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Mjuua agapanthi gen. et sp. nov., a biotrophic mycoparasite of *Fusarium* spp.

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Abstract: *Fusarium agapanthi* is newly reported from the centre of origin of *Agapanthus* in South Africa, where it is associated with dead flower stalks of *Agapanthus praecox*. *Mjuua agapanthi*, a rare hyphomycete with a morphology corresponding to asexual morphs of *Pyxidiophora*, was isolated as mycoparasitic on *F. agapanthi*, along with bacteria that co-occurred in synnematal heads of *M. agapanthi.* Germinating conidia of *M. agapanthi* were observed to parasitise germinating conidia of *F. agapanthi*. Although *M. agapanthi* could not be cultivated on its own, the association with *Fusarium* proved to not be restricted to *F. agapanthi*, as it could also be cultivated with other *Fusarium* spp. *Mjuua agapanthi* is a member of *Pyxidiophorales*, an order of obligate insect parasitic microfungi. The exact role of the bacteria in synnematal heads of *M. agapanthi* remains to be further elucidated, although one bacterium, *Alsobacter metallidurans*, appeared to cause lysis of the synnematal conidial cell walls. This discovery suggests that many unculturable obligate biotrophic microbes can probably be cultivated if co-cultivated with their respective hosts.

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Dedication: For MJW: "*I love a good story*".

INTRODUCTION

Fusarium agapanthi was initially described from Italy and Australia, where it was associated with leaf and stem spot of *Agapanthus* (African lily) (*Agapanthus praecox*) (Edwards *et al.* 2016). Guarnaccia *et al.* (2019) later proved pathogenicity of *F. agapanthi* to *A. praecox* and *A. africanus* in Italy, suggesting that it may have a wider *Agapanthus* host range than initially assumed.

Although *Agapanthus* is indigenous to South Africa, the plant is much sought after as ornamental, and as such can commonly be found on most continents with moderate climates. *Agapanthus* is known to host several endophytic fungi, some of which are phytopathogenic, and have been introduced, along with its host, to multiple continents. For example, *Teratosphaeria agapanthi*, originally described from South Africa in 1880 (as *Sphaerella agapanthi*), was recently reported from Portugal (Crous *et al.* 2011), as well as Australia, La Réunion, and the USA (Crous *et al.* 2020). Of note was the fact that *F. agapanthi* was placed as a monotypic lineage in the American clade of the *Fusarium fujikuroi* species complex (Edwards *et al.* 2016), which posed the question, "Did the pathogen simply jump onto *Agapanthus*, or could it have been introduced along with its host from South Africa?" The initial aim of this study was thus to sample symptomatic *Agapanthus* from South Africa to determine if *F. agapanthi* could also be present in the centre of origin of *Agapanthus*. During isolations a mycophilic fungus was obtained, and found to be a biotrophic mycoparasite of *Fusarium* spp. As the fungus appeared to be an undescribed taxon, the present study aimed to describe it, and resolve its ecology.

MATERIALS AND METHODS

Isolates

Initial samples were collected in the Western Cape Province of South Africa during April and November 2023, and consisted of dead *Agapanthus praecox* flower stalks that showed a pink to red discolouration in the bottom 3–10 cm (no leaf spot or root rot symptoms were observed). Samples were placed in damp chambers, and single conidial colonies established on Petri dishes containing 2 % malt extract agar (MEA) as described by Crous *et al.* (2019).

Soil samples were collected adjacent to *Agapanthus* plants, and fungal isolations followed previously established methods (Groenewald *et al.* 2018, Giraldo *et al.* 2019, Crous *et al.* 2021a). Colonies were sub-cultured on 2 % potato dextrose agar (PDA), oatmeal agar (OA), MEA and synthetic nutrient-poor agar (SNA) (Crous *et al.* 2019), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Bacteria were cultivated on R2A (Reasoners agar), low nutrient agar used to study (potable) water bacteria, TSA Trytic Soy agar, TSB Trytic

Soy Broth undiluted and 1:10 diluted with demi water, and VY/2 and CY medium for predatory bacteria (Reichenbach 2006). Whatman no 1 filterpaper was used to filtrate fungal suspension to enable isolation of slow growing bacteria. Plates and liquid cultures with bacteria were incubated at temperatures between 18 °C and 30 °C under aerobic conditions. Reference strains and specimens of the studied fungi are maintained in the culture collection and fungarium (CBS), and bacteria in the Netherlands Culture Collection of Bacteria (NCCB) of the Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands.

DNA extraction, amplification (PCR) and phylogeny

Fungal mycelium from fusarioid isolates was scraped from the surface of monosporic agar cultures with a sterile scalpel and the genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's protocols. Additionally, to obtain pure genomic DNA of a putative mycoparasitic fungus growing in association with *Fusarium* spp. and in order to confirm the identity of the different morphologies observed in culture, clusters of approximately 10–20 conidia were taken with a sterile needle, and with the aid of a Zeiss Discovery V20 dissecting microscope, from either aerial conidial chains and conidial heads formed on synnemata. DNA was isolated using the EZNA Forensic DNA reagent set (Omega Bio-Tek, Norcross, GA, USA) according to manufacturer's standard protocol. The latter procedure was repeated once. Partial fragments of the 18S (SSU) and 28S (LSU) nrRNA genes, and the complete internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS) of the nrDNA operon were sequenced for the putative mycoparasitic using the primer pairs NS1/NS4 for SSU (White *et al.* 1990), LR0R/LR5 for LSU (Vilgalys & Hester 1990, Vilgalys & Sun 1994), and ITS5/ITS4 for ITS (White *et al.* 1990). Partial fragments of the DNA-directed RNA polymerase II second largest subunit gene (*rpb2*), and the translation elongation factor 1-alpha gene (*tef1*) were amplified for fusarioid isolates using the primer pairs RPB2-5f2/ fRPB2-7cr (Liu *et al.* 1999, Reeb *et al.* 2004) and EF-1/EF-2 (O'Donnell *et al.* 1998), respectively. Conditions for PCR followed White *et al.* (1990) and Sandoval-Denis & Crous (2018). The resulting fragments were sequenced in both directions using the respective PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA); DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (Sigma-Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The DNA sequences were analysed and consensus sequences were computed using Geneious Prime v. 2023.2.1 (http://www.geneious.com, Kearse *et al.* 2012).

The sequences for each gene region were subjected to megablast searches (Zhang *et al.* 2000) to identify closely related sequences in the FusarioidID database (ww.fusarium.org) and NCBI's GenBank nucleotide database. Maximum-likelihood (ML) phylogenetic trees were generated using IQ-TREE v. 2.1.3 (Nguyen *et al.* 2015) and branch support values were calculated with 1 000 non-parametric bootstrap (BS) replicates and optimal modelfinding according to ModelFinder (Kalyaanamoorthy *et al.* 2017) as implemented in IQ-TREE. Bayesian analyses were performed with MrBayes v. 3.2.7a (Ronquist *et al.* 2012), executed on the CIPRES Science Gateway portal (https://www.phylo.org; Miller *et al.* 2012). The best evolutionary model for each gene partition was calculated using MrModeltest v. 2.3 (Nylander 2004), and the analyses included four parallel runs of 50 M generations, with a sampling frequency of every 1 000 generations. The 50 % majority rule consensus trees and posterior probability (PP) values were calculated after discarding the initial 25 % of saved trees as the 'burn-in' phase. Individual gene phylogenies were checked for topological conflicts between significantly supported clades (ML-BS ≥ 70 %, BI-PP ≥ 0.95), after which the different gene datasets were concatenated (Mason-Gamer & Kellogg 1996, Wiens 1998).

All resulting trees were printed with FigTree v.1.4.4 (Rambaut 2018) and the layout of the trees was done with Adobe Illustrator 2024 v. 28.2. Sequences derived in this study were submitted to GenBank.

For bacteria, DNA sequencing was performed directly from fungal synnemata and from a culture suspension of in MilliQ water. The presence of endosymbiont bacteria was examined by sequencing the bacterial small-subunit (16S) rRNA. Both fungal DNA and fungal cultures were used as a template to detect the presence of bacterial DNA. A suspension from a fungal culture was made in 500 µL sterile demi water and was incubated at 100 °C for 10 min. Amplification reactions were performed with primers 16S500F (5'-tggagagtttgatcctggctcag-3') and 16S500R (5'-taccgcggctgctggcac-3') (Hall *et al.* 2003). The mastermix contained 14.65 µL nuclease-free water, 2.5 µL 10× NH_4 buffer, 0.75 µL 50 mM $MgCl_2$, 1 µL 5 mM dNTPs, 1 µL 10 pmol/µL 16S500bpF, 1 µL 10 pmol/µL 16S500bpR, and 0.1 µL 5 U/µL Biotaq solution. The amplification reaction was as follows: pre-denaturation at 94 °C for 2 min and 36 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, amplification at 72 °C for 2 min, with final elongation step at 72 °C for 5 min. The resulting fragments were sequenced in both directions using the respective PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA). Amplification products were purified using magnetic beads with an automated robot. Sequencing reactions were performed on an ABI Prism 3730xl sequencer (Dolatabadi *et al.* 2016)

Morphology

Slide preparations from colonies sporulating on SNA were mounted in water (see Crous *et al.* 2021b). Observations were made with a Nikon SMZ25 dissection microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and images recorded on a Nikon DS-Ri2 camera with associated software. Low-temperature scanning electron microscopy (SEM) was performed by cutting out 5 × 5 mm^2 relevant parts (as judged by a dissecting microscope) from plates on SNA after 7 and 14 d of cultivation using a surgical blade. Pieces of agar were transferred into a copper cup (10 mm diam, 8 mm deep) and the agar blocks were glued to the copper using KP-Cryoblock frozen tissue medium (Klinipath). The copper cup was placed within a Petri dish with agar to prevent aerial hyphae from drying and collapsing. The sample was snap-frozen in nitrogen slush and immediately transferred to a JSM-IT200 scanning electron microscope (JEOL) equipped with an Oxford CT1500 Cryo station for cryo-electron microscopy (cryoSEM). The sample was sputter-coated with a gold target for 90 s with ideally 0.1–0.2 mBar argon flow. If the gold layer was too thin, a second or third coating session was done. Electron micrographs

were acquired using an acceleration voltage of 2 kV (uncoated samples) and 5 kV (for gold-coated samples) using several scanning speeds dependent on the sample. Taxonomic novelties were submitted to MycoBank (www.MycoBank.org; Crous *et al.* 2004).

Fungal interaction

To determine if there was a volatile metabolite involved between *M. agapanthi* and *F. agapanthi*, 6-cm-diam Petri dishes were individually inoculated with either *F. agapanthi* (CPC 46094), *F. fredkrugeri* (CPC 33747), *F. mexicanum* (NRRL 53147), or *F. ophioides* (CBS 118512). Additional dishes were inoculated with either synnematal conidia, or conidia from dry chains of *M. agapanthi* (CPC 46094). *Fusarium* plates were positioned in different combinations with plates of *M. agapanthi*, without lids, inside larger 15-cm-diam Petri dishes and the whole set-up was sealed.

In a second experiment Petri dishes were separately inoculated with a conidial suspension of *M. agapanthi*, and subsequently inoculated with one of the respective *Fusarium* spp. in a V-pattern across the dish, to determine if physical contact was needed between *M. agapanthi* and a *Fusarium* sp. to stimulate growth of *M. agapanthi*.

RESULTS

Morphology

A *Fusarium* sp. was consistently isolated from the dead flower stalks of *Agapanthus praecox* and soil samples. In some specimens, cultures were also obtained of a synnematous fungus with prominent, erect, white synnemata. Colonies of

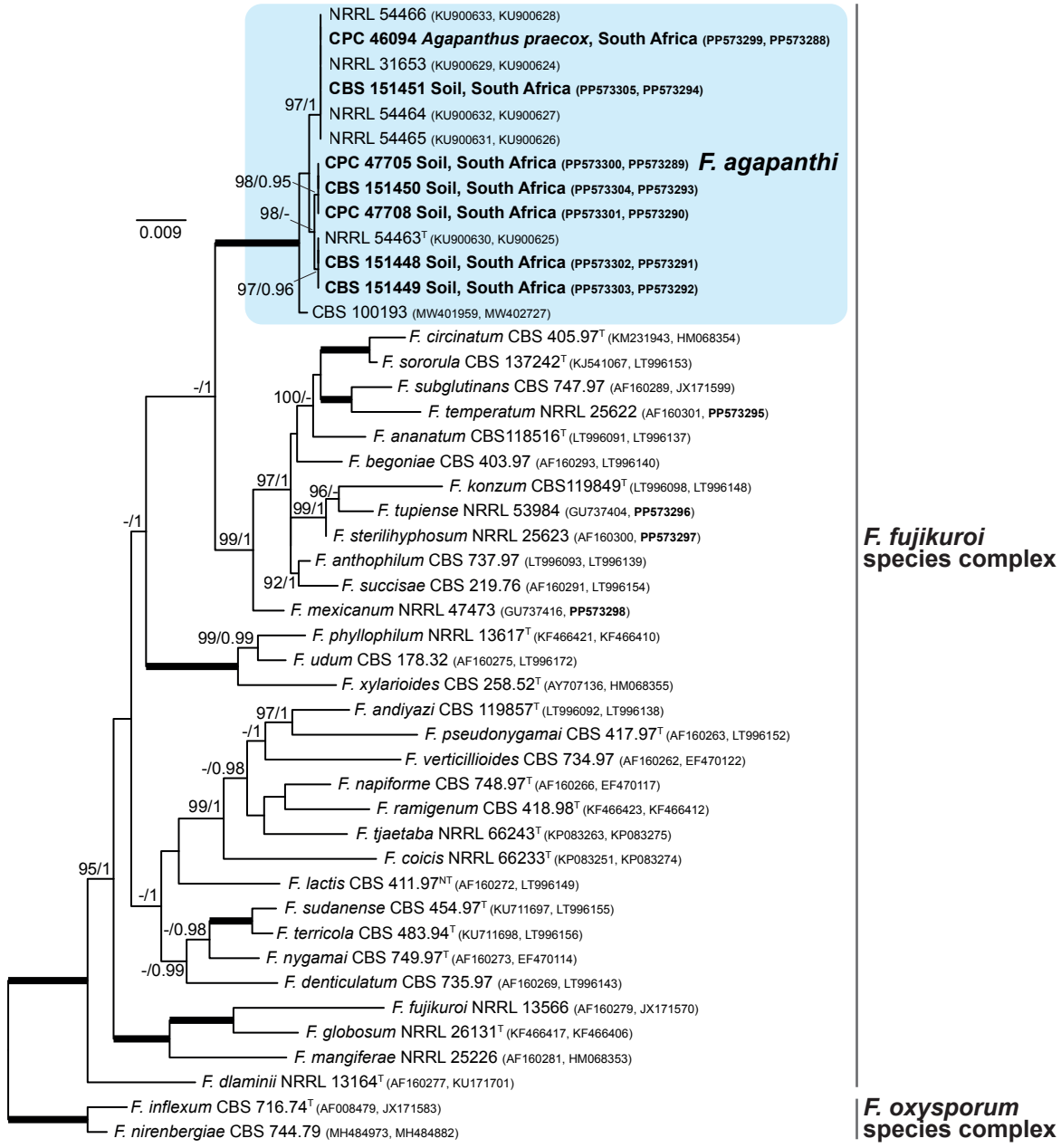

Fig. 1. IQ-TREE-ML phylogeny reconstructed using combined *RPB1* and *TEF1* sequences of representative species of the *Fusarium fujikuroi* and *F. oxysporum* species complexes. Branch length are proportional to phylogenetic distance. Numbers at nodes indicate ML-BS values ≥ 95 % and PP ≥ 0.95. GenBank accession numbers (*RPB1* and *TEF1*, respectively), are shown between parenthesis. *Fusarium* isolates from South African *Agapanthus* and associated soil samples, DNA sequences newly generated in this study and fully supported branches (ML-BS = 100 %, PP = 1) are indicated in **bold**.

the latter proved to be contaminated with the *Fusarium* sp. (microconidia commonly sporulated from microconidiophores on the sides of the synnemata), as well as a bacterium, which co-occurred among the mucoid conidial droplets at the tips of the synnemata.

BLAST results and phylogeny

Pairwise DNA alignments in the FusarioidID database (www.fusarium.org) showed that seven *Fusarium* strains isolated from *A. praecox* lesions or associated soil samples displayed 99.78 % and 100 % sequence similarity for *rpb2* and *tef1*, respectively, with the ex-type culture of *F. agapanthi* (NRRL 54463, GenBank accession numbers KU900625 and MN193856). Phylogenetic analyses based on partial *rpb2* and *tef1* sequences were conducted to confirm the identity of *Fusarium* isolates from *Agapanthus*. The analyses included sequences from 46 isolates encompassing type and reference cultures of 32 selected taxa of the *Fusarium fujikuroi* species complex, plus two outgroup taxa (*Fusarium nirenbergiae* CBS 744.79 and *Fusarium inflexum* CBS 716.74). Individual and combined gene phylogenies by both ML and Bayesian analyses resulted in identical topologies, thus Fig. 1 shows the ML topology with both ML-BS and PP support values. Six *Fusarium* isolates from soil and one from *A. praecox* were confirmed to belong to *F. agapanthi*.

Ribosomal DNA sequences (SSU, LSU and ITS) derived from the diverse conidial types [conidia produced on dry aerial chains (CPC 46094a) and synnemata (CPC 46094b)] were 100 % identical, showing no traces of contamination according to electropherograms, confirming the different conidial ontogenies as belonging to the same individual. BLAST searches of all three gene fragments indicated a genetic affinity to members of the *Pyxidiophoraceae* (*Pyxidiophorales*, *Laboulbeniomycetes*). Closest hits using the SSU sequence had highest similarity to *Pyxidiophora corallisetosa* (strain MG206c, GenBank MZ621256, 100 % sequence similarity, *Pyxidiophora*

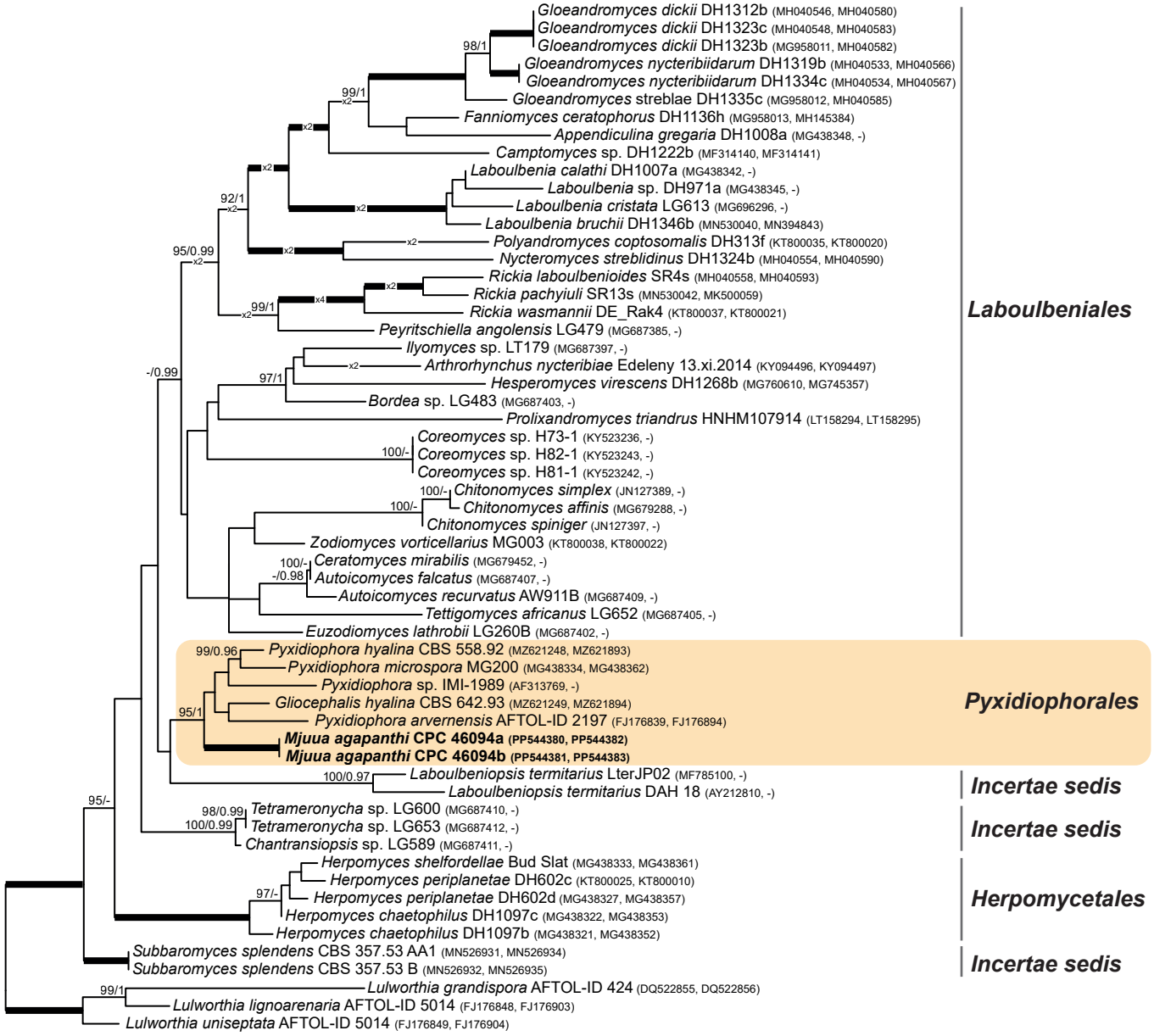

Fig. 2. IQ-TREE-ML phylogeny reconstructed using combined SSU and LSU sequences of representative taxa of the *Laboulbeniomycetes* and *Sordariomycetes*. Branch length are proportional to phylogenetic distance. Numbers at nodes indicate ML-BS values ≥ 95 % and PP ≥ 0.95. GenBank accession numbers (SSU and ITS, respectively), are shown between parenthesis. Isolates from South African *Agapanthus*, DNA sequences newly generated in this study and fully supported branches (ML-BS = 100 %, PP = 1) are indicated in **bold**.

schoenoplecti (strain NN047773, GenBank OP114670; 99 % sequence similarity), and *Pyxidiophora avernensis* (strain CBS 657.82, GenBank MZ621251; 99 % sequence similarity). Closest hits using the LSU sequence had highest similarity to *P. avernensis* (strain CBS 253.81, GenBank MZ621895; 92.38 % sequence similarity), *Pyxidiophora hyalina* (strain CBS 558.92, GenBank MZ621893, 92.24 % sequence similarity, and *P. corallisetosa* (strain MG206c, GenBank MZ621902; 92.13 % sequence similarity). Closest hits using the ITS sequence had highest similarity to an unidentified "*Chalara*" sp. (strain OTU693, GenBank MK018978; 94.67 % sequence similarity), *Pyxidiophora microspora* (strain MG200k, GenBank MZ642304, 82.4 % sequence similarity, and *Pyxidiophora nyctalidis* (strain MG219k, GenBank MZ642307; 82.35 % sequence similarity). A phylogeny of the *Laboulbeniomycetes* based on LSU and SSU sequences was constructed based on Blackwell *et al.* (2020) and Haelewaters *et al.* (2021) (Fig. 2). ITS data were excluded from the final analysis due to the scarcity of sequences available in databases for this fungal group. The analysis included sequences from 55 strains representing 29 genera of the *Herpomycetales*, *Laboulleniales*, *Pyxidiophorales*, and three related groups of uncertain affiliation, plus three *Lulworthia* spp. (*Lulworthiales*) as outgroup taxa (*L. grandispora* AFTOL-ID 424, *L. lignoarenaria* AFTOL-ID 5014 and *L. uniseptata* AFTOL-ID 5014). Isolate CPC 46094 consistently grouped within the *Pyxidiophorales* (and its single family *Pyxidiophoraceae*), resolved as a fully supported lineage (ML-BS 100 %, PP 1), basal to an unsupported clade containing representatives of the genera *Pyxidiophora* and *Gliocephalis hyalina.*

The bacterium *Alsobacter metallidurans* (NCCB 101035) was cultivated from the filtered mixed fungal culture (CPC 46094 = CBS 151304) after 4 d of incubation on R2A agar at 30 °C. This isolate from the alphaproteobacteria class exhibited the lysis of fungal cell walls in culture. Several other bacteria were cultivated from the mixed culture (CPC 47603 = CBS 151407) on TSA. These included *Rahnella aquatilis / R. aceris* (NCCB 101030); *Pseudomonas* sp. (NCCB 101031), which exhibited the highest partial 16SrRNA similarity (98.6 %) with "*Pseudomonas pratensis*", followed by "*P. massiliensis"* (97.6 %); and *Achromobacter* sp. (NCCB 101032), having a 98.8 % match with *Achromobacter spanius / A. mucicolens*. The bacteria from the second collection (CPC 47603 = CBS 151407) did however not exhibit the lysis of fungal cell walls as observed in the first mixed culture (CPC 46094 = CBS 151304).

Fungal interaction

No growth of *M. agapanthi* was observed when open, 6-cm-diam Petri dishes colonised by *Fusarium* spp. were positioned adjacent to dishes of *M. agapanthi* in different combinations inside sealed, larger 15-cm-diam Petri dishes. However, Petri dishes that were separately inoculated with a conidial suspension of *M. agapanthi*, and subsequently inoculated with the respective *Fusarium* spp. in a V-pattern across the dish resulted in good growth (both synnematal and microconidial morphs) along the *Fusarium* inoculation line, confirming the fact that physical contact is needed between *M. agapanthi* and a *Fusarium* sp. to stimulate growth of *M. agapanthi* (Fig. 3R, S). Successful co-cultivation was not restricted to *F. agapanthi* but was also achieved with the other *Fusarium* spp. tested.

Taxonomy

Mjuua Crous & Sand.-Den., ***gen. nov.*** MycoBank MB 852966.

Etymology: Named in honour of Michael John Wingfield (MJW), pronounced as "M-J-double u", on the occasion of his 70th birthday (21 April 2024).

Classification: *Pyxidiophoraceae*, *Pyxidiophorales*, *Laboulbeniomycetes*.
Synnematal morph: *Synnemata* erect, arising from submerged hyphae, hyaline, smooth, straight, consisting of numerous tightly aggregated conidiophores. *Conidiogenous cells* terminal on septate, cylindrical, hyaline, smooth conidiophores, with each terminal cell giving rise to 1–3 conidiogenous cells, subcylindrical, hyaline, smooth, phialidic, but with indistinct collarette. *Conidia* hyaline, smooth, aseptate, globose to clavate, apex obtuse, base truncate, solitary, aggregating in mucoid mass. *Microconidial morph*: *Microconidiophores* arising from submerged hyphae, reduced to conidiogenous cells or short conidiophores, hyaline, smooth, subcylindrical, 0–2-septate, giving rise to 1–3 conidiogenous cells, subcylindrical to subulate, hyaline, smooth, phialidic, giving rise to long chains of unbranched conidia. *Conidia* aseptate, hyaline, smooth, fusoid with truncate ends that appear slightly darkened and refractive.

Type species: *Mjuua agapanthi* Crous & Sand.-Den.

Mjuua agapanthi Crous & Sand.-Den., ***sp. nov.*** MycoBank MB 852967. Figs 3–6.

Etymology: Name refers to the host plant genus it occurs on, *Agapanthus*, along with its *Fusarium* host.

Synnematal morph: *Synnemata* erect, arising from submerged hyphae, hyaline, smooth, straight, up to 60 µm diam, 300 µm tall, consisting of numerous tightly aggregated conidiophores (Fig. 6). *Conidiogenous cells* terminal on septate, cylindrical, hyaline, smooth conidiophores, with each terminal cell giving rise to 1–3 conidiogenous cells, subcylindrical, hyaline, smooth, phialidic, but with indistinct collarette, 20–35 × 2–3 µm (Fig. 6). *Conidia* hyaline, smooth, aseptate, globose to clavate (Fig. 5), apex obtuse, base truncate with minute marginal frill [only seen under SEM (Fig. 6A), not light microscopy], solitary, aggregating in mucoid mass, 5–7 × 3–4 µm. *Microconidial morph*: *Microconidiophores* arising from submerged hyphae, reduced to conidiogenous cells or short conidiophores (Figs 3M–O, 6D), hyaline, smooth, subcylindrical, 0–2-septate, 20–60 × 3–4 µm, giving rise to 1–3 conidiogenous cells, subcylindrical to subulate, hyaline, smooth, 20–30 × 3–4 µm, phialidic, but collarette indistinct, giving rise to long unbranched chains of conidia. *Conidia* aseptate, hyaline, smooth, fusoid with truncate ends (Fig. 4B), that appear slightly darkened and refractive, (7–)10–11(–13) × (2.5)3(–3.5) µm.

Typus: **South Africa**, Western Cape Province, Cape Town, Kirstenbosch, on dead flower stalks of *Agapanthus praecox* (*Amaryllidaceae*), Apr. 2023, *P.W. Crous* (**holotype** CBS H- 25357, culture ex-type CPC 46094 = CBS 151304) (ex-holotype culture includes *F. agapanthi*, which is essential to allow *M. agapanthi* to grow, and a bacterium).

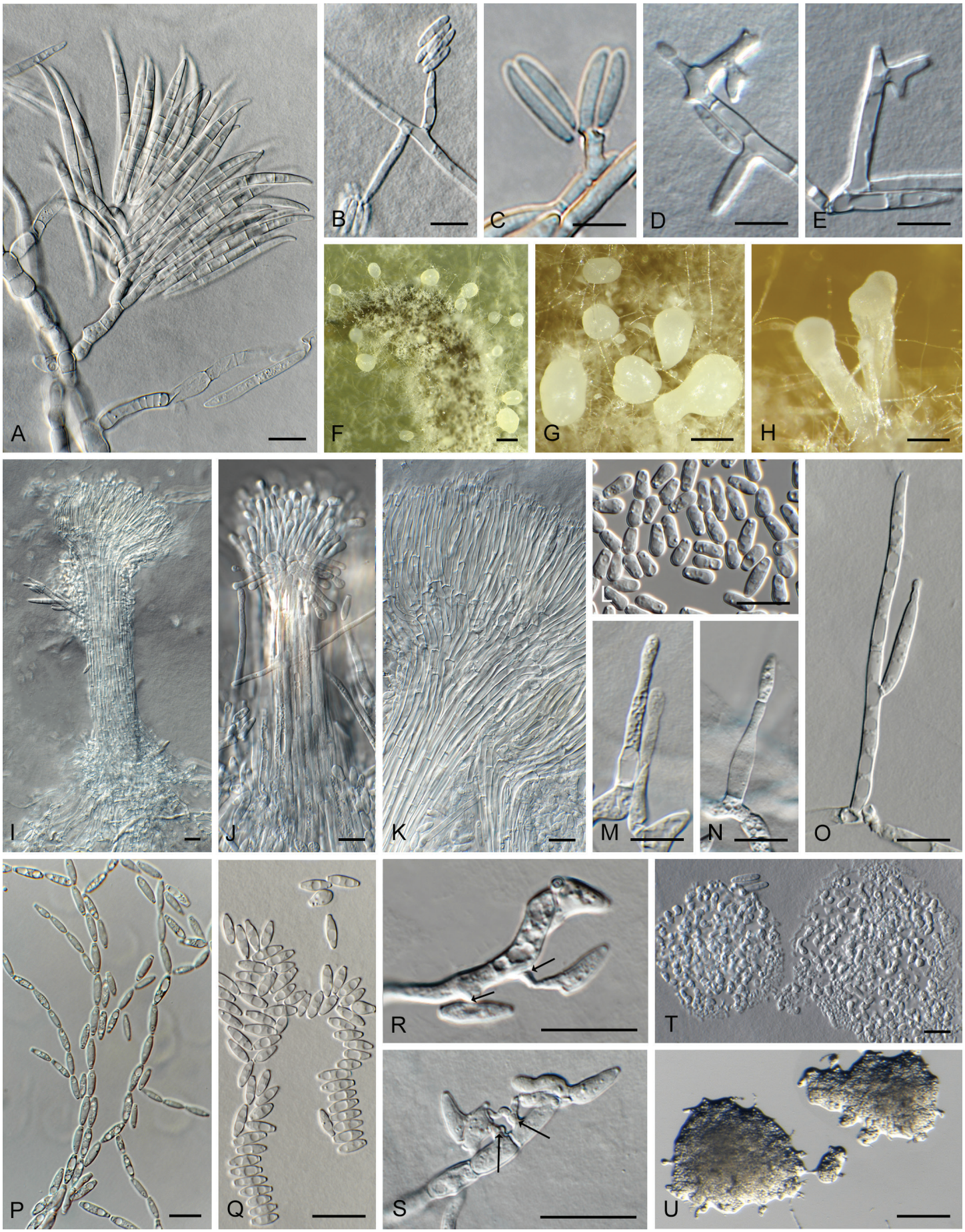

Fig. 3. A–E. *Fusarium agapanthi*. **A.** Macroconidia. **B–E.** Polyphialides with microconidia. **F–U.** *Mjuua agapanthi*. **F–K.** Synnemata, with phialides visible in J and K. **L.** Synnematal conidia. **M–O.** Philides that give rise to chains of microconidia. **P, Q.** Microconidia. **R, S.** Microconidia of *M. agapanthi* anastomosing with macroconidia of *F. agapanthi* (arrows). **T.** Conidia from synnematal head undergoing lysis due to bacterium. **U.** Colonies of *M. agapanthi* forming sporodochia, but not growing due to the absence of *F. agapanthi*. Scale bars: F–H = 60 µm; U = 100 µm; all others = 10 µm.

Additional isolate examined: **South Africa**, Western Cape Province, Cape Town, Kirstenbosch, on dead flower stalks of *A. praecox*, Nov. 2023, *P.W. Crous*, culture CPC 47603 = CBS 151407 (culture includes *F. agapanthi*, which is essential to allow *M. agapanthi* to grow, and a bacterium).

Notes: *Mjuua* is a genus in the *Pyxidiophoraceae* (*Pyxidiophorales*, *Laboulbeniomycetes*). Although all sexual morphs in the family have to date been treated as *Pyxidiophora*, it appears that this might be a generic complex, linked to different asexual morphs. Species of *Pyxidiophora* have been linked to up to three distinct morphs (Malloch 1995), namely *Gliocephalis*, *Thaxteriola*, *Pleurocatena*, and now also *Mjuua* (see discussion in Jacobs *et al.* 2005, Blackwell *et al.* 2020). Based on Malloch (1995), should a sexual morph of *Mjuua* be found, it would most likely be associated with an insect host.

Kirschner (2003) described two species of *Pyxidiophora* that were associated with bark beetles, colonising the sapwood and secondary phloem of conifers in Europe. Although these species could not be cultivated, he described their asexual morphs (as gabarnaudia-like), which closely resemble the genus *Mjuua* which is newly introduced here. In a subsequent study, Gams & Arnold (2007) validated the hyphomycete genus *Pleurocatena* (based on *P. acicularis*, CBS 180.94), as asexual morph linked to the *Pyxidiophora* complex. The conidiogenesis of the phialides and catenate conidia closely resemble that of *Mjuua,* except that the latter lacks aculeate setae adjacent to its conidiogenous cells. The *Pyxidiophora* asexual morphs depicted by Kirschner (2003) are similar to *Mjuua*, although they lack a synnematous synasexual morph, and are also distinct from *Gabarnaudia* (Samson 1974), thus presumably represent another lineage in the *Pyxidiophora* generic complex.

During their studies of *Pyxidiophora*, Blackwell & Malloch (1989) observed some species of *Pyxidiophora* that formed synnemata, but these had percurrently proliferating graphium-like conidiogenous cells, unlike those of *Mjuua* (Fig. 6), and usually developed perithecial ascomata a few days later. In the present study however, no sexual morph was observed in culture or on plant material, nor was an insect association observed in nature (only numerous nematodes were observed on the *Agapanthus* stems). Further studies are therefore required to further elucidate the ecology of *Mjuua* and its associated fungal and bacterial partners.

DISCUSSION

Fusarium agapanthi

Fusarium agapanthi has been associated with symptomatic *Agapanthus* in Australia and Italy but is not known from this host at the centre of origin of *Agapanthus*, South Africa. Because *Agapanthus* has been shown to harbour fungal pathogens with an endophytic growth phase, the aim of this study was thus to sample *Agapanthus* plants and surrounding soil from South Africa to determine if *F. agapanthi* could also be present in this country. *Agapanthus* plants were sampled on two occasions in Kirstenbosch, Cape Town, namely during April and November 2023. Although plants appeared healthy, bulbs of *Agapanthus praecox* with dead flower stalks that showed a pink to red

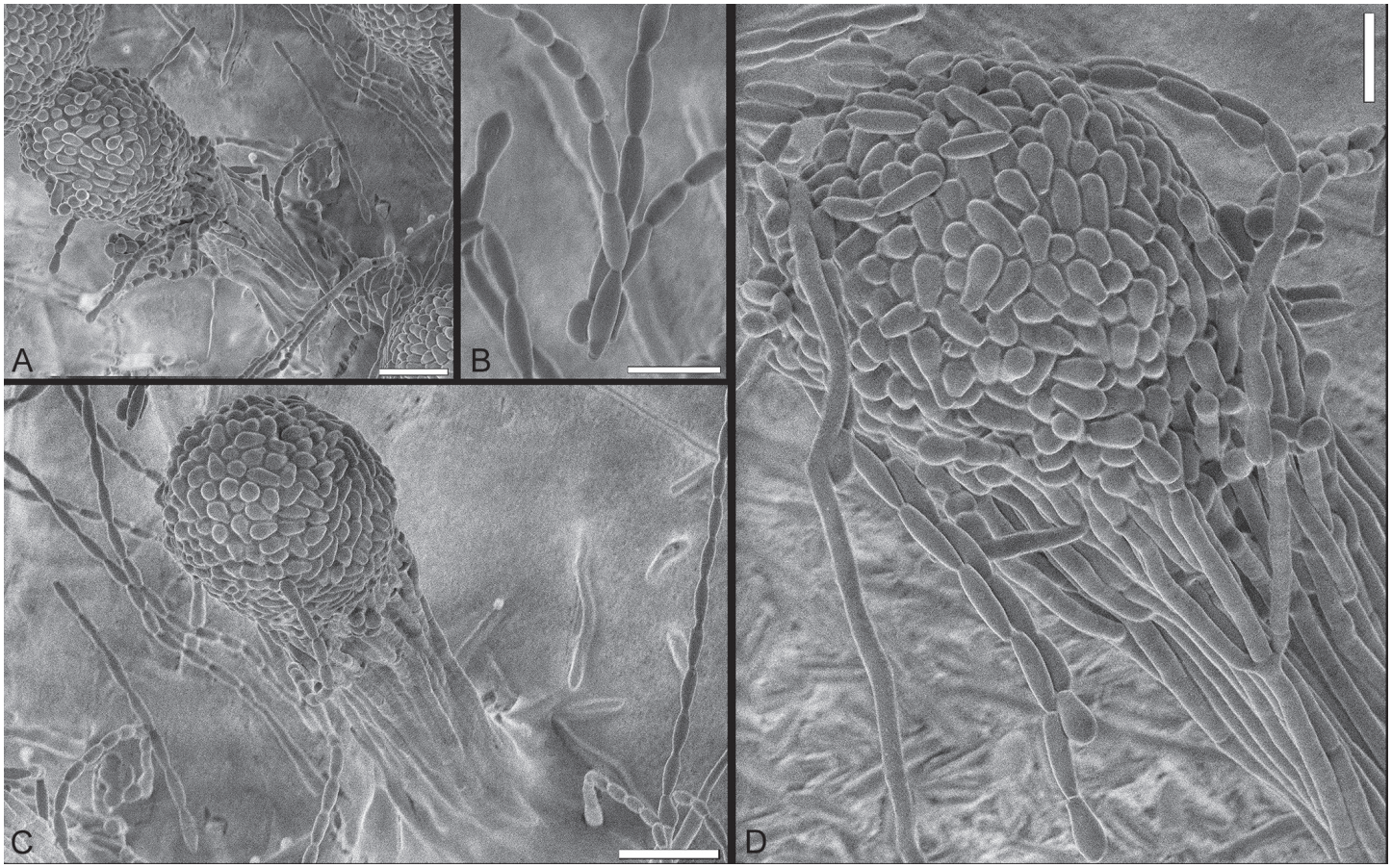

Fig. 4. Uncoated samples of *Mjuua agapanthi* after 1 wk of cultivation on SNA. **A, C.** Synnemata accompanied with conidial chains of the microconidial morph. **B.** Chains of microconidia. **D.** Detail of synnema in which the conidiophore chains of the microconidial morph are originating from branches on a conidiophore very close to the stalk of the synnema. Scale bars: B, D = 10 µm; A, C = 20 µm.

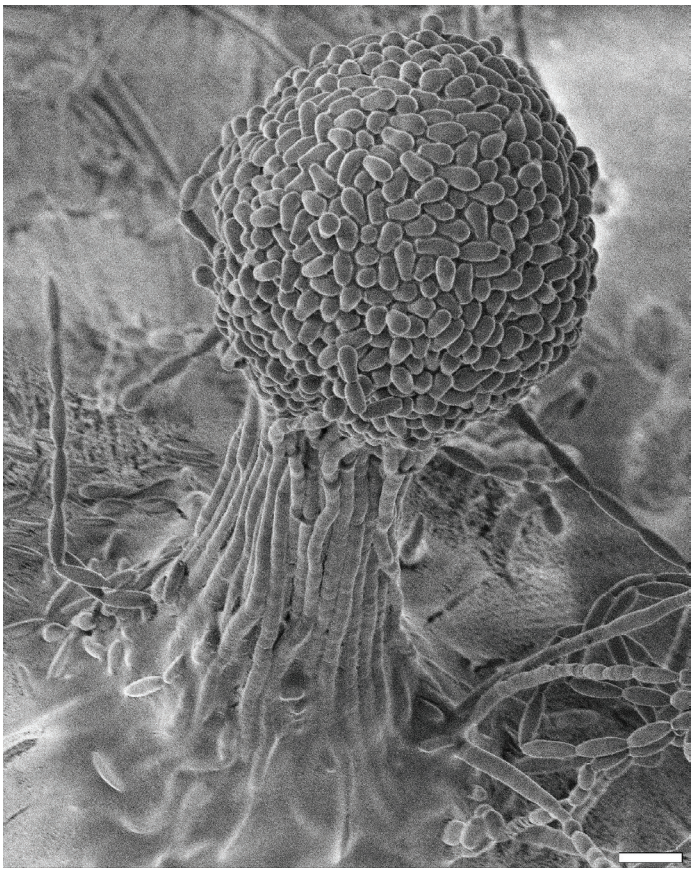

Fig. 5. Overview of an entire synnema of *Mjuua agapanthi* as a stack of three pictures. Note the composition of the stalk arising from the agar surface, the accompanying conidial chains and the characteristic bulbous shape of the conidia on top of the structure. Scale bar = 10 µm.

discolouration in the bottom 3–10 cm were found to be infected with *F. agapanthi*. Furthermore, fungal isolations from soil dilutions resulted in several fusaria being isolated, including *F. agapanthi*, proving that the pathogen is also well established in soil surrounding the *Agapanthus* plants.

Mjuua agapanthi

During isolations from plant material, a hyaline, synnematous fungus with a microconidial morph giving rise to long, unbranched conidial chains was also observed, which based on DNA data and morphology proved to represent a new species and genus, described here as *Mjuua agapanthi*. Despite attempts to derive axenic single-spore cultures of the latter, *M. agapanthi* could not be successfully cultivated without *F. agapanthi*, of which it proved to be a biotrophic mycoparasite. Furthermore, colonies of *M. agapanthi* were associated with various bacterial species that co-occur among the globose conidia in its synnematal heads; the role of the latter is unclear, but it cannot be excluded that the bacteria are more than mere contaminants, as *Alsobacter metallidurans* from the dual culture (CPC 46094 = CBS 151304) appeared to degrade conidial cell walls on synnematal heads (Fig. 3T). Without the *Fusarium* host, conidia of *M. agapanthi* (synnematal, and microconidial) do germinate, but do not form mycelium, but rather germinate to form slimy sporodochia (Fig. 3U), giving rise to chains of microconidia via microcyclic conidiation, or via short hyphae that form conidiogenous cells.
Although *F. agapanthi* was isolated from soil samples adjacent to *Agapanthus* plants, these isolates were obtained as pure cultures, without the presence of *M. agapanthi*, which was

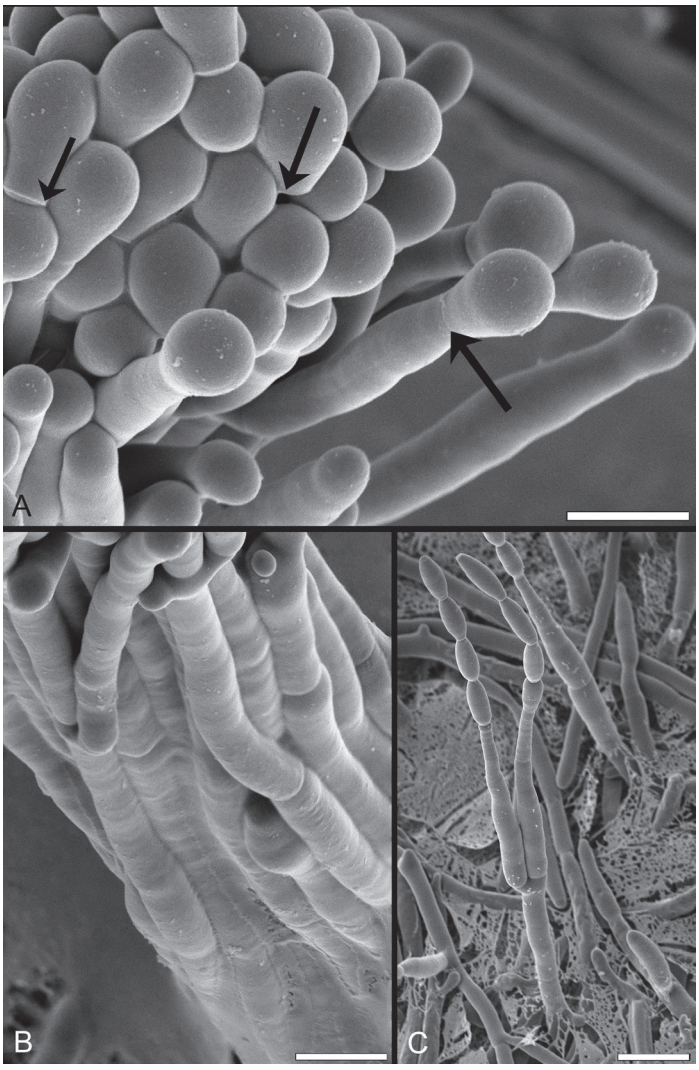

Fig. 6. Gold-coated samples of *Mjuua agapanthi* after 1 wk of cultivation on SNA. **A.** Details of spore formation on the synnema. At the right side first swelling of the conidiogenous cell is visible, including the formation of a first septum delineating the young conidium (arrow). Further stages show fully formed conidia with a stalk-like end and a conspicuous large scar (arrows). **B.** Details of the synnematal stalk with aggregated conidiophores. **C.** Conidiophore with microconidial morph arising from branched conidiophore. Conidiogenous cells exhibit a characteristic long-tapered neck. Scale bars: A, B = 5 µm; C = 10 µm.

only associated with some *F. agapanthi* isolates occurring on *Agapanthus* plants. The association between *F. agapanthi* and *M. agapanthi* seems well-established, as they were again isolated as co-occurring on *Agapanthus* plants in the same area during the second sampling trip in November 2023. However, The *Fusarium* association also proved to not be restricted to *F. agapanthi* only, as successful co-cultivation was also achieved with the other fusaria tested. Results from this study suggest that many unculturable obligate biotrophic microbes remain unstudied, given present established protocols. However, it is possible that some could be cultivated if they were co-cultivated with their respective hosts.

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