FUSE

Fungal Systematics and Evolution

VOLUME 4
DECEMBER 2019
PAGES 183–200

doi.org/10.3114/fuse.2019.04.10

Neotypification of *Fusarium chlamydosporum* - a reappraisal of a clinically important species complex

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Key words:
chlamydospores
clinical isolates
morphology
mycotoxin
new taxa
systematics

Abstract: *Fusarium chlamydosporum* represents a well-defined morpho-species of both phytopathological and clinical importance. Presently, five phylo-species lacking Latin binomials have been resolved in the *F. chlamydosporum* species complex (FCSC). Naming these phylo-species is complicated due to the lack of type material for *F. chlamydosporum*. Over the years a number of *F. chlamydosporum* isolates (which were formerly identified based on morphology only) have been accessioned in the culture collection of the Westerdijk Fungal Biodiversity Institute. The present study was undertaken to correctly identify these '*F. chlamydosporum'* isolates based on multilocus phylogenetic inference supported by morphological characteristics. Closer scrutiny of the metadata associated with one of these isolates allowed us to propose a neotype for *F. chlamydosporum*. Phylogenetic inference revealed the presence of nine phylo-species within the FCSC in this study. Of these, eight could be provided with names supported by subtle morphological characters. In addition, a new species, as *F. nodosum*, is introduced in the *F. sambucinum* species complex and *F. chlamydosporum var. fuscum* is raised to species level, as *F. coffeatum,* in the *F. incarnatum-equiseti* species complex (FIESC).

Effectively published online: 4 July 2019.

INTRODUCTION

Fusarium chlamydosporum represents a well-defined morpho-species (Gerlach & Nirenberg 1982, Leslie & Summerell 2006, O'Donnell *et al*. 2009, 2018) of both phytopathological and clinical importance (Leslie & Summerell 2006, O'Donnell *et al*. 2009). This species is characterised by its difficulty in forming sporodochia (requires exposure to UV-light; Gerlach & Nirenberg 1982), abundant and rapid formation of large chlamydospores, production of 3–5-septate macroconidia (*i.e.* sporodochial conidia), 0–2-septate microconidia (*i.e.* aerial conidia) and the production of a bright pink to dark wine-red pigment on various culture media (Wollenweber & Reinking 1925, 1935, Reinking & Wollenweber 1927, Gerlach & Nirenberg 1982, Leslie & Summerell 2006). Wollenweber & Reinking (1925) first introduced this species, isolated from the exterior of the pseudostem of *Musa sampientum,* collected in Tela, Honduras. They further classified this species as a member of the section *Sporotrichiella*, which also included *F. poae* and *F. sporotrichioides* at that time. Presently, various unnamed phylo-species (FCSC 1–5) and *F. nelsonii* (O'Donnell *et al*. 2009, 2018) constitute the *F. chlamydosporum* species complex (FCSC), sister to the *F. aywerte* (FASC; Laurence *et al*. 2016), *F. incarnatum-equiseti* (FIESC) and *F. sambucinum* (FSAMSC) species complexes (O'Donnell *et al*. 2013).

Fusarium chlamydosporum is commonly isolated from soils and grains in arid and semi-arid regions (Burgess & Summerell 1992, Kanaan & Bahkali 1993, Sangalang *et al*. 1995), and from plant material displaying disease symptoms that include crown rot (Du *et al*. 2017), blight (Satou *et al*. 2001), damping-off (Engelbrecht *et al*. 1983, Lazreg *et al.* 2013) and stem canker (Fugro 1999). This species has also been implicated in human and animal fusarioses (Kiehn *et al*. 1985, Martino *et al*. 1994, Segal *et al*. 1998, Kluger *et al*. 2004, Azor *et al*. 2009, O'Donnell *et al*. 2009) and together with members of the FIESC, account for approximately 15 % of fusarioses in the USA (O'Donnell *et al*. 2009). As with most *Fusarium* spp. associated with human fusarioses (Al-Hatmi *et al*. 2016), treatment of *F. chlamydosporum* infection is complicated due to multidrug-resistance, but amphotericin B and posaconazole have been shown to be effective (Pujol *et al*. 1997, Azor *et al*. 2009). In addition, several strains of *F. chlamydosporum* are known to produce the mycotoxins beauvericin, butanolide, moniliformin, trichothecene (Rabie *et al*. 1978, 1982, Marasas *et al*. 1984, O'Donnell *et al*. 2018), other secondary metabolites such as chlamydosporol (Savard *et al*. 1990), chitinase (Mathivanan *et al*. 1998), cellulase (Qin *et al*. 2010), and other unnamed compounds (Soumya *et al*. 2018, Wang *et al*. 2018). Recently, Soumya *et al*. (2018) isolated and characterised the red pigment produced by *F. chlamydosporum* in culture, and found that this long-chain hydrocarbon with unsaturated groups possess cytotoxicity towards human breast adenocarcinoma cells MCF-7, and could be exploited in cancer therapeutics as well as in the cosmetic industry.

The first critical multilocus phylogenetic study to include a large number of *F. chlamydosporum* isolates by O'Donnell *et al*. (2009) revealed four phylo-species (FCSC 1–4) within a group of clinical and environmental isolates initially identified as *F. chlamydosporum*, one of which included the ex-type of *F. nelsonii* (as FCSC 4; O'Donnell *et al*. 2009). Following this study, O'Donnell *et al.* (2018) identified a fifth phylo-species that was able to produce the mycotoxins beauvericin, butanolide and moniliformin. However, both studies refrained from providing names to the four unnamed phylo-species (FCSC 1–3 & 5) as no type material was available for *F. chlamydosporum s. str.* to serve as reference point. Over the years, a number of *F. chlamydosporum* isolates (which were formerly identified based on morphology only) have been accessioned in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute (WI), Utrecht, The Netherlands. However, given the paucity of key informative morphological features of especially *Fusarium* spp. (Nirenberg 1990, Lombard *et al*. 2019), the present study was undertaken to correctly identify these '*F. chlamydosporum*' isolates based on multilocus phylogenetic inference supported by morphological characteristics.

MATERIALS AND METHODS

Isolates

Fusarium isolates (Table 1), initially identified and treated as *F. chlamydosporum*, were obtained from the culture collection (CBS) of the WI in Utrecht, The Netherlands.

DNA isolation, PCR and sequencing

Total genomic DNA was extracted from 7-d-old isolates grown at 24 °C on potato dextrose agar (PDA; recipe in Crous *et al*. 2019) using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions. Partial gene sequences were determined for the calmodulin (*cmdA*), RNA polymerase largest (*rpb1*) & second largest subunit (*rpb2*), and translation elongation factor 1-alpha (*tef1*), using PCR protocols and primer pairs described elsewhere (O'Donnell *et al*. 1998, 2009, 2010, Lombard *et al*. 2019). Integrity of the sequences was ensured by sequencing the amplicons in both directions using the same primer pairs as were used for amplification. Consensus sequences for each locus were assembled in Geneious R11 (Kearse *et al*. 2012). All sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Initial analyses based on pairwise alignments and BLASTN searches on the *Fusarium*-MLST (www.wi.knaw.nl/fusarium/), *Fusarium*-ID (http://isolate.fusariumdb.org/guide.php; Geiser *et al.* 2004) and NCBI's GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) databases were done using *rbp2* and *tef1* partial sequences. Based on these comparisons, sequences of relevant *Fusarium* species/strains were retrieved (Table 1) and alignments of the individual loci were determined using MAFFT v. 7.110 (Katoh *et al*. 2017) and manually corrected where necessary. Three independent phylogenetic algorithms, Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian inference (BI), were employed for phylogenetic analyses. Phylogenetic analyses were conducted of the individual loci and then as a multilocus sequence dataset that included partial sequences of the four genes determined here.

For BI and ML, the best evolutionary models for each locus were determined using MrModeltest v. 2 (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) was used for BI to generate phylogenetic trees under optimal criteria for each locus. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was initiated in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies was below 0.01 with trees saved every 1 000 generations. The first 25 % of saved trees were discarded as the 'burn-in' phase and posterior probabilities (PP) were determined from the remaining trees.

The ML analyses were performed using RAxML-NG v. 0.6.0 (Kozlov *et al*. 2018) to obtain another measure of branch support. The robustness of the analysis was evaluated by bootstrap support (BS) with the number of bootstrap replicates automatically determined by the software. For MP, analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 1 000 random addition sequences. Tree-bisection-reconnection was used, with branch swapping option set on 'best trees' only. All characters were weighted equally and alignment gaps treated as fifth state. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap (BS) analyses (Hillis & Bull 1993) were based on 1 000 replications. Alignments and phylogenetic trees derived from this study were uploaded to TreeBASE (S24459; www.treebase.org).

Morphological characterisation

All isolates were characterised following the protocols described by Leslie & Summerell (2006) and Lombard *et al*. (2019) using PDA, oatmeal agar (OA, recipe in Crous *et al*. 2019), synthetic nutrient-poor agar (SNA; Nirenberg 1976) and carnation leaf agar (CLA; Fisher *et al.* 1982). Colony morphology, pigmentation, odour and growth rates were evaluated on PDA after 7 d at 24 °C using a 12/12 h light/dark cycle with near UV and white fluorescent light. Colour notations were done using the colour charts of Rayner (1970). Micromorphological characters were examined using water as mounting medium on a Zeiss Axioskop 2 *plus* with Differential Interference Contrast (DIC) optics and a Nikon AZ100 dissecting microscope both fitted with Nikon DS-Ri2 high definition colour digital cameras to photo-document fungal structures. Measurements were taken using the Nikon software NIS-elements D v. 4.50 and the 95 % confidence levels were determined for the conidial measurements with extremes given in parentheses. For all other fungal structures examined, only the extremes are presented. To facilitate the comparison of relevant micro- and macroconidial features, composite photo plates were assembled from separate photographs using PhotoShop CSS.

Table 1. Details of *Fusarium* strains included in the phylogenetic analyses.

				GenBank accession				
Species	**Culture accession[1]**	**Host/substrate**	**Origin**	***cmdA***	***rpb1***	***rpb2***	***tef1***	**Reference**
F. acacia-mearnsii	NRRL 26755 = CBS 110255 = MRC 5122	*Acacia mearnsii*	South Africa	–	KM361640	KM361658	AF212449	O'Donnell *et al.* (2000), Aoki *et al.* (2015)
F. armeniacum	NRRL 6227 = ATCC 36781 = FRC R-5319 = MRC 1783	Fescue hay	USA	–	JX171446	JX171560	HM744692	O'Donnell *et al.* (2013), Yli-Mattila *et al.* (2011)
	NRRL 29133 = CBS 485.94 = NRRL 26847 = NRRL 26908	Unknown	Australia	–	–	HQ154448	HM744659	Yli-Mattila *et al.* (2011)
	NRRL 31970 = FRC R-1957	Soil	Australia	–	–	HQ154453	HM744664	Yli-Mattila *et al.* (2011)
	NRRL 43641	Horse eye	USA	GQ505398	HM347192	GQ505494	GQ505430	O'Donnell *et al.* (2009, 2010)
F. asiaticum	NRRL 13818 = CBS 110257 = FRC R-5469 = MRC 1963 = NRRL 31547^{T}	*Hordeum vulgare*	Japan	–	JX171459	JX171573	AF212451	O'Donnell *et al.* (2000, 2013)
F. atrovinosum	CBS 445.67 = BBA 10357 = DSM 62169 = IMI 096270 = NRRL 26852 = NRRL 26913^{T}	*Triticum aestivum*	Australia	MN120693	MN120713	–	MN120752	Present study
	CBS 130394	Human leg	USA	MN120694	MN120714	MN120734	MN120753	Present study
	NRRL 13444	Soil	Australia	GQ505373	JX171454	GQ505467	GQ505403	O'Donnell *et al.* (2000, 2013)
	NRRL 34013	Human toenail	USA	GQ505378	–	GQ505472	GQ505408	O'Donnell *et al.* (2009)
	NRRL 34015	Human eye	USA	GQ505380	–	GQ505474	GQ505410	O'Donnell *et al.* (2009)
	NRRL 34016	Human leg	USA	GQ505381	HM347170	GQ505475	GQ505411	O'Donnell *et al.* (2009, 2010)
	NRRL 34021	Human lung	USA	GQ505385	–	GQ505479	GQ505415	O'Donnell *et al.* (2009)
	NRRL 34023	Human finger	USA	GQ505387	–	GQ505481	GQ505417	O'Donnell *et al.* (2009)
	NRRL 43627	Human bronchial lavage	USA	GQ505392	–	GQ505487	GQ505423	O'Donnell *et al.* (2009)
	NRRL 43630	Human sputum	USA	GQ505395	–	GQ505490	GQ505426	O'Donnell *et al.* (2009)
F. aywerte	NRRL 25410^{T}	Soil	Australia	KU171417	JX171513	JX171626	KU171717	O'Donnell *et al.* (2013), Brown & Proctor (2016)
	RBG5743	Soil	Australia	–	KP083273	KP083278	KP083250	Laurence *et al.* (2016)
F. boothii	NRRL 26916 = ATCC 24373 = CBS 316.73 = IMI 160243 = NRRL 26855^{T}	*Zea mays*	South Africa	–	KM361641	KM361659	AF212444	O'Donnell *et al.* (2000), Aoki *et al.* (2015)
F. brachygibbosum	NRRL 34033	Human foot	USA	GQ505388	HM347172	GQ505482	GQ505418	O'Donnell *et al.* (2009, 2010)
F. cerealis	NRRL 25491 = CBS 589.93	*Iris hollandica*	Netherlands	–	MG282371	MG282400	AF212465	O'Donnell *et al.* (2000), Waalwijk *et al.* (2018)
F. chlamydosporum	CBS 145.25 = NRRL 26912^{NT}	*Musa sapientum*	Honduras	MN120695	MN120715	MN120735	MN120754	Present study
	CBS 615.87 = NRRL 28578	*Colocasia esculenta*	Cuba	GQ505375	JX171526	GQ505469	GQ505405	O'Donnell *et al.* (2009, 2013)
	CBS 677.77 = NRRL 36539	Soil	Solomon Islands	GQ505391	MN120716	GQ505486	GQ505422	O'Donnell *et al.* (2009)

Table 1. (Continued).

Species	Culture accession[1]	Host/substrate	Origin	GenBank accession				Reference
				cmdA	*rpb1*	*rpb2*	*tef1*	
	NRRL 32521	Human	USA	GQ505376	–	GQ505470	GQ505406	O'Donnell *et al*. (2009)
	NRRL 34012	Human toe	USA	GQ505377	–	GQ505471	GQ505407	O'Donnell *et al*. (2009)
	NRRL 34014	Human sinus	USA	GQ505379	–	GQ505473	GQ505409	O'Donnell *et al*. (2009)
	NRRL 34017	Human sinus	USA	GQ505382	–	GQ505476	GQ505412	O'Donnell *et al*. (2009)
	NRRL 34018	Human arm	USA	GQ505383	–	GQ505477	GQ505413	O'Donnell *et al*. (2009)
	NRRL 34019	Human eye	USA	GQ505384	–	GQ505478	GQ505414	O'Donnell *et al*. (2009)
	NRRL 34022	Human sinus	USA	GQ505386	–	GQ505480	GQ505416	O'Donnell *et al*. (2009)
	NRRL 43628	Human finger	USA	GQ505393	–	GQ505488	GQ505424	O'Donnell *et al*. (2009)
	NRRL 43629	Human blood	USA	GQ505394	HM347186	GQ505489	GQ505425	O'Donnell *et al*. (2009, 2010)
	NRRL 43632	Human eye	USA	GQ505396	–	GQ505492	GQ505428	O'Donnell *et al*. (2009)
	NRRL 43633	Human sinus	USA	GQ505397	–	GQ505493	GQ505429	O'Donnell *et al*. (2009)
	NRRL 45992	Human leg	USA	GQ505399	–	GQ505495	GQ505431	O'Donnell *et al*. (2009)
	NRRL 52797	*Scirtothrips dorsalis*	India	–	JF741015	JF741190	JF740865	O'Donnell *et al*. (2012)
F. coffeatum	CBS 635.76 = BBA 62053 = NRRL 20841^T	*Cynodon lemfuensis*	South Africa	MN120696	MN120717	MN120736	MN120755	Present study
	CBS 430.81 = NRRL 28577	Grave stone	Romania	MN120697	–	MN120737	MN120756	Present study
F. culmorum	NRRL 25475 = CBS 417.86 = FRC R-8504 = IMI 309344	*Hordeum vulgare*	Denmark	–	JX171515	JX171628	AF212463	O'Donnell *et al*. (2000, 2013)
F. graminearum	NRRL 31084 = CBS 123657	*Zea mays*	USA	–	JX171531	JX171644	HM744693	O'Donnell *et al*. (2013), Yli-Mattila *et al*. (2011)
	NRRL 36905	*Triticum aestivum*	USA	–	KM361646	KM361664	DQ459742	Starkey *et al*. (2007), Aoki *et al*. (2015)
F. humicola	CBS 124.73 = ATCC 24372 = IMI 128101 = NRRL 25535^T	Soil	Pakistan	MN120698	MN120718	MN120738	MN120757	Present study
	CBS 491.77 = NRRL 36495	Soil	Kuwait	GQ505390	MN120719	GQ505485	GQ505421	O'Donnell *et al*. (2009)
F. lacertarum	NRRL 20423 = ATCC 42771 = CBS 130185 = IMI 300797^T	Lizard skin	India	GQ505505	JX171467	JX171581	GQ505593	O'Donnell *et al*. (2009, 2013)
	CBS 127131	Soil	USA	MN120699	MN120720	MN120739	MN120758	Present study
	NRRL 43680	Contact lens fluid	USA	–	–	EF470046	EF453007	O'Donnell *et al*. (2007)
F. langsethiae	NRRL 53409	*Hordeum vulgare*	Finland	–	–	HQ154455	HM744667	Yli-Mattila *et al*. (2011)
	NRRL 53411	*Avena sativa*	Finland	–	–	HQ154457	HM744669	Yli-Mattila *et al*. (2011)
	NRRL 53417	*Avena sativa*	Finland	–	KT597713	HQ154460	HM744672	Yli-Mattila *et al*. (2011), Rocha *et al*. (2015)
	NRRL 53436	*Hordeum vulgare*	Russia	–	–	HQ154476	HM744688	Yli-Mattila *et al*. (2011)
	NRRL 54940	*Avena sativa*	Norway	–	JX171550	JX171662	–	O'Donnell *et al*. (2013)

Table 1. (Continued).

Species	Culture accession[1]	Host/substrate	Origin	GenBank accession				Reference
				cmdA	*rpb1*	*rpb2*	*tef1*	
F. lunulosporum	NRRL 13393 = BBA 62459 = CBS 636.76 = FRC R-5822 = IMI 322097^{T}	*Citrus paradisi*	South Africa	–	KM361637	KM361655	AF212467	O'Donnell *et al.* (2000), Aoki *et al.* (2015)
F. microconidium	CBS 119843 = MRC 8391	Unknown	Unknown	MN120700	MN120721	–	MN120759	Present study
F. nelsonii	CBS 119876 = FRC R-8670 = MRC 4570^{T}	Plant debris	South Africa	MN120701	MN120722	MN120740	MN120760	Present study
	CBS 119877 = MRC 8520	Unknown	Unknown	MN120702	MN120723	MN120741	MN120761	Present study
F. nodosum	CBS 200.63	*Arachis hypogaea*	Portugal	MN120703	MN120724	MN120742	MN120762	Present study
	CBS 201.63^{T}	*Arachis hypogaea*	Portugal	MN120704	MN120725	MN120743	MN120763	Present study
	CBS 698. 74	*Arundo donax*	France	MN120705	MN120726	MN120744	MN120764	Present study
	CBS 119844 = BBA 62170 = MRC 1798	Unknown	Unknown	MN120706	MN120727	–	MN120765	Present study
	CBS 131779	*Triticum aestivum*	Iran	–	–	MN120745	MN120766	Present study
F. oxysporum	CBS 144143^{T}	*Solanum tuberosum*	Germany	MH484771	–	MH484953	MH485044	Lombard *et al.* (2019)
F. peruvianum	CBS 511.75^{T}	*Gossypium* sp.	Peru	MN120707	MN120728	MN120746	MN120767	Present study
F. poae	NRRL 66297		–	–	MG282363	MG282392	–	Waalwijk *et al.* (2018)
	NRRL 13714 = MRC 2181	*Triticum aestivum*	Canada	–	JX171458	JX171572	–	O'Donnell *et al.* (2013)
F. pseudograminearum	NRRL 28062 = CBS 109956 = FRC R-5291 = MAFF 237835^{T}	*Hordeum vulgare*	Australia	–	JX171524	JX171637	AF212468	O'Donnell *et al.* (2000, 2013)
F. sibiricum	NRRL 53429	*Avena sativa*	Russia	–	–	HQ154471	HM744683	Yli-Mattila *et al.* (2011)
	NRRL 53430^{T}	*Avena sativa*	Russia	–	–	HQ154472	HM744684	Yli-Mattila *et al.* (2011)
	NRRL 53431 = CBS 140945	*Avena sativa*	Russia	–	–	HQ154473	HM744685	Yli-Mattila *et al.* (2011)
F. spinosum	CBS 122438	Galia melon	Brazil (via Netherlands)	MN120708	MN120729	MN120747	MN120768	Present study
	NRRL 43631	Human leg	USA	–	HM347187	GQ505491	GQ505427	O'Donnell *et al.* (2009, 2010)
F. sporodochiale	CBS 199.63 = MUCL 6771	Termitary	Unknown	MN120709	MN120730	MN120748	MN120769	Present study
	CBS 220.61 = ATCC 14167 = MUCL 8047 = NRRL 20842^{T}	Soil	South Africa	MN120710	MN120731	MN120749	MN120770	Present study
F. sporotrichioides	CBS 462.94	*Glycosmis citrifolia*	Austria	MN120711	MN120732	MN120750	MN120771	Present study
	NRRL 3299 = ATCC 24631 = CBS 119840 = FRC T-423 = MRC 1768	*Zea mays*	France	–	JX171444	GQ915498	GQ915514	Proctor *et al.* (2009), O'Donnell *et al.* (2013)
	NRRL 29977	Unknown	Yugoslavia	–	KT597711	HQ154451	HM744662	Yli-Mattila *et al.* (2011), Rocha *et al.* (2015)
	NRRL 52928	Unknown	Turkey	–	–	JF741195	JF740870	O'Donnell *et al.* (2012)

Table 1. (Continued).

Species	Culture accession[1]	Host/substrate	Origin	GenBank accession				Reference
				cmdA	*rpb1*	*rpb2*	*tef1*	
	NRRL 52934	Unknown	Turkey	–	–	JF741201	JF740876	O'Donnell *et al.* (2012)
	NRRL 53434	*Avena sativa*	Russia	–	–	HQ154475	HM744687	Yli-Mattila *et al.* (2011)
F. tjaynera	NRRL 66246 = RBG5367[T]	*Triodia microstachya*	Australia	–	KP083268	KP083279	KP083266	Laurence *et al.* (2016)
	NRRL 66247 = RBG5366	*Sorghum intrans*	Australia	–	–	–	KP083266	Laurence *et al.* (2016)
F. venenatum	NRRL 22196 = BBA 65031	*Zea mays*	Germany	–	JX171494	JX171607	–	O'Donnell *et al.* (2013)
FIESC 24	CBS 101138 = BBA 70869	*Phaseolus vulgaris*	Turkey	MN120712	MN120733	MN120751	MN120772	Present study
	NRRL 52777	*Eurygaster* sp.	Turkey	–	JF741006	JF741171	JF740845	O'Donnell *et al.* (2012)
	NRRL 25080	*Nilaparvata lugens*	China	–	–	JF741041	JF740711	O'Donnell *et al.* (2012)
Fusarium sp.	NRRL 13338	Soil	Australia	GQ505372	JX171447	JX171561	GQ505402	O'Donnell *et al.* (2009, 2013)

[1]ATCC: American Type Culture Collection, USA; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany; CBS: Westerdijk Fungal Biodiverity Institute (WFBI), Utrecht, The Netherlands; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; FRC: Fusarium Research Center, Penn State University, Pennsylvania; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; MRC: National Research Institute for Nutritional Diseases, Tygerberg, South Africa; MAFF: Genetic Resources Center, National Agriculture and Food Research Organization (NARO), NARO Genebank, Microorganism Section, Japan; MUCL: Mycothéque de l'Université Catholique de Louvian, Belgium; NRRL: Agricultural Research Service Culture Collection, USA; RBG: Royal Botanic and Domain Trust, Sydney, Australia. [T] Ex-type culture; [NT]Neotype.

RESULTS

Phylogenetic analyses

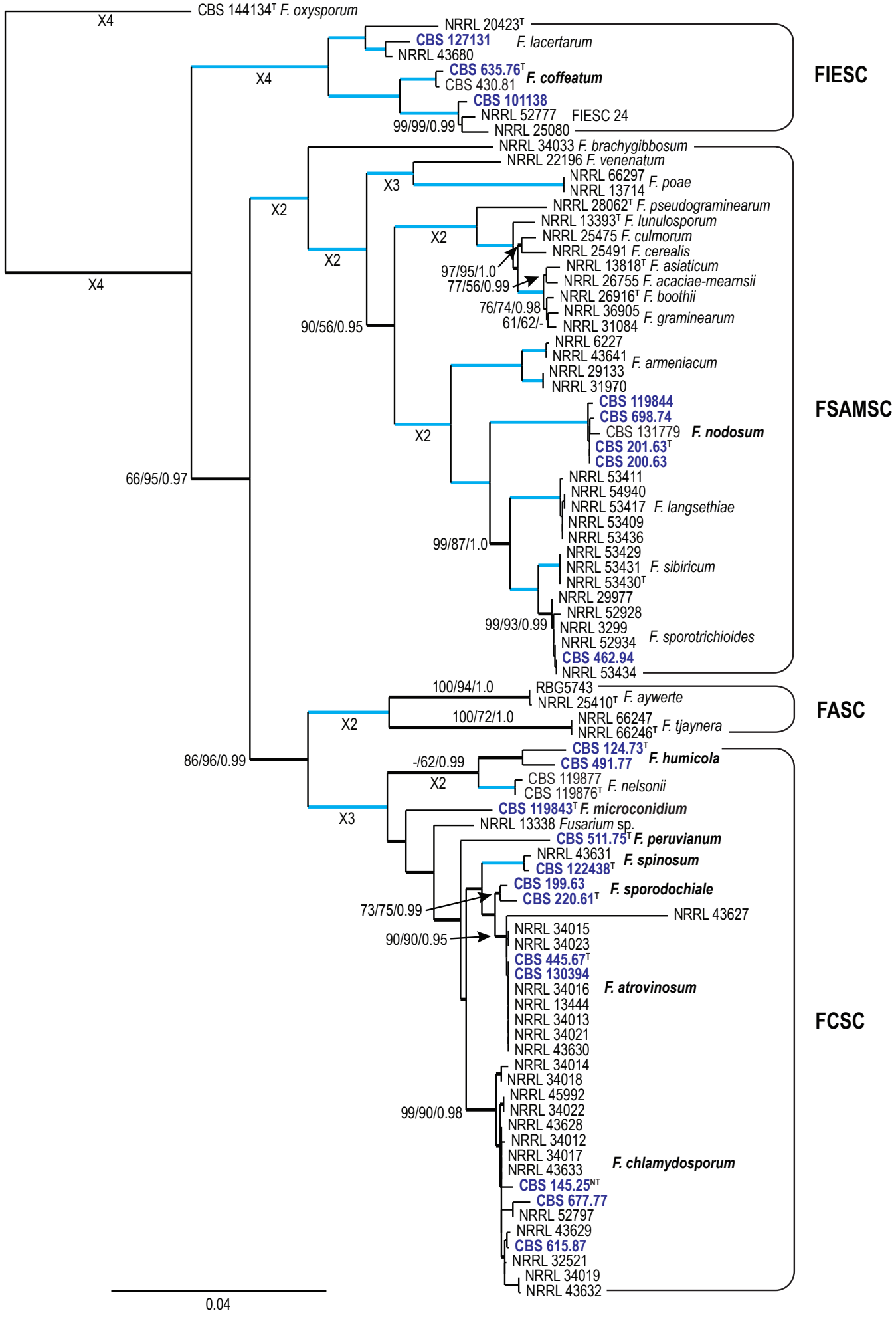
Approximately 500—650 bases were determined for *cmdA* and *tef1*, 1 845 bases for *rpb1* and 1 800 bases for *rpb2*. Sequence comparisons of the *rpb2* and *tef1* gene regions generated in this study against those in the *Fusarium*-MLST, *Fusarium*-ID and GenBank databases revealed that only 14 isolates belonged to the FCSC. Of the remaining 9 isolates, three were identified as members of the *F. incarnatum-equiseti* species complex (FIESC) and six belonged in the *F. sambucinum* species complex (FSAMSC).

For the BI and ML analyses, a K80 model for *cmdA*, a GTR+I+G model for *rbp1*, an HKY+G+I model for *rpb2* and an HKY+G for *tef1* were selected and incorporated into the analyses. The ML tree topology confirmed the tree topologies obtained from the BI and MP analyses, and therefore, only the ML tree is presented.

The combined four loci sequence dataset included 85 ingroup taxa with *F. oxysporum* (CBS 144134) as outgroup taxon. The dataset consisted of 4 875 characters including gaps. Of these characters, 3 267 were constant, 289 parsimony-uninformative and 1 319 parsimony-informative. The BI lasted for 18.8 M generations, and the consensus tree and posterior probabilities (PP) were calculated from 281 350 trees left after 93 782 were discarded as the 'burn-in' phase. The MP analysis yielded 1 000 trees (TL = 3 742; CI = 0.590; RI = 0.911; RC = 0.538) and a single best ML tree with -InL = -24632.989217 (Fig. 1).

In the phylogenetic tree (Fig. 1), the isolates thought to represent *F. chlamydosporum* clustered in three species complexes that included the FCSC, FIESC and FSAMSC. Three isolates clustered in the FIESC; CBS 127131 clustered in the *F. lacertarum* clade, CBS 635.76 (ex-type of *F. chlamydosporum var. fuscum*) clustered in the FIESC 28 clade, and CBS 101138 clustered in the FIESC 24 clade (O'Donnell *et al.* 2009, Wang *et al.* 2019). Six isolates clustered within the FSAMSC clade, of which CBS 462.94 clustered within the *F. sporotrichioides* clade. The remaining five isolates (CBS 200.63, 201.63, 698.74, 119844 & 131779) formed a highly-supported (ML- & MP-BS = 100, PP = 1.0) clade

Fig. 1. The ML consensus tree inferred from the combined *cmdA, rpb1, rpb2* and *tef1* sequence alignment. Thickened branches indicate branches present in the ML, MP and Bayesian consensus trees. Blue thickened lines indicate branches with full support (ML & MP BS = 100, PP = 1.0) with support values of other branches indicated at the branches. The tree is rooted to *Fusarium oxysporum* (CBS 144143). The scale bar indicates 0.04 expected changes per site. Isolates in dark blue were preserved in the CBS collection as *F. chlamydosporum*. Species complexes are indicated on the right following O'Donnell *et al.* (2013) and Laurence *et al.* (2016). Neo- and ex-types are indicated as [T] and [NT], respectively.



closely related but distinct from the *F. langsethiae, F. sibiricum* and *F. sporotrichioides* clades. Fourteen isolates clustered in the FCSC clade, of which three isolates (CBS 145.25, 615.87 & 677.77) clustered in the FCSC 1 (*sensu* O'Donnell *et al*. 2009), two (CBS 445.67 & 130394) in FCSC 2 (*sensu* O'Donnell *et al*. 2009), and one (CBS 122438) in FCSC 3 (*sensu* O'Donnell *et al*. 2009). Two isolates (CBS 199.63 & 220.61) formed a well-supported (ML-BS = 73, MP-BS = 75, PP = 0.99) distinct clade, sister to the FCSC 2 clade. Both isolates CBS 511.75 & 119843 formed two unique single lineages with the last four isolates (CBS 124.73, 491.77, 119876 & 119877) forming a distinct unique and supported (MP-BS = 62, PP = 0.99) clade in the FCSC.

Taxonomy

The following species are recognised as new within the FCSC and FSAMSC based on phylogenetic inference and morphological comparisons. In addition, *F. chlamydosporum var. fuscum* is raised to species level, as *F. coffeatum*, in the FIESC based on the placement of the ex-type strain in the phylogenetic inference and a neotype is designated for *F. chlamydosporum*. The single lineage represented by NRRL 13338 is not treated here, as the strain was not available to us at the time of this study.

Fusarium atrovinosum L. Lombard & Crous, ***sp. nov.*** MycoBank MB831559. Fig. 2.

Etymology: Named after the dark wine-red (dark vinaceous) reverse colouration of the PDA on which this fungus is grown.

Diagnosis: Only producing 0–1-septate aerial conidia (*i.e.* microconidia) on rarely branched polyphialides in culture with abundant chlamydospores.

Typus: **Australia**, from *Triticum aestivum*, 1961, *W.L. Gordon* (**holotype** CBS-H 24015 designated here, culture ex-type CBS 445.67 = BBA 10357 = DSM 62169 = IMI 096270 = NRRL 26852 = NRRL 26913).

Conidiophores carried on aerial mycelium 20–40 µm tall, unbranched or rarely irregularly or sympodially branched, bearing a terminal single phialide or whorl of 2–3 phialides; *aerial phialides* polyphialidic, subulate to subcylindrical, smooth- and thin-walled, 9–23 × 2–4 µm, periclinal thickening inconspicuous or absent; *aerial conidia* forming small false heads on the phialide tips, hyaline, fusiform to ellipsoidal to obovoid, smooth- and thin-walled, 0–1(–2)-septate; 0-septate conidia: 7–11(–15) × 2–4(–5) µm (av. 9 × 3 µm); 1-septate conidia: (11–)13–17(–20) × 4–6 µm (av. 15 × 5 µm); 2-septate conidia: (12–)14–18(–20) × 4–5 µm (av. 16 × 5 µm). *Sporodochia* not observed. *Chlamydospores* abundant, globose to subglobose, thick-walled, smooth to slightly verrucose, 12–22 µm diam, formed terminally or intercalarily in chains of three or more.

Culture characteristics: Colonies on PDA reaching 90 mm at 24 °C after 7 d. Colony surface greyish rose to vinaceous to buff in the centre, with abundant aerial mycelium, dense, woolly to cottony. Odour absent. Reverse livid red to dark vinaceous. On SNA, colonies membranous to woolly, white to pale rosy buff, with abundant sporulation on the surface giving a powdery appearance; reverse pale rosy buff. On CLA, aerial mycelium abundant, white, lacking sporodochia on the carnation leaf pieces. On OA, colonies woolly to cottony, buff in the centre becoming rosy vinaceous towards margins, appearing powdery.

Notes: *Fusarium atrovinosum* represents the clade FCSC 2 *sensu* O'Donnell *et al*. (2009). This species is closely related to *F. chlamydosporum, F. spinosum* and *F. sporodochiale* and can be distinguished from these three species by the lack of monophialides on the aerial mycelium. Additionally, *F. atrovinosum* did not produce any sporodochia on the carnation

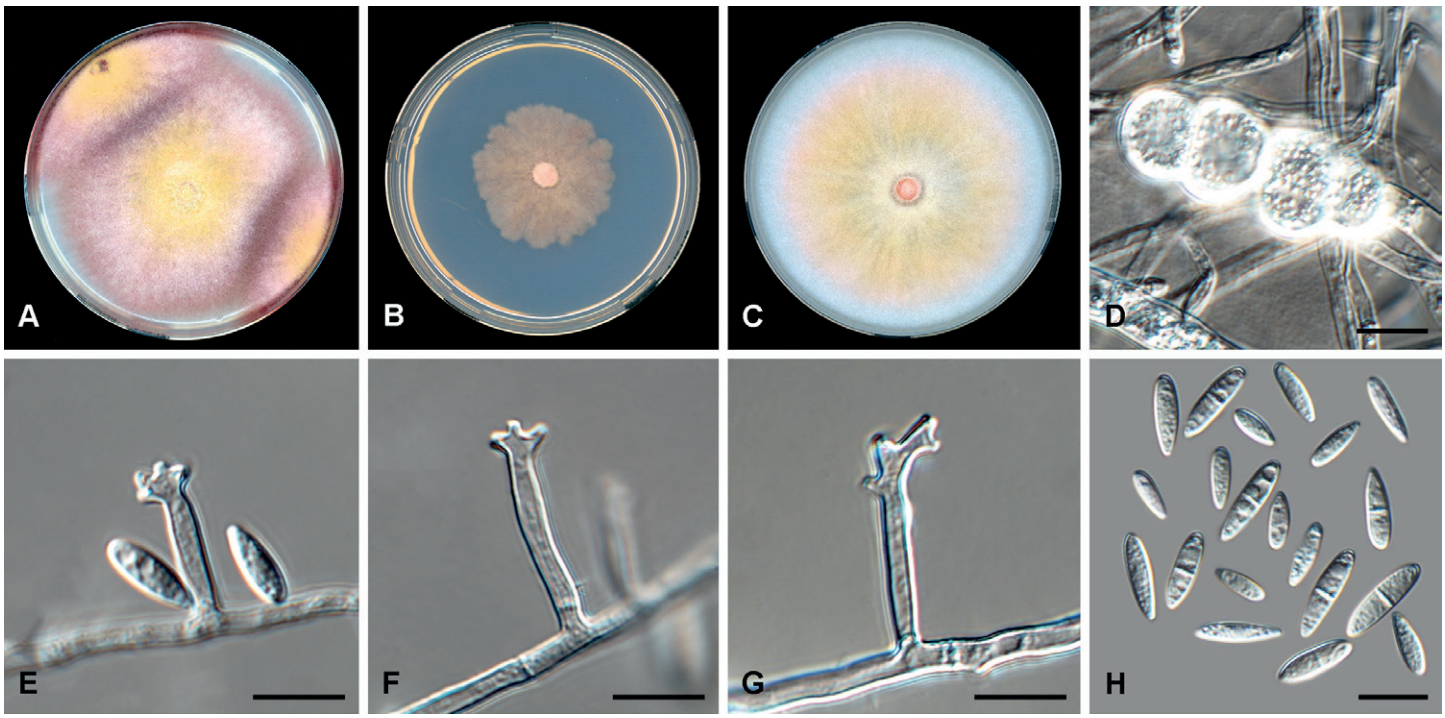


Fig. 2. *Fusarium atrovinosum* (CBS 445.67). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Chlamydospores on SNA. **E–G.** Polyphialides on aerial mycelium. **H.** Aerial conidia. Scale bars = 10 µm.

leaf pieces but did produce abundant chlamydospores, further distinguishing it from *F. sporodochiale*.

Fusarium chlamydosporum Wollenw. & Reinking, Phytopathology 15: 156. 1925.
Synonyms: *Fusarium sporotrichioides var. chlamydosporum* (Wollenw. & Reinking) Joffe, *Mycopath. Mycol. Appl*. **53**: 211. 1974.
Dactylium fusarioides Gonz. *et al*., *Boln. Real Soc. Españ. Hist. Nat., Biol*. **27**: 280. 1928.
Fusarium fusarioides (Gonz., *et al*.) C. Booth, *The genus* Fusarium: 88. 1971.
Fusarium sporotrichioides subsp. *minus* (Wollenw.) Riallo, *Fungi of the genus* Fusarium: 196. 1950.
Fusarium sporotrichiella var. sporotrichioides Bilai, *Fusarii*: 277. 1955.
Pseudofusarium purpureum Matsush., *Microfungi Solomon Isl. Papua-New Guinea (Osaka)*: 47. 1971.

Neotypus: **Honduras**, Tela, from pseudostem of *Musa sapientum*, *H.W. Wollenweber* & *O.A. Reinking* [**neotype** CBS 145.25 designated here (as metabolic inactive specimen), culture ex-neotype CBS 145.25 = NRRL 26912; MBT387601].

Descriptions and illustrations: Reinking & Wollenweber (1927), Wollenweber & Reinking (1925, 1935).

Notes: A letter from C.L. Shear (dated 23 January 1925) addressed to Prof. dr J. Westerdijk, director of the Centraalbureau voor Schimmelcultures (now WI), indicated that CBS 145.25 (as no. 871) is *F. chlamydosporum* (as "*F. chlamydosporum* n. sp.") isolated from banana collected in Tela, Honduras. He further confirmed that this isolate was identified by H.W. Wollenweber and O.A. Reinking. However, it is not clearly indicated whether this isolate represents the ex-type. Therefore, based on the matching geography, host and date, we designate this isolate as neotype of *F. chlamydosporum*.

Fusarium coffeatum L. Lombard & Crous, ***stat. et. nom. nov.*** MycoBank MB831560.
Basionym: *Fusarium chlamydosporum var. fuscum* Gerlach, *Phytopath. Z.* **90**: 41. 1977.

Etymology: Name refers to the characteristic coffee-brown pigmentation produced in cultures of this fungus.

Descriptions and illustrations: Gerlach (1977), Gerlach & Nirenberg (1982).

Notes: Gerlach (1977) and Gerlach & Nirenberg (1982) distinguished *F. chlamydosporum var. fuscum* from *F. chlamydosporum var. chlamydosporum* based on the beige to coffee-brown pigmentation in culture of the former variety, compared to the red pigment produced by the latter. Phylogenetic inference and sequence comparisons with the *Fusarium* databases and GenBank, showed that the ex-type (CBS 635.76; Fig. 1) of *F. chlamydosporum var. fuscum* belongs in the FIESC, clustering in the yet unnamed FIESC 28 clade (Wang *et al*. 2019). Therefore, this variety is raised to species level with a new name as the name *F. fuscum* is already occupied.

Fusarium humicola L. Lombard & Crous, ***sp. nov.*** MycoBank MB83156. Fig. 3.

Etymology: Named after the substrate, soil, from which the majority of the isolates of this species were isolated.

Diagnosis: Sporodochial conidia mostly straight but slightly curved at both ends; aerial conidia mostly 0–1-septate; chlamydospores not formed.

Typus: **Pakistan**, from soil, date unknown, *S.I. Ahmed* (**holotype** CBS-H 24016 designated here, culture ex-type CBS 124.73 = ATCC 24372 = IMI 128101 = NRRL 25535).

Conidiophores borne on aerial mycelium 40–120 µm tall, verticillately branched, rarely unbranched, bearing a terminal single phialide or whorl of 2–3 phialides; *aerial phialides* mono- and polyphialidic, subulate to subcylindrical, smooth- and thin-walled, 10–35 × 3–6 µm, periclinal thickening inconspicuous or absent; *aerial conidia* forming small false heads on the tips of the phialides, hyaline, ellipsoidal to obovoid, smooth- and thin-walled, 0–3-septate; 0-septate conidia: (6–)7–11(–16) × (2–)3–5(–6) µm (av. 9 × 4 µm); 1-septate conidia: (10–)11–15(–18) × 4–6 µm (av. 13 × 5 µm); 2-septate conidia: (15–)16–18(–19) × 4–5 µm (av. 17 × 5 µm); 3-septate conidia: (17–)18–24(–26) × 4–6 µm (av. 21 × 5 µm). *Sporodochia* pale luteous to pale salmon, formed sparsely on carnation leaves. *Sporodochial conidiophores* verticillately branched and densely packed, consisting of a short, smooth- and thin-walled stipe bearing apical whorls of 2–4 monophialides; *sporodochial phialides* subulate to subcylindrical, 10–25 × 3–5 µm, smooth- and thin-walled, sometimes showing a reduced and flared collarette. *Sporodochial conidia* falcate, mostly straight with dorsiventrally curved apical and basal cells, tapering towards both ends, with a blunt to papillate, curved apical cell and a blunt and distinctly foot-like basal cell, 3–5-septate, hyaline, smooth- and thin-walled; 3-septate conidia: (30–)34–40(–44) × 4–6 µm (av. 37 × 5 µm); 4-septate conidia: (33–)37–45(–50) × 4–6 µm (av. 41 × 5 µm); 5-septate conidia: (43–)47–55(–59) × 4–6(–7) µm (av. 51 × 5 µm). *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 75–85 mm at 24 °C after 7 d. Colony surface fulvous to ochreous in the centre becoming vinaceous to livid red towards the margin, with moderate aerial mycelium, dense, woolly to cottony. Odour absent. Reverse dark vinaceous to vinaceous. On SNA reaching 45–60 mm at 24 °C after 7 d, colonies membranous, greyish rose to rosy vinaceous, margin entire to undulate; reverse greyish rose to rosy vinaceous. On CLA, aerial mycelium sparse with abundant pale luteous to pale salmon sporodochia forming on the carnation leaves. On OA, colonies reaching 90 mm at 24 °C after 7 d, membranous to cottony, centre rosy vinaceous to greyish rose becoming honey to buff towards the margins; margins entire, reverse honey to buff.

Additional material examined: **Kuwait**, from soil, date unknown, *A.F. Moustafa*, CBS 491.77.

Notes: *Fusarium humicola* is closely related to *F. nelsonii* in the FCSC. *Fusarium nelsonii* produces more strongly curved and smaller sporodochial conidia (20–42 × 4–6 µm; Marasas *et al.* 1998) than those of *F. humicola* (30–59 × 4–6 µm overall).

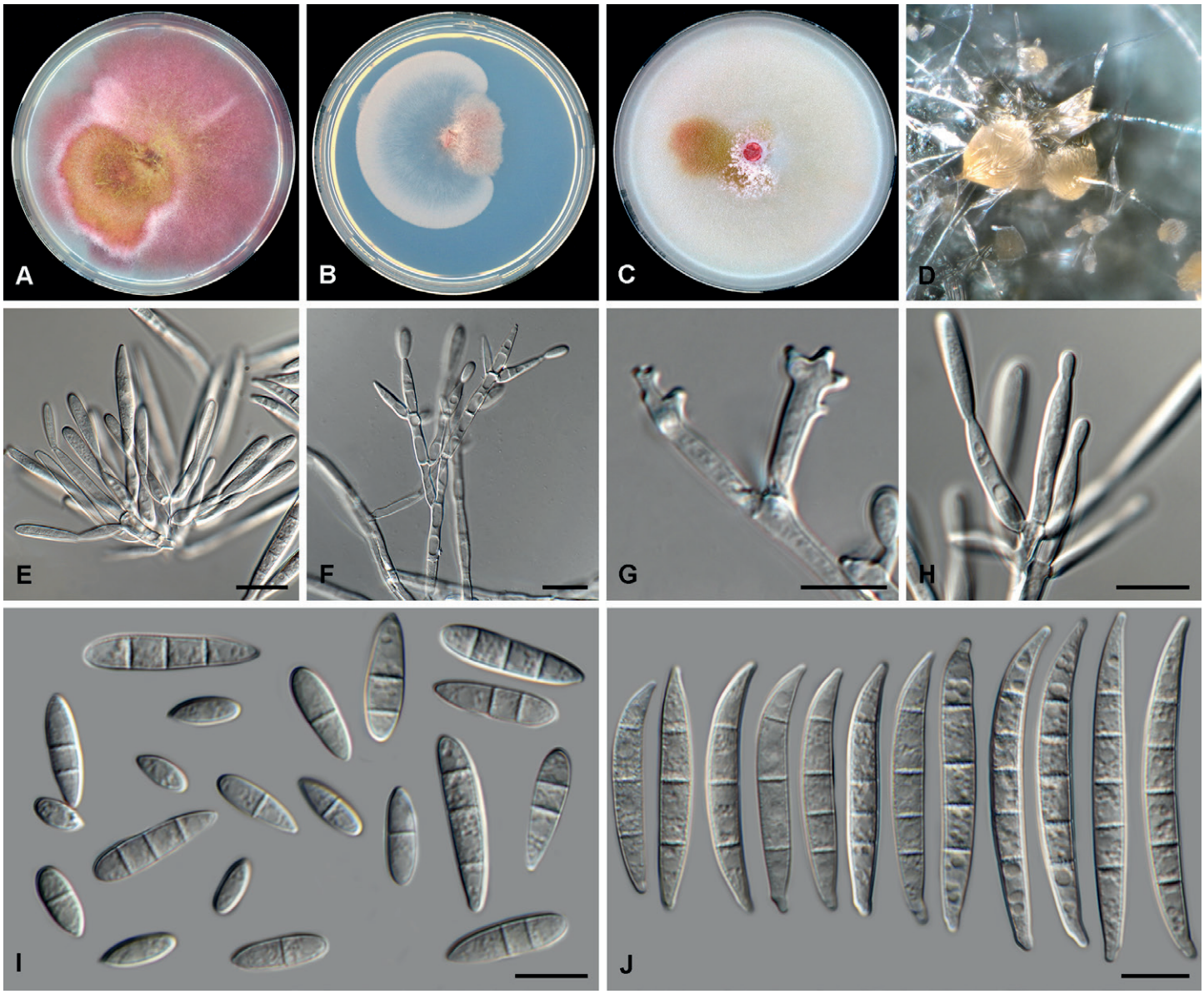


Fig. 3. *Fusarium humicola* (CBS 124.73). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Sporodochia on carnation leaf pieces. **E.** Sporodochial conidiophores. **F.** Conidiophores on aerial mycelium. **G.** Polyphialides. **H.** Monophialides. **I.** Aerial conidia. **J.** Sporodochial conidia. Scale bars = 10 µm.

Additionally, *F. humicola* did not produce any chlamydospores, even after 4 wk on SNA, whereas *F. nelsonii* produces these rapidly and abundantly (Leslie & Summerell 2006).

Fusarium microconidium L. Lombard & Crous, ***sp. nov.*** MycoBank MB831562. Fig. 4.

Etymology: Named after the only conidial form, microconidia (*i.e.* aerial conidia), produced in culture.

Diagnosis: Only producing 0–1-septate aerial conidia (*i.e.* microconidia) in culture and no sporodochial conidia (*i.e.* macroconidia) or chlamydospores.

Typus: **Unknown**, unknown collector, date and substrate, *deposited by W.F.O. Marasas* (**holotype** CBS-H 24017 designated here, culture ex-type CBS 119843 = MRC 8391 = KSU 11396).

Conidiophores borne on aerial mycelium, 20–40 µm tall, irregularly or sympodially branched or unbranched, bearing a terminal single phialide or whorl of 2–4 phialides; *aerial phialides* mono- and polyphialidic, subulate to subcylindrical, smooth- and thin-walled, 11–26 × 2–5 µm, periclinal thickening inconspicuous or absent; *monophialides* carried singly directly on aerial mycelium; *polyphialides* borne on branched conidiophores; *aerial conidia* forming small false heads on the tips of the phialides, hyaline, fusiform to ellipsoidal to obovoid, smooth- and thin-walled, 0–1-septate; 0-septate conidia: (6–)7–11(–13) × 4–5(–6) µm (av. 9 × 4 µm); 1-septate conidia: (11–)13–15(–16) × 4–6 µm (av. 14 × 5 µm). *Sporodochia* and *chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 90 mm at 24 °C after 7 d. Colony surface rose to rosy vinaceous to pale luteous in the centre, with abundant aerial mycelium, dense, woolly to cottony. Odour absent. Reverse livid red to dark vinaceous. On SNA, colonies membranous to woolly, white to pale rosy buff, with abundant sporulation on the surface giving a powdery appearance; reverse pale rosy buff. On CLA, aerial mycelium abundant, white, lacking sporodochia on the carnation leaf pieces. On OA, colonies membranous to cottony, white to buff with rosy flames towards margins, appearing wet.

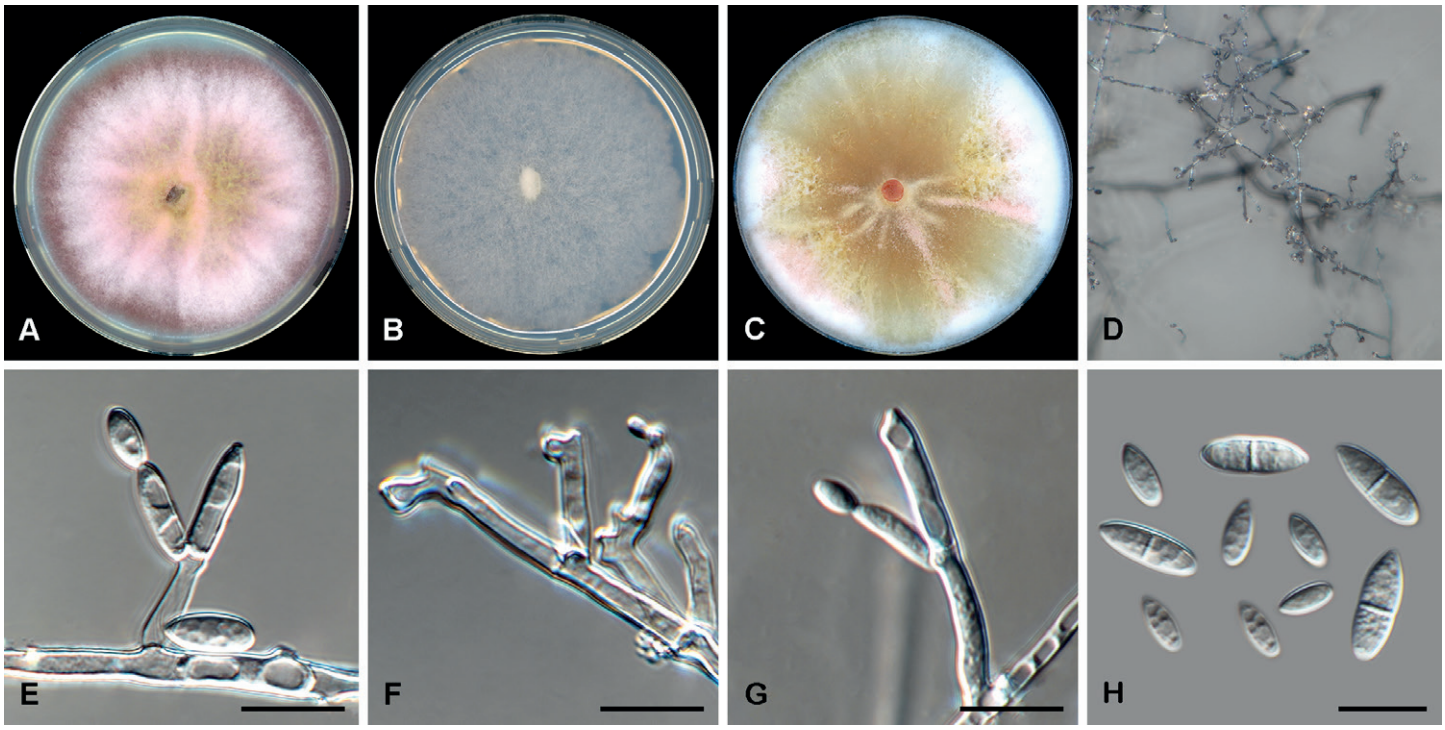


Fig. 4. *Fusarium microconidium* (CBS 119843). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Aerial mycelium with conidiophores on SNA. **E–G.** Mono- and polyphialides on aerial mycelium. **H.** Aerial conidia. Scale bars = 10 µm.

Notes: *Fusarium microconidium* represents a unique single lineage in the FCSC. This species is distinguished from other species in the FCSC based on the production of predominantly aseptate aerial conidia (*i.e.* microconidia) and lack of sporodochia and chlamydospores.

Fusarium nodosum L. Lombard & Crous, ***sp. nov.*** MycoBank MB831653. Fig. 5.

Etymology: Named after the knotted appearance of the polyphialidic aerial conidiophores.

Diagnosis: Rarely producing globose aerial conidia (microconidia).

Typus: **Portugal**, Lisbon, stored seed of *Arachis hypogaea*, 19 Dec. 1961, *C.M. Baeta Neves* (**holotype** CBS-H 24018 designated here, culture ex-type CBS 201.63).

Conidiophores borne on aerial mycelium, 10–65 µm tall, irregularly or sympodially branched or rarely unbranched, bearing a terminal single phialide or whorl of 2–4 phialides; *aerial phialides* mono- and polyphialidic, subulate to subcylindrical, smooth- and thin-walled, 10–22 × 3–4 µm, periclinal thickening inconspicuous or absent; *aerial conidia* forming small false heads on the phialide tips, hyaline, ellipsoidal to obovoid, rarely globose, smooth- and thin-walled, 0–1-septate; 0-septate conidia: (6–)9–13(–15) × 4–5 µm (av. 11 × 4 µm); 1-septate conidia: (11–)13–19(–21) × 2–4 µm (av. 16 × 5 µm). *Sporodochia* pale luteous to pale orange, formed abundantly on carnation leaves. *Sporodochial conidiophores* verticillately branched and densely packed, consisting of a short, smooth- and thin-walled stipe bearing apical whorls of 2–4 monophialides; *sporodochial phialides* subulate to subcylindrical, 10–21 × 3–5 µm, smooth- and thin-walled, sometimes showing a reduced and flared collarette. *Sporodochial conidia* falcate, curved dorsiventrally, broadening in the upper third, tapering towards both ends, with a blunt to papillate, curved apical cell and a blunt and distinctly foot-like basal cell, (1–)3–5-septate, hyaline, smooth- and thin-walled; 1-septate conidia: (24–)26–36(–38) × 4–6 µm (av. 31 × 5 µm); 2-septate conidia: (21–)24–30(–32) × 4–6 µm (av. 27 × 5 µm); 3-septate conidia: (26–)28–36(–40) × 5–7 µm (av. 32 × 6 µm); 4-septate conidia: (34–)36–42(–50) × (4–)5–7 µm (av. 39 × 6 µm); 5-septate conidia: (37–)40–44(–47) × 5–7 µm (av. 42 × 6 µm). *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 90 mm at 24 °C after 7 d. Colony surface rose to rosy vinaceous to sulphur yellow, with abundant aerial mycelium, dense, woolly to cottony. Odour absent. Reverse livid red to rose. On SNA, colonies membranous to woolly, white to pale rosy buff, with abundant sporulation on the surface giving a powdery appearance; reverse pale rosy buff. On CLA, aerial mycelium sparse with abundant pale luteous to pale orange sporodochia forming on the carnation leaves. On OA, colonies membranous to cottony, white to rosy buff, with abundant sporulation on substrate giving a powdery appearance.

Additional materials examined: **France**, Cassis, stem of *Arundo donax*, Oct. 1974, *W. Gams*, CBS 698.74. **Iran**, Golestan, Kalaleh, from wheat, *M. Davari*, CBS 131779. **Portugal**, Lisabon, stored seed of *Arachis hypogaea*, 19 Dec. 1961, *C.M. Baeta Neves*, CBS 200.63. **Unknown** locality, substrate and date, *W.F.O. Marasas*, CBS 119844 = BBA 62170 = MRC 1798.

Notes: *Fusarium nodosum* is closely related to *F. armeniacum*, *F. langsethiae, F. sibiricum* and *F. sporotrichioides* in the FSAMSC. *Fusarium armeniacum* characteristically does not produce polyphialidic conidiogenous cells (Burgess *et al*. 1993), distinguishing this species from *F. nodosum*. The remaining three species readily produce abundant globose aerial conidia (*i.e.* microconidia), which were rarely seen for *F. nodosum*.

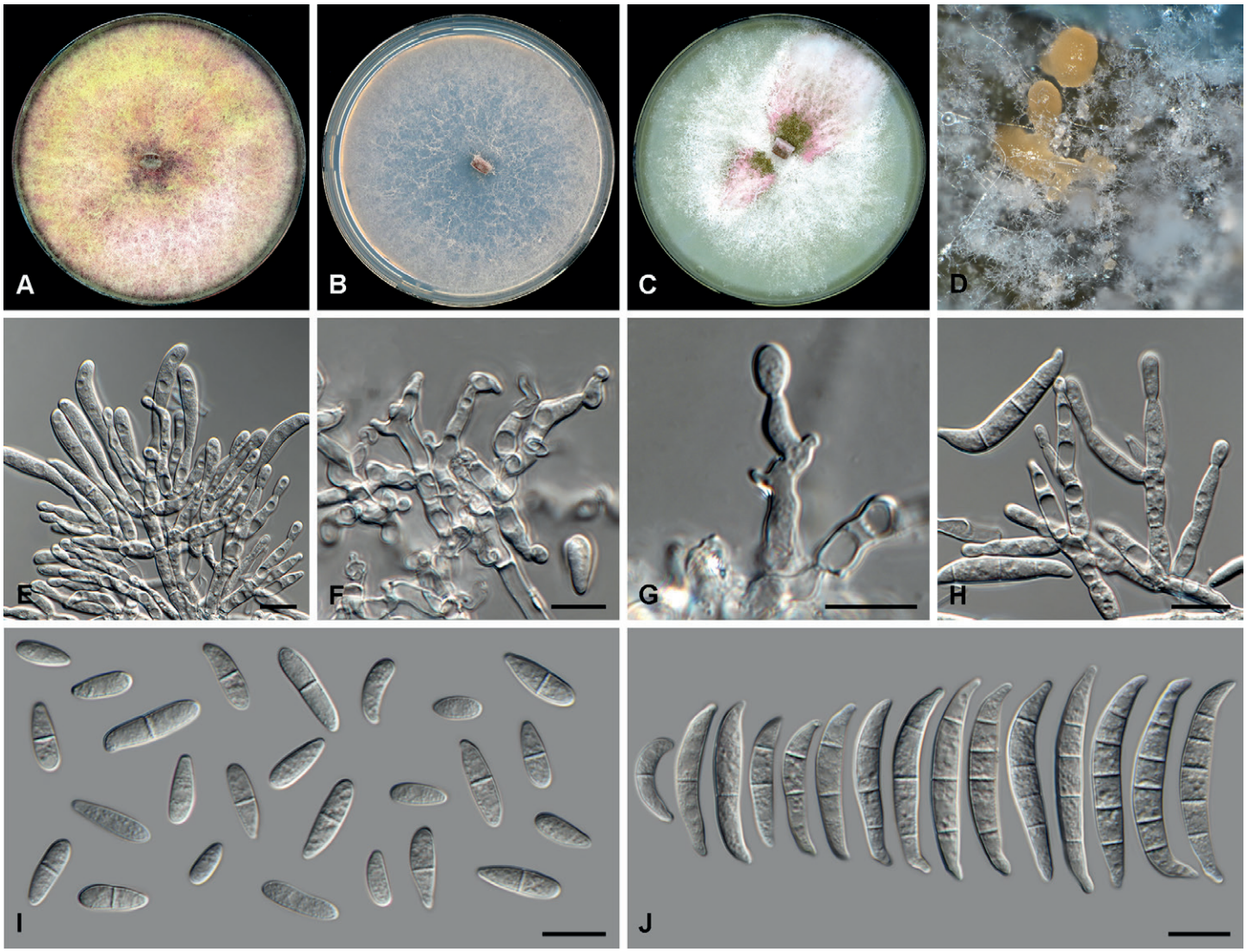


Fig. 5. *Fusarium nodosum* (CBS 201.63). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Sporodochia on carnation leaf pieces. **E.** Sporodochial conidiophores. **F, G.** Polyphialides on aerial mycelium. **H.** Monophialides on aerial mycelium. **I.** Aerial conidia. **J.** Sporodochial conidia. Scale bars = 10 µm.

Fusarium peruvianum L. Lombard & Crous, ***sp. nov.*** MycoBank MB831564. Fig. 6.

Etymology: Named after Peru, from where this fungus was collected.

Diagnosis: Producing both falcate (*i.e.* macroconidia) and ellipsoidal to obovoid (*i.e.* microconidia) aerial conidia on predominantly polyphialidic conidiogenous cells borne on aerial mycelium, lacking sporodochia, but readily producing chlamydospores.

Typus: **Peru**, from *Gossypium* sp. seedling, date unknown, *J.H. van Emden* (**holotype** CBS-H 24019 designated here, culture ex-type CBS 511.75).

Conidiophores borne on aerial mycelium, 10–85 µm tall, irregularly or sympodially branched, rarely unbranched, bearing a terminal whorl of 2–4 phialides; *aerial phialides* polyphialidic, rarely monophialidic, subulate to subcylindrical, smooth- and thin-walled, 14–28 × 2–5 µm, periclinal thickening inconspicuous or absent; *aerial conidia* forming small false heads on the tips of the phialides, hyaline, smooth- and thin-walled, of two types: (a) ellipsoidal to obovoid, 0–3(–4)-septate; 0-septate conidia: (9–)10–14(–15) × (3–)4–6 µm (av. 12 × 5 µm); 1-septate conidia: (12–)13–17(–19) × 4–6 µm (av. 15 × 5 µm); 2-septate conidia: 17–21(–24) × 5–7 µm (av. 19 × 6 µm); 3-septate conidia: (18–)19–23(–26) × (5–)6(–7) µm (av. 21 × 6 µm); 4-septate conidia: 28 × 6 µm; (b) falcate, fusiform to falcate, straight or gently dorsiventrally curved, with an indistinct papillate to notched basal cell, 3–4(–5)-septate; 3-septate conidia: (29–)33–39(–41) × 4–6 µm (av. 36 × 5 µm); 4-septate conidia: (32–)37–45(–51) × 4–6 µm (av. 41 × 5 µm); 5-septate conidia: (40–)41–49(–50) × 5–6 µm (av. 45 × 5 µm). *Sporodochia* not observed. *Chlamydospores* abundant, formed singly or in pairs, carried terminally or intercalarily, globose to subglobose, 10–25 µm diam, thick-walled, smooth to slightly verrucose.

Culture characteristics: Colonies on PDA reaching 90 mm at 24 °C after 7 d. Colony surface fulvous to ochreous in the centre becoming coral to vinaceous towards the margin, with abundant aerial mycelium, dense, woolly to cottony, sometimes granular due to abundant sporulation on medium surface. Odour absent. Reverse livid red to dark vinaceous. On SNA, colonies membranous

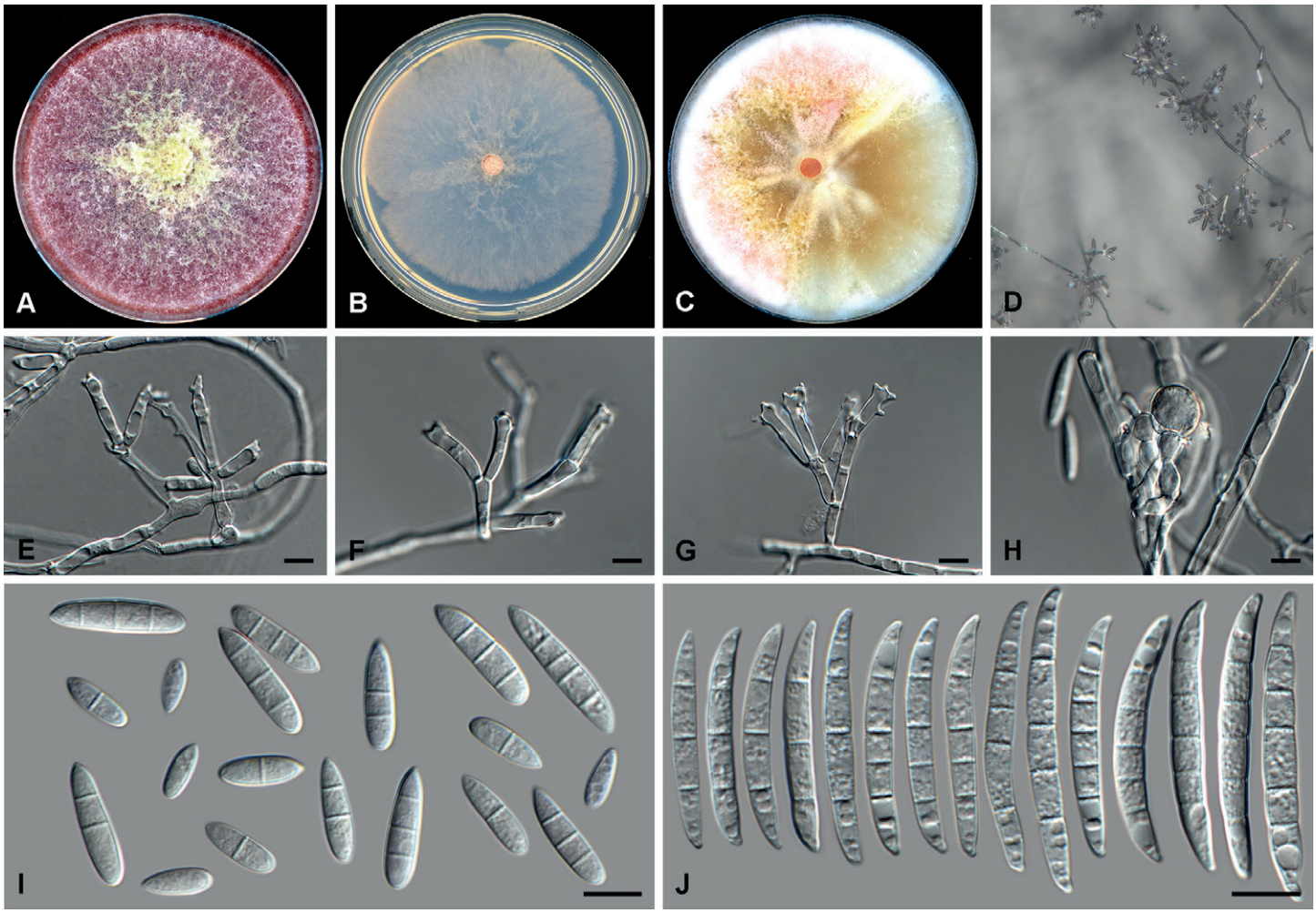


Fig. 6. *Fusarium peruvianum* (CBS 511.75). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Aerial mycelium with conidiophores on SNA. **E–G.** Mono- and polyphialides on aerial mycelium. **H.** Chlamydospores. **I.** Ellipsoidal to obovoid aerial conidia. **J.** Falcate aerial conidia. Scale bars = 10 µm.

to woolly, white, with abundant sporulation on the surface giving a powdery appearance; reverse colourless. On CLA, white aerial mycelium abundant, lacking sporodochia on carnation leaves. On OA, colonies cottony, ochreous to luteous in the centre with pale rosy vinaceous to rose flames, with abundant sporulation on substrate giving a powdery appearance.

Notes: *Fusarium peruvianum* represents the second unique single lineage in the FCSC. This species can be distinguished from other species in the FCSC based on the formation of falcate aerial conidia (*i.e.* macroconidia) on all substrates examined. Furthermore, *F. peruvianum* produced 4-septate obovoid aerial conidia (*i.e.* microconidia), a characteristic not observed for any of the other species in the FCSC studied here.

Fusarium spinosum L. Lombard, Houbraken & Crous, ***sp. nov.*** MycoBank MB831565. Fig. 7.

Etymology: Name refers to the "thorny" appearance of the polyphialides borne on the aerial mycelium.

Diagnosis: Only producing 3-septate, falcate aerial conidia (*i.e.* macroconidia) in culture, lacking sporodochia.

Typus: **Brazil**, from *Galia melon* imported into the Netherlands, 2007, *J. Houbraken* (**holotype** CBS-H 24020 designated here, culture ex-type CBS 122438).

Conidiophores borne on aerial mycelium 8–55 µm tall, irregularly or sympodially branched or unbranched, bearing a lateral single phialide or terminal whorl of 2–4 phialides; *aerial phialides* mono- and polyphialidic, subulate to subcylindrical, smooth- and thin-walled, 10–35 × 3–6 µm, periclinal thickening inconspicuous or absent; *monophialides* carried singly directly on aerial mycelium; *polyphialides* borne on branched conidiophores; *aerial conidia* forming small false heads on the tips of the phialides, hyaline, of two types: (a) fusiform to ellipsoidal to obovoid, straight to slightly curved, smooth- and thin-walled, 0–3-septate; 0-septate conidia: 11–17(–21) × 3–5 µm (av. 14 × 4 µm); 1-septate conidia: (12–)13–19(–24) × 3–5 µm (av. 16 × 4 µm); 2-septate conidia: (17–)18–22(–28) × 4–6 µm (av. 20 × 5 µm); 3-septate conidia: (19–)20–22(–29) × 4–6 µm (av. 21 × 5 µm); (b) falcate, slightly dorsiventrally curved, 3-septate, with an indistinct papillate to notched basal cell, (22–)24–32(–36) × 4–6 µm (av. 28 × 5 µm). *Sporodochia* not observed. *Chlamydospores* abundant, globose to subglobose, thick-walled, smooth to slightly verrucose, 12–24 µm diam, borne terminally or carried intercalarily, single or in chains.

Culture characteristics: Colonies on PDA reaching 90 mm at 24 °C after 7 d. Colony surface rose to rosy vinaceous to pale luteous in the centre, with abundant aerial mycelium, dense, woolly to cottony. Odour absent. Reverse fulvous to ochreous with rosy vinaceous flames. On SNA, colonies membranous to woolly, white to pale rosy buff, with abundant sporulation on

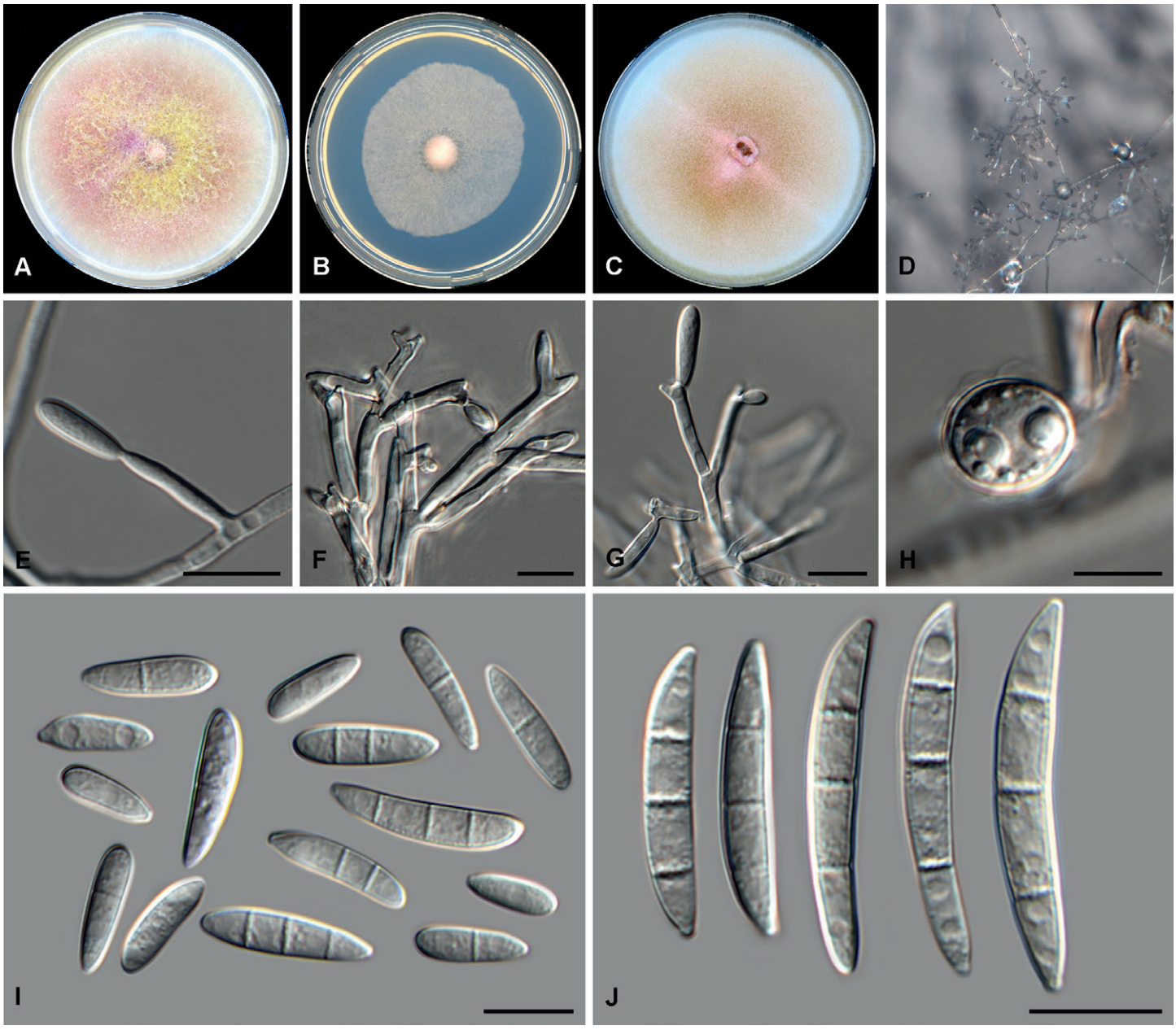


Fig. 7. *Fusarium spinosum* (CBS 122438). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Aerial mycelium with conidiophores on SNA. **E.** Monophialide on aerial mycelium. **F, G.** Polyphialides on aerial mycelium. **H.** Chlamydospore. **I.** Ellipsoidal to obovoid aerial conidia. **J.** Falcate aerial conidia. Scale bars = 10 µm.

the surface giving a powdery appearance; reverse pale rosy buff. On CLA, aerial mycelium abundant, white, lacking sporodochia on the carnation leaf pieces. On OA, colonies membranous to cottony, white to buff with rosy flames towards margins, with powdery appearance due to abundant sporulation on medium surface.

Notes: *Fusarium spinosum* represents the FCSC 3 *sensu* O'Donnell *et al*. (2009). This species is distinguished from other species in the FCSC by only forming 3-septate, falcate aerial conidia (*i.e.* macroconidia).

Fusarium sporodochiale L. Lombard & Crous, ***sp. nov.*** MycoBank MB831566. Fig. 8.

Etymology: Named after the abundant sporodochia this species produces on carnation leaf pieces.

Diagnosis: Producing up to 10-septate sporodochial conidia (*i.e.* macroconidia) and aseptate, rarely 1-septate aerial conidia (*i.e.* microconidia).

Typus: **South Africa**, Gauteng, Johannesburg, from soil, 29 May 1955, *D. Ordman* (**holotype** CBS H-12681 designated here, culture ex-type CBS 220.61 = ATCC 14167 = MUCL 8047 = NRRL 20842).

Conidiophores borne on aerial mycelium, 10–35 µm tall, irregularly or sympodially branched or unbranched, bearing a lateral single phialide or terminal whorl of 2–4 phialides; *aerial phialides* polyphialidic, rarely monophialidic, subulate to subcylindrical, smooth- and thin-walled, 11–23 × 2–4 µm, periclinal thickening inconspicuous or absent; *aerial conidia* forming small false heads on the tips of the phialides, hyaline, fusiform to ellipsoidal to obovoid, smooth- and thin-walled, aseptate, rarely 1-septate; 0-septate conidia: (7–)8–12(–13)

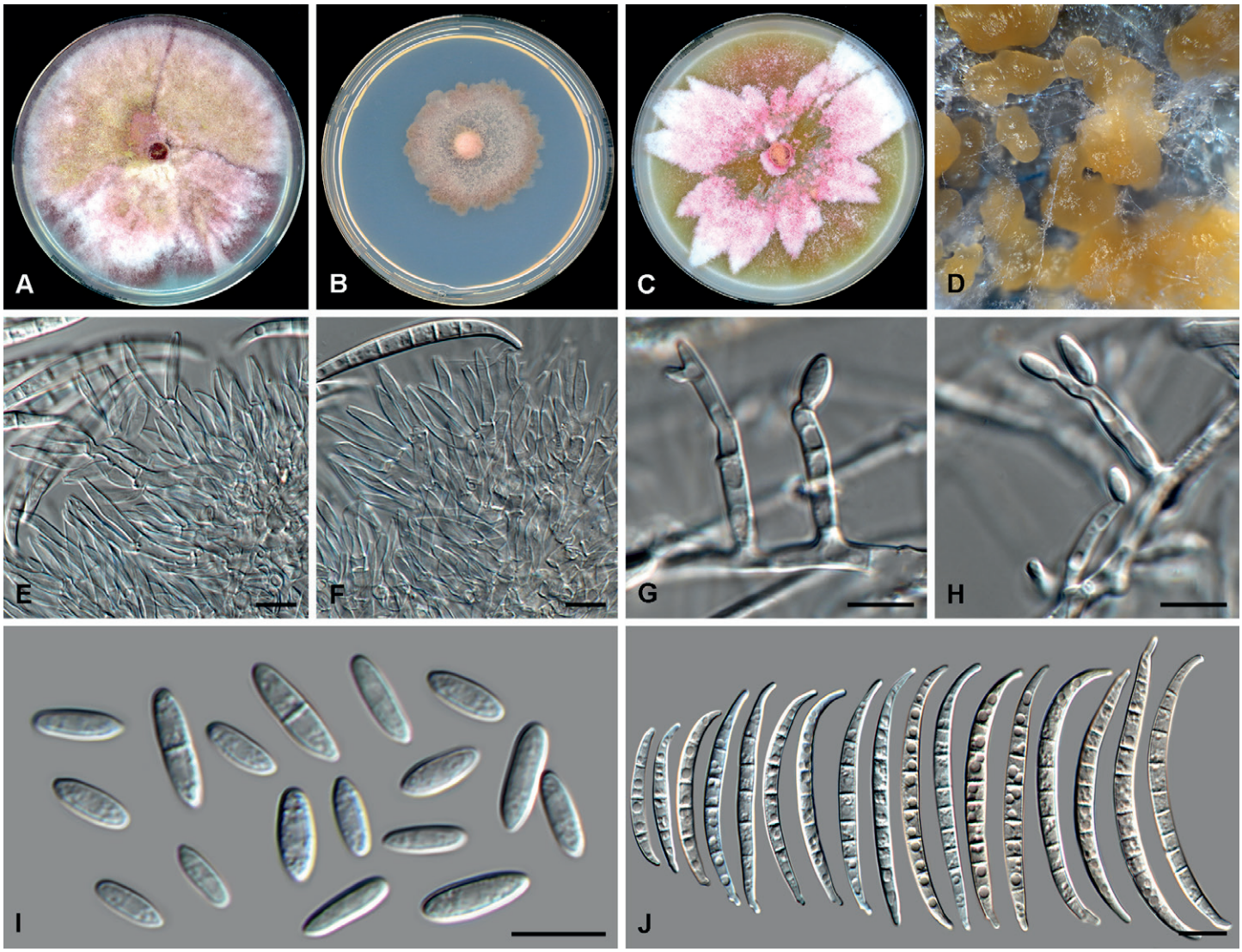


Fig. 8. *Fusarium sporodochiale* (CBS 220.61). **A.** Colony on PDA. **B.** Colony on SNA. **C.** Colony on OA. **D.** Sporodochia on carnation leaf pieces. **E, F.** Sporodochial conidiophores. **G, H.** Mono- and polyphialides on aerial mycelium. **I.** Aerial conidia. **J.** Sporodochial conidia. Scale bars = 10 µm.

× 2–4(–5) µm (av. 10 × 3 µm); 1-septate conidia: 11–17(–21) × 3–5 µm (av. 14 × 3 µm). *Sporodochia* pale luteous to pale orange, formed abundantly on carnation leaves and on media surfaces. *Sporodochial conidiophores* verticillately branched and densely packed, consisting of a short, smooth- and thin-walled stipe bearing apical whorls of 2–4 monophialides; *sporodochial phialides* subulate to subcylindrical, 11–25 × 2–4 µm, smooth- and thin-walled, sometimes showing a reduced and flared collarette. *Sporodochial conidia* falcate, slightly to strongly dorsiventrally curved, tapering towards both ends, with an elongated, strongly curved apical cell and a blunt and distinct foot-like basal cell, (1–)5–6(–10)-septate, hyaline, smooth- and thin-walled; 3-septate conidia: (31–)32–40(–42) × 4–5 µm (av. 36 × 4 µm); 4-septate conidia: (38–)41–49(–53) × 3–5 µm (av. 45 × 5 µm); 5-septate conidia: (45–)50–58(–61) × 4–6(–7) µm (av. 54 × 5 µm); 6-septate conidia: (51–)54–63(–71) × 4–6 µm (av. 59 × 5 µm); 7-septate conidia: (52–)56–66(–72) × 4–6 µm (av. 61 × 5 µm) ; 8-septate conidia: (56–)57–63(–72) × 4–6 µm (av. 61 × 5 µm). *Chlamydospores* not observed.

Culture characteristics: Colonies on PDA reaching 85–90 mm at 24 °C after 7 d. Colony surface rose to rosy vinaceous to sulphur yellow, with abundant aerial mycelium, dense, woolly to cottony. Odour absent. Reverse livid red to dark vinaceous. On SNA, colonies woolly, surface and reverse pale rosy buff. On CLA, aerial mycelium sparse with abundant pale luteous to pale orange sporodochia forming on the carnation leaves and surrounding medium surface. On OA, colonies membranous with cottony, rosy buff flames of aerial mycelium, with abundant sporulation.

Additional material examined: **Germany**, Berlin, from a termitary, date unknown, *W. Kerner*, CBS 199.63 = MUCL 6771.

Notes: *Fusarium sporodochiale* is a morphologically unique member of the FCSC, as this species can produce up to 10-septate sporodochial conidia (*i.e.* macroconidia). Additionally, the apical cell of the sporodochial conidia of *F. sporodochiale* is more elongated than those noted for *F. chlamydosporum* (Leslie & Summerell 2006) or any other species in this complex. A unique feature of this species is the abundance of sporodochia formed, not only on the carnation leaf pieces, but also on the medium surface.

DISCUSSION

A key component of modern taxonomic studies of the genus *Fusarium* is multilocus phylogenetic inference due to the numerous cryptic species now known to be present in the various species complexes. Therefore, the availability of type material plays a vital role in providing stability to a dynamic taxonomic system as is seen in *Fusarium* literature today. The FCSC is no exception as at least four unnamed phylo-species have been identified in the past (O'Donnell *et al*. 2009, 2018), which were initially identified as *F. chlamydosporum*.

Phylogenetic inference in this study resolved four additional phylo-species to the five already resolved by O'Donnell *et al*. (2009, 2018), of which three could be provided with names (*F. humicola*, *F. microconidium* and *F. peruvianum*) here, and one single lineage (NRRL 13338) initially treated as *F. nelsonii* (O'Donnell *et al*. 2009), remaining to be named. Neotypification of *F. chlamydosporum* in this study has allowed us to provide names for the remaining unnamed phylo-species: FCSC 1 = *F. chlamydosporum*; FCSC 2 = *F. atrovinosum*; FCSC 3 = *F. spinosum*; FCSC 5 = *F. sporodochiale*.

The ex-neotype strain (CBS 145.25) of *F. chlamydosporum* was found in this study to have deteriorated since 1925, and produced only a few aerial conidia (*i.e.* microconidia) on CLA, and none on PDA, SNA or OA. The same was observed for strains CBS 615.87 and CBS 677.77, indicating that strains of this species could deteriorate quickly during long-term storage. Booth (1971) also studied the (now) ex-neotype of *F. chlamydosporum* and concluded that this species is a *nomen confusum* as he was unable to distinguish it from *F. camptoceras* at that time. Gerlach & Nirenberg (1982) accepted *F. chlamydosporum* as a distinct species and rejected Booth's (1971) argument. However, Marasas *et al*. (1998) provided an emended description for *F. camptoceras*, clearly distinguishing it from *F. chlamydosporum*. The *F. chlamydosporum* clade (FCSC 1) included for the most part clinical isolates, but also isolates obtained from plants (banana and taro), thrips and soil (Table 1), indicating that this species has a broad ecological range. The remaining clinical isolates clustered in the *F. atrovinosum* (eight isolates) and *F. spinosum* (one isolate) clades. Both these latter species also included isolates obtained from plants and soil, reflective of a possible broader ecological range. The number of clinical isolates in each of these three species may not be a true reflection of their ecology, as this only represents the sample of sequence data available in public databases such as GenBank, FUSARIUM-ID and *Fusarium* MLST.

Isolates CBS 511.75, CBS 119843 and NRRL 13338 were resolved as single lineages in this study. All three these single lineages were also resolved in the individual analyses of the four loci used in this study (results not shown). Therefore, we introduced the names *F. microconidium* (CBS 119843) and *F. peruvianum* (CBS 511.75) for two of these single lineages, with a name pending for NRRL 13338 following morphological analysis.

Pairwise sequence comparisons of the *tef1* and *rpb2* sequences of MRC 35 (MH582448 & MH582208, respectively) and MRC 117 (MH582447 & MH 582074, respectively), identified by O'Donnell *et al*. (2018) as FCSC 5, with those of the ex-type of *F. sporodochiale* (CBS 220.61) showed 99 % sequence similarity for both loci compared to the 96 % similarity found with the neo/ex-type isolates of *F. atrovinosum* (CBS 445.67), *F. chlamydosporum* (CBS 145.25) and *F. spinosum* (CBS 122438), which were the closest phylogenetic neighbours. Therefore, we are able to link both CBS 220.61 and CBS 199.63 to FCSC 5 in this study. The *tef1* and *rpb2* sequences for both MRC 35 and MRC 117 were not available at the time, and could therefore not be included in this study.

To our knowledge, the ex-type strain of *F. chlamydosporum var. fuscum* (CBS 635.76; Gerlach 1977) has not yet been included in any phylogenetic study until now. However, it was surprising to observe its placement in the FIESC, clustering with CBS 430.81, an isolate known to represent the phylo-species FIESC 28 (O'Donnell *et al*. 2009). As no Latin name has yet been assigned to FIESC 28, we decided to raise this variety to species level with a new name, *F. coffeatum*. Two additional isolates preserved as *F. chlamydosporum* in the CBS culture collection also clustered within the FIESC. Isolate CBS 127131 proved to belong in the *F. lacertarum* clade, whereas CBS 101138 clustered within the FIESC 24 clade (O'Donnell *et al*. 2009). Both these isolates failed to produce sporodochia on CLA under UV-illumination, but produced abundant aerial conidia (*i.e.* microconidia), chlamydospores and a dark red pigmentation on the various media used here, similar to those associated with *F. chlamydosporum*. These characteristics probably resulted in the erroneous identification of these isolates.

Several isolates also clustered within the FSAMSC, with CBS 462.94 falling within the *F. sporotrichioides* clade. This isolate also failed to produce sporodochia on CLA but produced abundant aerial conidia (*i.e.* microconidia) and the characteristic red pigment in culture. However, no chlamydospores were observed. Either this isolate has been misidentified or became contaminated with *F. sporotrichioides* over time. The remaining four "*F. chlamydosporum*" isolates (CBS 200.63, CBS 201.63, CBS 698.74 & CBS 119844) formed a highly supported clade, distinct from the *F. armeniacum*, *F. langsethiae*, *F. sibiricum* and *F. sporotrichioides* clades, and were named as *F. nodosum*. The *F. nodosum* clade also included an isolate (CBS 131779) previously identified as *F. sporotrichioides* (Davari *et al*. 2013). It is not clear why these isolates were initially preserved in the CBS culture collection under the name *F. chlamydosporum*. The most noticeable overlapping character observed for these isolates with *F. chlamydosporum*, was the production of dark red pigments on PDA. These isolates all readily produced abundant sporodochia on CLA and no chlamydospores were found.

The FCSC now includes nine phylo-species, for which eight were provided with Latin binomials in this study. Although subtle morphological differences could be found among these eight newly named taxa, phylogenetic inference using the recommended *Fusarium* identification gene regions *rpb1, rpb2* and *tef1* should be used for accurate identification (O'Donnell *et al*. 2015).

ACKNOWLEDGEMENTS

The authors thank the technical staff, A. van Iperen, D. Vos-Kleyn and Y. Vlug for their valuable assistance with cultures.

REFERENCES

Al-Hatmi AMS, Hagen F, Menken SBJ, *et al*. (2016). Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to 2015. *Emerging Microbes & Infections* **5**: e124.

Aoki T, Vaughan MM, McCormick SP, *et al*. (2015). *Fusarium dactylidis sp. nov.*, a novel nivalenol toxin-producing species sister to *F. pseudograminearum* isolated from orchard grass (*Dactylis glomerata*) in Oregon and New Zealand. *Mycologia* **107**: 409–418.

Azor M, Gené J, Cano J, *et al*. (2009). Less-frequent *Fusarium* species of clinical interest: correlation between morphological and molecular identification and antifungal susceptibility. *Journal of Clinical Microbiology* **47**: 1463–1468.

Booth C (1971). *The genus* Fusarium. *Commonwealth Mycological Institute, Kew, Surrey, UK*.

Brown DW, Proctor RH (2016). Insights into natural products biosynthesis from analysis of 490 polyketide synthases from *Fusarium*. *Fungal Genetics and Biology* **89**: 37–51.

Burgess LW, Forbes GA, Windels C, *et al.* (1993). Characterization and distribution of *Fusarium acuminatum* subsp. *armeniacum subsp. nov*. *Mycologia* **15**: 119–124.

Burgess LW, Summerell BA (1992). Mycogeography of *Fusarium*: survey of *Fusarium* species in subtropical and semi-arid grassland soils from Queensland, Australia. *Mycological Research* **96**: 780–784.

Crous PW, Verkley GJM, Groenewald JZ, *et al.* (*eds*.) (2019). *Fungal Biodiversity*. Westerdijk Laboratory Manual Series. Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Davari M, Wei SH, Babay-Ahari A, *et al*. (2013). Geographic differences in trichothecene chemotypes of *Fusarium graminearum* in the northwest and north of Iran. *World Mycotoxin Journal* **6**: 137–150.

Du YX, Chen FR, Shi NN, *et al*. (2017). First report of *Fusarium chlamydosporum* causing banana crown rot in Fujian Province, China. *Plant Disease* **101**: 1048.

Engelbrecht MC, Smit WA, Knox-Davies PS (1983). Damping-off of rooibos tea, *Aspalathus linearis*. *Phytophylactica* **15**: 121–124.

Fisher NL, Burgess LW, Toussoun TA, *et al.* (1982). Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* **72**: 151–153.

Fugro PA (1999) A new disease of okra (*Abelmoschus esculentus* L.) in India. *Journal of Mycology and Plant Pathology* **29**: 264.

Geiser DM, Jiménez-Gasco M del M, Kang S, *et al*. (2004). FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology* **110**: 473–479.

Gerlach W (1977). Drei neue varietäten von *Fusarium merismoides, F. larvarum* und *F. chlamydosporum. Phytopathologische Zeitschrift* **90**: 31–42.

Gerlach W, Nirenberg H (1982). The genus *Fusarium* – a pictorial atlas. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* **209**: 1–406.

Hillis DM, Bull JJ (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.

Kanaan YM, Bahkali AH (1993). Frequency and cellulolytic activity of seed-borne *Fusarium* species isolated from Sausi Arabian cereal cultivars. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **100**: 291–298.

Katoh K, Rozewicki J, Yamada KD (2017). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* doi: 10.1093/bib/bbx108.

Kearse M, Moir R, Wilson A, *et al.* (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Kiehn TE, Nelson PE, Bernard EM, *et al*. (1985). Catheter-associated fungemia caused by *Fusarium chlamydosporum* in a patient with lymphocytic lymphoma. *Journal of Clinical Microbiology* **21**: 501–504.

Kozlov AM, Darriba D, Flouri T, *et al*. (2018). RaxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *bioRxiv* doi: http://dx.doi.org/10.1101/447110.

Kluger EK, Della Torre PK, Martin P, *et al*. (2004). Concurrent *Fusarium chlamydosporum* and *Microsphaeropsis arundinis* infections in a cat. *Journal of Feline Medicine and Surgery* **6**: 271–277.

Laurence MH, Walsh JL, Shuttleworth LA, *et al*. (2016). Six novel species of *Fusarium* from natural ecosystems in Australia. *Fungal Diversity* **77**: 349–366.

Lazreg F, Belabid L, Sanchez J, *et al.* (2013). First report of *Fusarium chlamydosporum* causing damping-off disease on Aleppo pine in Algeria. *Plant Disease* **97**: 1506.

Leslie JF, Summerell BA (2006). *The* Fusarium *Laboratory Manual*. Blackwell Publishing, Ames.

Lombard L, Sandoval-Denis M, Lamprecht S, *et al.* (2019). Epitypification of *Fusarium oxysporum* – clearing the taxonomic chaos. *Persoonia* **43**: 1–47.

Marasas WFO, Nelson PE, Toussoun TA (1984). *Toxigenic* Fusarium *species: Identity and mycotoxicology*. The Pennsylvania State University Press, University Park, Pennsylvania.

Marasas WFO, Rheeder JP, Logrieco A, *et al*. (1998). *Fusarium nelsonii* and *F. musarum*: Two new species in section *Arthrosporiella* related to *F. camptoceras*. *Mycologia* **90**: 505–513.

Martino P, Gastaldi R, Raccah R, *et al*. (1994). Clinical patterns of *Fusarium* infections in immunocompromised patients. *Journal of Infection* **28**: 7–15.

Mathivanan N, Kabilan V, Murugesan K (1998). Purification, characterization, and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccinia arachidis. Canadian Journal of Microbiology* **44**: 646–651.

Nirenberg HI (1976). Unterschungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. *Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* **169**: 1–117.

Nirenberg HI (1990). Recent advances in the taxonomy of *Fusarium*. *Studies in Mycology* **32**: 91–101.

Nylander JAA (2004). *MrModeltest v. 2.* Programme distributed by the author. Evolutionary Biology Centre, Uppsala University.

O'Donnell K, Sarver BAJ, Brandt M, *et al*. (2007). Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *Journal of Clinical Microbiology* **45**: 2235–2248.

O'Donnell K, Gueidan C, Sink S, *et al.* (2009). A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology* **46**: 936–948.

O'Donnell K, Humber RA, Geiser DM, *et al*. (2012). Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium MLST*. *Mycologia* **104**: 427–445.

O'Donnell K, Kistler HC, Cigelnik E, *et al*. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 2044–2049.

O'Donnell K, Kistler HC, Tacke BK, *et al.* (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus

causing wheat scab. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 7905–7910.

O'Donnell K, McCormick SP, Busman M, *et al*. (2018). Marasas *et al*. 1984 "Toxigenic *Fusarium* species: Identity and mycotoxicology" revisited. *Mycologia* **110**: 1058–1080.

O'Donnell K, Rooney AP, Proctor RH, *et al*. (2013). Phylogenetic analyses of *RPB1* and *RPB2* support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genetics and Biology* **52**: 20–31.

O'Donnell K, Sutton DA, Rinaldi MG, *et al.* (2009). Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology* **47**: 3851–3861.

O'Donnell K, Sutton DA, Rinaldi MG, *et al.* (2010). Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. *Journal of Clinical Microbiology* **48**: 3708–3718.

O'Donnell K, Ward TJ, Robert VARG, *et al*. (2015). DNA sequence-based identification of *Fusarium*: Current status and future directions. *Phytoparasitica* **43**: 583–595.

Proctor RH, McCormick SP, Alexander NJ, *et al*. (2009). Evidence that a secondary metabolic biosynthetic gene cluster has grown by gene relocation during evolution of the filamentous fungus *Fusarium*. *Molecular Microbiology* **74**: 1128–1142.

Pujol I, Guarro J, Gené J, *et al*. (1997). *In vitro* antifungal susceptibility of clinical and environmental *Fusarium* spp. strains. *Journal of Antimicrobial Chemotherapy* **39**: 163–167.

Qin Y, He H, Li N, *et al*. (2010). Isolation and characterization of a thermostable cellulase-producing *Fusarium chlamydosporum. World Journal of Microbiology and Biotechnology* **26**: 1991–1997.

Rabie CJ, Lübben A, Louw AI, *et al.* (1978). Moniliformin, a mycotoxin from *Fusarium fusarioides*. *Journal of Agricultural and Food Chemistry* **26**: 375–379.

Rabie CJ, Marasas WFO, Thiel PG, *et al*. (1982). Moniliformin production and toxicity of different *Fusarium* species from southern Africa. *Applied and Environmental Microbiology* **43**: 517–521.

Rayner RW (1970). A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, UK.

Reinking OA, Wollenweber HW (1927). Tropical fusaria. *The Philippine Journal of Science* **32**: 103–253.

Rocha LO, Laurence MH, Proctor RH, *et al*. (2015). Variation in type A trichothecene production and trichothecene biosynthetic genes in *Fusarium goolgardi* from natural ecosystems of Australia. *Toxins* **7**: 4577–4594.

Ronquist F, Huelsenbeck JP (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Sangalang AE, Burgess LW, Backhouse D, *et al*. (1995). Mycogeography of *Fusarium* species in soils from tropical, arid and Mediterranean regions of Australia. *Mycological Research* **99**: 523–528.

Satou M, Ichinoe M, Fukumoto F, *et al*. (2001). Fusarium blight of kangaroo paw (*Anigozanthos* spp.) caused by *Fusarium chlamydosporum* and *Fusarium semitectum*. *Journal of Phytopathology* **149**: 203–206.

Savard ME, Miller JD, Salleh B, *et al*. (1990). Chlamydosporol, a new metabolite from *Fusarium chlamydosporum. Mycopathologia* **110**: 177–181.

Segal BH, Walsh TJ, Liu JM, *et al*. (2009). Invasive infection with *Fusarium chlamydosporum* in a patient with aplastic anemia. *Journal of Clinical Microbiology* **36**: 1772–1776.

Soumya K, Murthy KN, Sreelatha GL, *et al*. (2018). Characterization of a red pigment from *Fusarium chlamydosporum* exhibiting selective cytotoxicity against human breast cancer MCF-7 cell lines. *Journal of Applied Microbiology* **125**: 148–158.

Starkey DE, Ward TJ, Aoki T, *et al.* (2007). Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. *Fungal Genetics and Biology* **44**: 1191–1204.

Swofford DL (2003). *PAUP*. Phylogenetic analysis using parsimony (*and other methods), v. 4.0b10.* Computer programme. Sinauer Associates, Sunderland, Massachusetts, USA.

Waalwijk C, Taga M, Zheng S-L, *et al*. (2018). Karyotype evolution in *Fusarium*. *IMA Fungus* **9**: 13–26.

Wang MM, Chen Q, Diao YZ, *et al*. (2019). *Fusarium incarnatum-equiseti* complex in China. *Persoonia* **43**: 70–89.

Wang Z-F, Zhang W, Xiao L, *et al*. (2018). Characterization and bioactive potentials of secondary metabolites from *Fusarium chlamydosporum. Natural Product Research* DOI: 10.1080/14786419.2018.1508142.

Wollenweber HW, Reinking OA (1925). *Aliquot fusaria tropicalia nova vel revisa*. *Phytopathology* **15**: 155–169.

Wollenweber HW, Reinking OA (1935). *Die Fusarien, ihre Beschreibung, Schadwirkung und Bekampfung*. Paul Parey, Berlin.

Yli-Mattila T, Ward TJ, O'Donnell K, *et al*. (2011). *Fusarium sibiricum sp. nov.,* a novel type A trichothecene-producing *Fusarium* from northern Asia closely related to *F. sporotrichioides* and *F. langsethiae*. *International Journal of Food Microbiology* **147**: 58–68.