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New endemic *Fusarium* species hitch-hiking with pathogenic *Fusarium* strains causing Panama disease in small-holder banana plots in Indonesia

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Key words

Indonesia
new species
non-pathogenic
phylogeny
species complex

Abstract *Fusarium* species are well known for their abundance, diversity and cosmopolitan life style. Many members of the genus *Fusarium* are associated with plant hosts, either as plant pathogens, secondary invaders, saprotrophs, and/or endophytes. We previously studied the diversity of *Fusarium* species in the *Fusarium oxysporum* species complex (FOSC) associated with Fusarium wilt of banana in Indonesia. In that study, several *Fusarium* species not belonging to the FOSC were found to be associated with Fusarium wilt of banana. These *Fusarium* isolates belonged to three *Fusarium* species complexes, which included the *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex (FIESC) and the *Fusarium sambucinum* species complex (FSSC). Using a multi-gene phylogeny that included partial fragments of the beta-tubulin (*tub*), calmodulin (*cmdA*), translation elongation factor 1-alpha (*tef1*), the internal transcribed spacer region of the rDNA (ITS), the large subunit of the rDNA (LSU), plus the RNA polymerase II large subunit (*rpb1*) and second largest subunit (*rpb2*) genes, we were able to identify and characterise several of these as new *Fusarium* species in the respective species complexes identified in this study.

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INTRODUCTION

Fusarium is one of the most diverse fungal genera that has been given much attention by mycologists and plant pathologists (Snyder & Hansen 1940, Nelson et al. 1983, Geiser et al. 2013, Aoki et al. 2014, 2018). Its global distribution, ability to adapt to manifold climatic conditions, and colonisation of a wide number of ecological niches and hosts, makes the diversity and abundance of *Fusarium* species unparalleled (Booth 1971, Gerlach & Nirenberg 1982, Geiser et al. 2013, Aoki et al. 2014). The genus *Fusarium* includes some of the most devastating plant pathogens, affecting many agronomical crops. Two of its species, *Fusarium graminearum* and *F. oxysporum*, were included in the top 10 list of fungal plant pathogens regarded as important in terms of scientific and economic impact (Dean et al. 2012, Geiser et al. 2013, Aoki et al. 2014).

Besides their role as plant pathogens, *Fusarium* species are also known as endophytes or saprophytic colonisers (Leslie et al. 1990, Bacon & Yates 2006). Many different *Fusarium* species are associated with symptomatic and asymptomatic plants (Leslie et al. 1990, Wang et al. 2004, Pinaria et al. 2010), although their role as pathogens can sometimes be difficult to determine via pathogenicity tests. However, many *Fusarium* species have not been associated with any disease symptoms on plants (Wang et al. 2004, Pinaria et al. 2010). Therefore, they are considered as endophytes and their association with their known host plants is difficult to discern (Kuldau & Yates 2000).

A complex of *Fusarium* spp. in the *Fusarium oxysporum* species complex (FOSC) is causing Fusarium wilt on banana (Maryani et al. 2019), also known as Panama disease (Stover 1962). The ability of these notorious fungi to infect a wide range of banana varieties has resulted in substantial economic strain in several banana producing regions (Ploetz et al. 2015, http://fusariumwilt.org/). Several studies acknowledged the diversity of *Fusarium* spp. pathogenic on banana and their worldwide distribution, thus recognising the threat to global banana cultivation (Ploetz 2006a, Ordonez et al. 2015, Maryani et al. 2019). However, to our knowledge, no study has been done to assess which other *Fusarium* species might be associated with Fusarium wilt on bananas.

In this study, we report *Fusarium* species hitch-hiking with pathogenic *Fusarium* spp. causing Panama disease, isolated from local banana varieties in Indonesia. Therefore, we aim to characterise these non-*Fusarium oxysporum* isolates, based on multi-gene phylogenetic inference, supported by morphological observations.

MATERIALS AND METHODS

Isolates

Isolates were obtained from the pseudostems of local banana plants clearly displaying symptoms of Fusarium wilt, which were sampled in small-holder backyard plantations across Indonesia in 2014–2015 (Maryani et al. 2019). The dried pseudostem samples were cut into pieces of 2 × 3 cm and plated on Komada medium (Komada 1975). Single-spore isolates were derived from resulting fungal colonies, and transferred to potato dextrose agar (PDA), on which they were maintained as working cultures, or stored in 20 % (v/v) glycerol at -80 °C for long term

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Table 1 *Fusarium* species recovered from pseudostems of banana with Fusarium wilt symptoms in Indonesia, with details information on origin, year of collection and GenBank/ENA accession numbers.

Species name	Strain number[1]	Location	Host[2]	Host genotype[2]	Year collected	GenBank/ENA accession number[3]						
						cal	ITS	LSU	*rpb1*	*rpb2*	*tef1*	*tub*
Fusarium desaboruense	InaCC F950^T	Sikka, Flores	*Musa* sp. var. Pisang Kepok	ABB	2015	–	–	–	LS479870	LS479852	–	LS479435
	InaCC F951	Sikka, Flores	*Musa* sp. var. Pisang Kepok	ABB	2015	–	–	–	LS479871	LS479853	–	LS479436
	InaCC F952	Sikka, Flores	*Musa* sp. var. Pisang Kepok	ABB	2015	–	–	–	LS479872	LS479854	–	LS479437
F. kotabaruense	InaCC F963^T	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Awak	ABB	2015	LS479429	LS479417	LS479890	LS479875	LS479859	LS479445	–
F. longipes	InaCC F974	Katingan, Central Kalimantan	*Musa* sp. var. Pisang Awak	ABB	2014	–	–	–	LS479880	LS479866	LS479451	–
F. lumajangense	InaCC F872^T	Kendal, Central Java	*Musa* sp. var. Pisang Raja Nangka	AAB	2014	–	–	–	–	LS479850	LS479441	LS479433
	InaCC F993	Lumajang, East Java	*Musa acuminata* var. Pisang Mas Kirana	AA	2014	–	–	–	–	LS479851	LS479442	LS479434
F. proliferatum	InaCC F962	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	2014	–	–	–	–	LS479868	LS479453	LS479439
	InaCC F992	Lumajang, East Java	*Musa acuminata* var. Pisang Mas Kirana	AA	2014	–	–	–	LS479882	LS479869	LS479454	LS479440
F. sulawense	InaCC F940^T	Bone, South Sulawesi	*Musa acuminata* var. Pisang Cere	AAA	2015	LS479422	LS479410	LS479883	–	LS479855	LS479443	–
	InaCC F941	Bone, South Sulawesi	*Musa acuminata* var. Pisang Cere	AAA	2015	LS479423	LS479411	LS479884	–	LS479856	LS479444	–
	Indo167	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Kepok	ABB	2015	LS479424	LS479412	LS479885	LS479874	LS479858	–	–
	InaCC F964	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Awak	ABB	2014	LS479425	LS479413	LS479886	LS479876	LS479860	LS479446	–
	Indo186	Banjar, South Kalimantan	*Musa* sp. var. Pisang Kepok	ABB	2014	LS479426	LS479414	LS479887	LS479878	LS479864	LS479449	–
	Indo188	Benajam, East Kalimantan	*Musa* sp. var. Pisang Awak	ABB	2014	LS479427	LS479415	LS479888	LS479879	LS479865	LS479450	–
F. tanahbumbuense	InaCC F965^T	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	2014	LS479432	LS479420	LS479893	LS479877	LS479863	LS479448	–
F. verticilloides	InaCC F991	Bondowoso, East Java	*Musa* sp. var. Pisang Kepok	ABB	2014	LS479421	–	–	LS479881	LS479867	LS479452	LS479438
Fusarium sp. FIESC 29	Indo174	Kota Baru, South Kalimantan	*Musa* sp. var. Pisang Awak	ABB	2014	LS479430	LS479418	LS479891	–	LS479861	–	–
Fusarium sp. FIESC 30	Indo175	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	2014	LS479431	LS479419	LS479892	–	LS479862	LS479447	–
Fusarium sp. FIESC 33	Indo161	Kota Baru, South Kalimantan	*Musa acuminata* var. Pisang Talas	AA	2014	LS479428	LS479416	LS479889	LS479873	LS479857	–	–

[1] InaCC: Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia; Indo: Collection of N. Maryani; ^{T}: ex-type strain.

[2] According to https://www.crop-diversity.org/mgis/taxonomy.

[3] *cal*: calmodulin; ITS: internal transcribed spacer region of the rDNA. LSU: large subunit of the rDNA; *rpb1*: RNA polymerase largest subunit gene; *rpb2*: RNA polymerase second largest subunit gene; *tef1*: translation elongation factor 1-alpha gene; *tub*: beta-tubulin.

preservation. All isolates were deposited in the Indonesian Culture Collection (InaCC) Cibinong, Indonesia.

Morphological characterisation

Morphological characterisations of the *Fusarium* species were performed on PDA for colony growth rates, pigmentation and production of aerial conidia; carnation leaf agar (CLA; Fisher et al. 1982) for formation of sporodochia and sporodochial conidia, and synthetic low-nutrient agar (SNA; Nirenberg 1981) for chlamydospores. To induce sporulation, cultures were incubated under continuous white light (Osram L18W/840 Cool White) for 7 d at 25 °C. Growth rates of all isolates were determined on PDA after 7 d incubation at 25 °C in the dark. Colony colour notation followed the mycological colour charts of Rayner (1970). Morphological characters were examined after mounting fungal structures in sterile water and observed using light microscopy (Nikon Eclipse 80i microscope) with Differential Interference Contrast (DIC) optics and a Nikon AZ100 stereomicroscope, both equipped with Nikon DS-Ri2 high definition colour digital cameras. Photographs and measurements were taken using the Nikon software NIS-elements D software v. 4.50. The length and width of at least 30 conidiogenous cells and 50 conidia were measured, and the mean values, standard deviation (SD) with maximum-minimum values were calculated. All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous et al. 2004).

DNA isolation, amplification and analyses

Genomic DNA was isolated using the DNA Wizard Magnetic DNA Purification System for Food kit (Promega, USA). Partial gene sequences were determined for the RNA polymerase largest subunit gene (*rpb1*) using primers RPB1-Fa and RPB1-G2R (O'Donnell et al. 2010), RNA polymerase second largest subunit gene (*rpb2*) using primers RPB2-5f2 and RPB2-7cr (O'Donnell et al. 2010), the translation elongation factor 1-alpha gene (*tef1*) using primers EF1 and EF2 (O'Donnell et al. 1998a), calmodulin (*cmdA*) CAL-228F and CAL-2RD (Carbone & Kohn 1999, Quaedvlieg et al. 2011), beta-tubulin (*tub*) using primers TUB-T1 and TUB-4RD (O'Donnell & Cigelnik 1997, Woudenberg et al. 2009), the internal transcribed spacer region (ITS) using primers ITS4 and ITS5 (White et al. 1990) and the large subunit of the ribosomal DNA (LSU) using primers LR0R and LR5 (Rehner & Samuels 1994, Vilgalys & Hester 1990). PCR conditions followed those described by Lombard et al. (2015). Amplicons were sequenced in both directions using the same primer pairs as were used for amplification to ensure integrity of the sequences. Consensus sequences were analysed and assembled using MEGA v. 7 (Kumar et al. 2016). Subsequent alignments for each individual locus were generated using MAFFT v. 7.110 (Katoh et al. 2017) and manually corrected if necessary. The individual sequences generated in this study were compared with those maintained in the *Fusarium*-MLST database (http://www.westerdijkinstitute.nl/fusarium/) and GenBank, and relevant sequences were included in the subsequent phylogenetic inferences.

Phylogenetic analyses were based on Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was performed using RAxML v. 8 (randomised accelerated (sic) maximum likelihood for high performance computing; Stamatakis 2014) through RAxML BlackBox (https://raxml-ng.vital-it.ch/#/) or the CIPRES science gateway portal (Miller et al. 2012). To assess the robustness of the analyses, the Bootstrap support (BS) was determined automatically by the software using default parameters. The BI analysis was performed using MrBayes v. 3.2.6 (Ronquist et al. 2012) on the CIPRES science gateway portal (Miller et al. 2012), using four Markov chain Monte Carlo (MCMC) chains starting from a random tree topology. The MCMC

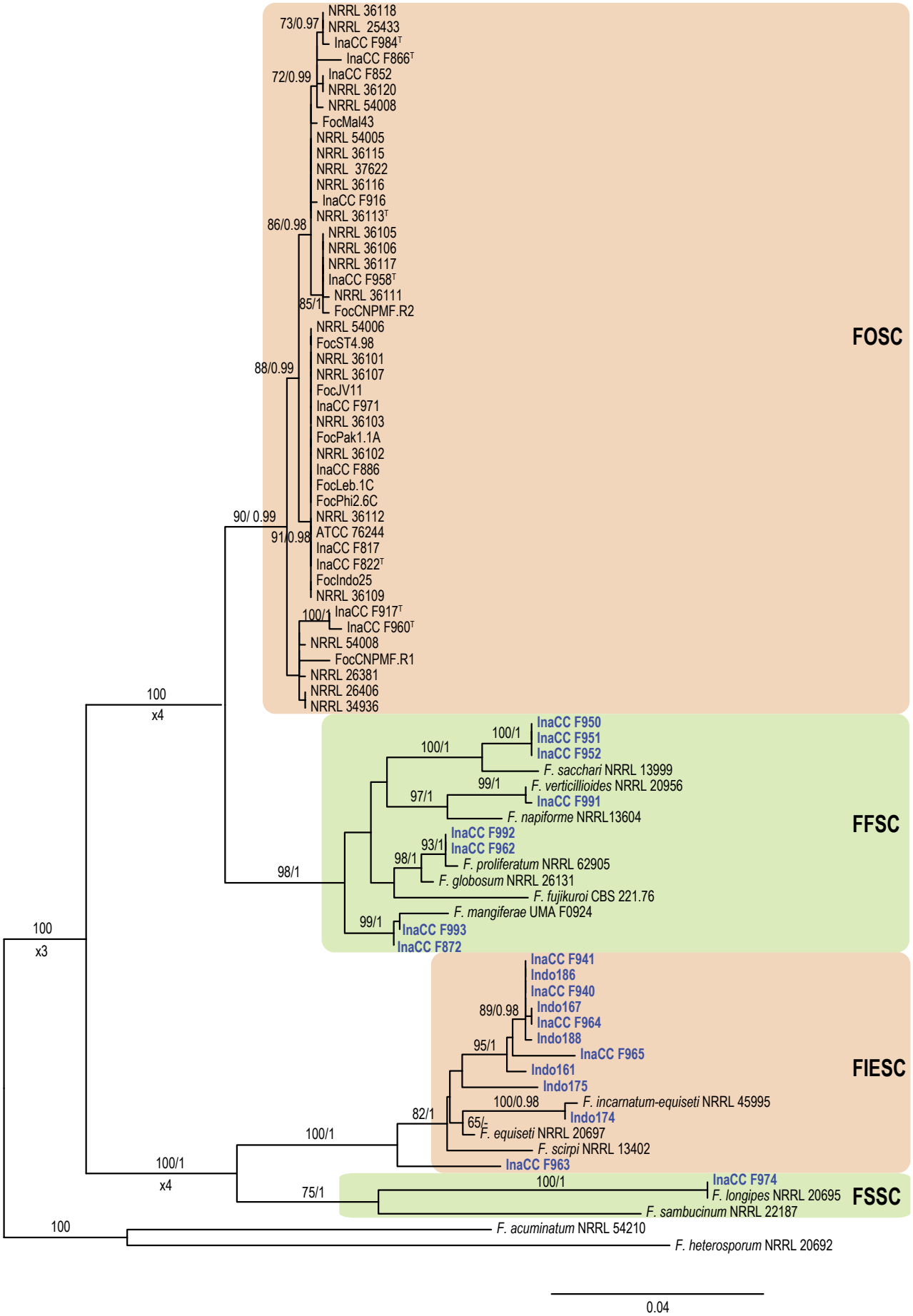

Fig. 1 Maximum likelihood tree inferred using the *rpb2* gene region of the Indonesian isolates in the *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium sambucinum* species complex (FSSC), and *Fusarium oxysporum* species complex (FOSC) isolates from a previous study (Maryani et al. 2019). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium acuminatum* (NRRL 54210) and *Fusarium heterosporum* (NRRL 20692).

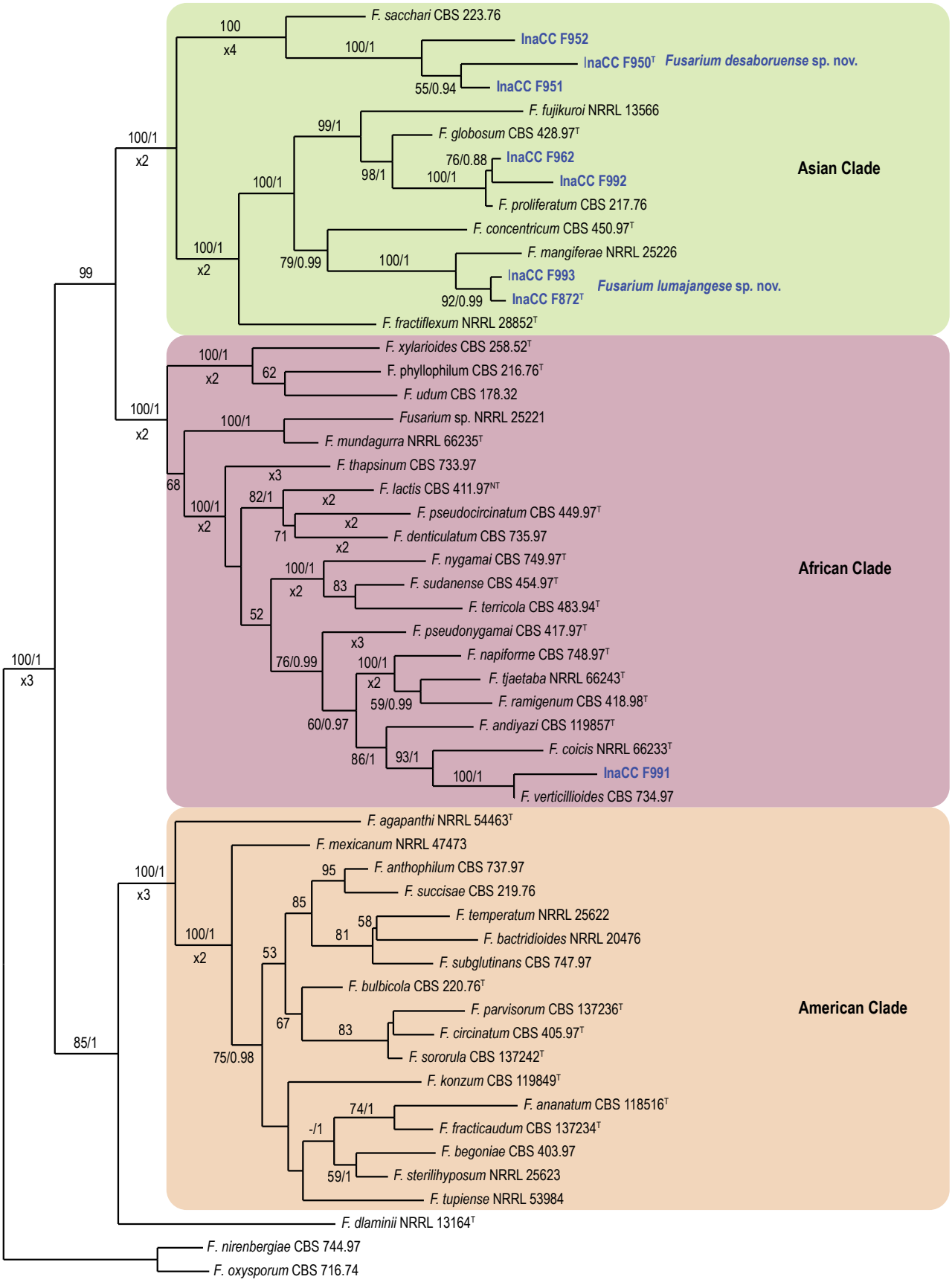

Fig. 2 Maximum likelihood tree inferred from the combined *cmdA*, *tef1*, *tub*, *rpb1*, and *rpb2* sequence datasets of the *Fusarium fujikuroi* species complex (FFSC) including eight Indonesian isolates (indicated in blue). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium nirenbergiae* (CBS 744.97) and *F. oxysporum* (CBS 716.74).

Table 2 *Fusarium* species included in this study.

Species	Strain number[1]	Further classification	Country	Host	GenBank/ENA accession number[2]						
					cal	ITS	LSU	*rpb1*	*rpb2*	*tef1*	*tub*
Fusarium acuminatum	NRRL 54210				–	–	–	–	GQ505484	–	–
F. agapanthi	NRRL 54463^{T}		Australia	*Agapanthus* sp.	KU900611	–	–	KU900620	KU900625	KU900630	KU900635
F. ananatum	CBS 118516^{T}		South Africa	*Ananas comosus* fruit	LT996175	–	–	LT996188	LT996137	LT996091	LT996112
F. andiyazi	CBS 119857^{T} = NRRL 21727		South Africa	*Sorghum bicolor* soil debris	LT996176	–	–	LT996189	LT996138	LT996092	LT996113
F. anthophilum	CBS 737.97 = NRRL 13602		Germany	*Hippeastrum* sp.	LT996177	–	–	LT996190	LT996139	LT996093	LT996114
F. armeniacum	NRRL 6227		USA	Fescue hay	–	–	–	JX171446	JX171560	–	–
F. asiaticum	CBS 110257 = NRRL 13818		Japan	Barley	–	–	–	JX171459	JX171573	–	–
F. bactridioides	NRRL 20476		USA	*Cronartium conigenum*	AF158343	–	–	–	–	AF160290	U34434
F. begoniae	CBS 403.97^{T} = NRRL 25300		Germany	*Begonia* elatior hybrid	AF158346	–	–	LT996191	LT996140	AF160293	U61543
F. bulbicola	CBS 220.76^{T} = NRRL 13618		Germany	*Nerine bowdenii*	KF466327	–	–	KF466394	KF466404	KF466415	KF466437
F. cf. *compactum*	NRRL 13829		Japan	River sediments	–	–	–	JX171460	JX171574	–	–
F. circinatum	CBS 405.97^{T} = NRRL 25331		USA	*Pinus radiata*	KM231393	–	–	JX171510	HM068354	KM231943	KM232080
F. coicis	NRRL 66233^{T}		Australia	*Coix gasteenii*	LT996178	–	–	KP083269	KP083274	KP083251	LT996115
F. concentricum	CBS 450.97^{T} = NRRL 25181		Costa Rica	*Musa sapientum* fruit	AF158335	–	–	LT996192	JF741086	AF160282	U61548
F. cugenangense	InaCC F984^{T}	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	–	–	–	–	LS479308	–	–
	NRRL 36118	f. sp. *cubense*	Thailand	*Musa* sp. var. Kluai nam wa	–	–	–	–	LS479221	–	–
	NRRL 25433	f. sp. *vasinvectum*	China	*Gosypium* sp.	–	–	–	–	LS479202	–	–
F. culmorum	CBS 417.86 = NRRL 25475		Denmark	Moldy barley kernel	–	–	–	JX171515	JX171628	–	–
F. denticulatum	CBS 735.97 = NRRL 25302		USA	*Ipomoea batatas*	AF158322	–	–	LT996195	LT996143	AF160269	U61550
F. dlaminii	CBS 119860^{T} = NRRL 13164		South Africa	Soil debris in cornfield	AF158330	–	–	KU171681	KU171701	AF160277	U34430
F. duoseptatum	InaCC F916	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	–	–	–	–	LS479239	–	–
	FocMal43	f. sp. *cubense*	Malaysia	*Musa* sp. var. Pisang Rastali	–	–	–	–	LS479207	–	–
	NRRL 36115	f. sp. *cubense*	Malaysia	*Musa acuminata* var. Pisang Ambon	–	–	–	–	LS479218	–	–
	NRRL 36116	f. sp. *cubense*	Malaysia	*Musa* sp. var. Pisang Keling	–	–	–	–	LS479219	–	–
F. equiseti	CBS 107.07 = NRRL 36136	FIESC 14a			GQ505556	GQ505733	GQ505733	–	GQ505822	GQ505644	–
	CBS 185.34 = NRRL 36321	FIESC 14a	Netherlands	Soil	GQ505559	GQ505736	GQ505736	–	GQ505825	GQ505647	–
	CBS 307.94^{NT} = NRRL 26419	FIESC 14a	Germany	Soil	GQ505511	GQ505688	GQ505688	–	GQ505777	GQ505599	–
	CBS 414.86 = NRRL 36466	FIESC 14a	Denmark	Potato peel	GQ505565	GQ505742	GQ505742	–	GQ505831	GQ505653	–
F. fracticaudum	CBS 137234^{PT}		Colombia	*Pinus maximonoii* stem	LT996179	–	–	LT996196	LT996144	KJ541059	KJ541051
F. fractiflexum	NRRL 28852^{T}		Japan	*Cymbidium* sp.	AF158341	–	–	LT575064	LT575064	AF160288	AF160315
F. fujikuroi	NRRL 13566		China	*Oryza sativa*	AF158332	U34557	U34528	JX171456	JX171570	AF160279	U34415
	CBS 221.76				–	–	–	–	KU604255	–	–
F. globosum	CBS 428.97^{T} = NRRL 26131		South Africa	*Zea mays*	KF466329	–	–	KF466396	KF466406	KF466417	KF466439
F. goolgardi	NRRL 66250^{T} = RBG 5411		Australia	*Xanthorrhoea glauca*	–	–	–	KP083270	KP083280	–	–
F. graminearum	CBS 123657 = NRRL 31084		USA	Corn	–	–	–	JX171531	JX171644	–	–
F. grosmichelii	InaCC F852	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Ambon Lumut	–	–	–	–	LS479342	–	–
	NRRL 36120	f. sp. *cubense*	Thailand	*Musa sapientum*	–	–	–	–	LS479222	–	–
F. heterosporum	NRRL 20692		Ethiopia	*Cynodon dactylon*	–	–	–	–	JX171593	–	–
F. hexaseptatum	InaCC F866^{T}	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Ambon Kuning	–	–	–	–	LS479359	–	–
F. kalimantanense	InaCC F917^{T}		Indonesia	*Musa acuminata* var. Pisang Ambon	–	–	–	–	LS479241	–	–
F. konzum	CBS 119849^{T}		USA	*Sorghastrum nuttans*	LT996182	–	–	LT996200	LT996148	LT996098	LT996118
F. kyushuense	NRRL 25349		Japan	*Triticum aestivum*	–	–	–	–	GQ915492	–	–
F. lacertarum	CBS 102300 = NRRL 36123	FIESC 4b			GQ505555	GQ505732	GQ505732	–	JX171581	GQ505593	–
	CBS 130185^{T} = NRRL 20423	FIESC 4a	India	Lizard skin	GQ505505	GQ505682	GQ505682	–	GQ505821	GQ505643	–
F. lactis	CBS 411.97^{NT} = NRRL 25200		USA	*Ficus carica*	AF158325	–	–	LT996201	LT996149	AF160272	U61551
F. langsethiae	NRRL 54940		Norway	Oats	–	–	–	JX171550	JX171662	–	–
F. longipes	NRRL 13368		Australia	Soil	–	–	–	JX171448	JX171562	–	–
	NRRL 20695				–	–	–	–	GQ915493	–	–
F. mangiferae	NRRL 25226		Israel	*Mangifera indica*	AF158334	–	–	JX171509	HM068353	AF160281	U61561
	UMAF0924			*Mangifera indica*	–	–	–	–	KP753442	–	–

Table 2 (cont.)

Species	Strain number[1]	Further classification	Country	Host	GenBank/ENA accession number[2]						
					cal	ITS	LSU	*rpb1*	*rpb2*	*tef1*	*tub*
F. mexicanum	NRRL 47473		Mexico	*Mangifera indica* inflorescence	GU737389	–	–	Not public	Not public	GU737416	GU737308
F. mundagurra	NRRL 66235 = RGB 5717^T		Australia	Soil	–	–	–	KP083272	KP083276	KP083256	–
F. napiforme	CBS 748.97^T = NRRL 13604		Namibia	*Pennisetum typhoides*	AF158319	–	–	HM347136	EF470117	AF160266	U34428
F. nygamai	CBS 749.97^T = NRRL 13448		Australia	*Sorghum bicolor* necrotic root	AF158326	–	–	LT996202	EF470114	AF160273	U34426
F. odoratissimum	InaCC F817	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	–	–	–	–	LS479304	–	–
	InaCC F822^T	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Raja	–	–	–	–	LS479386	–	–
	NRRL 54006	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Manurung	–	–	–	–	LS479198	–	–
	FocJV11	f. sp. *cubense*	Jordan	*Musa acuminata* var. Cavendish	–	–	–	–	LS479205	–	–
	FocLeb1.2C	f. sp. *cubense*	Lebanon	*Musa acuminata* var. Cavendish	–	–	–	–	LS479206	–	–
	NRRL 36102	f. sp. *cubense*	China	*Musa acuminata* var. Cavendish	–	–	–	–	LS479209	–	–
	FocPak1.1A	f. sp. *cubense*	Pakistan	*Musa acuminata* var. Cavendish	–	–	–	–	LS479223	–	–
	FocPhi2.6C	f. sp. *cubense*	The Philippines	*Musa acuminata* var. Cavendish	–	–	–	–	LS479224	–	–
F. oxysporum	CBS 716.74		Germany	*Vicia faba*	AF158366	–	–	JX171469	JX171583	AF008479	U34435
	CBS 744.97		USA	*Pseudotsuga menziesii*	AF158365	–	–	LT996203	LT575065	AF160312	U34424
	NRRL 26381	f. sp. *lycopersici*	USA	*Solanum lycopersicum*	–	–	–	–	LS479195	–	–
	NRRL 54002			Soil	–	–	–	–	LS479194	–	–
	FocCNPMF.R1	f. sp. *cubense*	Brazil	*Musa* sp. var. Silk	–	–	–	–	LS479196	–	–
	NRRL 34936	f. sp. *lycopersici*	Spain	*Solanum lycopersicum*	–	–	–	–	LS479200	–	–
	NRRL 26406	f. sp. *melonis*		*Cucumis melo*	–	–	–	–	LS479201	–	–
F. palustre	NRRL 54056^T		USA	*Spartina alterniflora*	–	–	–	KT597718	KT597731	–	–
F. parvisorum	CBS 137236^T		Colombia	*Pinus patula* roots	LT996183	–	–	–	LT996150	KJ541060	KJ541055
F. phialoporum	InaCC F971	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Awak	–	–	–	–	LS479292	–	–
	FocST4.98	f. sp. *cubense*	Spain	*Musa acuminata* var. Dwarf Cavendish	–	–	–	–	LS479227	–	–
	FocIndo25	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Ambon	–	–	–	–	LS479204	–	–
	NRRL 36101	f. sp. *cubense*	Australia	*Musa* sp. var. Mons Mari	–	–	–	–	LS479208	–	–
	NRRL 36103	f. sp. *cubense*	The Philippines	*Musa acuminata* var. Cavendish	–	–	–	–	LS479210	–	–
	NRRL 36109	f. sp. *cubense*	Australia	*Musa acuminata* var. SH3142	–	–	–	–	LS479214	–	–
	NRRL 36112	f. sp. *cubense*	South Africa	*Musa acuminata* var. Cavendish	–	–	–	–	LS479216	–	–
F. phyllophilum	CBS 216.76^T = NRRL 13617		Italy	*Dracaena deremensis* leaf	KF466333	–	–	KF466399	KF466410	KF466421	KF466443
F. poae	NRRL 13714				–	–	–	JX171458	JX171572	–	–
F. proliferatum	CBS 217.76 = NRRL 22944		Germany	*Cattleya* pseudobulb, hybrid	AF158333	–	–	JX171504	HM068352	AF160280	U34416
	NRRL 62905				–	–	–	–	KU171707	–	–
F. pseudocircinatum	CBS 449.97^T = NRRL 22946		Ghana	*Solanum* sp.	AF158324	–	–	LT996204	LT996151	AF160271	U34427
F. pseudograminearum	CBS 109956^T = NRRL 28062		Australia	*Hordeum vulgare* crowns	–	–	–	JX171524	JX171637	–	–
F. pseudonygamai	CBS 417.97^T = NRRL 13592		Nigeria	*Pennisetum typhoides*	AF158316	–	–	LT996205	LT996152	AF160263	U34421
F. purpurascens	InaCC F886	f. sp. *cubense*	Indonesia	*Musa* sp. var. Pisang Kepok	–	–	–	–	LS479385	–	–
	ATCC 76244	f. sp. *cubense*	USA	*Musa acuminata* var. Apple	–	–	–	–	LS479199	–	–
	NRRL 36107	f. sp. *cubense*	Honduras	*Musa* sp. var. Maqueno	–	–	–	–	LS479213	–	–
F. ramigenum	CBS 418.98^T = NRRL 25208		USA	*Ficus carica*	KF466335	–	–	KF466401	KF466412	KF466423	KF466445
F. sacchari	CBS 223.76 = NRRL 13999		India	*Saccharum officinarum*	AF158331	–	–	JX171466	JX171580	AF160278	U34414
F. sambucinum	NRRL 22187 = NRRL 20727		England	*Solanum* sp.	–	–	–	JX171493	JX171606	–	–
F. sangayamense	InaCC F960^T		Indonesia	*Musa* sp. var. Pisang Kepok	–	–	–	–	LS479283	–	–
F. scirpi	CBS 447.84 = NRRL 36478	FIESC 9a	Australia	Pasture soil	GQ505566	GQ505743	GQ505743	–	GQ505832	GQ505654	–
	CBS 448.84 = NRRL 29134	FIESC 9a	Australia	Pasture soil	GQ505517	GQ505694	GQ505694	–	GQ505783	GQ505605	–
	CBS 610.95 = NRRL 26922	FIESC 9c	France	Soil	GQ505513	GQ505690	GQ505690	–	GQ505779	GQ505601	–
	NRRL 13402	FIESC 9b	Australia	Pine nursery soil	GQ505504	GQ505681	GQ505681	–	JX171566	GQ505592	–
F. sibiricum	NRRL 53430^T		Russia	*Avena sativa*	–	–	–	–	HQ154472	–	–
F. sororula	CBS 137242^T		Colombia	*Pinus patula* stems	LT996184	–	–	LT996206	LT996153	KJ541067	KJ541057
F. tardichlamydosporum	InaCC F958^T	f. sp. *cubense*	Indonesia	*Musa acuminata* var. Pisang Barangan	–	–	–	–	LS479280	–	–
	FocCNPMF.R2	f. sp. *cubense*	Brazil	*Musa* sp. var. Monthan	–	–	–	–	LS479197	–	–
	NRRL 36105	f. sp. *cubense*	Honduras	*Musa* sp. var. Bluggoe	–	–	–	–	LS479211	–	–

Table 2 (cont.)

Species	Strain number[1]	Further classification	Country	Host	GenBank/ENA accession number[2]						
					cal	ITS	LSU	*rpb1*	*rpb2*	*tef1*	*tub*
F. tardichlamydosporum (cont.)	NRRL 36106	f. sp. *cubense*	Australia	*Musa acuminata* var. Lady Finger	–	–	–	–	LS479212	–	–
	NRRL 36111	f. sp. *cubense*	Australia	*Musa* sp. var. Bluggoe	–	–	–	–	LS479215	–	–
	NRRL 36117	f. sp. *cubense*	Malaysia	*Musa* sp. var. Pisang Awak Legor	–	–	–	–	LS479220	–	–
F. tardicrescens	NRRL 36113[T]	f. sp. *cubense*	Malawi	*Musa* sp. var. Harare	–	–	–	–	LS479217	–	–
	NRRL 37622	f. sp. *pisi*		*Cicer* sp.	–	–	–	–	LS479203	–	–
	NRRL 54008	f. sp. *conglutinans*	Brazil	Silk	–	–	–	–	LS479225	–	–
	NRRL 54005	f. sp. *raphani*		*Raphanus* sp.	–	–	–	–	LS479226	–	–
Fusarium sp.	NRRL 3020	FIESC 10a			GQ505498	GQ505675	GQ505675	–	GQ505764	GQ505586	–
	NRRL 3214	FIESC 10a			GQ505499	GQ505676	GQ505676	–	GQ505765	GQ505587	–
	NRRL 5537	FIESC 8a	USA	Fescue hay	GQ505500	GQ505677	GQ505677	–	GQ505766	GQ505588	–
	NRRL 6548	FIESC 12a	Germany	*Hordeum vulgare* seedling	GQ505501	GQ505678	GQ505678	–	GQ505767	GQ505589	–
	NRRL 13335	FIESC 21a			GQ505502	GQ505679	GQ505679	–	GQ505768	GQ505590	–
	NRRL 20722	FIESC 27a	Kenya	*Pyrethrum* sp.	GQ505507	GQ505684	GQ505684	–	GQ505773	GQ505595	–
	NRRL 22244	FIESC 25a	China	Rice	GQ505508	GQ505685	GQ505685	–	GQ505774	GQ505596	–
	NRRL 25221		Zimbabwe		–	–	–	–	–	AF160268	–
	NRRL 25795	FIESC 5c	Germany	*Disphyma crassifolium* seed	GQ505509	GQ505686	GQ505686	–	GQ505775	GQ505597	–
	NRRL 26417	FIESC 26a	Cuba	Plant leaf litter	GQ505510	GQ505687	GQ505687	–	GQ505776	GQ505598	–
	NRRL 26921	FIESC 12a	Germany	Culm base of *Triticum aestivum*	GQ505512	GQ505689	GQ505689	–	GQ505778	GQ505600	–
	NRRL 28029	FIESC 3b	USA		GQ505514	GQ505691	GQ505691	–	GQ505780	GQ505602	–
	NRRL 28577	FIESC 28a	Romania	Grave stone	GQ505515	GQ505692	GQ505692	–	GQ505781	GQ505603	–
	NRRL 28714	FIESC 26b			GQ505516	GQ505693	GQ505693	–	GQ505782	GQ505604	–
	NRRL 31008		Australia	Soil	–	–	–	JX171529	JX171642	–	–
	NRRL 31011	FIESC 12a	Germany	*Thuja* sp.	GQ505518	GQ505695	GQ505695	–	GQ505784	GQ505606	–
	NRRL 31160	FIESC 15c	USA	Human lung	GQ505519	GQ505696	GQ505696	–	GQ505785	GQ505607	–
	NRRL 31167	FIESC 18a	USA	Human sputum	GQ505520	GQ505697	GQ505697	–	GQ505786	GQ505608	–
	NRRL 32175	FIESC 15a	USA	Human sputum	GQ505521	GQ505698	GQ505698	–	GQ505787	GQ505609	–
	NRRL 32181	FIESC 15c	USA	Human blood	GQ505522	GQ505699	GQ505699	–	GQ505788	GQ505610	–
	NRRL 32182	FIESC 15b	USA	Human blood	GQ505523	GQ505700	GQ505700	–	GQ505789	GQ505611	–
	NRRL 32522	FIESC 18b	USA	Human diabetic cellulitis	GQ505524	GQ505701	GQ505701	–	GQ505790	GQ505612	–
	NRRL 32864	FIESC 17a	USA	Human	GQ505525	GQ505702	GQ505702	–	GQ505791	GQ505613	–
	NRRL 32865	FIESC 21b	Brazil	Human endocarditis	GQ505526	GQ505703	GQ505703	–	GQ505792	GQ505614	–
	NRRL 32866	FIESC 23a	USA	Human cancer patient	GQ505527	GQ505704	GQ505704	–	GQ505793	GQ505615	–
	NRRL 32867	FIESC 23a	USA	Human	GQ505528	GQ505705	GQ505705	–	GQ505794	GQ505616	–
	NRRL 32868	FIESC 25c	USA	Human blood	GQ505529	GQ505706	GQ505706	–	GQ505795	GQ505617	–
	NRRL 32869	FIESC 15c	USA	Human cancer patient	GQ505530	GQ505707	GQ505707	–	GQ505796	GQ505618	–
	NRRL 32871	FIESC 5a	USA	Human abscess	GQ505531	GQ505708	GQ505708	–	GQ505797	GQ505619	–
	NRRL 32994	FIESC 15c	USA	Human ethmoid sinus	GQ505533	GQ505710	GQ505710	–	GQ505799	GQ505621	–
	NRRL 32995	FIESC 15c	USA	Human sinus	GQ505534	GQ505711	GQ505711	–	GQ505800	GQ505622	–
	NRRL 32996	FIESC 15c	USA	Human leg wound	GQ505535	GQ505712	GQ505712	–	GQ505801	GQ505623	–
	NRRL 32997	FIESC 7a	USA	Human toenail	GQ505536	GQ505713	GQ505713	–	GQ505802	GQ505624	–
	NRRL 34001	FIESC 15e	USA	Human foot wound	GQ505537	GQ505714	GQ505714	–	GQ505803	GQ505625	–
	NRRL 34002	FIESC 22a	USA	Human ethmoid sinus	GQ505538	GQ505715	GQ505715	–	GQ505804	GQ505626	–
	NRRL 34003	FIESC 20a	USA	Human sputum	GQ505539	GQ505716	GQ505716	–	GQ505805	GQ505627	–
	NRRL 34004	FIESC 16a	USA	Human BAL	GQ505540	GQ505717	GQ505717	–	GQ505806	GQ505628	–
	NRRL 34005	FIESC 24a	USA	Human intravitreal fluid	GQ505541	GQ505718	GQ505718	–	GQ505807	GQ505629	–
	NRRL 34006	FIESC 15a	USA	Human eye	GQ505542	GQ505719	GQ505719	–	GQ505808	GQ505630	–
	NRRL 34007	FIESC 15a	USA	Human sputum	GQ505543	GQ505720	GQ505720	–	GQ505809	GQ505631	–
	NRRL 34008	FIESC 15d	USA	Human lung	GQ505544	GQ505721	GQ505721	–	GQ505810	GQ505632	–
	NRRL 34010	FIESC 15c	USA	Human maxullary sinus	GQ505545	GQ505722	GQ505722	–	GQ505811	GQ505633	–
	NRRL 34011	FIESC 15a	USA	Human sputum	GQ505546	GQ505723	GQ505723	–	GQ505812	GQ505634	–
	NRRL 34032	FIESC 5a	USA	Human abscess	GQ505547	GQ505724	GQ505724	–	GQ505813	GQ505635	–

Table 2 (cont.)

Species	Strain number[1]	Further classification	Country	Host	GenBank/ENA accession number[2]						
					cal	ITS	LSU	*rpb1*	*rpb2*	*tef1*	*tub*
Fusarium sp. (cont.)	NRRL 34034	FIESC 1c	USA	Human leg	GQ505548	GQ505725	GQ505725	–	GQ505814	GQ505636	–
	NRRL 34035	FIESC 5d	USA	Human sinus	GQ505549	GQ505726	GQ505726	–	GQ505815	GQ505637	–
	NRRL 34037	FIESC 5b	USA	Human abscess	GQ505550	GQ505727	GQ505727	–	GQ505816	GQ505638	–
	NRRL 34039	FIESC 1b	USA	Human	GQ505551	GQ505728	GQ505728	–	GQ505817	GQ505639	–
	NRRL 34056	FIESC 16b	USA	Human bronchial wash	GQ505552	GQ505729	GQ505729	–	GQ505818	GQ505640	–
	NRRL 34059	FIESC 16c	USA	Human blood	GQ505553	GQ505730	GQ505730	–	GQ505819	GQ505641	–
	NRRL 34070	FIESC 17c	USA	Tortoise	GQ505554	GQ505731	GQ505731	–	GQ505820	GQ505642	–
	NRRL 36269	FIESC 12b	Croatia	*Pinus nigra* seesling	GQ505557	GQ505734	GQ505734	–	GQ505823	GQ505645	–
	NRRL 36318	FIESC 3a			GQ505558	GQ505735	GQ505735	–	GQ505824	GQ505646	–
	NRRL 36323	FIESC 3a	England	Cotton yarn	GQ505560	GQ505737	GQ505737	–	GQ505826	GQ505648	–
	NRRL 36351				–	–	–	–	GQ915484	–	–
	NRRL 36372	FIESC 11a	Netherlands Antilles	Air	GQ505561	GQ505738	GQ505738	–	GQ505827	GQ505649	–
	NRRL 36392	FIESC 12c	Germany	Seedling	GQ505562	GQ505739	GQ505739	–	GQ505828	GQ505650	–
	NRRL 36401	FIESC 2a	Mozambique	Cotton	GQ505563	GQ505740	GQ505740	–	GQ505829	GQ505651	–
	NRRL 36448	FIESC 2b	Sudan	*Phaseolus vulgaris* seed	GQ505564	GQ505741	GQ505741	–	GQ505830	GQ505652	–
	NRRL 36548	FIESC 17b	Congo	Banana	GQ505567	GQ505744	GQ505744	–	GQ505833	GQ505655	–
	NRRL 36575	FIESC 20b	USA	*Juniperus chinensis* leaf	GQ505568	GQ505745	GQ505745	–	GQ505834	GQ505656	–
	NRRL 43297	FIESC 24b	USA	*Spartina* rhizomes	GQ505569	GQ505746	GQ505746	–	GQ505835	GQ505657	–
	NRRL 43619	FIESC 15a	USA	Human finger	GQ505570	GQ505748	GQ505748	–	GQ505837	GQ505659	–
	NRRL 43622	FIESC 15c	USA	Human lung	GQ505571	GQ505749	GQ505749	–	GQ505838	GQ505660	–
	NRRL 43635	FIESC 13a	USA	Horse	GQ505573	GQ505751	GQ505751	–	GQ505840	GQ505662	–
	NRRL 43638	FIESC 6a	USA	Manatee	GQ505576	GQ505754	GQ505754	–	GQ505843	GQ505665	–
	NRRL 43639	FIESC 19a	USA	Manatee	GQ505577	GQ505755	GQ505755	–	GQ505844	GQ505666	–
	NRRL 43640	FIESC 1a	USA	Dog nose	GQ505578	GQ505756	GQ505756	–	GQ505845	GQ505667	–
	NRRL 43694	FIESC 6a	USA	Human eye	GQ505579	GQ505757	GQ505757	–	GQ505846	GQ505668	–
	NRRL 43730	FIESC 16c	USA	Contact lens	GQ505580	GQ505758	GQ505758	–	GQ505847	GQ505669	–
	NRRL 45995	FIESC 5b	USA	Human abscess	GQ505581	GQ505759	GQ505759	–	GQ505848	GQ505670	–
	NRRL 45997	FIESC 5f	USA	Human sinus	GQ505583	GQ505761	GQ505761	–	GQ505850	GQ505672	–
	NRRL 45998	FIESC 6b	USA	Human toe	GQ505584	GQ505762	GQ505762	–	GQ505851	GQ505673	–
F. sporotrichioides	NRRL 3299		USA	Corn	–	–	–	JX171444	HQ154454	–	–
F. sterilihyposum	NRRL 25623		South Africa	Mango	AF158353	–	–	Not public	Not public	AF160300	AF160316
F. subglutinans	CBS 747.97 = NRRL 22016		USA	Corn	AF158342	–	–	JX171486	JX171599	AF160289	U34417
F. succisae	CBS 219.76 = NRRL 13613		Germany	*Succisa pratensis* flower	AF158344	–	–	LT996207	LT996154	AF160291	U34419
F. sudanense	CBS 454.97^{T} = NRRL 25451		Sudan	*Striga hermonthica*	LT996185	–	–	LT996208	LT996155	KU711697	KU603909
F. temperatum	NRRL 25622 = NRRL 26616		South Africa	*Zea mays*	AF158354	–	–	Not public	Not public	AF16030	AF160317
F. terricola	CBS 483.94^{T}		Australia	Soil	KU603951	–	–	LT996209	LT996156	KU711698	KU603908
F. thapsinum	CBS 733.97 = NRRL 22045		South Africa	*Sorghum bicolor*	LT996186	–	–	JX171487	JX171600	AF160270	U34418
F. tjaetaba	NRRL 66243^{T}		Australia	*Sorghum interjectum*	LT996187	–	–	KP083267	KP083275	KP083263	GU737296
F. tupiense	NRRL 53984		Brazil	*Mangifera indica*	GU737377	–	–	Not public	Not public	GU737404	GU737296
F. udum	CBS 178.32 = NRRL 22949		Germany	*Lactarius pubescens*	AF158328	–	–	LT996220	LT996172	AF160275	U34433
F. venenatum	CBS 458.93^{T}		Austria	Winter wheat halm base	–	–	–	–	KM232382	–	–
F. verticillioides	CBS 734.97 = NRRL 22172		Germany	*Zea mays*	AF158315	–	–	LT996221	EF470122	AF160262	U34413
	NRRL 20956			*Zea mays*	–	–	–	–	JX171598	–	–
F. xylarioides	CBS 258.52 = NRRL 25486		Ivory Coast	*Coffea* trunk	–	–	–	JX171517	HM068355	AY707136	AY707118

[1] ATCC: American Type Culture Collection, USA; CBS: collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; Foc: collection of Wageningen Plant Research, Wageningen University, The Netherlands; InaCC: Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia; NRRL: Agricultural Research Service Culture Collection, USA; RBG: Royal Botanical Gardens Trust, Sydney, New South Wales, Australia; UMAF: Microbiology and Plant Pathology Laboratory Collection, University of Malaga, Spain; PT: ex-paratype culture; T: ex-type culture; NT: neotype.

[2] *cal*: calmodulin; ITS: internal transcribed spacer region of the rDNA; LSU: large subunit of the rDNA; *rpb1*: RNA polymerase largest subunit gene; *rpb2*: RNA polymerase second largest subunit gene; *tef1*: translation elongation factor 1-alpha gene; *tub*: beta-tubulin; Sequences marked as 'Not public' were obtained from Kerry O'Donnell's alignment datasets.

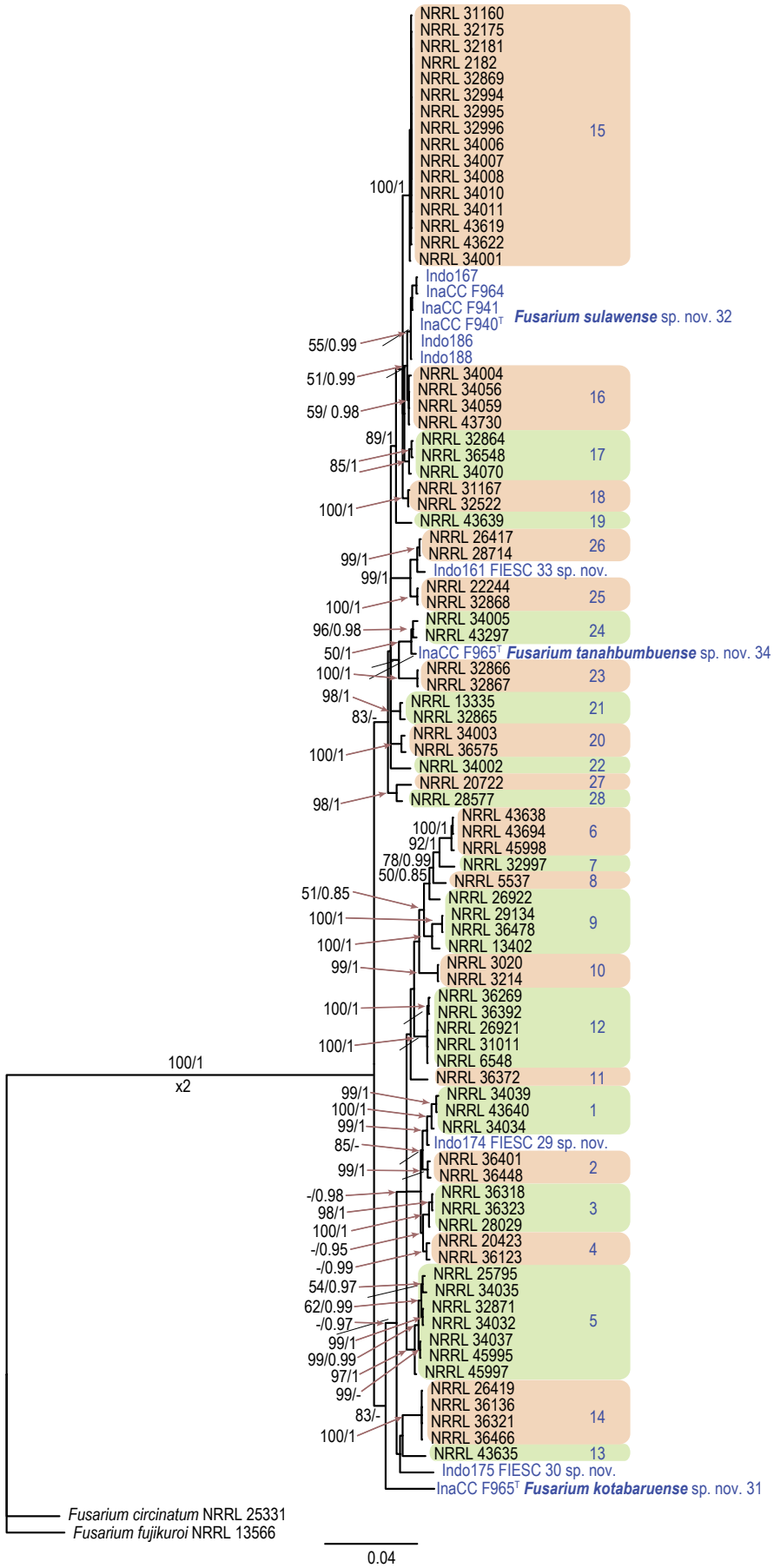

Fig. 3 Maximum likelihood tree inferred from the combined *cmdA*, ITS, *rpb2*, *tef1*, and LSU sequence datasets of the *Fusarium incarnatum-equiseti* species complex (FIESC) including 11 Indonesian isolates (indicated in blue). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium circinatum* (NRRL 25331) and *Fusarium fujikuroi* (NRRL 13566).

analyses lasted until the average standard deviations of split frequencies were below 0.01 with phylogenies saved every 1 000 generations. The first 25 % of saved trees were discarded as the 'burn-in' phase and the 50 % consensus trees and posterior probabilities (PP) were determined from the remaining trees. All the sequences generated in this study were deposited in GenBank and the European Nucleotide Archive (ENA) and the alignments in TreeBASE.

Pathogenicity

Representative isolates from the different *Fusarium* species were selected for pathogenicity assays. *Fusarium odoratissimum*, Tropical Race 4 (TR4) isolate InaCC F856, was used as a positive control, and negative controls were treated with sterile water only. Two to three-month-old banana plants of the Cavendish variety Grand Naine were used in green house controlled conditions (constant day temperature of 25 °C, night temperature of 23 °C, ambient light until max. 16 h, and a relative humidity of ≥ 75 %). Preparation of the fungal inoculum, pathogenicity tests and severity scoring followed the protocol of Maryani et al. (2019). Five plant replicates were included for each isolate tested and 7 wk after inoculation disease severity was evaluated by scoring external foliage and internal corm symptoms.

RESULTS

In total, 20 isolates were identified that did not belong to the *Fusarium oxysporum* species complex (FOSC). These isolates were recovered from 13 banana varieties from the islands of Flores, Java, Kalimantan, and Sulawesi (Table 1). An initial preliminary phylogenetic inference based on *rpb2* sequence data, demonstrated that most isolates belonged to the *Fusarium incarnatum-equiseti* species complex (FIESC, 11 isolates), followed by the *F. fujikuroi* species complex (FFSC, eight isolates), and the *F. sambucinum* species complex (FSSC, one isolate) (Fig. 1). Nine isolates in FIESC originated from Kalimantan, isolated from *Musa* sp. variety Pisang Awak (ABB), Pisang Kepok (ABB), and Pisang Talas (AA) and two isolates from Sulawesi, isolated from *Musa acuminata* var. Pisang Cere (AAA). The majority of the isolates in FFSC were isolated from bananas varieties in Java. The only isolate in the FSSC was isolated from the variety Pisang Awak (ABB) in Central Kalimantan. *Fusarium* isolates belonging to different species complexes were in some cases recovered from the same sample: isolate InaCC F962 in the FFSC and isolate Indo175 in the FIESC were isolated from the same sample of *Musa acuminata* var. Pisang Talas (AA) from South Kalimantan. In the FFSC, isolate InaCC F993 and Indo 213 were also isolated from a sample of *Musa acuminata* var. Pisang Mas Kirana (AA) from East Java. Additionally, different banana varieties were found to be associated with the same *Fusarium* species (Table 1).

Fusarium fujikuroi species complex (FFSC) phylogeny

The eight isolates belonging to the FFSC were further analysed using a multi-gene phylogeny based on *cmdA*, *rpb1*, *rpb2*, *tef1*, and *tub*. The final alignment included 4 795 characters (*cmdA* 545, *rpb1* 1534, *rpb2* 1551, *tef* 677 and *tub* 488) including alignment gaps, and encompassed 54 isolates, with two outgroup taxa (*F. oxysporum* CBS 716.74 and CBS 744.97) (Table 2).

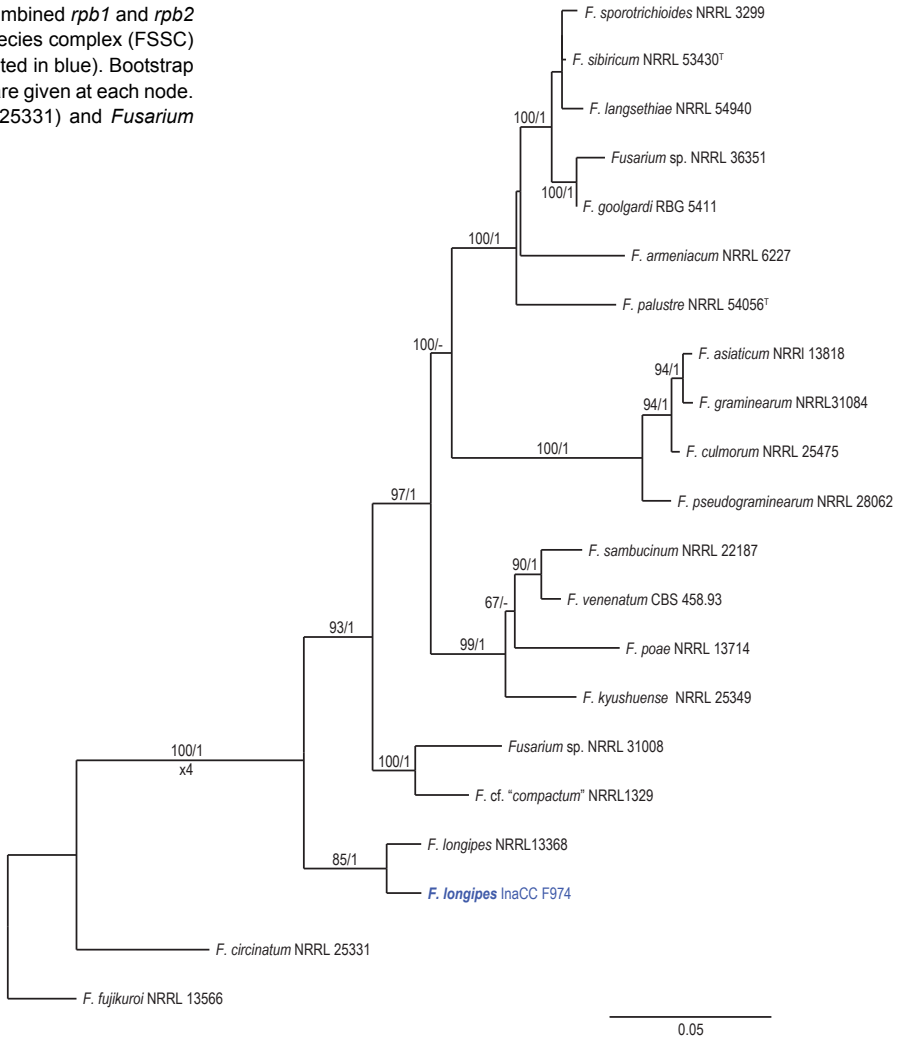

Fig. 4 Maximum likelihood tree inferred from the combined *rpb1* and *rpb2* sequence datasets of the *Fusarium sambucinum* species complex (FSSC) including one Indonesian isolate InaCC F974 (indicated in blue). Bootstrap support values and Bayesian posterior probabilities are given at each node. The tree is rooted to *Fusarium circinatum* (NRRL 25331) and *Fusarium fujikuroi* (NRRL 13566).

The analysis was consistently able to distinguish the three biogeographical clades known as the African, American and Asian clades sensu O'Donnell et al. (1998a). All of the Indonesian isolates clustered within the Asian clade of FFSC except for isolate InaCC F991, identified as *F. verticilloides*, and clustered within the African clade (Fig. 2). According to the multi-gene analysis, two isolates (InaCC F962 and InaCC F992) were identified as *F. proliferatum*, while two new phylogenetic species were recognised among the Indonesian isolates. Isolates InaCC F872 and InaCC F993, from central and East Java, respectively, clustered in a distinct, highly supported clade (96 bs/0.99 pp) closely related to *F. mangiferae*. Isolates InaCC F950–152, formed a distinct group (100 bs/1.0 pp), closely related to, but genetically distinct from *F. sacchari*.

Fusarium incarnatum-equiseti species complex (FIESC) phylogeny

The 11 isolates belonging to the FIESC were assessed using a more inclusive analysis based on five loci (*cmdA*, ITS, LSU, *rpb2* and *tef1*; Fig. 3). The alignment consisted of a total 2 746 characters (*cmdA* 653, ITS 510, LSU 562, *rpb2* 597 and *tef1* 424), from 93 isolates, including all the phylogenetic clades known in this species complex plus two outgroup taxa (*Fusarium circinatum* NRRL 25331 and *F. fujikuroi* NRRL 13566). Multi-gene phylogenetic inference was able to recognise six new phylogenetic species in the FIESC. The number of new phylogenetic species recognised is equally distributed in the incarnatum clade and the equiseti clade (three new phylospecies each) sensu O'Donnell et al. (2009). In the *incarnatum* clade, isolates InaCC F940, InaCC F941, Indo167, InaCC F964, Indo186, and Indo188 clustered in a distinct clade (55 bp/0.99 pp) closely related to the phylogenetic species FIESC-16 which is introduced here as phylogenetic species FIESC-32. These isolates were obtained from five different banana variety hosts in Sulawesi and Kalimantan. The other two new species in the *incarnatum* clade are monotypic lineages represented by isolate Indo161 (99 bp/1 pp) closely related to FIESC-26 and isolate InaCC F965 (50 bp/1 pp) closely related to FIESC-24, introduced as phylogenetic species FIESC-33 and FIESC-34, respectively. In the *equiseti* clade, three isolates: Indo174 (99 bp/1 pp) closely related to FIESC-1; Indo175 (-/1 pp) and InaCC F963 (55 bp/1 pp), both isolates closely related to FIESC-13, formed monotypic lineages which are introduced here as FIESC-29, FIESC-30, and FIESC-31, respectively. These phylogenetic species were isolated from two banana varieties in relatively close proximity in South Kalimantan.

Fusarium sambucinum species complex (FSSC) phylogeny

The single Indonesian isolate in the FSSC was further analysed using a two-gene phylogeny based on *rpb1* and *rpb2* sequences. The analysis included a total of 2 461 characters (*rpb1* 854 and *rpb2* 1607) from a total of 21 isolates representing the FSSC and two outgroup taxa (*F. circinatum* NRRL 25331 and *F. fujikuroi* NRRL 13566). Isolate InaCC F974 was identified as *F. longipes* (Fig. 4) based on phylogenetic inference.

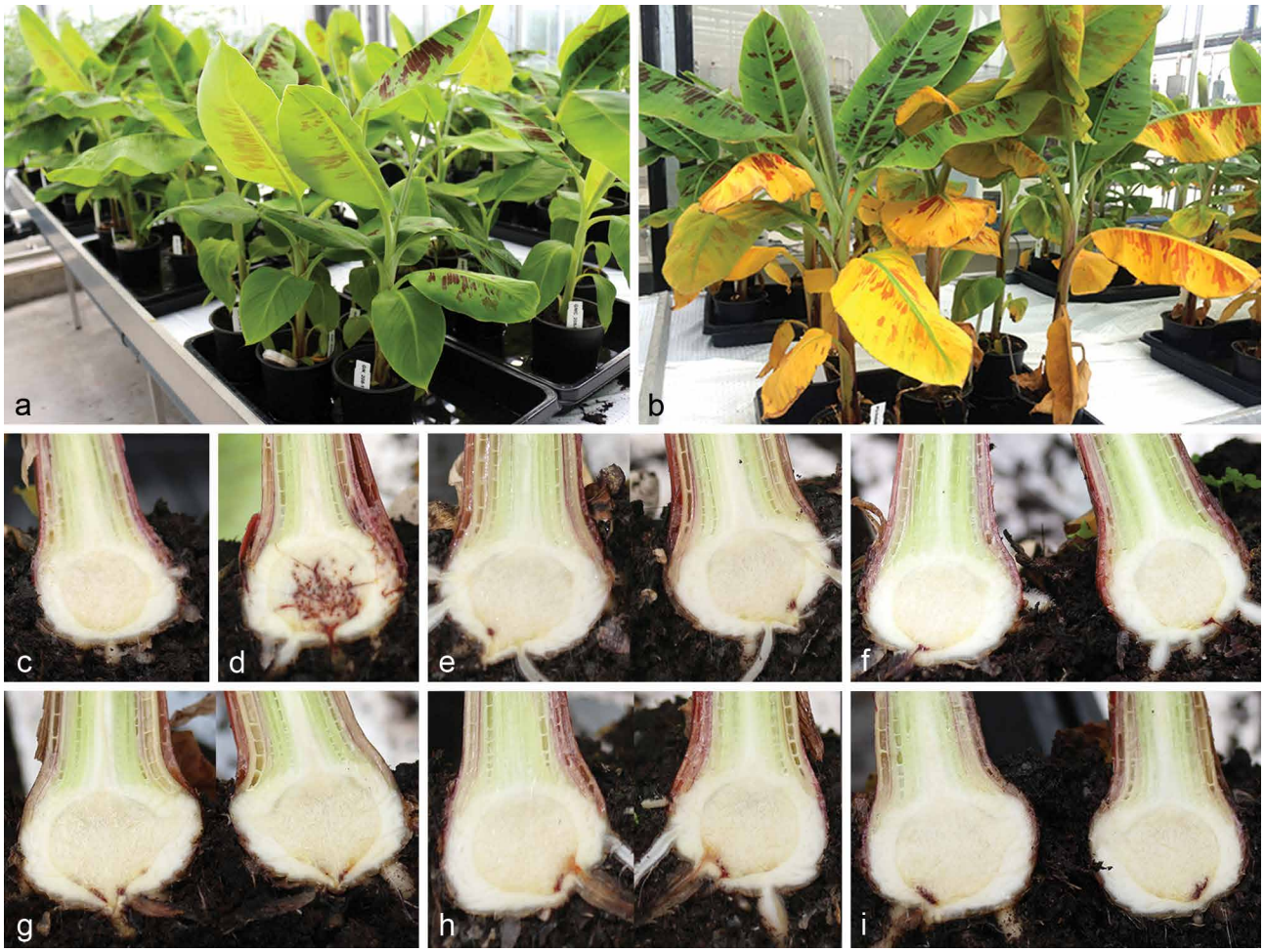

Fig. 5 Pathogenicity test of *Fusarium* spp. that belong to other species complexes. a. Plants before inoculation; b. wilting symptom caused by *Fusarium odoratissimum* InaCC F856, seven weeks after inoculation; c. control; d. positive control *Fusarium odoratissimum* (InaCC F856); e. *Fusarium proliferatum* (InaCC F992); f. *Fusarium desaboruense* (InaCC F950); g. *Fusarium lumajangense* (InaCC F872^{T}); h. *Fusarium longipes* (InaCC F974); i. FIESC (Indo161); j. *Fusarium lumajangense* (InaCC F993).

Pathogenicity

Representative isolates from each species complex were tested for their pathogenicity against banana variety Cavendish (Fig. 5). Selected isolates included InaCC F872, InaCC F950, and InaCC F992 (FFSC), InaCC F962 (FIESC), InaCC F974 (FSSC). None of the isolates was able to cause any disease symptoms in the inoculated plants. All of the isolates tested caused only slight discoloration in the corm without any further disease development.

Taxonomy

The *Fusarium* species in each complex and novel species identified in this study are described below.

Fusarium lumajangense N. Maryani, Sand.-Den., L. Lombard, Kema & Crous, *sp. nov.* — MycoBank MB828960; Fig. 6

Etymology. Name refers to Lumajang, the region from where this species was collected in Indonesia.

Typus. Indonesia, Desa Kandang Kepus, Kecamatan Senduro, Lumajang, East Java (E113°4'157" S8°4'46"), in infected pseudostem of *Musa acuminata* var. Pisang Mas Kirana (AA), 17 July 2014, *N. Maryani* (holotype specimen and culture, InaCC F872, preserved in metabolically inactive state).

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium, septate, branching profusely, irregularly or sympodially or reduced to solitary conidiogenous cells formed laterally on aerial hyphae; *conidiogenous cells* mono- or polyphialidic, acute, subulate or subcylindrical, smooth- and thin-walled (6–)10–22.5(–31.5) × 2–3(–4) µm, formed terminally and singly on conidiophores or intercalary, often proliferating percurrently; periclinal thickening inconspicuous or absent; *conidia* of two types: a) (microconidia) ovoid to ellipsoid, smooth- and thin-walled, (6–)9–18(–23) × (2–)3(–5) µm (av. 13 × 4 µm), 0–1-septate, arranged in false heads on monophialides; and b) (macroconidia) falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, (1–2–)3-septate, formed on polyphialides; 1-septate conidia 18.5 × 3.5 µm; 2-septate conidia 40 × 4 µm; 3-septate conidia (26–)29–39.5(–44.5) × (3–)3.5–4.5(–5.5) µm; av. (18.5–)28–39.5(–44.5) × (3–)3.5–4.5(–5.5) µm. *Sporodochia* formed abundantly on surface of carnation leaves after 7 d, pale orange to orange. *Conidiophores* on sporodochia, septate, mostly unbranched or rarely sparsely and irregularly branched, bearing terminal monophialides, carried singly or grouped in verticillately branched; *conidiogenous cells* monophialidic, ampulliform, doliiform to subcylindrical, smooth- and thin-walled, (11.5–)12.5–18.5(–23.5) × (2–)3–4(–4.5) µm, proliferating percurrently several times, with short collarets and inconspicuous periclinal thickening; *sporodochial conidia* falcate, apical cells gently curved, papillate, basal cells slightly curved, foot-shaped, 3–5-septate: 3-septate conidia, (30–)34.5–46.5(–54) × 3.5–4.5 µm; 4-septate conidia, 41–48(–52.5) × (3–)3.5–4.5 µm; 5-septate conidia, (42.5–)45–53(–56) × 3.5–4.5 µm; av. (30–)40–50.5(–56) × (3–)3.5–4(–4.5) µm. *Chlamydospores* not observed.

Culture characteristics — Colony on PDA showing optimal growth at 25 °C with an average growth rate of 3.5–4.6 mm/d. Colony reverse, lilac to violet becoming white towards the margin, later becoming dark purple with time. Colony surface dry, white becoming livid purple towards the margin, turning completely purple with age. Aerial mycelium abundant, cottony, with moderate sporulation and lacking exudates.

Geography & Host — Lumajang, East Java, *Musa acuminata*. var. Pisang Mas Kirana (AA).

Pathogenicity — Non-pathogenic on Cavendish (AAA).

Additional material examined. Indonesia, Desa Kandang Kepus, Kecamatan Senduro, Lumajang, East Java (E113°4'157" S8°4'46"), in infected pseudostem of *Musa acuminata* var. Pisang Mas Kirana (AA), 17 July 2014, *N. Maryani* (InaCC F993).

Notes — *Fusarium lumajangense* exhibits similar morphological features to *F. mangiferae* (Britz et al. 2002), also clustering in a sister relationship with the latter species. However, besides its clear phylogenetic delimitation, the polyphialides found in *F. lumajangense* commonly present two conidiogenous loci.

Fusarium desaboruense N. Maryani, Sand.-Den., L. Lombard, Kema & Crous, *sp. nov.* — MycoBank MB828961; Fig. 7

Etymology. Name refers to Desa Boru, the village from where this species was collected in Indonesia.

Typus. Indonesia, Desa Boru, Kecamatan Waigate, Sikka Flores, East Nusa Tenggara (E122°22'7" S8°36'49"), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 17 Aug. 2015, *N. Maryani* (holotype specimen and culture, InaCC F951, preserved in metabolically inactive state).

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA and SNA, less frequent on CLA, septate, sparingly or profusely branching irregularly or sympodially, rarely reduced to solitary conidiogenous cells, formed laterally on aerial hyphae; *conidiogenous cells* mono- or polyphialidic, acute, subulate or subcylindrical, smooth- and thin-walled (6–)15–33(–44) × (2–)2.5–4(–7) µm (av. 21.5 × 3 µm), formed terminally, singly or in whorls on conidiophores or intercalary, proliferating percurrently, periclinal thickening inconspicuous or absent; *conidia* of two types: a) (microconidia) ovoid to ellipsoid, smooth- and thin-walled, (10–)11–16(–18) × (4–)6(–7) µm (av. 13 × 5 µm), 0–1-septate, arranged in false heads on monophialides; and b) (macroconidia) falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, 1–3-septate, formed on polyphialides: 1-septate conidia 22.5–26(–27) × 3.4–4 µm; 2-septate conidia (21.5–)22–26 × 3–4.5 µm; 3-septate conidia (23–)24.5–34(–37) × 3–4.5 µm; av. (21.5–)22–30.5(–37) × 3–4.5 µm. *Sporodochia* formed abundantly on CLA after 7 d, pale orange to orange. *Conidiophores* in sporodochia unbranched, rarely laterally branched up to two times; *conidiogenous cells* monophialidic, smooth- and thin-walled (15.5–)16.5–24(–29) × (2.5–)3–4 µm (av. 20 × 3.5 µm), solitary, terminal or lateral, or in terminal groups of up to three conidiogenous cells, with minute collarettes and periclinal thickening; *sporodochial conidia* falcate, apical cells gently curved, papillate, basal cells gently curved, foot-shaped, 1–3(–4)-septate: 1-septate conidia (14.5–)15–20.5(–22) × 3.5–4.5 µm; 2-septate conidia (20.5–)21.5–24 × 3.5–4.5(–5) µm; 3-septate conidia (21–)24–29(–31.5) × (3.5–)4–5(–5.5) µm; 4-septate conidia 34 × 5.5 µm; av. (14.5–)20–28(–34.5) × (3.5–)4–5(–5.5) µm. *Chlamydospores* not observed.

Culture characteristics — Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.9–5.2 mm/d. Colony reverse, pale violet becoming white towards the margins, turning violet with age and pigmented. Colony surface cottony, pale violet, becoming white with age, immersed mycelium becoming purple and lacking exudates. Aerial mycelium abundant, cottony, with abundant sporulation.

Geography & Host — Sikka Flores, East Nusa Tenggara, *Musa* sp. var. Pisang Kepok (ABB).

Pathogenicity — Not pathogenic on Cavendish (AAA).

Additional materials examined. Indonesia, Desa Boru, Kecamatan Waigate, Sikka Flores, East Nusa Tenggara (E122°22'7" S8°36'49"), on infected pseudostem of *Musa* sp. var. Pisang Kepok (ABB), 17 Aug. 2015, *N. Maryani* (InaCC F950, InaCC F952).

Notes — Morphologically very similar to *F. sacchari* (Leslie & Summerell 2006) and *F. subglutinans* (Nelson et al. 1983),

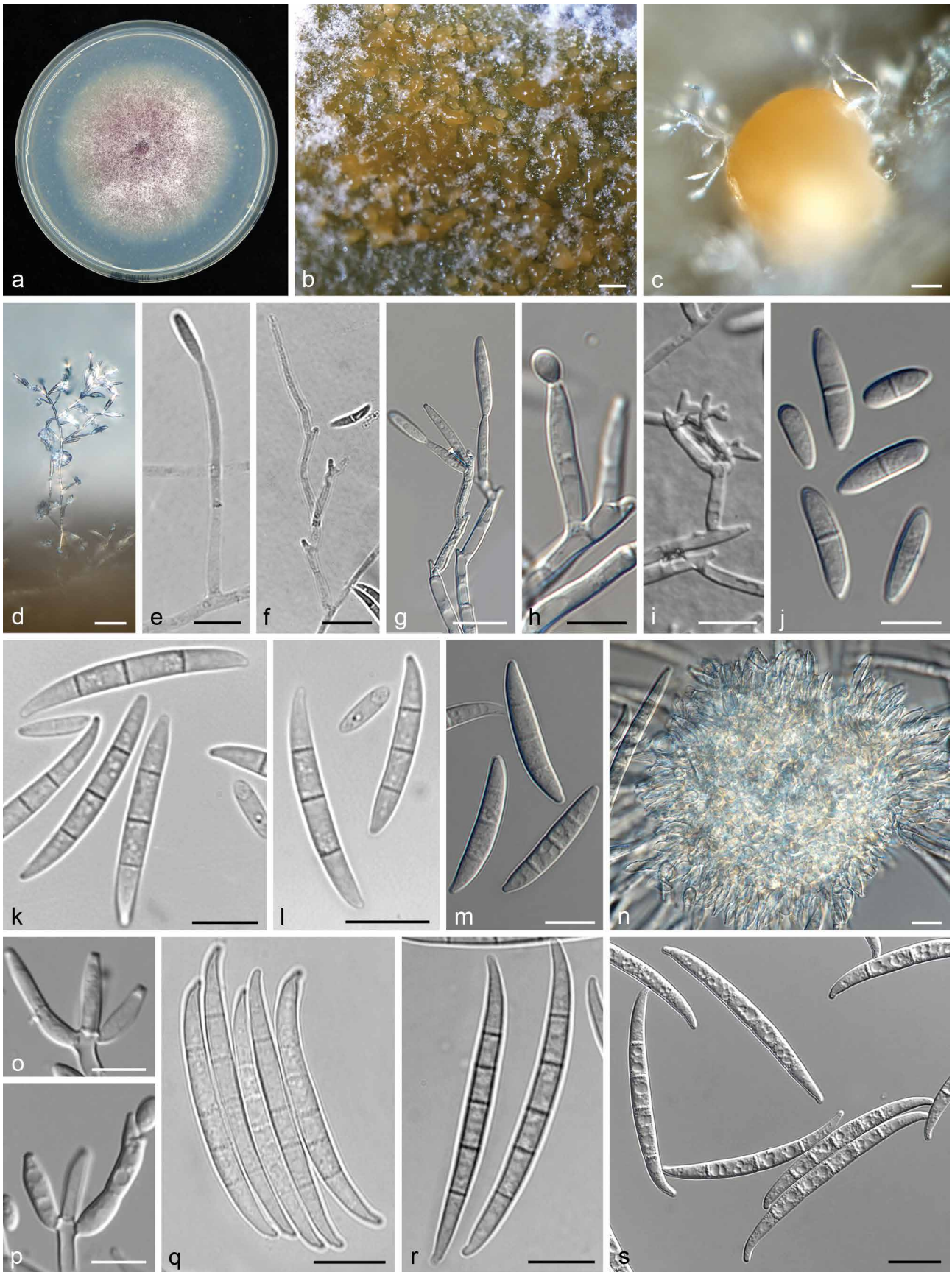

Fig. 6 *Fusarium lumajangense* (ex-type InaCC F993). a. Culture grown on PDA; b–c. sporodochia on carnation leaves; d–i. aerial conidiophores and phialides; j–m. aerial conidia; n–p. sporodochial conidiophores and phialides; q–s. sporodochial conidia. — Scale bars: b–d = 50 µm; e = 5 µm; f–s = 10 µm.

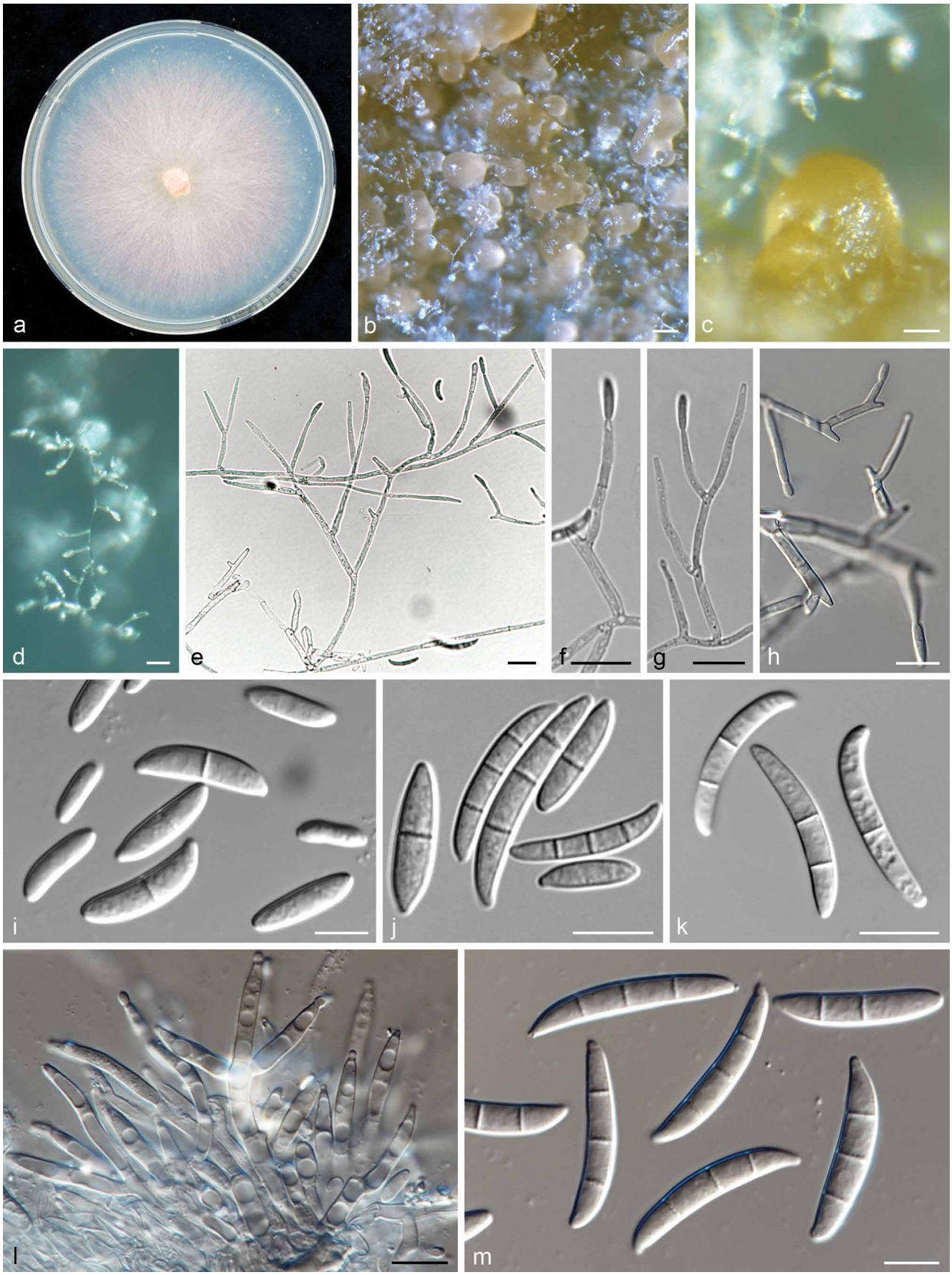

Fig. 7 *Fusarium desaboruense* (ex-type InaCC F950). a. Culture grown on PDA; b–c. sporodochia on carnation leaves; d–h. aerial conidiophores and conidiogenous cells; i–k. aerial conidia; l. sporodochial conidiophores and phialides; m. sporodochial conidia. — Scale bars: b–d = 20 µm; e–m = 10 µm.

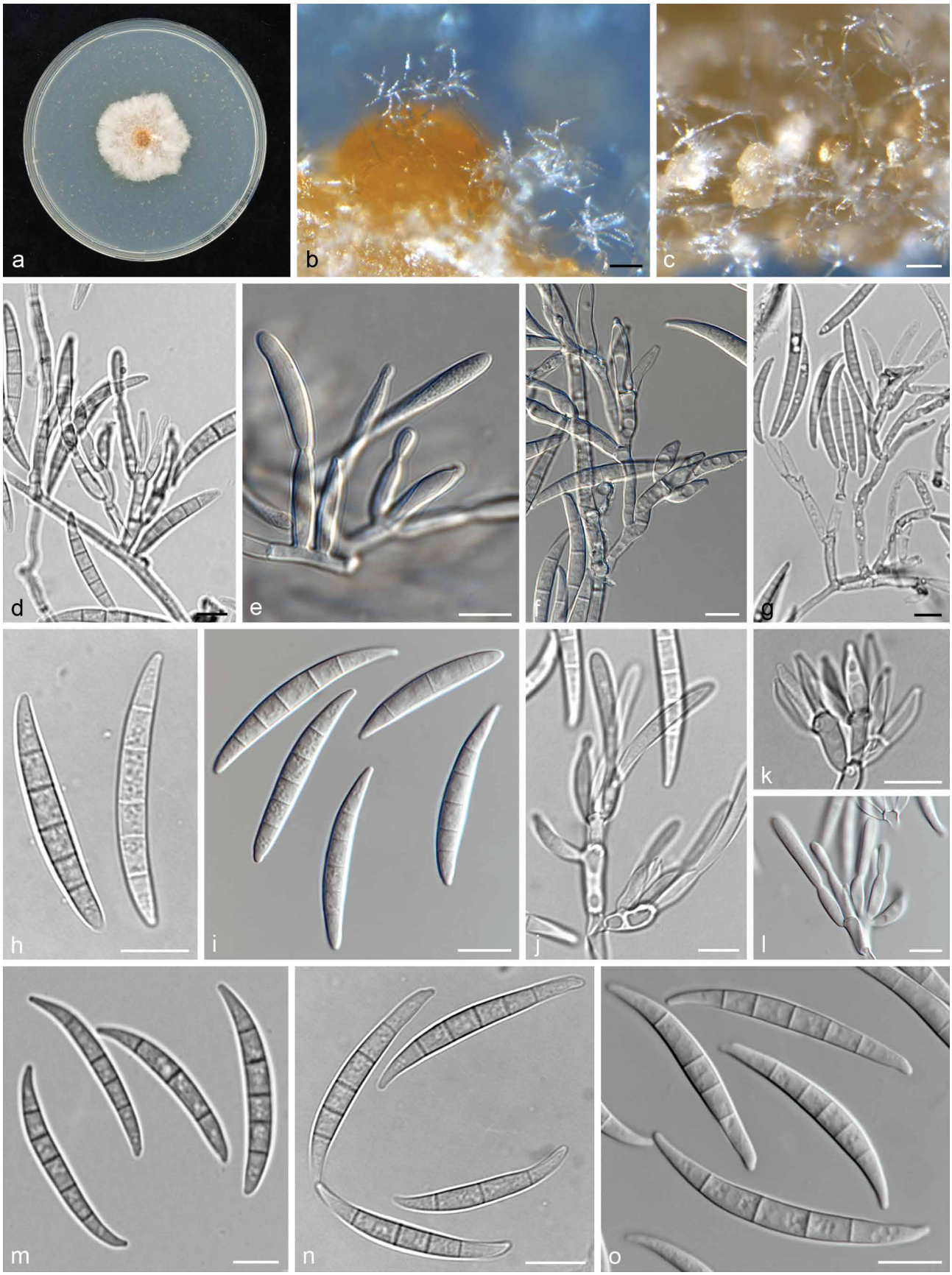

Fig. 8 *Fusarium tanahbumbuense* (ex-type InaCC F965). a. Culture grown on PDA; b–c. sporodochia on carnation leaves; d–g. aerial conidiophores and conidiogenous cells; h–i. aerial conidia; j–l. sporodochial conidiophores and conidiogenous cells; m–o. sporodochial conidia. — Scale bars: b–c = 50 µm; d–o = 10 µm.

except that this species produces sporodochia abundantly under regular culturing conditions. *Fusarium desaboruense* can be distinguished by the septation of its macroconidia (1–4-septate) and microconidia (1–3-septate), not observed in *F. saccari* (Leslie & Summerell 2006). Phylogenetic analyses of partial *rpb2* gene sequences recognised this species as distinct from *F. sacchari* with strong support of BP 99 %.

Fusarium tanahbumbuense N. Maryani, Sand.-Den., L. Lombard, Kema & Crous, *sp. nov.* — MycoBank MB828962; Fig. 8

Etymology. Name refers to Tanah Bumbu, the region from where this species was collected in Indonesia.

Typus. Indonesia, Desa Betung, Kecamatan Kusan Hilir, Tanah Bumbu, Kalimantan Selatan (E115°37'477" S3°50'77"), on infected pseudostem of *Musa* sp. var. Pisang Hawa (ABB), 20 June 2014, *N. Maryani* (holotype specimen and culture, InaCC F965, preserved in metabolically inactive state).

Sporulation abundant from conidiophores borne on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA, SNA, and CLA, septate, irregularly of verticillately branched; conidiogenous cells monophialidic or polyphialidic, subulate or subcylindrical, smooth- and thin-walled, (11–)13–24(–38) × (4–)5–6(–7) µm (av. 19 × 6 µm), formed terminally, singly or in groups of up to three cells on a stipe, or carried singly and laterally on aerial mycelium, collarettes and periclinal thickening inconspicuous or absent; *conidia* of one type (macroconidia) falcate and multiseptate, apical cells conical to papillate, basal cells indistinct or foot-shaped, 3–5-septate, formed on both mono- and polyphialides, 3-septate conidia, 31–36(–38.5) × 3.5–5(–5.5) µm; 4-septate conidia, (31–)33.5–43.5(–48) × 3.5–5(–5.5) µm; 5-septate conidia, (30–)37–45(–47) × 4–5.5(–6) µm; av. (30–)34.5–44(–48) × (3.5–)4–5.5(–6) µm. *Sporodochia* formed abundantly on CLA after 7 d, pale orange; *conidiophores* in sporodochia irregularly

Fig. 9 *Fusarium sulawense* (ex-type InaCC F964). a. Culture grown on PDA; b–c. sporodochia on carnation leaves; d–h. aerial conidiophores and conidiogenous cells; i. aerial conidia; j–k. sporodochial conidiophores and conidiogenous cells; l–m. sporodochial conidia. — Scale bars: b–c = 50 µm; d–g, i–m = 10 µm; h = 5 µm.

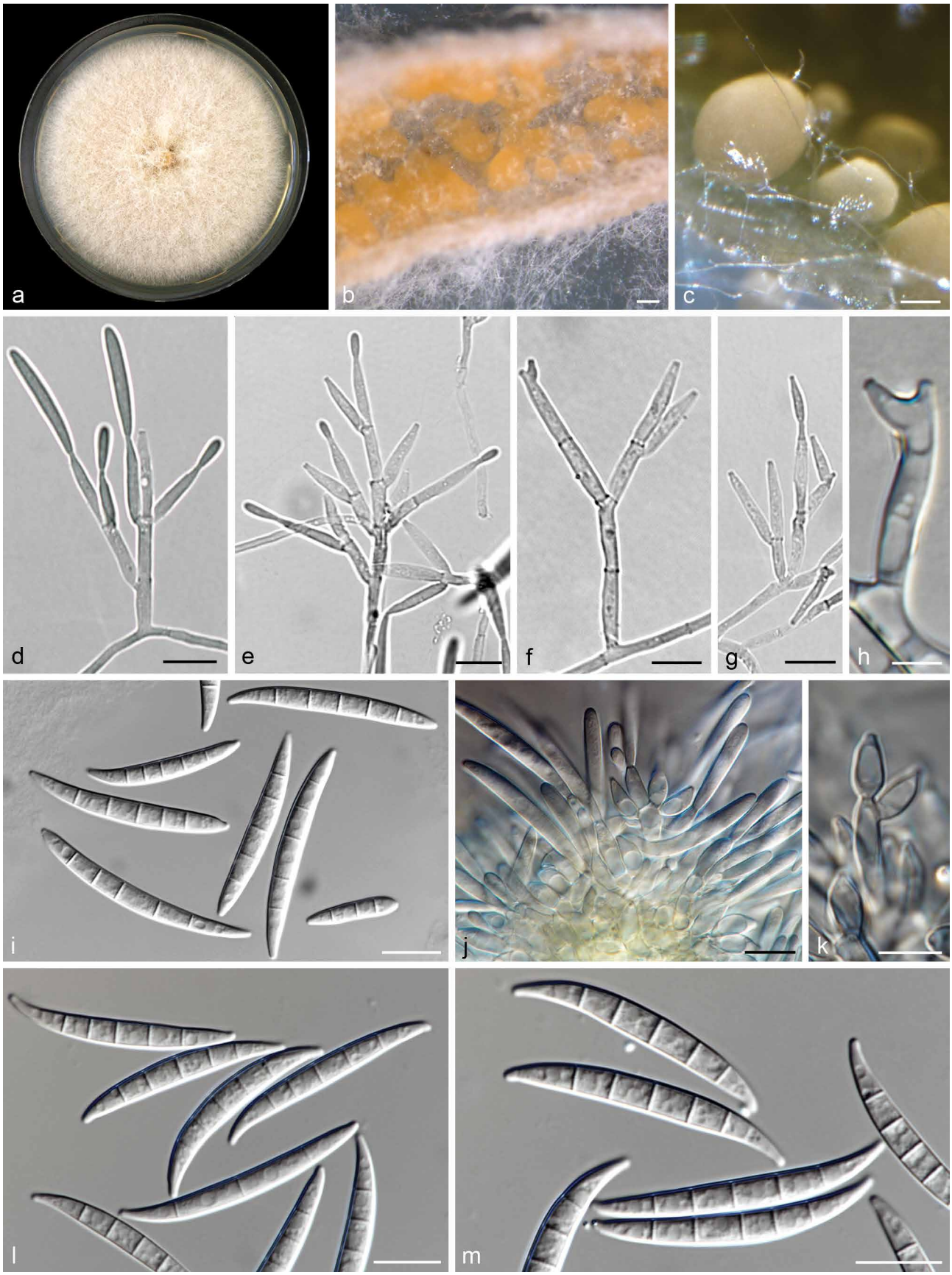

Fig. 10 *Fusarium kotabaruense* (ex-type InaCC F963). a. Culture grown on PDA; b. mycelium on carnation leaves; c–h. conidiophores and conidiogenous cells; i–k. conidia. — Scale bars: b = 200 µm; c–d = 50 µm; e–f, h–k = 10 µm; g = 5 µm.

and laterally branched; *conidiogenous cells* monophialidic, doliiform to ampulliform, smooth- and thin-walled, (9.5–)10–13(–15) × (2.5–)3–4 µm (av. 11.5 × 3.5 µm), collarettes or periclinal thickening inconspicuous or absent; *sporodochial conidia* falcate, apical cells gently curved, papillate; basal cells slightly curved, foot-shaped, (2–)3–5-septate: 2-septate conidia, 40.5 × 4.5 µm; 3-septate conidia, (25.5–)29–36.5(–41) × 3.5–4.5 µm; 4-septate conidia, (32.5–)34–40(–46) × 3.5–4.5(–5) µm; 5-septate conidia, (36–)37–43.5(–49) × 3.5–4.5(–5) µm; av. (25.5–)32–41.5(–49) × 3.5–5 µm. *Chlamydospores* not observed.

Culture characteristics — Colony on PDA showing optimal growth at 25 °C with an average growth rate of 1.3–2.2 mm/d. Colony reverse, rosy buff becoming white towards the margins, turning cinnamon to fawn with age and pigmented. Colony surface cottony, rosy buff becoming white towards the margin, turning hazel with age. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates.

Geography & Host — Tanah Bumbu, South Kalimantan, *Musa* sp. var. Pisang Hawa (ABB).

Pathogenicity — NA.

Notes — *Fusarium tanahbumbuense* can be distinguished from the fungus illustrated as *F. semitectum* by Leslie & Summerell (2006) and Nelson et al. (1983) by the absence of microconidia and chlamydospores. The polyphialides observed for this species also greatly differed from those that have been observed for *F. semitectum* which have 3–5 openings (Nelson et al. 1983).

Fusarium sulawense N. Maryani, Sand.-Den., L. Lombard, Kema & Crous, *sp. nov.* — MycoBank MB828963; Fig. 9

Etymology. Name refers to Sulawesi, the island from where this species was collected in Indonesia.

Typus. Indonesia, Desa Seli, Kecamatan Bengo, Bone, Sulawesi Selatan (E120°1'12.8" S4°37'26"), on infected pseudostem of *Musa acuminata* var. Pisang Cere (AAA), 12 Aug. 2015, *N. Maryani* (holotype specimen and culture, InaCC F940, preserved in metabolically inactive state).

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA and SNA, less frequent on CLA, septate, irregularly or verticillately branched; *conidiogenous cells* mono- or polyphialidic, subulate to subcylindrical, smooth- and thin-walled, (8.5–)14–22.5(–27) × (2–)2.5–4(–4.5) µm (av. 18 × 3 µm), formed singly, laterally or terminally, or more often in groups of 2–3 cells, sometimes proliferating percurrently, collarettes and periclinal thickening inconspicuous or absent; *conidia* of one type (macroconidia), falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, 3–5(–9)-septate, formed on both mono- and polyphialides, 3-septate conidia, 20.5–47.5(–55) × 3.5–5 µm; 5-septate conidia, (33.5–)39.5–48(–50.5) × (4–)4.5–5.5 µm; 6-septate conidia, 51.5 × 6 µm; 9-septate conidia, 67 × 5.5 µm; av. (20.5–)36–51(–67.5) × (3.5–)4–5.5(–6) µm. *Sporodochia* formed rarely on CLA after 7 d, pale orange; *conidiophores* in sporodochia unbranched or irregularly branched, densely packed, bearing terminal clusters of 2–5 conidiogenous cells; *conidiogenous cells* monophialidic, short ampulliform, smooth- and thin-walled, (8.5–)9–11.5(–13) × (3–)3.5–5(–5.5) µm (av. 10.5 × 4.5 µm) with a minute collarette and inconspicuous periclinal thickening; *sporodochial conidia* falcate, apical cells gently curved, papillate; basal cells slightly curved, foot-shaped, (3–)5(–6)-septate: 3-septate conidia, (29.5–)30–44 × 4–4.5 µm; 4-septate conidia, 30 × 5.5 µm; 5-septate conidia, (30–)36–41.5(–43.5) × (3.5–)4–5(–5.5) µm; 6-septate conidia 43.5 × 5 µm; av. (30–)36–41.5(–44) × (3.5–)4–5(–5.5) µm. *Chlamydospores* not observed.

Culture characteristics — Colony on PDA showing optimal growth at 25 °C with an average growth rate of 5.2–6.0 mm/d. Colony reverse rosy buff becoming white towards the margins. Colony surface dry, cottony, saffron. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates.

Geography & Host — Bone, South Sulawesi, *Musa acuminata* var. Pisang Cere (AAA).

Pathogenicity — Non-pathogenic on Cavendish (AAA).

Additional material examined. Indonesia, Desa Sungai Birah, Kecamatan Pamukan Barat, Kota Baru, Kalimantan Selatan (E115°59'982" S2°22'883"), on infected pseudostem of *Musa* var. Pisang Hawa (ABB), 19 June 2014, *N. Maryani* (InaCC F964).

Notes — *Fusarium sulawense* is relatively fast growing (av. 5.2–6.0 mm/d) compared to its sister species in the Incarnatum clade, FIESC-34 (av. 1.3–2.2 mm/d). Members of this species were recovered from different banana varieties in the Kalimantan and Sulawesi islands of Indonesia.

Fusarium kotabaruense N. Maryani, Sand.-Den., L. Lombard, Kema & Crous, *sp. nov.* — MycoBank MB828964; Fig. 10

Etymology. Name refers to Kota Baru one of the nine regencies in the Indonesian province of South Kalimantan.

Typus. Indonesia, Desa Sungai Birah, Kecamatan Pamukan Barat, Kota Baru, Kalimantan Selatan (E115°59'982" S2°22'883"), on infected pseudostem of *Musa* var. Pisang Hawa (ABB), 19 June 2014, *N. Maryani* (holotype specimen and culture, InaCC F963, preserved in metabolically inactive state).

Sporulation abundant from conidiophores carried on aerial mycelium. *Conidiophores* on aerial mycelium abundant on PDA and SNA, less frequent on CLA, septate, irregularly branching; *conidiogenous cells* mono- or polyphialidic, subulate to subcylindrical, smooth- and thin-walled, (15–)19–33(–40) × 4–7 µm (av. 26 × 5 µm), forming terminally, singly or in verticillately branched conidiophores, less commonly laterally or intercalary, proliferating percurrently, periclinal thickening inconspicuous or absent; falcate and multiseptate, apical cells papillate, basal cells indistinct or foot-shaped, (2–)3–5(–7)-septate, formed on both mono- and polyphialides: 2-septate conidia, (21–)21.5–25(–26) × 5–6 µm; 3-septate conidia, (24.5–)28–35(–36.5) × 5.5–6.5(–7) µm; 4-septate conidia, (32–)34–39.5(–41.5) × 5.5–6.5(–7) µm; 5-septate conidia, (34.5–)36–42.5(–45) × (5–)5.5–6.5(–7.5) µm; 6-septate conidia, 39–40.5 × 5.5–7 µm; 7-septate conidia, (38.5–)39.5–44(–45) × 6–7 µm; av. (21–)31.5–41.5(–45) × (5–)5.5–6.5(–7.5) µm. *Sporodochia* and *chlamydospores* not observed.

Culture characteristics — Colony on PDA showing optimal growth at 25 °C with an average growth rate of 5.0–6.85 mm/d. Colony reverse rosy buff. Colony surface cottony rosy buff. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates.

Geography & Host — Kota Baru, South Kalimantan, *Musa* sp. var. Pisang Hawa (ABB).

Pathogenicity — Non-pathogenic on Cavendish (AAA).

Notes — *Fusarium kotabaruense* represents a species in the Equiseti clade of the FIESC and relatively fast growing (5.0–6.85 mm/d). Most distinguishing characteristic of this species is the absence of sporodochia on CLA culture. However, aerial conidiophores are abundant with conidia produced with high variability in its septation, (0–)3–5(–7)-septate.

Fusarium longipes Wollenw. & Reinking, Phytopathology 15: 160. 1925 — Fig. 11

Sporulation abundant from conidiophores carried on aerial mycelium and from sporodochia. *Conidiophores* on aerial mycelium abundant on PDA and SNA, rare on CLA, septate, branching irregularly, mostly reduced to solitary conidiogenous cells

formed singly and laterally on aerial hyphae; *conidiogenous cells* monophialidic, doliiform to ampulliform, smooth- and thin-walled, (7–)10–13(–15) × 3–4(–5) µm (av. 12 × 6 µm), formed laterally on aerial hyphae or clustering terminally on conidiophores, with a minute collarette; *conidia* (microconidia) obovoid to ellipsoid, rough- and thin-walled, (7–)10–19(–23) × (3–)4(–5) µm (av. 15 × 4 µm), 0–2-septate, arranged in false heads on monophialides. *Sporodochia* formed abundantly on CLA after 7 d, bright orange, later turning red to purple; *conidiophores* in sporodochia highly irregularly or verticillately branched, sympodially to solitary conidiogenous cells; *conidiogenous cells* monophialidic, doliiform, ampulliform to subcylindrical, 7–11(–14) × (2–)2.5–3.5(–4) µm (av. 9.5 × 3 µm), with inconspicuous collarets; *sporodochial conidia* falcate, apical cells strongly curved, tapering and whip-like with rounded apex, basal cells foot-shaped and elongated, (3–)4–5-septate: 3-septate conidia, 28.5 × 3.5 µm; 4-septate conidia, (37–)38–43(–43.5) × 4.5–5.5 µm; 5-septate conidia, (37–)42–49.5(–53.5) × (3.5–)4.5–5(–6) µm; av. (28.5–)40.5–49.5(–53.5) × (3–)4–5(–6) µm. *Chlamydospores* ellipsoid, sub-globose to globose, formed intercalary or terminal, single or in pairs, or in clumps, (7–)10–13(–15) × (7–)9–13(–14) µm (av. 12 × 11 µm), brown, rough-walled.

Culture characteristics — Colony on PDA showing optimal growth at 25 °C with an average growth rate of 4.2–4.9 mm/d. Colony reverse livid red becoming white towards the margin, becoming completely livid red to bay with age. Colony surface cottony greyish rose becoming vinaceous with age and white toward the margins. Aerial mycelium abundant, cottony, with high sporulation and lacking exudates. Sporodochia formed abundantly on CLA after 7 d, pale orange to orange.

Geography & Host — Katingan, Central Kalimantan, *Musa* sp. var. Pisang Awak (ABB).

Pathogenicity — Non-pathogenic on Cavendish (AAA).

Material examined. Indonesia, Desa Tewang Menyangen, T. Sangalang, Katingan, Central Kalimantan (E113°6'552" S1°41'83"), on infected pseudostem of *Musa* var. Pisang Awak (ABB), 23 June 2014, *N. Maryani* (specimen and culture, InaCC F974, preserved in metabolically inactive state).

Notes — This banana isolate of *F. longipes* displays some unique characteristics which differ slightly from *F. longipes vide* Leslie & Summerell (2006), which include the presence of

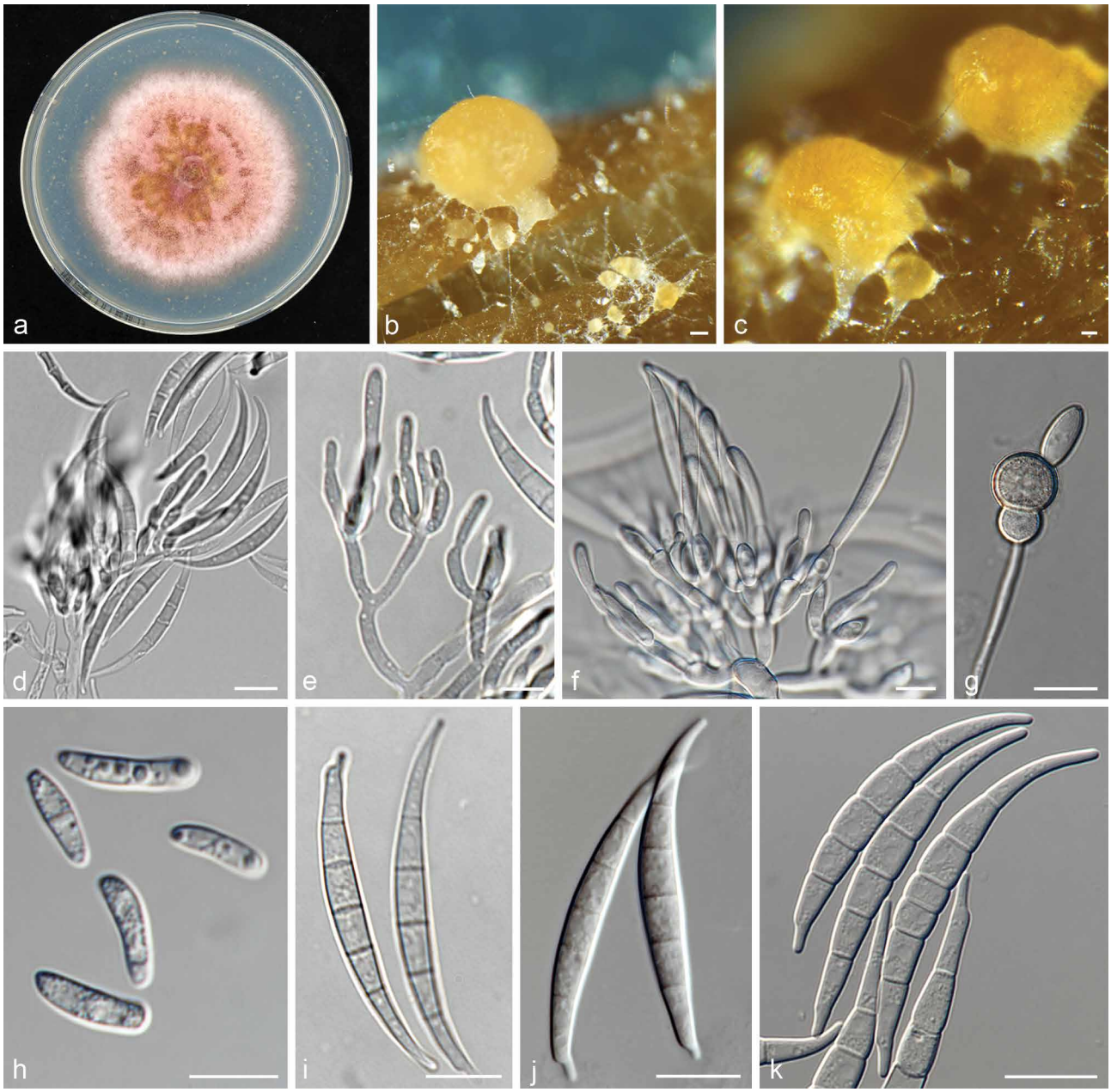

Fig. 11 *Fusarium longipes* (InaCC F974). a. Culture grown on PDA; b–c. sporodochia on carnation leaves; d. sporodochial conidiophores; e–f. branched conidiophores; g. falcate-shaped macroconidia; h. microconidia; i. chlamydospores. — Scale bars: b–k = 10 µm.

microconidia and chlamydospores. This species is more similar to *F. equiseti* as described by Nelson et al. (1983), except for the length of the long curvature of the macroconidia. Additionally, the chlamydospore formation also differs from the original description of *F. longipes*.

DISCUSSION

This study further expands our knowledge on the diversity of *Fusarium* species isolated from banana plants displaying symptoms of Fusarium wilt in Indonesia, the centre of origin for this economically important crop. It is not surprising that 90 % of the isolates recovered from the samples were members of FOSC, as the diseased pseudostem of banana served as source of isolation (Maryani et al. 2019). However, the remaining isolates were tentatively identified as members of other *Fusarium* species complexes, which included the FIESC, FSSC, and FFSC. Remarkably, only *Fusarium* species were isolated, while no other fungal genera could be recovered from the banana samples. This indicates a marked dominance of *Fusarium* in diseased banana plants. It is well known that *Fusarium* is commonly associated with higher plants, being ubiquitous in terrestrial ecosystems, especially in the tropics, where most diseases on perennial crops are induced by this genus (Ploetz 2006b). It has also been suggested that for any *Fusarium* associated disease found in plants, many other *Fusarium* species also reside in the same host as endophytes (Leslie & Summerell 2006). Moreover, the samples were collected from locations in Indonesia where bananas are grown in mixed backyard ecosystems with other tropical crops (Maryani et al. 2019). This ecological niche enhanced the chance that a much higher diversity of *Fusarium* species would be discovered than expected.

We were able to identify a total of 20 isolates collected from pseudostems of banana plants displaying symptoms of Fusarium wilt that did not belong to FOSC. These isolates were found to belong to three different *Fusarium* species complexes of which eight represented novel phylogenetic species in the FFSC and FIESC. Information regarding *Fusarium* spp. other than *F. oxysporum* in banana is scarce, since the majority of studies point to the specific detection and control of pathogenic strain of *F. oxysporum* (O'Donnell et al. 1998b, Ordonez et al. 2015, Ploetz et al. 2015, Maryani et al. 2019). However, some studies have reported an abundance of *Fusarium* species in asymptomatic banana plant organs. Zakaria & Rahman (2011) identified *F. oxysporum*, *F. semitectum* and *F. solani* (current name *Neocosmospora solani*) in healthy roots of wild banana plants (*Musa acuminata*) in Malaysia and *Fusarium concentricum* was reported in *Musa sapientum* from Costa Rica (Nirenberg & O'Donnell 1998). Moreover, a higher diversity of *Fusarium* species has been reported from banana fruits, which included *F. chlamydosporum*, *F. equiseti*, *F. proliferatum*, *F. sacchari*, *F. subglutinans*, and *F. verticilloides* (Jimenez et al. 1993, Moretti et al. 2004, Zheng et al. 2012). Two of these species, *F. proliferatum* and *F. verticilloides*, were also found in this study.

Pathogenicity tests demonstrated that the Indonesian isolates were not pathogenic on the Cavendish banana variety Grand Naine. Moreover, our results indicate that these species more likely play an endophytic role, which is consistent with previous knowledge on asymptomatic/healthy banana plants (Zakaria & Rahman 2011). A similar case has been reported on vanilla stem rot disease in Indonesia. Pinaria et al. (2010) isolated 12 *Fusarium* species from symptomatic vanilla stems. Pathogenicity tests indicated that none of these caused any disease on vanilla plants, with the exception of *F. oxysporum* f. sp. *vanillae*. In another study, *F. oxysporum* f. sp. *vasinfectum* was found to be the only species that caused Fusarium wilt of cotton amongst 20 *Fusarium* species isolated from wild *Gossypium* in Australia (Wang et al. 2004).

The highest diversity of isolates obtained in this study belonged to the FIESC. This species complex displays a remarkable abundance of phylogenetic species diversity which include both animal and plant associated pathogens, plant endophytes and soil inhabitants (Leslie & Summerell 2006, O'Donnell et al. 2009, Villani et al. 2016). Many of the FIESC have been isolated from various plants displaying disease symptoms, but their pathogenicity was never established (Leslie & Summerell 2006). Previous studies have reported the presence of FIESC in banana fruits and roots, as well as causing storage rot of bananas (Leslie & Summerell 2006, Zakaria & Rahman 2011, Zheng et al. 2012). However, this study represents the first report of FIESC from the pseudostem of bananas, indicating that members of this species complex have been isolated from every part of the banana plant. Thus far, species of the FIESC have been found to be more abundant in banana fruit, indicating a hemibiotrophic fungal lifestyle in plants (Bacon & Yates 2006), and therefore these are often found in stored banana fruits, which are a very suitable environment for toxin producing fungal species like most FIESC members (Desjardins 2006).

The second most diverse *Fusarium* species complex found in this study was the FFSC. Five species where identified from banana, including the common plant pathogenic species *F. proliferatum* and *F. verticilloides*. Additionally, two novel species, *F. lumajangense* and *F. desaboruense*, were also identified in this study. The FFSC is known to include species able to cause disease in a variety of important agronomic crops, especially in the tropics (O'Donnell et al. 1998b). Each of the novel species identified in this complex were closely related to recognized plant pathogens: *F. lumajangense* is phylogenetically and morphologically closely related to *F. mangiferae*, a species causing mango-malformation on mango (*Mangifera indica*), and *F. desaboruense* is closely related to *F. sacchari*, the causal agent of 'pokkah boeng' disease on sugarcane (Handojo et al. 1989, Britz et al. 2002). The plant pathogenic species *F. proliferatum*, a well-known pathogen on maize, sorgum, mango, and asparagus, and *F. verticilloides*, a pathogen on maize (Handojo et al. 1989, Britz et al. 2002, Ploetz 2006b) and notorious producer of fumonisins (Desjardin 2006), were isolated at low frequency. Interestingly, all the hosts mentioned above are present in Indonesia as important cultivated crops. Moreover, Indonesian bananas are mainly produced in small scale household plantations and co-cultivated with other crops such as rice, maize, sugarcane, and other perennial tropical crops (Maryani et al. 2019). This complex agroecosystem from which our banana samples were obtained might explain the presence of FFSC species in banana plants affected by Fusarium wilt.

Members of the FFSC isolated in this study were not pathogenic to the banana variety Cavendish. *Fusarium fujikuroi*, *F. sacchari*, *F. subglutinans*, and *F. verticilloides* have been reported from rice affected by 'Bakanae' disease, although, only *F. fujikuroi*, is known to cause the disease (Zainudin et al. 2008, Amatulli et al. 2010). A similar set of species in FFSC was also found in sugarcane, maize, and vanilla (Ploetz 2006b, Pinaria et al. 2010), although their association with these crops, without inducing disease, is still unknown. Moreover, their presence suggests an endophytic life style, causing no harm to the host plants or perhaps acting as secondary invaders or saprobes as the isolates were obtained from diseased plants. However, banana plants might serve as an intermediate host, as suggested by Handojo et al. (1989) for 'Pokkah boeng' disease on sugarcane.

A single isolate was found to belong to the FSSC, identified as *F. longipes* based on phylogenetic inference, a species abundant in tropical areas as a soil inhabitant or as a saprophyte

(Backhouse & Burgess 1995, Onyike & Nelson 1993). However, to our knowledge, our finding is the first report of this species from banana since the report of Reinking & Wollenweber (1927). They described *F. longipes* from mature living leaves of *Musa sapientum* in Honduras. Here, however, this species was cultured from the diseased pseudostem of banana variety Pisang Awak (ABB) on Kalimantan. This species appears to be commonly recovered from both healthy and diseased plants, suggesting that *F. longipes* could be endophytic in banana. This hypothesis was also further supported by the pathogenicity test conducted in this study. *Fusarium longipes* is known to be isolated more frequent during a higher rainfall period and under high temperatures (Burgess et al. 1988, Backhouse & Burgess 1995). This is consistent with our findings where *F. longipes* was recovered from banana plants growing at a relatively high temperature (35 °C) and humidity (62 %). With morphological distinctions from the previous description of *F. longipes*, InaCC F974 found in this study might represent a novel species. More isolates and additional gene regions are needed to capture the possible diversity in morphology and phylogenetic relationships.

Our current study highlights the diversity of *Fusarium* species in banana plants exhibiting Fusarium wilt. While only *Fusarium* spp. in the FOSC has been shown to be a true pathogen (Stover 1962, Maryani et al. 2019), the role of the remaining species in banana plants requires further investigation. Whether these *Fusarium* species are true endophytes of the various varieties of banana sampled in this study, possible saprophytes or secondary pathogens should still be determined experimentally. Isolation from asymptomatic plants of similar banana varieties would provide possible evidence of an endophytic lifestyle of the *Fusarium* species reported here. Moreover, the pathogenicity of each species on their respective host varieties needs to be tested in the future. Such studies would also reveal whether banana plants serve as intermediate hosts for a particular *Fusarium* species. Lastly, there is no doubt that tropical areas including Indonesia should receive more attention when studying *Fusarium* biodiversity.

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APPENDIX

Recently Maryani et al. (2019) recognised nine independent genetic lineages in a collection of *Fusarium oxysporum* f. sp. *cubense* isolates obtained from Indonesia, one of which was named *F. tardicrescens*. However, the holotype was incorrectly cited rendering the species invalid. *Fusarium tardicrescens* is therefore validated here.

Fusarium tardicrescens N. Maryani, L. Lombard, Kema & Crous, *sp. nov*. — MycoBank MB828959

Synonym: *Fusarium tardicrescens* N. Maryani et al., Stud. Mycol. 92: 185. 2019. Nom. inval., Art. 40.7 (Shenzhen).

Typus. Malawi, Karonga, Misuku Hills, *Musa sapientum* cv. Harare, 1989, RC Ploetz (holotype specimen and culture, CBS 102024, preserved in metabolically inactive state).

Description & Illustrations — Maryani et al. (2019).