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SHORT COMMUNICATION

The critically endangered geophyte *Gladiolus aureus* threatened by a wilt disease associated with *Fusarium libertatis*

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Abstract

Gladiolus aureus is a geophyte species endemic to the South African Cape Peninsula. Pressures including land-use change and invasion by alien plants have caused severe population declines, leading to its classification as Critically Endangered (CR). Kirstenbosch National Botanical Garden (NBG) maintains the largest *ex situ* collection of this species. During plant health surveys conducted as part of a sentinel plant research project, a recurring wilt disease was observed in the *ex situ* collection. Symptomatic plants were sampled and fungal isolations consistently yielded a *Fusarium* species, identified as *F. libertatis,* as confirmed through DNA sequencing and phylogenetic analyses. Dormant corms donated by Kirstenbosch NBG for pathogenicity tests were asymptomatically colonised by the same fungus, suggesting that *F. libertatis* may be widespread in the collection and may be moved through contaminated planting material as well as growing media. This study highlights the challenges posed by plant pathogens to the conservation of threatened plant taxa and underscores the limited disease management tools available in *ex situ* collections.

Keywords Botanical gardens · *Ex situ* · Fusarium wilt · Plant conservation

South Africa is renowned for its exceptional biodiversity, including a remarkable richness of geophytic plants (Procheş et al. 2006). Within this group, the genus *Gladiolus* is particularly diverse, with 166 of the 296 currently accepted species native to the country. Most South African *Gladiolus* species are endemic (138, 83%; SANBI 2024) and have restricted areas of occupation. These characteristics, combined with additional pressures such as invasive plant species and land-use change, place many *Gladiolus* species at heightened risk of extinction.

Gladiolus aureus is a Critically Endangered (CR) species endemic to the Cape Peninsula (Victor and Duncan 2010). Historically, the species was known from only two extant populations (sites around Kommetjie and Simon's Town), however, one of these (Simon's Town) was subsequently declared extinct. The remaining population (Kommetjie) has been in continuous decline since the 1960s (Duncan and Ashton 2005; Victor and Duncan 2010) and is now thought to have also disappeared (G. Duncan, *pers. obs.*) suggesting the species is Extinct in the Wild (EW). *Ex situ* collections were established at Kirstenbosch National Botanical Garden (NBG) between 1975 and 1976 (Duncan and Ashton 2005) and currently represent the largest living collection of *G. aureus*.

In 2022, during plant health monitoring activities at Kirstenbosch NBG as part of the FABI–SANBI sentinel plant project (https://www.fabinet.up.ac.za/index.php/sentinel-plant-network), a recurring disease affecting *ex situ G. aureus* plants was noted (G. Duncan, *pers. obs.*). Symptomatic plants of different age classes exhibited corm rot and wilting (Fig. 1a, b). Two samples, each consisting of entire symptomatic adult plants (Fig. 1b), were collected and processed in the laboratories at FABI (https://www.fabinet.up.ac.za) within 48 h of collection. Based on the observed symptomatology and previous reports of Fusarium wilt affecting geophytic species, isolation procedures specifically targeted for this group of fungi were included.

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Fig. 1 Symptoms and signs of Fusarium wilt on *Gladiolus aureus*. **a–b**. Wilting and corm rot observed in plants from the geophyte *ex situ* collections at Kirstenbosch National Botanical Garden (NBG). **c–d**. Corm rot and mycelial growth on two-year-old corm-propagated plants at the Forestry and Agricultural Biotechnology Institute (FABI)

For fungal isolations, samples were surface disinfected by immersion in 70% ethanol for 30 s, 5% sodium hypochlorite for 1 min, and then rinsed with sterile distilled water. Small sections of corm tissue and basal portions of leaves showing discolouration were dissected and plated into Petri dishes containing 2% malt extract agar (MEA; 20 g/L malt extract, 20 g/L agar; Biolab) and *Fusarium* selective medium (FSM; 1.5% peptone, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 2% Difco agar [Biolab, Midrand, South Africa], 0.1% pentachloronitrobenzene [PCNB], 0.01% streptomycin sulphate) (Leslie and Summerell 2008). Plates were incubated for 2–6 days, after which fungal growth was examined. *Fusarium* colonies were consistently and exclusively recovered from all isolations from both plants. Individual colonies were subcultured onto fresh MEA plates and incubated for up to seven days, after which single-spore isolates were obtained. Representative single-spore cultures were deposited in the culture collection (CMW) at FABI.

For species-level identification, DNA sequencing was carried out for two isolates (CMW61820 and CMW61822, one per plant). Genomic DNA was extracted from mycelium harvested from actively growing colonies using the Prepman® Ultra Sample Preparation Reagent kit (Thermo Fisher Scientific, Waltham, MA). Three gene regions were amplified: elongation factor 1-α (*tef1*), RNA polymerase II second largest unit (*rpb2*) and calmodulin (*CaM*), using primers EF1 and EF2 (O'Donnell et al. 2008), RPB2-5f2/RPB2-7cR (O'Donnell et al. 2007) and CL1/CL2 (O'Donnell et al. 2000), respectively. PCR products were cleaned using ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™, Thermo Fisher). The BigDye™ Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, USA) was used to prepare amplicons for sequencing in both directions. Sanger sequencing was conducted at the sequencing facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Resulting sequences were assembled using CLC Main Workbench v. 21.0.3.

The generated DNA sequences (GenBank Accession Numbers: PX964305–PX964313) were submitted

to GenBank's BLAST utility (https://blast.ncbi.nlm.nih.gov/Blast.cgi), filtering for sequences from type material. Sequences for *tef1* from our isolates resulted in 100% identity exclusively with sequences from *F. hoodiae* isolates (MH485020). In contrast, *rpb2* sequences showed the closest matches (99.8–100%) with sequences from *F. libertatis* (MH484932, MH484933), while *Cam* sequences resulted in 100% identity with sequences from a range of species in the *Fusarium oxysporum* Species Complex (FOSC). Sequences were also submitted for polyphasic identification through the FUSARIOID-ID database (Westerdijk Fungal Biodiversity Institute; https://www.fusarium.org/page/Homepage), which resulted in the closest match (99.96% similarity) with *F. libertatis* (LLC1736).

Phylogenetic analyses were conducted for each of the three gene regions individually, as well as for a combined dataset. Sequence datasets for species within the FOSC were constructed for each gene region. Sequences were aligned using the MAFFT v.7 online server (https://mafft.cbrc.jp/alignment/server/), manually inspected and trimmed in MEGA v7.0.26 and concatenated using CLC Main Workbench v. 21.0.3. Maximum likelihood trees were inferred using the IQ-TREE online server (http://iqtree.cibiv.univie.ac.at/) for each gene region independently and for the concatenated dataset (using partitioned data). Resulting trees were visualised and edited using FigTree v.1.4.4 and Affinity Designer v. 1.10.5.1342.

Phylogenetic analyses based on individual gene regions yielded results consistent with BLAST analyses. When *tef1* sequences were analysed, isolates grouped with *F. hoodiae* and *F. irdabamae*; with *rpb2* they grouped with *F. libertatis*, and when *Cam* was used, they clustered with multiple species within the FOSC. Despite these differences, the concatenated phylogeny (Fig. 2) resolved the isolates in a highly supported clade along with *F. libertatis* isolates. For the purpose of this study, the isolates were therefore identified as *F. libertatis*. The discordance among single-gene phylogenies may reflect historical introgression or hybridisation with related species, including *F. hoodiae*. Importantly, our results indicate that reliance on *tef1* alone could lead to misidentification within this group, highlighting *rpb2* as a more reliable marker for accurate identification.

To test the pathogenicity of our isolates on *G. aureus*, dormant corms were obtained from Kirstenbosch NBG for experimental testing. Ten corms were transported to FABI facilities and set in a sand–compost mixture in late October 2023. From March 2024, the corms were watered twice weekly, and by April all had sprouted and appeared healthy. By June 2024, however, all plants began to decline before they could be inoculated. White mycelium emerged from the corms (Fig. 1c, d), and isolations were performed following the methods described above. *Fusarium*-like colonies were recovered from all corms, and the same procedures were used to identify them to species level. One isolate (CMW68301) was selected for sequencing of the same gene regions.

Results of phylogenetic analysis (Fig. 2), including the isolate obtained from the FABI-grown plants, confirmed it was identical to those obtained from the Kirstenbosch NBG collection. These results indicate a strong association between *F. libertatis* and the corm rot and wilting observed in the *ex situ* collections. Furthermore, they also demonstrate that *F. libertatis* can survive in asymptomatic dormant corms and may contribute to disease development upon sprouting. Based on these findings and the known dispersal mechanisms of *Fusarium* species, the fungus is likely spread via contaminated corms and growing media. It is important to note that pathogenicity could not be experimentally confirmed through completion of Koch's postulates in this study. All corms intended for inoculation developed symptoms before inoculation and yielded the same fungus. Thus, although the consistent recovery of *F. libertatis* from symptomatic plants and asymptomatic corms supports a strong association with the observed wilt disease, its causal role remains to be confirmed. Future studies should therefore aim to test pathogenicity using verified pathogen-free corms or plants grown from seed.

Despite its association with corm rot and wilting in *ex situ* collections of *G. aureus* documented in this study, information on *F. libertatis* as a plant pathogen, as well as on its ecology and origin, remains limited. *Fusarium libertatis* was described in 2019 from rock surfaces on Robben Island, South Africa (Lombard et al. 2019). Although the original description included an isolate obtained from *Aspalathus* sp., no disease symptoms were reported for that host. Subsequently, *F. libertatis* was reported from soil in sorghum fields in Ethiopia (Lombard et al. 2022) and from greenhouse-grown jade plants (*Crassula ovata*) showing vascular wilt symptoms in the USA (Elmer et al. 2023). The latter represents the only previous report of *F. libertatis* causing plant disease. Nevertheless, the limited information currently available does not provide strong evidence that *F. libertatis* constitutes an invasive alien pathogen; its origin remains uncertain (i.e., it may be regarded as cryptogenic), and its suggested impact on *G. aureus* appears restricted to plants under cultivation in enclosed environments. At present, these factors do not support prioritisation for formal risk analysis as an alien taxon.

The occurrence of a wilt disease in *ex situ* collections of *G. aureus* is, however, particularly concerning given the threatened status of this species. The recurrent nature of the disease compromises long-term *ex situ* conservation efforts and poses a risk of progressive loss of genetic diversity within the collection, potentially leading to the loss of

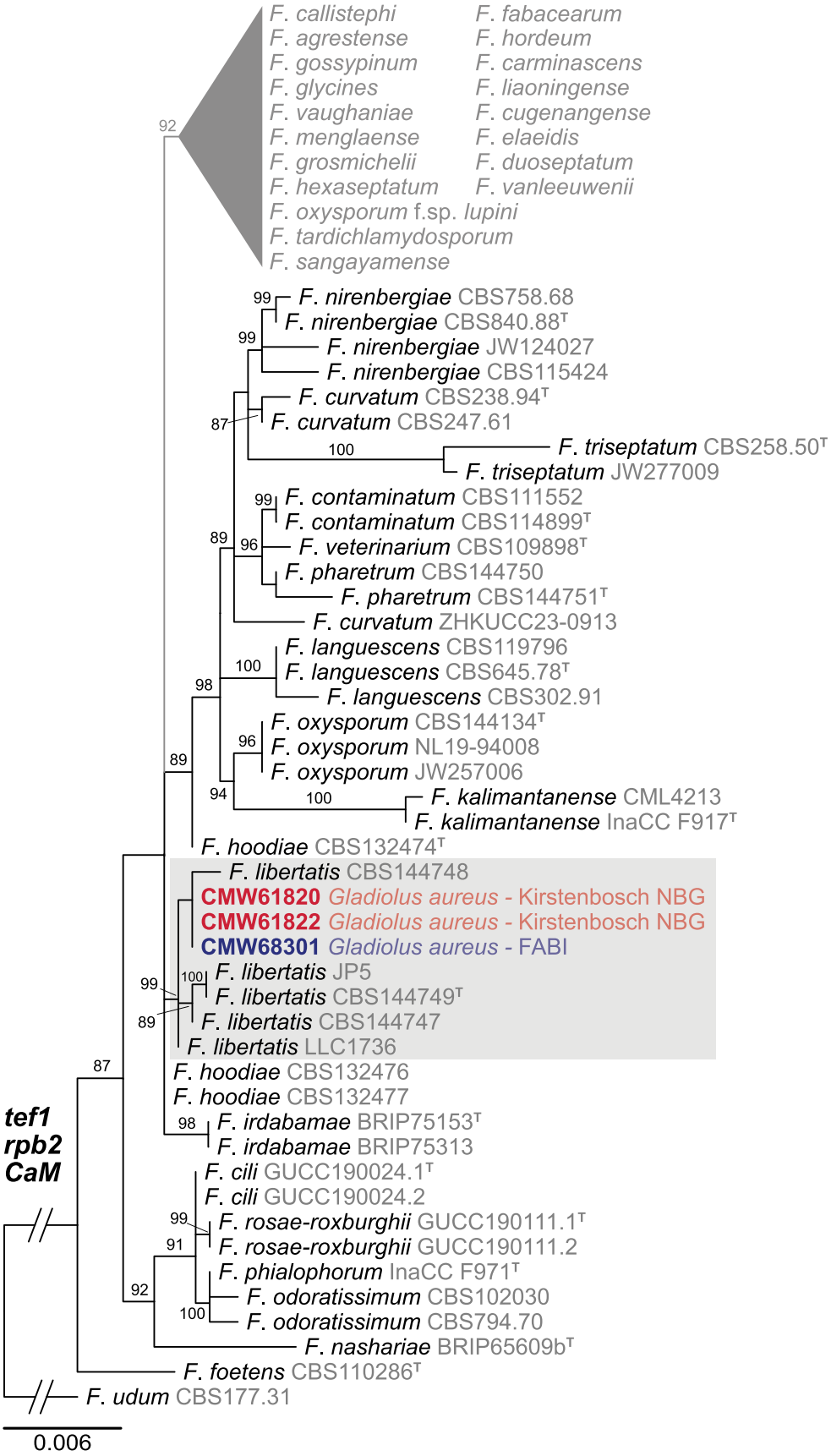

Fig. 2 Maximum likelihood phylogenetic tree based on concatenated *tef1*, *rpb2*, and *Cam* gene regions for species within the *Fusarium oxysporum* species complex (FOSC). Isolates obtained in this study are highlighted in red and blue. Sampling locations are indicated together with isolates names. Numbers on branches represent bootstrap support values (1000 repetitions), only values of 80% or higher are shown

irreplaceable germplasm. Furthermore, the presence of a fungal pathogen may limit the feasibility of reintroducing *G. aureus* populations into the wild, as it carries the risk of inadvertently introducing this microbe into sensitive areas where it may not yet be present. Given that the most recent conservation assessment of *G. aureus* was conducted over a decade ago, and that the species is now suspected to be extinct in the wild, our findings support the need for a re-evaluation of its conservation status, explicitly incorporating emerging biotic threats such as plant diseases that were not previously considered.

Wilt diseases, such as those caused by *Fusarium* spp. in controlled environments (e.g., greenhouses), have been extensively studied in agricultural and horticultural systems, resulting in the development of a range of management strategies. However, in conservation settings, the application of these approaches is often constrained by regulatory restrictions on chemical use, including fungicide registration, and by limited infrastructure to ensure the effective sanitation of propagation material and growing medium (e.g., soil pasteurization). This study highlights a critical gap between existing plant health knowledge and its practical implementation in sensitive collections. It also emphasizes a need for improved institutional mechanisms and operational flexibility within existing regulatory frameworks to enable timely access to appropriate preventative and curative management tools for conservation purposes.

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Data availability All isolates obtained in this study were deposited in the Culture Collection (CMW) at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. All DNA sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/), accessions PX964305–PX964313.

Declarations

Ethical declaration Not applicable.

Competing interests The authors declare no competing interests.

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