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ORIGINAL ARTICLE

Fusarium species from tropical grasses in Brazil and description of two new taxa

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Abstract

Fusarium isolates were obtained from asymptomatic seeds of wild grasses collected in six regions of Brazil. Eleven phylogenetic species were identified among 41 isolates based on sequences of *EF-1α*. These are members of the *F. fujikuroi* (FFSC, *n* = 24), *F. incarnatum-equiseti* (FIESC, *n* = 13), and *F. chlamydosporum* (FCSC, *n* = 5) species complexes that encompass known plant pathogens, mycotoxigenic species, and endophytes. Phylogenetic analyses based on *EF-1α*, *RPB2*, and *TUB* revealed two new species, *F. caapi* and *F. brachiariae*, that belong to the African clade of the FFSC and share main morphological features of *F. mundagurra* and *F. nygamai*. Another encountered isolate formed a singleton phylogenetic lineage within the FIESC. This survey shows that naturally occurring and cultivated grasses not only harbor a high diversity of known species, which are pathogens of maize, sorghum, rice, and sugarcane, but also novel *Fusarium* species.

Keywords *Brachiaria* · Endophyte · *Fusarium chlamydosporum* species complex · *Fusarium fujikuroi* species complex · *Fusarium incarnatum-equiseti* species complex · *Panicum maximum* · Two new species

Introduction

The genus *Fusarium* encompasses a variety of species that are pathogenic to plants, humans, and domestic animals and capable of producing bioactive secondary metabolites, including mycotoxins (Leslie and Summerell 2006; Kvas et al. 2009; Proctor et al. 2013). These fungi are also common endophytes of native and cultivated plants and especially grasses (Leslie and Summerell 2006; Kvas et al. 2009). The wide diversity of grass endophytes includes species from the following *Fusarium* species complexes: *F. chlamydosporum* (FCSC), *F. fujikuroi* (FFSC), *F. incarnatum-equiseti* (FIESC), *F. oxysporum* (FOSC), and *F. sambucinum*(FSAMSC)(Bentley et al. 2007; Nor Azliza et al. 2014; Kago et al. 2016; Laurence et al. 2016; Chehri et al. 2017).

Surveys of grass endophytes conducted in natural ecosystems have resulted in the discovery of new *Fusarium* species, especially those from the FFSC. *Fusarium konzum* was described from native grasses, mainly *Andropogon* and *Sorghastrum* species from prairies in Kansas, USA (Zeller et al. 2003). *Fusarium gaditjirrii* was reported from the grasses *Heteropogon triticeus* and *Themeda triandra* growing in the savannahs of Australia (Phan et al. 2004). *Fusarium lyarnte*, *F. tjaetaba*, and *F. werrikimbe* were described in association with *Sorghum interjectum* and *Sorghum leiocladum*, and *F. coicis* was isolated from *Coix gasteenii*(Walsh et al. 2010; Laurence et al. 2016). Known pathogenic and mycotoxigenic species also exist as endophytes in wild grasses. *Fusarium sacchari*, the main causal agent of pokkah boeng disease of sugarcane, was the predominant endophyte of *Oryza*

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australiensis in Australia (Petrovic et al. 2013). *Fusarium verticillioides*, *F. proliferatum*, *F. andiyazi*, and *F. thapsinum* are known producers of fumonisins and moniliformin and found in asymptomatic native grasses in the USA (Leslie et al. 2004). *Fusarium fujikuroi* causes bakanae disease in rice and was isolated from aquatic *Echinochloa* plants collected near rice fields (Carter et al. 2008). These results suggest that native or introduced grasses can act as inoculum reservoirs of known or unknown and potentially plant pathogenics and mycotoxigenic *Fusarium* species (Leslie et al. 2004).

Plants of *Brachiaria* (syn. *Urochloa*, "surinam grass") and *Panicum maximum* (syn. *Megathyrsus maximus*, "guinea grass") originate from Africa and Australia and are widely used as forage grasses in Brazil. Consequently, they are potentially invasive in natural grasslands. These grasses are also used for the recovery of degraded areas and prevention of erosive processes, in consortium with crops, such as maize and sorghum, and employed as source of straw in no-tillage agricultural systems (Timossi et al. 2007; Borghi et al. 2013a, b). There are few reports of endophytic *Fusarium* species associated with *Brachiaria* and *Panicum*, and reported identifications are usually limited to genus level (e.g., Mallmann et al. 2013; Kago et al. 2016; Teasdale et al. 2018). Since these forage grasses are frequently used in consortia with important crop plants, inventories were initiated to explore whether they may harbor plant pathogenic or mycotoxigenic *Fusarium* species. Endophytic *Fusarium* isolates from seeds of *Brachiaria* spp. and *Panicum maximum* collected in several geographic locations in Brazil were investigated using *EF-1α* and multilocus phylogenetic analyses coupled to morphological characterization. Known species from the FFSC, FIESC, and FCSC were found, together with two taxonomical novelties described herein.

Materials and methods

Fungal isolates

Seeds collected in full maturity were disinfested in 70% alcohol for 30 s and hypochlorite 2% for 2 min and washed in sterile water and dried on filter paper. Around 100 seeds from each sample were macerated in a crucible; the small fragments were transferred to five Petri plates (6 cm diam.) containing 2% malt extract agar (20 g of malt extract L^{-1}; HiMedia Laboratories, Mumbai, India) and incubated at 25 °C for 4–7 days with 12 h light/12 h dark cycle. Individual conidia from selected *Fusarium* colonies were subcultured using a micromanipulator. The isolates are deposited in the Coleção Micológica de Lavras (CML), Departamento de Fitopatologia, Universidade Federal de Lavras, Minas Gerais, Brazil (http://www.dfp.ufla.br/cml/) (Table 1). Types were deposited at Herbarium UB, Universidade de Brasília.

PCR, sequencing, and phylogenetic analyses

Isolates were grown in malt extract broth for 4 days under agitation of 100 rpm on an orbital shaker at room temperature. DNA was extracted from mycelia using the Wizard® Genomic DNA Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions. DNA concentration was estimated using NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, USA). Fragments of the translation elongation factor 1-alpha (*EF-1α*) gene were amplified with primer pair EF-1/EF-2 or EF-3/EF-22 (O'Donnell et al. 1998, 2008) for all isolates, following the cycle conditions described by O'Donnell et al. (2008). Portions of the RNA polymerase second largest subunit (*RPB2*, using primer pair 5F2/7cR, Sung et al. 2007; Liu et al. 1999) and beta-tubulin (*TUB*, T1/T2, O'Donnell and Cigelnik 1997) genes were amplified for selected FFSC isolates according to O'Donnell et al. (2008) and O'Donnell and Cigelnik (1997). PCR reactions were performed with GoTaq® Colorless Master Mix (Promega, Madison, USA), purified with Wizard® SV Gel and PCR Clean-up System kit (Promega). Sequencing of PCR products was done with primers used for amplifications. SeqAssem program was used to assemble and edit the sequences (Hepperle 2004), which were deposited in GenBank (Table 1). Alignments were generated using CLUSTALW as implemented in the MEGAX software (Kumar et al. 2018). DNA sequences from selected *Fusarium* species were downloaded from GenBank and added to the alignments (Supplementary Table 1). The alignments were deposited in TreeBASE (www.treebase.org; study number: S26843). Aligned sequences consisted of 97 parsimony-informativepositions/450 bp(FFSC) and 110/522 (FIESC and FCSC) for *EF-1α*, 110/794 for *RPB2*, and 76/528 for *TUB*. Phylogenetic analyses were performed for each gene partition and for the concatenated dataset of FFSC isolates. Maximum parsimony (MP) and maximum likelihood (ML) methods were performed in MEGAX software with 1000 bootstrap replications. Bayesian inference (BI) analyses were performed with using MrBayes 3.2.7 (Ronquist et al. 2012) with two independent analyses run for 1,000,000 generations, sampled every 500 generations, after discarding 25% of initial trees. The best-fitted models of nucleotide substitution used in the ML and BI analyses, estimated using jModelTest (Darriba et al. 2012), were K2

Table 1 *Fusarium* species isolated from asymptomatic seeds of *Brachiaria* spp. and *Panicum maximum* used in the current study

Species complex[a]	Species	CML[b]	Host	Geographic origin[c]	Year	Mating type[d]	GenBank accession no.		
							EF-1α	RPB2	TUB
FFSC	*F. brachiariae* sp. nov.	3032 T	*B. decumbens*	Campo Grande MS	2012	1	MT901348	MT901314	MT901321
		3163	*B. decumbens*	Campo Grande MS	2012	1	MT901349	MT901315	MT901322
	F. caapi sp. nov.	3657 T	*B. brizantha*	Guaíra SP	2016	1	MT901350	MT901316	MT901323
		3658	*B. brizantha*	Guaíra SP	2016	2	MT901351	MT901317	MT901324
		3659	*B. brizantha*	Guaíra SP	2016	2	MT901352	MT901318	MT901325
		3660	*B. brizantha*	Guaíra SP	2016	1	MT901353	MT901319	MT901326
		3881	*B. brizantha*	Montes Claros MG	2017	2	MT901354	MT901320	MT901327
	F. fujikuroi	3035	*B. brizantha*	Campo Grande MS	2012		MH187953		
	F. madaense	3040	*B. decumbens*	Lavras MG	2013		MT901355		
		3041	*B. brizantha*	Lavras MG	2012		MT901356		
		3044	*B. brizantha*	Lavras MG	2013		MK895713		
		3656	*B. brizantha*	Porto Alegre RS	2016		MT901357		
		3036	*B. brizantha*	Campo Grande MS	2012		MT901358		
		3038	*B. brizantha*	Lavras MG	2013		MT901359		
		3052	*B. decumbens*	Água Clara MS	2013		MT901360		
	F. thapsinum	3039	*B. brizantha*	Lavras MG	2013		MH187954		
		3164	*B. brizantha*	Campo Grande MS	2013		MT901361		
		3045	*B. decumbens*	Lavras MG	2012		MT901362		
		3046	*B. brizantha*	Lavras MG	2013		MT901363		
		3047	*B. brizantha*	Rondonópolis MT	2013		MT901364		
	F. verticillioides	3033	*B. decumbens*	Lavras MG	2012		MT901365		
		3043	*B. brizantha*	Lavras MG	2012		MT901366		
		3664	*B. decumbens*	Rondonópolis MT	2013		MT901367		
FIESC	*F. duofalcatisporum* (FIESC 2)[e]	3636	*B. brizantha*	Rondonópolis MT	2016		MT901337		
		4090	*P. maximum*	Porto Alegre RS	2016		MT901338		
	F. lacertarum (FIESC 4)	4102	*B. brizantha*	Porto Alegre RS	2016		MT901339		
		4103	*B. brizantha*	Goiânia GO	2016		MT901340		
		4104	*B. brizantha*	Porto Alegre RS	2016		MT901341		
	F. hainanense (FIESC 26)	4105	*P. maximum*	Porto Alegre RS	2016		MT901330		
		4106	*P. maximum*	Porto Alegre RS	2016		MT901331		
		4084	*P. maximum*	Porto Alegre RS	2016		MT901332		
		4107	*P. maximum*	Porto Alegre RS	2016		MT901333		
		4109	*P. maximum*	Porto Alegre RS	2016		MT901334		
		4110	*P. maximum*	Porto Alegre RS	2016		MT901335		
		4113	*P. maximum*	Porto Alegre RS	2016		MT901336		
	Fusarium sp.	4092	*P. maximum*	Porto Alegre RS	2016		MT901342		
FCSC	*F. chlamydosporum* (FCSC 1)	3665	*P. maximum*	Porto Alegre RS	2016		MT901343		
		3666	*P. maximum*	Porto Alegre RS	2016		MT901344		
		4096	*P. maximum*	Porto Alegre RS	2016		MT901345		
		3667	*P. maximum*	Porto Alegre RS	2016		MT901346		
		4098	*P. maximum*	Porto Alegre RS	2016		MT901347		

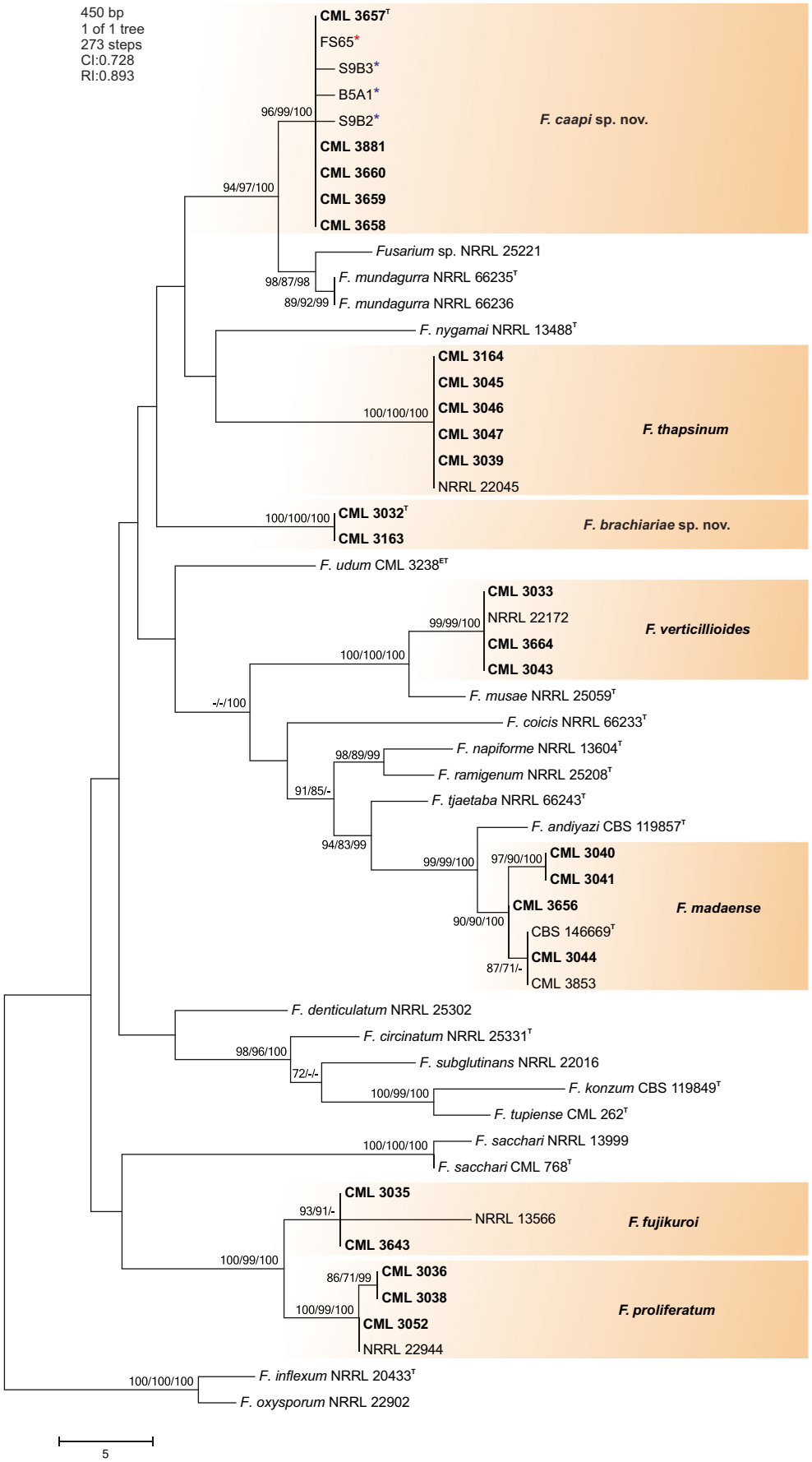
[a] FFSC, *Fusarium fujikuroi* species complex; FIESC, *F. incarnatum-equiseti* species complex; FCSC, *F. chlamydosporum* species complex

[b] CML, Coleção Micológica de Lavras, Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. T, ex-type strain

[c] States of Brazil: GO, Goiás; MG, Minas Gerais; MS, Mato Grosso do Sul; MT, Mato Grosso; RS, Rio Grande do Sul; SP, São Paulo

[d] Mating type identified by PCR: MAT-1 = 1; MAT-2 = 2

[e] Arabic numerals to identify phylogenetic species within FCSC and FIESC designated by O'Donnell et al. (2008)


450 bp
1 of 1 tree
273 steps
CI:0.728
RI:0.893
CML 3657T
FS65*
S9B3*
B5A1*
S9B2*
CML 3881
CML 3660
CML 3659
CML 3658
F. caapi sp. nov.
96/99/100
94/97/100
Fusarium sp. NRRL 25221
F. mundagurra NRRL 66235T
F. mundagurra NRRL 66236
98/87/98
89/92/99
F. nygamai NRRL 13488T
CML 3164
CML 3045
CML 3046
CML 3047
CML 3039
NRRL 22045
F. thapsinum
100/100/100
CML 3032T
CML 3163
F. brachiariae sp. nov.
100/100/100
F. udum CML 3238ET
CML 3033
NRRL 22172
CML 3664
CML 3043
F. verticillioides
99/99/100
100/100/100
F. musae NRRL 25059T
-/-/100
F. coicis NRRL 66233T
F. napiforme NRRL 13604T
F. ramigenum NRRL 25208T
98/89/99
91/85/-
F. tjaetaba NRRL 66243T
F. andiyazi CBS 119857T
94/83/99
CML 3040
CML 3041
CML 3656
CBS 146669T
CML 3044
CML 3853
F. madaense
97/90/100
99/99/100
90/90/100
87/71/-
F. denticulatum NRRL 25302
F. circinatum NRRL 25331T
F. subglutinans NRRL 22016
F. konzum CBS 119849T
F. tupiense CML 262T
98/96/100
72/-/-
100/99/100
F. sacchari NRRL 13999
F. sacchari CML 768T
100/100/100
CML 3035
NRRL 13566
CML 3643
F. fujikuroi
93/91/-
100/99/100
CML 3036
CML 3038
CML 3052
NRRL 22944
F. proliferatum
86/71/99
100/99/100
F. inflexum NRRL 20433T
F. oxysporum NRRL 22902
100/100/100
5

◀ **Fig. 1** Maximum parsimony phylogenetic tree inferred from partial *EF-1α* sequences showing the phylogenetic relatedness of *Fusarium* species associated with *Brachiaria* spp. with other species of the *Fusarium fujikuroi* species complex. Bootstrap values ≥ 70% (MP and maximum likelihood) and posterior probability ≥ 95% (Bayesian inference) are shown at the internodes. Ex-type strains are indicated with T and ex-epitype strains with ET. Isolates of *F. caapi* with blue asterisks were obtained from rice root in Kenya and with red asterisks from a clinical indoor environment in Brazil. *Fusarium inflexum* (NRRL 20433) and *F. oxysporum* (NRRL 22902) were used as outgroup

+ G for *EF-1α*, *RPB2*, and *TUB*(FFSC) and GTR + G for *EF-1α* (FIESC and FCSC).

Morphological characterization

The isolates were characterized morphologically according to Leslie and Summerell (2006) and Zeller et al. (2003). The morphological characteristics of micro- and macroconidia such as shape and size (25–30 measurements per isolate),

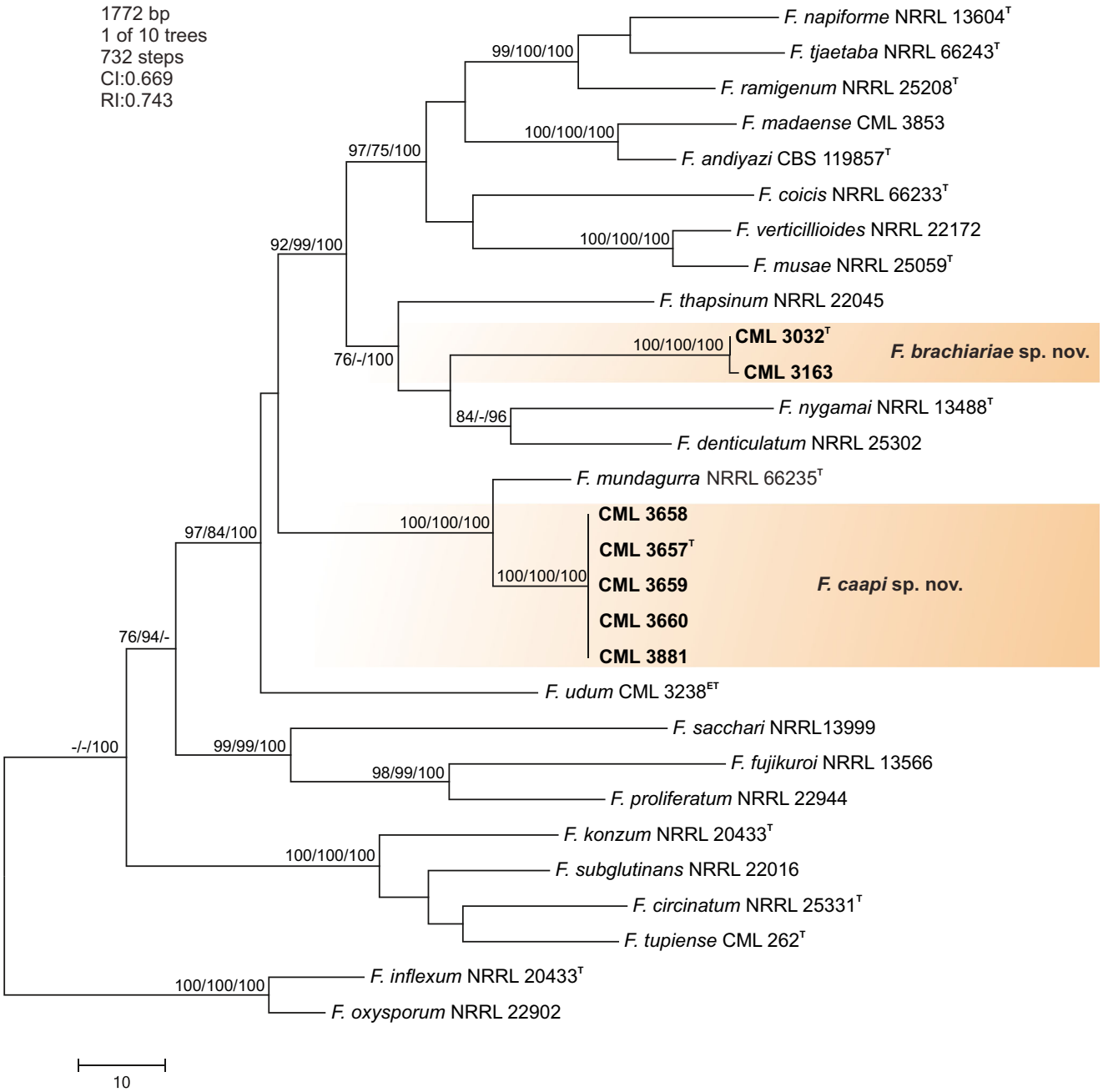
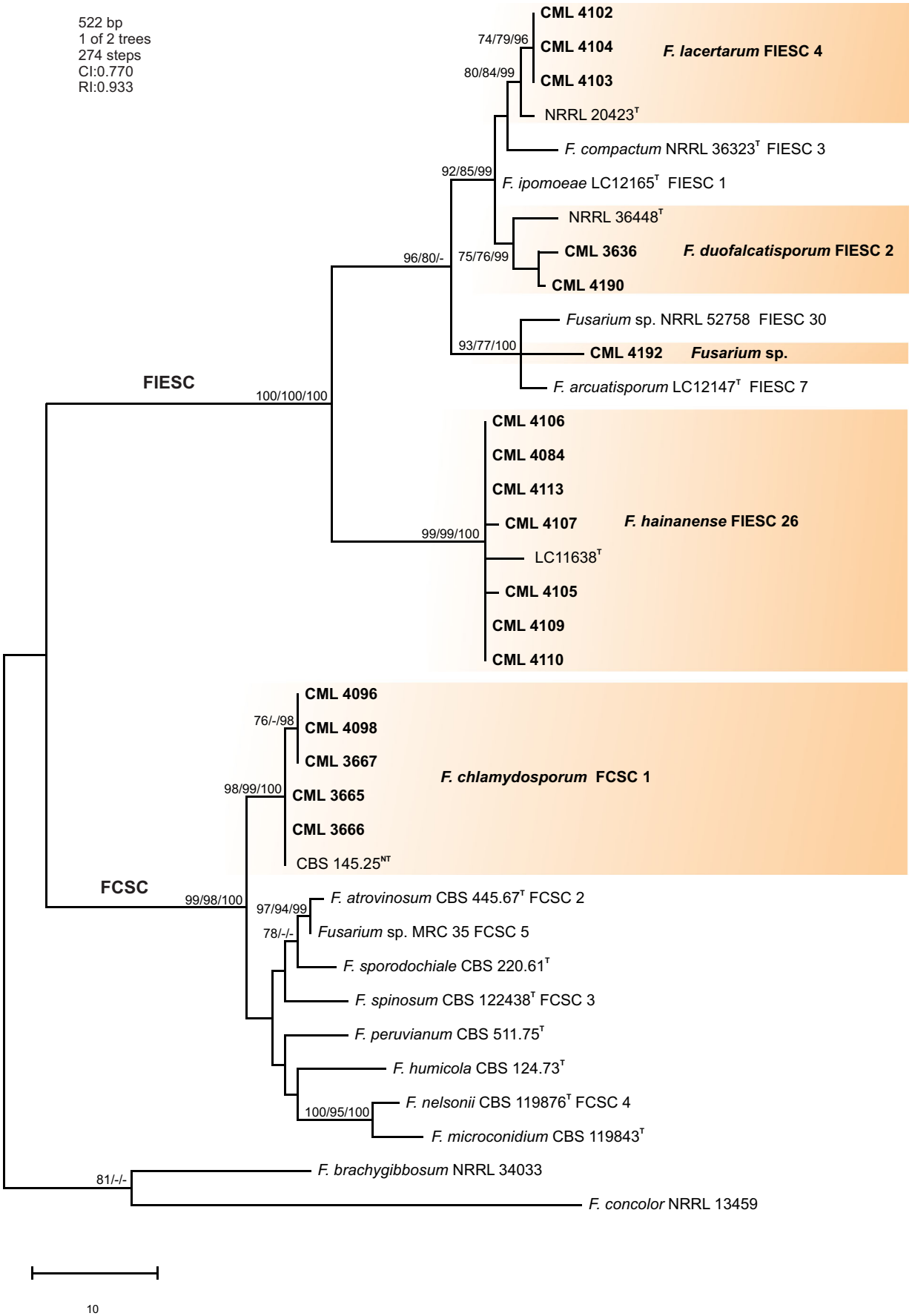


Fig. 2 Maximum parsimony phylogenetic tree inferred from partial *EF-1α*, *TUB*, and *RPB2* sequences showing the phylogenetic relatedness of *Fusarium* species associated with *Brachiaria* spp. with other species of the *Fusarium fujikuroi* species complex. Bootstrap values ≥ 70% (MP and maximum likelihood) and posterior probability ≥ 95% (Bayesian inference) are shown at the internodes. Ex-type strains are indicated with T and ex-epitype strains with ET. *Fusarium inflexum* (NRRL 20433) and *F. oxysporum* (NRRL 22902) were used as outgroup


522 bp
1 of 2 trees
274 steps
CI:0.770
RI:0.933
CML 4102
74/79/96
CML 4104
F. lacertarum FIESC 4
80/84/99
CML 4103
NRRL 20423T
F. compactum NRRL 36323T FIESC 3
92/85/99
F. ipomoeae LC12165T FIESC 1
NRRL 36448T
96/80/-
75/76/99
CML 3636
F. duofalcatisporum FIESC 2
CML 4190
Fusarium sp. NRRL 52758 FIESC 30
93/77/100
CML 4192
Fusarium sp.
FIESC
F. arcuatisporum LC12147T FIESC 7
100/100/100
CML 4106
CML 4084
CML 4113
CML 4107
F. hainanense FIESC 26
99/99/100
LC11638T
CML 4105
CML 4109
CML 4110
CML 4096
76/-/98
CML 4098
CML 3667
F. chlamydosporum FCSC 1
98/99/100
CML 3665
CML 3666
CBS 145.25NT
FCSC
99/98/100
97/94/99
F. atrovinosum CBS 445.67T FCSC 2
78/-/-
Fusarium sp. MRC 35 FCSC 5
F. sporodochiale CBS 220.61T
F. spinosum CBS 122438T FCSC 3
F. peruvianum CBS 511.75T
F. humicola CBS 124.73T
100/95/100
F. nelsonii CBS 119876T FCSC 4
F. microconidium CBS 119843T
81/-/-
F. brachygibbosum NRRL 34033
F. concolor NRRL 13459
10

◀ **Fig. 3** Maximum parsimony phylogenetic tree inferred from partial *EF-1α* sequences showing the phylogenetic relatedness of *Fusarium* species associated with *Brachiaria* spp. with other species of the *Fusarium incarnatum-equiseti* and *F. chlamydosporum* species complexes. Bootstrap values ≥ 70% (MP and maximum likelihood) and posterior probability ≥ 95% (Bayesian inference) are shown at the internodes. Ex-type strains are indicated with T and ex-neotype strains with NT. *Fusarium brachygibbosum* (NRRL 34033) and *F. concolor* (NRRL 13459) were used as outgroup

arrangement of conidiogenous cells, and presence or absence of conidial chains and chlamydospores were examined after growing the isolates on synthetic nutrient-poor agar with carnation leaf pieces for 10 to 14 days at 20 °C. The colony growth radius was assessed after 4 days and mycelium and colony color (surface and reverse) at 25 °C on potato dextrose agar (PDA, Merck, Darmstadt, Germany) in the dark.

Mating type and sexual compatibility test

The mating types of the isolates representing the new species were determined by PCR, using the protocols described by Steenkamp et al. (2000). Crosses were conducted between isolates from opposite mating types as described by Klittich and Leslie (1988). The isolates were tested as both female and male parent. Crosses were also carried out between the isolates of the new species and the tester isolates of other biological species from the FFSC (Supplementary Table 2).

Results

Phylogenetic analyses

Phylogenetic analysis based on the *EF-1α* gene showed that 23 isolates belong to the FFSC (Fig. 1). Sixteen isolates were identified as *F. thapsinum* ($n = 5$), *F. madaense* ($n = 4$), *F. verticillioides* ($n = 3$), *F. proliferatum* ($n = 3$), and *F. fujikuroi* ($n = 1$). Two monophyletic groups, composed by two and five isolates from this study, did not correspond to any known species within the FFSC (Figs. 1 and 2) and are here described as *F. caapi* and *F. brachiariae*. *EF-1α* sequences from three rice root endophytes collected in Kenya and one strain from a clinical environment in Brazil grouped within the *F. caapi* clade (Fig. 1). Phylogenetic analyses of *RPB2*, *TUB*(Supplementary Figs. 1 and 2), and the combined dataset (*EF-1α*, *RPB2* and *TUB*) (Fig. 2) confirmed the exclusive monophyly of both new species and their placement in the African clade of the FFSC. *Fusarium caapi* is closely related to *F. mundagurra* and *Fusarium* sp. NRRL 25221, while *F. brachiariae* is only distantly related to *F. denticulatum*, *F. thapsinum*, and *F. nygamai*(Fig. 2).

The remaining 18 isolates were identified as members of the FIESC or FCSC (Fig. 3). Three species were placed in the FIESC: *F. hainanense* ($n = 7$), *F. lacertarum* ($n = 3$), and *F. duofalcatisporum* ($n = 2$). The isolate CML 4092 formed a clade with *F. arcuatisporum* and FIESC 30 and could represent a new phylogenetic species. *Fusarium chlamydosporum* was the only species identified within the FCSC (Fig. 3).

Mating type identification and sexual stage induction

Two isolates of *F. caapi* were identified as MAT-1 and three as MAT-2. Both isolates of *F. brachiariae* were identified as MAT-1(Table 1). Perithecia were not formed in crosses between isolates of *F. caapi* or between isolates from both new species and tester strains from nine known biological species within the FFSC.

Taxonomy

Fusarium brachiariae M. M. Costa, M. P. Melo, F. S. Carmo & L. H. Pfenning, sp. nov. Fig. 4

MycoBank: MB 836892

Etymology: Refers to *Brachiaria*, the genus, from which this fungus was isolated.

Typification: BRAZIL. Mato Grosso do Sul: Campo Grande, a dried culture on SNA of a strain isolated from *Brachiaria decumbens* seed, 2012, *M. P. Melo* (holotype UB 24188). Ex-type culture CML 3032. GenBank accession numbers: *EF-1α* = MT901348; *TUB* = MT901321; and *RPB2* = MT901314.

Colonies on PDA reaching 3.4 cm at 25 °C in 4 days. Aerial mycelium floccose. Colony color cream, reverse white with violet color. Odor absent. On SNA, microconidia hyaline, oval to clavate, 0 to 1 septate, mostly 0 septate: 2–7(− 11) × 1.5–3 μm, 1-septate: 5–14 × 1.8–4.5 μm, produced in false heads and in short chains, arising from mono- and polyphialides with up to three openings, phialides 12.5–30.5 × 2–3.5 μm in size, formed on simple or branched conidiophores. Sporodochia on SNA abundant, forming within 10 days on carnation leaf pieces and occasionally on the agar surface, producing orange conidial masses. Macroconidia relatively slender with a significant curvature, with evident apical and basal cells, 3–4(− 6) septate, 3-septate: 21–48 × 2.5–5 μm, 4-septate: 30–43 × 2.5–5 μm, 5-septate: 45–48 × 2.5–5 μm, 6-septate: 45–65 × 2.5–5 μm. Chlamydospores formed abundantly within 1 week, singly or in chains, thin-walled, terminal or intercalary, globose, subglobose, cylindrical to subcylindrical, 3.5–20.2 × 5–15.5 μm.

Host: Brachiaria decumbens

Known distribution: Brazil

Other specimen examined. BRAZIL, Mato Grosso do Sul: Campo Grande, from seeds of *Brachiaria decumbens*, 2016, *M. P. Melo* (CML 3163).

Fusarium caapi M. M. Costa, M. P. Melo, F. S. Carmo & L. H. Pfenning, sp. nov. Fig. 5

MycoBank: MB 836897

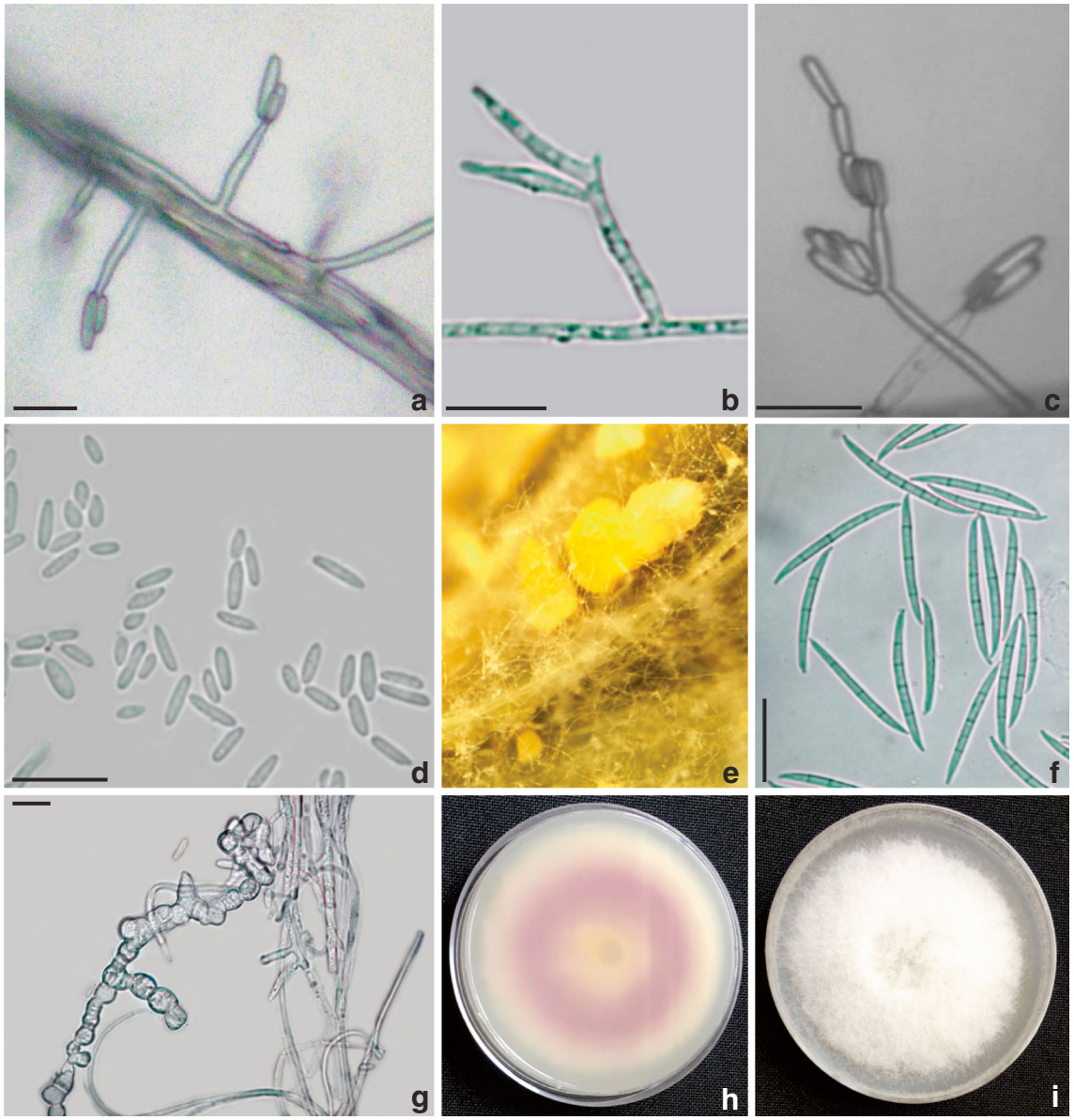


Fig. 4 Morphological characters of the asexual stage of *Fusarium brachiariae.* **a** Monophialides. **b** Polyphialide. **c** Microconidia in short chains. **d** 0-septate microconidia. **e** Sporodochia formed on carnation leaf on SNA**. f** 3–5 septate macroconidia. **g** Chlamydospores in chains**. h–i** Reverse and obverse of PDA colony incubated for 14 days at 25 °C. Bars: **a–d** = 20 μm and **f–g** = 30 μm

Etymology: caapi meaning grass or slender leaf in *tupi guarani*, an extinct language of the Tupinambá Brazilian Indians.

Typification: BRAZIL. São Paulo: Guaíra, a dried culture on SNA of a strain isolated from *Brachiaria brizantha* seed, 2016, *F. S. Carmo.* (holotype UB 24189). Ex-type culture CML 3657. GenBank accession numbers: *EF-1α* = MT901350; *TUB* = MT901323; and *RPB2* = MT901316.

Colonies on PDA reaching 3.8 cm at 25 °C in 4 days. Aerial mycelium floccose, with a powdery appearance. Colony color cream, reverse white to gray. Odor absent. On SNA, microconidia hyaline, oval to obovate, 0 to 1 septate, mostly 0 septate. 0-septate: 2–5–(11) × 1.5–3 μm, 1-septate: 7.5–12.5 × 2.5–5.5 μm, produced in false heads or short chains arising from mono- and polyphialides, phialides 17.5–32.5 × 2–5 μm in size, formed on simple or branched conidiophores. Sporodochia abundant, forming within 10 days on carnation leaf pieces and occasionally on the agar surface, producing orange conidial masses. Macroconidia straight to slightly curved, relatively slender and thin walled, with hardly evident apical and basal cells, 3–5(− 6) septate, mostly 3-septate: 3-septate: 20–47.5 × 2.5–5 μm, 4-septate: 35–50 × 2.5–5 μm, 5-septate and 6-septate: 45–52.5 × 2.5–5. Chlamydospores produced abundantly within 1 week, in chains, thin-walled, terminal or

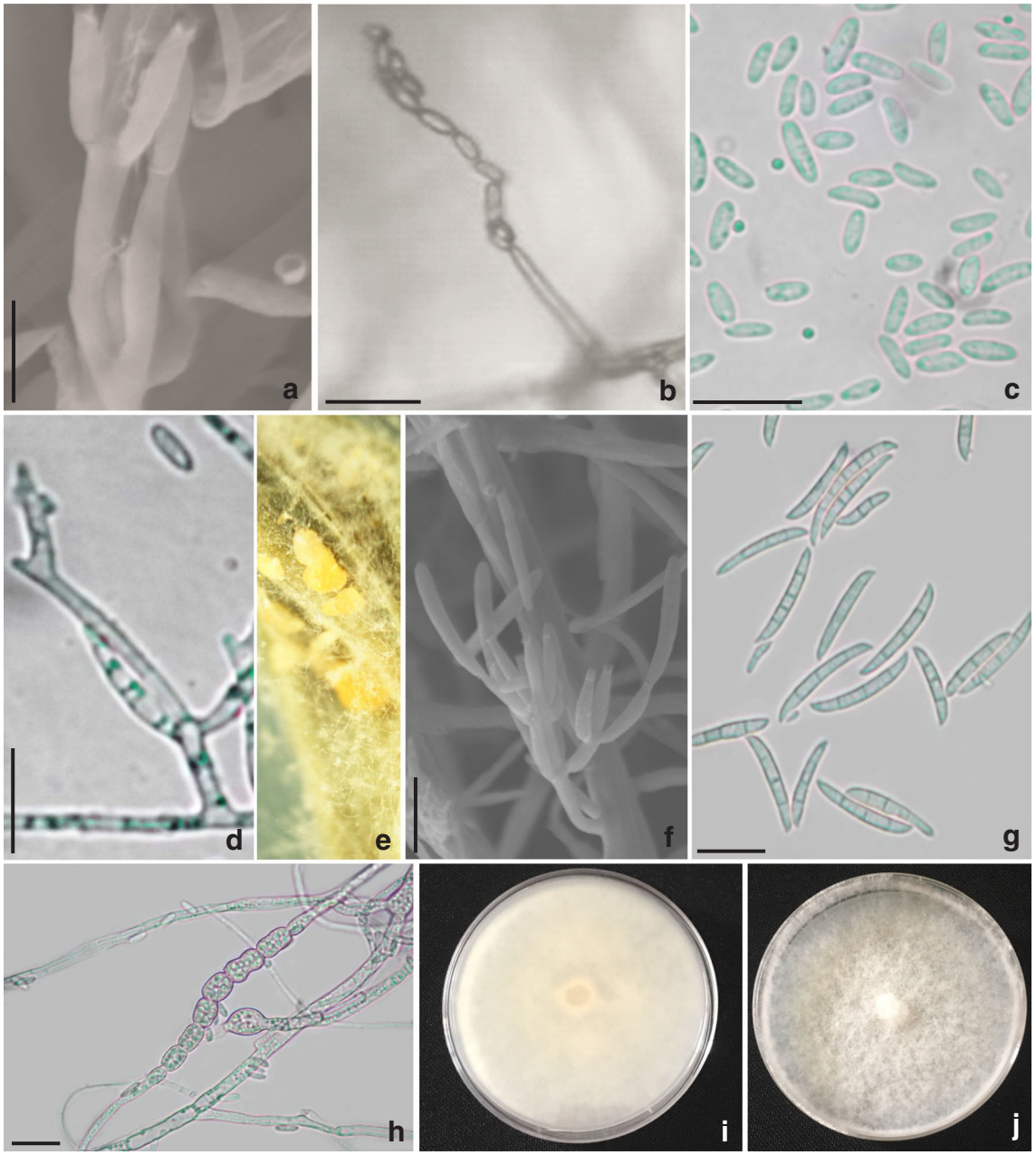


Fig. 5 Morphological characters of the asexual stage of *Fusarium caapi*. **a** Monophialides on conidiophores. **b** Microconidia in short chains. **c** 0-septate microconidia. **d** Polyphialide. **e** Sporodochia formed on carnation leaf on SNA. **f** Sporodochia. **g** 3–6 septate macroconidia. **h** Chlamydospores. **i**–**j** Reverse and obverse of PDA colony incubated for 14 days at 25 °C. Bars: **a**–**c** = 20 μm, **d** = 10 μm, and **f**–**h** = 30 μm

intercalary, globose, subglobose, cylindrical to subcylindrical, 4.5–22.2 × 5–18.5 μm.

Host: Brachiaria brizantha, *Oryza sativa* and indoor air in a clinical environment

Known distribution: Brazil and Kenya

Other specimens examined. BRAZIL, São Paulo: Guaíra, from seeds of *Brachiaria brizantha*, 2016, *F. S. Carmo* (CML 3660, CML 3658, CML 3659); Minas Gerais: Montes Claros, from seeds of *Brachiaria brizantha*, 2017, *F. S. Rocha* (CML 3881).

Discussion

In this study, eleven phylogenetic species from three *Fusarium* species complexes, *F. fujikuroi*, *F. incarnatum-equiseti*, and *F. chlamydosporum*, were isolated from apparently healthy seeds of *Brachiaria* spp. and *Panicum maximum* collected in different geographic regions of Brazil. Most FCSC and FIESC isolates were obtained from Rio Grande do Sul state in southern Brazil, and two FIESC isolates were encountered from seeds collected in the Central-West region

of the country (Goiás and Mato Grosso states). In contrast, only one FFSC isolate was obtained from the South Region, eight from the Central-West, and 14 from the Southeast Region of Brazil. While FCSC and FIESC were predominant in *Panicum maximum*, FFSC isolates were only found in association with *Brachiaria* spp. Despite the observed variations in the species complex composition and in the number of isolates, the small sampling size prevents the establishment of more clear relationships between host species and/or location and the prevalence of particular *Fusarium* species. Larger inventories are thus required for resolving grass host range and geographic distribution especially of the here newly described *Fusarium* species.

Phylogenetic analyses of three gene regions supported the identification of two new species from the FFSC. *Fusarium brachiariae* and *F. caapi* belong to the African clade, and Africa is the geographical origin of *Brachiaria* species. This is consistent with the phylogeographic hypothesis that species in the FFSC are distributed into three major clades (African, American, and Asian), representing the origin of *Fusarium* species and their plant hosts (O'Donnell et al. 1998). *Fusarium caapi* is phylogenetically close to *F. mundagurra*, which was described from soil and *Mangifera indica* in Australia (Laurence et al. 2016). The phylogenetic affinities of *F. brachiariae* to other species within the FFSC are less clearly defined. Three endophytic strains from rice root in Kenya (Pili et al. 2016) and one strain from hospital indoor air in Brazil (Moretti et al. 2018) were identified as *F. caapi*. It is thus possible that *F. caapi* was introduced in Brazil via propagating material of *Brachiaria* brought from Africa and is now distributed in the environment. *Fusarium caapi* and *F. brachiariae* are likely associated with other substrates and broadly distributed in Brazil.

Fusarium caapi and *F. brachiariae* differ in shape and size of the macroconidia. Macroconidia of *F. brachiariae* are larger and more elongated and have more prominent apical cells than in *F. caapi.* Colonies of *F. brachiariae* isolates were white or violet on PDA medium, whereas colonies of *F. caapi* have a cream color. Both species are morphologically similar to *F. mundagurra* and *F. nygamai* that produce conidia in short chains arising from polyphialides and abundant chlamydospores (Burgess and Trimboli 1986; Laurence et al. 2016). Chlamydospore production is restricted to few species of the FFSC, among them *F. acutatum*, *F. mundagurra*, *F. napiforme*, *F. nygamai*, *F. udum*, and *F. xylarioides*, all belonging to the African clade (Leslie and Summerell 2006; Laurence et al. 2016; Pfenning et al. 2019). No fertile crosses were obtained between *F. caapi* isolates. Likewise, it was not possible to cross the *F. brachiariae* isolates, as both have the same mating type. On the other hand, *F. caapi* and *F. brachiariae* did not cross with the tester isolates of any other FFSC species used in this work, confirming the reproductive isolation of the known mating populations. It remains unclear if the sexual stage of *F. brachiariae* and *F. caapi* exists in nature.

Other members of the FFSC isolated from *Brachiaria* in this survey, i.e., *F. fujikuroi*, *F. madaense*, *F. proliferatum*, *F. thapsinum*, and *F. verticillioides*, are important pathogens of maize, rice, sugarcane, and millet worldwide, and some are producers of fumonisins and moniliformin (Leslie et al. 2004, 2005; Costa et al. 2019; Ezekiel et al. 2020; Nicolli et al. 2020). *Fusarium madaense* has been isolated in Brazil as an endophyte in maize, rice, sorghum, finger millet, and pearl millet and as food contaminants in African countries (Ezekiel et al. 2020; Nicolli et al. 2020; Costa et al. unpublished). *Fusarium fujikuroi*, *F. verticillioides*, *F. proliferatum*, and *F. thapsinum* have been isolated as endophytes of native grasses in different countries (Leslie et al. 2004; Carter et al. 2008; Nor Azliza et al. 2014).

Species from FCSC and FIESC are endophytes or opportunistic pathogens of cultivated plants (Leslie and Summerell 2006). Members of both complexes are frequently associated with native and wild grasses (Bentley et al. 2007; Nor Azliza et al. 2014; Elmer et al. 2016) and commonly isolated from wheat, maize, rice, barley, and oat (Villani et al. 2016; Moreira et al. 2020). FIESC species can produce a dozen of mycotoxins, mainly trichothecenes and zearalenone (Villani et al. 2016; O'Donnell et al. 2018), while members from the FCSC may produce B-trichothecenes (O'Donnell et al. 2013). Although these complexes are rarely associated with cattle poisoning, their presence in wild *Brachiaria* and *Panicum* suggests the possibility of mycotoxin contamination when these grasses are used for animal feed (Botha et al. 2014).

All *Fusarium* isolates were recovered from seeds, suggesting that inflorescences could be the sites of infection by airborne conidia. Maize, sorghum, rice, and sugarcane are often planted near or in consortium with *Brachiaria* and *Panicum* grasses and could serve as inoculum sources, or the other way around. Inflorescences act as sites of infection for *F. culmorum*, *F. graminearum*, and *F. verticillioides* inducing ear rot disease in maize (Duncan and Howard 2010; Oldenburg and Ellner 2015). Systemic colonization of aerial tissues is another way, by which endophytic Fusaria could reach the seeds of grasses in the field, as described for *F. verticillioides* in maize (Duncan and Howard 2010).

This is the first survey of *Fusarium* species associated with *Brachiaria* spp. and *Panicum maximum* in Brazil, in which known pathogens and potential mycotoxin producers were recovered from these important plants of agronomic and livestock use. Even though these grass species are not native to Brazil, they are present throughout the territory and part of the country's natural vegetation.

Over the years, the interest in investigating *Fusarium* species associated with asymptomatic native or introduced plants has increased (Leslie et al. 2004; Phan et al. 2004; Walsh et al. 2010; Laurence et al. 2016). Our results from a relatively

small sampling reinforce the understanding that *Fusarium* species known as plant pathogens are also endophytes in noncrop hosts (Phan et al. 2004). Furthermore, this survey confirms that wild grasses are sources of unknown fungi and harbor a high diversity of *Fusarium* species (Walsh et al. 2010). Thus, an even greater diversity of *Fusarium* species may be recovered from grasslands and from other unexplored ecosystems in Brazil where *Brachiaria*, *Panicum*, and other seeded forages occur along with native or naturally occurring species.

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Authors' contributions Marileide M. Costa and Ludwig H. Pfenning designed the project, supervised its execution, wrote the first draft of the manuscript, and prepared the taxonomic descriptions. Maruzanete P. Melo, Filipe S. Carmo, Elaine A. Guimarães, and Fernando S. Rocha contributed with collection of material, isolation, identification, and preservation of fungal species and the elaboration of the discussion. Gláucia M. Moreira, Sarah S. Costa, and Lucas M. Abreu contributed with sequence analysis, preparation of phylogenetic trees, and overall analysis of the data. All authors commented on previous versions of the manuscript and approved the final version. Therefore, they had full access to all the data obtained in this study and take responsibility for the integrity and security of the data.

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Data availability Biological reference material is deposited and available in official collections and DNA sequences and alignments at GenBank and TreeBASE, respectively.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable.

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