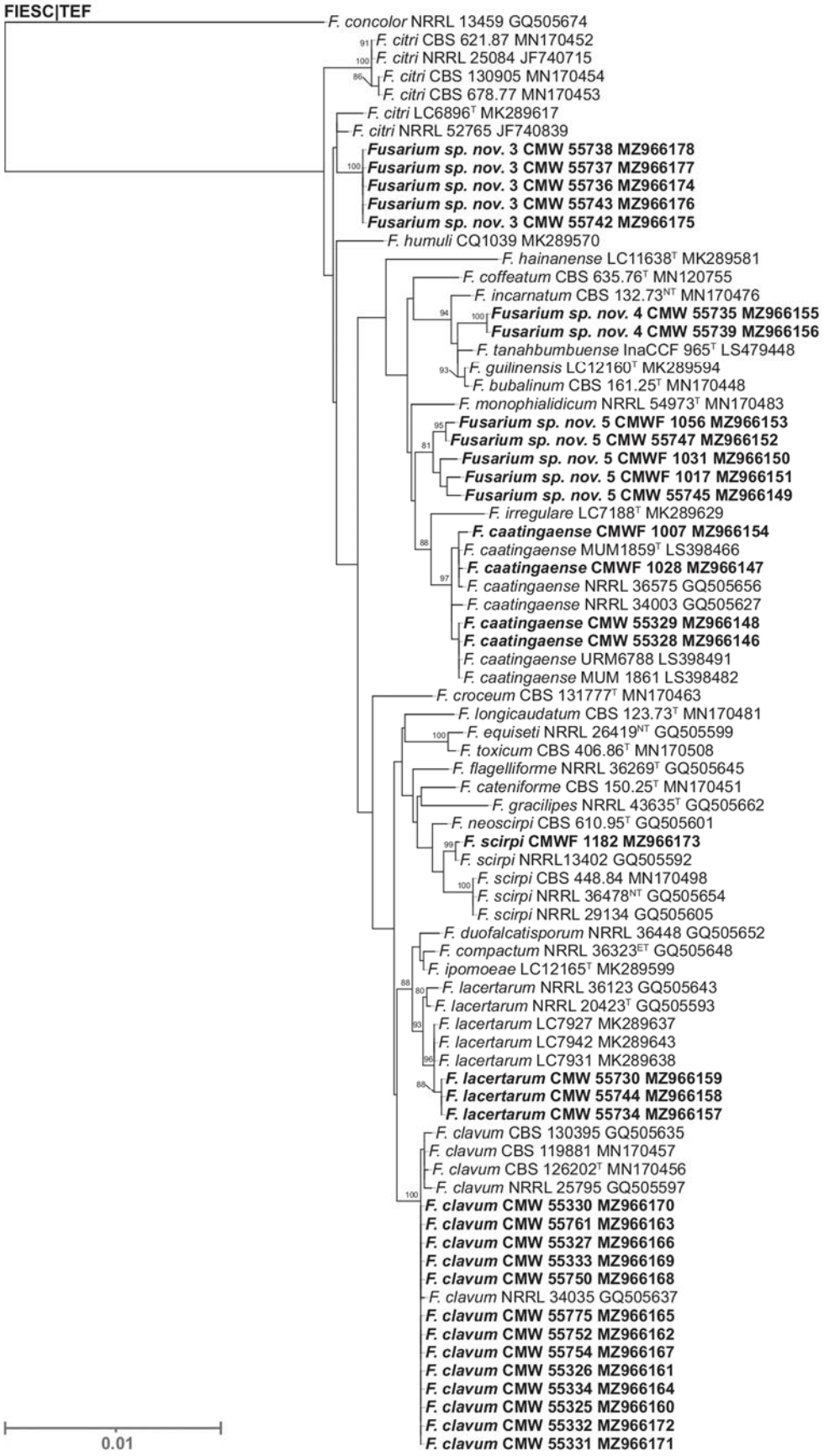
0.01

Fig. 3. ML tree based on *TEF1α* showing identities and diversity of *Fusarium* species in FOSC associated with *Syzgium cordatum*. Bootstrap values ≥80% are shown above branches. Sequences obtained from ex-type cultures are indicated by ^{T}, epi-type cultures indicated by ^{ET}. Sequences obtained from strains during this study are indicated by bold text. The tree was rooted to *F. foetens* (CBS 120665).

Phylogenetic analysis of the FOSC based on *TEF1α* (Fig. 3) did not provide sufficient resolution to robustly delineate groups representative of species. Species delineation, based on *TEF1α* alone, was also not clear for species obtained from the Maryani et al. (2019) and Lombard et al. (2019a) datasets. The generalized name *Fusarium oxysporum sensu lato* was therefore used for the isolates obtained in this study.

The *TEF1α* phylogeny separated the 33 FIESC isolates into seven well-supported and exclusive groups representing existing or new species in this complex (Figure 4). Of the seven groups, three and four formed respectively part of the so-called *F. equiseti* and *F. incarnatum* clades of the complex (O'Donnell et al., 2009; O'Donnell et al., 2012). In the *F. incarnatum* clade, four isolates arising from this study grouped wit*h F. caatingaense* previously referred to as the FIESC20 clade (Xia et al., 2019), as well as three unique clusters that likely represent new species (i.e., *Fusarium* sp. nov. 3 represented by five isolates, Fusarium sp. nov. 4 represented by two isolates and *Fusarium* sp. nov. 5 represented by five isolates). Of the *F. equiseti* clade isolates, 13 isolates resolved as *F. clavum* (previously referred to as FIESC5) (O'Donnell et al., 2009), one isolate was likely conspecific with *F. scirpi*, while three isolates formed a group with *F. lacertarum*, previously known as FIESC 4 (Xia et al., 2019).


FIESC|TEF
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F. citri NRRL 25084 JF740715
F. citri CBS 130905 MN170454
F. citri CBS 678.77 MN170453
F. citri LC6896T MK289617
F. citri NRRL 52765 JF740839
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Fusarium sp. nov. 3 CMW 55743 MZ966176
Fusarium sp. nov. 3 CMW 55742 MZ966175
F. humuli CQ1039 MK289570
F. hainanense LC11638T MK289581
F. coffeatum CBS 635.76T MN120755
F. incarnatum CBS 132.73NT MN170476
Fusarium sp. nov. 4 CMW 55735 MZ966155
Fusarium sp. nov. 4 CMW 55739 MZ966156
F. tanahbumbuense InaCCF 965T LS479448
F. guilinensis LC12160T MK289594
F. bubalinum CBS 161.25T MN170448
F. monophialidicum NRRL 54973T MN170483
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Fusarium sp. nov. 5 CMWF 1017 MZ966151
Fusarium sp. nov. 5 CMW 55745 MZ966149
F. irregulare LC7188T MK289629
F. caatingaense CMWF 1007 MZ966154
F. caatingaense MUM1859T LS398466
F. caatingaense CMWF 1028 MZ966147
F. caatingaense NRRL 36575 GQ505656
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F. caatingaense CMW 55329 MZ966148
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F. caatingaense MUM 1861 LS398482
F. croceum CBS 131777T MN170463
F. longicaudatum CBS 123.73T MN170481
F. equiseti NRRL 26419NT GQ505599
F. toxicum CBS 406.86T MN170508
F. flagelliforme NRRL 36269T GQ505645
F. cateniforme CBS 150.25T MN170451
F. gracilipes NRRL 43635T GQ505662
F. neoscirpi CBS 610.95T GQ505601
F. scirpi CMWF 1182 MZ966173
F. scirpi NRRL13402 GQ505592
F. scirpi CBS 448.84 MN170498
F. scirpi NRRL 36478NT GQ505654
F. scirpi NRRL 29134 GQ505605
F. duofalcatisporum NRRL 36448 GQ505652
F. compactum NRRL 36323ET GQ505648
F. ipomoeae LC12165T MK289599
F. lacertarum NRRL 36123 GQ505643
F. lacertarum NRRL 20423T GQ505593
F. lacertarum LC7927 MK289637
F. lacertarum LC7942 MK289643
F. lacertarum LC7931 MK289638
F. lacertarum CMW 55730 MZ966159
F. lacertarum CMW 55744 MZ966158
F. lacertarum CMW 55734 MZ966157
F. clavum CBS 130395 GQ505635
F. clavum CBS 119881 MN170457
F. clavum CBS 126202T MN170456
F. clavum NRRL 25795 GQ505597
F. clavum CMW 55330 MZ966170
F. clavum CMW 55761 MZ966163
F. clavum CMW 55327 MZ966166
F. clavum CMW 55333 MZ966169
F. clavum CMW 55750 MZ966168
F. clavum NRRL 34035 GQ505637
F. clavum CMW 55775 MZ966165
F. clavum CMW 55752 MZ966162
F. clavum CMW 55754 MZ966167
F. clavum CMW 55326 MZ966161
F. clavum CMW 55334 MZ966164
F. clavum CMW 55325 MZ966160
F. clavum CMW 55332 MZ966172
F. clavum CMW 55331 MZ966171
0.01

Fig. 4. ML tree based on *TEF1α* showing identities and diversity of *Fusarium* species in FIESC associated with *Syzgium cordatum*. Bootstrap values ≥80% are shown above branches. Sequences obtained from ex-type cultures are indicated by ^{T}, epi-type cultures indicated by ^{ET}, neo-type cultures indicated by ^{NT}. GenBank accession numbers are shown next to the culture number with the accession numbers. The sequences created during this study are indicated by bold text. The tree was rooted to *F. concolor* (NRRL 13459).

Phylogenetic analysis for the single isolate residing in the FCSC grouped with *F. sporodochiale* CBS 199.63, MRC 117 and MRC 35 (Figure 5). *Fusarium sporodochiale* was described by Lombard et al. (2019b) and the ex-type was isolated from soil in South Africa, however the ecology of the species is not well studied.

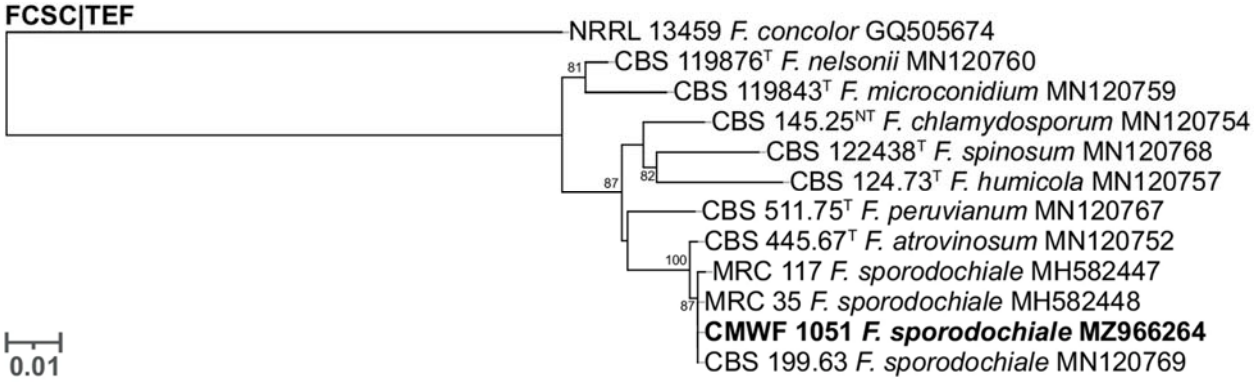

Fig. 5. ML tree based on *TEF1α* showing identities and diversity of *Fusarium* species in FCSC associated with *Syzgium cordatum*. Bootstrap values ≥70% are shown above branches. Sequences obtained from ex-type cultures are indicated by ^{T}, neo-type cultures indicated by ^{NT}. GenBank accession numbers are shown next to the culture number with the accession numbers. The sequences created during this study are indicated by bold text. The tree was rooted to *F. concolor* (NRRL 1345)

Overall, the phylogenetic analyses revealed that the 118 isolates collected from both healthy and malformed *S. cordatum* inflorescences represented at least 15 species and/or lineages of which five represent new species. Four species (*F. annulatum*, *F. oxysporum*, *F. caatingaense*, *Fusarium* sp. nov. 5) was isolated from trees sampled from at least three or all four sampling regions and multiple isolates were obtained for each species. With regards to sampling area, the greatest number of species was obtained from KwaZulu-Natal with 7 species isolated from malformed and 1 from healthy inflorescences, with 6 of these species also found in both tissue types (Table 3). Species residing in the FOSC and FIESC were isolated from both healthy and malformed

Table 3 Number of isolates from malformed and healthy inflorescences

From: Diversity of *Fusarium* species associated with healthy and malformed *Syzygium cordatum* inflorescences in South Africa

Species Complex[a]	Species Identity[b]	COLLECTION SITES[c]				
		KZN (HI)	KZN (MI)	M (MI)	WC(MI)	Gauteng (MI)
FFSC	*F. fujikuroi*	0	11	0	0	1
	F. annulatum	0	13	1	1	1
	F. verticillioides	0	1	0	0	0
	F. sacchari	0	1	0	0	0
	Fusarium sp. nov. 1	0	3	0	0	0
	Fusarium sp. nov. 2	0	5	0	0	1
FOSC	*F. oxysporum*	8	30	0	7	0
FIESC	*F. clavum*	5	8	0	0	0
	F. caatingaense	1	2	0	1	0
	F. scirpi	0	0	0	1	0
	F. lacertarum	1	2	0	0	0
	Fusarium sp. nov. 3	2	3	0	0	0
	Fusarium sp. nov. 4	0	2	0	0	0
	Fusarium sp. nov. 5	1	2	0	2	0
FCSC	*F. sporodochiale*	1	0	0	0	0
	Total	**19**	**83**	**1**	**12**	**3**

[a] FFSC denotes the *F. fujikuroi* species complex, FOSC denotes the *F. oxysporum* species complex, FIESC denotes the *F. incarnatum-equiseti* species complex and FCSC denotes the *F. chylamydosporum* species complex.

[b] Species were assigned according to their phylogenetic placement based on *TEF1*α

[c] Collection sites across South Africa with KZN representing the KwaZulu-Natal isolates from 2008 and 2016 and M and WC representing isolates sampled from Mpumalanga and the Western Cape during the 2008 survey, respectively. Tissue indicates whether the inflorescence was malformed or healthy (HI = healthy inflorescence, MI= malformed inflorescence).

inflorescences, whereas species residing in the FFSC were isolated only from malformed inflorescences. Finally, the single species in FCSP was isolated from healthy inflorescences.

Discussion

Results of this study showed that *S. cordatum* inflorescences represent a remarkable reservoir of *Fusarium* species diversity. Overall, the 118 *Fusarium* isolates collected, represented 15 species residing in four different species complexes and five of these species are new to science. The number of species and the number of novel species probably also represents an underestimate of the diversity from this unusual niche. This is particularly notable when considering that samples were not collected from all areas of South Africa where these trees are found.

The most abundant *Fusarium* isolates arising from this study were members of the FOSC. Species residing in this complex are typically grouped in *formae speciales* and races that represent plant pathogenic and host cultivar associations (Summerell, 2019). Many of these groups have, however, evolved multiple times independently and numerous *formae speciales* or groups of these are polyphyletic (Summerell, 2019). To resolve some of the taxonomic questions linked to the polyphyletic nature of these groups, a number of new *F. oxysporum* species have been described (Maryani et al., 2019; Lombard et al., 2019b) although providing names for *formae speciales* is not broadly supported (Summerell, 2019). Our analyses, however, did not provide robust resolution for the species in this complex (Fig. 3), we therefore kept the broader naming (*sensu lato*) for the *Fusarium oxysporum* isolates.

The larger number of FOSC isolates obtained in this study was not surprising since species in this complex has wide geographic distributions and are found in both cultivated and uncultivated soils as well as under different climatic conditions (Edel-Hermann and Lecomte, 2018). In addition, species in this group have wide host ranges that include both monocotyledon and dicotyledon plants (Edel-Hermann and Lecomte, 2019). As pathogens, they cause wilts, root, stem, and crown rots as well as vascular diseases on economically important plants (Booth, 1971; Leslie and Summerell, 2006a; Summerell, 2019; Edel-Hermann and Lecomte, 2018). Some isolates residing in the FOSC were obtained from both malformed and healthy tissues. The role of FOSC taxa in *S. cordatum* malformation is however not yet clear, but it is relevant that members of this complex

have previously been associated with malformed inflorescences of *Cicer arietinum* (Chickpea) (Arunodhayam et al., 2014; Haseeb et al., 2014) and *M. indica* (Liew et al., 2016).

The second highest number of *Fusarium* isolates obtained from the *S. cordatum* inflorescences resided in the FFSC. Species in this complex include numerous plant pathogens and important mycotoxin producers (Leslie and Summerell, 2006b; Windels, 2000; Yilmaz et al., 2021). Most FFSC isolates represented *F. annulatum* and *F. fujikuroi*, which were obtained from all sampling sites and from both the 2008 and 2016 KwaZulu-Natal surveys. *Fusarium annulatum* (as *F. proliferatum*) has been reported as the causal agent of MMD in China (Zhan et al., 2010; Lv et al. ,2010), Egypt (Wafaa et al., 2010; Haggag, 2010) and Malaysia (Nor et al., 2013). Likewise, *Fusarium fujikuroi* has been associated with MMD in Australia (Liew et al., 2016), although its role in malformation is currently unknown. Species in this complex were also the only collections found exclusively on malformed inflorescences. The possibility that they or either of the two new species delineated here are involved in the development of malformed *S. cordatum* inflorescences needs to be considered in future studies.

Several members in the FIESC were recovered from healthy and malformed inflorescences. Some species in this complex are associated with diseases in agriculturally important crops causing cankers and rots (Villani et al., 2016), while others are ubiquitous, cosmopolitan, and soil-inhabiting as plant saprotrophs and some are endophytes found in healthy plant tissues (Burgess and Summerell, 1992, Leslie and Summerell, 2006a). Interestingly, *Fusarium scirpi,* has been isolated from malformed *M. indica* inflorescence tissue, but was not linked to the epidemiology of MMD disease and is rather considered as an environmental species (Liew et al., 2016). Three novel species residing in this complex arose from this study and their possible role in malformation disease needs to be determined.

One isolate residing in the FCSC was obtained in this study. Little is known regarding the biology of *F. sporodochiale*, which is a relatively newly discovered species that have previously been isolated from soil and termite mounds (Lombard et al., 2019b).

Overall, the results from this study serve as the first important step towards the characterization of floral malformation on *S. cordatum*. Malformation appears to be associated with a number of species including at least five novel *Fusarium* species residing in four different species complexes. The new species should be formally described in future studies. However, as a priority, the species residing in the FFSC should be characterized in more detail and subjected to pathogenicity studies since they were the taxa found only in malformed inflorescences. An important component of future studies must also be to consider other biotic and/or abiotic factors associated with a floral malformation in *S. cordatum* including the effects of climate change as temperature and drought stress have been linked to malformation previously (Chimonidou-Pavlidou, 2004). It will also be important to consider the possible role of other biotic factors such as insects (Takei et al., 2015; Wool, 2004), mites (Jeppson et al., 1975; Lindquist et al., 1996) nematodes (Todd and Atkins, 1958; Khan et al., 2012), phytoplasmas (Weisburg et al., 1989) and viruses (Qazi et al., 2007) that have previously been linked to malformations in inflorescences in other species. In terms of biotic factors, the priority focus should be on *Eriophyoid* mite species that were commonly observed on malformed inflorescences. Their presence could increase disease severity, similarly to what has been reported for *Fusarium mangiferae* and floral and bud malformations in mangoes (Gamliel-Atinsky et al., 2009; Gamliel-Atinsky et al., 2010).

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