Mycological Progress (2025) 24:38
https://doi.org/10.1007/s11557-025-02059-2

ORIGINAL ARTICLE

Capturing the fungal diversity hidden in Eastern Cape dairy pastures

Claudette Dewing[1] · **Neriman Yilmaz**[1] · **Emma T. Steenkamp**[1] · **Brenda D. Wingfield**[1] · **Cobus M. Visagie**[1]

Received: 3 December 2024 / Revised: 13 May 2025 / Accepted: 29 May 2025
© The Author(s) 2025

Abstract

Fungi in dairy pastures impact cattle health, yet the diversity of fungal species present in South African pastures remains understudied. Following an outbreak of Sporidesmin-Induced Liver Disease (SILD; caused by the mycotoxin sporidesmin produced by *Pseudopithomyces toxicarius*) in the Eastern Cape in 2020, we collected mixed pasture samples from 14 dairy farms affected by this disease. Our aim was to investigate what fungal species are present in communities and whether species like *Ps. toxicarius* are present that may play a role in cattle health. A total of 708 strains were isolated from 95 mixed pasture samples and identified based on DNA sequence data to 132 species representing 55 genera. *Fusarium* was the most isolated (207 strains; 21 species; 55 samples), followed by *Penicillium* (75 strains; 22 species; 27 samples), *Pseudopithomyces* (69 strains; 2 species; 21 samples), *Cladosporium* (54 strains; 6 species; 23 samples), *Epicoccum* (52 strains; 6 species; 24 samples) and *Bipolaris* (38 strains; 3 species; 19 samples). Several strains could not be identified and represent potentially new or previously uncharacterised species. Additionally, phylogenetic analyses revealed the presence of *Ps. palmicola* and *Ps. toxicarius* in the Eastern Cape dairy pastures. Our findings underscore the ecological complexity of pasture environments and raise important questions about the role of fungal diversity in livestock health.

Keywords Cattle health · Facial eczema · Fungal diversity · Mycotoxins · *Pithomyces chartarum*

Introduction

The Eastern Cape province of South Africa has a diverse agricultural landscape, and plays an important role in the country's citrus, wool, and dairy industries. Grasslands and pastures are important in supporting the province's livestock sector, providing forage that sustains the largest proportion of South Africa's goats (39%), sheep (30%) and cattle (25%) populations (Department of Agriculture Land Reform and Rural Development 2023). The Eastern Cape, Western Cape and KwaZulu-Natal provinces are the joint largest milk producers, with each contributing around 29% of South Africa's total milk production (Milk Producers' Organisation 2024). The milk industry in South Africa contributes about 0.4% to global milk production while also creating jobs, supporting rural developments and promoting food security (Smith 2021).

Dairy pastures in the Eastern Cape are typically planted with kikuyu (*Cenchrus clandestinus*), annual ryegrass (*Lolium multiflorum*) and perennial ryegrass (*Lolium perenne*) (Truter et al. 2015). These species are selected for their adaptability to the local climate and their nutritional value and quality, including factors like taste and yield (Truter et al. 2015; MilkSA 2023). Kikuyu is known for its lower digestibility compared to other grasses, such as perennial ryegrass, which can limit its forage quality (Marais 2001; Van der Colf et al. 2015). To overcome this, pastures are often overseeded with ryegrass and clover to enhance productivity and quality (Botha et al. 2008b, a; Swanepoel et al. 2014; Van der Colf et al. 2015; MilkSA 2024). These mixed pastures combine the benefits of both grass species, aiming to extend the grazing season and improve pasture quality in order to enhance cattle health and optimal milk production (Botha et al. 2008b, a; Van der Colf et al. 2015).

Grasses are colonised by a wide range of fungi (Sánchez Márquez et al. 2012), some of which are associated with diseases in cattle. Notable examples include kikuyu poisoning (Bourke 2007; Di Menna et al. 2009) and

Section Editor: Tanay Bose

Cobus M. Visagie
cobus.visagie@fabi.up.ac.za

[1] Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa

Published online: 21 June 2025

Sporidesmin-Induced Liver Disease (SILD, previously known as facial eczema or pithomycotoxicosis).

Kikuyu poisoning is a ruminitis syndrome in cattle resulting in dehydration, forestomach necrosis, neuromuscular symptoms, tremors, and incoordination (Bryson 1982; Newsholme et al. 1983; Kellerman et al. 2005; Bourke 2007). Since the first outbreak of kikuyu poisoning, it was hypothesised that kikuyu poisoning may present a mycotoxicosis. In Australia, *Fusarium torulosum* was consistently isolated from pastures where cattle displayed kikuyu poisoning symptoms (Martinovich et al. 1972; Bourke 2007; Ryley et al. 2007). This fungus can produce wortmannin and butenolide, compounds linked to similar disease symptoms to kikuyu poisoning (Tookey et al. 1972; Abbas and Mirocha 1988; Gunther et al. 1989; Bourke 2007). In South Africa, Botha et al. (2014a) surveyed Eastern Cape dairy pastures during several kikuyu poisoning outbreaks between 2008–2010. Although *F. torulosum* was not detected during the survey, several other *Fusarium* species were identified, including *F. culmorum, F. redolens, F. oxysporum,* and species from the *Fusarium incarnatum-equiseti* species complex (FIESC) (Botha et al. 2014a). These species can produce various mycotoxins such as aurofusarin, beauvericin, butenolide, culmorin, deoxynivalenol, enniatins, fusaric acid, fusarins, fumonisins, moniliformin, nivalenol and zearalenone (Munkvold et al. 2021).

SILD is a secondary photosensitisation of ruminants caused by the mycotoxin sporidesmin produced by the fungus *Pseudopithomyces toxicarius* (formerly considered *Ps. chartarum*) (Weir et al. 2025). It occurs when phylloerythrin, a chlorophyll by-product, accumulates in the bloodstream due to impaired breakdown, leading to sunlight sensitivity, sunburn and skin lesions, alongside symptoms like reduced fertility, weight loss, poor growth and decreased milk production (Kellerman et al. 2005; Di Menna et al. 2009). SILD has been reported in Argentina, Australia, China, France, South Africa, the Netherlands, Portugal, Spain, Turkey, the United States and Uruguay (Marasas et al. 1972; Riet and Diaz 1974; Edwards et al. 1983; Bezille et al. 1984; Hansen et al. 1994; Fuertes et al. 2004; Pinto et al. 2005; Van Wuijckhuise et al. 2006; Ozmen et al. 2008; Riet-Correa et al. 2013; Liu et al. 2023; Davis et al. 2025). In New Zealand, SILD has caused significant production losses for over a century, exceeding NZD 100 million annually (Pollock et al. 2015). While the majority of *Ps. toxicarius* isolates from New Zealand produce sporidesmin (Weir et al. 2025), the proportion of sporidesmin-producing isolates in other countries are often lower or is unknown (Di Menna et al. 2009). In South Africa, SILD was first reported in sheep (Marasas et al. 1972). This was followed by more reports from sheep and other animals like dairy cows (Van der Merwe et al. 1979; De Wet and Erasmus 1984; Kellerman and Coetzer 1985; Davis et al. 2025). Considering the recent taxonomic revision of *Ps. chartarum* and the introduction of the toxin-producing *Ps. toxicarius* (Weir et al. 2025), knowledge on what species occurs in South African dairy pastures and the risk they pose to cattle health are unknown.

Recent increases in SILD reports from Eastern Cape dairy farms suggest growing awareness about the disease. However, knowledge on the fungal communities associated with dairy pastures in the region remains limited. The study aimed to survey Eastern Cape dairy pastures affected by SILD to determine the presence of *Pseudopithomyces* species and identify other fungi that may impact cattle health.

Materials and methods

Sampling and isolations

A total of 95 mixed grass pasture samples (mainly consisting of kikuyu and annual and perennial ryegrass) were collected across 14 dairy farms in the Eastern Cape province of South Africa during May 2020 from where cattle displayed potential SILD symptoms. Samples were kept dry in brown paper bags at low temperatures. For isolations, plant material was cut into 4 mm pieces and plated directly onto potato dextrose agar (PDA; 213400; Thermo Fisher Scientific, USA), dichloran 18% glycerol agar (DG18; CM0729B; Thermo Fisher Scientific, USA) and water agar (WA). Chloramphenicol (100 ppm) was added to both the PDA and WA to prevent bacterial growth. The plates were incubated for 7–10 d at 25 °C and checked regularly for fungal growth. Colonies were transferred to pure cultures on quarter strength PDA supplemented with chloramphenicol (100 ppm). Where possible, strains were identified to genus level based on their morphological characteristics. All strains were accessioned in a working culture collection (CN) of the Applied Mycology group at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa. Cultures were preserved as agar blocks or spore suspensions in cryovials containing 10% glycerol and stored at −80 °C. Representative strains were deposited to the CMW and CMW-IA culture collections of FABI (Table S1).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from 7–10 d old colonies. DNA from pigmented fungi was extracted using the Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, USA) following the manufacturer's protocol. PrepMan Ultra Sample Preparation Reagent (Thermo Fisher Scientific, USA) was used for *Fusarium* and other hyaline fungi following the manufacturer's instructions.

PCR amplifications were prepared in 25 µL total volumes containing 5 µL BioLine 5X MyTaq Reaction Buffer

(Meridian BioScience, USA), 0.50 µL of each primer (10 µM), 17.85 µL demineralised sterile water and 0.15 µL BioLine MyTaq DNA Polymerase (Meridian BioScience, USA). Loci targeted are those known to provide species level identifications in respective genera and included the nuclear ribosomal internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) and the large subunit of the nuclear ribosomal RNA (LSU) for strains that could not be identified to a genus based on morphology (Schoch et al. 2012), β-tubulin (*BenA*) for *Penicillium* (Visagie et al. 2014), calmodulin (*CaM*) for *Aspergillus* (Samson et al. 2014), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) for *Alternaria* (Woudenberg et al. 2013) and other *Pleosporales* (Manamgoda et al. 2012; Hernández-Restrepo et al. 2018), RNA polymerase second largest subunit (*RPB2*) for *Epicoccum, Neoascochyta* and *Pseudopithomyces* (Hou et al. 2020) and translation elongation factor 1-α (*TEF*) for *Cladosporium* (Bensch et al. 2012), *Fusarium* (O'Donnell et al. 2015) and *Trichoderma* (Samuels 2006). Information regarding the primers and PCR conditions used during this study were obtained from previous studies (Vilgalys and Hester 1990; Rehner and Samuels 1994; Glass and Donaldson 1995; Masclaux et al. 1995; O'Donnell and Cigelnik 1997; De Hoog and Van den Ende 1998; O'Donnell et al. 1998; Berbee et al. 1999; Carbone and Kohn 1999; Liu et al. 1999; Reeb et al. 2004; Hong et al. 2006; Yilmaz et al. 2021) and are provided in Table 1.

Amplified fragments were purified using the ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) and sequenced in both directions using the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, USA) with the same primers used for PCR amplification. Reactions were analysed on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, USA) at the DNA sequencing facility at the University of Pretoria. Contigs were assembled and edited in Geneious Prime v. 2019.0.4 (BioMatters Ltd., New Zealand). Newly generated sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/), with accession numbers provided in Table S1.

BLASTn analysis was performed to compare obtained sequences against the NCBI (National Centre for

Table 1 Primers and PCR conditions used in this study

Locus	Target genus	PCR amplification protocol	Primer	Primer sequence 5'–3'	Reference
BenA	*Penicillium*	94 °C 5 min; 35 cycles of 94 °C 45 s, 56 °C 45 s, 72 °C 60 s; 72 °C 7 min; 10 °C ∞	T10	ACGATAGGTTCACCTCCAGAC	O'Donnell and Cigelnik (1997)
			Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	Glass and Donaldson (1995)
CaM	*Aspergillus*	94 °C 5 min; 35 cycles of 94 °C 45 s, 56 °C 45 s, 72 °C 60 s; 72 °C 7 min; 10 °C ∞	cmd5	CCGAGTACAAGGARGCCTTC	Hong et al. (2006)
			cmd6	CCGATRGAGGTCATRACGTGG	
GAPDH	*Alternaria*/other *Pleosporales*	94 °C 5 min; 30 cycles of 94 °C 45 s, 52 °C 60 s, 72 °C 60 s; 72 °C 7 min; 10 °C soak	gdp1	CAACGGCTTCGGTCGCATTG	Berbee et al. (1999)
			gdp2	GCCAAGCAGTTGGTTGTGC	
ITS	General	94 °C 5 min; 35 cycles of 94 °C 45 s, 55 °C 45 s, 72 °C 60 s; 72 °C 7 min; 10 °C ∞	V9G	TTACGTCCCTGCCCTTTGTA	De Hoog and Van den Ende (1998)
			LS266	GCATTCCCAAACAACTCGACTC	Masclaux et al. (1995)
TEF	*Cladosporium*/*Trichoderma*	94 °C 5 min; 35 cycles of 94 °C 45 s, 52 °C 45 s, 72 °C 60 s; 72 °C 7 min; 10 °C ∞	728F	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn (1999)
			986R	TACTTGAAGGAACCCTTACC	
	Fusarium	94 °C 5 min; 35 cycles of 94 °C 45 s, 53 °C 45 s, 72 °C 60 s; 72 °C 7 min; 10 °C ∞	EF1	ATGGGTAAGGARGACAAGAC	Yilmaz et al. (2021); O'Donnell et al. (1998)
			EF2	GGARGTACCAGTSATCATG	
RPB2	*Epicoccum*/*Pseudopithomyces*/ *Neoascochyta*	95 °C 5 min; 40 cycles of 94 °C 30 s, 51 °C 90 s, 68 °C 2 min; 68 °C 5 min; 10 °C ∞	5F2	GGGGWGAYCAGAAGAAGGC	Reeb et al. (2004)
			7Cr	CCCATRGCTTGYTTRCCCAT	Liu et al. (1999)
LSU	General	94 °C 5 min; 40 cycles of 94 °C 45 s, 48 °C 30 s, 72 °C 90 s; 72 °C 6 min; 10 °C ∞	LR5	ATCCTGAGGGAAACTTC	Vilgalys and Hester (1990)
			LROR	GTACCCGCTGAACTTAAGC	Rehner and Samuels (1994)

Biotechnology Information, USA) GenBank nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain identifications. All ITS comparisons were made against the NCBI Fungal ITS Reference Sequence Targeted Loci Project database (Schoch et al. 2014). For *Aspergillus* and *Penicillium* strains, identifications were made based on comparisons with a locally curated DNA sequence database largely based on Houbraken et al. (2020), *Alternaria* against data largely based on Woudenberg et al. (2013), *Cladosporium* against data largely based on Bensch et al. (2012), *Trichoderma* against data largely based on Bissett et al. (2015) and *Fusarium* was identified using data on www.fusarium.org. All additional data files containing the reference datasets, alignments, and tree files were uploaded to Figshare (https://doi.org/10.6084/m9.figshare.27936900) hosted by the University of Pretoria research data repository.

Phylogenetic analysis of *Pseudopithomyces*

To assess the phylogenetic relationships for our *Pseudopithomyces* strains, we compiled a reference sequence dataset consisting of ITS, *RPB2*, *ACT* (actin), *GAPDH* and *TEF* sequences based on the dataset of Weir et al. (2025) (see Table S2). Sequence alignments were performed using MAFFT v. 7.427 (Katoh and Standley 2013) with the G-INS-I option selected. Alignments were concatenated with each gene region treated as distinct partitions in the subsequent analysis. A Maximum Likelihood (ML) tree was made in IQ-TREE v. 2.1.2 (Nguyen et al. 2015) applying the most suitable model parameters calculated using ModelFinder (Kalyaanamoorthy et al. 2017) incorporated into IQ-TREE. Support in nodes were calculated with an ultrafast bootstrap analysis with 10,000 replicates. The tree was visualised in FigTree v. 1.4.4 and edited for publication using Affinity Designer v. 1.6.1 (Serif (Europe) Ltd, Nottingham, UK).

Results

Fungal identifications

Isolations from the 95 mixed pasture samples resulted in 708 strains accessioned and stored in the FABI culture collection. We generated 886 new DNA reference sequences and submitted them to GenBank [ITS (*n* = 328), *BenA* (*n* = 80), *CaM* (*n* = 22), *GAPDH* (*n* = 48), LSU (*n* = 39), *RPB2* (*n* = 88) and *TEF* (*n* = 281)] (Table S1).

Strains were identified to 132 species, representing 55 genera, 38 families, 15 orders and seven classes. The fungal community is presented as a pie chart with the proportion sizes calculated corresponding to the number of strains isolated per taxon (Fig. 1). The numbers represented with each taxon are given as ‘x (y), z’: x = the number of strains; y = the number of species identified; and z = the number of samples that a taxon was isolated from (Fig. 1). At the genus level, *Fusarium* (55/95 samples; 207 strains), *Penicillium* (27/95; 75 strains), *Pseudopithomyces* (21/95; 69 strains), *Cladosporium* (23/95; 54 strains), *Epicoccum* (24/95; 52 strains) and *Bipolaris* (19/95; 38 strains) were the most frequently isolated fungi in mixed pastures. *Penicillium* had the highest species diversity (*n* = 22), followed by *Fusarium* (*n* = 21), *Epicoccum* (*n* = 6), *Cladosporium* (*n* = 6), *Bipolaris* (*n* = 3) and *Pseudopithomyces* (*n* = 2). The most common species were *Fusarium croceum* [*Fusarium incarnatum-equiseti* species complex (FIESC); 32/95; 56 strains], *Pseudopithomyces toxicarius* (20/95; 66 strains), *Fusarium mariecurieae* (FIESC; 20/95; 33 strains), *Epicoccum italicum* (18/95; 26 strains), *Cladosporium pseudocladosporioides* (15/95; 31 strains), *Fusarium culmorum* [*Fusarium sambucinum* species complex (FSAMSC); 15/95; 22 strains], *Bipolaris zeae* (13/95; 29 strains), *Penicillium rubens* (section *Chrysogena*; 13/95; 17 strains), *Fusarium pascuum* (FIESC; 12/95; 17 strains) and *Fusarium clavus* (FIESC; 10/95; 19 strains).

Several strains could not be accurately identified using morphological or DNA sequence data and may represent undescribed species. These include *Absidia* (*n* = 3, 1 species), *Alternaria* (*n* = 18, 1 species), *Ceratobasidium* (*n* = 1, 1 species), *Chaetomium* (*n* = 2, 1 species), *Cladosporium* (*n* = 2, 1 species), *Epicoccum* (*n* = 2, 1 species), *Fusarium* (*n* = 4, 3 species), *Geotrichum* (*n* = 5, 1 species), *Neoacremonium* (*n* = 1, 1 species), *Neoascochyta* (*n* = 12, 2 species), *Neurospora* (*n* = 1, 1 species), *Nigrospora* (*n* = 1, 1 species), *Pararamichloridium* (*n* = 1, 1 species), *Penicillium* (*n* = 5, 3 species), *Preussia* (*n* = 9, 2 species), *Roussoella* (*n* = 1, 1 species), *Sordaria* (*n* = 1, 1 species), *Sphaeronaemella* (*n* = 1, 1 species), *Trichoderma* (*n* = 4, 1 species) and *Valsonectria* (*n* = 2, 1 species). Results from homology searches of their sequences against NCBI’s Fungal ITS Reference Sequence Targeted Loci Project database are listed in Table 2.

Some strains (CN049F9 and CN103F1) could only be identified at the order level within *Pleosporales*. These strains will be subjected to further investigation in the future.

Phylogenetic analyses of *Pseudopithomyces*

The aligned dataset contained 68 ingroup taxa, with *Bimuria novae-zelandiae* (ICMP24821^T) selected as the outgroup (Fig. 2). The alignment was 6,343 bp long (*ACT* = 1,604; *GAPDH* = 1,254; ITS = 551; *RPB2* = 953; *TEF* = 1,981). The most suitable nucleotide substitution models were K2P + FQ + G for ITS, TN + F + G for *ACT*, *GAPDH*, TN + F + I + G for *TEF*, and TNe + FQ + G for *RPB2*. Our strains clearly belong to *Ps. toxicarius* (*n* = 66) and *Ps. palmicola* (*n* = 3).

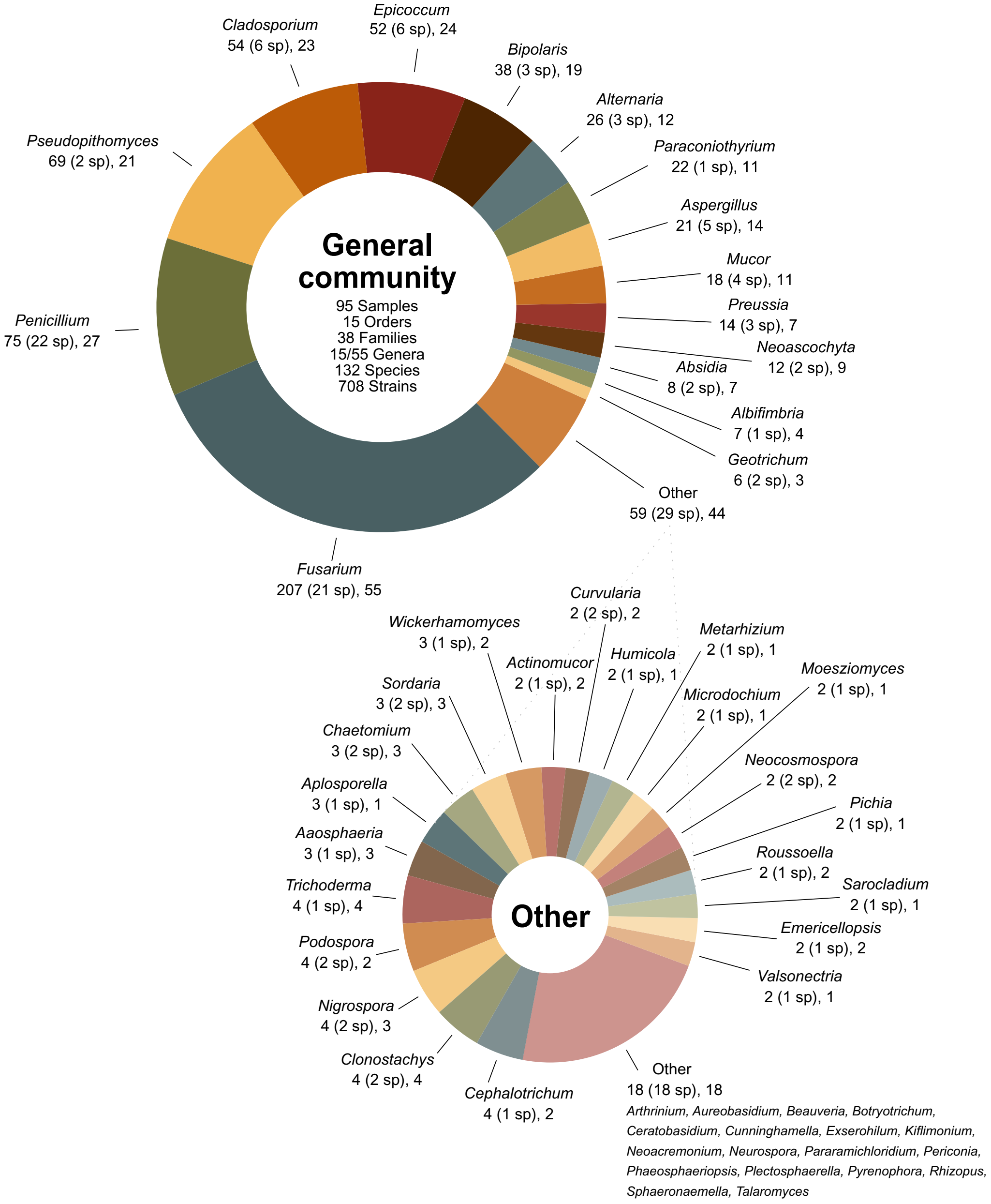

Fig. 1 Pie chart summarising the fungal community associated with dairy pastures in the Eastern Cape. The proportions are based on the number of strains isolated for each genus. The numbers in the legends stand for [x (y), z] x = the number of strains; y = the number of species identified; and z = the number of samples that a taxon was isolated from

Discussion

Dairy pastures can harbour a wide variety of fungi. In this study, we characterised the fungal community of 95 mixed grass samples from dairy pastures collected at 14 farms in the Eastern Cape of South Africa. The samples were collected when cattle showed signs of SILD symptoms in May 2020. The fungal communities detected were relatively diverse, with a total of 132 species identified across 55 genera. The most commonly detected genera were *Fusarium, Penicillium, Pseudopithomyces, Cladosporium, Epicoccum* and *Bipolaris.* Additionally, several isolates require further study that we could not identify with confidence at species level. These isolates may potentially belong to new species, represent new sequence variation within a species, or be described species that have not yet been sequenced. Two of these were recently described as new *Penicillium* species, *Pe. pascuigraminis* and *Pe. viridipigmentum*, in Visagie et al. (2024b).

Table 2 Data obtained from homology searches using ITS sequences against NCBI's Fungal ITS Reference Sequence Targeted Loci Project database

Genus	Strains	Closest species	GenBank information
Absidia	CMW 61050; CMW 61068; CN049G6	*Absidia aguabelensis*	strain URM 8213, GenBank NR_189383.1; Identities = 552/635 (87%), 31 gaps (4%)
Ceratobasidium	CN071G9	*Ceratobasidium ramicola*	strain CBS 133.82, GenBank NR_138368.1; Identities = 516/584 (88%), 26 gaps (4%)
Chaetomium	CN040E2; CMW 61055	*Chaetomium spirochaete*	strain CBS 730.84, GenBank NR_144823.1; Identities = 615/616 (99%), no gaps
Epicoccum	CMW 61078; CN049H1	*Epicoccum* sp. 1	strain NV-2016, GenBank LT593066.1; Identities = 798/825 (97%), no gaps
Geotrichum	CMW 61038; CMW 61356; CN050A4; CN050A6; CN056A6	*Geotrichum pandrosion*	strain BRIP 74954a, GenBank NR_189975.1; Identities = 228/231 (99%), no gaps
Neoacremonium	CMW 60726	*Neoacremonium vitellinum*	strain CBS 792.69, GenBank NG_058556.1; Identities = 224/228 (98%), no gaps
Neoascochyta	CN041C6; CMW 61348; CMW 61349; CN049G8; CN098F7; CN099B6	*Neoascochyta argentina*	strain CBS 112524, GenBank MT018302.1; Identities = 540/595 (91%), no gaps
	CN049H8; CN049H9; CN099A9	*Neoascochyta* sp. 3 LH-2020	strain CBS 140547, GenBank MT018308.1; Identities = 590/596 (99%), no gaps
Neurospora	CN110D3	*Gelasinospora brevispora*	strain CBS 547.94, GenBank NR_159858.1; Identities = 687/695 (99%), two gaps (0%)
Nigrospora	CMW 61524	*Nigrospora sacchari-officinarum*	strain CGMCC 3.19335, GenBank NR_165926.1; Identities = 530/536 (99%), one gap (0%)
Pararamichloridium	CN107C2	*Pararamichloridium aquisubtropicum*	strain GZAAS 21–0382, GenBank NR_185675.1; Identities = 591/602 (98%), five gaps (0%)
Preussia	CMW 61034; CMW 61041; CN048G1; CN049C4; CN049D9	*Preussia polymorpha*	strain CBS 117679, GenBank NR_137729.1; Identities = 387/414 (93%), nine gaps (2%)
	CMW 61079	*Preussia persica*	strain CBS 117680, GenBank NR_137730.1, Identities = 468/474 (99%), one gap (0%)
	CN115F1; CN115F2; CN115F3	*Preussia procaviae*	strain CBS 146827, GenBank NR_171769.1; Identities = 513/546 (94%), 12 gaps (2%)
Roussoella	CMW 61022; CN048E3	*Roussoella chinensis*	strain HKAS 125555, GenBank NR_189903.1; Identities = 451/471 (96%), three gaps (0%)
Sordaria	CMW 61042	*Sordaria equicola*	strain CBS 146992, GenBank NR_173047.1; Identities = 568/573 (99%), two gaps (0%)
Sphaeronaemella	CN128H6	*Candidozyma duobushaemuli*	strain CBS 7798, GenBank NR_130694.1; Identities = 264/337 (78%), 12 gaps (3%)
Valsonectria	CN041F7; CN041F8	*Valsonectria whitehaneyae*	strain BRIP 69038b, GenBank NR_191303.1; Identities = 610/644 (95%), nine gaps (1%)

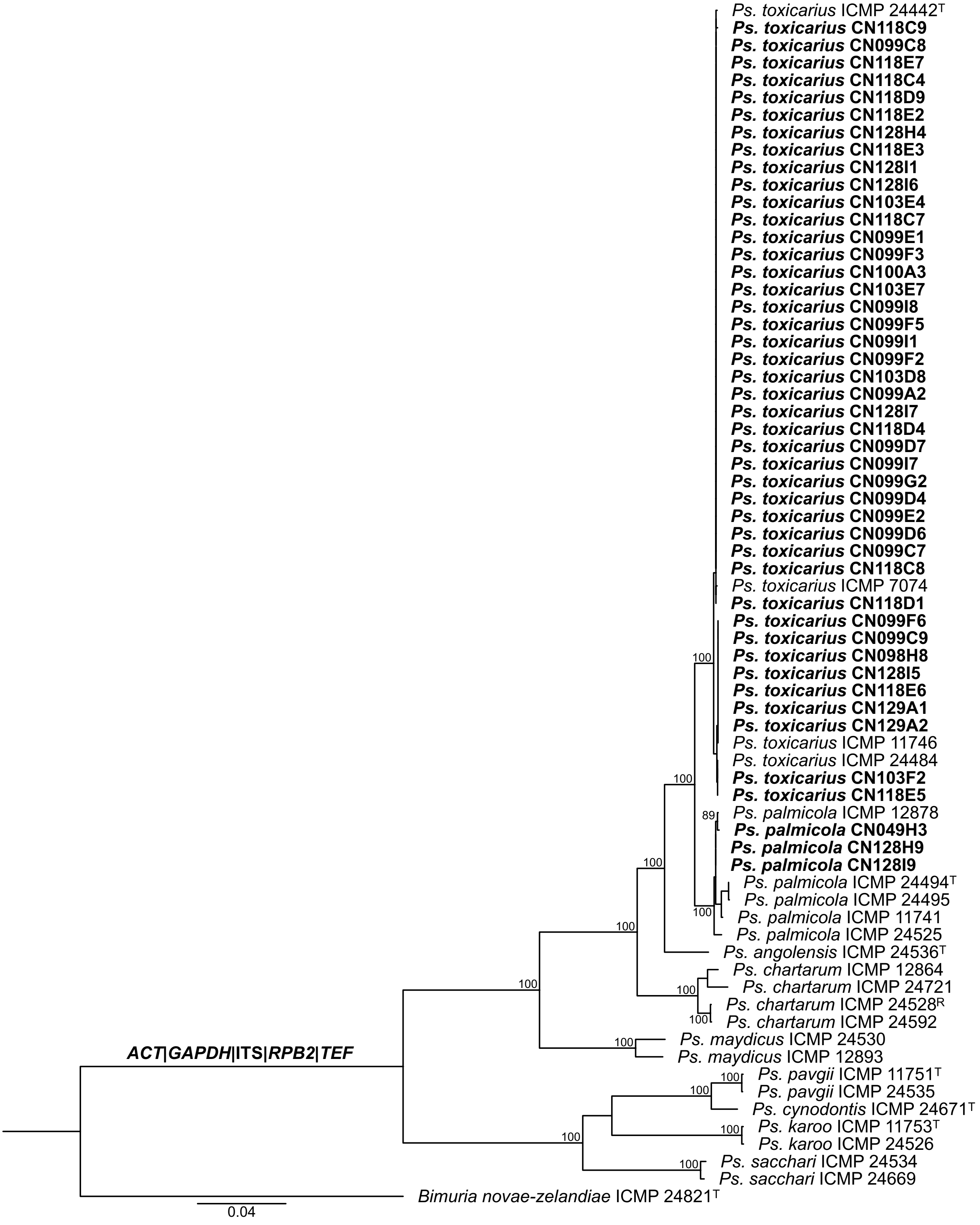

Fig. 2 Maximum likelihood tree of *Pseudopithomyces* based on a concatenated dataset of *ACT*, *GAPDH*, ITS, *RPB2* and *TEF*. Strains of species isolated from this study are shown in bold text. The tree was rooted to *Bimuria novae-zelandiae*. Branch support in nodes higher than 80% are indicated at relevant branches (^{T}= ex-type; ^{R}= reference strain)

The causal agent of SILD, *Ps. toxicarius*, was detected from 21 samples collected at two out of 14 farms. The taxonomy of *Pseudopithomyces*, and especially *Ps. chartarum*, had been complex until the recent revision by Weir et al. (2025). Previously, Da Cunha et al. (2014) and Pratibha and Prabhugaonkar (2015) showed that the genus *Pithomyces* was polyphyletic, with species resolved across several families in *Pleosporales*. The type of *Pithomyces*, *Pithomyces flavus*, belong to *Astrosphaeriellaceae* (Pratibha and Prabhugaonkar 2015), while *Leptosphaerulina chartarum*, erroneously thought to be the sexual morph of *P. chartarum* (Roux 1986), was shown to be a distant relative in *Didymellaceae* (Da Cunha et al. 2014). *Pithomyces chartarum*, *P. maydicus* and *P. sacchari* formed a unique group in *Didymosphaeriaceae*, with these species transferred into the newly described *Pseudopithomyces* (Ariyawansa et al. 2015). *Pseudopithomyces chartarum* was the designated type, with strain MUCL 15905 used as a reference strain, as the holotype (FH00781082) is a dried specimen on paper that has no living culture or sequence available. Weir et al. (2025) found that *Ps. chartarum* strains resolved in two clades in their phylogenetic analyses. The clade containing MUCL 15905 was found to mostly not produce sporidesmin and was considered to represent *Ps. chartarum*. The sporidesmin-producing clade was described as *Ps. toxicarius*, now considered the primary causative species responsible for SILD in New Zealand. Our limited survey suggests that *Ps. toxicarius* is similarly the causative species in South Africa, with *Ps. palmicola* not reported to produce sporidesmin.

Fusarium was the most commonly isolated genus, recovered from 55 of the 95 samples collected across 13 of the 14 dairy pastures. We expected a high number of *Fusarium*, belonging to several species, as it is known to colonise grasses (*Poaceae*) and can function in this niche as either an endophyte or plant pathogen (Leslie and Summerell 2006; Bentley et al. 2007; Nor Azliza et al. 2014; Laurence et al. 2016; Chehri et al. 2017). Most of the identified *Fusarium* species belonged to the FIESC, including *F. brevicaudatum, F. clavus, F. coffeatum, F. croceum* and three new species described as *F. cumulatum*, *F. mariecurieae* and *F. pascuum* by Dewing et al. (2025). Other species identified included those from the FSAMSC (*F. culmorum, F. graminearum, F. poae*), *Fusarium oxysporum* species complex (FOSC) (*F. fabacearum, F. glycines, F. wimaladesilvae* and two potentially new species), *Fusarium chlamydosporum* species complex (FCSC) (*F. chlamydosporum*) and *Fusarium fujikuroi* species complex (FFSC) (*F. terricola*). In line with our data, Botha et al. (2014a) previously reported the presence of *F. culmorum* and *F. oxysporum* in Eastern Cape kikuyu pastures, while Gabbedy et al. (1974) and Newsholme et al. (1983) reported *Fusarium* isolated from kikuyu from Australia and South Africa, respectively, identifying it only to the genus level. *Fusarium torulosum*, which was reported by Ryley et al. (2007) to be linked to kikuyu poisoning in Australia, was not isolated during our survey. We also did not isolate the previously reported *F. verticillioides* (= *F. moniliforme*) (Newsholme et al. 1983), *F. incarnatum* (= *F. semitectum*) and *F. subglutinans* (≡ *Fusarium moniliforme* var. *subglutinans*) (Wong et al. 1987) from Australian kikuyu grass. Some earlier studies from South Africa (Van der Merwe et al. 1979) and Australia (Peet et al. 1990) did not detect *Fusarium* in their surveys of grass pastures. In contrast, we identified several species not previously reported from pastures, including *F. brevicaudatum*, *F. cerealis*, *F. chlamydosorum*, *F. clavus, F. coffeatum*, *F. croceum*, *F. graminearum, F. poae* and *F. terricola.* Several of the species we identified are reported as mycotoxin producers (Villani et al. 2019; Munkvold et al. 2021).

Some mycotoxins produced by *Fusarium clavus* (zearalenone), *F. culmorum* (butenolide, deoxynivalenol, zearalenone), *F. graminearum* (butenolide, deoxynivalenol, zearalenone) and *F. poae* (butenolide) have been linked to cattle diseases (Tookey et al. 1972; Trenholm et al. 1985; Weaver et al. 1986; Mathur et al. 2001; Munkvold et al. 2021). Deoxynivalenol exposure can lead to abortion and chronic toxicity, manifesting as stunted growth, reduced weight gain, weakened immunity, and increased susceptibility to disease (Trenholm et al. 1985; Biscoto et al. 2022; Toutounchi et al. 2022). Zearalenone has been shown to induce hypoestrogenism, leading to issues such as infertility (Weaver et al. 1986; Fushimi et al. 2015; Gruber-Dorninger et al. 2021). Additionally, butenolide has the potential to cause lethal acute inflammation in the forestomach of cattle, a condition typically observed during kikuyu poisoning (Tookey et al. 1972). Although some mycotoxins produced by certain *Fusarium* species have been reported to be associated with kikuyu poisoning (Tookey et al. 1972; Ryley et al. 2007), no kikuyu poisoning symptoms were observed in the cattle during our survey in the Eastern Cape, and *F. torulosum* was not isolated during our survey. The lack of kikuyu poisoning symptoms during our survey highlights the fact that the presence of mycotoxigenic fungi does not necessarily provide evidence of mycotoxin production, while mycotoxin occurrence and concentration levels can vary considerably under suitable conditions (Gallo et al. 2015). Despite an increased awareness of *Fusarium* mycotoxin occurrences in pastures, data remain very limited to accurately assess the risk of *Fusarium* mycotoxin exposure in cattle.

Other mycotoxigenic species affecting cattle health and productivity were also identified in Eastern Cape pastures. *Aspergillus clavatus* is a known producer of patulin (Lopez-Diaz and Flannigan 1997; Varga et al. 2007; Houbraken et al. 2020), while *Penicillium* species such as *Pe. chrysogenum, Pe. coprophilum* and *Pe. crustosum* produce roquefortine C (El-banna et al. 1987; Frisvad and Filtenborg 1989; Häggblom 1990; Frisvad et al. 2004; Houbraken et al. 2012, 2016). Roquefortine C is a very common compound produced by many *Penicillium* species, including the blue cheese related *Pe. roqueforti* (Frisvad and Samson 2004). *Penicillium crustosum* also produces penitrem A–G (Frisvad et al. 2004; Botha et al. 2019), while *Pe. citrinum* produces citrinin (Lai et al. 2013). These mycotoxins have been shown to adversely affect cattle health, causing a range of issues such as fever, diarrhoea and skin irritation (citrinin); neurotoxicosis (patulin and penitrem A); incoordination and diaphoresis (penitrem A) and reversible paralytic effects (roquefortine C) (Häggblom 1990; Griffiths and Done 1991; Sabater-Vilar et al. 2004; Botha et al. 2014b, 2019). We did not confirm whether these compounds are produced by the strains isolated, but we can confirm that these strains were not widespread in the pastures based on the low numbers they were isolated from in our study. For example, *As. clavatus* was only represented by 12 strains, *Pe. crustosum* by 11 strains, *Pe. chrysogenum* by two strains and both *Pe. coprophilum* and *Pe. citrinum* by one strain each. Although these strains were present in low numbers, prolonged exposure to low levels of these mycotoxins could still negatively impact cattle health. The presence of these mycotoxigenic species in Eastern Cape pastures emphasises the need for vigilant monitoring and management practices to mitigate their impact on cattle health and productivity.

Our study reports more diverse communities than previous surveys conducted during outbreaks of SILD or kikuyu poisoning (Marasas et al. 1972; Bryson and Newsholme 1978; Van der Merwe et al. 1979; Newsholme et al. 1983; Wong et al. 1987; Ryley et al. 2007; Botha et al. 2014a). This could be attributed to earlier species identifications that relied on morphological characters, which often led to misidentifications, especially in groups containing cryptic species, thus underestimating overall fungal diversity. The incorporation of DNA sequencing in modern taxonomy has resulted in an increased number of species described in several genera (Hawksworth and Lücking 2017). In particular, genera such as *Aspergillus*, *Fusarium* and *Penicillium* have seen a sharp increase in the rate of species descriptions in the past decade, with many that are considered cryptic (Houbraken et al. 2020; Crous et al. 2021; Visagie et al. 2024a). For example, the FOSC was recently studied by Lombard et al. (2019) who provided new species names for the 15 lineages that they considered represented cryptic species. These findings highlight the importance of incorporating molecular techniques such as DNA sequencing to accurately assess fungal diversity in general, particularly in complex genera like *Fusarium*.

Our study provides baseline knowledge of the fungi present in Eastern Cape dairy pastures. With the use of molecular data, we discovered a more diverse fungal community than previously reported, including 28 potentially undescribed species. We also confirmed the presence of several mycotoxigenic species from the genera *Aspergillus, Fusarium*, *Penicillium* and *Pseudopithomyces*, which are known to potentially impact cattle health. Furthermore, we confirmed the presence of *Ps. toxicarius* in dairy pastures of the Eastern Cape, the causative species of SILD. Future work will focus on: 1) describing the new species identified in our study, 2) investigating their mycotoxin production potential and 3) analysing the secondary metabolite profiles of these new species to determine their potential effects on cattle health.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-025-02059-2.

Acknowledgements We would like to thank Anthony Davis and Jan Myburgh, who provided the samples analysed in this study. We are also grateful to Bevan Weir and Christine Voisey, for the discussions and sharing of data related to *Pseudopithomyces*. We acknowledge Kerri-Anne van Gaalen for isolating some of the strains used in this study.

Author contributions Claudette Dewing performed experiments, analysed the data and wrote the manuscript. Neriman Yilmaz, Emma T. Steenkamp, Brenda D. Wingfield and Cobus M. Visagie conceived and helped design the experiments. The original draft was composed by Claudette Dewing and reviewed and edited by Neriman Yilmaz, Emma T. Steenkamp, Brenda D. Wingfield and Cobus M. Visagie. All authors read and approved the final manuscript. Resources were provided by Neriman Yilmaz, Emma T. Steenkamp, Brenda D. Wingfield and Cobus M. Visagie. Funding was acquired by Neriman Yilmaz, and Cobus M. Visagie.

Funding Open access funding provided by University of Pretoria. We received funding from the National Research Foundation (NRF) of South Africa (grant number: 137791), the European Union's Horizon 2020 research and innovation program (RISE) under the Marie Skłodowska-Curie grant agreement No. 101008129, project acronym "Mycobiomics", and the Future Leaders—African Independent Research fellowship program (FLAIR, FLR\R1\201831).

Data availability All sequence data generated for this work can be accessed via GenBank: https://www.ncbi.nlm.nih.gov/genbank/. Other additional data files containing the reference datasets, alignments, and tree files were uploaded to Figshare (https://doi.org/10.6084/m9.figshare.27936900).

Declarations

Ethical approval Ethical approval for this study is not applicable.

Consent to participate All authors consent to participate in this study.

Consent to publish All authors consent to publication of this study.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abbas HK, Mirocha CJ (1988) Isolation and purification of a hemorrhagic factor (wortmannin) from *Fusarium oxysporum* (N17B). Appl Environ Microbiol 54:1268–1274. https://doi.org/10.1128/aem.54.5.1268-1274.1988

Ariyawansa HA, Hyde KD, Jayasiri SC, Buyck B, Chethana KWT, Dai DQ, Dai YC, Daranagama DA, Jayawardena RS, Lücking R, Ghobad-Nejhad M, Niskanen T, Thambugala KM, Voigt K, Zhao RL, Li G-J, Doilom M, Boonmee S, Yang ZL, Cai Q, Cui Y-Y, Bahkali AH, Chen J, Cui BK, Chen JJ, Dayarathne MC, Dissanayake AJ, Ekanayaka AH, Hashimoto A, Hongsanan S, Jones EBG, Larsson E, Li WJ, Li Q-R, Liu JK, Luo ZL, Maharachchikumbura SSN, Mapook A, McKenzie EHC, Norphanphoun C, Konta S, Pang KL, Perera RH, Phookamsak R, Phukhamsakda C, Pinruan U, Randrianjohany E, Singtripop C, Tanaka K, Tian CM, Tibpromma S, Abdel-Wahab MA, Wanasinghe DN, Wijayawardene NN, Zhang J-F, Zhang H, Abdel-Aziz FA, Wedin M, Westberg M, Ammirati JF, Bulgakov TS, Lima DX, Callaghan TM, Callac P, Chang C-H, Coca LF, Dal-Forno M, Dollhofer V, Fliegerová K, Greiner K, Griffith GW, Ho H-M, Hofstetter V, Jeewon R, Kang JC, Wen T-C, Kirk PM, Kytövuori I, Lawrey JD, Xing J, Li H, Liu ZY, Liu XZ, Liimatainen K, Lumbsch HT, Matsumura M, Moncada B, Nuankaew S, Parnmen S, De Azevedo SALCM, Sommai S, Song Y, De Souza CAF, De Souza-Motta CM, Su HY, Suetrong S, Wang Y, Wei S-F, Wen TC, Yuan HS et al (2015) Fungal diversity notes 111–252—Taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers 75:27–274. https://doi.org/10.1007/s13225-015-0346-5

Bensch K, Braun U, Groenewald JZ, Crous PW (2012) The genus *Cladosporium*. Stud Mycol 72:1–401. https://doi.org/10.3114/sim0003

Bentley AR, Petrovic T, Griffiths SP, Burgess LW, Summerell BA (2007) Crop pathogens and other *Fusarium* species associated with *Austrostipa aristiglumis*. Australas Plant Pathol 36:434–438. https://doi.org/10.1071/AP07047

Berbee ML, Pirseyedi M, Hubbard S (1999) *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91:964–977. https://doi.org/10.1080/00275514.1999.12061106

Bezille P, Braun JP, Jl B (1984) First identification of facial eczema in sheep in Europe. Epidemiological, clinical and biological aspects. Recueil Med Vet 160:339–347

Biscoto GL, Salvato LA, Alvarenga ÉR, Dias RR, Pinheiro GR, Rodrigues MP, Pinto PN, Freitas RP, Keller KM (2022) Mycotoxins in cattle feed and feed ingredients in Brazil: a five-year survey. Toxins 14:552. https://doi.org/10.3390/toxins14080552

Bissett J, Gams W, Jaklitsch W, Samuels GJ (2015) Accepted *Trichoderma* names in the year 2015. IMA Fungus 6:263–295. https://doi.org/10.5598/imafungus.2015.06.02.02

Botha PR, Meeske R, Snyman HA (2008a) Kikuyu over-sown with ryegrass and clover: grazing capacity, milk production and milk composition. Afr J Range Forage Sci 25:103–110. https://doi.org/10.2989/AJRF.2008.25.3.2.599

Botha PR, Meeske R, Snyman HA (2008b) Kikuyu over-sown with ryegrass and clover: dry matter production, botanical composition and nutritional value. Afr J Range Forage Sci 25:93–101. https://doi.org/10.2989/AJRF.2008.25.3.1.598

Botha CJ, Truter M, Jacobs A (2014a) *Fusarium* species isolated from *Pennisetum clandestinum* collected during outbreaks of kikuyu poisoning in cattle in South Africa. Onderstepoort J Vet Res 81:e1–e8. https://doi.org/10.4102/ojvr.v81i1.803

Botha CJ, Legg MJ, Truter M, Sulyok M (2014b) Multitoxin analysis of *Aspergillus clavatus*-infected feed samples implicated in two outbreaks of neuromycotoxicosis in cattle in South Africa. Onderstepoort J Vet Res 81:e1–e6. https://doi.org/10.4102/ojvr.v81i1.848

Botha CJ, Visagie CM, Sulyok M (2019) Putative neuromycotoxicoses in an adult male following ingestion of moldy walnuts. Mycotoxin Res 35:9–16. https://doi.org/10.1007/s12550-018-0326-1

Bourke CA (2007) A review of kikuyu grass (*Pennisetum clandestinum*) poisoning in cattle. Aust Vet J 85:261–267. https://doi.org/10.1111/j.1751-0813.2007.00168.x

Bryson RW (1982) Kikuyu poisoning and the army worm. J S Afr Vet Assoc 53:161–165

Bryson RW, Newsholme SJ (1978) Kikuyu grass poisoning of cattle in Natal. J South Afr Vet Assoc 49:19–21. https://doi.org/10.10520/AJA00382809_4112

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556. https://doi.org/10.1080/00275514.1999.12061051

Chehri K, Hajeb S, Maassoumi S (2017) Morphological and molecular identification and PCR amplification to determine the toxigenic potential of *Fusarium graminearum* species complex (FGSC) isolated from wild grasses in Iran. J Agric Sci Technol 19:1617–1629

Crous PW, Lombard L, Sandoval-Denis M, Seifert KA, Schroers HJ, Chaverri P, Gené J, Guarro J, Hirooka Y, Bensch K, Kema GHJ, Lamprecht SC, Cai L, Rossman AY, Stadler M, Summerbell RC, Taylor JW, Ploch S, Visagie CM, Yilmaz N, Frisvad JC, Abdel-Azeem AM, Abdollahzadeh J, Abdolrasouli A, Akulov A, Alberts JF, Araújo JPM, Ariyawansa HA, Bakhshi M, Bendiksby M, Ben Hadj Amor A, Bezerra JDP, Boekhout T, Câmara MPS, Carbia M, Cardinali G, Castañeda-Ruiz RF, Celis A, Chaturvedi V, Collemare J, Croll D, Damm U, Decock CA, De Vries RP, Ezekiel CN, Fan XL, Fernández NB, Gaya E, González CD, Gramaje D, Groenewald JZ, Grube M, Guevara-Suarez M, Gupta VK, Guarnaccia V, Haddaji A, Hagen F, Haelewaters D, Hansen K, Hashimoto A, Hernández-Restrepo M, Houbraken J, Hubka V, Hyde KD, Iturriaga T, Jeewon R, Johnston PR, Jurjević Ž, Karalti İ, Korsten L, Kuramae EE, Kušan I, Labuda R, Lawrence DP, Lee HB, Lechat C, Li HY, Litovka YA, Maharachchikumbura SSN, Marin-Felix Y, Matio Kemkuignou B, Matočec N, McTaggart AR, Mlčoch P, Mugnai L, Nakashima C, Nilsson RH, Noumeur SR, Pavlov IN, Peralta MP, Phillips AJL, Pitt JI, Polizzi G, Quaedvlieg W, Rajeshkumar KC, Restrepo S, Rhaiem A, Robert J, Robert V, Rodrigues AM et al (2021) *Fusarium*: more than a node or a foot-shaped basal cell. Stud Mycol 98:100116. https://doi.org/10.1016/j.simyco.2021.100116

Da Cunha KC, Sutton DA, Gené J, Cano J, Capilla J, Madrid H, Decock C, Wiederhold NP, Guarro J (2014) *Pithomyces* species (*Montagnulaceae*) from clinical specimens: identification and antifungal susceptibility profiles. Med Mycol 52:748–757. https://doi.org/10.1093/mmy/myu044

Davis AJ, Jordaan DS, Myburgh JG (2025) First report of a sporidesmin toxicity (Facial Eczema) outbreak in a South African dairy herd. J S Afr Vet Assoc 96:33–38. https://doi.org/10.36303/jsava.662

De Hoog GS, Van den Ende AHGG (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. Mycoses 41:183–189. https://doi.org/10.1111/j.1439-0507.1998.tb00321.x

De Wet JA, Erasmus JA (1984) Suspected facial eczema in sheep in the central Orange Free State. J S Afr Vet Assoc 55:199–200

Department of Agriculture Land Reform and Rural Development (2023) Newsletter: National livestock statistics https://old.dalrrd.gov.za/Home/Crop-Estimates/Statistical-Information/Livestock. Accessed on 15 April 2024

Dewing A, Visagie CM, Steenkamp ET, Wingfield BD, Yilmaz N (2025) Three new species of *Fusarium* (*Nectriaceae, Hypocreales)* isolated from Eastern Cape dairy pastures in South Africa. MycoKeys 115:241–271

Di Menna ME, Smith BL, Miles CO (2009) A history of facial eczema (pithomycotoxicosis) research. N Z J Agric Res 52:345–376. https://doi.org/10.1080/00288230909510519

Edwards J, Richards R, Love R, Harrison M, Gwynn R (1983) An abattoir survey of the prevalence of facial eczema in sheep in Western Australia. Aust Vet J 60:157. https://doi.org/10.1111/j.1751-0813.1983.tb05937.x

El-banna AA, Pitt JI, Leistner L (1987) Production of mycotoxins by *Penicillium* species. Syst Appl Microbiol 10:42–46. https://doi.org/10.1016/S0723-2020(87)80008-5

Frisvad JC, Filtenborg O (1989) Terverticillate Penicillia: chemotaxonomy and mycotoxin production. Mycologia 81:837–861. https://doi.org/10.1080/00275514.1989.12025674

Frisvad JC, Samson RA (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud Mycol 49:1–174

Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004) Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Stud Mycol 49:201–241

Fuertes M, Martínez A, Moreno O, Benavides J, Menéndez J, García Marín J, Pérez V, Ferreras M (2004) Estudio clínico y lesional de un brote de fotosensibilización ovina de origen hepatógeno en Asturias. Producción Ovina y Caprina: XXIX Jornadas Científicas. VIII Internacionales de la Sociedad Española de Ovinotecnia y Caprinotecnia. Sociedad Española de Ovinotecnia y Caprinotecnia SEOC

Fushimi Y, Takagi M, Monniaux D, Uno S, Kokushi E, Shinya U, Kawashima C, Otoi T, Deguchi E, Fink-Gremmels J (2015) Effects of dietary contamination by zearalenone and its metabolites on serum anti-müllerian hormone: impact on the reproductive performance of breeding cows. Reprod Domest Anim 50:834–839. https://doi.org/10.1111/rda.12599

Gabbedy BJ, Gwynn R, Hopkinson WI, Kay BE, Wood PM (1974) Kikuyu poisoning of cattle in Western Australia. Aust Vet J 50:369–370. https://doi.org/10.1111/j.1751-0813.1974.tb14116.x

Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF (2015) Review on mycotoxin issues in ruminants: occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. Toxins 7:3057–3111. https://doi.org/10.3390/toxins7083057

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Griffiths IB, Done SH (1991) Citrinin as a possible cause of the pruritus, pyrexia, haemorrhagic syndrome in cattle. Vet Rec 129:113–117. https://doi.org/10.1136/vr.129.6.113

Gruber-Dorninger C, Faas J, Doupovec B, Aleschko M, Stoiber C, Höbartner-Gußl A, Schöndorfer K, Killinger M, Zebeli Q, Schatzmayr D (2021) Metabolism of zearalenone in the rumen of dairy cows with and without application of a zearalenone-degrading enzyme. Toxins 13:84. https://doi.org/10.3390/toxins13020084

Gunther R, Abbas HK, Mirocha CJ (1989) Acute pathological effects on rats of orally administered wortmannin-containing preparations and purified wortmannin from *Fusarium oxysporum*. Food Chem Toxicol 27:173–179. https://doi.org/10.1016/0278-6915(89)90066-5

Häggblom P (1990) Isolation of roquefortine C from feed grain. Appl Environ Microbiol 56:2924–2926. https://doi.org/10.1128/aem.56.9.2924-2926.1990

Hansen D, McCoy R, Hedstrom O, Snyder S, Ballerstedt P (1994) Photosensitization associated with exposure to *Pithomyces chartarum* in lambs. J Am Vet Med Assoc 204:1668–1671

Hawksworth DL, Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectr 5. https://doi.org/10.1128/microbiolspec.FUNK-0052-2016

Hernández-Restrepo M, Madrid H, Tan YP, Da Cunha KC, Gené J, Guarro J, Crous PW (2018) Multi-locus phylogeny and taxonomy of *Exserohilum*. Persoonia 41:71–108. https://doi.org/10.3767/persoonia.2018.41.05

Hong SB, Cho HS, Shin HD, Frisvad JC, Samson RA (2006) Novel *Neosartorya* species isolated from soil in Korea. Int J Syst Evol Microbiol 56:477–486. https://doi.org/10.1099/ijs.0.63980-0

Hou LW, Groenewald JZ, Pfenning LH, Yarden O, Crous PW, Cai L (2020) The phoma-like dilemma. Stud Mycol 96:309–396. https://doi.org/10.1016/j.simyco.2020.05.001

Houbraken J, Frisvad JC, Seifert KA, Overy DP, Tuthill DM, Valdez JG, Samson RA (2012) New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*. Persoonia 29:78–100. https://doi.org/10.3767/003158512x660571

Houbraken J, Wang L, Lee HB, Frisvad JC (2016) New sections in *Penicillium* containing novel species producing patulin, pyripyropens or other bioactive compounds. Persoonia 36:299–314. https://doi.org/10.3767/003158516X692040

Houbraken J, Kocsubé S, Visagie CM, Yilmaz N, Wang XC, Meijer M, Kraak B, Hubka V, Bensch K, Samson RA, Frisvad JC (2020) Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (*Eurotiales*): An overview of families, genera, subgenera, sections, series and species. Stud Mycol 95:5–169. https://doi.org/10.1016/j.simyco.2020.05.002

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Kellerman TS, Coetzer JA (1985) Hepatogenous photosensitivity diseases in South Africa. Onderstepoort J Vet Res 52:157–173

Kellerman TS, Coetzer JAW, Naudé TW, Botha CJ (2005) Plant poisonings and mycotoxicoses of livestock in Southern Africa. Oxford University Press, Cape Town

Lai D, Brötz-Oesterhelt H, Müller WE, Wray V, Proksch P (2013) Bioactive polyketides and alkaloids from *Penicillium citrinum*, a fungal endophyte isolated from *Ocimum tenuiflorum*. Fitoterapia 91:100–106. https://doi.org/10.1016/j.fitote.2013.08.017

Laurence MH, Walsh JL, Shuttleworth LA, Robinson DM, Johansen RM, Petrovic T, Vu TT, Burgess LW, Summerell BA, Liew EC (2016) Six novel species of *Fusarium* from natural ecosystems in Australia. Fungal Diversity 77:349–366. https://doi.org/10.1007/s13225-015-0337-6

Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual. Blackwell Professional Publishing. Ames, Iowa. https://doi.org/10.1002/9780470278376

Liu Y, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16:1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Liu L, Zhang J, Qu W, Wang Y, Yan G, Li Z, Zhang Y, Zhang H (2023) Fungi, including *Pithomyces chartarum* cause facial eczema and inflammation in grazing sheep in Western China. Microb Pathog 185:106451. https://doi.org/10.1016/j.micpath.2023.106451

Lombard L, Sandoval-Denis M, Lamprecht SC, Crous PW (2019) Epitypification of *Fusarium oxysporum* - clearing the taxonomic chaos. Persoonia 43:1–47. https://doi.org/10.3767/persoonia.2019.43.01

Lopez-Diaz TM, Flannigan B (1997) Production of patulin and cytochalasin E by *Aspergillus clavatus* during malting of barley and wheat. Int J Food Microbiol 35:129–136. https://doi.org/10.1016/S0168-1605(96)01211-1

Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD (2012) A phylogenetic and taxonomic re-evaluation of the *Bipolaris* - *Cochliobolus* - *Curvularia* complex. Fungal Divers 56:131–144. https://doi.org/10.1007/s13225-012-0189-2

Marais JP (2001) Factors affecting the nutritive value of kikuyu grass (*Pennisetum clandestinum*) - a review. Trop Grassl 35:65–84

Marasas WF, Adelaar TF, Kellerman TS, Minné JA, Van Rensburg IB, Burroughs GW (1972) First report of facial eczema in sheep in South Africa. Onderstepoort J Vet Res 39:107–112

Martinovich D, Mortimer PH, di Menna ME (1972) Similarities between so-called kikuyu poisoning of cattle and two experimental mycotoxicoses. N Z Vet J 20:57–58. https://doi.org/10.1080/00480169.1972.34008

Masclaux F, Guého E, De Hoog GS, Christen R (1995) Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. J Med Vet Mycol 33:327–338. https://doi.org/10.1080/02681219580000651

Mathur S, Constable PD, Eppley RM, Waggoner AL, Tumbleson ME, Haschek WM (2001) Fumonisin B1 is hepatotoxic and nephrotoxic in milk-fed calves. Toxicol Sci 60:385–396. https://doi.org/10.1093/toxsci/60.2.385

Milk Producers' Organisation (2024) Lacto data. https://www.mpo.co.za/news-trends/lacto-data/. Accessed 15 April 2024

MilkSA (2023) Kikuyu for dairying https://milksa.co.za/research/dairy-rd-in-sa/kikuyu-dairying. Accessed 18 January 2024

MilkSA (2024) Productivity of kikuyu–ryegrass pasture as affected by method of cultivation https://milksa.co.za/research/dairy-rd-in-sa/productivity-kikuyu%E2%80%93ryegrass-pasture-affected-method-cultivation. Accessed 21 July 2024

Munkvold GP, Proctor RH, Moretti A (2021) Mycotoxin production in *Fusarium* according to contemporary species concepts. Annu Rev Phytopathol 59:373–402. https://doi.org/10.1146/annurev-phyto-020620-102825

Newsholme SJ, Kellerman TS, Van der Westhuizen GCA, Soley JT (1983) Intoxication of cattle on kikuyu grass following army worm (*Spodoptera exempta*) invasion. Onderstepoort J Vet Res 50:157–167

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300

Nor Azliza I, Hafizi R, Nurhazrati M, Salleh B (2014) Production of major mycotoxins by *Fusarium* species isolated from wild grasses in peninsular Malaysia. Sains Malaysiana 43:89–94

O'Donnell K, Ward TJ, Robert VARG, Crous PW, Geiser DM, Kang S (2015) DNA sequence-based identification of *Fusarium*: Current status and future directions. Phytoparasitica 43:583–595. https://doi.org/10.1007/s12600-015-0484-z

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116. https://doi.org/10.1006/mpev.1996.0376

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Nat Acad Sci USA 95:2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Ozmen O, Sahinduran S, Haligur M, Albay MK (2008) Clinicopathological studies on facial eczema outbreak in sheep in Southwest Turkey. Trop Anim Health Prod 40:545–551. https://doi.org/10.1007/s11250-008-9132-7

Peet RL, Dickson J, Hare M (1990) Kikuyu poisoning in goats and sheep. Aust Vet J 67:229–230. https://doi.org/10.1111/j.1751-0813.1990.tb07769.x

Pinto C, Santos V, Dinis J, Peleteiro M, Fitzgerald J, Hawkes A, Smith B (2005) Pithomycotoxicosis (facial eczema) in ruminants in the Azores, Portugal. Vet Rec 157:805–810. https://doi.org/10.1136/vr.157.25.805

Pollock ML, Wishart H, Holland JP, Malone FE, Waterhouse A (2015) Photosensitisation of livestock grazing *Narthecium ossifragum*: Current knowledge and future directions. Vet J 206:275–283. https://doi.org/10.1016/j.tvjl.2015.07.022

Pratibha J, Prabhugaonkar A (2015) Multi-gene phylogeny of *Pithomyces* with the sexual morph of *P. flavus* Berk. & Broome. Phytotaxa 218:84–90. https://doi.org/10.11646/phytotaxa.218.1.7

Reeb V, Lutzoni F, Roux C (2004) Contribution of *RPB2* to multilocus phylogenetic studies of the euascomycetes (*Pezizomycotina*, *Fungi*) with special emphasis on the lichen-forming *Acarosporaceae* and evolution of polyspory. Mol Phylogenet Evol 32:1036–1060. https://doi.org/10.1016/j.ympev.2004.04.012

Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res 98:625–634. https://doi.org/10.1016/S0953-7562(09)80409-7

Riet A, Diaz L (1974) El hongo *Pithomyces chartarum* asociado con casos de fotosensibilización hepatógena en bovinos. Presented at the INTA–CNPGL Exchange Meeting, 19–21 June, Paysandú

Riet-Correa F, Rivero R, Odriozola E, Adrien MdL, Medeiros RM, Schild AL (2013) Mycotoxicoses of ruminants and horses. J Vet Diagn Invest 25:692–708. https://doi.org/10.1177/10406387135045

Roux C (1986) *Leptosphaerulina chartarum* sp.nov., the teleomorph of *Pithomyces chartarum*. Trans Br Mycol Soc 86:319–323. https://doi.org/10.1016/S0007-1536(86)80163-2

Ryley MJ, Bourke CA, Liew ECY, Summerell BA (2007) Is *Fusarium torulosum* the causal agent of kikuyu poisoning in Australia? Aust Plant Dis Notes 2:133–135. https://doi.org/10.1071/DN07053

Sabater-Vilar M, Maas RF, De Bosschere H, Ducatelle R, Fink-Gremmels J (2004) Patulin produced by an *Aspergillus clavatus* isolated from feed containing malting residues associated with a lethal neurotoxicosis in cattle. Mycopathologia 158:419–426. https://doi.org/10.1007/s11046-005-2877-x

Samson RA, Visagie CM, Houbraken J, Hong S-B, Hubka V, Klaassen CH, Perrone G, Seifert KA, Susca A, Tanney JB (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol 78:141–173

Samuels GJ (2006) *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96:195–206. https://doi.org/10.1094/phyto-96-0195

Sánchez Márquez S, Bills GF, Herrero N, Zabalgogeazcoa Í (2012) Non-systemic fungal endophytes of grasses. Fungal Ecol 5:289–297. https://doi.org/10.1016/j.funeco.2010.12.001

Schoch C, Seifert K, Huhndorf S, Robert V, Spouge J, Levesque C, Chen W (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Nat Acad Sci USA 109:6241–6246. https://doi.org/10.1073/pnas.1117018109

Schoch CL, Robbertse B, Robert V, Vu D, Cardinali G, Irinyi L, Meyer W, Nilsson RH, Hughes K, Miller AN, Kirk PM, Abarenkov K, Aime MC, Ariyawansa HA, Bidartondo M, Boekhout T, Buyck B, Cai Q, Chen J, Crespo A, Crous PW, Damm U, De Beer ZW, Dentinger BT, Divakar PK, Dueñas M, Feau N, Fliegerova K, García MA, Ge ZW, Griffith GW, Groenewald JZ, Groenewald M, Grube M, Gryzenhout M, Gueidan C, Guo L, Hambleton S, Hamelin R, Hansen K, Hofstetter V, Hong SB, Houbraken J, Hyde KD, Inderbitzin P, Johnston PR, Karunarathna SC, Kõljalg U, Kovács GM, Kraichak E, Krizsan K, Kurtzman CP, Larsson KH, Leavitt S, Letcher PM, Liimatainen K, Liu JK, Lodge DJ, Luangsa-ard JJ, Lumbsch HT, Maharachchikumbura SS, Manamgoda D, Martín MP, Minnis AM, Moncalvo JM, Mulè G, Nakasone KK, Niskanen T, Olariaga I, Papp T, Petkovits T, Pino-Bodas R, Powell MJ, Raja HA, Redecker D, Sarmiento-Ramirez JM, Seifert KA, Shrestha B, Stenroos S, Stielow B, Suh SO, Tanaka K, Tedersoo L, Telleria MT, Udayanga D, Untereiner WA, Diéguez Uribeondo J, Subbarao KV, Vágvölgyi C, Visagie C, Voigt K, Walker DM, Weir BS, Weiß M, Wijayawardene NN, Wingfield MJ, Xu JP, Yang ZL, Zhang N, Zhuang WY et al (2014) Finding needles in haystacks: Linking scientific names, reference specimens and molecular data for Fungi. Database 2014: bau061. https://doi.org/10.1093/database/bau061

Smith G (2021) South Africa's dairy sector strives to shake off its past. https://www.darigold.com/the-daily-churn/page/3/. Accessed 12 April 2024

Swanepoel PA, Botha PR, Snyman HA, Du Preez CC (2014) Impact of cultivation method on productivity and botanical composition of a kikuyu–ryegrass pasture. Afr J Range Forage Sci 31:215–220. https://doi.org/10.2989/10220119.2014.903999

Tookey HL, Yates SG, Ellis JJ, Grove MD, Nichols RE (1972) Toxic effects of a butenolide mycotoxin and of *Fusarium tricinctum* cultures in cattle. J Am Vet Med Assoc 160:1522–1526

Toutounchi NS, Braber S, Van't Land B, Thijssen S, Garssen J, Folkerts G, Hogenkamp A (2022) Deoxynivalenol exposure during pregnancy has adverse effects on placental structure and immunity in mice model. Reprod Toxicol 112:109-118. https://doi.org/10.1016/j.reprotox.2022.07.002

Trenholm H, Thompson B, Martin K, Greenhalgh R, McAllister A (1985) Ingestion of vomitoxin (deoxynivalenol)-contaminated wheat by nonlactating dairy cows. J Dairy Sci 68:1000–1005

Truter WF, Botha PR, Dannhauser CS, Maasdorp BV, Miles N, Smith A, Snyman HA, Tainton NM (2015) Southern African pasture and forage science entering the 21st century: past to present Afr J Range Forage Sci 32:73-89. https://doi.org/10.2989/10220119.2015.1054429

Van der Colf J, Botha PR, Meeske R, Truter WF (2015) Grazing capacity, milk production and milk composition of kikuyu oversown with annual or perennial ryegrass. Afr J Range Forage Sci 32:143–151. https://doi.org/10.2989/10220119.2015.1052096

Van der Merwe WJ, Eicker A, Marasas WF, Kellerman TS (1979) Aerospora of an *Eragrostis curvula* pasture in South Africa. Onderstepoort J Vet Res 46:19–25

Van Wuijckhuise L, Snoep J, Cremers G, Duvivier A, Groeneveld A, Ottens W, Van Der Sar S (2006) First case of pithomycotoxicosis (facial eczema) in the Netherlands. Tijdschr Diergeneeskd 131:858–861

Varga J, Due M, Frisvad JC, Samson RA (2007) Taxonomic revision of *Aspergillus* section *Clavati* based on molecular, morphological and physiological data. Stud Mycol 59:89–106. https://doi.org/10.3114/sim.2007.59.11

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Villani A, Proctor RH, Kim H-S, Brown DW, Logrieco AF, Amatulli MT, Moretti A, Susca A (2019) Variation in secondary metabolite production potential in the *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes. BMC Genomics 20:314. https://doi.org/10.1186/s12864-019-5567-7

Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CH, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA (2014) Identification and nomenclature of the genus *Penicillium*. Stud Mycol 78:343–371. https://doi.org/10.1016/j.simyco.2014.09.001

Visagie CM, Yilmaz N, Kocsubé S, Frisvad JC, Hubka V, Samson RA, Houbraken J (2024a) A review of recently introduced *Aspergillus*, *Penicillium*, *Talaromyces* and other Eurotiales species. Stud Mycol 107:1–66. https://doi.org/10.3114/sim.2024.107.01

Visagie CM, Yilmaz N, Allison JD, Barreto RW, Boekhout T, Boers J, Delgado MA, Dewing C, Fitza KNE, Furtado ECA, Gaya E, Hill R, Hobden A, Hu DM, Hülsewig T, Khonsanit A, Luangsa-Ard JJ, Mthembu A, Pereira CM, Price JL, Pringle A, Qikani N, Sandoval-Denis M, Schumacher RK, Seifert KA, Slippers B, Tennakoon DS, Thanakitpipattana D, Van Vuuren NI, Groenewald JZ, Crous PW (2024b) New and interesting fungi. 7. Fungal Syst Evol 13:441–494. https://doi.org/10.3114/fuse.2024.13.12

Weaver G, Kurtz H, Behrens J, Robison T, Seguin B, Bates F, Mirocha C (1986) Effect of zearalenone on the fertility of virgin dairy heifers. Am J Vet Res 47:1395–1397

Weir BS, Sidhu JS, Brosnahan CL, Lee D, Maclean PH, Park D, Jauregui R, Johnson RD, Petterson ME, Williams AFR, Morse NR, Sprosen JM, Lim Y-W, Bridgeman BJ, Walker TJ, Kumar S, Mace WJ, Prakash S, Liu X, Hume DE, Couldrey C, Beever RE, Voisey CR (2025) Global diversity analysis of plant-associated *Pseudopithomyces* fungi reveals a new species producing the toxin associated with facial eczema in livestock: *Pseudopithomyces toxicarius* sp. nov. Stud Mycol 112:39–73. https://doi.org/10.3114/sim.2025.112.02

Wong PT, Roth IJ, Jackson AR (1987) Kikuyu poisoning of cattle in New South Wales and its relationship to pasture fungi on kikuyu grass (*Pennisetum clandestinum*). Austrian Veterinary Journal 64:229–232. https://doi.org/10.1111/j.1751-0813.1987.tb09688.x

Woudenberg JH, Groenewald JZ, Binder M, Crous PW (2013) *Alternaria* redefined. Stud Mycol 75:171–212. https://doi.org/10.3114/sim0015

Yilmaz N, Sandoval-Denis M, Lombard L, Visagie CM, Wingfield BD, Crous PW (2021) Redefining species limits in the *Fusarium fujikuroi* species complex. Persoonia 46:129–162. https://doi.org/10.3767/persoonia.2021.46.05

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.