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# First report of various *Fusarium* species from the Stevenson-Hamilton Supersite granite catena system in the Kruger National Park, South Africa

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## Introduction

The Kruger National Park (KNP) covers the north-eastern part of southern Africa (Carruthers 2017) and is also linked with the Gonarezhou National Park (Zimbabwe) and the Limpopo National Park (Mozambique) as the Great Limpopo Transfrontier Park. The KNP is part of the Kruger to Canyons Biosphere area designated by the United Nations Educational, Scientific and Cultural Organization (UNESCO) as an International Man and Biosphere Reserve (the 'Biosphere') (http://www.unesco.org/new/en/natural-sciences/environment/ecological-sciences/biosphere-reserves/africa/south-africa/kruger-to-canyons/). The Stevenson-Hamilton Supersite, where this study was conducted, is part of four research 'supersites' in the KNP, with each representing distinct geological, climatic and linked biodiversity patterns (Smit et al. 2013).

The foundational biological information regarding soil biota in South Africa was recently assessed, and it included soil fungi (Janion-Scheepers et al. 2016). These authors reported that despite South Africa being only 0.8% of the earth's terrestrial area, it contains nearly 1.8% of the world's described soil species. Areas such as the Nama-Karoo, Northern Cape and Eastern Cape are undersampled for most taxa as well as natural soils in biodiversity hotspots. Similarly, the KNP with its diverse ecosystems is not well explored.

The fungal genus *Fusarium* has a cosmopolitan distribution and includes a vast number of species. These species are commonly recovered from a variety of substrates including soil, air, water and decaying plant materials (Leslie & Summerell 2006). They have diverse ecosystem functions in soils and are also able to colonise living tissues of plants and animals, including humans, acting as endophytes (microbial organisms existing inside plant tissues), secondary invaders or becoming devastating plant pathogens (Nelson, Dignani & Anaissie 1994). In addition to their ability to colonise a multiplicity of habitats, *Fusarium* species are present in almost any ecosystem in the world (Leslie & Summerell 2006).

A number of genera representing previously known *Fusarium* species were established based on deoxyribonucleic acid (DNA) sequence data (Lombard et al. 2015). For instance, the *Fusarium solani* species complex (FSSC) was proposed to be the genus *Neocosmospora* (Lombard et al. 2015). However, because of the close association with the name *Fusarium* and the fact that these names serve a large community of end-users, that is, plant pathologists, quarantine officers, veterinarians and medical practitioners, a different system was proposed where the name *Fusarium* was kept, for instance, for the FSSC (Geiser et al. 2013). The resulting confusion is evident as a number of new species in the complex kept the name of *Fusarium*, for example, *F. euwallaceae* (Freeman et al. 2014), which is the pathogenic fungus associated with the devastating polyphagous Shothole Borer. These taxa are usually included in *Fusarium* research.

A multidisciplinary study was conducted in the KNP to study the structure and biodiversity, and the various possible biotic and abiotic interactions of a catena or hill slope ecosystem on the Stevenson-Hamilton Supersite (25°06′28.6S, 31°34′41.9E and 25°06′25.7S, 31°34′33.7E). The aim of



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this study was to establish a baseline on the species diversity of *Fusarium* occurring at the particular supersite in order to possibly use species in *Fusarium* and closely related genera, which include specialised and generalist species, as a possible focus group to study interactions with the various biotic and abiotic factors in the catena system.

Previous studies have already described five new species with representative isolates from the KNP. These included *F. nygamai* (Burgess & Trimboli 1986) and *F. fredkrugeri* (Sandoval-Denis et al. 2018) in the FFSC, *F. polyphialidicum* (Marasas et al. 1986) in the *Fusarium concolor* species complex (FCOSC), *F. convolutans* in the *Fusarium buharicum* species complex (FBSC) and *F. transvaalense* in the *Fusarium sambucinum* species complex (FSaSC) from soils (Sandoval-Denis et al. 2018). Jacobs-Venter et al. (2018a) separated *F. polyphialidicum* strains into three species, namely *F. concolor, F. babinda* and *F. austroafricanum*, and confirmed that *F. polyphialidicum* is synonymous with *F. concolor*. *F. fredkrugeri*, *F. convolutans* and *F. transvaalense* originated from the Stevenson-Hamilton Supersite.

In this study, soil and rhizosphere samples from various plants in the Stevenson-Hamilton Supersite, which has a distinct geology and hydrology, were obtained. Isolations from these samples revealed a large collection of *Fusarium* isolates. The aim of this study was to identify these fusaria. As information on microbes, including fungi, is scarce for the KNP, and basically non-existent for the supersite, the study will contribute pioneering and invaluable biodiversity data on these ill-studied organisms that will be informative and useful for the management and conservation of the KNP.

# Materials and methods

## Sampling

The study was conducted in the Southern Granites 'Supersite' catena close to the Stevenson-Hamilton Memorial (Smit et al. 2013). Four random soil samples to a depth of 5 cm were taken for each of the components of the catena system in a transect of more or less 500 m following Theron, Van Aardt and Du Preez (2020). Furthermore, roots of *Pogonarthria squarrosa* (sickle grass, Poaceae, Poales), *Sporobolus nitens* (curly leaved drop seed grass, Poaceae) and *Schkuhria pinnata* (dwarf marigold, Asteraceae, Asterales), which included some of the dominant plants in the catena (Theron et al. 2020), were collected. Topsoil was deliberately included with the assumption that the soils will contain soil-associated fusaria as well as spores that were aerially distributed from plants in the area. The soils were transported cold to the laboratory, where soil dilution series were made on Rose Bengal–glycerine–urea medium (Leslie & Summerell 2006) and 20% potato dextrose agar (PDA; Biolab, Merck, South Africa). Roots were suspended in sterile water and shaken, and the soil solution was then used in a dilution series. Colonies resembling the cultural morphotypes of *Fusarium* species were purified from the primary plates by making single spore cultures from colonies on SNA medium (Leslie & Summerell 2011). Cultures were deposited in the National Collection of Fungi (PREM), Biosystematics Division, Agricultural Research Council (ARC), Pretoria, South Africa (Table 1).

## Deoxyribonucleic acid sequence-based characterisation

Inqaba Biotec (Pretoria, South Africa) extracted DNA from the scraped mycelium of 1-week-old cultures grown on PDA, and the translation elongation factor 1α gene region (TEF1α) was amplified and sequenced using primers EF1 and EF2 (O'Donnell et al. 2008). Sequences obtained were viewed and edited with Geneious 7.1.9 (Biomatter, Auckland).

Sequences were grouped into *Fusarium* species complexes using a skeleton sequence data set representing all species complexes in *Fusarium* and genera previously named as *Fusarium*, as well as species grouping outside species complexes (data not shown). After the appropriate complex or closest related species has been identified, sequences were included in separate DNA data sets representing all known and vouchered sequences of the particular group or complex. All alignments were performed in Mafft 7.0 (http://mafft.cbrc.jp/alignment/software/) with the L-INS-I option selected (Katoh et al. 2005). The alignments were corrected manually where needed.

Representative sequences with a high identity to the FFSC were aligned with all currently recognised species and phylogenetic lineages in the FFSC (Edwards et al. 2016; Geiser et al. 2013; Herron et al. 2015; Moroti et al. 2016). Similarly representative sequences with a high identity to the *Fusarium chlamydosporum* species complex (FCSC) (Lombard et al. 2019a; O'Donnell et al. 2009b) and *Fusarium oxysporum* species complex (FOSC) (Laurence et al. 2014; Lombard et al. 2019b; O'Donnell et al. 2009a) were aligned in data sets linked to the listed references. Sequences of the FSSC (O'Donnell et al. 2008) that are now known as *Neocosmospora* (Lombard et al. 2015) were also used. Maximum likelihood analyses were conducted in MEGA v. 7 using the models assigned to each data set and with a 1000 Bootstrap replicates to determine the support of the branches.

## Ethical considerations

Ethical approval for the multidisciplinary project as a whole was obtained from the Interfaculty Animal Ethics Committee at the University of the Free State (UFS-AED2019/0121). SANParks permit numbers for collection of soil for lab analyses and vegetation for identification purposes are SK069, SK2095 and SK054.

# Results

## Deoxyribonucleic acid sequence-based characterisation

Isolates (109) characterised in this study from the catena system represented four species complexes, namely FFSC, FCSC, FSSC and FOSC, and originated from the rhizospheres

**TABLE 1:** List of *Fusarium* isolates identified in this study that originated from the Stevenson-Hamilton catena in Kruger National Park, South Africa.

| PPRI number† | Identification | Collectors | Isolator | Host scientific name | Substrate/niche | Date of collection | DNA Data Bank of Japan number‡ |
|---|---|---|---|---|---|---|---|
| 20296 | *Fusarium proliferatum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521472 |
| 20281 | *F. nygamai* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521459 |
| 20306 | *F. nygamai* | E. Theron & J. du Preez | M. Gryzenhout | *Schkuhria pinnata* | Rhizosphere | 01 March 2015 | LC521479 |
| 20610 | *Neocosmospora vasinfecta* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521484 |
| 19535 | *Neocosmospora vasinfecta* | P.A.L. le Roux | M Gryzenhout | | Soil | 9-15 May 2014 | LC521384 |
| 19537 | *Neocosmospora vasinfecta* | P.A.L. le Roux | M Gryzenhout | | Soil | 9-15 May 2014 | LC521386 |
| 19521 | *F. cf. chlamydosporum* | P.A.L. le Roux | M Gryzenhout | | Soil | 9-15 May 2014 | LC521379 |
| 20270 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521450 |
| 20240 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521424 |
| 20245 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521428 |
| 20216 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521401 |
| 20653 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521487 |
| 20644 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521486 |
| 20616 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521485 |
| 20307 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Schkuhria pinnata* | Rhizosphere | 01 March 2015 | LC521480 |
| 20295 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521471 |
| 20294 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521470 |
| 20264 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521445 |
| 20258 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521440 |
| 20257 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521439 |
| 20243 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521426 |
| 20231 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521416 |
| 20228 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521413 |
| 20224 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521409 |
| 20219 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521404 |
| 20215 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521400 |
| 20213 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521398 |
| 20272 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521451 |
| 20267 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521447 |
| 20262 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521444 |
| 20237 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521421 |
| 20248 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521430 |
| 20246 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521429 |
| 20244 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521427 |
| 20242 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521425 |
| 20238 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521422 |
| 20221 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521406 |
| 20217 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521402 |
| 20214 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521399 |
| 20212 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521397 |
| 20210 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521395 |
| 20208 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521393 |
| 20206 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521392 |
| 20203 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521389 |
| 20202 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521388 |
| 19522 | *F. cf. chlamydosporum* | P.A.L. le Roux | M Gryzenhout | | Soil | 9-15 May 2014 | LC521380 |
| 19523 | *F. cf. chlamydosporum* | P.A.L. le Roux | M Gryzenhout | | Soil | 9-15 May 2014 | LC521381 |
| 19530 | *F. cf. chlamydosporum* | P.A.L. le Roux | M Gryzenhout | | Soil | 9-15 May 2014 | LC521383 |
| 20249 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521431 |
| 20251 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521433 |
| 20252 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521434 |
| 20253 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521435 |
| 20254 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521436 |
| 20256 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521438 |
| 20259 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521441 |
| 20260 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521442 |
| 20261 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521443 |
| 20269 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521449 |
| 20273 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521452 |
| 20274 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521453 |

Table 1 continues on the next page →

**TABLE 1 (Continues...):** List of *Fusarium* isolates identified in this study that originated from the Stevenson-Hamilton catena in Kruger National Park, South Africa.

| PPRI number† | Identification | Collectors | Isolator | Host scientific name | Substrate/niche | Date of collection | DNA Data Bank of Japan number‡ |
|---|---|---|---|---|---|---|---|
| 20275 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521454 |
| 20290 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521466 |
| 20292 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521468 |
| 20657 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521488 |
| 20660 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521489 |
| 20209 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521394 |
| 20233 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521418 |
| 20235 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521419 |
| 20227 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521412 |
| 20250 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521432 |
| 20223 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521408 |
| 20308 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Schkuhria pinnata* | Rhizosphere | 01 March 2015 | LC521481 |
| 20225 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521410 |
| 20266 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521446 |
| 20239 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521423 |
| 20201 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521387 |
| 20229 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521414 |
| 20211 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521396 |
| 20305 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Schkuhria pinnata* | Rhizosphere | 01 March 2015 | LC521478 |
| 20230 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521415 |
| 20220 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521405 |
| 20222 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521407 |
| 20226 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521411 |
| 20232 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521417 |
| 20276 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521455 |
| 20278 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521456 |
| 20280 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521458 |
| 20282 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521460 |
| 20609 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521483 |
| 20279 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521457 |
| 20255 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521437 |
| 20218 | *F. cf. chlamydosporum* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521403 |
| 20293 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521469 |
| 20291 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521467 |
| 20205 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521391 |
| 20284 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521462 |
| 20283 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521461 |
| 20300 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521475 |
| 20298 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521473 |
| 20299 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521474 |
| 20304 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521477 |
| 20285 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521463 |
| 20303 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521476 |
| 20287 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521464 |
| 20289 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Pogonarthria squarrosa* | Rhizosphere | 01 March 2015 | LC521465 |
| 20204 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Sporobolus nitens* | Rhizosphere | 01 March 2015 | LC521390 |
| 20309 | *F. oxysporum sensu lato* | E. Theron & J. du Preez | M. Gryzenhout | *Schkuhria pinnata* | Rhizosphere | 01 March 2015 | LC521482 |

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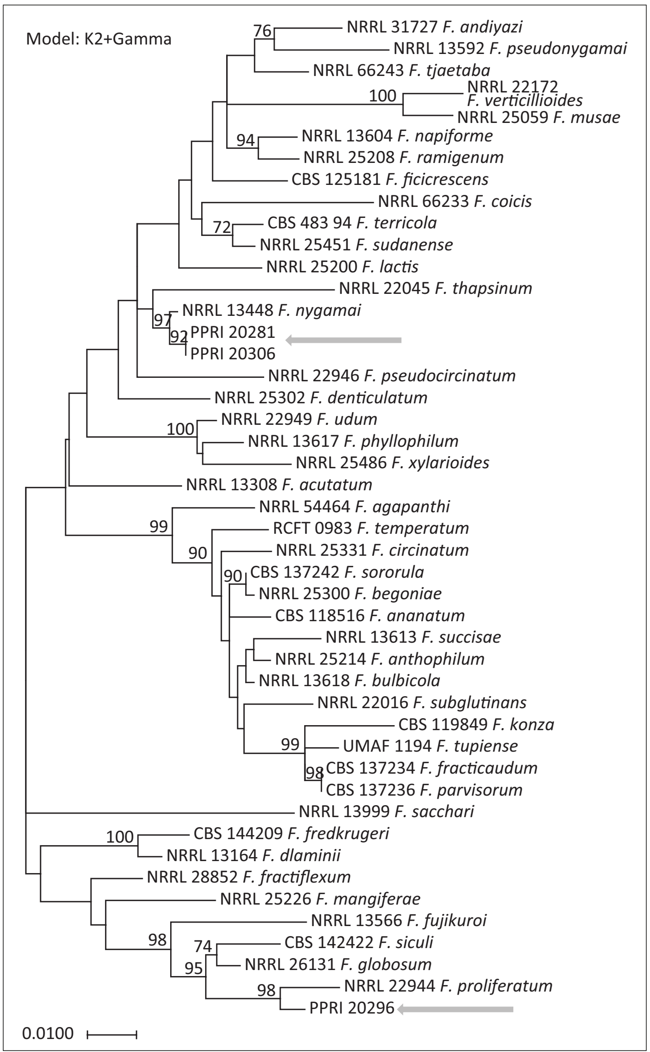
of all three plants and the topsoil (Table 1). Each of these complexes includes a diversity of species. In the FFSC, isolate PPRI 20296 was identified as *F. proliferatum* (Bootstrap support 98%), and isolates PPRI 20281 and PPRI 201306 were identified as *F. nygamai* (Bootstrap support 97%) (Figure 1). Isolates PPRI 20610, PPRI 19535 and PPRI 19537 were grouped in the clade of *N. vasinfecta* (Figure 2) in the FSSC (Bootstrap support of 97%) and grouped into two haplotype groups. Isolates from the KNP that were grouped in the FCSC (Table 1) constituted a very large number of isolates that did not group with any of the previously known lineages or newly described species (Figure 3). Between-isolate variation seven haplotypes was seen that could be indicative of more possible cryptic species or significant population structure. Based on the TEF sequence data alone, all of the novel species described (Lombard et al. 2019a) in the FOSC could not be resolved but isolates (Table 1) formed four haplotypes that grouped together with *F. callistephi* and *F. fabacearum*, and isolates from Australia (Figure 4).



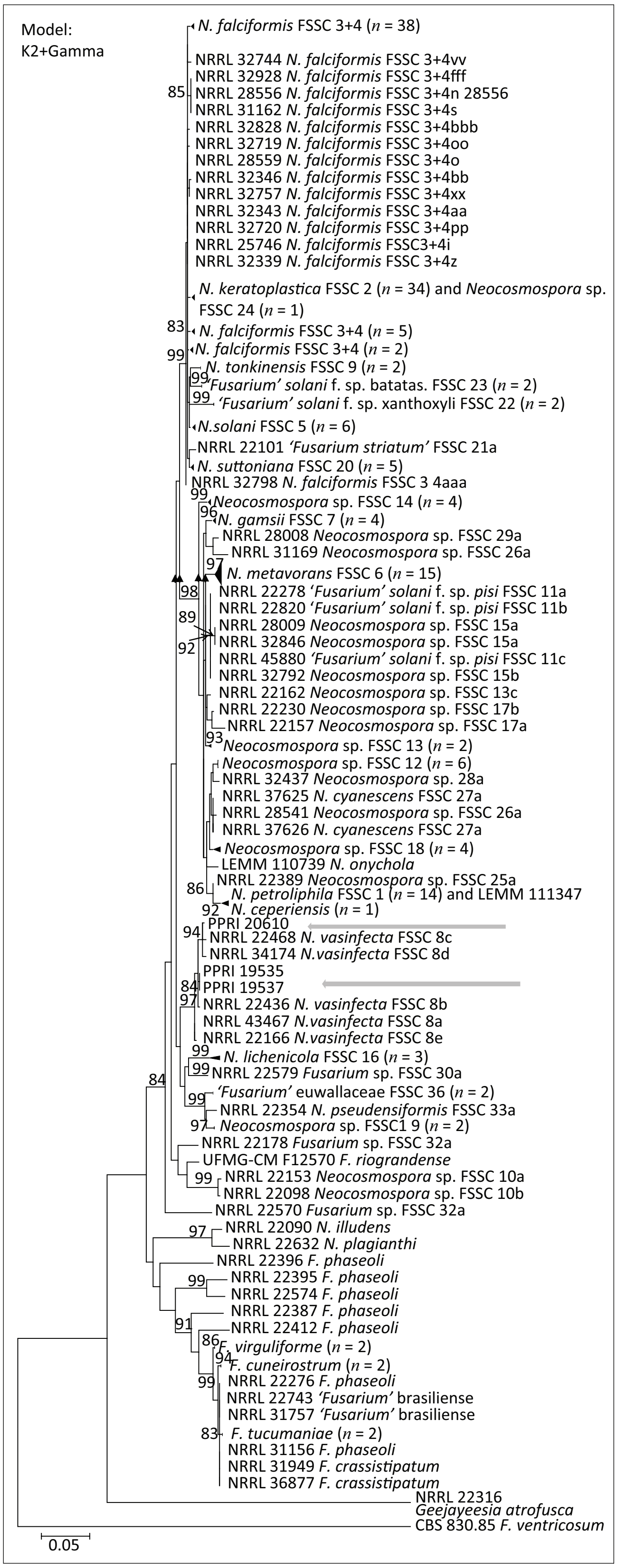

NRRL, National Center for Agricultural Utilization Research; CBS, CBS-KNAW Culture Collection, Westerdijk Fungal Biodiversity Institute; PPRI, Plant Protection Research Institute; RCFT, Rio Cuarto *Fusarium temperatum*; UMAF, University of Málaga.

**FIGURE 1:** Unrooted maximum likelihood phylogram of all currently sequenced *Fusarium* species in the *Fusarium fujikuroi* species complex based on translation elongation factor 1-a gene sequences. Bootstrap support values higher than 80% are shown on the branches. The evolutionary model applied to the analysis is indicated on the figure. Isolates from this study are indicated with PPRI numbers.

# Discussion

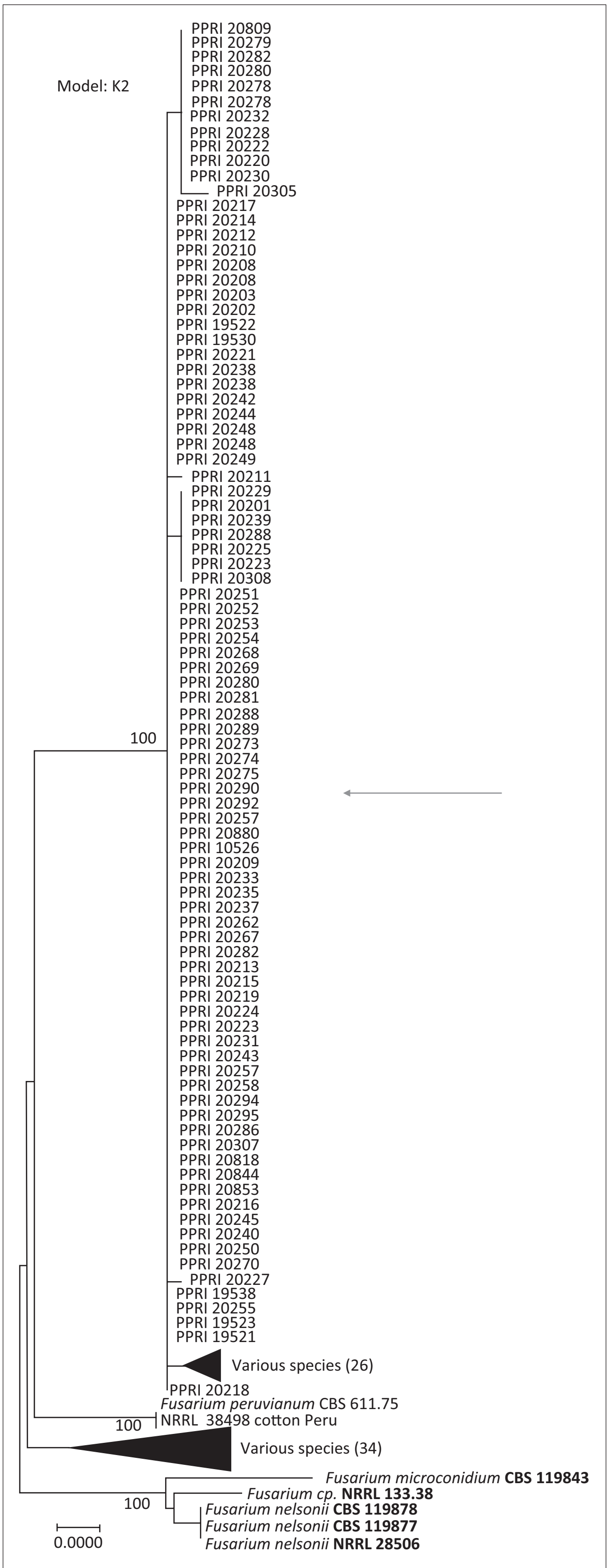
This study represents the first report of *F. proliferatum* (FFSC), *N. vasinfectum* that was previously known as *F. cosmosporiellum* in the FSSC (Geiser et al. 2013) and *F. oxysporum sensu lato* (FOSC) from soils in the Stevenson-Hamilton Granite Supersite in the KNP. Possible new species in the FCSC were also detected. The presence of *F. nygamai* (FFSC) was confirmed. Together with other species previously described from the KNP (*F. fredkrugeri*, *F. convalutum*, *F. transvaalense* and *F. concolor* as *F. polyphialidicum*), there are thus at least nine *Fusarium* species present in the KNP.

*Fusarium nygamai*, *F. proliferatum*, *F. oxysporum sensu lato*, *F. chlamydosporum*, *N. vasinfectum* and *F. concolor* are species that have a world-wide occurrence, including South Africa (Jacobs et al. 2018a; Leslie & Summerell 2006). They are associated with various plant hosts as well as soils and can also produce mycotoxins in food commodities or be



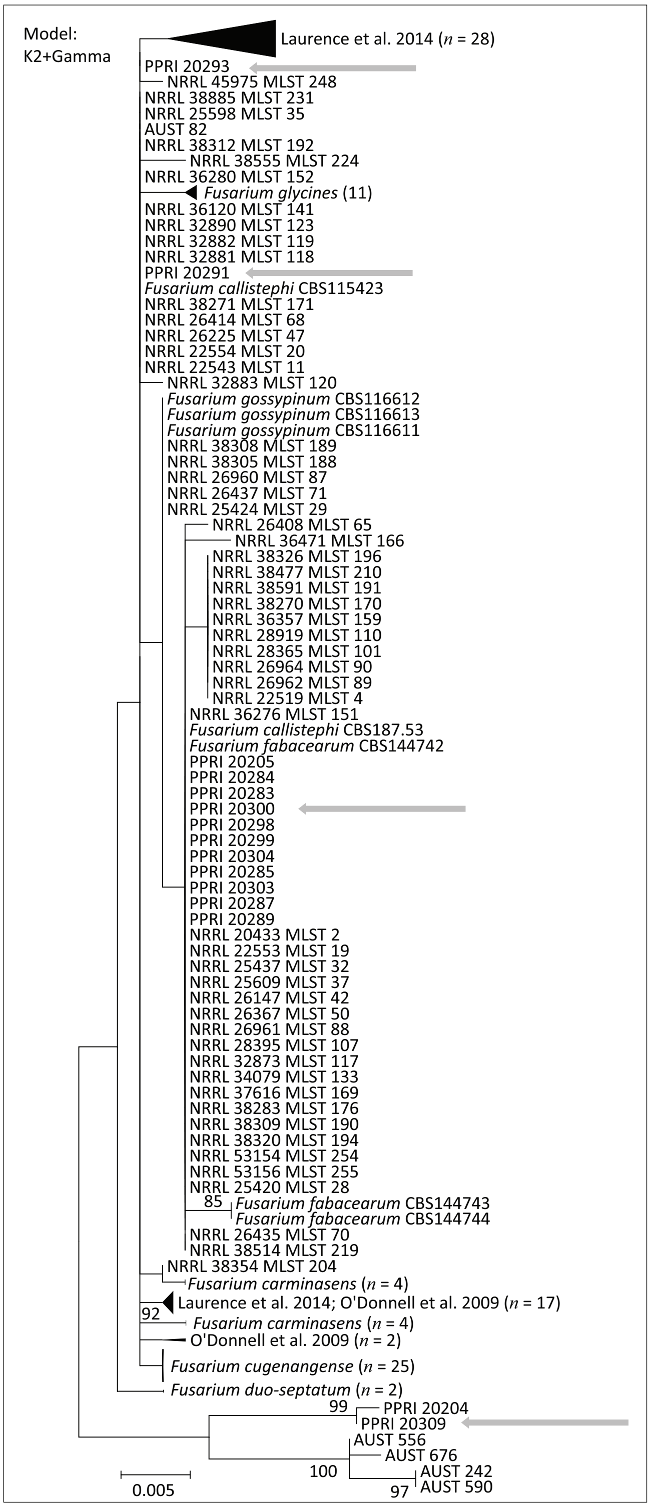

PPRI, Plant Protection Research Institute; FSSC, fusarium solani species complex; NRRL, National Center for Agricultural Utilization Research; LEMM, Laboratory of Molecular Microbial Ecology; UFMG-CM, Centro de Microscopia, Universidade Federal de Minas.

**FIGURE 2:** Unrooted maximum likelihood phylogram of all currently sequenced *Fusarium* species in the *Fusarium solani* species complex (also referred to as *Neocosmospora* with some species named as *Neocosmospora*) based on translation elongation factor 1-a gene sequences. Bootstrap support values higher than 80% are shown on the branches. The evolutionary model applied to the analysis is indicated on the figure. Isolates from this study are indicated with PPRI numbers.




PPRI, Plant Protection Research Institute; PPRI, Plant Protection Research Institute; NRRL, National Center for Agricultural Utilization Research; MLST, Multilocus Sequence Type; CBS, CBS-KNAW Culture Collection, Westerdijk Fungal Biodiversity Institute; NRRL, National Center for Agricultural Utilization Research.

**FIGURE 3:** Unrooted maximum likelihood phylogram of all currently sequenced *Fusarium* species in the *Fusarium chlamydosporum* species complex based on translation elongation factor 1-a gene sequences. Bootstrap support values higher than 80% are shown on the branches. The evolutionary model applied to the analysis is indicated on the figure. Isolates from this study are indicated with PPRI numbers.




PPRI, Plant Protection Research Institute; NRRL, National Center for Agricultural Utilization Research; MLST, Multilocus Sequence Type; CBS, CBS-KNAW Culture Collection, Westerdijk Fungal Biodiversity Institute; AUST, Australia, used by Laurence et al. (2014).

**FIGURE 4:** Unrooted maximum likelihood phylogram of all currently sequenced *Fusarium* species in the *Fusarium oxysporum* species complex based on translation elongation factor 1-a gene sequences. Bootstrap support values higher than 80% are shown on the branches. The evolutionary model applied to the analysis is indicated on the figure. Isolates from this study are indicated with PPRI numbers.

associated with diseases of animals or humans (Leslie & Summerell 2006). The new species *F. fredkrugeri*, *F. convalutum* and *F. transvaalense* have only recently been described and have most likely not yet been discovered elsewhere. Because these species are generalists that can be isolated from various substrates and plant hosts, these species most likely are not suitable to represent a target group to study unique species associations within a catena system.

The majority of strains (90) obtained from the KNP sample sites belonged to *F. chlamydosporum* species complex. A four-locus typing scheme (O'Donnell et al. 2009b) revealed MLST and species within the species complex, and Lombard et al. (2019a) recently published the description of numerous new species in the complex. Isolates obtained from this study appear to represent new species. As before, a number of new species from the KNP are yet to be described.

What is notable is that several undescribed *Fusarium* species have been discovered in the KNP. Previously, *F. nygamai*, *F. polyphialidicum*, *F. fredkrugeri*, *F. convalutum* and *F. transvaalense* were described from the KNP, while possible new species have also been characterised in this study. Since their description, *F. nygamai* and *F. concolor* (also as the synonym *F. polyphialidicum*) have been discovered from across the world (Leslie & Summerell 2006), suggesting a wide substrate, host and geographical range despite being first described from a national conservation park in South Africa. The number of undescribed species of *Fusarium* in the KNP is not surprising because the biodiversity of *Fusarium* and closely allied genera that were previously called *Fusarium* is largely untouched. This is especially so in pristine natural areas (Jacobs et al. 2018b), because most *Fusarium* research in South Africa is focused on agricultural problems or animal and human health issues caused by *Fusarium* species.

## Conclusion

The KNP plays an important role in not only protecting the native ecosystems present in that area and the animals and plants they contain but also protecting *Fusarium* species that occur in South Africa, of which some are new to science. The ecological roles of these species in numerous ecosystems are, however, still unknown, and further studies on their impact on ecosystem services and function must be pursued. Such studies are important because Gryzenhout et al. (2020) showed through environmental sequencing that *Fusarium* species are one of the dominant groups found within the soil-plant root zones of plants occurring in the Stevenson-Hamilton Granite Supersite. Further sequencing of additional genes, as what has been done in this study, will provide a better estimation on species level of the species that could be involved.

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### Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

### Authors' contributions

The authors directly participated in the study design, execution and interpretation of the research. All authors contributed equally to this research work.

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### Data availability

Data are available from the corresponding author on request.

### Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliate agency of the authors.

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