FUSE
Fungal Systematics and Evolution
VOLUME 8
DECEMBER 2021
PAGES 101–127

doi.org/10.3114/fuse.2021.08.09

Citizen science project reveals novel fusarioid fungi (*Nectriaceae, Sordariomycetes*) from urban soils

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Key words:
biodiversity
Fusarium
multi-gene phylogeny
new taxa
systematics

Abstract: Soil fungi play a crucial role in soil quality and fertility in being able to break down organic matter but are frequently also observed to play a role as important plant pathogens. As part of a Citizen Science Project initiated by the Westerdijk Fungal Biodiversity Institute and the Utrecht University Museum, which aimed to describe novel fungal species from Dutch garden soil, the diversity of fusarioid fungi (*Fusarium* and other fusarioid genera), which are members of *Nectriaceae* (*Hypocreales*) was investigated. Preliminary analyses of ITS and LSU sequences from more than 4 750 isolates obtained indicated that 109 strains belong to this generic complex. Based on multi-locus phylogenies of combinations of *cmdA*, *tef1*, *rpb1*, *rpb2* and *tub2* alignments, and morphological characteristics, 25 species were identified, namely 22 in *Fusarium* and three in *Neocosmospora.* Furthermore*,* two species were described as new namely *F. vanleeuwenii* from the *Fusarium oxysporum* species complex (FOSC), and *F. wereldwijsianum* from the *Fusarium incarnatum-equiseti* species complex (FIESC)*.* Other species encountered in this study include in the FOSC: *F. curvatum, F. nirenbergiae, F. oxysporum* and three undescribed *Fusarium* spp.; in the FIESC: *F. clavus, F. croceum, F. equiseti, F. flagelliforme* and *F. toxicum*; *Fusarium tricinctum* species complex: *F. flocciferum* and *F. torulosum*; the *Fusarium sambucinum* species complex: *F. culmorum* and *F. graminearum*; the *Fusarium redolens* species complex: *F. redolens*; and the *Fusarium fujikuroi* species complex: *F. verticillioides.* Three species of *Neocosmospora* were encountered, namely *N. solani*, *N. stercicola* and *N. tonkinensis.* Although soil fungal diversity has been well studied in the Netherlands, this study revealed two new species, and eight new records: *F. clavus, F. croceum, F. flagelliforme, F. odoratissimum, F. tardicrescens*, *F. toxicum, F. triseptatum* and *N. stercicola*.

Citation: Crous PW, Hernández-Restrepo M, van Iperen AL, Starink-Willemse M, Sandoval-Denis M, Groenewald JZ (2021). Citizen science project reveals novel fusarioid fungi (*Nectriaceae*, *Sordariomycetes*) from urban soils. *Fungal Systematics and Evolution* **8**: 101–127. doi: 10.3114/fuse.2021.08.09
Received: 13 August 2021; **Accepted:** 28 September 2021; **Effectively published online:** 12 October 2021
Corresponding editor: L. Cai

INTRODUCTION

Fusarium and allied fusarioid genera in *Nectriaceae* are highly diverse in morphology and ecology, and have a worldwide distribution, commonly occurring on plants and plant products, in air, water and soil. Macroconidia are typically borne in sporodochia, and taxa have in the past been identified as *Fusarium* if their macroconidia were curved, septate, had a pointed apex, and basal cell with a foot-like notch near the attachment point (Wollenweber & Reinking 1935, Snyder & Hansen 1940, Geiser *et al*. 2021). However, recent studies have shown that this morphology has evolved several times within *Sordariomycetes*, and that within *Nectriaceae* alone up to 20 genera share the fusarioid macromorphology. These genera are distinct phylogenetically and biologically, and have sexual morphs other than *Gibberella*, which is restricted to *Fusarium s. str*. (Gräfenhan *et al.* 2011, Rossman & Seifert 2011, Schroers *et al.* 2011, Rossman *et al*. 2013, Lombard *et al.* 2015, Sandoval-Denis *et al.* 2019, Crous *et al.* 2021a).

Species of fusarioid fungi can produce several different spore types, namely macro-, meso- and microconidia, ascospores and chlamydospores (Crous *et al.* 2021b). Chlamydospores can occur singly or in clusters, forming microsclerotia that have thick, pigmented, smooth to rough walls. They form in hyphae or conidia, either terminally or intercalary, and are the resting spores that make fusarioid taxa highly adapted to survive in soils for extended periods of time. In agricultural soils, chlamydospores commonly occur in plant debris of previous crops, awaiting fresh nutrients and favourable conditions to reactivate (Couteaudier & Alabouvette 1990).

The genus *Fusarium s. str*. contains 17 species complexes that correlate to different phylogenetic lineages (Crous *et al.* 2021b). Common soil-borne fusarioid fungi include the *Fusarium oxysporum* species complex (FOSC; Lombard *et al*. 2019) and species of *Neocosmospora* (formerly known as the *Fusarium solani* species complex; Sandoval-Denis *et al*. 2018, 2019). The FOSC contains many plant pathogenic taxa, several of which are host specific, which paved the way for "special forms" to be recognised as "*formae speciales*", and "races" to help distinguish them (Snyder & Hansen 1940). Such *formae speciales*, however, are frequently seen to represent distinct phylogenetic species

(Lombard *et al*. 2019, Maryani *et al.* 2019a, b). Despite this terminology being a dated approach to dealing with the diversity in *Fusarium*, plant pathologists still use it to help distinguish the diversity they encounter in the field, and more than 144 *f. spp.* have been named in the FOSC to date (Lombard *et al.* 2019), with additional subspecific classifications including haplotypes, races and vegetative compatibility groups also being used.

Species of *Fusarium* produce a range of trichothecenes (mycotoxins) in different ecological niches, that are of concern to animal and human health when such contaminated products are consumed (O'Donnell *et al.* 2018). These compounds are common throughout *Fusarium s. str*. and are observed in well-known plant pathogenic species such as *F. culmorum*, *F. graminearum*, *F. sporotrichioides* and *F. tricinctum* (Bamburg *et al.* 1968, Tatsuno *et al*. 1968, Yoshizawa & Morooka 1973, Jiménez *et al*. 1997), but again absent from species of *Neocosmospora* (Crous *et al.* 2021b). Because of the threat and great losses caused by soilborne fusarioid fungi in plant, human and animal health, it is imperative that we gain knowledge of the diversity of fusarioid fungi in soil to better understand their function and impact in different terrestrial ecosystems.

The present Citizen Science Project was initiated by the Westerdijk Fungal Biodiversity Institute (WI) and the Utrecht University Museum, aiming to investigate the diversity of fungi in Dutch garden soil collected by children in their home gardens and schoolgrounds from different regions in the Netherlands (Crous *et al.* 2017, 2018, 2021a; Groenewald *et al.* 2018, Giraldo *et al*. 2019, Hernández-Restrepo *et al.* 2020, Hou *et al*. 2020). During this project thousands of isolates were obtained from 404 soil samples. Of these, 109 isolates were found to represent fusarioid fungi, and selected for this study. The aim of the present study was to investigate the diversity of fusarioid fungi from Dutch garden soil, describe and illustrate novel species, and compare them with known taxa.

MATERIALS AND METHODS

Isolates

Soil samples collected from garden soils in the urban environment followed the methods of Groenewald *et al.* (2018) and Giraldo *et al*. (2019). Colonies were sub-cultured on 2 % potato-dextrose agar (PDA), oatmeal agar (OA), malt extract agar (MEA) (Crous *et al*. 2019b), synthetic nutrient-poor agar (SNA; Nirenberg 1976), carnation leaf agar (CLA; Fisher *et al*. 1982), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains and specimens of the studied fungi are maintained in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands.

DNA extraction, amplification (PCR) and phylogeny

Protocols for genomic DNA isolation, PCR amplification of partial calmodulin (*cmdA*) gene, internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS), partial 28S nrRNA gene (LSU), DNA-directed RNA polymerase II largest (*rpb1*) and second largest subunit (*rpb2*) genes, and translation elongation factor 1-alpha (*tef1*) gene, and sequencing of the novel strains (Table 1) followed Crous *et al.* (2021b). The two parts of *rpb2* listed in Table 1 corresponded to the sequences generated using primer pairs RPB2-5f2 / fRPB2-7cR and fRPB2-7cf / RPB2-11ar (see Crous *et al.* (2021b) for primer details). Partial beta-tubulin (*tub2*) gene sequences were not generated during the course of this study.

Initial identifications to genus level were made using megablast searches (Zhang *et al*. 2000) of the ITS sequences against NCBI's GenBank nucleotide database, after which *tef1* sequences were used to further identify the *Fusarium* species complexes. Reference sequences (Supplementary Table S1) from Crous *et al.* (2021b) and based on megablast searches were then used to construct single-gene and multi-gene alignments for *Neocosmospora* and the different *Fusarium* species complexes. Phylogenetic analyses using RAxML Blackbox v. 1.0.0 (https://raxml-ng.vital-it.ch/#/; Kozlov *et al.* 2019), IQ-TREE v. 2.1.3 (Nguyen *et al*. 2015, Minh *et al*. 2020) and MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003) followed Crous *et al.* (2021b), with the exception that trees were saved every 10 or 100 generations (Table 2). All resulting trees were printed with Geneious v. 11.1.5 and the layout of the trees was done in Adobe Illustrator v. CC 2018.

Morphology

Slide preparations were mounted in water, from colonies sporulating on CLA, following the protocols described by Crous *et al*. (2021b). Observations were made with a Nikon SMZ25 dissection-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and images recorded on a Nikon DS-Ri2 camera with associated software. Colony characters and pigment production were noted after 7 d of growth on MEA, PDA and OA incubated at 25 °C. Colony colours (surface and reverse) were scored using the colour charts of Rayner (1970).

RESULTS

Phylogeny

Six multigene alignments were generated in the present study and subjected to the three phylogenetic analyses described above. Statistical values for the alignments and phylogenetic trees are summarised in Table 2. Sequences derived in this study were deposited in GenBank (Table 1), the alignments in TreeBASE (www.treebase.org; study number 28680), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al*. 2004).

Fusarium citricola and *F. tricinctum* species complexes (Fig. 1): Novel isolates from Dutch soils clustered with three known species, namely *F. acuminatum*, *F. flocciferum* and *F. torulosum* (all three in the *F. tricinctum* species complex). The three phylogenetic analyses (RAxML, IQ-TREE, and MrBayes) overall displayed the same species clades and mainly differed with regards to the backbone relationships between species clades/ lineages [data not shown, trees available in TreeBASE and support and posterior probability (PP) values are superimposed on the presented figure]. The loci *cmdA* and *rpb1* are not well-represented in the dataset, with roughly half of the strains having a sequence present (Tables 1, 2, Supplementary Table S1).

Fusarium incarnatum-equiseti species complex (Fig. 2): Novel isolates from Dutch soils clustered with five known species, namely *F. clavus*, *F. croceum*, *F. equiseti*, *F. flagelliforme* and *F. toxicum*, as

Table 1. Collection details and GenBank accession numbers of isolates treated in this study, and associated ex-type strains where applicable. Species names in bold highlight taxonomic novelties. The ITS and LSU sequences were not used in analyses but are provided for completeness.

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	*rpb2* part 1	*rpb2* part 2	*cmdA*	*rpb1*	ITS	LSU
<u>*Fusarium fujikuroi* species complex</u>											
Fusarium verticillioides	JW 145017	Netherlands: Soil	A.E. Jansen; 2017	–	MZ921825	MZ921693	–	MZ921513	MZ921624	–	MZ890483
<u>*Fusarium incarnatum-equiseti* species complex</u>											
Fusarium clavus	JW 288002	Netherlands: Soil	Group 8, OBS de Toonladder; 2017	–	MZ921826	MZ921694	–	MZ921514	MZ921625	–	MZ890484
	NL19-041003	Netherlands: Soil	L. Oegema, R. van Stee & D. Kwast; 2019	RSG Simon Vestdijk	MZ921827	MZ921695	–	MZ921515	–	MZ890340	MZ890485
	NL19-048011	Netherlands: Soil	S. Goinga & J. de Groot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921828	MZ921696	–	MZ921516	–	MZ890341	MZ890486
	NL19-056012	Netherlands: Soil	S. Verhage, S. Moens & K. Basting; 29 Oct. 2019	Zwin college	MZ921829	MZ921697	–	MZ921517	–	MZ890342	MZ890487
	NL19-056013	Netherlands: Soil	S. Verhage, S. Moens & K. Basting; 29 Oct. 2019	Zwin college	MZ921830	MZ921698	–	MZ921518	–	MZ890343	MZ890488
Fusarium croceum	NL19-059006	Netherlands: Soil	A. van Strien, I. Beemsterboer & S. Groosman; 23 Oct. 2019	Zwin college	MZ921831	MZ921699	–	MZ921519	–	MZ890344	MZ890489
	NL19-060011	Netherlands: Soil	T. Vercruisse; 27 Oct. 2019	Zwin college	MZ921832	MZ921700	–	MZ921520	–	MZ890345	MZ890490
Fusarium equiseti	CBS 148218 = NL19-25004	Netherlands: Soil	C. Dijkstra & L. Kruit; 6 Jun. 2019	Het Hogeland College Warffum	MZ921833	MZ921701	–	MZ921521	–	MZ890346	MZ890491
	CBS 148383 = NL19-008004	Netherlands: Soil	S. de Boer; 17 Dec. 2019	GSG 't Schylger Jouw	MZ921834	MZ921702	–	MZ921522	–	MZ890347	MZ890492
	NL19-045005	Netherlands: Soil	E.-A. Duinstra, R. Jagersma & M. Postmus; 9 Oct. 2019	RSG Simon Vestdijk	MZ921835	MZ921703	–	MZ921523	MZ921626	MZ890348	MZ890493
	NL19-047003	Netherlands: Soil	S. Kuiper, N. Zijlstra & E. Schot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921836	MZ921704	–	MZ921524	MZ921627	MZ890349	–
	NL19-059004	Netherlands: Soil	A. van Strien, I. Beemsterboer & S. Groosman; 23 Oct. 2019	Zwin college	MZ921837	MZ921705	–	MZ921525	–	MZ890350	MZ890494
	NL19-97009	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921838	MZ921706	–	MZ921526	MZ921628	MZ890351	MZ890495
Fusarium flagelliforme	NL19-041004	Netherlands: Soil	L. Oegema, R. van Stee & D. Kwast; 2019	RSG Simon Vestdijk	MZ921839	MZ921707	–	MZ921527	MZ921629	MZ890352	MZ890496

Table 1. (Continued).

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	***rpb2* part 1**	***rpb2* part 2**	***cmdA***	***rpb1***	**ITS**	**LSU**
	NL19-047004	Netherlands: Soil	S. Kuiper, N. Zijlstra & E. Schot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921840	MZ921708	–	MZ921528	–	MZ890353	MZ890497
	NL19-050003	Netherlands: Soil	T. van der Schoot & J. Koel; 10 Oct. 2019	RSG Simon Vestdijk	MZ921841	MZ921709	–	MZ921529	–	MZ890354	MZ890498
	NL19-052002	Netherlands: Soil	M. Stellemans, L. de Winde & N. Quist; 21 Oct. 2019	Zwin college	MZ921842	MZ921710	–	MZ921530	–	MZ890355	MZ890499
	NL19-068002	Netherlands: Soil	S. Walraven & M. Bekooy; 28 Oct. 2019	Zwin college	MZ921843	MZ921711	–	MZ921531	–	MZ890356	MZ890500
	NL19-97010	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921844	MZ921712	–	MZ921532	MZ921630	MZ890357	MZ890501
Fusarium toxicum	NL19-041005	Netherlands: Soil	L. Oegema, R. van Stee & D. Kwast; 2019	RSG Simon Vestdijk	MZ921845	MZ921713	–	MZ921533	MZ921631	MZ890358	MZ890502
	NL19-041006	Netherlands: Soil	L. Oegema, R. van Stee & D. Kwast; 2019	RSG Simon Vestdijk	MZ921846	MZ921714	–	MZ921534	MZ921632	MZ890359	MZ890503
	NL19-050001	Netherlands: Soil	T. van der Schoot & J. Koel; 10 Oct. 2019	RSG Simon Vestdijk	MZ921847	MZ921715	–	MZ921535	MZ921633	MZ890360	MZ890504
***Fusarium wereldwijsianum* sp. nov.**	CBS 148219 = NL19-99002	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921848	MZ921716	–	MZ921536	MZ921634	MZ890361	MZ890505
	CBS 148220 = NL19-99003	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921849	MZ921717	–	MZ921537	MZ921635	MZ890362	MZ890506
	CBS 148244 = NL19-94009, ex-type	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921850	MZ921718	–	MZ921538	MZ921636	MZ890363	MZ890507
	CBS 148385 = NL19-057012	Netherlands: Soil	F. Guilliet, T. Bron & I. Geernaert; Oct. 2019	Zwin college	MZ921851	MZ921719	–	MZ921539	–	MZ890364	MZ890508
	CBS 148386 = NL19-059003	Netherlands: Soil	A. van Strien, I. Beemsterboer & S. Groosman; 23 Oct. 2019	Zwin college	MZ921852	MZ921720	–	MZ921540	–	MZ890365	MZ890509
<u>*Fusarium oxysporum* species complex</u>											
Fusarium curvatum	JW 39001	Netherlands: Soil	R. Ramanand; 2017	–	MZ921853	MZ921721	–	MZ921541	–	MZ890366	MZ890510
Fusarium nirenbergiae	CBS 148373 = JW 5042	Netherlands: Soil	F. & R. Niemeijer; 2017	–	MZ921867	MZ921735	–	MZ921555	MZ921646	MZ890378	MZ890522
	CBS 148379 = JW 124027	Netherlands: Soil	S. Vermeulen; 2017	–	MZ921868	MZ921736	–	MZ921556	MZ921647	–	MZ890523
	CBS 148381 = JW 288013	Netherlands: Soil	Group 8, OBS de Toonladder; 2017	–	MZ921870	MZ921738	–	MZ921558	–	–	MZ890525

Table 1. (Continued).

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	***rpb2* part 1**	***rpb2* part 2**	***cmdA***	***rpb1***	**ITS**	**LSU**
	CBS 148382 = JW 289011	Netherlands: Soil	KMN Spelerij; 2017	–	MZ921871	MZ921739	–	MZ921559	–	–	MZ890526
	CBS 148384 = NL19-048010	Netherlands: Soil	S. Goinga & J. de Groot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921873	MZ921741	–	MZ921561	–	MZ890381	MZ890528
	CBS 148387 = NL19-100010	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921875	MZ921744	–	MZ921564	–	MZ890384	MZ890531
	CBS 148388 = BE19-004016	Belgium: Soil	T. Antheunis; 2019	Viso Cor Mariae	MZ921866	MZ921734	–	MZ921554	–	MZ890377	MZ890521
	JW 192006	Netherlands: Soil	L. Borsboom; 2017	–	MZ921869	MZ921737	–	MZ921557	–	MZ890379	MZ890524
	NL19-045004	Netherlands: Soil	E.-A. Duinstra, R. Jagersma & M. Postmus; 9 Oct. 2019	RSG Simon Vestdijk	MZ921872	MZ921740	–	MZ921560	MZ921648	MZ890380	MZ890527
	NL19-053002	Netherlands: Soil	L. van Eetveldt, G. Jones & F. Walraven; 25 Oct. 2019	Zwin college	MZ921874	MZ921742	–	MZ921562	–	MZ890382	MZ890529
	NL19-053003	Netherlands: Soil	L. van Eetveldt, G. Jones & F. Walraven; 25 Oct. 2019	Zwin college	–	MZ921743	–	MZ921563	MZ921649	MZ890383	MZ890530
	NL19-28011	Netherlands: Soil	H. Meertens & D. Zaagman; 6 Jun. 2019	Het Hogeland College Warffum	MZ921876	MZ921745	–	MZ921565	MZ921650	MZ890385	MZ890532
	NL19-91009	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921877	MZ921746	–	MZ921566	MZ921651	MZ890386	MZ890533
	NL19-91010	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921878	MZ921747	–	MZ921567	MZ921652	MZ890387	MZ890534
	NL19-99011	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921879	MZ921748	–	MZ921568	MZ921653	MZ890388	MZ890535
Fusarium odoratissimum	JW 54001	Netherlands: Soil	I., M. & L. Zoert; 2017	–	MZ921880	MZ921749	–	MZ921569	MZ921654	–	–
Fusarium oxysporum	JW 11005	Netherlands: Soil	M. Francisca; 2017	–	MZ921881	MZ921750	–	MZ921570	MZ921655	MZ890389	MZ890536
	JW 231014	Netherlands: Soil	D. Pol, R. Verf, J. Wilks & M. de Ruiter; 2017	–	MZ921882	MZ921751	–	MZ921571	MZ921656	MZ890390	–
	JW 257006	Netherlands: Soil	KSU de Achtbaan; 2017	–	MZ921883	MZ921752	–	MZ921572	MZ921657	–	MZ890537
	NL19-94002	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921884	MZ921753	–	MZ921573	MZ921658	MZ890391	MZ890538
	NL19-94008	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921885	MZ921754	–	MZ921574	MZ921659	MZ890392	MZ890539

Table 1. (Continued).

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	*rpb2* part 1	*rpb2* part 2	*cmdA*	*rpb1*	ITS	LSU
Fusarium sp. 1	CBS 148204 = JW 191014	Netherlands: Soil	T. & K. Wesselink; 2017	–	MZ921858	MZ921726	–	MZ921546	MZ921641	MZ890371	MZ890514
	CBS 148216 = JW 53002	Netherlands: Soil	K. Brennand; 2017	–	MZ921863	MZ921731	–	MZ921551	MZ921645	–	–
	CBS 148217 = NL19-25001	Netherlands: Soil	C. Dijkstra & L. Kruit; 6 Jun. 2019	Het Hogeland College Warffum	MZ921864	MZ921732	–	MZ921552	–	MZ890375	MZ890519
Fusarium sp. 2	CBS 130323 = NRRL 26677	Australia: Subungual debris of 40-year-old female with nail infection	Unknown	–	MH485018	MH484927	–	MH484745	–	–	–
	CBS 148185 = JW 1072	Netherlands: Soil	J. van Dijk; 2017	–	MZ921854	MZ921722	–	MZ921542	MZ921637	MZ890367	MZ890511
	CBS 128.81 = BBA 63925 = NRRL 36233	USA: *Chrysanthemum* sp.	Unknown		MH484975	MH484884	–	MH484702	–	–	–
	CBS 680.89 = IPO 11179 = NRRL 26221	Netherlands: *Cucumis sativus*, in greenhouse on rockwool	N. Hubbeling; –	–	MH484980	MH484889	–	MH484707	–	–	–
Fusarium sp. 3	CBS 148198 = JW 4030	Netherlands: Soil	F. Wiegerinck; 2017	–	MZ921855	MZ921723	–	MZ921543	MZ921638	MZ890368	MZ890512
	CBS 148199 = JW 9002	Netherlands: Soil	A.-S. den Boer; 2017	–	MZ921856	MZ921724	–	MZ921544	MZ921639	MZ890369	MZ890513
	CBS 148200 = JW 10005	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921857	MZ921725	–	MZ921545	MZ921640	MZ890370	–
	CBS 148205 = JW 204009	Netherlands: Soil	I. Kleij; 2017	–	MZ921859	MZ921727	–	MZ921547	MZ921642	–	MZ890515
	CBS 148206 = JW 210014	Netherlands: Soil	N. Keij; 2017	–	MZ921860	MZ921728	–	MZ921548	MZ921643	MZ890372	MZ890516
	CBS 148207 = JW 210019	Netherlands: Soil	N. Keij; 2017	–	MZ921861	MZ921729	–	MZ921549	MZ921644	MZ890373	MZ890517
	CBS 148208 = JW 231016	Netherlands: Soil	D. Pol, R. Verf, J. Wilks & M. de Ruiter; 2017	–	MZ921862	MZ921730	–	MZ921550	–	MZ890374	MZ890518
	CBS 148222 = BE19-004006	Belgium: Soil	T. Antheunis; 2019	Viso Cor Mariae	MZ921865	MZ921733	–	MZ921553	–	MZ890376	MZ890520
Fusarium tardicrescens	JW 6021	Netherlands: Soil	H.W. Vos; 2017	–	MZ921886	MZ921755	–	MZ921575	MZ921660	MZ890393	MZ890540
	JW 6043	Netherlands: Soil	H.W. Vos; 2017	–	MZ921887	MZ921756	–	MZ921576	MZ921661	MZ890394	–
Fusarium triseptatum	CBS 148380 = JW 277008	Netherlands: Soil	Lukasschool; 2017	–	MZ921888	MZ921757	–	MZ921577	MZ921662	–	MZ890541
	JW 277009	Netherlands: Soil	Lukasschool; 2017	–	MZ921889	MZ921758	–	MZ921578	–	–	MZ890542

Table 1. (Continued).

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	*rpb2* part 1	*rpb2* part 2	*cmdA*	*rpb1*	ITS	LSU
***Fusarium vanleeuwenii* sp. nov.**	CBS 148372 = JW 10008, ex-type	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921896	MZ921765	–	MZ921585	MZ921669	MZ890401	–
	CBS 148374 = JW 10001	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921890	MZ921759	–	MZ921579	MZ921663	MZ890395	MZ890543
	CBS 148375 = JW 10003	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921892	MZ921761	–	MZ921581	MZ921665	MZ890397	–
	CBS 148376 = JW 10004	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921893	MZ921762	–	MZ921582	MZ921666	MZ890398	–
	CBS 148377 = JW 10006	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921894	MZ921763	–	MZ921583	MZ921667	MZ890399	–
	CBS 148378 = JW 10007	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921895	MZ921764	–	MZ921584	MZ921668	MZ890400	–
	JW 10002	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921891	MZ921760	–	MZ921580	MZ921664	MZ890396	MZ890544
	JW 10009	Netherlands: Soil	M.J. van Leeuwen; 2017	–	MZ921897	MZ921766	–	MZ921586	MZ921670	MZ890402	–
<u>*Fusarium redolens* species complex</u>											
Fusarium redolens	NL19-003007	Netherlands: Soil	B. Wulp; 17 Dec. 2019	GSG ’t Schylger Jouw	MZ921898	MZ921767		–	MZ921671	MZ890403	MZ890545
<u>*Fusarium sambucinum* species complex</u>											
Fusarium culmorum	BE19-002002	Belgium: Soil	S. Vanopbroeke; 2019	Viso Cor Mariae	MZ921899	MZ921768	MZ921802	MZ921587	–	MZ890404	MZ890546
	BE19-009002	Belgium: Soil	N. Caen; 2019	Viso Cor Mariae	MZ921900	MZ921769	MZ921803	MZ921588	–	MZ890405	MZ890547
	NL19-047005	Netherlands: Soil	S. Kuiper, N. Zijlstra & E. Schot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921901	MZ921770	MZ921804	MZ921589	–	MZ890406	MZ890548
	NL19-060003	Netherlands: Soil	T. Vercruisse; 27 Oct. 2019	Zwin college	MZ921902	MZ921771	MZ921805	MZ921590	–	MZ890407	MZ890549
	NL19-076001	Netherlands: Soil	W. Vercouteren, S. Meas & R. Verhije; 6 Nov. 2019	Zwin college	MZ921903	MZ921772	MZ921806	MZ921591	–	MZ890408	MZ890550
	NL19-25005	Netherlands: Soil	C. Dijkstra & L. Kruit; 6 Jun. 2019	Het Hogeland College Warffum	MZ921904	MZ921773	MZ921807	–	–	MZ890409	MZ890551
	NL19-93013	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921905	MZ921774	MZ921808	MZ921592	MZ921672	MZ890410	MZ890552
Fusarium graminearum	NL19-100008	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921906	MZ921775	MZ921809	MZ921593	MZ921673	MZ890411	MZ890553

Table 1. (Continued).

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	*rpb2* part 1	*rpb2* part 2	*cmdA*	*rpb1*	ITS	LSU
<u>*Fusarium tricinctum* species complex</u>											
Fusarium acuminatum	JW 288021	Netherlands: Soil	Group 8, OBS de Toonladder; 2017	–	MZ921907	MZ921776	MZ921810	MZ921594	MZ921674	–	MZ890554
	JW 289003	Netherlands: Soil	KMN Spelerij; 2017	–	MZ921908	MZ921777	MZ921811	MZ921595	MZ921675	–	MZ890555
	NL19-048014	Netherlands: Soil	S. Goinga & J. de Groot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921909	MZ921778	MZ921812	MZ921596	–	MZ890412	MZ890556
	NL19-077002	Netherlands: Soil	R. van der Wel & T. Wolfret; 5 Nov. 2019	Zwin college	MZ921910	MZ921779	MZ921813	MZ921597	–	MZ890413	MZ890557
Fusarium flocciferum	CBS 143231 = JW 14004	Netherlands: Soil	D. Peters; 2017	–	MG386159	MG386149	MG386149	MZ921598	MG386138	MG386078	MG386131
	CBS 143667 = JW 14005, ex-type of *F. petersiae*	Netherlands: Soil	D. Peters; 2017	–	MG386160	MG386150	MG386150	MZ921599	MG386139	MG386079	MG386132
	CBS 147837 = NL19-100011	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	–	MZ921780	MZ921814	MZ921600	–	MZ890416	MZ890558
	CBS 821.68 = NRRL 28450, ex-epitype	Germany: Greenhouse soil	D. Bredemeier; 1966	–	MW928837	MW928824	MW928824	–	MW928807	–	–
	JW 5026	Netherlands: Soil	F. & R. Niemeijer; 2017	–	MZ921911	MZ921781	MZ921815	MZ921601	MZ921676	MZ890417	MZ890559
	JW 18005	Netherlands: Soil	W. van der Heijden; 2017	–	MZ921912	MZ921782	MZ921816	MZ921602	MZ921677	MZ890418	–
	JW 248008	Netherlands: Soil	J.-W. Koolen; 2017	–	MZ921913	MZ921783	MZ921817	MZ921603	MZ921678	MZ890419	MZ890560
	JW 267001	Netherlands: Soil	Basisschool de Baanbreker; 2017	–	MZ921914	MZ921784	MZ921818	MZ921604	–	–	MZ890561
	NL19-048012	Netherlands: Soil	S. Goinga & J. de Groot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921915	MZ921785	MZ921819	MZ921605	–	MZ890420	MZ890562
	NL19-048013	Netherlands: Soil	S. Goinga & J. de Groot; 10 Oct. 2019	RSG Simon Vestdijk	MZ921916	MZ921786	MZ921820	MZ921606	–	MZ890421	MZ890563
	NL19-97008	Netherlands: Soil	S. Frederikze, J. Mes & S. El Maghnouji; 31 Jul. 2019	ACB Wereldwijs	MZ921917	MZ921787	MZ921821	MZ921607	MZ921679	MZ890422	MZ890564
Fusarium torulosum	JW 24001	Netherlands: Soil	J. van der Stel; 2017	–	MZ921918	MZ921788	MZ921822	MZ921608	MZ921680	MZ890423	MZ890565
<u>*Neocosmospora*</u>											
Neocosmospora solani	JW 1075	Netherlands: Soil	J. van Dijk; 2017	–	MZ921919	MZ921789	–	MZ921609	MZ921681	MZ890424	MZ890566
	JW 14011	Netherlands: Soil	D. Peters; 2017	–	MZ921920	MZ921790	–	MZ921610	–	MZ890425	MZ890567
	JW 191039	Netherlands: Soil	T. & K. Wesselink; 2017	–	MZ921921	MZ921791	–	MZ921611	MZ921682	MZ890426	MZ890568

Table 1. (Continued).

Species complex and Species	Culture or working collection number(s)	Country and Substrate	Collector(s) and Collection date	School or educational institution	GenBank accession number(s)[1]						
					tef1	***rpb2* part 1**	***rpb2* part 2**	***cmdA***	***rpb1***	**ITS**	**LSU**
	JW 232018	Netherlands: Soil	M. van Meijl; 2017	–	MZ921922	MZ921792	–	MZ921612	–	MZ890427	MZ890569
	JW 288011	Netherlands: Soil	Group 8, OBS de Toonladder; 2017	–	MZ921923	MZ921793	–	MZ921613	MZ921683	–	MZ890570
Neocosmospora stercicola	JW 1093	Netherlands: Soil	J. van Dijk; 2017	–	MZ921924	MZ921794	–	MZ921614	MZ921684	MZ890428	MZ890571
	JW 75001	Netherlands: Soil	O. Terpstra; 2017	–	MZ921925	MZ921795	–	MZ921615	MZ921685	MZ890429	MZ890572
	JW 235004	Netherlands: Soil	T. Tuinier; 2017	–	MZ921926	MZ921796	–	MZ921616	MZ921686	MZ890430	MZ890573
	JW 235009	Netherlands: Soil	T. Tuinier; 2017	–	MZ921927	MZ921797	–	MZ921617	MZ921687	MZ890431	MZ890574
Neocosmospora tonkinensis	JW 234010	Netherlands: Soil	T. Vanmeulebrouk; 2017	–	MZ921928	MZ921798	–	MZ921618	MZ921688	–	MZ890575
	JW 236012	Netherlands: Soil	A. Vanmeulebrouk; 2017	–	–	MZ921799	–	MZ921619	–	–	MZ890576

[1] *cmdA*: partial calmodulin gene; ITS: internal transcribed spacer regions with intervening 5.8S nrRNA gene; *rpb1*: partial DNA-directed RNA polymerase II largest subunit gene; *rpb2*: partial DNA-directed RNA polymerase II second largest subunit gene; *tef1*: partial translation elongation factor 1-alpha gene; *tub2*: partial beta-tubulin gene.

Table 2. Summary of phylogenetic information for the different analyses in this study[1].

Analysis	Locus[2]	Number of strains (incl. outgroup)	Length incl. gaps	BI unique site patterns	Model (AIC)	Model (BIC)	BI sample frequency	Number of sampled trees (BI)	ML -InL (R)	ML -InL (IQ-TREE)
Fusarium citricola & *F. tricinctum* species complexes	*cmdA*	23	669	118	SYM+G	K2P+G4	–	–	–	-1717.014
	rpb1	27	1 787	370	GTR+I	TNe+G4	–	–	–	-4717.229
	rpb2 (part 1)	42	910	226	SYM+G	TNe+G4	–	–	–	-2795.892
	rpb2 (part 2)	34	628	104	GTR+G	TNe+G4	–	–	–	-1747.846
	tef1	41	756	256	GTR+I	TIM2e+G4	–	–	–	-2469.915
	Combined	45	4 750	1 074	–	–	10	78 002	-13596.815643	-13617.725
Fusarium incarnatum-equiseti species complex	*cmdA*	72	661	157	SYM+G	TNe+R3	–	–	–	-2121.606
	rpb1	29	1 729	226	SYM+I	TNe+R2	–	–		-4384.505
	rpb2 (part 1)	73	886	174	GTR+I	TNe+G4	–	–		-2770.072
	tef1	73	743	262	GTR+G	TNe+R3	–	–		-3166.37
	Combined	73	4 019	819	–	–	10	1 285 502	-13545.426546	-13066.849

Table 2. (Continued).

Analysis	Locus[2]	Number of strains (incl. outgroup)	Length incl. gaps	BI unique site patterns	Model (AIC)	Model (BIC)	BI sample frequency	Number of sampled trees (BI)	ML -lnL (R)	ML -lnL (IQ-TREE)
Fusarium oxysporum species complex	*cmdA*	117	608	53	K80	K2P	–	–	–	-1074.196
	rpb1	73	1451	216	SYM+I+G	TNe+R2	–	–	–	-3381.188
	rpb2 (part 1)	154	882	111	HKY+G	K2P+I	–	–	–	-2070.032
	tef1	154	584	158	HKY+G	TNe+G4	–	–	–	-1733.616
	tub2	74	577	141	SYM+G	TIMe+R2	–	–	–	-1812.746
	Combined	155	4 102	679	–	–	100	80 178	-10424.792230	-10.414.344
Fusarium redolens & *F. fujikuroi* species complexes	*cmdA*	17	690	149	SYM+I	TIM3e+G4	–	–	–	-1842.997
	rpb1	29	1 788	329	SYM+I	TNe+G4	–	–	–	-5168.810
	rpb2 (part 1)	34	904	297	SYM+I+G	TIM2e+I+G4	–	–	–	-4009.430
	tef1	33	763	323	GTR+G	TIM2e+G4	–	–	–	-3494.236
	Combined	34	4 145	1 098	–	–	10	64 502	-15323.869379	-15336.259
Fusarium sambucinum species complex	*cmdA*	14	661	71	SYM	TNe+I	–	–	–	-1499.250
	rpb1	33	1 793	240	SYM+I	TNe+G4	–	–	–	-4257.654
	rpb2 (part 1)	38	905	212	SYM+G	TIM2e+I	–	–	–	-2940.257
	rpb2 (part 2)	38	629	133	GTR+G	TNe+I	–	–	–	-2089.607
	tef1	38	755	197	GTR+G	TIM2e+R2	–	–	–	-2285.191
	Combined	39	4 743	853	–	–	10	30 752	-13596.206653	-13604.873
Neocosmospora	*cmdA*	42	674	221	SYM+I+G	K2P+G4	–	–	–	-2585.283
	rpb1	42	1 687	524	GTR+I+G	TIM3e+I+G4	–	–	–	-6296.579
	rpb2 (part 1)	69	866	241	SYM+G	TNe+G4	–	–	–	-2990.034
	tef1	76	752	300	GTR+G	TN+F+G4	–	–	–	-3000.621
	Combined	77	3 979	1 286	–	–	10	859 502	-15410.126704	-15411.266

[1] BI: Bayesian inference; Model (AIC): Evolutionary model selected by MrModeltest under the Akaike Information Criterion; Model (BIC): Evolutionary model selected by ModelFinder in IQ-TREE; BI sample frequency: Number of nth generations sampled; ML -lnL (R): Log-likelihood of final tree in RAxML; ML -lnL(IQ-TREE): Log-likelihood of consensus tree in IQ-TREE.

[2] *cmdA*: partial calmodulin gene, *tef1*: partial translation elongation factor 1-alpha gene; *rpb1*: partial DNA-directed RNA polymerase II largest subunit gene; *rpb2*: partial DNA-directed RNA polymerase II second largest subunit gene; *tub2*: partial beta-tubulin gene.

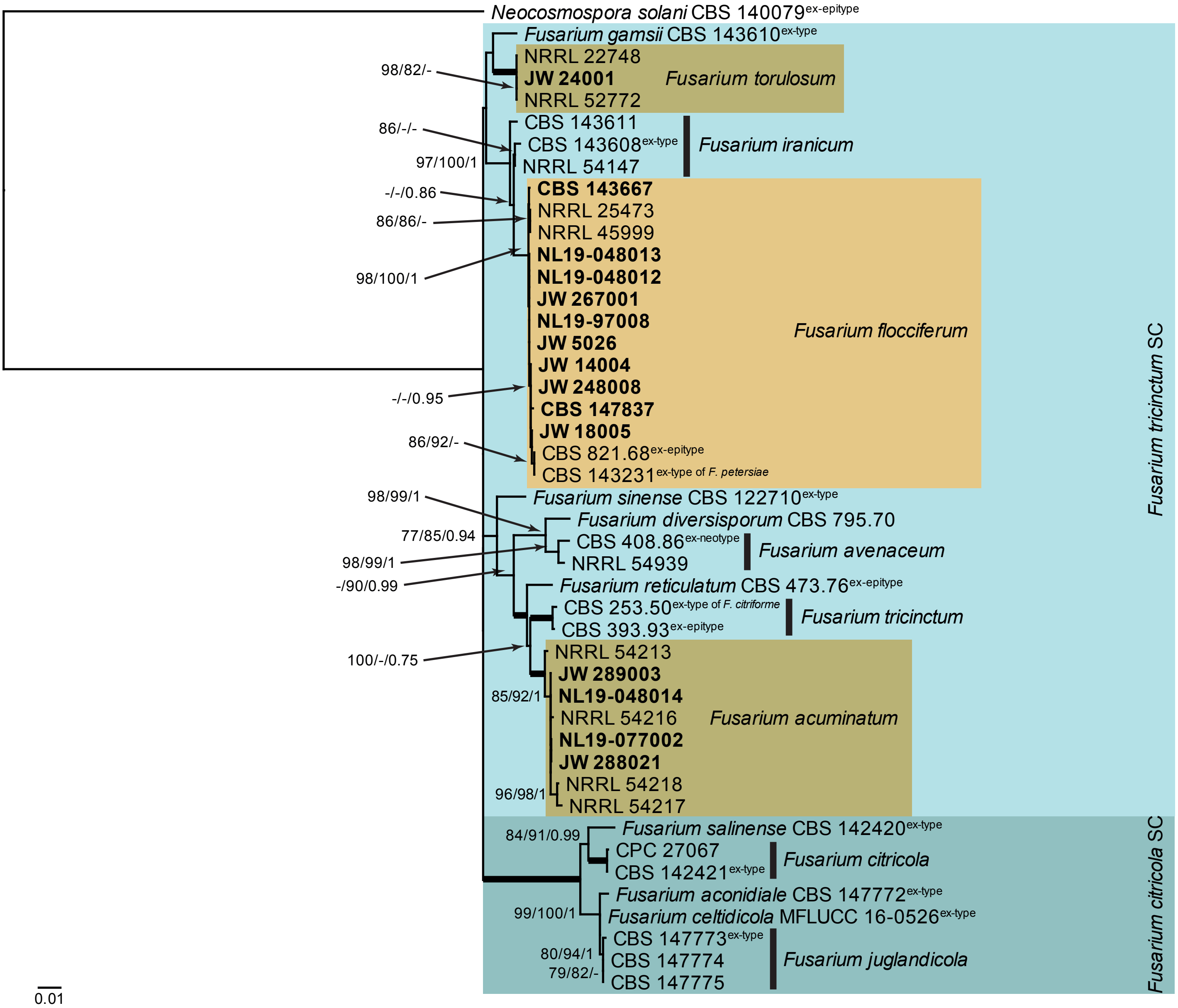

Fig 1. The RAxML consensus tree inferred from the combined *F. citricola*/*tricinctum* species complexes *tef1*, *rpb2* (parts 1 and 2), *rpb1* and *cmdA* sequence alignment. Thickened lines indicate branches with full support (RAxML & IQ-TREE bootstrap = 100 %; PP = 1.0) with support values of other branches indicated at the branches (RAxML > 74 % / IQ-TREE > 84 % / PP > 0.74). The tree is rooted to *Neocosmospora solani* (CBS 140079, ex-epitype culture). The scale bar indicates the number of expected changes per site. Species complexes are indicated on the right and highlighted with coloured blocks. Species clades containing the novel citizen science strains (in bold) are highlighted with coloured blocks.

well as a species clade not associated with any known species. The three phylogenetic analyses (RAxML, IQ-TREE and MrBayes) overall displayed the same species clades and mainly differed with regards to the backbone relationships between species clades/lineages (data not shown, trees available in TreeBASE and support and PP values are superimposed on the presented figure). The locus *rpb1* is not well-represented in the dataset, with less than half of the strains having a sequence present (Tables 1, 2, Supplementary Table S1).

Fusarium oxysporum species complex (Fig. 3): Novel isolates from Dutch soils clustered with six known species, namely *F. curvatum*, *F. nirenbergiae*, *F. odoratissimum*, *F. oxysporum* and *F. triseptatum*, as well as four species clades not associated with any known species. The three phylogenetic analyses (RAxML, IQ-TREE and MrBayes) overall displayed the same species clades and mainly differed with regards to the backbone relationships between species clades/lineages (data not shown, trees available in TreeBASE and support and PP values are superimposed on the presented figure). The loci *rpb1* and *tub2* are not well-represented in the dataset, with roughly half of the strains having a sequence present (Tables 1, 2, Supplementary Table S1).

Fusarium fujikuroi and *F. redolens* species complexes (Fig. 4): Novel isolates from Dutch soils clustered with two known species, namely *F. redolens* (*F. redolens* species complex) and *F. verticillioides* (*F. fujikuroi* species complex). The three phylogenetic analyses (RAxML, IQ-TREE and MrBayes) had the same overall topology and same species clades/lineages (data not shown, trees available in TreeBASE and support and PP values are superimposed on the presented figure). The locus *cmdA* is not well-represented in the dataset, with roughly half of the strains having a sequence present (Tables 1, 2, Supplementary Table S1).

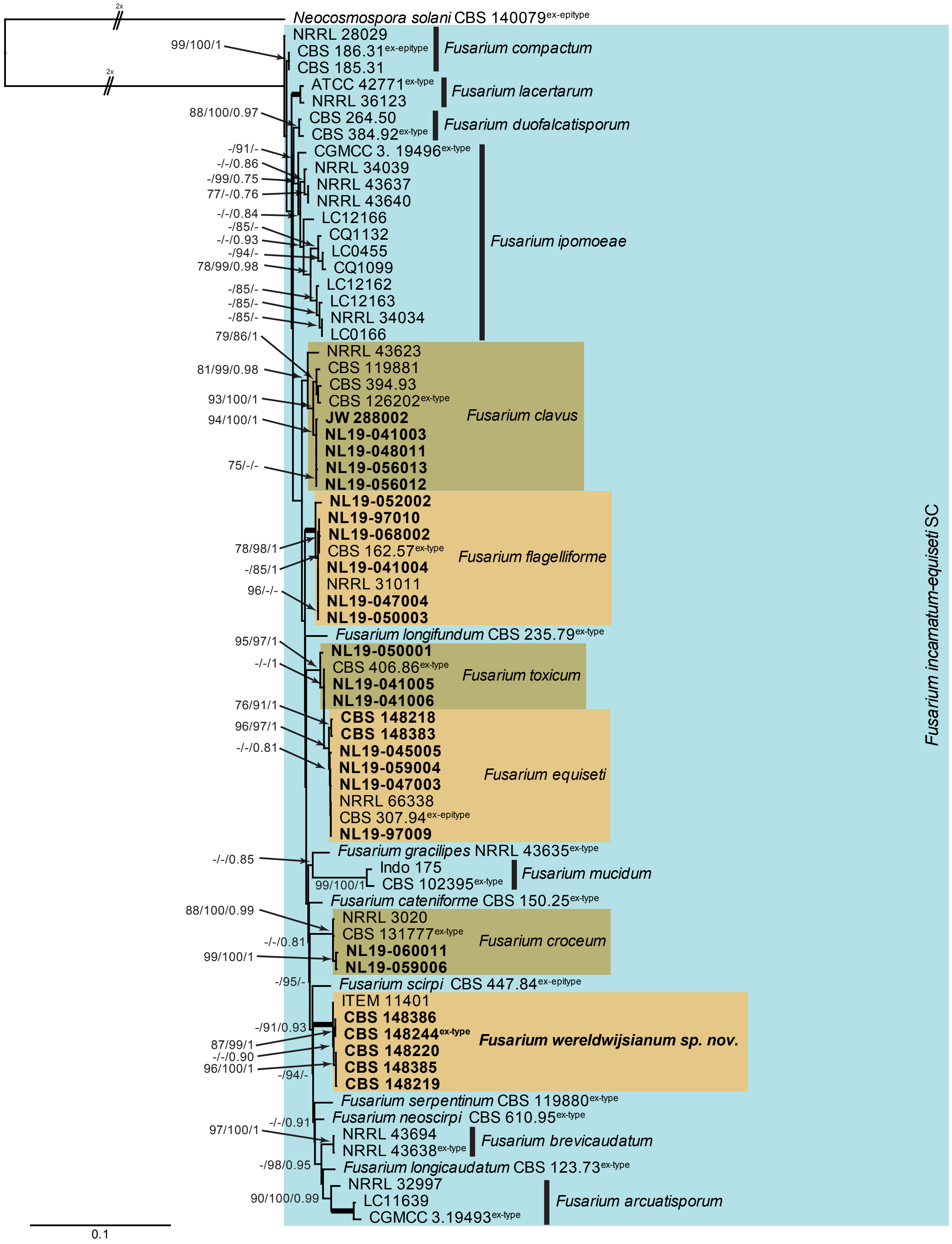

Fig 2. The RAxML consensus tree inferred from the combined *F. incarnatum-equiseti* species complex *tef1*, *rpb2* (first part), *cmdA* and *rpb1* sequence alignment. Thickened lines indicate branches with full support (RAxML & IQ-TREE bootstrap = 100 %; PP = 1.0) with support values of other branches indicated at the branches (RAxML > 74 % / IQ-TREE > 84 % / PP > 0.74). The tree is rooted to *Neocosmospora solani* (CBS 140079, ex-epitype culture) and the two basal branches were halved to facilitate layout. The scale bar indicates the number of expected changes per site. The *F. incarnatum-equiseti* species complex is indicated on the right and highlighted with a coloured block. Species clades containing the novel citizen science strains (in bold) are highlighted with coloured blocks and the novelty described in the present study is printed in bold font.

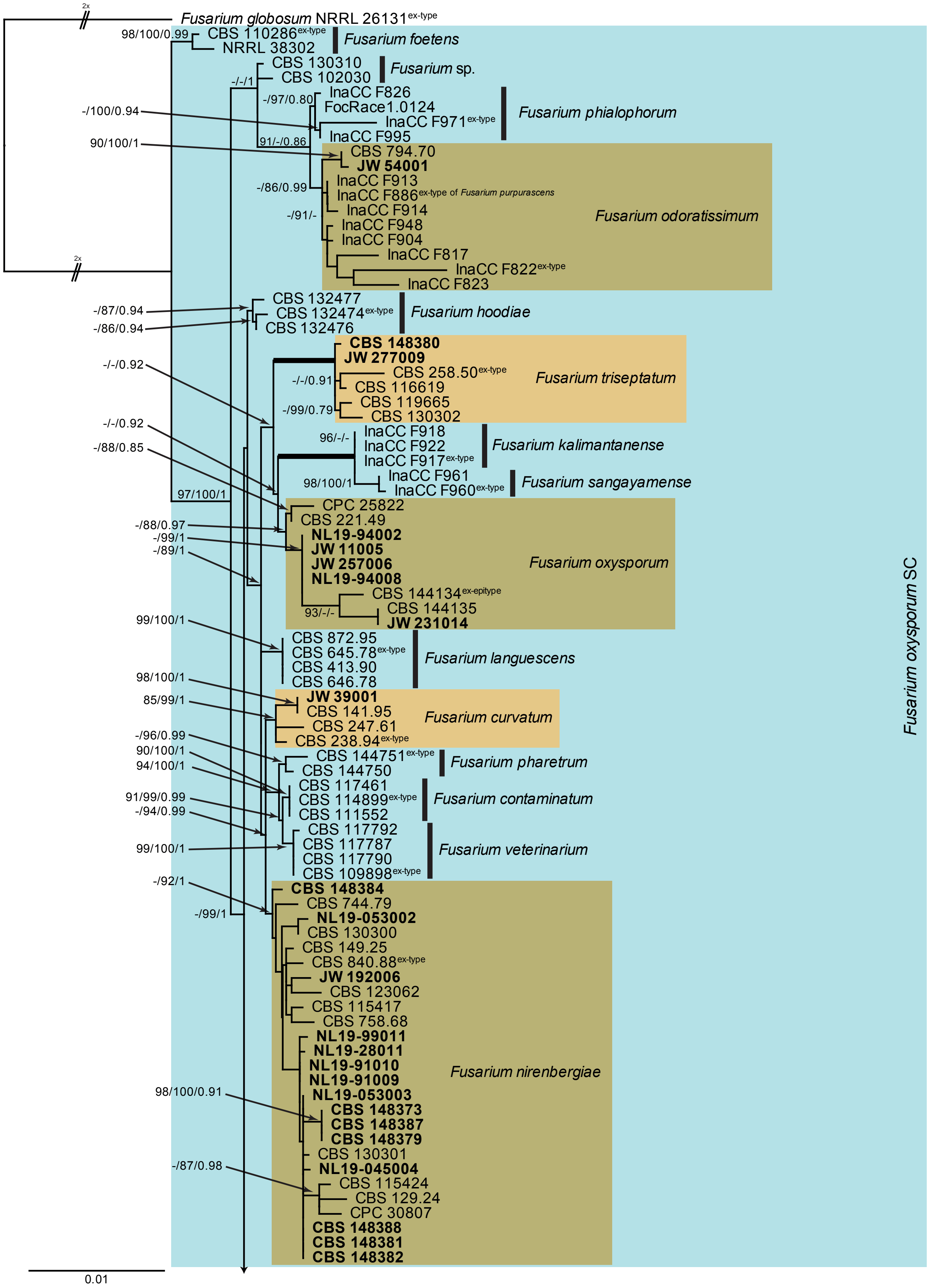

Fig 3. The RAxML consensus tree inferred from the combined *F. oxysporum* species complex *tef1*, *rpb2* (first part), *tub2*, *cmdA* and *rpb1* sequence alignment. Thickened lines indicate branches with full support (RAxML & IQ-TREE bootstrap = 100 %; PP = 1.0) with support values of other branches indicated at the branches (RAxML > 74 % / IQ-TREE > 84 % / PP > 0.74). The tree is rooted to *Fusarium globosum* (NRRL 26131) and the two basal branches were halved to facilitate layout. The scale bar indicates the number of expected changes per site. The *F. oxysporum* species complex is indicated on the right and highlighted with a coloured block. Species clades containing the novel citizen science strains (in bold) are highlighted with coloured blocks and the novelty described in the present study is printed in bold font.

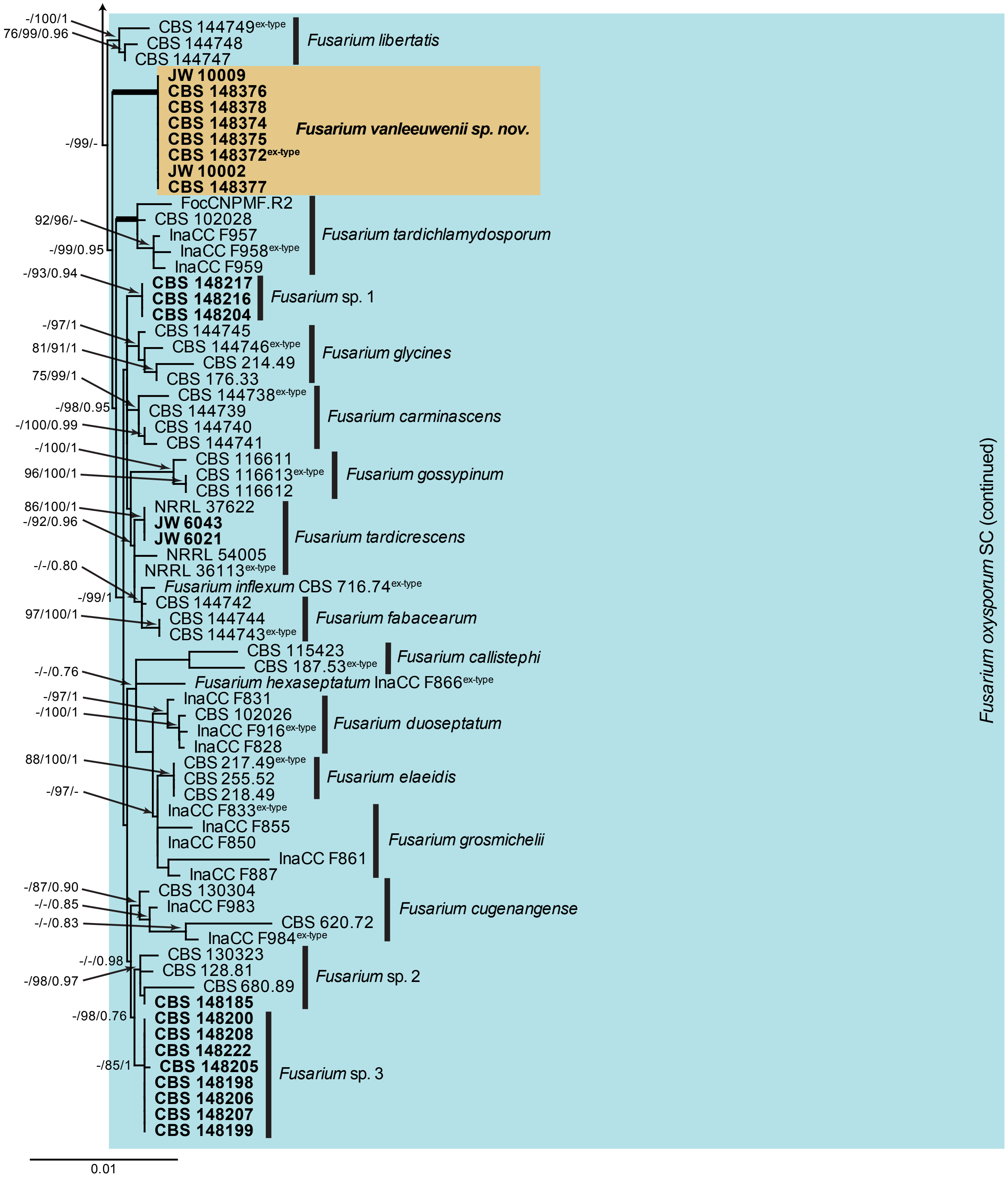

Fig 3. (Continued).

Fusarium sambucinum species complex (Fig. 5): Novel isolates from Dutch soils clustered with two known species, namely *F. culmorum* and *F. graminearum*. The three phylogenetic analyses (RAxML, IQ-TREE and MrBayes) overall displayed the same species clades and the Bayesian phylogeny mainly differed with regards to the backbone relationships between species clades/lineages in the lower half of the tree (data not shown, trees available in TreeBASE and support and PP values are superimposed on the presented figure). The locus *cmdA* is not well-represented in the dataset, with less than half of the strains having a sequence present (Tables 1, 2, Supplementary Table S1).

Neocosmospora (Fig. 6): Novel isolates from Dutch soils clustered with three known species, namely *N. solani*, *N. stercicola* and *N. tonkinensis*. The three phylogenetic analyses

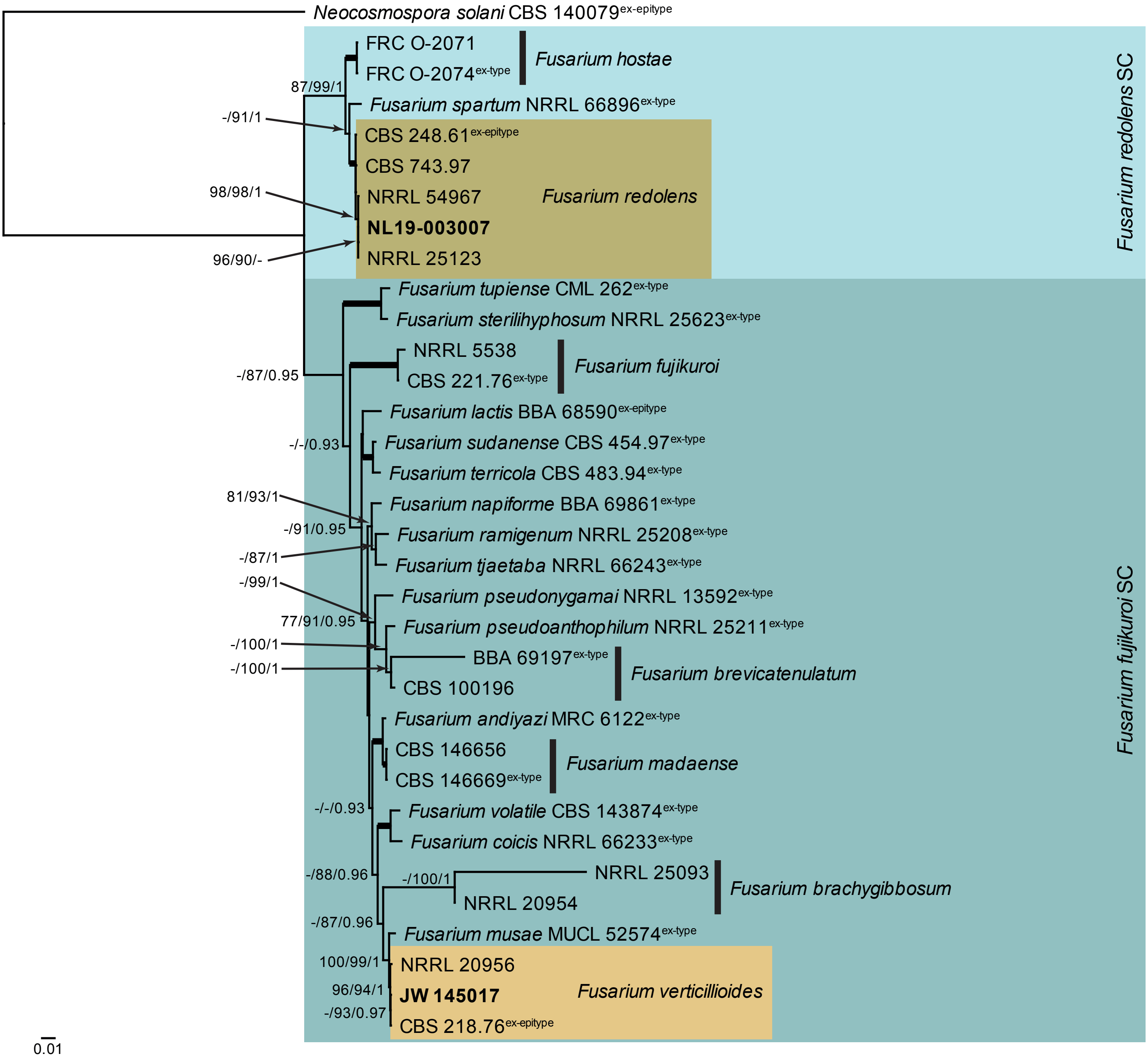

Fig 4. The RAxML consensus tree inferred from the combined *F. redolens*/*fujikuroi* species complexes *tef1*, *rpb2* (first part), *rpb1* and *cmdA* sequence alignment. Thickened lines indicate branches with full support (RAxML & IQ-TREE bootstrap = 100 %; PP = 1.0) with support values of other branches indicated at the branches (RAxML > 74 % / IQ-TREE > 84 % / PP > 0.74). The tree is rooted to *Neocosmospora solani* (CBS 140079, ex-epitype culture). The scale bar indicates the number of expected changes per site. Species complexes are indicated on the right and highlighted with coloured blocks. Species clades containing the novel citizen science strains (in bold) are highlighted with coloured blocks.

(RAxML, IQ-TREE and MrBayes) had the same overall topology, except for swapping around between *N. rectiphora* and *N. vasinfecta* as being the most basal species, and had the same species clades/lineages (data not shown, trees available in TreeBASE and support and PP values are superimposed on the presented figure). The loci *cmdA* and *rpb1* are not well-represented in the dataset, with roughly half of the strains having a sequence present (Tables 1, 2, Supplementary Table S1).

Based on these phylogenetic trees, several taxonomic decisions were made, and the individual and combined trees are discussed under the Notes in the Taxonomy section below, where applicable.

Taxonomy

Fusarium flocciferum Corda, in Sturm, Deutschl. Fl., Abt. 3, Pilze Deutschl. 2: 17. 1828.
New synonym: *Fusarium petersiae* L. Lombard, Persoonia 39: 457. 2017.
Additional synonyms see Crous *et al.* (2021b)

Material examined: **Germany**, from greenhouse soil, 1966, *D. Bredemeier*, ex-epitype culture of *F. flocciferum* CBS 821.68 = NRRL 28450. **Netherlands**, Friesland Province, Harlingen, from soil, 10 Oct. 2019, *S. Goinga & J. de Groot*, cultures NL19-048012, NL19-048013; Gelderland Province, Arnhem, from soil, Mar. 2017, *D. Peters* (holotype of *F. petersiae* CBS

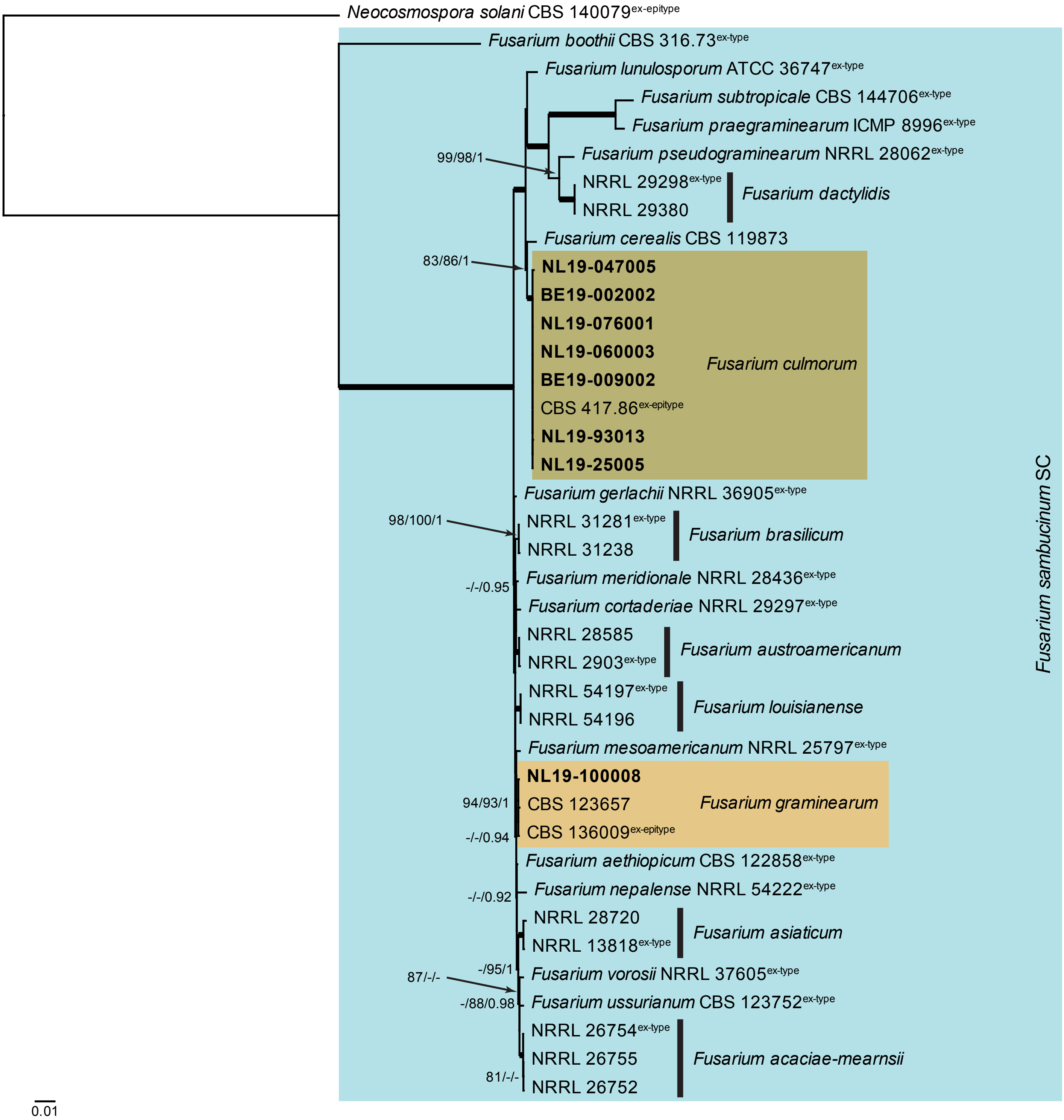

Fig 5. The RAxML consensus tree inferred from the combined *F. sambucinum* species complex *tef1*, *rpb2* (parts 1 and 2), *rpb1* and *cmdA* sequence alignment. Thickened lines indicate branches with full support (RAxML & IQ-TREE bootstrap = 100 %; PP = 1.0) with support values of other branches indicated at the branches (RAxML > 74 % / IQ-TREE > 84 % / PP > 0.74). The tree is rooted to *Neocosmospora solani* (CBS 140079, ex-epitype culture). The scale bar indicates the number of expected changes per site. The *Fusarium sambucinum* species complex is indicated on the right and highlighted with a coloured block. Species clades containing the novel citizen science strains (in bold) are highlighted with coloured blocks.

H-23233, culture ex-type CBS 143231 = JW 14004); *ibid.*, culture JW 14005 = CBS 143667; Nijmegen, from soil, 2017, *J.W. Koolen*, culture JW 248008; North Brabant Province, Valkenswaard, from soil, 2017, *W. van der Heijden*, culture JW 18005; Utrecht Province, Utrecht, from soil, 2017, students of Basisschool de Baanbreker, culture JW267001; Bilthoven, Planetenplein, from garden soil, 31 Jul. 2019, *S. Frederikze, J. Mes & S. Maghnouji*, cultures NL19-97008, NL19-100011 = CBS 147837; Nieuwegein, from soil, 2017, *F. & R. Niemeijer*, culture JW 5026.

Notes: *Fusarium petersiae* was described from soil collected in this citizen science project (Crous *et al.* 2017). In the original publication, it was distinguished from *F. flocciferum* by the formation of sporodochia, up to 5-septate macroconidia, and the lack of conidiophores in aerial mycelium. *Fusarium flocciferum* was originally circumscribed as lacking sporodochia in culture and producing abundant 1–3-septate macroconidia on aerial conidiophores (Booth 1971). As we have shown here (Fig. 1),

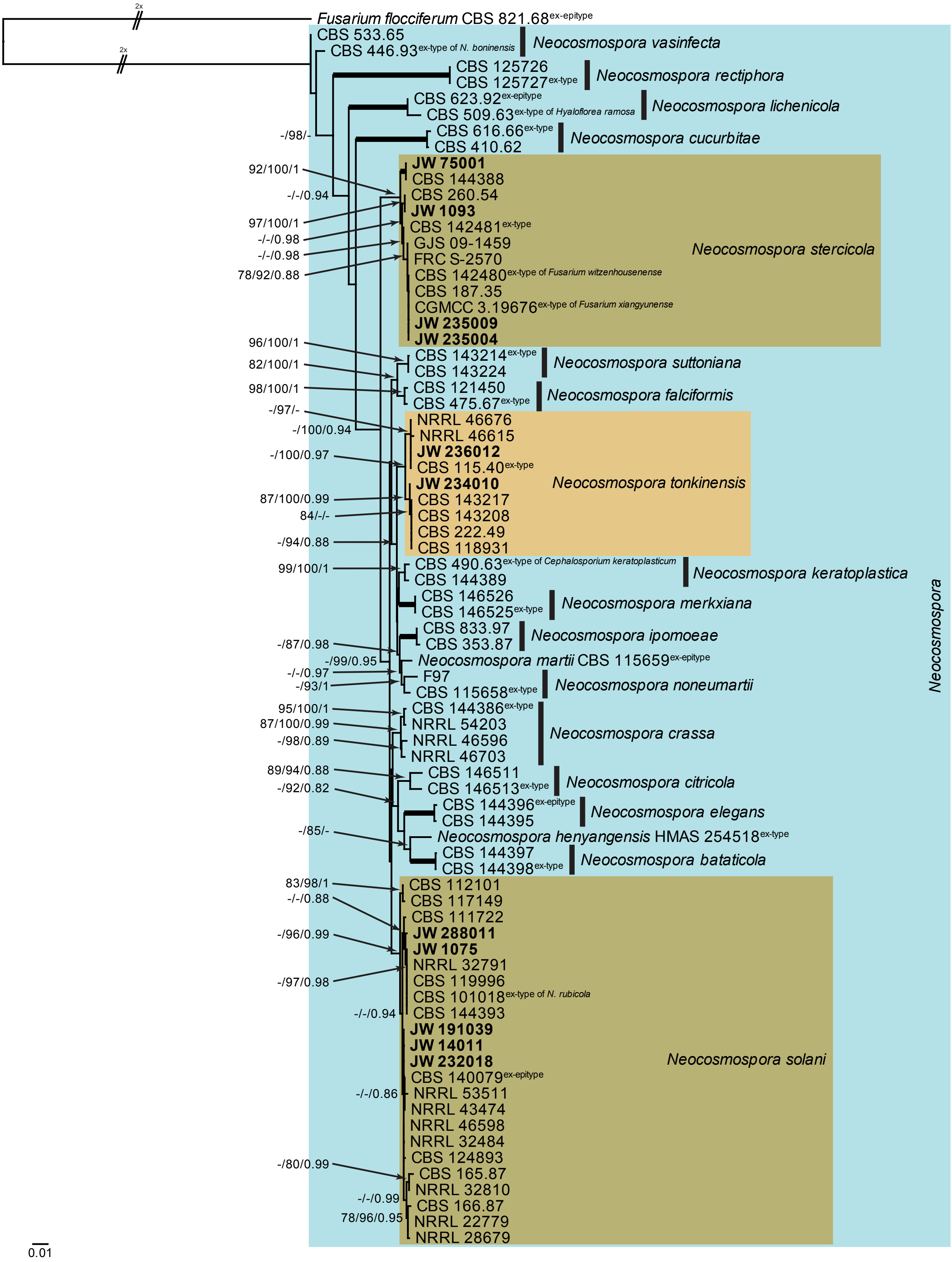

Fig 6. The RAxML consensus tree inferred from the combined *Neocosmospora tef1*, *rpb2* (first part), *rpb1* and *cmdA* sequence alignment. Thickened lines indicate branches with full support (RAxML & IQ-TREE bootstrap = 100 %; PP = 1.0) with support values of other branches indicated at the branches (RAxML > 74 % / IQ-TREE > 84 % / PP > 0.74). The tree is rooted to *Fusarium flocciferum* (CBS 821.68, ex-epitype culture) and the two basal branches were halved to facilitate layout. The scale bar indicates the number of expected changes per site. The genus *Neocosmospora* is indicated on the right and highlighted with a coloured block. Species clades containing the novel citizen science strains (in bold) are highlighted with coloured blocks.

however, *F. petersiae* (CBS 143231) is phylogenetically identical to *F. flocciferum* (ex-type CBS 821.68) and is therefore reduced to synonymy.

***Fusarium* sp. 1.** Fig. 7.

CBS 148217 (= NL19-25001): *Aerial conidiophores* sparingly branched, with terminal or intercalary conidiogenous cells, giving rise to macro- and microconidia; *aerial conidiogenous cells* monophialidic, subulate to subcylindrical, smooth and thin-walled, 5–30 × 2–3.5 µm, with flared collarette and minute periclinal thickening at apex. *Microconidia* aggregating in false heads, ellipsoid to subcylindrical, falcate, 0–1-septate, 5–20 × 3–4 µm. *Sporodochia* pale luteous to orange, abundant on CLA. *Sporodochial conidiophores* densely aggregated, verticillately branched, consisting of a short stipe bearing whorls of 2–3 monophialides; *sporodochial conidiogenous cells* monophialidic, subulate to subcylindrical, 10–15 × 4–5 µm, smooth- and thin-walled, with periclinal thickening at apex and minute, flared collarette. *Sporodochial conidia* falcate, curved dorsiventrally, sides almost parallel, tapering towards both ends; apical cell papillate and curved; basal cell foot-shaped, notch poorly developed, 3(–5)-septate, hyaline, smooth-walled, guttulate; 3-septate conidia (33–)43–45(–48) × (3.5–)4(–5) µm, 5-septate conidia rare, up to 60 µm long. *Chlamydospores* not observed.

Culture characteristics: Colonies spreading, with cottony aerial mycelium. On PDA surface and reverse pale vinaceous. On OA surface pale vinaceous, reverse rosy buff.

Isolates examined: **Netherlands**, Groningen Province, Warffum, from garden soil, 6 Jun. 2019, *C. Dijkstra & L. Kruit*, culture NL19-25001 = CBS 148217; Limburg Province, Ell, 2017, *K. Brennand*, culture JW 53002 = CBS 148216; Utrecht Province, Amersfoort, 2017, *T. & K. Wesselink*, culture JW 191014 = CBS 148204.

Notes: *Fusarium* sp. 1 (CBS 148217) is related (Fig. 3) to *F. tardichlamydosporum* [macroconidia (36–)37–43(–45) × (4–)5–6(–7) µm (av. 40 × 5 µm), 3–5-septate; Maryani *et al.* 2019a], *F. carminascens* [3-septate macroconidia: (21–)26–36(–40) × 3–5 µm (av. 31 × 4 µm); 4-septate macroconidia: (31–)33–43(–44) × 4–5 µm (av. 38 × 4 µm); Lombard et al. 2019]; and *F. vanleeuwenii* [3-septate macroconidia (32–)45–50(–52) × (3.5–)4(–4.5) µm, 4–5-septate conidia 52–60 × 4.5–5 µm, 7–8-septate conidia rare, 65–75 × 5–6 µm] in the FOSC (see elsewhere in this paper). It is morphologically distinct from these species based on the dimensions of its macroconidia. The species is undisguisable from other included species on *cmdA* (intermingled with numerous species), *rpb1* (intermingled with *F. keijii* and *F. joseae*), *rpb2* (intermingled with numerous species), and *tef1* (intermingled with *F. cugenangense*), and can best be identified using a multi-gene phylogenetic analysis. No *tub2* sequences were available for comparison. The species clade is well-supported in two of the analyses (IQ-TREE bootstrap support value = 99 %; Bayesian PP = 0.95). This species is unnamed at present, pending further data.

***Fusarium* sp. 2.** Fig. 8.

CBS 148185 (= JW 1072): *Aerial conidiophores* sparingly branched, 2–20 µm tall, mostly reduced to conidiogenous cells on hyphae; *aerial conidiogenous cells* monophialidic, subulate to subcylindrical, smooth and thin-walled, 2–20 × 2–6 µm, with flared collarette and minute periclinal thickening at apex. *Microconidia* aggregating in false heads, falcate, subcylindrical to reniform, (0–)1(–2)-septate, (10–)13–15(–20) × (3–)3.5–4 µm. *Sporodochia* pale luteous, abundant on CLA. *Sporodochial conidiophores* densely aggregated, verticillately branched, consisting of a short stipe bearing whorls of 2–3 monophialides; *sporodochial conidiogenous cells* monophialidic, subulate to subcylindrical, 9–22 × 3–5 µm, smooth- and thin-walled, with periclinal thickening at apex and minute, flared collarette. *Sporodochial conidia* falcate, moderately curved dorsiventrally, sides almost parallel, tapering towards both ends; apical cell blunt to papillate and curved; basal cell foot-shaped, notch poorly developed, 3(–6)-septate, hyaline, smooth-walled, guttulate; 3-septate conidia (30–)38–43(–47) × 4–5(–6) µm, 4-septate conidia 45–47 × 4.5–5 µm, 5-septate conidia 50–65 × 5 µm. *Chlamydospores* not observed.

Culture characteristics: Colonies flat, spreading, with cottony aerial mycelium. On PDA surface rosy vinaceous, reverse greyish rose. On OA surface and reverse greyish rose.

Isolates examined: **Australia**, Subungual debris of 40-year-old female with nail infection, collection date unknown, collector unknown, culture CBS 130323 =NRRL 26677. **Netherlands**, North Holland Province, Amsterdam, from garden soil, Mar. 2017, *J.F.T.M. van Dijk,* culture CBS 148185 = JW 1072; Zuid-Holland Province, Nootdorp*, Cucumis sativus*, in greenhouse on rockwool, No. 1979, collection date unknown, *N. Hubbeling*, culture CBS 680.89 = IPO 11179 = NRRL 26221. **USA**, on *Chrysantemum* sp., collection date unknown, collector unknown, culture CBS 128.81 =NRRL 36233 = BBA63925.

Notes: *Fusarium s*p. 2. (CBS 148185) is related (Fig. 3) to *F. cugenangense* (FOSC; associated with banana, but non-pathogenic on Gros Michel (AAA) and Cavendish (AAA); Maryani *et al.* 2019a) and *Fusarium* sp. 3 (see below). It is distinguished morphologically from *F. cugenangense* which has smaller micro- (av. 12 × 5 µm), and larger macroconidia (44–)47–54(–57) × (5–)6–7(–8) µm (av. 53 × 7 µm), 3–6-septate (Maryani *et al.* 2019a). *Fusarium* sp. 3 is similar to *Fusarium* sp. 2, but has larger macroconidia, *e.g.* 3-septate macroconidia (33–)43–50(–55) × (3.5–)4(–4.5) µm, 5-septate macroconidia 65–75 × 4–5 µm, and produces chlamydospores. This species can readily be distinguished from other included species based on *tef1*, but is undisguisable from other included species on *cmdA*, *rpb1*, *rpb2* and *tub2*. This species clade is supported in two of the analyses (IQ-TREE bootstrap support value = 94 %; Bayesian PP = 0.98), but is left unnamed, pending further data.

***Fusarium* sp. 3.** Fig. 9.

CBS 148207 (= JW 210019): *Aerial conidiophores* sparingly branched, mostly reduced to monophialides; *aerial conidiogenous cells* monophialidic, subcylindrical, smooth and thin-walled, 2–15 × 3–4 µm, with minute collarette at apex. *Microconidia* aggregating in false heads, ellipsoid to subcylindrical, falcate, 0–1-septate, (8–)10–17(–28) × (2.5–)3(–3.5) µm. *Sporodochia* pale white, sparse on CLA. *Sporodochial conidiophores* densely aggregated, verticillately branched, consisting of a short stipe bearing whorls of 2–3 monophialides; *sporodochial conidiogenous cells* monophialidic, subulate to subcylindrical, 5–15 × 3–5 µm, smooth- and thin-walled, with

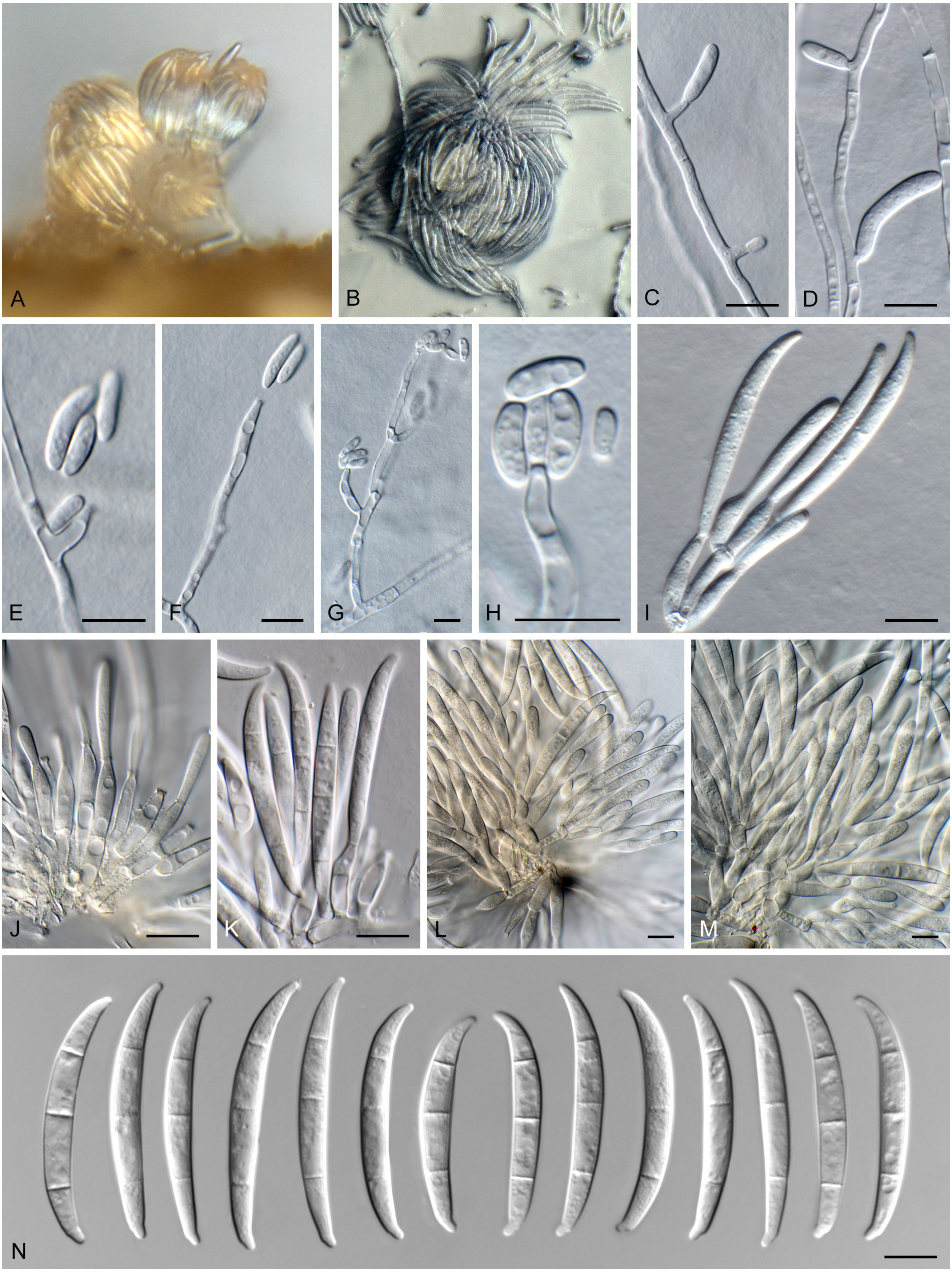

Fig. 7. *Fusarium* sp. 1 (CBS 148217). **A.** Sporodochium on CLA. **B.** Sporodochium on SNA. **C–H.** Aerial conidiophores with microconidia. **I–M.** Sporodochial conidiophores. **N.** Macroconidia. Scale bars = 10 µm.

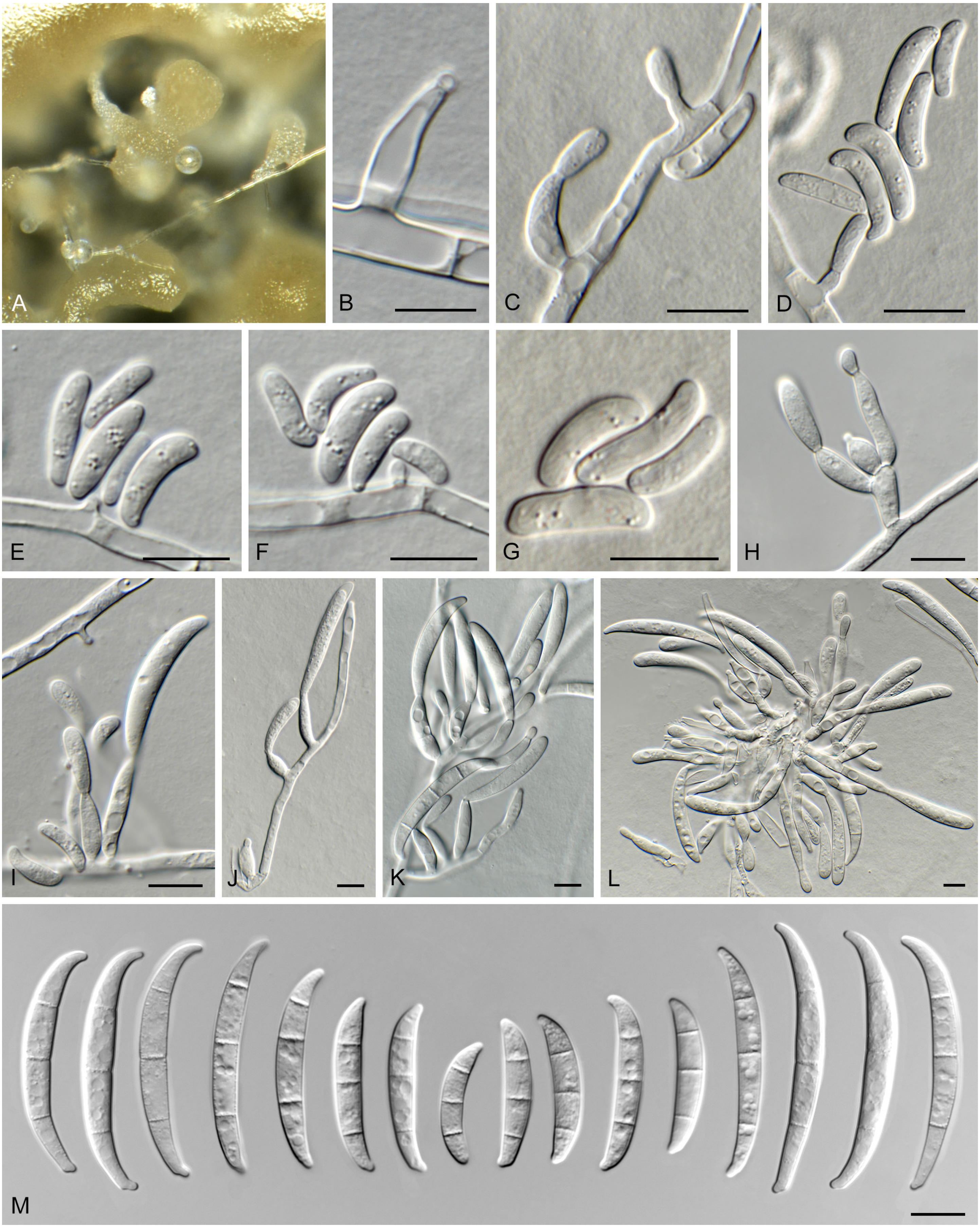

Fig. 8. *Fusarium* sp. 2 (CBS 148185). **A.** Sporodochia on CLA. **B–H.** Aerial conidiophores with microconidia. **I, J.** Aerial conidiophores with macroconidia. **K, L.** Sporodochial conidiophores. **M.** Macroconidia. Scale bars = 10 µm.

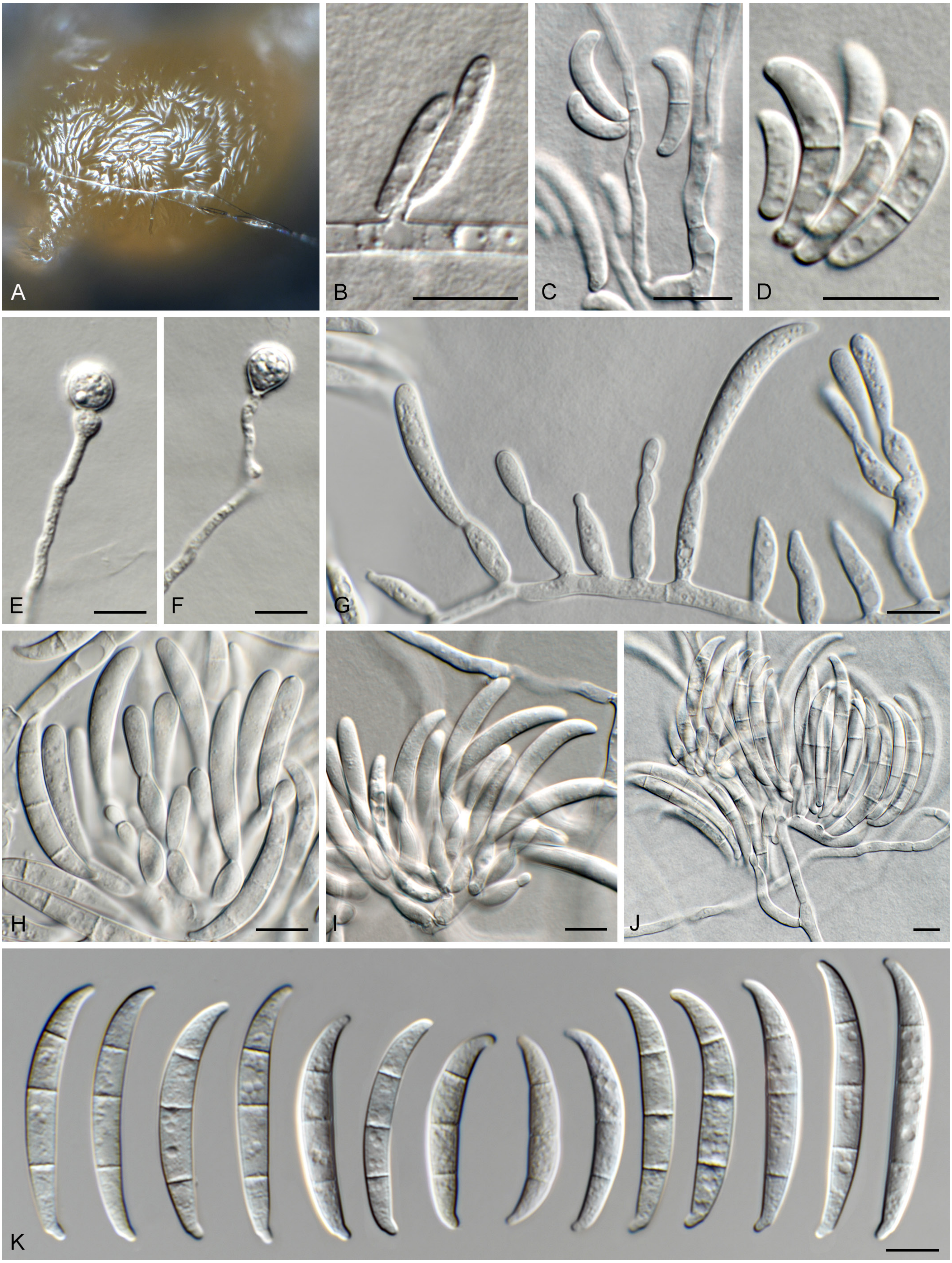

Fig. 9. *Fusarium* sp. 3 (CBS 148207). **A.** Sporodochium on CLA. **B, C, G.** Aerial conidiophores with conidia. **D.** Microconidia. **E, F.** Chlamydospores. **H–J.** Sporodochial conidiophores. **K.** Macroconidia. Scale bars = 10 µm.

periclinal thickening at apex and minute, flared collarette. *Sporodochial conidia* straight to falcate, curved dorsiventrally, sides almost parallel, tapering towards both ends; apical cell blunt or papillate and curved; basal cell foot-shaped, notch poorly developed, 3(–5)-septate, hyaline, smooth-walled, guttulate; 3-septate conidia (33–)43–50(–55) × (3.5–)4(–4.5) µm, 5-septate conidia rare, 65–75 × 4–5 µm. *Chlamydospores* sparingly formed on CLA, subglobose to globose, pale brown, thick-walled, terminal or intercalary, 6–8 µm diam.

Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium. On PDA surface and reverse pale vinaceous. On OA surface and reverse livid vinaceous.

Isolates examined: **Belgium**, East Flanders, Brakel, from garden soil, 2019, *T. Antheunis*, culture BE 19_004006 = CBS 148222. **Netherlands**, Friesland Province, Heerenveen, from garden soil, 2017, *N. Keij*, culture JW 210019 = CBS 148207; Friesland Province, Heerenveen, from garden soil, 2017, *N. Keij*, culture JW 210014 = CBS 148206; Friesland Province, Leeuwarden, from garden soil, 2017, *D. Pol, R. Verf, J. Wilks & M. de Ruiter*, culture JW 231016 = CBS 148208; Gelderland Province, Geldermalsen, from garden soil, 2017, *A.-S. den Boer,* culture JW 9002 = CBS 148199; Gelderland Province, Culemborg, from garden soil, 2017, *I. Kleij*, culture JW 204009 = CBS 148205; Utrecht Province, Amersfoort, from garden soil, 2017, *F. Wiegerinck*, culture JW 4030 = CBS 148198; Utrecht Province, Utrecht, from garden soil, 2017, *M.J. van Leeuwen*, culture JW 10005 = CBS 148200.

Notes: *Fusarium* sp. 3 (CBS 148207) is closely related (Fig. 3) to *Fusarium* sp. 2 [3-septate macroconidia (30–)38–43(–47) × 4–5(–6) µm] in the FOSC, and can be distinguished morphologically in having larger 3-septate macroconidia, and in producing chlamydospores, which were not observed in *Fusarium* sp. 2. This species can readily be distinguished from other included species based on *cmdA* and *tef1*, but is undisguisable from other included species on *rpb1* and *rpb2*. No *tub2* sequences were available for comparison. The species clade is poorly to fully supported in two of the analyses (IQ-TREE bootstrap support value = 85 %; Bayesian PP = 1), but is left unnamed, pending further data.

Fusarium vanleeuwenii Crous & Sand.-Den., ***sp. nov.*** MycoBank MB 840894. Fig. 10.

Etymology: Named after the collector, Maurits Jesse van Leeuwen. This sample was collected during a Citizen Science project of the Westerdijk Fungal Biodiversity Institute.

Typus: **Netherlands**, Utrecht Province, Utrecht, from garden soil, 2017, *M.J. van Leeuwen*, (**holotype** CBS H-24786, culture ex-type CBS 148372 = JW 10008).

Aerial conidiophores irregularly branched, up to 70 µm tall, or reduced to conidiogenous cells on hyphae; conidiogenous cells monophialidic, subulate to subcylindrical, smooth and thin-walled in branched clusters, 10–25 × 4–5 µm; at times reduced to conidiogenous pegs on hyphae, erect, 2–10 × 1.5–2.5 µm, with flared collarette and minute periclinal thickening at apex. *Microconidia* aggregating in mucoid droplets, 0(–2)-septate, ellipsoid to subcylindrical, reniform to somewhat falcate, apical cell becoming hooked, guttulate, (7–)10–14(–18) × 2.5–4 µm. *Sporodochial conidiophores* in moderate numbers on CLA, pale yellow, densely aggregated, irregularly branched, typically in whorls of 2–4 phialides; *sporodochial conidiogenous cells* monophialidic, subulate to subcylindrical, 9–18 × 3–4.5 µm, with periclinal thickening at apex and inconspicuous collarette. *Sporodochial conidia* falcate, moderately curved, more so on outer than inner plane, widest in middle; apical cell papillate to hooked; basal cell foot-shaped, notch poorly developed, (1–)3(–8)-septate, hyaline, smooth-walled, guttulate; 1-septate conidia 15–20 × 3–4 µm, 2-septate conidia 20–25 × 3–4 µm, 3-septate conidia (32–)45–50(–52) × (3.5–)4(–4.5) µm, 4–5-septate conidia 52–60 × 4.5–5 µm, 7–8-septate conidia rare, 65–75 × 5–6 µm. *Chlamydospores* sparse after 1 wk, globose to subglobose, 7–8 µm diam, formed terminally or intercalary, single, smooth-walled, subhyaline.

Culture characteristics: Colonies erumpent, spreading, covering dish in 7 d, with moderate aerial mycelium. On PDA surface vinaceous, reverse rosy vinaceous. On OA surface livid red, reverse greyish rose. On MEA surface and reverse dark vinaceous.

Additional isolates examined: **Netherlands**, Utrecht Province, Utrecht, from garden soil, 2017, *M.J. van Leeuwen*, cultures CBS 148374 = JW 10001, JW 10002, CBS 148375 = JW 10003, CBS 148376 = JW 10004, CBS 148377 = JW 10006, CBS 148378 = JW 10007, JW 10009.

Notes: *Fusarium vanleeuwenii* is distantly related (Fig. 3) to *F. tardichlamydosporum*, a species in the FOSC associated with Panama disease of banana, pathogenic on Gros Michel (AAA) (Foc-Race1) (Maryani *et al.* 2019a). Morphologically, the two species are very similar, but *F. tardichlamydosporum* has smaller micro- (3–)5–9(–15) × (2–)5(–9) µm, and macroconidia (36–)37–43(–45) × (4–)5–6(–7) µm (av. 40 × 5 µm), 3–5-septate (Maryani *et al.* 2019a).

Fusarium vanleeuwenii is characteristic in that it has sparse chlamydospores, the aerial conidiophores are reduced to conidiogenous pegs on hyphae, and the reniform microconidia tend to have hooked apical cells. This species can readily be distinguished from other included species based on *cmdA*, *rpb1*, and *rpb2*, but is intermingled with *F. foetens* and *F. oxysporum* on *tef1*. No *tub2* sequences were available for comparison. The species clade is fully supported in all analyses (RAxML bootstrap support value = 100 %; IQ-TREE bootstrap support value = 100 %; Bayesian PP = 1).

Fusarium wereldwijsianum Crous & Sand.-Den., ***sp. nov.*** MycoBank MB 840895. Fig. 11.

Etymology: Named after the school “Wereldwijs” (Bilthoven, the Netherlands) where the sample was collected. This sample was collected during a Citizen Science project of the Westerdijk Fungal Biodiversity Institute.

Typus: **Netherlands**, Utrecht Province, Bilthoven, Planetenplein, from garden soil, 31 Jul. 2019, *S. Frederikze, J. Mes & S. Maghnouji* (holotype CBS H-24787, culture ex-type CBS 148244 = NL19-94009).

Aerial conidiophores sparingly branched, 5–20 µm tall, bearing terminal and lateral monophialides, but mostly reduced to conidiogenous cells on hyphae; *aerial conidiogenous cells* monophialidic, subulate to subcylindrical, smooth and thin-walled, 5–15 × 3.5–4 µm, with flared collarette and minute

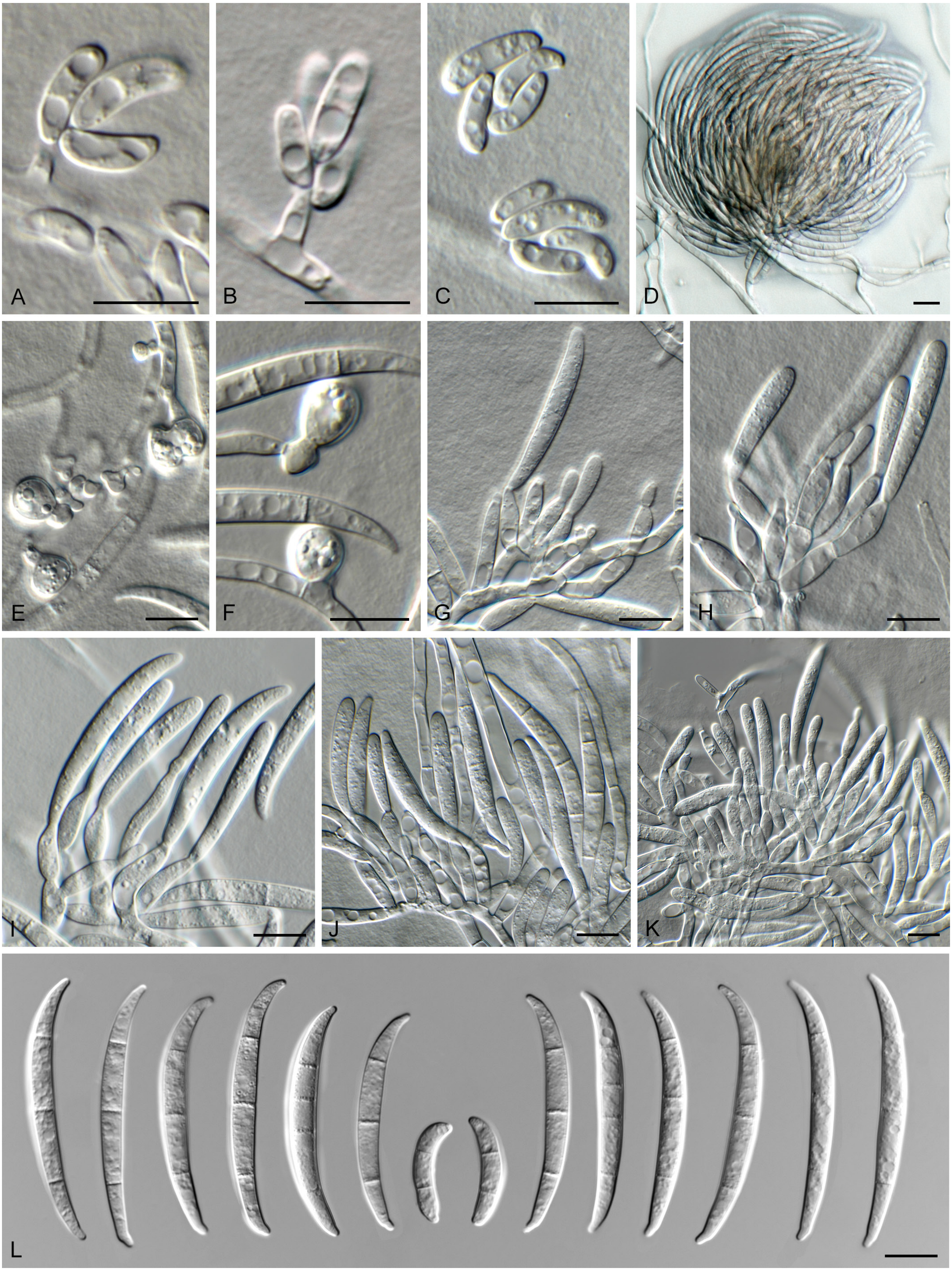

Fig. 10. *Fusarium vanleeuwenii* (CBS 148372). **A–C.** Aerial conidiophores with microconidia. **D.** Sporodochium on SNA. **E, F.** Chlamydospores. **G–K.** Sporodochial conidiophores. **L.** Macroconidia. Scale bars = 10 µm.

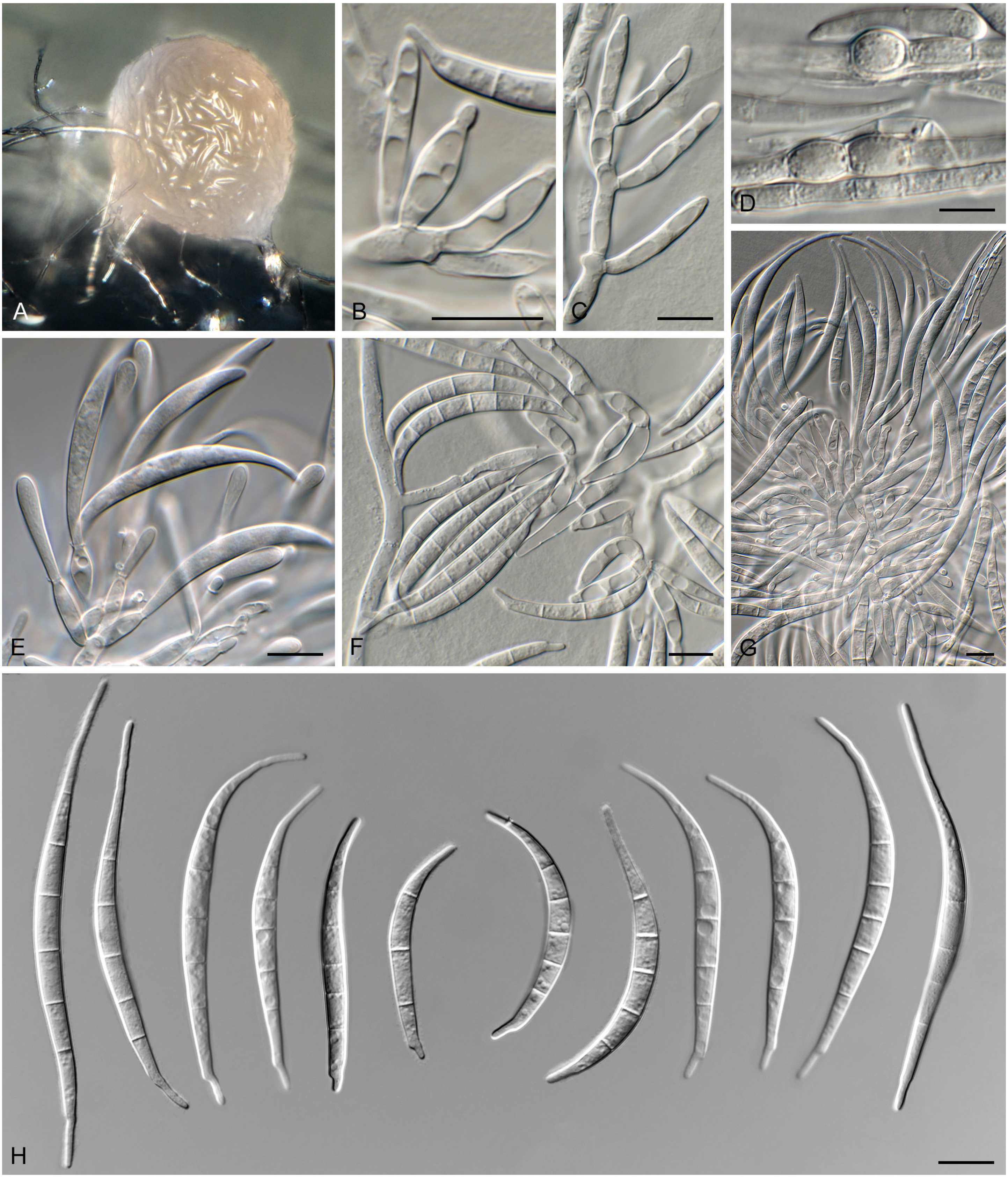

Fig. 11. *Fusarium wereldwijsianum* (CBS 148244). **A.** Sporodochium on CLA. **B, C, E–G.** Sporodochial conidiophores. **D.** Chlamydospores. **H.** Macroconidia. Scale bars = 10 µm.

periclinal thickening at apex. *Aerial conidia* aggregating in false heads, falcate, 1–3-septate, apex obtuse to acutely rounded, base obtuse to notched, (16–)20–22(–25) × 3–3.5(–4) µm. *Sporodochia* orange, abundant on CLA. *Sporodochial conidiophores* densely aggregated, verticillately branched, consisting of a short stipe bearing whorls of 2–4 monophialides; *sporodochial conidiogenous cells* monophialidic, subulate to subcylindrical, 10–20 × 3.5–4 µm, smooth- and thin-walled, with periclinal thickening at apex and minute, flared collarette. *Sporodochial conidia* falcate, curved dorsiventrally, tapering towards both ends; apical cell elongated, curved, whip-like; basal cell foot-shaped, notch well developed, 3(–5)-septate, hyaline, smooth-walled, guttulate; 3-septate conidia (40–)45–60(–65) × 4(–5) µm, 5-septate conidia (45–)55–65 × 4–4.5(–5) µm. *Chlamydospores* on SNA after 1 wk sparse, solitary, intercalary or terminal, subglobose, 6–8 µm diam, becoming brown with age.

Culture characteristics: Colonies spreading, with cottony aerial mycelium. On PDA surface and reverse rosy buff. On OA surface buff to rosy buff, reverse rosy buff to rosy vinaceous.

Additional isolates examined: **Netherlands**, Utrecht Province, Bilthoven, Planetenplein, 31 Jul. 2019, *S. Frederikze, J. Mes & S. Maghnouji*, cultures cultures CBS 148219 = NL19-99003, CBS 148220 = NL19-99002; Zeeland Province, Oostburg, 23 Oct. 2019, *A. van Strien, I. Beemsterboer & S. Groosman,* culture CBS 148386 = NL19-059003; Zeeland Province, Oostburg, Oct. 2019, *F. Guilliet, T. Bron & I. Geernaert,* culture CBS 148385 = NL19-057012.

Notes: *Fusarium wereldwijsianum* is a member of the *F. incarnatum-equiseti* species complex (FIESC; Wang *et al.* 2019, Xia *et al.* 2019), clustering among *F. scirpi, F. serpentinum* and *F. neoscirpi* (Fig. 2). It can be distinguished morphologically from *F. scirpi* which commonly has polyphialides, and 6–7-septate macroconidia (Leslie & Summerell 2006). *Fusarium wereldwijsianum* is further distinguished from *F. neoscirpi* which has smaller macroconidia [3-septate conidia: (28–)32–42(–46) × 4–5 µm (av. 37 × 4 µm); 5-septate conidia: (47–)50–58(–64) × 4–6 µm (av. 54 × 5 µm); Xia *et al.* 2019], and lacks chlamydospores. It is also distinct from *F. serpentinum* which has larger, (3–)5–7(–8)-septate macroconidia [3-septate conidia: (42–)43–51(–54) × 4–6 µm; 5-septate conidia: (57–)67–85(–92) × 4–6 µm; Xia *et al.* 2019]. *Fusarium wereldwijsianum* can readily be distinguished from other included species based on *cmdA*, *rpb1*, and *tef1*, but less readily so on *rpb2*. The species clade is fully supported in all analyses (RAxML bootstrap support value = 100 %; IQ-TREE bootstrap support value = 100 %; Bayesian PP = 1).

DISCUSSION

The present study focused on fusarioid fungi that were isolated from soil in the Netherlands during a Citizen Science project, which already has revealed numerous new species of filamentous fungi and yeasts (Crous *et al*. 2017, 2018, Groenewald *et al.* 2018, Giraldo *et al.* 2019, Hou *et al*. 2020, Crous *et al.* 2021a).

Fusarium and allied fusarioid genera are common soil inhabitants, and therefore it should not be seen as surprising that the present study identified 25 taxa, including 22 *Fusarium* spp., and three species of *Neocosmospora*. One new species was described from the FOSC, namely *F. vanleeuwenii,* and one from the FIESC, namely *F. wereldwijsianum.* Furthermore, *F. petersiae* (Crous *et al*. 2017) was also reduced to synonymy under *F. flocciferum*, which was found to be morphologically more variable than suspected when it was first described (Booth 1971).

Although the various soil samples were collected from garden soils in the urban environment, it was somewhat surprising to also encounter a well-known pathogen of banana, such as *F. odoratissimum* (syn. *F. purpurascens sensu* Crous *et al.* 2021b). Some Dutch isolates clustered with named subclades such as *F. callistephi* (CBS 187.53) or *F. tardicrescens* (JW 6021, JW 6043) (Maryani *et al*. 2019a), or appeared to represent new taxa, which we prefer to leave unnamed for now, pending more data to help resolve species boundaries within this clade. The identification of JW 6021 and JW 6043 as *F. tardicrescens* is based on the *rpb1* and *tef1* association with strain NRRL 37622 (see TreeBASE), a strain previously identified as belonging to that species (Maryani *et al*. 2019a).

Other species isolated that belong to the FOSC include: *F. curvatum*, described from *Beaucarnia* sp. and *Hedera helix* in the Netherlands, but also known from *Matthiola incana* in Germany (Lombard *et al.* 2019); *F. nirenbergiae,* described from *Dianthus caryophyllus* and *Solanum lycopersicum* in the Netherlands, but also known from numerous other plant and animal hosts, including humans, in countries such as Brazil, Italy, South Africa and the USA (Lombard *et al.* 2019); *F. oxysporum,* originally described from a rotten tuber of *Solanum tuberosum*, but having a wide host range with a worldwide distribution (Lombard *et al.* 2019), and *F. triseptatum,* known from hosts such as *Ipomoea batatas*, humans (USA), wilted *Gossypium hirsutum* (Ivory Coast), and sago starch (Papua New Guinea) (Lombard *et al.* 2019).

Five species from the FIESC isolated include: *F. clavus,* known from desert soil in Namibia, but also from various plant hosts in Germany, Iran, Russia and the USA (Xia *et al.* 2019); *F. croceum,* described from soil in the Czech Republic, but also known from *Triticum* in Iran (Xia *et al*. 2019); *F. equiseti,* a saprobe or secondary invader, common in cool to temperate or hot and arid climates (Leslie & Summerell 2006); *F. flagelliforme* known from *Pinus nigra* seedlings in Croatia, and various plant hosts in Germany (Xia *et al.* 2019), and *F. toxicum,* known from soil collected in Germany, but also isolated from a dog in the USA (Xia *et al.* 2019).

The *Fusarium tricinctum* species complex (FTSC) was represented by three species: *F. acuminatum,* a soil saprobe associated with roots and crowns of plants in temperate regions (Leslie & Summerell 2006), *F. torulosum,* occurring in soil in temperate regions, and from a number of plant hosts including cereals, tomatoes, beet root and trees (Leslie & Summerell 2006), and *F. flocciferum,* a common species in temperate regions, occurring in soil, and roots, fruits, stems and twigs of various plant hosts in Europe, North America and Iran (Gerlach & Nirenberg 1982, Torbati *et al.* 2018). The *Fusarium sambucinum* species complex (FSAMSC) was represented by two species: *Fusarium culmorum*, a species commonly found in temperate climates, associated with cereal crowns and grain, and plant debris in soil, and *F. graminearum*, a species primarily associated with maize, wheat and barley, but also other plant hosts (Leslie & Summerell 2006). The *Fusarium redolens* species complex (FRSC) was represented by a single species, *F. redolens*, which is a common soilborne fungus found in temperate areas. Likewise, the *Fusarium fujikuroi* species complex (FFSC) was also associated with a single species, *F. verticillioides*, which is a common pathogen of maize with a worldwide distribution (Leslie & Summerell 2006).

Finally, three species of *Neocosmospora* were also encountered in this study. These include *N. solani*, a common soil inhabitant, which is known from several plant species and has a global distribution. Less well-known species include *N. stercicola*, known from soil, and various other plant hosts in Europe (Sandoval-Denis *et al.* 2019), and *N. tonkinensis*, known from *Musa sapientum* in Vietnam, and various plant hosts in Europe, including *Euphorbia fulgens* in the Netherlands, and a turtle head lesion and human cornea in the USA (Sandoval-Denis 2019).

These findings underline the fact that fusarioid fungi are common soil inhabitants and are generally widely distributed. The ability of these fungi to produce chlamydospores (resting spores) in hyphae, macroconidia, and plant debris, make them well suited to survive adverse conditions for extended periods of time in the soil environment. Although many are saprobic, they appear to also can switch to an opportunistic or pathogenic lifestyle under more favourable conditions, and once in contact with their ideal host(s). It is therefore probable that several of the species described here as presumed saprobes, will in time be shown to be pathogens under favourable conditions.

In conclusion, this study has revealed a high number of fusarioid taxa in the urban soil environment, underlining the importance of this substrate for the discovery of novel taxa, and for gaining a better understanding of species diversity of fusarioid taxa in soil.

ACKNOWLEDGEMENTS

This study was financially supported by the Utrecht University Museum and the Royal Dutch Academy of Arts and Sciences for promoting the Citizen Science project, and for providing a platform to facilitate interaction with various Dutch primary schools. We are grateful to all the children and parents who participated in this project, collecting samples in their gardens and submitting them to the Westerdijk Institute for analyses. We are thankful to the staff from the Westerdijk Institute: Manon Verweij, Karin Schagen and Mariëtte Oosterwegel, for promoting the project and establishing communication with the collectors and schools. We also thank Marjan Vermaas for assistance with the photographic plates.

Conflict of interest: The authors declare that there is no conflict of interest.

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Supplementary Material: http://fuse-journal.org/

Table S1. Collection details and GenBank accession numbers of strains used in the phylogenetic trees.