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ORIGINAL ARTICLE

Diversity of *Fusarium* associated with banana wilt in eastern Democratic Republic of Congo

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Abstract

Fusarium wilt of banana (FWB) is a major agricultural threat worldwide, historically attributed to several races of *Fusarium oxysporum* f. sp. *cubense* (Foc). The updated taxonomic scheme tends to group these races of Foc into distinct species. In addition to the *F. oxysporum* species complex (FOSC), a few species within the *Fusarium fujikuroi* species complex (FFSC) have occasionally been reported on bananas. In eastern Democratic Republic of Congo, FWB has been reported across North and South Kivu. This study aims to assess the diversity of *Fusarium* locally associated with FWB through morphological, phylogenetic, and pathogenic characterization. Seventy *Fusarium* strains were isolated from pseudostems showing infection of vascular tissues. Morphological characterization was performed on synthetic low-nutrient agar, potato dextrose agar, and carnation leaf agar. Pathogenicity was assessed on Cavendish and Gros-Michel in greenhouse conditions. Molecular phylogenetic relationships were inferred based on DNA sequence data from partial translation elongation factor 1-alpha and the second-largest subunit of RNA polymerase. To confirm species identity, partial calmodulin and beta-tubulin gene regions were also sequenced for some strains. Of the 70 *Fusarium* strains analyzed, 31 belonged to the FOSC, including 28 strains identified as *F. tardichlamydosporum* and one strain (F141) identified as *F. duoseptatum*, both previously recognized as Foc Race 1. Two additional FOSC strains (F93 and F146) formed a distinct clade, separate from the newly defined Foc species. No isolate was identified as *F. odoratissimum* (Foc TR4). Thirty-five strains were classified as *F. joanfreemaniae*, a member of the *F. fujikuroi* species complex. In addition, *F. solani*, *F. andiyazi*, and *F. incarnatum* have been reported. *Fusarium tardichlamydosporum* and *F. duoseptatum* strains induced symptoms under all tested conditions. Inversely, *F. joanfreemaniae* caused symptoms on Gros Michel and Cavendish only under stressed, injured roots, indicating a weak or opportunistic role. This highlights a multi-species disease complex with implications for resistance-based management.

Keywords Vascular wilt · *Musa* · Plant disease complex · Co-infections · Phylogenetic analysis · Morphological characterization · *Fusarium joanfreemaniae*

Introduction

Bananas and plantains (*Musa* spp.) are among the most widely produced and traded fruits worldwide (Evans et al. 2020). They are cultivated in all tropical and subtropical regions and represent a staple food and a source of income for over 400 million people in the world (Magdama et al. 2020; Madalla et al. 2023). Banana consumption in central and southern Africa is among the highest in the world (Akankwasa et al. 2021; Martínez-de la Parte et al. 2024). The fruit is sold in local markets mainly as cooked (plantains) but also as dessert bananas (Ndayihanzamaso et al. 2020; Mukeshambala et al. 2024). Some varieties are

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used also to make homemade beer (Karangwa et al. 2016; Marimo et al. 2020).

In the Democratic Republic of Congo (D.R.C.), bananas and plantains plantations covered 1.2 million hectares in 2018, and represented one of the most important staple crops (FAO stat, 2024). However, the local production remains low, averaging 10 tons per hectare, compared to 50 tons per hectare in some regions of the world (Beed et al. 2010). This low productivity is due to several factors, including diseases (Drenth & Kema 2021; Mukeshambala et al. 2024).

Among banana diseases, *Fusarium* wilt of banana (FWB), known as Panama disease, is one of the most devastating diseases worldwide (Chittarath et al. 2022; Tshikhudo et al. 2025). It caused the loss of several thousand hectares of Gros Michel bananas in the mid-twentieth century (Ghag et al. 2015). FWB is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). The fungus attacks plants at the root system, damaging the xylem and pseudovessels, disrupting water and nutrients transport (Stover 1962; Pegg et al. 2019).

Fusarium oxysporum f. sp. *cubense* belongs to a group of cosmopolitan fungi known as the *Fusarium oxysporum* species complex (FOSC) (Pegg et al. 2019; EFSA PLH Panel et al. 2022; Chittarath et al. 2022). Four races were historically defined within Foc (Ploetz 2006) but only R1, R2, and R4 infect banana cultivars. Race 1 affects "Gros Michel" and other dessert bananas from the AAA and AAB genomic groups, whereas Race 2 targets "Bluggoe" cultivars from the ABB subgroup and Race 4 affects many cultivars, particularly those in the Cavendish subgroup, which are resistant to Race 1. Race 3 is associated with *Heliconia* spp. and is no longer considered pathogenic to bananas (Ploetz 2015a; Warman and Aitken 2018). Race 4 is further subdivided into two groups: subtropical Race 4 (SR4), found in subtropical regions, and tropical Race 4 (TR4) (Ploetz 2015a; Garcia et al. 2018; EFSA PLH Panel et al. 2022). The subtropical Race 4 affects Cavendish plants only under predisposing conditions such as cold winter temperatures, soil water saturation, or drought and is not reported to infect Cavendish in tropical environments. In contrast, TR4 can infect Cavendish plants regardless of environmental conditions or the physiological status of the host (González-Arriagada et al. 2024; Dita et al. 2018; Warman & Aitken 2018; Pegg et al. 2019).

The TR4 is the most concerning threat nowadays. It first appeared in south-east Asia and is now spreading worldwide. Losses caused by this race are estimated to exceed 100,000 hectares of banana plantation worldwide (Ordonez et al. 2015; EFSA PLH Panel et al., 2022). In Africa, it has been diagnosed in Mozambique in 2013, in Mayotte in 2021, and in the Grande Comoros Island in 2023 (Aguayo et al. 2021; EFSA PLH Panel et al. 2022; Mmadi et al. 2023). The discovery of Cavendish varieties resistant to Race 1 helped control the epidemic until the appearance of Race 4.

The ability of Foc to produce chlamydospores that can survive in the soil for several years and the presence of different cultivar-specific races make the disease highly damaging and difficult to control (Dita et al. 2018; Pegg et al. 2019). Effective management of FWB is not yet fully available. Control generally relies on preventing the introduction of inoculum into uninfected areas and the use of resistant cultivars (Ploetz 2015b). However, the use of resistant cultivars is often limited by the spread of different Foc races and the genetic evolution of strains within Foc and other *Fusarium* species. Understanding the genetic and pathological diversity would enable the development of sustainable management approaches for FWB (Maryani et al. 2019).

Early studies of Foc diversity focused on variation in pathogenicity (Stover and Simmonds 1987). Different Foc races and strains exhibit varying levels of virulence and host specificity (Edel-Hermann and Lecomte 2019). However, pathogenicity alone does not provide a comprehensive understanding of diversity within and between races (Liu et al. 2019). In addition, pathogenicity tests can be influenced by factors such as host age, inoculation method, and environmental conditions (Karangwa et al. 2018).

Other studies have focused on vegetative compatibility, which represents the natural ability of filamentous fungi to fuse their hyphae by anastomosis when they are genetically similar (Glass et al. 2000; Mostert et al. 2022). Within Foc, 24 vegetative compatibility groups (VCGs) have been identified (Fourie et al. 2009; Fourie et al. 2011; Thangavelu et al. 2024)**.** Visser et al. (2010) and Martínez-de la Parte et al. (2024) showed that vegetative compatibility is a useful way of dividing the Foc into genetically isolated groups, but it does not measure genetic relatedness between isolates and VCGs are phenotypic markers that may be under selection pressure.

Studies on the genetic diversity and evolutionary relationships of Foc have progressed considerably over the last two decades and now incorporate molecular and phylogenetic methods (O'Donnell et al. 1998; Maryani et al. 2019; Martínez-de la Parte et al. 2024; Roberts et al. 2024). Morphological observations, virulence studies, and phylogenetic inferences based on the elongation factor 1-alpha (TEF-1α) gene and the large subunits of RNA polymerase II (RPB1 and RPB2) have made it possible to rethink the different Foc races and redefine them as distinct species (Maryani et al. 2019). The lineage traditionally recognized as Foc Race 1 now encompasses several distinct species, namely *F. purpurascens*, *F. hexaseptatum*, *F. phialophorum*, *F. grosmichelii*, *F. duoseptatum*, and *F. tardichlamydosporum*. In contrast, Race 2 corresponds to *F. cugenangense*, while Race 4 has been reassigned to *F. odoratissimum*. Furthermore, *F.*

tardicrescens was assigned to a lineage for which no formal race designation had previously been defined (Maryani et al. 2019; Thi et al. 2022). However, this new taxonomic scheme is still debated. Bedoya et al. (2021) argue that further studies integrating multiple data are essential to refine the classification of Foc. However, other studies, following the approach of Maryani et al. (2019), have facilitated the characterization and analysis of *Fusarium* strain diversity in different regions of the world (Ujat et al. 2021; Kema et al. 2021; Thi et al. 2022; Van Westerhoven et al. 2023).

While Foc is the primary causal agent of FWB, other *Fusarium* species may also be associated with the disease, either as opportunistic pathogens or as contributors to disease severity in co-infections. Some species from the *F. fujikuroi* species complex (FFSC), such as *F. sacchari*, *F. verticillioides*, *F. mindanaoense*, *F. fujikuroi*, *F. mangiferae*, *F. musae*, *F. proliferatum*, and *F. joanfreemaniae* have been reported in association with bananas (Karangwa et al. 2018; Huang et al. 2019; Maryani et al. 2019; Ujat et al. 2021; Thi et al. 2022; Nozawa et al. 2023; dos Santos Santana et al. 2026). *Fusarium triseptatum* and *F. solani*, which do not belong to the FFSC, have also been observed in diseased plants. Pathogenicity to banana has been experimentally confirmed for *F. mindanaoense* (Nozawa et al. 2023), *F. verticillioides* (M108), *F. triseptatum* (dos Santos Santana et al. 2026), and *F. sacchari* (M7) (Maldonado-Bonilla et al. 2019; dos Santos Santana et al. 2026).

A major challenge in combating FWB in eastern D.R.C. is the limited understanding of the diversity of *Fusarium* species associated with the disease. Studies conducted by Karangwa et al. (2018) showed the presence of Foc Races 1 and 2 in the Great Lakes region of Africa (Eastern D.R.C., Rwanda, Burundi). However, in the D.R.C., studies on FWB remain scarce, with little data available on the genetic diversity, pathogenicity, and distribution of races or species. This gap presents a major obstacle to the implementation of effective control measures including the development of disease-resistant varieties. Furthermore, understanding the diversity of *Fusarium* species is essential for assessing the potential introduction or emergence of TR4 in the region. Early detection and characterization of virulent strains can help policymakers, researchers, and farmers to implement proactive measures to prevent large-scale outbreaks. Given the significance of bananas for food security, addressing this knowledge gap through scientific research is crucial for ensuring the sustainability of banana production in the eastern D.R.C.

The aim of this study was to assess the diversity of *Fusarium* species associated with banana wilt in eastern D.R.C. using an integrated morphological, molecular, and pathological approach, with pathogenicity assessed by two banana inoculation methods.

Materials and methods

Study area and sample collection

Surveys of banana plants affected by *Fusarium* wilt were conducted in May and June 2019 across six territories in eastern D.R.C., in South Kivu (Kabare, Walungu, Kalehe, and Idjwi) and North Kivu province (Masisi and Rutshuru) (Fig. 1). These areas were selected based on their importance in banana production.

Fusarium-infected banana plants were identified in the field by observing characteristic symptoms, including yellowing of older leaf margins, leaf collapse at the petioles, and discoloration and splitting of pseudostems (Stover 1962; Ploetz 2015a). The pseudostems of diseased plants were cut, and discolored brownish vascular tissue was sampled, placed on sterile filter paper in a paper envelope, labeled and transported to the laboratory under cool conditions. Global positioning coordinates were recorded for each sample collected. A total number of 151 samples were collected from the six territories, respectively, 31 in Idjwi, 26 in Kabare, 32 in Kalehe, 21 in Masisi, 24 in Rutshuru, and 17 in Walungu.

Banana cultivars were identified based on morphological descriptors according to Simmonds and Shepherd (1955) and the East African Banana Descriptor (Karamura et al. 2012), together with the vernacular names used by farmers.

Isolation and morphological characterization

Dried pseudostem samples were cut into 2- to 3-cm segments and surface-sterilized by immersion in 70% ethanol for 30 s, followed by treatment in a 1% sodium hypochlorite solution for 1 min. The segments were then rinsed five times in sterile distilled water and dried on sterile blotting paper. Three disinfected segments were placed onto synthetic low-nutrient agar (SNA) supplemented with 0.5% chloramphenicol and incubated at 25 °C in darkness for 4 days. Axenic cultures were obtained by collecting a small amount of conidia from SNA with an inoculation needle and streaking them onto water agar plates, which allowed the conidia to separate. After 24 h of incubation, plates were observed under an inverted microscope, and individual germinating conidia were collected and transferred to potato dextrose agar (PDA). Monosporic isolates were maintained in 15% (v/v) glycerol at − 80 °C. All isolates were deposited at the Laboratory of Plant Health, Applied Microbiology, Earth and Life Institute, UCLouvain, Belgium.

Morphological characterization of all *Fusarium* isolates was performed on carnation leaf agar (CLA), synthetic low-nutrient agar (SNA), and potato dextrose agar (PDA). Growth rates were measured after 7 days of incubation at

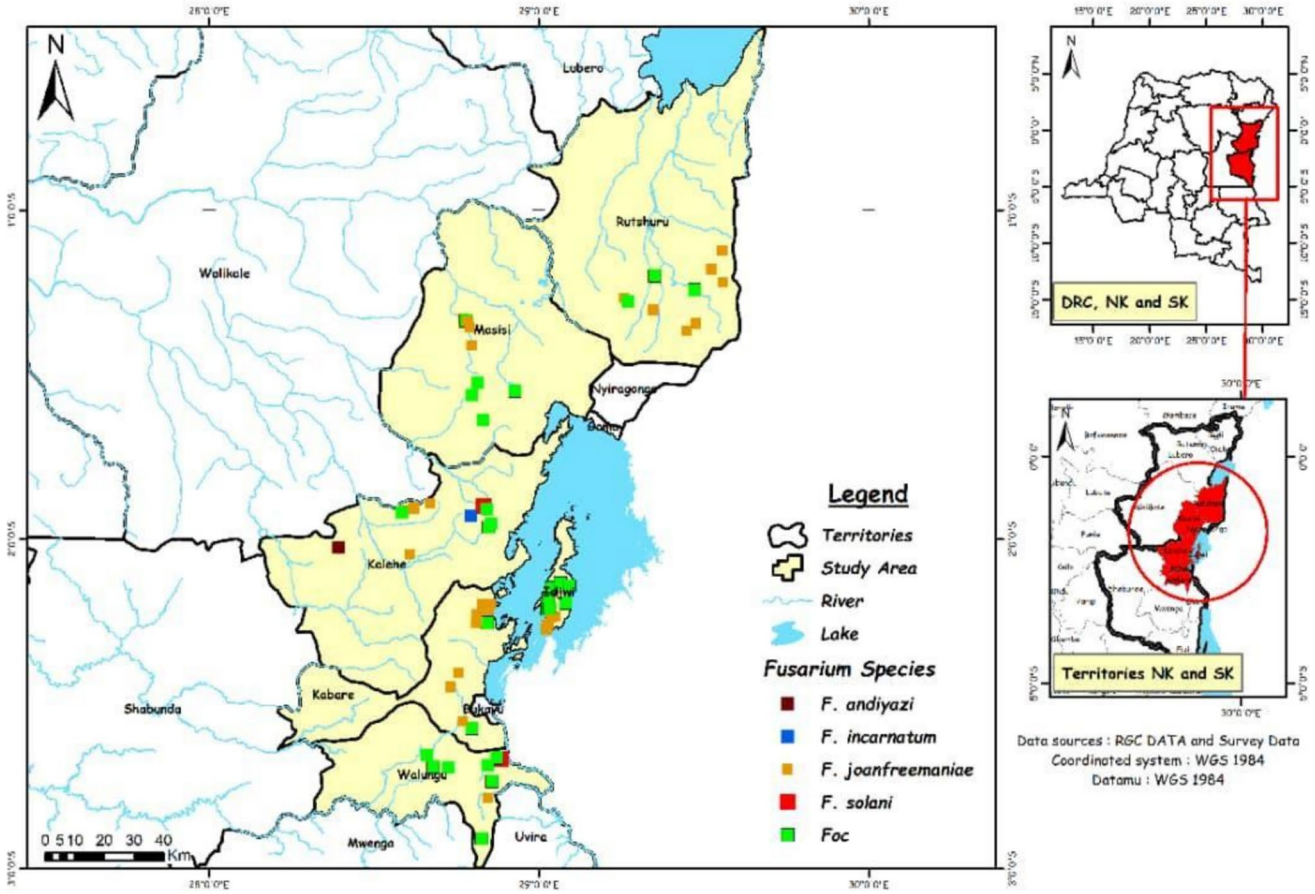

Fig. 1 Distribution of *Fusarium* species identified in South and North Kivu provinces, eastern Democratic Republic of the Congo. The map was generated using ArcMap 10.8.1

25 °C in the dark on PDA. Colonies developing on SNA were examined under a light microscope (Olympus BH-2) at × 400 magnification. Colony color and reverse colony color were recorded on PDA at room temperature. In the initial stage of cultivation, isolates were identified as *Fusarium* spp. based on hyphal morphology and the presence of different types of conidia, following the protocols and species descriptions of Maryani et al. (2019) and Thi et al. (2022).

Molecular identification and phylogenetic analysis of *Fusarium* strains

Fungal DNA was extracted using a cetyl trimethylammonium bromide (CTAB) method based on the study of Lin et al. (2013) and Thi et al. (2022). Amplification reactions of the translation elongation factor 1-alpha gene (TEF-1α) using primers EF1 (5′ATGGGTAAGGARGACAAGAC3′) and EF2 (5′GGARGTACCAGTSATCATGTT3′) (O'Donnell et al. 1998) and the RNA polymerase second-largest subunit gene (RPB2) using primers RPB2-5f2 (GGGGWGAYC AGAAGAAGGC) and RPB2-7cr (CCCATRGCTTGYTTR CCCAT) (O'Donnell et al. 2010), were performed using standard PCR (25 µL). Reactions were initiated with a 3-min heating at 95 °C followed by 30 cycles consisting of 95 °C for 30 s, 57 °C (TEF-1α), or 60 °C (RPB2) for 60 s, and 72 °C for 60 s. Reactions were terminated by incubation at 72 °C for 3 min. To confirm species identity within certain groups, the calmodulin (CAL) and β-tubulin (TUB) gene regions were also sequenced for a subset of strains using the following primer pairs: CL1D (5′-GAG TTCAAGGAGGCCTTCTC-3′) and CL2A (5′-TTTTTG CATCATGAGTTGGAC-3′) (O'Donnell et al. 2000), and T1 (5′-AACATGCGTGAGATTGTAAGT-3′) and T22 (5′-TCTGGATGTTGTTGGGAATCC-3′) (O'Donnell et al. 2000). PCR reactions for *Calmodulin* were initiated with a 5-min heating at 94 °C, followed by amplification for 30 cycles, including denaturation at 94 °C for 30 s, annealing at 52 °C for 1 min, extension at 72 °C for 1 min, and a final extension step of 7 min at 72 °C. PCR reactions for *β-tubulin* were initiated with a 3-min heating at 94 °C, followed by amplification for 30 cycles, including denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and 30 s, extension

at 72 °C for 2 min, and a final extension step of 10 min at 72 °C. PCR products were sequenced in both directions by Microsynth Seqlab GmbH (Germany) using standard Sanger sequencing protocols. Strain sequences have been deposited in GenBank, as listed in the Supplementary Table 1.

Sequence data for the 67 *Fusarium* reference strains, including TEF-1α sequences for all strains and RPB2, CAL, and TUB sequences where available, were extracted from GenBank via NCBI (Supplementary Table 2). Including the sequences from 70 of our characterized strains (Table 1 and Supplementary Table 1), the final data matrix comprised 137 strains. Sequences from each locus (TEF-1α, RPB2, CAL, and TUB) were automatically aligned in MEGA 11 (Tamura et al. 2021) using ClustalW (Larkin et al. 2007) and subsequently manually refined and assembled into a combined multilocus dataset in Phylogenetic Data Editor (PhyDE) version 0.9971 (Müller et al. 2010). Maximum Likelihood (ML) phylogenetic analyses were conducted using RAxML v.8 (Stamatakis 2014) under the GTR + GAMMA model of nucleotide substitution, which is widely applied in *Fusarium* multilocus phylogenies (O'Donnell et al. 2012). Node support was assessed with 1,000 rapid bootstrap replicates. Bootstrap values ≥ 70% were considered to indicate moderate support, and values ≥ 90% strong support. Phylogenetic trees were visualized and edited using FigTree v1.4.4. Phylogenetic trees were rooted using *F. dimerum* as the outgroup taxon (Maryani et al. 2019).

Pathogenicity assays

In vitro plants of cultivars Gros Michel and Cavendish (cv. 902) were obtained from Vitropic (France) and transferred to individual 1-L pots containing professional potting soil (DCM, De Ceuster Meststoffen, Belgium) that had been sterilized twice by autoclaving at 121 °C for 15 min. The plants were then placed in an environmentally controlled greenhouse compartment (28 ± 2 °C, 16-h light, 80% relative humidity). Plants were weaned under plastic for 2 weeks to maintain high-humidity conditions and then grown for 8 weeks prior to inoculation.

Based on the multilocus analysis, ten *Fusarium* strains (F19, F20, F28, F32, F37, F39, F52, F71, F132, and F141) were selected for pathogenicity assays. *Fusarium* strains were grown at 25 °C on PDA for 10 days. To collect the conidia, 3 mL of sterile water containing 0.05% Tween 20 was added to the Petri dishes, and the mycelium and spores were harvested. The conidia were obtained by filtration through a double layer of cheesecloth. The concentration of the suspension was determined using a Thoma cell (hemocytometer), and the inoculum was adjusted to 10^6 conidia/mL.

Two inoculation methods described by Garcia-Bastidas et al. (2019) were used: (1) dipping banana plants with trimmed roots into a spore suspension, and (2) pouring the inoculum directly onto the soil (pouring method). For the dipping method, banana plants were uprooted, their root systems rinsed with water, and the root tips wounded by removing approximately 10 cm. The roots were then soaked for 30 min in 500 mL of a solution containing 10^6 conidia/mL. After inoculation, the plants were transferred to 2-L pots. Roots of control plants were wounded in the same way and soaked in sterile distilled water. For the pouring method, a 500 mL solution of inoculum (10^6 conidia/mL) was poured into the pot containing the banana plantlet. In the control treatment, a solution of sterile distilled water was used instead. Each treatment was replicated four times.

The plants were grown at 28 °C and 80% relative humidity (Dita et al. 2011; Viljoen et al. 2017; Karangwa et al. 2018). They were watered with sterile water. After inoculation, the plants were observed and scored for disease symptoms and progression at weekly intervals. Plants were inspected externally and internally when they were completely rotten, or finally at 6 weeks after inoculation. For external symptoms, yellowing/wilting of leaves was scored on a 0–4 scale, where 0 = no wilting symptoms; 1 = 1–2 yellowing/wilting leaves; 2 = 3–4 yellowing/wilting leaves; 3 = 5 yellowing/wilting leaves; and 4 = more than 5 yellowing/wilting leaves (Kuswinanti et al. 2011). For internal assessment, plants were uprooted, cleaned, and cut longitudinally at the rhizome (corm). The Rhizome Discoloration (RD) was assessed based on the extent of internal tissue invasion in the pseudostem, using a scale from 0 to 4 as follows: 0 = no invasion of the pseudostem; 1 = invasion limited to the outer sheaths; 2 = slight invasion along the vascular bundles; 3 = invasion of most vascular bundles except the central cylinder; and 4 = complete invasion of all vascular bundles (Garcia-Bastidas et al. 2019). The Leaf Symptom Index (LSI, %) and the Rhizome Discoloration Index (RDI, %) were determined using the formula described by Willoquet et al. (2023).

$$LSI\ or\ RDI = \frac{\sum (ni\ x\ Si)x100}{(Nx\ Smax).}$$

where n_i is the number of plants or leaves with a given severity score, S_i is the severity score attributed, N is the total number of plants evaluated, and S_{max} is the maximum possible severity score.

To confirm pathogenicity, reisolations were performed from symptomatic tissues onto SNA medium.

Table 1 Morphological characteristics of *Fusarium* strains isolated in eastern Democratic Republic of the Congo

Code	Province	Territory	Cultivar	DRMG (PDA)	Observe color (PDA)	Reverse color (PDA)	PhialIdes (PDA)	NSMa (CLA)	NSMi (CLA)	DRMG (PDA)
F144	North Kivu	Masisi	Kisubi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F148	North Kivu	Masisi	Kisubi	*F. tardichamydosporium*	white to pink	Pink	SM	3–5	0–1	7
F45	North Kivu	Masisi	Kamera	*F. joanfreemaniae*	White	Pale yellow	Polyphialides	3	0–1	6
F65	North Kivu	Masisi	Gros-Michel	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F67	North Kivu	Masisi	Kisubi	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F70	North Kivu	Masisi	Kisubi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F74	North Kivu	Masisi	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F84	North Kivu	Masisi	Kamera	*F. tardichamydosporium*	White	White	SM	3–5	0–1	6
F51	North Kivu	Masisi	Kisamunyu	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F106	North Kivu	Rutshuru	Gros-Michel	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F128	North Kivu	Rutshuru	Cingurube	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F146	North Kivu	Rutshuru	Kamera	Foc	White	Central pink	SM	3	0–1	7
F20	North Kivu	Rutshuru	Kamera	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F22	North Kivu	Rutshuru	Kisamunyu	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F27	North Kivu	Rutshuru	Cingurube	*F. tardichamydosporium*	white to pink	Pink	SM	3–5	0–1	6
F46	North Kivu	Rutshuru	Kisamunyu	*F. joanfreemaniae*	White	Pale yellow	Polyphialides	3	0–1	6
F80	North Kivu	Rutshuru	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F99	North Kivu	Rutshuru	Kisubi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F82	North Kivu	Rutshuru	Kisamunyu	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F100	South Kivu	Idjwi	Kamera	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F101	South Kivu	Idjwi	Gros-Michel	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F11	South Kivu	Idjwi	Kamera	*F. tardichamydosporium*	White	White	SM	3–5	0–1	7
F119	South Kivu	Idjwi	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F149	South Kivu	Idjwi	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F16	South Kivu	Idjwi	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	7
F39	South Kivu	Idjwi	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F57	South Kivu	Idjwi	Gros-Michel	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F61	South Kivu	Idjwi	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F66	South Kivu	Idjwi	Gros-Michel	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	7
F71	South Kivu	Idjwi	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F76	South Kivu	Idjwi	Kisamunyu	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F79	South Kivu	Idjwi	Musheba	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F93	South Kivu	Idjwi	Kamera	Foc	White	Central pink	SM	3	0–1	7
F83	South Kivu	Idjwi	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F115	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	violet	Violet	Polyphialides	3	0–1	7
F122	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7

Table 1 (continued)

Code	Province	Territory	Cultivar	DRMG (PDA)	Observe color (PDA)	Reverse color (PDA)	PhialIdes (PDA)	NSMa (CLA)	NSMi (CLA)	DRMG (PDA)
F147	South Kivu	Kabare	Kisamunyu	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	7
F151	South Kivu	Kabare	Gros-Michel	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F26	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F29	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Violet	Violet	Polyphialides	3	0–1	6
F32	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	violet	Violet	Polyphialides	3	0–1	6
F54	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F56	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F88	South Kivu	Kabare	Gros-Michel	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F17	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Violet	Violet	Polyphialides	3	0–1	6
F92	South Kivu	Kabare	Magizi	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F38	South Kivu	Kabare	Gros-Michel	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	6
F103	South Kivu	Kalehe	Kisamunyu	*F. joanfreemaniae*	Violet	Violet	Polyphialides	3	0–1	7
F126	South Kivu	Kalehe	Kisamunyu	*F. solani*	White	White	LM	3	0–1	8
F150	South Kivu	Kalehe	Gros-Michel	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F19	South Kivu	Kalehe	Kisamunyu	*F. joanfreemaniae*	Violet	Violet	Polyphialides	3	0–1	6
F30	South Kivu	Kalehe	Kisamunyu	*F. tardichamydosporium*	white to pink	Pink	SM	3–5	0–1	7
F33	South Kivu	Kalehe	Gros-Michel	*F. tardichamydosporium*	white to pink	Pink	SM	3–5	0–1	6
F36	South Kivu	Kalehe	Kisubi	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F37	South Kivu	Kalehe	Gros-Michel	*F. andiyazi*	White	Central purple	SM	3	0	7
F52	South Kivu	Kalehe	Gros-Michel	*F. incarnatum*	White	White	SM	3–6	NA	8
F62	South Kivu	Kalehe	Gros-Michel	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	7
F69	South Kivu	Kalehe	Kisubi	*F. joanfreemaniae*	Violet	Violet	Polyphialides	3	0–1	6
F2	South Kivu	Kalehe	Gros-Michel	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F41	South Kivu	Kalehe	Kamera	*F. joanfreemaniae*	Violet	Violet	Polyphialides	3	0–1	7
F109	South Kivu	Walungu	Kisamunyu	*F. joanfreemaniae*	Light violet	Light violet	Polyphialides	3	0–1	7
F132	South Kivu	Walungu	Kamera	*F. solani*	White	White	LM	3	0–1	8
F141	South Kivu	Walungu	Kamera	*F, duoseptatum*	White	Central purple	SM	3–5	0–1	7
F145	South Kivu	Walungu	Kamera	*F. tardichamydosporium*	White	White	SM	3–5	0–1	6
F25	South Kivu	Walungu	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F28	South Kivu	Walungu	Kamera	*F. tardichamydosporium*	white to pink	Pink	SM	3–5	0–1	6
F72	South Kivu	Walungu	Kamera	*F. tardichamydosporium*	white to pink	Pink	SM	3–5	0–1	6
F73	South Kivu	Walungu	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	6
F9	South Kivu	Walungu	Kamera	*F. joanfreemaniae*	White	Pale yellow	Polyphialides	3	0–1	6
F113	South Kivu	Walungu	Kamera	*F. tardichamydosporium*	White	Central purple	SM	3–5	0–1	7

DRMG days to reach maximum growth on PDA, *NSMa* number of septa in macroconidia, *NSMi* number of septa in microconidia

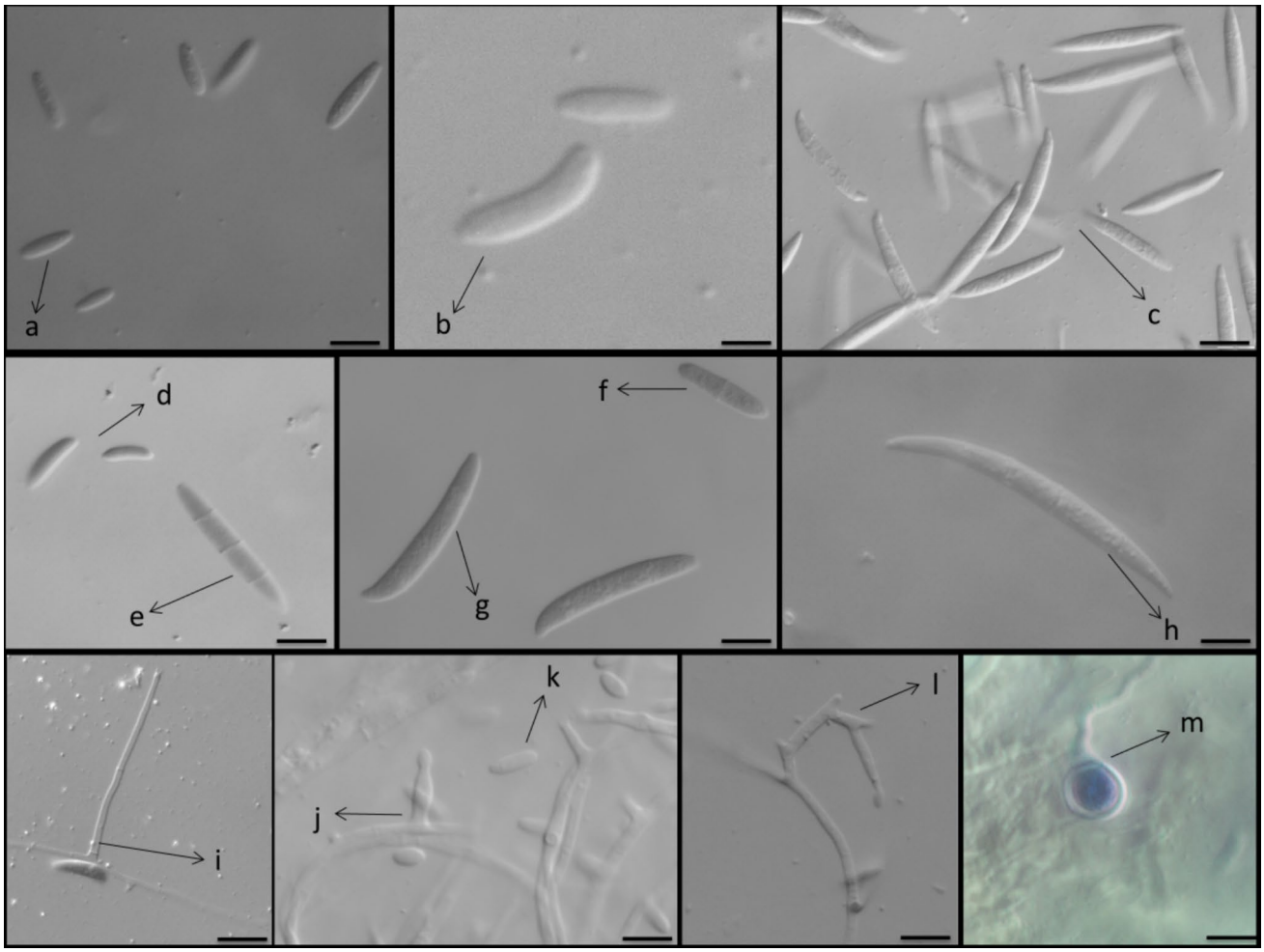

Fig. 2 *Fusarium* spores and phialides observed. Legend: a, b, d, f, and k, microconidia; c, e, g, and h, macroconidia; i, j, and l, phialides; m, chlamydospores

Results

Fusarium wilt distribution and sampling

The presence of FWB was observed in all areas of the South and North Kivu provinces in eastern D.R.C. A total of 70 strains were obtained from discolored vascular strands of the pseudostem collected from banana plants exhibiting typical symptoms (Table 1 and Fig. 1).

Banana cultivars affected by FWB include the dessert cultivars Gros Michel (AAA), Kamera (AAA) and Cingurube (AAA), the beer cultivars Magizi (AAA) and Kisukari (AAB), the cooking banana cultivar Kisamunyu (AAA), and the plantain cultivar Musheba (AAB). Some local East African Highland Banana (EAHB) cultivars, known as Matooke, such as Kisamunyu and Magizi, which are typically considered resistant to Races 1 and 2, showed symptoms of FWB.

Morphological characterization

All isolates were initially identified as belonging to the genus *Fusarium* based on their morphological characteristics. On SNA and CLA media, macroconidia were generally similar in shape, sickle-shaped with 3–6 septa. Microconidia varied in shape, ranging from oval to kidney-shaped, with 0–1 septum. Thirty-six strains produced microconidia in the aerial mycelium on polyphialides while thirty-two produced microconidia in false heads on short monophialides. Two strains produced microconidia on long monophialides. Spherical chlamydospores were observed only in some strains (Fig. 2 and Table 1). No strains produced the characteristic odor associated with Race 4 on different media.

On PDA medium, the cultures were mostly unpigmented, giving the colonies an overall white appearance, while the reverse side showed central pigmentation in shades of violet, pale yellow, purple, or pink, with the peripheral area remaining white. In terms of texture, most strains developed

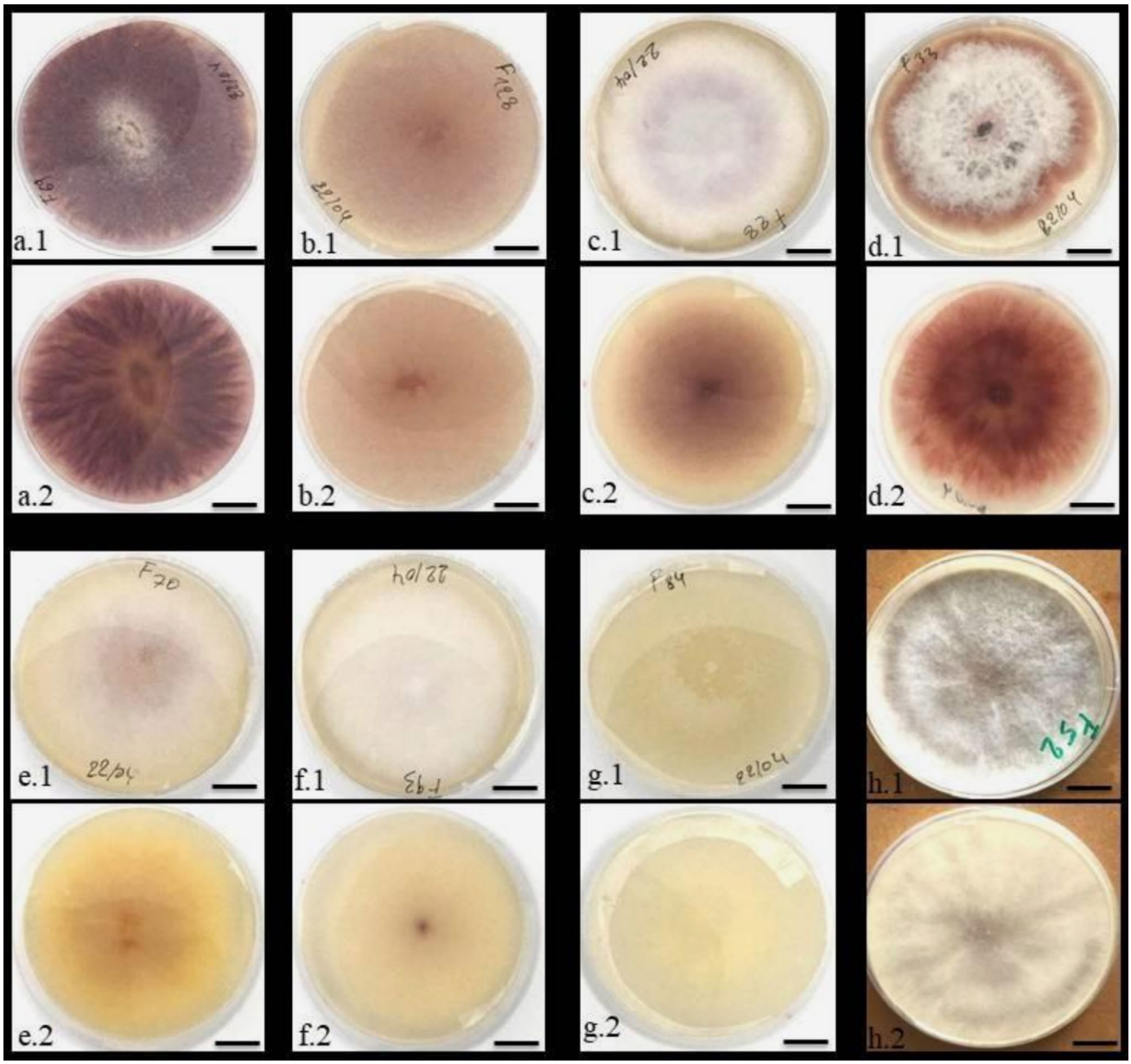

Fig. 3 Colony morphology of *Fusarium* isolates on PDA after 7 days of incubation at 25 °C in the dark. 1, front view; 2, reverse view; (a.1) and (a.2) show violet colonies; (b.1) and (b.2) show light violet; (c.1) is white, while (c.2) shows central purple coloration; (d.1) is white to pink, and (d.2) is pink; (e.1) is white, and (e.2) is pale yellow; (f.1) is white, while (f.2) shows central pink; (g.1) and (g.2) are white with less dense mycelia, and (h.1) and (h.2) are white with fluffy, cottony mycelia

raised, fluffy, cottony mycelia while a few strains produced less dense mycelia, with some showing a silky and spaced texture (Fig. 3 and Table 1).

Phylogenetic analysis

Phylogenetic analyses including the 70 *Fusarium* isolates from this study (Table 1) and 67 reference sequences from GenBank (Supplementary Table 2) revealed two main groups. Thirty-one strains belonged to the FOSC lineage. According to the classification and nomenclature proposed by Maryani et al. (2019), 28 FOSC strains clustered with reference sequences of *F. tardichlamydosporum* and one isolate (F141) clustered with reference strain of *F. duoseptatum*. Both species were previously classified as Foc Race 1. Two isolates, F93 and F146, formed their own distinct clade, separate from all other species within FOSC. *Fusarium odoratissimum* (TR4) was not detected in our sampling (Fig. 4A).

Thirty-five strains were attributed to *F. joanfreemaniae* (FFSC) on the basis of the TEF-1α phylogenetic studies

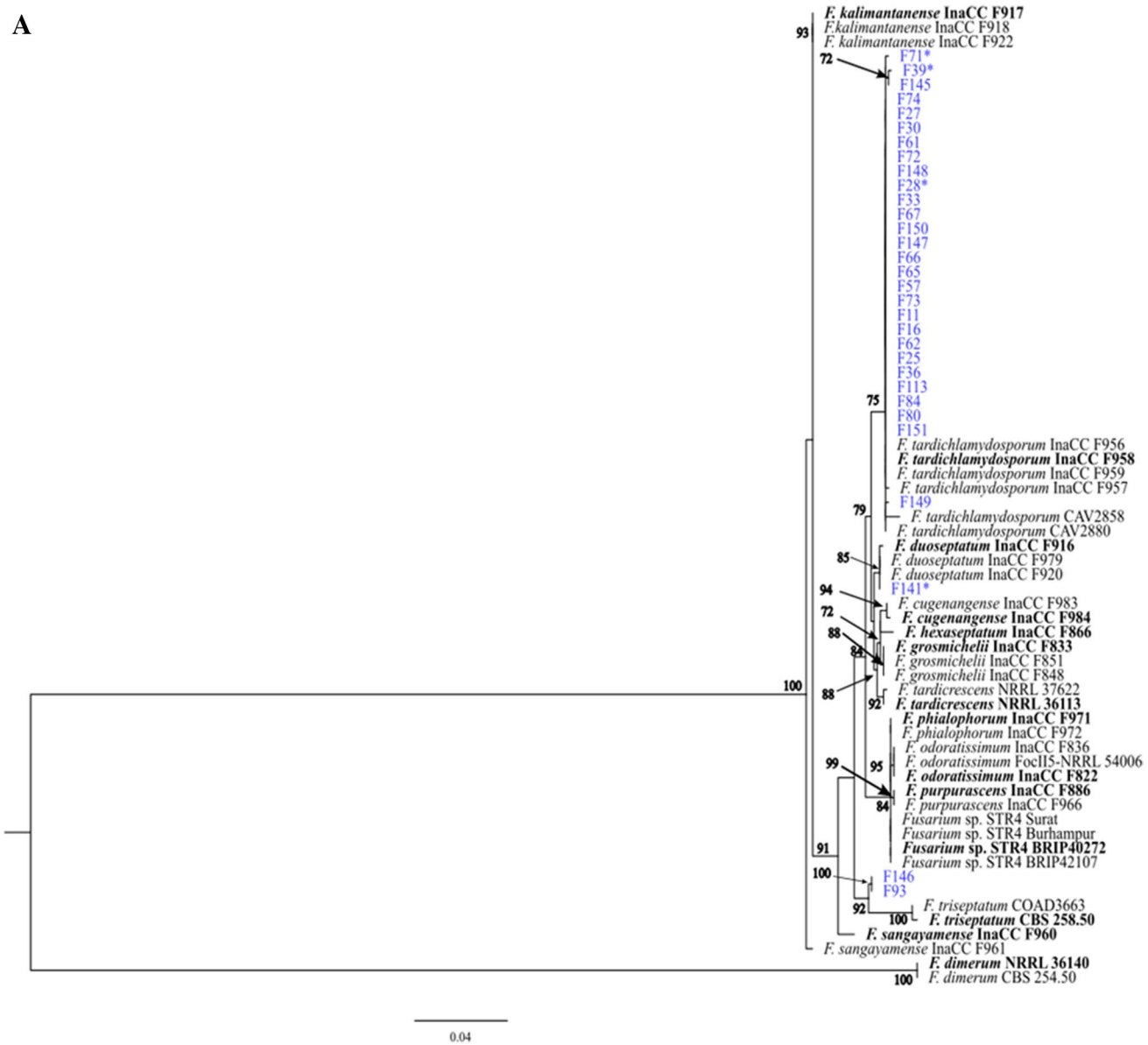

Fig. 4 **A** Maximum likelihood tree inferred from the combined TEF-1α and RPB2 gene sequences of 31 isolates from the D.R.C. and 37 reference sequences from GenBank, including members of the FOSC and *F. dimerum* as the outgroup. Isolates from eastern D.R.C. are indicated in blue, whereas type or ex-type strains are shown in bold. Isolates (CAV2858 and CAV2880) previously identified as *F. oxysporum* f. sp. *cubense* (Karangwa et al. 2018) were reassigned to *F. tardichlamydosporum* based on phylogenetic analysis. Isolates used in the pathogenicity assays are indicated with an asterisk (*). **B** Maximum likelihood tree inferred from the combined TEF-1α and RPB2 gene sequences of 36 isolates from the D.R.C. and 28 reference sequences from GenBank, including members of the FFSC and *F. dimerum* as the outgroup. Isolates from eastern D.R.C. are indicated in blue, whereas type or ex-type strains are shown in bold. Isolates previously identified as *F. sacchari* (CAV2804, CAV2837 and M7) by Karangwa et al. (2018) and Maldonado-Bonilla et al. (2019) were reassigned to *F. joanfreemaniae* based on phylogenetic analysis. Isolates used in the pathogenicity assays are indicated with an asterisk (*)

(Supplementary Fig. 2). However, our isolates from North and South Kivu presented nucleotide divergences at eight positions in the RPB2 gene alignment, resulting in two subclades (Fig. 4B and Supplementary Fig. 3), containing, respectively, 26 isolates and nine isolates. The sequences of calmodulin (ten isolates, five from each subclade) and of β-tubulin (six isolates, three from each subclade) did not show nucleotide variation.

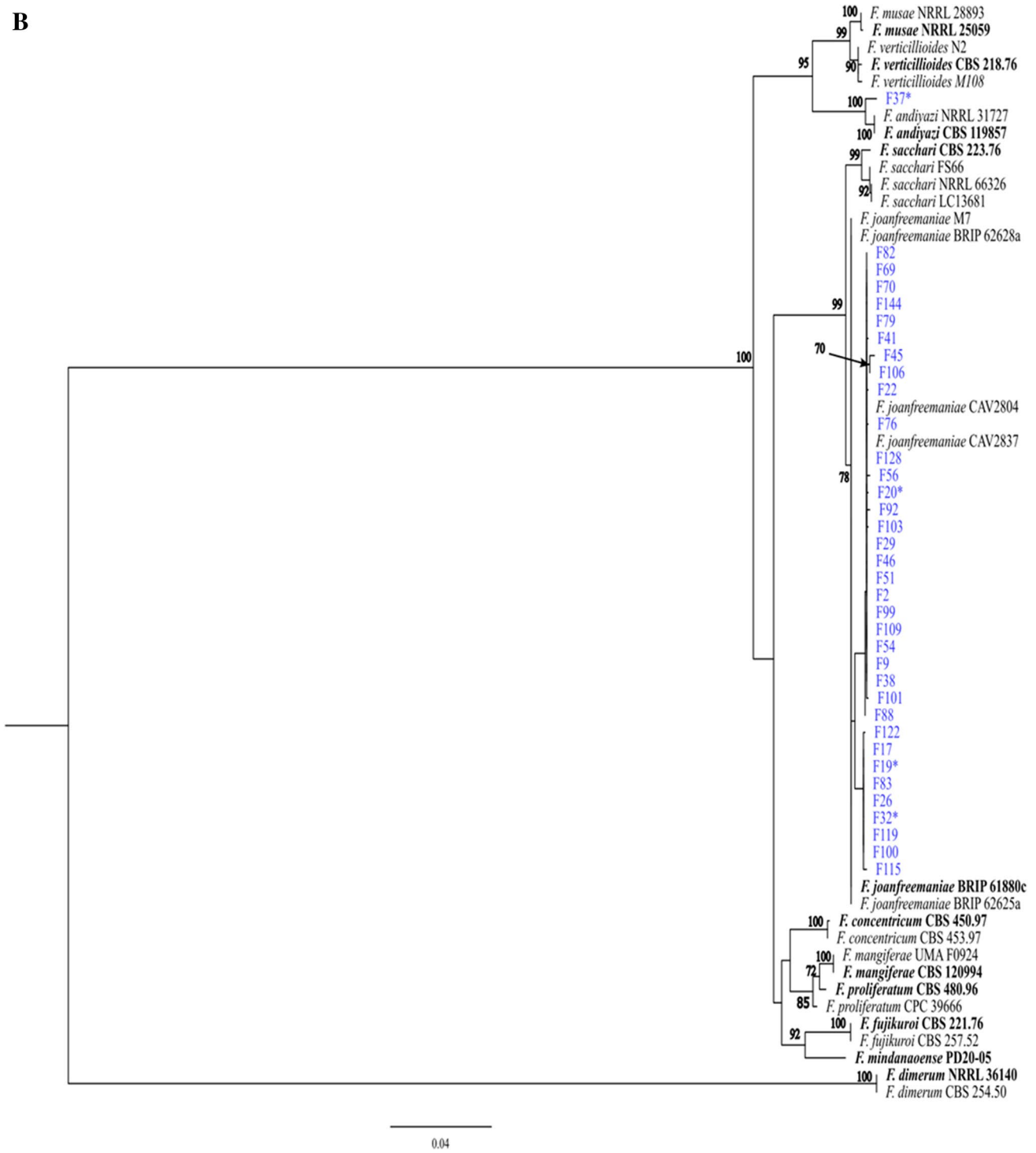

Fig. 4 (continued)

Four residual strains were identified as *F. solani* (two strains), *F. andiyazi* (one strain) and *F. incarnatum* (one strain) (Supplementary Fig. 1).

The dominant species, *F. tardichlamydosporum* and *F. joanfreemaniae*, were found at all surveyed sites in South and North Kivu, whereas the residual species were found in South Kivu (Fig. 1).

Fig. 5 Pathogenicity test of Foc and *F. joanfreemaniae* on banana plants (Gros Michel (GM) and Cavendish (CV) cultivars): **a** wounded GM (control), **b** wounded GM inoculated with Foc, **c** non-wounded GM inoculated with Foc, **d** wounded CV (control), **e** non-wounded CV (control), **f** wounded CV inoculated with *F. joanfreemaniae*, **g** GM tuber (control), **h** CV tuber (control), **i** CV tuber showing discolored tissues inoculated with Foc, and **j** CV tuber showing discolored tissues inoculated with *F. joanfreemaniae*

Pathogenicity test

The pathogenicity test showed that our strains of *F. tardichlamydosporum* and *F. duoseptatum* were pathogenic only to the Gros Michel cultivar, regardless of the inoculation method used. These results confirm their classification as Foc Race 1 (Fig. 5 and Table 2). *Fusarium joanfreemaniae* strains caused disease symptoms on both Gros Michel and Cavendish cultivars, but only when the banana roots were mechanically wounded. In contrast, *F. solani*, *F. andiyazi*, and *F. incarnatum* strains did not induce any symptoms, regardless of the inoculation method (Table 2). Disease severity indices (LSI and RDI) for *F. joanfreemaniae* were markedly lower than those observed for Foc R1 under identical conditions.

Discussion

FWB has been reported in the eastern Democratic Republic of Congo since 2000 (Rutherford 2001). It was also reported by Karangwa et al. (2016, 2018) in the Great Lakes region, who revealed the occurrence of Foc races 1 and 2. In this study, we performed the characterization of the *Fusarium* species involved in the disease in both North and South Kivu (Fig. 4A and 4B). In our 2019 survey in both South and North Kivu, FWB was detected on EAHB cultivars, which are, however, considered resistant to races 1 and 2.

Our results revealed the occurrence of *F. tardichlamydosporum* (Fig. 4A), which represents Foc Race 1 *pro parte*. The two strains of Foc Race 1 (CAV2858 and CAV2880) reported previously by Karangwa (2018) in the Great Lake region are here re-identified as *F. tardichlamydosporum*. This species appears to be the most widespread affecting bananas locally. It was also reported as dominant in other countries around the world. For instance, Thi et al. (2022) reported it in Vietnam where it accounted for 68% of the *Fusarium* strains associated with banana wilt. One strain (F141) was identified as *F. duoseptatum*, which was also classified as Foc Race 1 (Maryani et al. 2019), whereas two strains (F93 and F146) formed a distinct, unnamed clade (Fig. 4A) in the current taxonomic scheme of Foc (Maryani et al. 2019). Nucleotide sequence variability in the TEF-1α and RPB2 genes, compared with the reference strains proposed by Maryani et al. (2019), and in the CAL and TUB genes, compared with sequences available in

Table 2 Pathogenicity tests on Gros Michel (GM) and Cavendish (Cv) cultivars with wounded roots (wr) and unwounded roots (ur)

Strains	Codes	Leaf symptom index (LSI, %)				Rhizome discoloration index (RDI, %)			
		GMwr	GMur	Cvwr	Cvur	GMwr	GMur	Cvwr	Cvur
Control	T	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F. tardichlamydosporium (Foc-R1)	F28	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00
F. duoseptatum (Foc-R1)	F141	100.00	100.00	0.00	0.00	100.00	100.00	0.00	0.00
F. tardichlamydosporium (Foc-R1)	F39	100.00	100.00	1.25	0.00	100.00	100.00	0.00	0.00
F. tardichlamydosporium (Foc-R1)	F71	100.00	87.50	0.00	0.00	100.00	100.00	0.00	0.00
F. joanefreemaniae	F19	20.00	0.00	10.00	0.00	15.00	0.00	10.00	0.00
F. joanefreemaniae	F20	26.25	0.00	15.00	0.00	20.00	0.00	15.00	0.00
F. joanefreemaniae	F32	13.75	0.00	10.00	0.00	8.75	0.00	10.00	0.00
F. andayazi	F52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F. incarnatum	F132	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F. solani	F37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

GenBank, suggests that these two strains could represent an undescribed taxon. Overall, the isolates within the FOSC, including *F. tardichlamydosporum*, *F. duoseptatum*, and the unnamed clade, displayed morphological characteristics consistent with the FOSC, such as macroconidia shape and septation, and microconidia produced on polyphialides, as described by Maryani et al. (2019). Notably, *F. tardichlamydosporum* isolates generally produced chlamydospores after approximately 4 weeks, reflecting their late appearance under the culture conditions (Maryani et al. 2019).

The pathogenicity tests showed that our strains of *F. tardichlamydosporum* and *F. duoseptatum* were pathogenic only to the Gros Michel cultivar, regardless of the inoculation methods used. These results confirm their classification as Foc Race 1 (Ploetz 2015a).

Our results also report for the first time the presence of *F. joanfreemaniae* (FFSC) on bananas in eastern D.R.C. *Fusarium joanfreemaniae* is a species closely related to *F. sacchari*. It was first described as an endophytic species on bananas in 2015 (Tan and Shivas 2023). These authors differentiated *F. sacchari* from *F. joanfreemaniae* based on nucleotide variation in the TEF-1α gene. The variations observed in the RPB2, CAL, and TUB genes of our *F. joanfreemaniae* strains, compared to the reference strain of *F. sacchari* (CBS 223.76), confirm that they represent two distinct taxa. Our results also allowed the reidentification of two *F. sacchari* strains originating from the eastern D.R.C. (CAV2837) and from Burundi (CAV2804), reported by Karangwa et al. (2018), as *F. joanfreemaniae*. However, the virulence of these two strains was not tested (Karangwa et al. 2018). *Fusarium joanfreemaniae* accounted for 50% of the isolates obtained in this study and appears to be widely distributed across both North and South Kivu.

Han et al. (2025) also reported the presence of *F. joanfreemaniae* associated with banana wilt in China. They also distinguished *F. joanfreemaniae* from *F. sacchari* by sequencing the TEF-1α, RPB2, and TUB genes. Isolates of *F. joanfreemaniae* were distinguished morphologically by a high prevalence of polyphialides, characteristic of this species (Tan and Shivas 2023).

Species of the *F fujikuroi* species complex (FFSC) have been reported on bananas by Maryani et al. (2019), Huang et al. (2019), and Maldonado-Bonilla et al. (2019). Nozawa et al. (2023) identified *F. mindanaoense* as a pathogen of the Cavendish cultivar. Additionally, Maldonado-Bonilla et al. (2019) reported in southern Mexico two strains pathogenic to banana considered as *F. verticillioides* (M108) and *F. sacchari* (M7). Huang et al. (2019) also reported two strains (U34558 and AF160280) of *F. proliferatum* as pathogens of the Pisang Awak banana (*Musa* ABB) in China. More recently, dos Santos Santana et al. (2026) reported one strain of *F. sacchari* and one strain of *F. triseptatum* (a member of the FOSC) causing vascular wilt on banana plants in Brazil.

Our strains of *F. joanfreemaniae* were isolated from banana plants showing *Fusarium* wilt symptoms. However, several lines of evidence suggest that this species is a weak or opportunistic pathogen rather than a primary causal agent. In the pathogenicity assays performed in this study, *F. joanfreemaniae* caused symptoms only when roots were mechanically wounded, whereas Foc strains induced generalized wilting regardless of root injury. Moreover, disease severity (LSI and RDI) caused by *F. joanfreemaniae* was markedly lower than that caused by Foc R1. Importantly, other *Fusarium* species isolated in this study (*F. solani*, *F. andiyazi*, and *F. incarnatum*) did not induce symptoms even under the same wounding conditions. This suggests that *F. joanfreemaniae* possesses a limited capacity to colonize healthy banana tissues and pathogenicity is highly dependent on the health status of the plant. Consequently, the presence of *Fusarium* wilt symptoms associated with *F. joanfreemaniae* in EAHB cultivars (Mugisho et al. unpublished data) may reflect opportunistic colonization of injured tissue rather than primary pathogenicity. Root damage, which could occur in the field due to management practices or root-feeding insects such as *Cosmopolites sordidus*, is known to facilitate Foc infection (Sánchez et al. 2023; Médard et al. 2025). This highlights the need to consider mechanical damage and environmental factors when interpreting disease incidence linked to *F. joanfreemaniae*.

The pathogenicity of *F. joanfreemaniae* could mainly be explained by the presence of the SIX genes, which play a key role in pathogenicity. The genes SIX1, SIX2, SIX6, SIX7, SIX9, SIX11, and SIX13 have been identified in some species of the FFSC clade (Cui et al. 2021; Czislowski et al. 2018; Czislowski et al. 2021). Maldonado-Bonilla et al. (2019) detected the presence of the SIX1a gene in *F. sacchari* (M7) and the SIX1, SIX6, and SIX9 genes in *F. verticillioides* (M108). Their presence in certain strains of the FFSC clade could also play a key role in pathogenicity.

To date, 14 SIX genes have been identified in *F. oxysporum* (Czislowski et al. 2021). Several authors have shown the importance of the SIX1a and SIX6 genes in the pathogenicity of Foc in bananas. Cui et al. (2021) highlighted the possibility of gene transfer between *Fusarium* species, which could explain the evolution of pathogenicity in certain species of the FFSC. Van Dam and Rep (2017) reported that the SIX6 gene of *F. hostae* (HY9), a member of the FFSC clade, was horizontally transferred from Foc. Finally, the presence of SIX genes should not be interpreted as definitive evidence of pathogenicity. Homologs of SIX genes are known to occur in non-pathogenic endophytic *Fusarium* species. Further analyses would be required to determine which specific SIX genes

are present and whether they are functionally associated with virulence in *F. joanfreemaniae*.

Other species from the FFSC (*F. mangiferae*, *F. fujikuroi*, *F. proliferatum*, *F. sacchari*, and *F. verticillioides*) have been isolated from banana plants showing symptoms of wilt but also from asymptomatic banana as endophytes (Karangwa et al. 2018; Huang et al. 2019; Maryani et al. 2019; Ujat et al. 2021; Thi et al. 2022). Marasas et al. (2006), Ploetz (2006), and Qiu et al. (2023) reported that these species are associated also with other crops including maize, mango, rice, tomato, and sugarcane in tropical regions, and can act as pathogens on these hosts.

The strains of *F. solani*, *F. andiyazi*, and *F. incarnatum* isolated in this study did not cause any disease symptoms *in vitro*, regardless of the inoculation technique used. These species have previously been isolated from banana plants showing symptoms of *Fusarium* wilt by other researchers, but without reproducing the symptoms under controlled conditions (Maryani et al. 2019). They could be considered as secondary saprophytes or endophytes of banana (or weak latent/soil-borne pathogens).

In conclusion, this study demonstrated that banana wilt in South and North Kivu, eastern D.R.C. is primarily caused by *F. tardichlamydosporum* (FOSC, Foc Race 1 *pro parte*). *Fusarium joanfreemaniae* (FFSC) was also isolated at the same level of incidence. Although this species was associated with symptoms in several banana cultivars, including Cavendish, in our pathogenicity tests, this species was able to induce disease symptoms on banana plants only under stress conditions, such as mechanical root injury, and disease severity was substantially lower than that caused by Foc R1. These findings suggest that *F. joanfreemaniae* behaves as a weak or opportunistic pathogen, rather than a primary causal agent. Additionally, two further Foc strains could not be assigned to any of the recently established species of the Foc clades and may represent a distinct, undescribed taxon.

The Tropical Race 4 (TR4) was not detected during our surveys in the Great Lakes region. Nonetheless, it was detected in Mozambique in 2013, in Mayotte in 2021, and in the Grande Comore Island in 2023 (EFSA PLH Panel et al., 2022; Mmadi et al. 2023). Continuous monitoring in tropical Africa is therefore strongly recommended. Assessing the presence of the SIX genes in the Foc and *F. joanfreemaniae* strains could be crucial for understanding its evolutionary potential.

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Author contribution Janvier Mugisho Zirhumana: conceptualization, methodology, investigation, data curation, formal analysis, sequence analysis, and writing—original draft, review, and editing; Cony Decock: conceptualization, sequence analysis, and writing—review and editing; Marcelin Cokola Cuma: methodology and writing—review and editing; Espoir Bisimwa Basengere: conceptualization and writing—review and editing; Anne Legrève: conceptualization, methodology, supervision, and writing—review and editing.

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Data availability DNA sequences generated in this study have been deposited in the GenBank database under accession numbers PX309132–PX309291.

Declarations

Competing interests The authors declare no competing interests.

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