Persoonia 40, 2018: 1–25
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

ISSN (Online) 1878-9080
https://doi.org/10.3767/persoonia.2018.40.01

Symptomatic *Citrus* trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species

M. Sandoval-Denis[1,2], V. Guarnaccia[1], G. Polizzi[3], P.W. Crous[1,2,4]

Key words

Citrus canker
citrus dieback
morphology
multigene phylogeny
systematics

Abstract The diversity of fusaria in symptomatic *Citrus* trees in Greece, Italy and Spain was evaluated using morphological and molecular multi-locus analyses based on fragments of the calmodulin (*CAM*), intergenic spacer region of the rDNA (IGS), internal transcribed spacer region of the rDNA (ITS), large subunit of the rDNA (LSU), RNA polymerase largest subunit (*RPB1*), RNA polymerase second largest subunit (*RPB2*), translation elongation factor 1-alpha (*EF-1α*) and beta-tubulin (*TUB*) genes. A total of 11 species (six *Fusarium* spp., and five *Neocosmospora* spp.) were isolated from dry root rot, crown, trunk or twig canker or twig dieback of citrus trees. The most commonly isolated species were *Fusarium sarcochroum*, *F. oxysporum* and *Neocosmospora solani*. Three new *Fusarium* species are described, i.e., *F. citricola* and *F. salinense* belonging to the newly described *F. citricola* species complex; and *F. siculi* belonging to the *F. fujikuroi* species complex. Results of pathogenicity tests showed this new complex to include prominent canker causing agents affecting several *Citrus* spp. In addition, two new species are described in *Neocosmospora*, named *N. croci* and *N. macrospora*, the latter species being clearly differentiated from most members of this genus by producing large, up to nine-septate sporodochial conidia.

Article info Received: 3 March 2017; Accepted: 7 April 2017; Published: 1 June 2017.

INTRODUCTION

Fusarium (*Hypocreales*, *Nectriaceae*) is one of the most renowned genera in kingdom *Fungi*. It includes in its broad sense, a large number of morphologically and phylogenetically diverse fungi, commonly found as air-, soil- or water-borne saprobic organisms, and also found either in dead or living plant material as endophytes or epiphytes (Leslie & Summerell 2006, 2011, Aoki et al. 2014). Many *Fusarium* spp. are also important plant pathogens or secondary invaders with worldwide distribution, while numerous species are significant mycotoxigenic species or agents of devastating human and animal diseases, often isolated from immunocompromised hosts (O'Donnell et al. 2010, 2016, Aoki et al. 2014, Van Diepeningen et al. 2014).

First described by Link (1809) and typified by *Fusarium roseum* (presently *F. sambucinum* nom. cons.) (Gams et al. 1997), the generic and species concepts in *Fusarium* have endured significant changes since the cornerstone phenotypically-based taxonomic treatments that grouped species into sections, morphological varieties or forms and later in *formae speciales* based on pathogenicity and host ranges (Wollenweber & Reinking 1935, Snyder & Hansen 1940, Toussoun & Nelson 1976, Gerlach & Nirenberg 1982, Nelson et al. 1983, Burgess et al. 1988); and the following redistribution of species into complexes after the introduction of modern molecular tools (O'Donnell et al. 2000, 2013, Geiser et al. 2013, Aoki et al. 2014). Currently, more than 1 400 *Fusarium* names are listed in the Index Fungorum and MycoBank databases.

[1] Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands;
corresponding author e-mail: p.crous@westerdijkinstitute.nl.
[2] Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa.
[3] Dipartimento di Agricoltura, Alimentazione ed Ambiente, sezione Patologia Vegetale, University of Catania, Via S. Sofia 100, 95123 Catania, Italy.
[4] Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Gräfenhan et al. (2011) and Schroers et al. (2011) provided compelling phylogenetic evidence indicating that the traditional morphology-based concept of *Fusarium* is polyphyletic, suggesting the splicing of the genus into several linages, many of them linked to known distinct sexual-morphs. Contrary arguments were presented by Geiser et al. (2013), arguing for a wider definition of the genus in order to conserve the long standing use of *Fusarium* avoiding the exclusion of many agriculturally and medically relevant species, especially those in the *Fusarium solani* species complex (FSSC). More recently, Lombard et al. (2015) revised the generic limits of the *Nectriaceae* based on a 10-gene phylogenetic approach combined with morphological observations; as a result *Fusarium* was confined to species producing a *Gibberella* sexual morph (perithecial ascomata, white, yellow, orange to dark purple-black coloured with warty superficial peridium cells, forming (0–)1–3-septate, smooth, ellipsoidal ascospores) and in this new circumscription it includes at least 16 species complexes and numerous monotypic lineages (O'Donnell et al. 2013). *Neocosmospora* now includes one the most recognised groups of plant, human and animal pathogens previously assigned to the *Fusarium solani* species complex, characterised by forming yellow, orange or red-brown coloured perithecial sexual-morphs, with smooth to coarsely warted, large and angular superficial peridial cells, producing aseptate or 1-septate, globose to ellipsoidal, finely striate ascospores. Lastly, two new genera were proposed, *Bisifusarium* which encompasses asexual species previously included in the *Fusarium dimerum* species complex, including species associated with fruit rot and roots of *Citrus* spp. as well as clinically relevant fungi (Schroers et al. 2009), morphologically characterised by the lack of microconidia, a rather slow growth, forming slimy colonies on artificial media, and the production of short fusarium-like 0–1(–2)-septate macroconidia, while no sexual-morph has ever been described (Gerlach & Nirenberg 1982, Leslie & Summerell 2006, Schroers et al. 2009), and *Rectifusarium* to include species previously allocated to the *Fusarium ventricosum* species complex, characterised by the

absence of sporodochia and the production of wedge-shaped macroconidia, terminal chlamydospores and dark-red, smooth-walled perithecia, forming 1-septate and verrucose ascospores (Wollenweber 1913, Booth 1971).

Fusarium was recently included in the top 10 globally most important genera of plant pathogenic fungi, based on perceived scientific and economic importance, in particular because of the *F. graminearum* (FGSC) and *F. oxysporum* (FOSC) phylogenetic species complexes (Dean et al. 2012). Further impactful fusaria include *Fusarium subglutinans* and *F. verticillioides* as well as *Neocosmospora* (*Fusarium*) *solani* s.str., and other members of the *Neocosmospora solani* species complex (FSSC) (Zhang et al. 2006).

Citrus is one of the most important fruit crops worldwide, second only to apple (FAO 2016). European countries, especially Italy and Spain, are among the largest producers and exporters worldwide (FAO 2016). *Fusarium* species are commonly found in soils and plants of citrus, in both orchard and nursery environments, and have been reported to be associated with major diseases of citrus plants (Menge 1988, Derrick & Timmer 2000), connected to several symptoms, such as dry root rot, root rot, feeder root rot, wilt, twig dieback and citrus decline (Menge 1988, Spina et al. 2008). *Neocosmospora* (*Fusarium*) *solani* s.lat. is the causal organism of a disease named dry root rot of citrus. The association between stressed plants and *N. solani* can be destructive causing a sudden decline when the plant is weakened by factors such as root girdling or injuries, association with Phytophthora rot, grafting incompatibility, poor drainage, poor soil aeration, excess fertilizer or soil pH alteration (Menge 1988, Polizzi et al. 1992). Members of FOSC are associated with Fusarium wilt of various citrus hosts (Timmer et al. 1979, Timmer 1982). Chlorosis and epinasty of young leaves, wilt, leaf abscission and young twig dieback are the first symptoms of this vascular disease. Often gum exudation and vascular discoloration are observed on affected twigs (Timmer et al. 1979, Timmer 1982). *Fusarium equiseti* has been isolated from citrus roots in Florida (Smith et al. 1988), while *F. proliferatum*, *F. sambucinum* and *Neocosmospora* (*Fusarium*) *solani* were isolated from roots in citrus orchards in Greece (Malikoutsaki-Mathioudi et al. 1987). Moreover, *F. oxysporum* f. sp. *citri* was recently found causing wilt on citrus in Tunisia (Hannachi et al. 2014).

By contrast, positive ecological interactions between fusaria and *Citrus* spp. have been recorded for species formerly included in *Fusarium*, i.e., *Microcera coccophila* (Syn *Fusarium coccophilum*) and *Microcera larvarum* (Syn *Fusarium larvarum*), successfully employed as biocontrol agents against citrus fruit attacking armoured scales (McCoy et al. 2009, Dao et al. 2015, Moore & Duncan 2016).

While *Fusarium* taxonomy is actively changing, with numerous species being described each year mostly based in molecular phylogenetic approaches, just a handful of studies deal with the distribution of *Fusarium* spp. in *Citrus*, and there is scant data for the Mediterranean basin. During a recent survey to identify fungal pathogens associated with *Citrus* in Europe, several fusarium-like isolates were obtained from diverse symptomatic tissues. This study was conducted in order to fully characterise these isolates using morphological and molecular characters. Furthermore, many papers discuss the dilemma to reproduce *Fusarium* diseases of citrus via artificial inoculations because of an uncertain interaction with biotic and abiotic factors (Graham et al. 1985, Dandurand & Menge 1993). In the present study, we thus only tested those *Fusarium* spp. isolated from twig and trunk canker disease symptoms, to determine their ability to induce those same disease symptoms.

MATERIALS AND METHODS

Sampling

During 2015 and 2016 surveys were performed in important citrus-producing regions of Europe. Twigs, trunks and crown sections were collected from plants showing cankers, dry root rot, wilt and decline.

Fragments (5 × 5 mm) of symptomatic tissues were cut from the leading edges of lesions, surface-sterilised in a sodium hypochlorite solution (10 %) for 20 s, followed by 70 % ethanol for 30 s, and rinsed three times in sterilised water. Tissue fragments were dried in sterilised filter paper, placed on 2 % potato dextrose agar (PDA) amended with 100 µg/mL penicillin and 100 µg/mL streptomycin (PDA-PS) and incubated at 25 °C until characteristic *Fusarium* colonies were observed, after which pure cultures were obtained by transferring single conidia to fresh PDA.

Fungal isolates

A total of 39 fusarium-like isolates were obtained from symptomatic tissues of living *Citrus* spp. (Table 1).

Morphological characterisation

All isolates were characterised based on their cultural and morphological characteristics following protocols described by Aoki et al. (2003, 2005). Colony morphology, pigmentation, odour and growth rates were evaluated at 3, 4 and 7 d on PDA and oatmeal agar (OA) (recipes in Crous et al. 2009) at 25 °C with a 12/12 h cool fluorescent light/dark cycle, while colony colours were rated according to Rayner (1970). Mycelial growth rates were evaluated according to protocols described elsewhere (Aoki et al. 2013), with some modifications; briefly, cultures were prepared on PDA and OA by transferring agar blocks of approximately 5 × 5 mm from cultures on SNA. These cultures were incubated in the dark at temperatures ranging from 6–40 °C in 3 °C intervals and growth rates were recorded after 1, 4 and 7 d. Radial mycelial growth rates were calculated as mean values per day by measuring the difference in colony size in 16 directions around the colony, all measurements were made in duplicate. Morphological observations included the presence and characteristics of sporodochia, sporodochial and microconidial size, shape and degree of septation; disposition of the microconidia; conidiophore length and branching patterns, nature of the conidiogenous cells and presence or absence of chlamydospores using synthetic nutrient poor agar (SNA; Nirenberg 1976) with and without sterilised pieces of carnation leaves (Snyder & Hansen 1947, Fisher et al. 1982), incubated at room temperature (approximately 20 °C) (Leslie & Summerell 2006), using the same photoperiod described above. Micromorphological characteristics were examined and photo-documented using water as mounting medium on a Nikon Eclipse 80i microscope with Differential Interference Contrast (DIC) optics and a Nikon AZ100 stereomicroscope, both equipped with a Nikon DS-Ri2 high definition colour digital cameras. Photographs and measurements were taken using the Nikon software NIS-elements D software v. 4.50. The length and width of at least 30 conidiogenous cells and 50 conidia were measured, and the mean values, SD plus maximum-minimum values were calculated. To facilitate the comparison of relevant morphological features of the micro- and macroconidia, composite photo plates were assembled from separate photographs using PhotoShop CS5.1.

Table 1 Isolates form *Citrus* included in this study.

Species name[1]	Strain number[2]	Country and region	Source	Associated symptoms	GenBank accession number[3]							
					CAM	*EF-1α*	IGS	ITS	LSU	*RPB1*	*RPB2*	*TUB*
F. citricola	CPC 27067	Italy, Cosenza	*Citrus limon*	Twigs canker		LT746194		LT746242	LT746242	LT746287	LT746307	
	CPC 27069	Italy, Vibo Valentia	*Citrus sinensis*	Twigs canker		LT746195		LT746243	LT746243	LT746288	LT746308	
	CPC 27709	Italy, Taranto	*Citrus sinensis*	Trunk canker		LT746196		LT746244	LT746244	LT746289	LT746309	
	CPC 27805 = CBS 142421[T]	Italy, Cosenza	*Citrus reticulata*	Crown canker		LT746197		LT746245	LT746245	LT746290	LT746310	
	CPC 27813	Italy, Cosenza	*Citrus reticulata*	Crown canker		LT746198		LT746246	LT746246	LT746291	LT746311	
F. ensiforme	CPC 27190	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746199		LT746247	LT746247		LT746312	
	CPC 27191	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746200		LT746248	LT746248		LT746313	
F. oxysporum	CPC 27194	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746201	LT746233	LT746249	LT746249		LT746314	
	CPC 27196	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746202	LT746234	LT746250	LT746250		LT746315	
	CPC 27700	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746203	LT746235	LT746251	LT746251		LT746316	
	CPC 27701	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746204	LT746236	LT746252	LT746252		LT746317	
	CPC 27702	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746205	LT746237	LT746253	LT746253		LT746318	
	CPC 28190	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746206	LT746238	LT746254	LT746254		LT746319	
F. salinense	CPC 26403	Italy, Catania	*Citrus sinensis*	Twigs canker		LT746191		LT746239	LT746239	LT746284	LT746304	
	CPC 26457	Italy, Catania	*Citrus sinensis*	Twigs canker		LT746192		LT746240	LT746240	LT746285	LT746305	
	CPC 26973 = CBS 142420[T]	Italy, Leni, Messina	*Citrus sinensis*	Twigs canker		LT746193		LT746241	LT746241	LT746286	LT746306	
F. sarcochroum	CPC 26369	Italy, Catania	*Citrus limon*	Twigs dieback		LT746207		LT746255	LT746255	LT746292	LT746320	
	CPC 26370	Italy, Catania	*Citrus limon*	Twigs dieback		LT746208		LT746256	LT746256	LT746293	LT746321	
	CPC 26851	Greece, Missolonghi	*Citrus reticulata*	Trunk canker		LT746209		LT746257	LT746257	LT746294	LT746322	
	CPC 27921	Italy, Catania	*Citrus sinensis*	Trunk canker		LT746210		LT746258	LT746258	LT746295	LT746323	
	CPC 28075	Spain, Alginet	*Citrus reticulata*	Twigs dieback		LT746211		LT746259	LT746259	LT746296	LT746324	
	CPC 28116	Spain, Algemesi	*Citrus reticulata*	Twigs dieback		LT746212		LT746260	LT746260	LT746297	LT746325	
	CPC 28118	Spain, Castellò	*Citrus limon*	Twigs dieback		LT746213		LT746261	LT746261	LT746298	LT746326	
F. siculi	CPC 27188 = CBS 142422[T]	Italy, Catania	*Citrus sinensis*	Dry root rot	LT746189	LT746214		LT746262	LT746262	LT746299	LT746327	LT746346
	CPC 27189	Italy, Catania	*Citrus sinensis*	Dry root rot	LT746190	LT746215		LT746263	LT746263	LT746300	LT746328	LT746347
N. croci	CPC 27186 = CBS 142423[T]	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746216		LT746264	LT746264		LT746329	
	CPC 27187	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746217		LT746265	LT746265		LT746330	
N. macrospora	CPC 28191 = CBS 142424[T]	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746218		LT746266	LT746281		LT746331	
	CPC 28192	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746219		LT746267	LT746282		LT746332	
	CPC 28193	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746220		LT746268	LT746283		LT746333	
N. solani	CPC 27192	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746221		LT746269	LT746269		LT746334	
	CPC 27193	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746222		LT746270	LT746270		LT746335	
	CPC 27198	Italy, Catania	*Citrus sinensis*	Dry root rot		LT746223		LT746271	LT746271		LT746336	
	CPC 27199	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746224		LT746272	LT746272		LT746337	
	CPC 27200	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746225		LT746273	LT746273		LT746338	
	CPC 28189	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746226		LT746274	LT746274		LT746339	
Neocosmospora sp. FSSC 9	CPC 27195	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746227		LT746275	LT746275		LT746340	
Neocosmospora sp. FSSC 28	CPC 28194	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746228		LT746276	LT746276		LT746341	
	CPC 28195	Italy, Siracusa	*Citrus sinensis*	Dry root rot		LT746229		LT746277	LT746277		LT746342	

[1] *F*: *Fusarium*. *N*: *Neocosmospora*.

[2] [T] Ex-type strains; CPC: Culture collection of P.W. Crous, held at the Westerdijk Fungal Biodiversity Institute (formerly CBS-KNAW Fungal Biodiversity Centre), Utrecht, The Netherlands.

[3] *CAM*: Calmodulin; *EF-1α*: Translation elongation factor 1-alpha; IGS: Intergenic spacer region of the rDNA; ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: Partial large subunit of the rDNA; *RPB1*: RNA polymerase largest subunit; *RPB2*: RNA polymerase second largest subunit; *TUB*: Beta-tubulin.

Table 2 Origin, culture and sequence GenBank accession numbers of strains used for phylogenetic analyses.

Species name[1]	Strain number[2]	Country and source	GenBank accession number[3]						
			CAM	*EF-1α*	ITS	LSU	*RPB1*	*RPB2*	*TUB*
F. acuminatum	NRRL 36147 = CBS 109232	Unknown, human bronchial secretion	–	GQ505420	GQ505452	GQ505452	HM347174	GQ505484	–
	NRRL 52789	Taiwan, eggplant soil	–	JF740857	JF740933	JF740933	JF741010	JF741183	–
	NRRL 54210	Unknown	–	HM068308	HM068318	HM068318	–	HM068328	–
F. agapanthi	NRRL 54463^{T}	Australia, *Agapanthus* sp.	KU900611	KU900630	–	–	KU900620	KU900625	KU900635
F. ananatum	NRRL 22945 = CBS 184.29	England, *Ananas comosus*	–	KR071762	U34562	–	JX171505	–	–
	NRRL 53131	Italy, human	–	HM347128	–	–	HM347198	HM347213	–
F. andiyazi	NRRL 31727^{T} = CBS 119857	South Africa, *Sorghum bicolor* soil debris	–	KR071718	KR071651	–	–	KT154004	KP662894
F. anguioides	NRRL 25385^{NT} = ATCC 66485	China, soil in bamboo grove	–	–	–	–	–	JX171624	–
F. anthophilum	NRRL 13602 = CBS 737.97	Germany, *Hippeastrum* sp.	–	AF160292	–	–	–	–	U61541
	NRRL 25214	Germany, *Hippeastrum* sp.	KU171416	KF466414	–	–	KU171676	KU171696	KF466436
F. armeniacum	NRRL 6227 = ATCC 36781	USA, fescue hay	–	–	–	–	–	JX171560	–
F. asiaticum	NRRL 13818 = CBS 110257	Japan, barley	–	–	–	–	–	JX171573	–
F. avenaceum	FRC R-09495	USA, *Lisianthus* sp.	–	GQ915502	–	–	–	GQ915486	–
	NRRL 25128	Poland, *Hymenoptera ichneumonidae*	–	JF740751	JF740894	JF740894	JF740962	JF741079	–
	NRRL 25129	Poland, *Hymenoptera ichneumonidae*	–	JF740752	JF740895	JF740895	–	JF741080	–
	NRRL 25130	USA, egg mass from *Lymantria dispar*	–	JF740753	JF740896	JF740896	–	JF741081	–
	NRRL 54939	Finland, barley	–	–	–	–	JX171551	JX171663	–
F. babinda	NRRL 25539 = CBS 396.96	Australia, rainforest soil	–	–	–	–	–	KU171698	–
F. begoniae	NRRL 25300^{T} = CBS 403.97	Germany, *Begonia elatior* hybrid plant	–	AF160293	–	–	–	–	U61543
F. beomiforme	NRRL 25174 = CBS 740.97	New Caledonia, soil	–	–	–	–	–	JX171619	–
F. brasiliense	NRRL 22743	Brazil, *Glycine max*	–	EF408407	FJ919502	FJ919502	–	EU329525	–
F. buharicum	NRRL 13371 = CBS 796.70	Iran, *Hibiscus cannabinus*	–	–	–	–	JX171449	JX171563	–
F. bulbicola	NRRL 13618^{T} = CBS 220.76	Germany, *Nerine bowdenii*	KF466327	AF160294	U61676	–	KF466394	KF466404	KF466437
F. burgessii	CBS 125537T = RBG 5315	Australia, soil	–	–	–	–	–	HQ646393	–
F. circinatum	NRRL 25331^{T} = CBS 405.97	USA, Monterrey pine tree	AF158348	AF160295	NR120263	–	JX171510	JX171623	KM232080
F. coicis	NRRL 66233^{T}	Australia, *Coix gasteenii*	–	–	–	–	–	KP083274	–
F. concentricum	NRRL 25181^{T} = CBS 450.97	Costa Rica, *Musa sapientum*	–	AF160282	NR111886	–	–	–	U61548
F. concolor	NRRL 13459^{T} = CBS 961.87	South Africa, plant debris	–	–	–	–	–	JX171569	–
F. culmorum	NRRL 25475 = CBS 417.86	Denmark, barley kernel	–	–	–	–	–	JX171628	–
F. cuneirostrum	NRRL 31104	Japan, *Phaseolus vulgaris*	–	EF408413	FJ919509	FJ919509	–	EU329558	–
F. denticulatum	NRRL 25302 = CBS 735.97	USA, *Ipomoea batatas*	–	AF160269	–	–	–	–	U61550
F. dlaminii	NRRL 43665	USA, contact lens	–	–	–	–	–	EF470035	–
F. ensiforme	NRRL 28009 = CDC B-5543	USA, human eye	–	DQ246869	DQ094351	DQ236393	–	EF470136	–
	NRRL 32792	Japan, human	–	DQ247101	DQ094561	DQ236603	–	EU329621	–
F. equiseti	NRRL 20697 = CBS 245.61	Chile, *Beta vulgaris*	–	GQ505594	GQ505683	GQ505683	JX171481	JX171595	–
F. euwallaceae	NRRL 54723 = CBS 135855	Israel, beetle from avocado tree	–	JQ038008	JQ038015	JQ038015	–	JQ038029	–
	NRRL 54724 = CBS 135856	Israel, beetle from avocado tree	–	JQ038009	JQ038016	JQ038016	–	JQ038030	–
F. flocciferum	NRRL 25473 = CBS 831.85	Germany, *Triticum aestivum*	–	–	–	–	JX171514	JX171627	–
	NRRL 45999 = UTHSC 06-3449	USA, human scalp	–	GQ505433	GQ505465	GQ505465	HM347195	GQ505497	–
F. fractiflexum	NRRL 28852^{T}	Japan, *Cymbidium* sp.	AF158341	AF160288	AF158304	–	–	–	–
F. fujikuroi	NRRL 13566 = ATCC 38941	China, *Oryza sativa*	–	AF160279	U34557	–	JX171456	JX171570	–
F. gaditjirri	NRRL 45417 = FRC M-8754	Australia, *Heteropogon triticeus*	–	–	–	–	–	KU171704	–
F. globosum	CBS 429.97 = NRRL 26132	South Africa, *Zea mays* seed	–	**LT746230**	**LT746278**	–	**LT746301**	**LT746343**	**LT746348**
	CBS 430.97 = NRRL 26133	South Africa, *Zea mays* seed	–	**LT746231**	**LT746279**	–	**LT746302**	**LT746344**	**LT746349**
	CBS 431.97 = NRRL 26134	South Africa, *Zea mays* seed	–	**LT746232**	**LT746280**	–	**LT746303**	**LT746345**	**LT746350**
	NRRL 26131^{T} = CBS 428.97	South Africa, corn seed	KF466329	AF160285	–	–	KF466396	KF466406	KF466439
F. graminearum	NRRL 31084 = CBS 123657	USA, corn	–	–	–	–	–	JX171644	–
F. heterosporum	NRRL 20692 = CBS 737.79	Ethiopia, *Cynodon dactylon*	–	–	–	–	JX171479	JX171593	–
	NRRL 20693 = CBS 720.79	Netherlands, *Claviceps purpurea* on *Lolium perenne*	–	–	–	–	JX171480	JX171594	–

F. hostae	NRRL 29889 = FRC 0-2074	USA, *Hosta* sp.	–	–	–	–	–	JX171640	–
F. inflexum	NRRL 20433^{T} = CBS 716.74	Germany, *Vicia faba*	AF158366	AF008479	U34577	–	JX171469	JX171583	–
F. keratoplasticum	NRRL 22661^{T}	Japan, human eye	–	DQ246846	DQ094331	DQ236373	–	EU329524	–
	NRRL 28561	USA, human	–	DQ246902	DQ094375	DQ236417	–	EU329552	–
	NRRL 43433	USA, human eye	–	–	–	–	–	DQ790561	–
F. konzum	NRRL 53387	Brazil, *Araucaria angustifolia*	–	–	–	–	–	–	–
F. lacertarum	NRRL 20423 = CBS 130185	India, lizard skin	–	GQ505593	GQ505682	GQ505682	HM347137	JX171581	–
F. lactis	NRRL 25200^{NT} = CBS 411.97	USA, *Ficus carica*	AF158325	AF160272	NR111887	–	–	–	U61551
F. lateritium	FRC L101 = BBA 62455	Guinea, *Coffea canephora*	–	AY707163	–	–	–	–	–
	FRC L107	Zimbabwe, coffee	–	AY707164	–	–	–	–	–
	FRC L110	Papua New Guinea, coffee twig	–	AY707165	–	–	–	–	–
	FRC L112	Papua New Guinea, coffee twig	–	AY707166	–	–	–	–	–
	FRC L120	Unknown, coffee	–	AY707167	–	–	–	–	–
	FRC L200	Philippines, soil	–	AY707168	–	–	–	–	–
	FRC L375	Brazil, dry coffee berry	–	AY707169	–	–	–	–	–
	FRC L376	Brazil, coffee seed	–	AY707170	–	–	–	–	–
	FRC L402	Malawi, coffee bark	–	AY707171	–	–	–	–	–
	FRC L69	Zimbabwe, *Coffea arabica* berries	–	AY707155	–	–	–	–	–
	FRC L81	New Caledonia, orange twig	–	AY707156	–	–	–	–	–
	FRC L82	New Caledonia, orange twig	–	AY707157	–	–	–	–	–
	FRC L83	New Guinea, coffee berry	–	AY707158	–	–	–	–	–
	FRC L84	New Guinea, coffee berry	–	AY707159	–	–	–	–	–
	FRC L86	New Guinea, coffee berry	–	AY707160	–	–	–	–	–
	FRC L87	New Caledonia, coffee berry	–	AY707161	–	–	–	–	–
	FRC L95	Ethiopia, *Coffea arabica*	–	AY707162	–	–	–	–	–
	NRRL 13622	USA, elm tree	–	–	–	–	JX171457	JX171571	–
	NRRL 25197 = CBS 130184	Venezuela, *Bambusa vulgaris*	–	–	–	–	–	HM347207	–
	NRRL 25485 = CBS 746.79	New Zealand, *Citrus* sp.	–	AY707172	–	–	–	–	–
	NRRL 37021	New Guinea, coffee twig	–	–	–	–	JX504710	HM068358	–
F. lichenicola	NRRL 34123	India, human eye	–	DQ247192	DQ094645	DQ236687	–	EU329635	–
F. lyarnte	NRRL 54252 = CBS 125536	Australia, *Sorghum interjectum*	–	–	–	–	–	JX171661	–
F. mangiferae	NRRL 25226^{T} = BBA 69662	India, *Mangifera indica*	AF158334	AF160281	U61691	–	JX171509	JX171622	U61561
F. mexicanum	NRRL 47473	Mexico, mango inflorescence	GU737389	–	–	–	–	–	–
F. napiforme	NRRL 13604^{T} = CBS 748.97	Namibia, *Pennisetum typhoides*	HQ412325	AF160266	U34570	–	HM347136	EF470117	U34428
F. nelsonii	NRRL 13338	Australia, soil	–	GQ505402	GQ505434	GQ505434	JX171447	JX171561	–
F. nisikadoi	NRRL 25179 = CBS 742.97	Japan, *Phyllostachys nigra* var. *henonis*	–	–	–	–	–	JX171620	–
F. nurragi	NRRL 36452 = CBS 392.96	Australia, soil	–	–	–	–	JX171538	JX171650	–
F. nygamai	NRRL 13448^{T} = CBS 749.97	Australia, necrotic sorghum root	–	AF160273	NR_130698	–	–	EF470114	U34426
F. oxysporum	NRRL 22902 = IMI 375335	USA, Douglas fir seedling root	–	AF160312	U34566	–	–	–	U34424
	NRRL 25387 = ATCC 26225	New Zealand, human	–	HM347117	–	–	JX171512	HM347209	–
F. paranaense	CML1830^{T}	Brazil, soybean root	–	KF597797	–	–	–	KF680011	–
	CML1833	Brazil, soybean root	–	KF597798	–	–	–	KF680012	–
F. petroliphilum	NRRL 22141	New Zealand, cucurbit	–	AF178329	DQ094307	DQ236349	–	EU329491	–
	NRRL 43812 = CDC 2006743705	USA, contact lens solution	–	EF453054	EF453205	EF453205	–	EF470093	–
F. phyllophilum	NRRL 13617^{T} = CBS 216.76	Italy, *Dracaena deremensis*	KF466333	AF160274	U34574	–	KF466399	KF466410	KF466443
F. plagianthi	NRRL 22632	New Zealand, *Hoheria glabrata*	–	AF178354	AF178417	AF178386	–	JX171614	–
F. poae	NRRL 13714	Unknown	–	–	–	–	–	JX171572	–
F. proliferatum	NRRL 22944 = CBS 217.76	Germany, *Cymbidium* sp.	–	AF160280	U34558	–	JX171504	HM068352	U34416
F. pseudocircinatum	NRRL 22946^{T} = CBS 126.73	Ghana, *Solanum* sp.	–	AF160271	U34569	–	–	–	U34427
F. pseudonygamai	NRRL 13592^{T} = CBS 417.97	Nigeria, *Pennisetum typhoides*	AF158316	AF160263	NR_137162	–	–	–	U34421
F. ramigenum	NRRL 25208^{T} = CBS 418.97	USA, *Ficus carica*	KF466335	AF160267	NR111888	–	KF466401	KF466412	KF466445
F. redolens	NRRL 22901 = CBS 743.97	Canada, plant seedling, Douglas fir tree	–	–	–	–	–	KU171708	–
F. sacchari	NRRL 13999 = CBS 223.76	India, *Saccharum officinarum*	–	AF160278	U34556	–	JX171466	JX171580	–

Table 2 (cont.)

Species name[1]	Strain number[2]	Country and source	GenBank accession number[3]						
			CAM	*EF-1α*	ITS	LSU	*RPB1*	*RPB2*	*TUB*
F. sambucinum	NRRL 22187 = NRRL 20727	England, potato	–	–	–	–	–	JX171606	–
F. sarcochroum	NRRL 20472 = CBS 745.79	Switzerland, *Viscum album*	–	–	–	–	JX171472	JX171586	–
F. scirpi	NRRL 13402	Australia, pine nursery soil	–	GQ505592	GQ505681	GQ505681	JX171452	JX171566	–
Fusarium sp.	F201237	China, *Zanthoxylum bungeanum*	–	KM527105	–	–	–	KM520371	–
	NRRL 13444	Australia, corn soil	–	GQ505403	GQ505435	GQ505435	JX171454	GQ505467	–
	NRRL 25533	USA, *Lymantria dispar*	–	–	–	–	–	JX171631	–
	NRRL 26417 = CBS 544.96	Cuba, plant leaf litter	–	–	–	–	–	GQ505776	–
	NRRL 26756	South Africa, ornamental grass	–	AF160307	AF158310	–	–	–	AF160322
	NRRL 28578 = CBS 615.87	Cuba, *Colocasia esculenta*	–	GQ505405	GQ505437	GQ505437	JX171526	GQ505469	–
	NRRL 32175	Unknown	–	–	–	–	–	JX171645	–
	NRRL 34036 = UTHSC 01-1965	USA, human ethmoid sinus	–	–	–	–	HM347173	GQ505483	–
	NRRL 52714	Turkey, *Eurygaster* sp.	–	JF740796	JF740911	JF740911	JF740977	JF741122	–
	NRRL 52720	Turkey, *Eurygaster* sp.	–	JF740802	JF740914	JF740914	–	JF741128	–
	NRRL 52722	Turkey, *Eurygaster* sp.	–	JF740804	JF740915	JF740915	JF740980	JF741130	–
	NRRL 52727	Turkey, unknown	–	JF740807	JF740917	JF740917	JF740982	JF741133	–
	NRRL 52730	Turkey, unknown	–	JF740809	JF740918	JF740918	JF740984	JF741135	–
	NRRL 52933	Turkey, unknown	–	JF740875	JF740937	JF740937	JF741019	JF741200	–
F. sterilihyphosum	NRRL 25623^{T}	South Africa, mango	AF158353	AF160300	F158305	–	–	–	–
F. stilboides	NRRL 20429 = ATCC 15662	Nyasaland, coffee bark	–	–	–	–	JX171468	JX171582	–
F. striatum	NRRL 22101	Panama, cotton cloth	–	AF178333	AF178398	AF178367	–	EU329490	–
F. subglutinans	NRRL 22016^{T} = CBS 747.97	USA, corn	–	AF160289	U34559	–	JX171486	JX171599	–
F. sublunatum	NRRL 13384^{T} = CBS 189.34	Costa Rica, soil of banana plantation	–	–	–	–	–	JX171565	–
F. succisae	NRRL 13613 = CBS 219.76	Germany, *Succisa pratensis*	–	AF160291	U34561	–	–	–	U34419
F. thapsinum	NRRL 22045 = CBS 733.97	South Africa, *Sorghum bicolor*	–	AF160270	U34560	–	JX171487	JX171600	–
F. tjaetaba	NRRL 66243^{T}	Australia, *Sorghum interjectum*	–	–	–	–	–	KP083275	–
F. torreyae	NRRL 54149	USA, *Torreya taxifolia*	–	–	–	–	–	JX171660	–
F. torulosum	NRRL 22748 = NRRL 13919	Netherlands, *Buxus* sp.	–	–	–	–	JX171502	JX171615	–
	NRRL 52772	Norway, *Galleria mellonella* larva	–	JF740840	JF740926	JF740926	JF741003	JF741166	–
F. tricinctum	NRRL 25481^{T} = CBS 393.93	Germany, culm base of winter wheat cv diplomat	–	HM068307	HM068317	HM068317	JX171516	HM068327	–
F. tupiense	NRRL 53984^{T}	Brazil, *Mangifera indica*	GU737377	DQ452859	–	–	–	–	–
F. udum	NRRL 22949 = CBS 178.32	Germany, unknown	–	AF160275	U34575	–	–	–	U34433
F. venenatum	NRRL 22196 = BBA 65031	Germany, corn	–	–	–	–	–	JX171607	–
F. verrucosum	NRRL 22566 = BBA 64786	Venezuela, Bamboo culm	–	–	–	–	–	JX171613	–
F. verticillioides	NRRL 22172 = CBS 734.97	Germany, corn	–	AF160262	U34555	–	–	–	U34413
Fusicolla aquaeductuum	NRRL 20686 = CBS 734.79	Germany, drinking water	–	–	–	–	–	JX171590	–
Fusicolla sp.	NRRL 22136 = IMI 297027	India, waste water	–	–	–	–	–	JX171604	–
N. ambrosia	NRRL 20438 = IMI 296597	India, *Camellia sinensis*	–	AF178332	AF178397	DQ236357	–	JX171584	–
	NRRL 22346	India, *Camellia sinensis*	–	FJ240350	EU329669	EU329669	–	EU329503	–
N. falciformis	NRRL 32757	USA, sand	–	DQ247075	DQ094536	DQ236578	–	EU329614	–
	NRRL 32828	USA, human	–	DQ247135	DQ094594	DQ236636	–	EU329626	–
	NRRL 43441	USA, human eye	–	–	–	–	–	DQ790566	–
N. illudens	NRRL 22090	New Zealand, *Beilschmiedia tawa*	–	AF178326	AF178393	AF178362	–	JX171601	–
N. solani	NRRL 22389 = BBA 67587	USA, *Liriodendron tulipifera*	–	AF178340	AF178404	AF178373	–	EU329506	–
	NRRL 32846 = FRC S-1278	USA, human eye	–	–	–	–	–	FJ240410	–
	NRRL 52778	Syria, *Eurygaster* sp.	–	JF740846	JF740931	JF740931	JF741003	JF741172	–
	NRRL 52790	Turkey, *Eurygaster* sp.	–	JF740858	–	–	JF741011	JF741184	–
	NRRL 66304^{ET} = CBS 140079	Slovenia, *Solanum tuberosum*	–	KT313611	KT313633	KT313633	–	KT313623	–
	NRRL 32741	USA, human eye	–	DQ247061	DQ094522	DQ236564	–	EU329608	–

Neocosmospora sp.	FRC S 2432	USA, university building	–	JN235756	JN235326	JN235326	–	JN235941	–
	LEMM 110739	Colombia, human toenail	–	LN827969	LN828118	–	–	LN828057	–
	LEMM 111347	Colombia, human toenail	–	LN827970	LN828119	–	–	LN828058	–
	NRRL 22098	USA, cucurbit	–	AF178327	DQ094301	DQ236343	–	EU329489	–
	NRRL 22153	USA, cucurbit	–	AF178346	DQ094302	DQ236344	–	EU329492	–
	NRRL 22157 = ATCC 18689	Japan, *Morus alba*	–	AF178359	DQ094306	DQ236348	–	EU329493	–
	NRRL 22161 = ATCC 18692	Japan, *Robinea pseudoacacia*	–	AF178330	DQ094311	DQ236353	–	EU329494	–
	NRRL 22163	Japan, *Xanthoxylum piperitum*	–	AF178328	AF178394	AF178363	–	EU329496	–
	NRRL 22178	Venezuela, dicot tree	–	AF178334	AF178399	AF178368	–	EU329498	–
	NRRL 22230 = ATCC 44934	Japan, *Morus alba*	–	AF178358	DQ094305	DQ236347	–	EU329499	–
	NRRL 22354	French Guiana, bark	–	AF178338	AF178402	AF178371	–	EU329504	–
	NRRL 22400	USA, *Ipomoea batatas*	–	AF178343	DQ094303	DQ236345	–	EU329509	–
	NRRL 22570	Brazil, *Piper nigrum*	–	AF178360	AF178422	AF178391	–	EU329513	–
	NRRL 22579	Indonesia, bark	–	AF178352	AF178415	AF178384	–	EU329515	–
	NRRL 22586 = BBA 67586	USA, *Robinea pseudoacacia*	–	AF178353	DQ094312	DQ236354	–	EU329516	–
	NRRL 22642 = ATCC 38341	Japan, gill of *Penaeus japonicus*	–	DQ246844	DQ094329	DQ236371	–	EU329522	–
	NRRL 22782	Spain, human eye	–	DQ246850	EU329670	EU329670	–	EU329528	–
	NRRL 22820	USA, *Glycine max*	–	AF178355	DQ094310	DQ236352	–	EU329532	–
	NRRL 25137	Papua New Guinea, diseased cocoa pods	–	JF740757	JF740899	JF740899	–	JF741084	–
	NRRL 28001	USA, human	–	DQ246866	DQ094348	DQ236390	–	EF470129	–
	NRRL 28008 = CDC B-4701	USA, unknown	–	DQ246868	DQ094350	DQ236392	–	EF470135	–
	NRRL 28541 = UTHSC 98-1305	USA, synovial fluid	–	DQ246882	EU329674	EU329674	–	HM347151	–
	NRRL 31158	USA, human wound	–	DQ246916	DQ094389	DQ236431	–	EU329559	–
	NRRL 31169	USA, human oral wound	–	KR673963	DQ094396	DQ236438	–	KR673999	–
	NRRL 32301 = UTHSC 01-595	USA, human eye	–	DQ246929	EU329677	EU329677	–	EU329567	–
	NRRL 32437 = CBS 109028	Switzerland, human subcutaneous nodule	–	DQ246979	DQ094446	DQ236488	–	EU329581	–
	NRRL 32705	USA, human	–	DQ247025	DQ094488	DQ236530	–	EU329594	–
	NRRL 32736	USA, human eye	–	DQ247056	DQ094517	DQ236559	–	EU329605	–
	NRRL 32755	USA, turtle	–	DQ247073	DQ094534	DQ236576	–	EU329613	–
	NRRL 32770	USA, human eye	–	DQ247083	DQ094544	DQ236586	–	EU329615	–
	NRRL 32785	USA, human	–	DQ247094	FJ240371	FJ240371	–	EU329618	–
	NRRL 32821 = FRC S-1230	USA, turtle egg	–	DQ247128	DQ094587	DQ236629	–	EU329625	–
	NRRL 32858	USA, human	–	DQ247163	DQ094617	DQ236659	–	EU329630	–
	NRRL 37625	Netherlands, human	–	FJ24035	EU329684	EU329684	–	EU329637	–
	NRRL 43502	USA, human eye	–	DQ790488	DQ790532	DQ790532	–	DQ790576	–
	NRRL 45880	USA, *Pisum sativum*	–	FJ240352	EU329689	EU329689	–	JX171655	–
	NRRL 46703	Spain, nematode	–	HM347126	EU329712	EU329712	–	EU329661	–
	NRRL 46707 = FMR 8030	Brazil, human eye	–	HM347127	EU329716	EU329716	–	EU329665	–
	NRRL 52781	Benin, *Hypothenemus hampei*	–	JF740849	–	–	–	JF741175	–
	NRRL 54992 = UTHSC 09-1008	USA, Zebra shark	–	KC808213	KC808255	KC808255	–	KC808354	–
	NRRL 54993 = UTHSC 09-1009	USA, Zebra shark	–	KC808214	KC808256	KC808256	–	KC808355	–
	NRRL 62797	USA, unknown	–	KF906129	KF906130	KF906130	–	KF906132	–
N. vasinfecta	NRRL 22436	South Africa, soil	–	AF178348	AF178412	DQ236359	–	JX171610	–
	NRRL 43467 = CBS 130182	USA, human eye	–	EF452940	EF453092	EF453092	–	EF469979	–

[1] *F*: *Fusarium*. *N*: *Neocosmospora*.

[2] T: Ex-type, ET: Ex-epitype, NT: Ex-neotype. ATCC: American Type Culture Collection, Manasas, VA, USA; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CDC: Centers for Disease Control and Prevention, Atlanta, GA, USA; CML: Coleção Micológica de Lavras, MG, Brazil; F: Laboratory of Zhi-Min Cao, Northwest A&F University, Shaanxi, China; FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain; FRC: Fusarium Research Center, University Park, PA, USA; IMI: CABI Biosciences, Egham, Surrey, England; LEMM: Laboratorio Especializado de Micología Médica, Bogotá, Colombia; NRRL: Agricultural Research Service Culture Collection, NCAUR-ARS-USDA, Peoria, IL, USA; UTHSC: Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center, San Antonio, USA; RBG: Royal Botanic Gardens Trust, Sydney, New South Wales, Australia.

[3] *CAM*: Calmodulin; *EF-1α*: Translation elongation factor 1-alpha; ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: Partial large subunit of the rDNA; *RPB1*: RNA polymerase largest subunit; *RPB2*: RNA polymerase second largest subunit; *TUB*: Beta-tubulin. Sequences generated in this study appear in **bold**.

DNA isolation, PCR and sequencing

Isolates were grown for 7 d on PDA at 25 °C using a 12/12 h photoperiod. Total DNA extraction was performed from fresh mycelium scrapped from the colony surface using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions. Fragments of the calmodulin (*CAM*), the intergenic spacer region of the rDNA (IGS), the internal transcribed spacer region of the rDNA (ITS), a partial fragment of the large subunit of the rDNA (LSU) (spanning the variable domains D1 to D3), RNA polymerase largest subunit (*RPB1*), RNA polymerase second largest subunit (*RPB2*), the translation elongation factor 1-alpha (*EF-1α*) and beta-tubulin (*TUB*) genes were amplified and sequenced using PCR protocols described elsewhere (O'Donnell et al. 1998a, 2007, 2009a, b, 2010, Geiser et al. 2004) using the primer pairs CL1/CL2 for *CAM* (O'Donnell et al. 2009b), iNL11/iCNS1 and the internal sequencing primers NLa/CNSa for IGS (O'Donnell et al. 2009a), ITS4/ITS5 for ITS (White et al. 1990), LR0R/LR5 for LSU (Vilgalys & Hester 1990, Vilgalys & Sun 1994), Fa/G2R for *RPB1* (O'Donnell et al. 2010), 5f2/7cr plus 7cf/11ar for *RPB2* (O'Donnell et al. 2010), EF-1/EF-2 for *EF-1α* (O'Donnell et al. 1998b) and 2Fd/4Rd for *TUB* (Woudenberg et al. 2009). Consensus sequences were assembled from forward and reverse sequences using Seqman Pro v. 10.0.1 (DNASTAR, Madison, WI, USA). All sequences generated in this study were deposited in GenBank (Table 1). A further 585 DNA sequences representing 191 strains were retrieved from GenBank and included in the phylogenetic analyses (Table 2).

Phylogenetic analysis

Sequences of the individual loci were aligned using MAFFT on the web server of the European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/Tools/msa/mafft/) (Katoh & Standley 2013, Li et al. 2015), and the alignments were checked and manually corrected if necessary using MEGA v. 6.06 (Tamura et al. 2013). A first phylogenetic analysis was carried out using *RPB2* sequences in order to assess the isolate distribution on the different species complexes of *Fusarium* and fusarium-like genera. To establish the identity of the isolates to the species level, different phylogenetic analyses were conducted first individually for each locus and then as multilocus sequence analyses using the following loci combinations: *CAM*, *EF-1α*, ITS, *RPB1*, *RPB2* and *TUB* for members of the *Fusarium fujikuroi* species complex (FFSC) (O'Donnell et al. 2000, Edwards et al. 2016); *RPB1*, *RPB2* and *TUB*, for members of the *Fusarium lateritium* species complex (FLSC); *EF-1α*, ITS, LSU, *RPB1* and *RPB2* for isolates related with the *Fusarium tricinctum* species complex (FTSC); and lastly *EF-1α*, ITS, LSU and *RPB2* for isolates belonging to *Neocosmospora* (formerly known as the *Fusarium solani* species complex, FSSC) (O'Donnell et al. 2008, Lombard et al. 2015, Chitrampalam & Nelson 2016). Isolates belonging to the FOSC were characterised based on their haplotype distribution using a two-locus dataset that included *EF-1α* and IGS sequences following the procedures and alignments of O'Donnell et al. (2009a). Phylogenetic inference was based on three independent algorithms: Maximum Parsimony, RaxML and Bayesian analyses. Maximum Parsimony (MP) analyses were conducted using PAUP v. 4.0b10 (Swofford 2002). Heuristic searches were carried out with 1 000 random stepwise addition replicates, with tree bisection and reconstruction (TBR) branch swapping, with all characters treated as equally weighted and gaps treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC, respectively) were calculated. Statistical support for the branches was evaluated using a bootstrap analysis (BS) of 1 000 replicates.

RaxML (ML) and Bayesian analyses (BI) were run on the CIPRES Science Gateway portal (Miller et al. 2012) using RaxML v. 8.2.9 and MrBayes v. 3.2.6, respectively. Evolutionary models were calculated using MrModelTest v. 2.3 (Nylander 2004)

Table 3 Characteristics of the gene partitions used in this study.

Genus/species complex (SC)[1]	Locus[2]	Number of sites				Evolutionary model[3]
		Total	Constant	Variable	Parsimony informative	
Overview tree	*RPB2*	1559	882	670	607	GTR+I+G
F. citricola SC	*EF-1α*	532	335	194	164	GTR+G
	ITS	523	428	95	91	GTR+G
	LSU	524	481	43	39	HKY+I
	RPB1	605	419	186	141	SYM+G
	RPB2	1501	1005	496	454	GTR+I+G
F. fujikuroi SC	*CAM*	655	518	134	76	SYM+G
	EF-1α	455	316	134	67	SYM+G
	ITS	459	421	38	31	SYM+I
	RPB1	1279	1038	241	141	SYM+I+G
	RPB2	1640	1305	335	216	GTR+I+G
	TUB	507	387	119	59	SYM+G
F. oxysporum SC	*EF-1α*	621	483	138	97	NA
	IGS	2220	1422	744	552	NA
F. lateritium SC	*EF-1α*	562	435	125	85	GTR+G
	RPB1	628	508	120	61	SYM+G
	RPB2	696	540	156	77	GTR+I+G
N. solani SC	*EF-1α*	328	211	108	66	GTR+G
	ITS	503	372	127	101	GTR+I+G
	LSU	482	439	43	35	GTR+I+G
	RPB2	1648	1212	436	361	GTR+I+G

[1] *F*: *Fusarium*. *N*: *Neocosmospora*.

[2] *CAM*: Calmodulin; *EF-1α*: Translation elongation factor 1-alpha; IGS: Intergenic spacer region of the rDNA; ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: Partial large subunit of the rDNA; *RPB1*: RNA polymerase largest subunit; *RPB2*: RNA polymerase second largest subunit; *TUB*: Beta-tubulin.

[3] G: Gamma distributed rate variation among sites; GTR: Generalised time-reversible; HKY: Hasegawa-Kishino-Yano; I: Proportion of invariable sites; SYM: Symmetrical model.

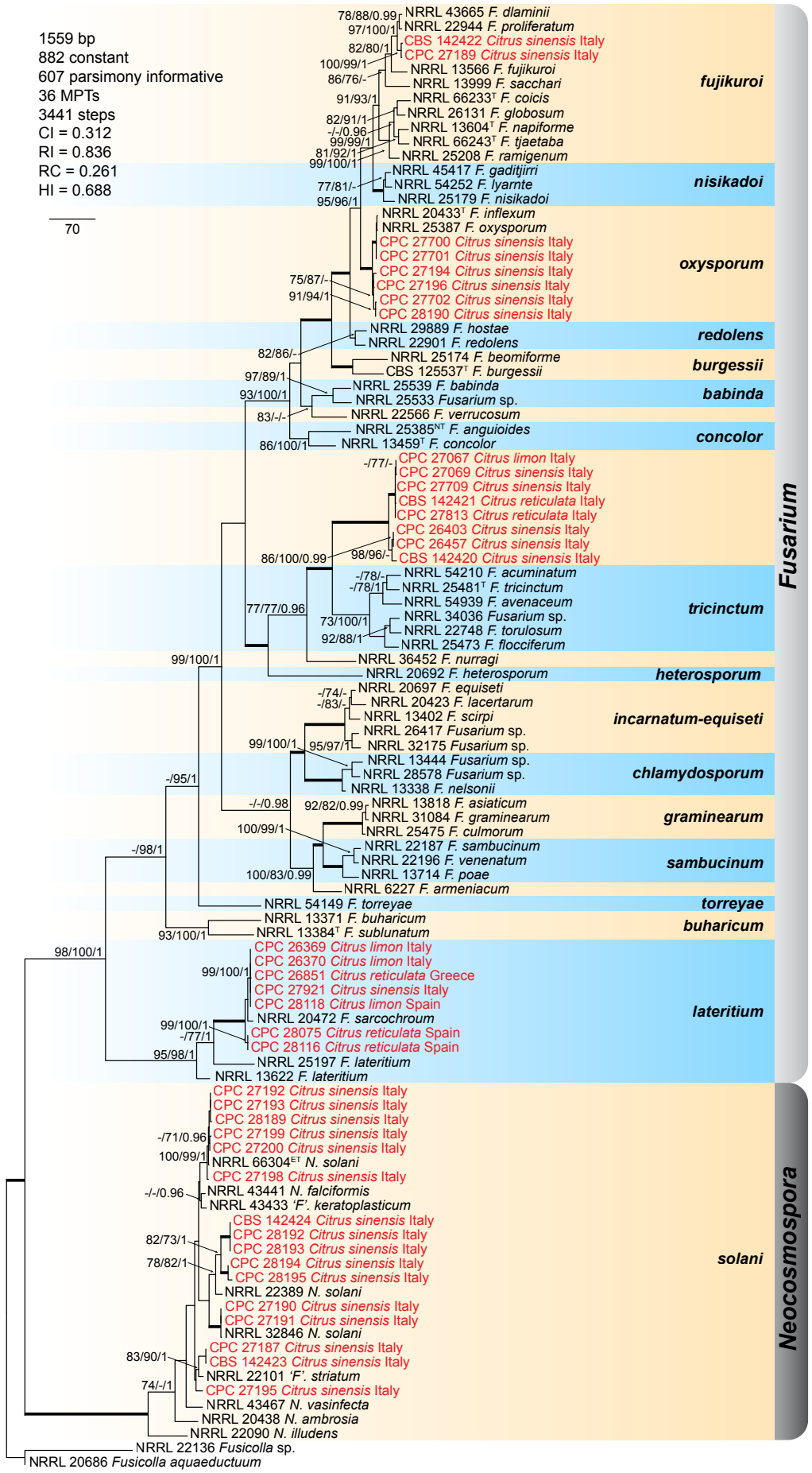

Fig. 1 One of 36 Maximum parsimony (MP) best-tree phylograms obtained from *RPB2* sequences of 99 strains from *Fusarium* and *Neocosmospora* species. Branch lengths are proportional to distance. Numbers on the nodes are MP and RaxML bootstrap values above 70 % and Bayesian posterior probability values above 0.95. Full supported branches and names of each species complex is indicated in **bold**. Isolates obtained from *Citrus* are indicated in red font. Species complexes not including *Citrus*-derived isolates were collapsed. Ex-type and ex-epitype and ex-neotype strains are indicated with ^{T}, ^{ET} and ^{NT}, respectively. The names of known species complexes are shown in **bold**. The tree was rooted to *Fusicolla aquaeductuum* (NRRL 20686) and *Fusicolla* sp. (NRRL 22136).

selecting the best-fit model for each data partition according to the Akaike criterion. The characteristics of the different gene partitions and evolutionary models employed in this study are summarised in Table 3. For ML analyses the default parameters were used and BS was carried out using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included two parallel runs of 5 000 000 generations, with the stop rule option and a sampling frequency set to each 1 000 generations. The 50 % majority rule consensus trees and posterior probability (PP) values were calculated after discarding the first 25 % of the samples as burn-in. The resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). The individual gene datasets were assessed for incongruence before being concatenated by checking their individual phylogenies for conflicts between clades with significant MP, ML and BI support (Mason-Gamer & Kellogg 1996, Wiens 1998). Alignments and phylogenetic trees derived from this study were uploaded to TreeBASE (www.treebase.org).

Genealogical concordance phylogenetic species recognition (GCPSR)

In order to determine the recombination level between the species newly proposed here and its closest phylogenetic relatives, pairwise homoplasy index (PHI) tests were performed using the respective concatenated multilocus datasets (Bruen et al. 2006). The tests were conducted using SplitsTree v. 4.14.4 (Huson & Bryant 2006) as described by Quaedvlieg et al. (2014). A PHI value below 0.05 ($\Phi w < 0.05$) indicated the presence of significant recombination in the dataset. In addition, split graphs were constructed for visualisation of the relationship between closely related species.

Pathogenicity tests

Pathogenicity tests with the fungal species isolated from twig- and trunk-cankers were performed to satisfy Koch's postulates. Six representative isolates were selected (*F. citricola*: CPC 27805, CPC 27709; *F. salinense*: CPC 26403, CPC 26973; *F. sarcochroum*: CPC 27921, CPC 28116). The isolates were inoculated on potted 1-yr-old healthy *Citrus limon* ('Femminello

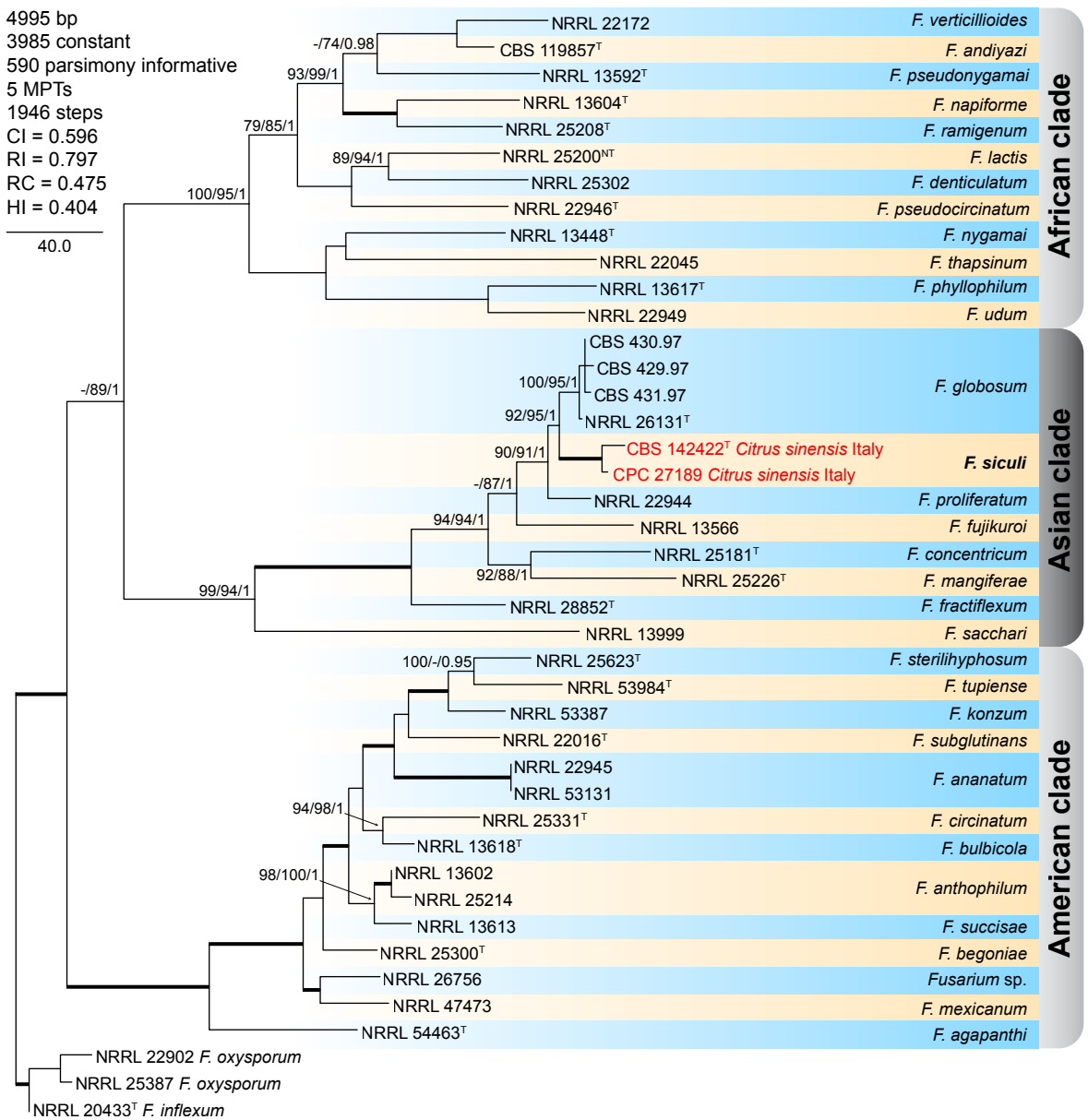

Fig. 2 One of five Maximum parsimony (MP) best-tree phylograms obtained from combined *CAM*, *EF-1α*, ITS, *RPB1*, *RPB2* and *TUB* sequences of 39 strains belonging to the *Fusarium fujikuroi* species complex. Branch lengths are proportional to distance. Numbers on the nodes are MP and RaxML bootstrap values above 70 % and Bayesian posterior probability values above 0.95. Full supported branches are indicated in **bold**. Isolates obtained from *Citrus* are indicated in red font. Ex-type and ex-neotype strains are indicated with T and NT, respectively. Names of newly proposed taxa are shown in **bold**. The tree was rooted to *Fusarium inflexum* (NRRL 20433) and *Fusarium oxysporum* (NRRL 22902, NRRL 25387).

Siracusano 2KR'), *C. sinensis* ('Tarocco') and *C. reticulata* ('Tardivo di Ciaculli') plants. Three plants for each isolate/citrus species combination were inoculated. Following the methods used in a recent citrus canker study (Adesemoye et al. 2014), five wounds per plant were made on twigs using a sterile blade. A 3-mm-diam mycelial plug from a 5–7-d-old culture growing on PDA was placed on each wound, and the inoculated area was covered with Parafilm® (American National Can, Chicago, IL, USA). The same number of wounds/plants were inoculated with sterile PDA plugs and served as controls. Inoculated plants and controls were incubated at 25 °C in moist chambers for 4 wk. Symptoms development was evaluated 4 wk after inoculation. In order to fulfil Koch's postulates, the inoculated fungi were re-isolated from twigs showing lesions and the identity of the re-isolated fungi was confirmed by sequencing the *RPB2* locus as described above.

RESULTS

In total 39 monosporic isolates resembling *Fusarium* spp. were collected from three *Citrus* species, i.e., *Citrus limon*, *C. reticulata* and *C. sinensis*. Most isolates were associated with dry root rot of orange trees, 10 isolates were recovered from twig- and trunk-cankers and five from twig dieback. The majority of isolates (35) were obtained from samples collected in Italy, while three and one isolate were recovered, respectively, in Spain and Greece (Table 1).

Phylogenetic identification

A first phylogenetic analysis based in *RPB2* sequences was conducted in order to position the isolates in the treated genera and their respective species complexes (Fig. 1). The analysis included sequences from 102 isolates spanning the different species complexes of the genera *Fusarium* and *Neocosmospora*, and two outgroup taxa (*Fusicolla aquaeductuum* NRRL 20686 and *Fusicolla* sp. NRRL 22136). From the 38 isolates obtained from *Citrus* species 23 belonged to *Fusarium* and were distributed in three known species complexes, i.e., FFSC (two isolates), FLSC (seven isolates) and FOSC (six isolates), eight isolates clustered in two clades forming a distinct, well-supported, unnamed lineage sister to the FTSC. The remaining 15 isolates nested within *Neocosmospora*, previously known as the *Fusarium solani* species complex (FSSC).

To further characterise the isolates belonging to FOSC, a haplotype distribution analysis was performed following O'Donnell et al. (2009a). The six *Fusarium* isolates from *Citrus* belonged to six different haplotypes. The genotypes of the isolates CPC 27194 and CPC 27196 were identical to the haplotypes 30 and 113 of *F. oxysporum* f. sp. *vasinfectum*, while each of four isolates (CPC 27700, 27701, 27702, 28190) corresponded to new genetically distinct populations in FOSC (data not shown).

Seven isolates belonging to the FLSC were identified as *Fusarium sarcochroum* based on a phylogenetic analysis comprising *EF-1α*, *RPB1* and *RPB2* loci (data not shown, all trees are available in TreeBASE).

The phylogenetic analysis of the isolates that belonged to the FFSC included sequences from six loci (*CAM*, *EF-1α*, ITS, *RPB1*, *RPB2* and *TUB*) and 42 isolates including the outgroup taxa (*F. inflexum* NRRL 20433, *F. oxysporum* NRRL 22902 and NRRL 25387), representing 33 taxa covering the three main phylogenetic clades known in this species complex (African, American and Asian clade sensu O'Donnell et al. 1998a) (Fig. 2). The two *Fusarium* isolates from *Citrus* (CPC 27188, 27189) clustered within the Asian clade of FFSC in a well-supported group sister to *F. globosum* and *F. proliferatum*. However, they were morphologically and genetically distinct from the latter species, as also confirmed by the PHI analysis (Φw = 1.0, Fig. 3a), and are described here as a new species, *F. siculi*.

In order to establish the phylogenetic position of the eight *Fusarium* isolates that formed a distinct new lineage in the original *RPB2* phylogeny, we carried out a more inclusive analysis, which included 3 685 bp from five loci (*EF-1α*, ITS, LSU, *RPB1* and *RPB2*) and 41 isolates representing 19 phylogenetic species, covering four known related species complexes of *Fusarium*, i.e., *F. chlamydosporum* species complex (FCSC),

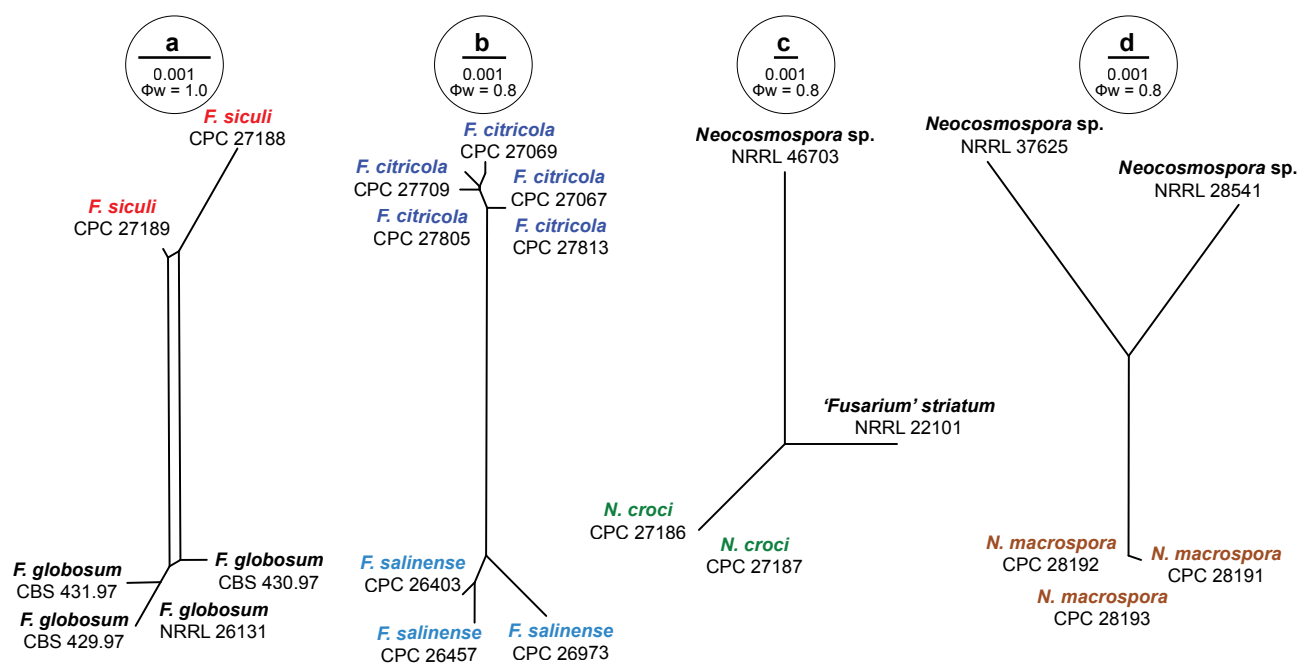

Fig. 3 Splitgraphs showing the results of the pairwise homoplasy index (PHI) test of newly described taxa and closely related species using both LogDet transformation and splits decomposition. PHI test results (Φw) < 0.05 indicate significant recombination within the dataset. a. *Fusarium siculi* sp. nov. in the *F. fujikuroi* species complex; b. *Fusarium salinense* and *F. citricola* sp. nov. in the *F. citricola* species complex; c, d. *Neocosmospora croci* and *N. macrospora* sp. nov., respectively, in *N. solani* species complex.

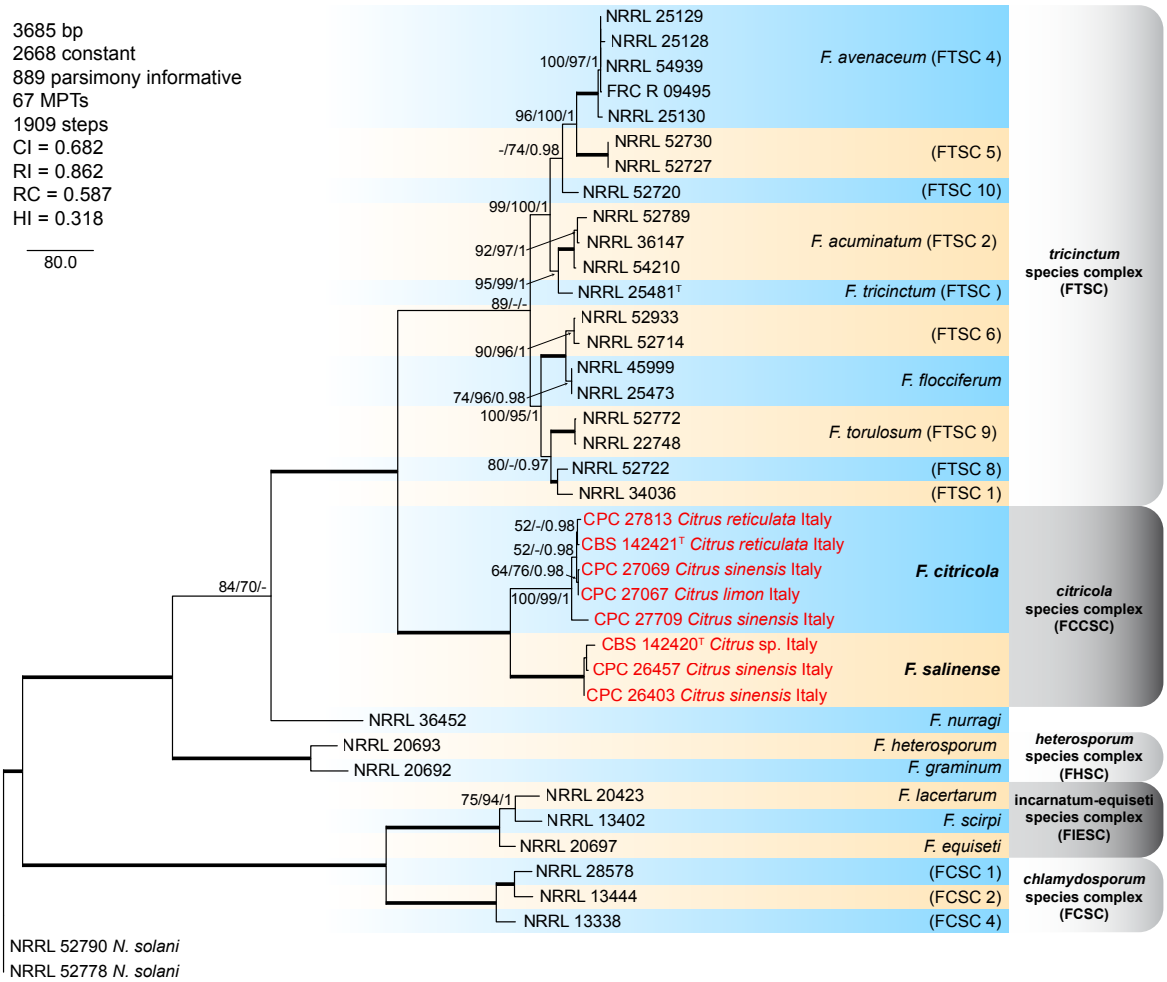

Fig. 4 One of 67 Maximum parsimony (MP) best-tree phylograms obtained from *EF-1α*, ITS, LSU, *RPB1* and *RPB2* sequences of 37 strains from *Fusarium* species. Branch lengths are proportional to distance. Numbers on the nodes are MP and RaxML bootstrap values above 70 % and Bayesian posterior probability values above 0.95. Full supported branches are indicated in **bold**. Isolates obtained from *Citrus* are indicated in red font. Names of newly proposed taxa are shown in **bold**. Ex-type are indicated with T. The tree was rooted to *Neocosmospora solani* (NRRL 52778, 52790).

F. heterosporum species complex (FHSC), *F. incarnatum-equiseti* species complex (FIESC) and FTSC; a representative of a known related single lineage (*F. nurragi*) plus two outgroup taxa. MP, ML and BI produced topologically similar trees, of which one of the most parsimonious trees is shown in Fig. 4. The analysis supported six different highly supported lineages which corresponded to *F. nurragi*, four *Fusarium* species complexes, i.e.; FCSC, FIESC, FHSC, FTSC and a new fully-supported lineage, phylogenetically and morphologically divergent from its sister clades, which is named here the *F. citricola* species complex (FCCSC). Within FCCSC, the isolates from *Citrus* grouped into two distinct highly supported phylogenetic clades as also confirmed by PHI analysis (Φw = 0.8 in both cases, Fig. 3b). These two clades are described below as the new species *F. citricola* and *F. salinense*.

The multilocus analysis of *Neocosmospora* encompassed 2 961 bp from four loci (*EF-1α*, ITS, LSU and *RPB2*) and 83 isolates spanning 47 known taxa and/or phylogenetic clades of this species complex (Fig. 5). The isolates from *Citrus* were distributed within four previously known clades: *N. solani* (six isolates), and the unnamed phylogenetic species FSSC 9 (one isolate), FSSC 28 and FSSC 15 (two isolates, each). Two isolates (CPC 27186, 27187) clustered in a new phylogenetic lineage sister to *F. striatum*, while three isolates (CPC 28191, 28192, 28193) formed a new lineage closely related to the phylogenetic species FSSC 26 and FSSC 27. The genealogical exclusivity of both new lineages was confirmed by the PHI test, showing no evidence of recombination (Φw = 1.0, Fig. 3c, d). They are described below as the new species *Neocosmospora croci* and *N. macrospora*.

Taxonomy

Fusarium citricola Guarnaccia, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB820246; Fig. 6

Etymology. Refers to *Citrus*, the host genus from which this fungus was isolated.

Colonies on PDA growing in the dark with an average radial growth rate of 2.9–4.7 and 2.5–4.2 mm/d at 21 and 24 °C, respectively (reaching 35–43 mm diam in 7 d at 24 °C). Colony surface pale luteous to pale yellow (orange to red when incubated in light), flat or slightly raised at the centre, radially striated, membranous to dusty, aerial mycelium scant or absent; colony margins irregular, lobate, serrate or filiform. Odour absent. Reverse pale luteous to straw. Diffusible pigment absent in the dark, an orange to red pigment sometimes present when incubated in the light. Colonies on OA incubated at 24 °C in the dark reaching a maximum of 60–62 mm diam at 7 d. Colony colour sulphur to pure yellow with white periphery, flat, radially finely striated, membranous and shiny to slightly velvety in the outer margins, aerial mycelium absent or scant, if present floccose, forming irregular rings at the periphery of the colony; margins regular, filiform. Reverse sulphur to pure yellow, without diffusible pigments. On SNA, hyphae hyaline, smooth-walled,

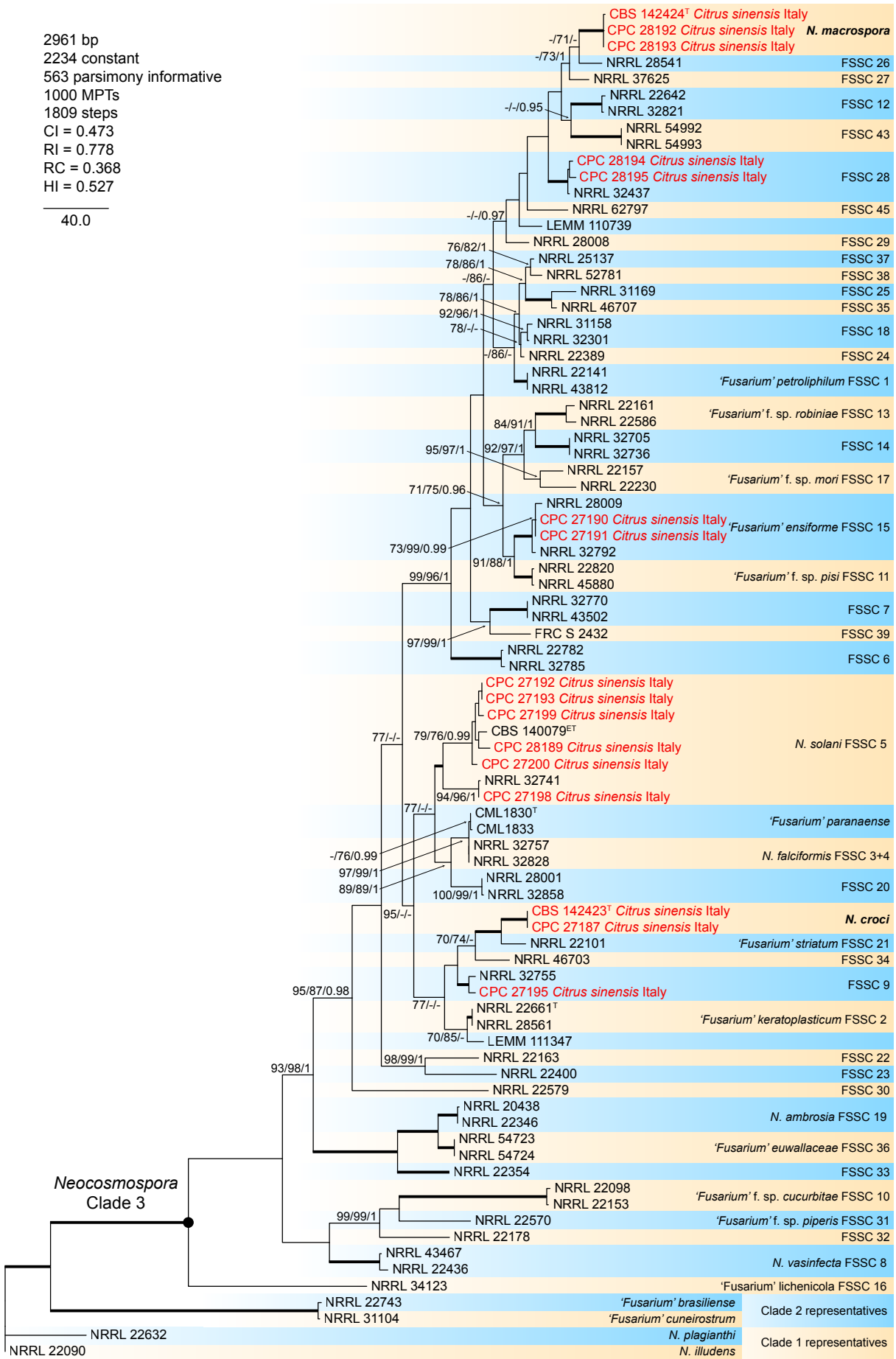

Fig. 5 One of 1 000 Maximum parsimony (MP) best-tree phylograms obtained from *EF-1α*, ITS, LSU and *RPB2* sequences of 83 strains from *Neocosmospora* species. Branch lengths are proportional to distance. Numbers on the nodes are MP and RaxML bootstrap values above 70 % and Bayesian posterior probability values above 0.95. Full supported branches are indicated in **bold**. Isolates obtained from *Citrus* are indicated in red font. Names of newly proposed taxa are shown in **bold**. Ex-type and ex-epitype strains are indicated with T and ET, respectively. The tree was rooted to *Fusarium illudens* (22090) and *Fusarium plagianthi* (NRRL 22632).

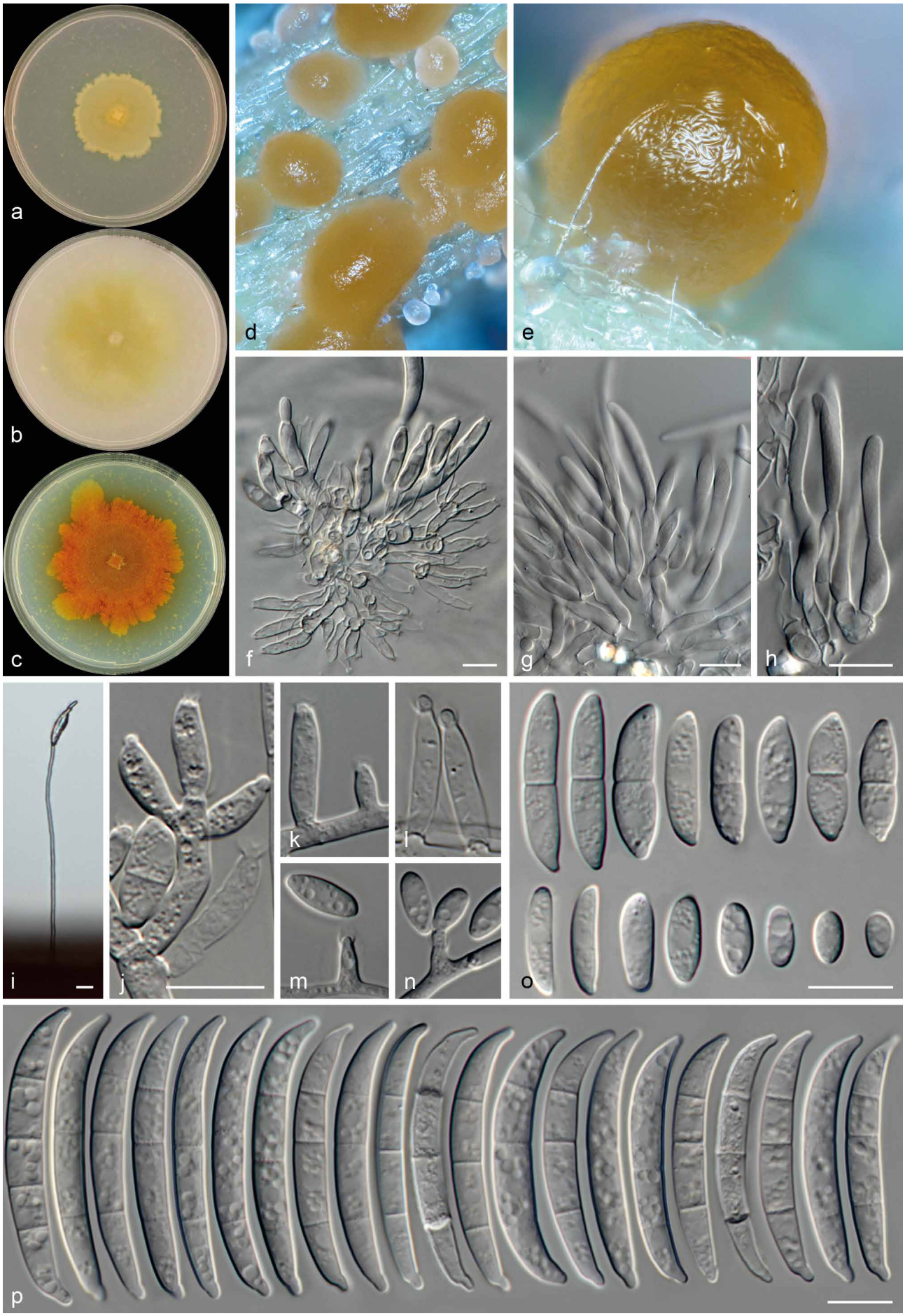

Fig. 6 *Fusarium citricola* CBS 142421. a–b. Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark; c. colony on PDA after 7 d at 24 °C under continuous white light; d–e. sporodochia formed on the surface of carnation leaves; f–h. sporodochial conidiophores and phialides; i–j. aerial conidiophores; k–n. aerial phialides; o. aerial conidia (microconidia); p. sporodochial conidia (macroconidia). — Scale bars = 10 µm (scale bar in j also applies to k–n).

1–10 µm wide. *Chlamydospores* absent. Sporulation abundant from sporodochia, rarely from conidiophores formed directly on the substrate mycelium. *Conidiophores* in the aerial mycelium 4–50 µm tall, unbranched or sparingly branched, bearing terminal or intercalary monophialides, often reduced to single phialides. *Phialides* subulate to subcylindrical, smooth- and thin-walled, 4–22.5 × 2–4.5 µm, without periclinal thickening; *conidia* hyaline, ellipsoidal to falcate, smooth- and thin-walled, 0–3-septate, (6.4–)9.9–22.9(–32.6) × (3.1–)3.9–5.2(–6.5) µm, forming small false heads on the tips of monophialides. *Sporodochia* bright orange coloured, formed abundantly on carnation leaves or the surface of the agar. *Conidiophores* in sporodochia 20–62.5 µm tall, verticillately branched and densely packed, bearing apical whorls of 2–3 monophialides or rarely single lateral monophialides; *sporodochial phialides* subulate to subcylindrical, 10–18 × 2.5–4 µm, smooth- and thin-walled, sometimes showing a reduced and somewhat flared collarette. *Sporodochial conidia* falcate, curved dorsiventrally with almost parallel sides tapering slightly towards both ends, with a blunt to papillate, curved apical cell and a foot-like basal cell, (1–)2–4(–6)-septate, commonly with one or more empty cells hyaline, thin- and smooth-walled. One-septate conidia: (35.5–)36.2–39.9 × 4.1–4.8 µm; two-septate conidia: (33.7–)34–37.9(–39.9) × 4.4–5.7(–6.2) µm; three-septate conidia: (27.5–)32.3–37.3(–40.5) × (3.8–)4.2–5.1(–6) µm; four-septate conidia: (32.1–)34.4–39.8(–42.5) × (4.1–)4.6–5.4(–5.7) µm; six-septate conidia: 39–41.9(–42.5) × (4.4–)4.6–5.5 µm.

Cardinal temperatures for growth — Minimum 12 °C, maximum 30 °C, optimal 18–21 °C.

Specimens examined. Italy, Cosenza, Rocca Imperiale, from *Citrus limon* twigs, 9 June 2015, *V. Guarnaccia* (CPC 27067); Taranto, Massafra, from *Citrus sinensis* twigs, 9 June 2015, *V. Guarnaccia* (CPC 27709); Cosenza, Rocca Imperiale, from *Citrus reticulata* 'Caffin' crown, 10 Aug. 2015, *V. Guarnaccia* (CBS H-23020, holotype, dried culture on SNA with carnation leaves, culture ex-type CBS 142421 = CPC 27805); Cosenza, Rocca Imperiale, from *Citrus reticulata* 'Caffin' crown, 1 Sept. 2015, *V. Guarnaccia* (CPC 27813).

Notes — *Fusarium citricola* was recovered from diverse *Citrus* species with advanced canker symptoms in Apulia and Calabria, Southern Italy. The role of this species in the canker disease was confirmed by pathogenicity tests.

Fusarium citricola has similar morphological characters to *F. salinense*, with both species forming the new lineage here named FCCSC (see general notes under *F. salinense*). The former species can be distinguished by its slightly smaller sporodochial conidia, often with a gentle and symmetrical dorsiventral curvature, produced on somewhat larger sporodochial phialides, and its 0–3-septate microconidia (vs the often asymmetrically curved macroconidia and 0–1(–2)-septate microconidia in *F. salinense*).

Fusarium salinense Sandoval-Denis, Guarnaccia & Polizzi, *sp. nov.* — MycoBank MB820245; Fig. 7

Etymology. Refers to Salina, one of the Aeolian Islands, in the north-eastern coast of Sicily, where the ex-type strain of this fungus was collected.

Colonies on PDA growing in the dark with an average radial growth rate of 3.1–4.7 and 2.8–5.2 mm/d at 21 and 24 °C, respectively (reaching 39–43 mm diam in 7 d at 24 °C). Colony surface pale luteous to sulphur yellow with white to pale luteous margins, flat, velvety to felty with abundant floccose aerial mycelium; colony margins irregular, undulate to lobate. Odour strongly mouldy. Reverse pale luteous to orange toward the centre of the colony. Yellow diffusible pigment sometimes present, while red colonies and diffusible pigments occur when incubated in light. Colonies on OA incubated at 24 °C in the dark reaching a maximum of 65–70 mm diam in 7 d. Colony colour pale luteous, flat, membranous to slightly velvety or cottony, aerial mycelium scarce or absent; margins regular, filiform. Reverse pale luteous without diffusible pigments. On SNA, growth almost entirely pionnotal; hyphae hyaline, smooth-walled, 1–10 µm wide. *Chlamydospores* absent, but rounded, thin-walled hyphal swellings sometimes present in old cultures. Sporulation abundant from sporodochia, rarely from conidiophores formed directly on the substrate mycelium. *Conidiophores* in the aerial mycelium 25–150 µm tall, irregularly branched, bearing terminal or lateral monophialides; *phialides* subulate, ampulliform, subcylindrical to doliiform, smooth- and thin-walled, often reduced to small phialidic pegs, 7.5–23 × 2.5–5 µm, without periclinal thickening; collarettes small and barely visible or lacking; *conidia* hyaline, oval, ellipsoidal to falcate, smooth- and thin-walled, 0–1(–2)-septate, (4.7–)9.2–17.2(–23) × (2.8–)4–5.5(–7) µm, single or forming small false heads. *Sporodochia* flesh, salmon to orange coloured, formed abundantly on the surface of the agar and on carnation leaves. *Conidiophores* in sporodochia 42.5–106 µm tall, densely and irregularly branched, often bi- or tri-verticillately, sometimes slightly stipitate, bearing 1–2 terminal, rarely lateral monophialides; *sporodochial phialides* subulate to subcylindrical, 10–22.5 × 2.5–4 µm, smooth- and thin-walled, often with a minute apical collarette. *Sporodochial conidia* falcate, slender, with a gentle curvature and nearly parallel dorsiventral lines or an unequal curvature, slightly more pronounced in the upper part of the spore, tapering slightly towards the basal end, with a papillate and curved apical cell and a barely notched to foot-like basal cell, (2–)3–4(–5)-septate, often showing one or more empty cells, hyaline, thin- and smooth-walled. Three-septate conidia: (19.8–)30.7–41.3(–45.6) × (2.8–)3.6–5.2(–6.2) µm; four-septate conidia: (36.5–)39–44.5(–45.4) × (4.1–)4.4–5.5(–6.1) µm; five-septate conidia: (41.8–)42.9–48(–49.1) × 5.5–5.8 (–5.9) µm.

Cardinal temperatures for growth — Minimum 12 °C, maximum 33 °C, optimal 21–24 °C.

Specimens examined. Italy, Sicily, Catania, Riposto, from *Citrus sinensis* 'Valencia' twigs, 2 Mar. 2015, *V. Guarnaccia* (CPC 26403); Sicily, Catania, Riposto, from *Citrus sinensis* 'Valencia' twigs, 2 Mar. 2015, *V. Guarnaccia* (CPC 26457); Sicily, Messina, Leni, from *Citrus sinensis* twigs, 5 June 2015, *V. Guarnaccia* (CBS H-23019, holotype, dried culture on SNA with carnation leaves, culture ex-type CBS 142420 = CPC 26973).

Notes — *Fusarium salinense* was isolated from two locations in close proximity in Sicily and Salina, one of the Aeolian Islands, which might suggest some level of geographical isolation restricted to the Tyrrhenian Sea. It was a prominent pathogen, producing canker symptoms on three different *Citrus* species.

Fusarium salinense and *F. citricola*, also described here, constitute the *Fusarium citricola* species complex (FCCSC), characterised by abundant production of bright orange sporodochia, the presence of red pigments when incubated under continuous white light and the reduced size of its aerial conidiophores and phialides. *Fusarium salinense* produces sparingly branched conidiophores in the aerial mycelium, especially in young cultures, but its growth soon becomes almost entirely pionnotal, while some aerial conidiation can still be observed from reduced phialides or phialidic pegs. The latter feature is somewhat reminiscent of *Bisifusarium* which, however, differs in the absence of microconidia and sporodochia, its distinctly shaped, curved and short macroconidia, and by presenting a yeast-like growth on PDA, also being phylogenetically distant (Schroers et al. 2009).

Other closely related taxa include species from the phylogenetically allied FTSC from which *F. salinense* differs by its gently curved macroconidia, and the absence of pyriform microconidia and chlamydospores. The shape and size of the macroconidia and the characteristics of the sporodochia also aligns *F. salinense* with species in the FCSC. However, a clear phylogenetic

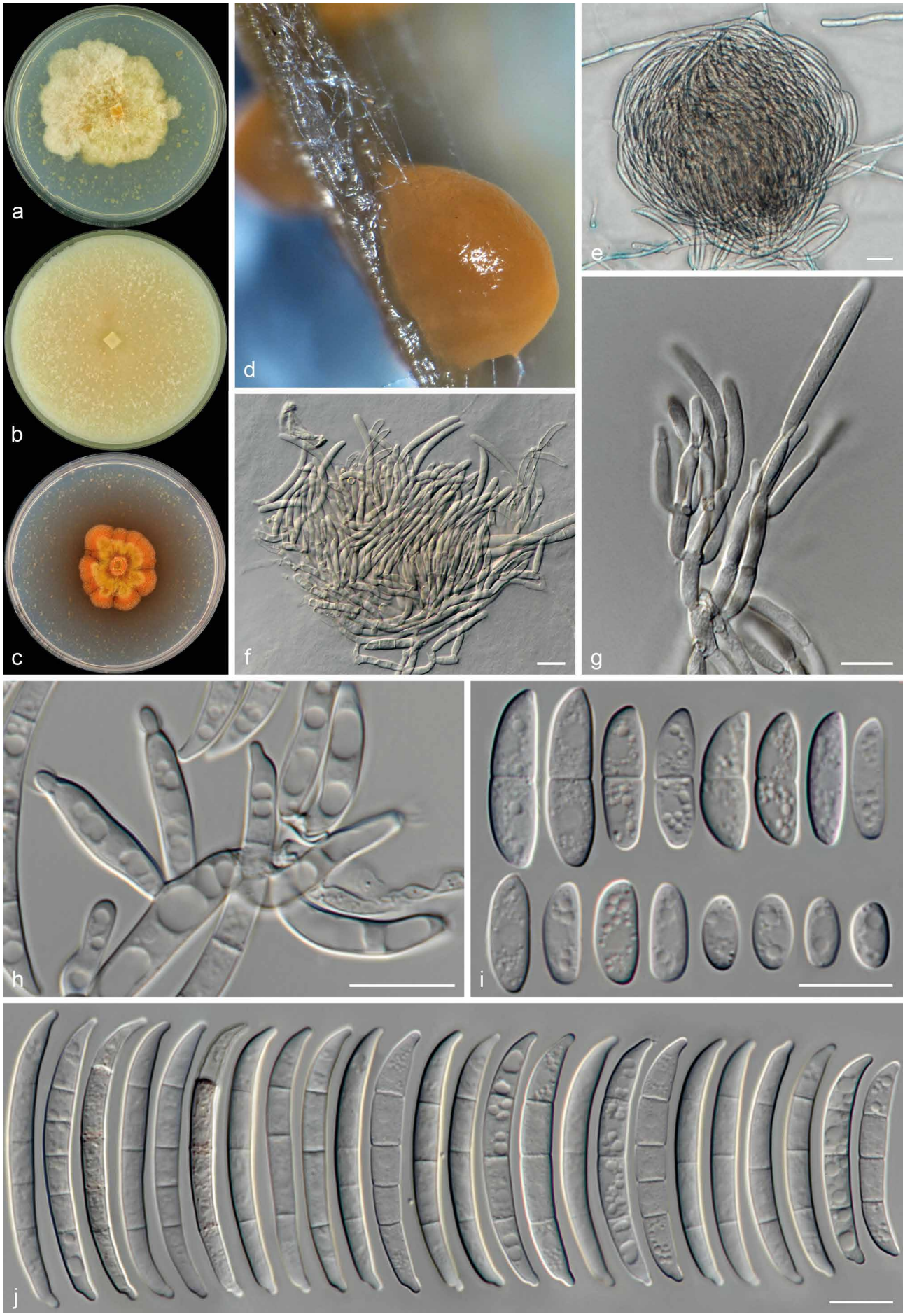

Fig. 7 *Fusarium salinense* CBS 142420. a–b. Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark; c. colony on PDA after 7 d at 24 °C under continuous white light; d. sporodochia formed on the surface of carnation leaves; e. sporodochia formed on the agar surface; f–g. sporodochial conidiophores; h. aerial phialides; i. aerial conidia (microconidia); j. sporodochial conidia (macroconidia). — Scale bars = 10 µm.

separation exists between the two species complexes as well as clear morphological differences as the rounded, almost papillate apical cell in *F. salinense* (vs pointed in FCSC), the scant production of microconidia and the absence of chlamydospores.

Fusarium salinense and its closest phylogenetic ally *F. citricola* can be distinguished by the formation, in the former species, of shorter sporodochial phialides and slightly longer and robust macroconidia often with an unequal dorsiventral curvature.

Fusarium siculi Sandoval-Denis, Guarnaccia & Polizzi, *sp. nov.* — MycoBank MB820248; Fig. 8

Etymology. From Latin *Siculi*, 'Sicels', an old italic tribe that inhabited Sicily, and from which the name of the island has derived.

Colonies on PDA growing in the dark with an average radial growth rate of 5.1–6.1 and 5.5–6.8 mm/d at 21 and 24 °C, respectively (reaching 77–90 mm diam in 7 d at 24 °C). Colony colour peach to pale rose with saffron margins, flat and radially striated, membranous with scant loose aerial mycelium. Odour strong, mouldy. Margins filiform to arachnoid. Reverse at first white, turning pale orange, luteous to scarlet coloured. Colonies on OA incubated at 24 °C in the dark reaching a maximum of 75–79 mm diam at 7 d. Colony colour salmon to coral in irregular patches, flat, membranous, aerial mycelium scantly present as patches or absent; margins regular and fimbriate. Reverse flesh, coral to pale rust coloured with slight production of a pale rust diffusible pigment. On SNA, hyphae hyaline, smooth-walled, 0.5–11.5 µm wide. *Chlamydospores* absent. Sporulation abundant from aerial conidiophores or sporodochia. *Conidiophores* in the aerial mycelium or erect, 47–165 × 2–5.5 µm, simple or sparsely branched, often branching verticillately or less common sympodially, bearing terminal mono- and polyphialides, or more rarely intercalary phialides; *phialides* short acicular, subulate to subcylindrical, smooth- and thin-walled, 16.5–33.5 × 2–4 µm, without periclinal thickening or distinct collarettes, rarely proliferating subapically; *conidia* subcylindrical to clavate, often with a somewhat flattened base, straight or slightly curved, smooth- and thin-walled, 0(–1)-septate, (5.3–)8.5–12.3(–16.8) × (2.3–)2.9–3.5(–3.8) µm, arranged in long basipetal chains that quickly collapse into false heads. *Sporodochia* saffron to apricot coloured, formed on the surface of carnation leaves and often almost completely covered by aerial mycelium. *Conidiophores* in sporodochia 29.5–45.5 µm tall, branched, mono- or biverticillate, bearing 1–2 terminal monophialides; *sporodochial phialides* subulate, lageniform or cylindrical, tapering abruptly toward apex, 9–22 × 2–4.5 µm often with a minute collarette; *sporodochial conidia* falcate, slender, straight or slightly curved, tapering towards both ends, with a blunt and often curved apical cell and a foot-like to slightly notched basal cell, 3–5-septate, hyaline, thin- and smooth-walled. Three-septate conidia: (27.1–)34.4–47.3(–56.1) × (3–)3.3–3.8(–4.4) µm; four-septate conidia: (41.4–)43.4–49.6(–50.8) × (3.4–)3.6–4.1 µm; five-septate conidia: (48–)48.3–53(–53.1) × 3.4–3.7(–3.8) µm.

Cardinal temperatures for growth — Minimum 12 °C, maximum 36 °C, optimal 21–27 °C.

Specimens examined. Italy, Sicily, Catania, Paternó, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CBS H-23021, holotype, dried culture on SNA with carnation leaves, culture ex-type CBS 142422 = CPC 27188); Sicily, Catania, Paternó, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CPC 28189).

Notes — *Fusarium siculi* is phylogenetically related to *F. globosum*, a species known from maize and wheat from Africa and Asia (Rheeder et al. 1996, Aoki & Nirenberg 1999). However, the two species are morphologically clearly differentiated by the presence of clavate and globose microconidia in *F. globosum*. It is known that the incubation conditions can influence conidial development in the latter species, with the production of globose conidia being suppressed by continuous exposure to black light (Aoki & Nirenberg 1999, Leslie & Summerell 2006). We confirmed the production of globose conidia by all *F. globosum* strains available in the CBS culture collection, including the ex-type strain (CBS 428.97) under the incubation conditions used in this study. Additionally, *F. siculi* can still easily be recognised considering the degree of septation of its clavate conidia (0–1-septate vs 0–3-septate in *F. globosum*). *Fusarium siculi* also resembles other species in FFSC producing mono- and polyphialides, and clavate, 0–1-septate microconidia arranged in chains and false heads like *F. fujikuroi*, *F. nygamai* or *F. pseudoanthophilum*. Nevertheless, *F. fujikuroi* and *F. pseudoanthophilum* produce additional obovoid to pyriform microconidia, a character not seen in *F. siculi*, while the latter species can be distinguished from *F. nygamai* by the absence of chlamydospores. In addition to the morphological differences and the clear phylogenetic delimitation, *F. siculi* differs in its host association, with none of the species mentioned above yet reported from *Citrus* (Farr & Rossman 2017).

Neocosmospora croci Guarnaccia, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB820251; Fig. 9

Etymology. From Latin *crocum* 'saffron', referring to the production of red diffusible pigments at high temperatures.

Colonies on PDA growing in the dark with an average radial growth rate of 2.5–3.8 and 2–4.8 mm/d at 21 and 24 °C, respectively (reaching 52–54 mm diam in 7 d at 24 °C). Colony colour at first white, becoming straw to pale buff; flat, at first membranous, becoming felty with scant aerial mycelium; margins regular and fimbriate; odour absent. Reverse white to straw coloured without diffusible pigments. A slight production of a pale saffron to saffron diffusible pigment may occur when incubated in the dark at 36 °C. Colonies on OA incubated at 24 °C in the dark reaching a maximum of 33–37 mm diam at 7 d. Colony colour at first white, becoming straw, flat, membranous and shiny, aerial mycelium absent; margins regular and fimbriate. Reverse white to pale luteous, without diffusible pigments. On SNA, hyphae hyaline, smooth-walled, 0.5–12 µm wide. *Chlamydospores* scarcely produced in hyphae, subglobose to globose, hyaline to subhyaline and smooth-walled, terminal and intercalary, often in pairs or in chains, 5–9.5 µm diam. Sporulation abundant from erect conidiophores formed on the agar surface or aggregated in sporodochia. *Conidiophores* in the aerial mycelium 54.5–94 × 3.5–5.5 µm, mostly unbranched, rarely basally dichotomously branched, forming monophialides on the apices; *phialides* slender, subulate to subcylindrical, monophialidic, smooth- and thin-walled, 18–63.5 × 2–5 µm, with slight periclinal thickening at the tip and a short flared apical collarette; *conidia* of two types: a) obovoid, ellipsoidal to cylindrical, sometimes gently curved becoming reniform to allantoid, hyaline, smooth and thin-walled, 0–1(–3)-septate, (5.2–)7.2–17.2(–33.9) × (2.4–)3.2–4.8(–6.5) µm, arranged in slimy heads at the tip of phialides; and b) cylindrical to falcate, formed on the agar surface and morphologically indistinguishable from sporodochial conidia. *Sporodochia* cream coloured, scantly produced on the surface of carnation leaves. *Conidiophores* in sporodochia 30–82 µm tall, irregularly branched, short stipitate, bearing terminal monophialides; *sporodochial phialides* subulate to subcylindrical, smooth- and thin-walled, 11.5–27.5 × 3.5–5.5 µm, with periclinal thickening and a small, flared collarette; *sporodochial conidia* cylindrical to falcate, gently curved with nearly symmetrical dorsal and ventral lines or slightly wider at the middle or apical part, typically with a blunt and almost rounded apical cell and a barely notched foot cell, 3–5-septate, hyaline, thick- and smooth-walled. Three-septate

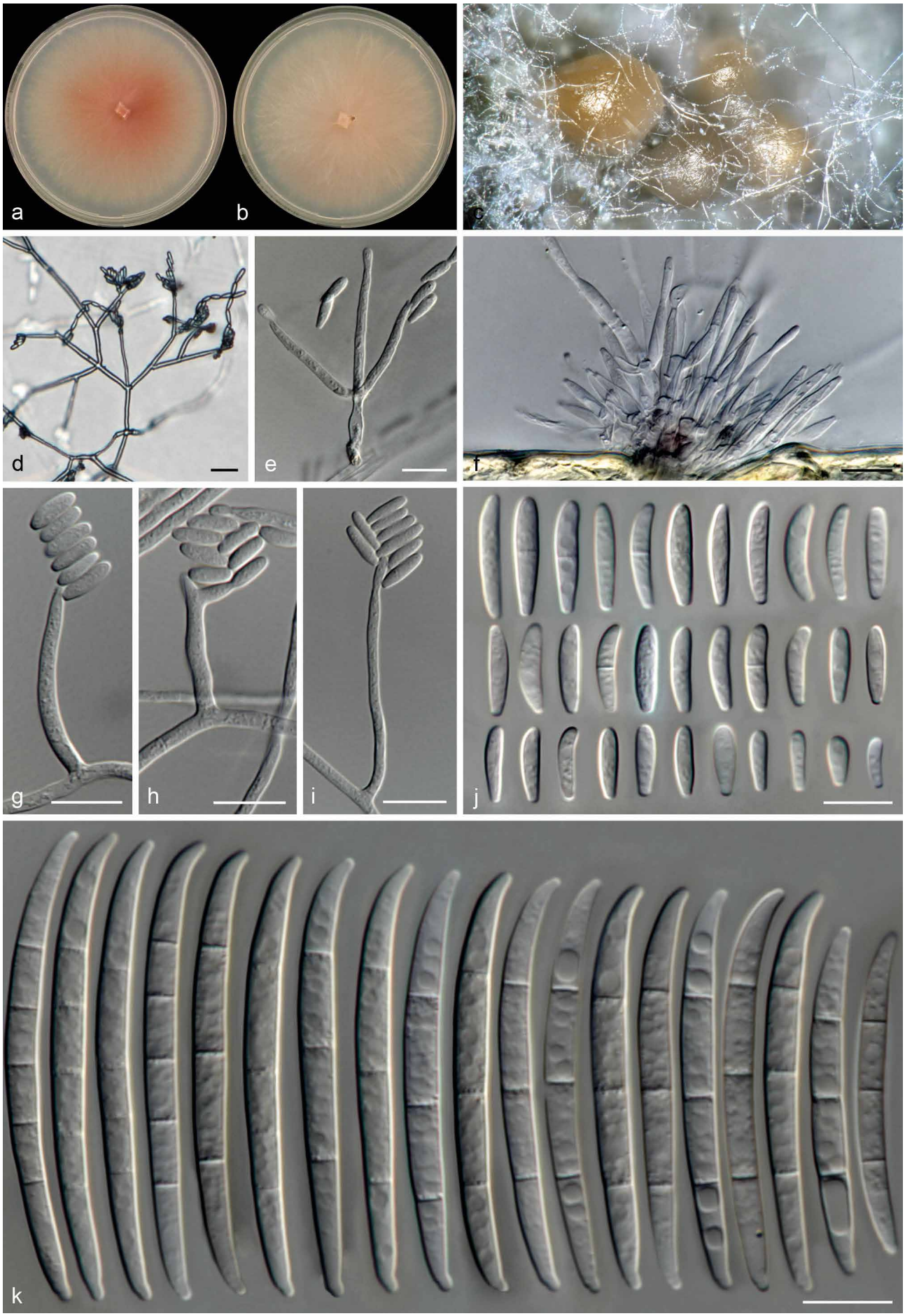

Fig. 8 *Fusarium siculi* CBS 142422. a–b. Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark; c. sporodochia formed on the surface of carnation leaves; d–e. aerial conidiophores; f. sporodochial conidiophores formed on the surface of carnation leaves; g–i. aerial phialides and conidia; j. aerial conidia (microconidia); k. sporodochial conidia (macroconidia). — Scale bars = 10 µm.

Fig. 9 *Neocosmospora croci* CBS 142423. a–b. Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark; c–d. sporodochia formed on the surface of carnation leaves; e–h. aerial conidiophores; i–j. sporodochial conidiophores and phialides; k–l. chlamydospores; m–o, aerial phialides and conidia; p. aerial conidia (microconidia); q. sporodochial conidia (macroconidia). — Scale bars: k, l = 5 µm, all others = 10 µm.

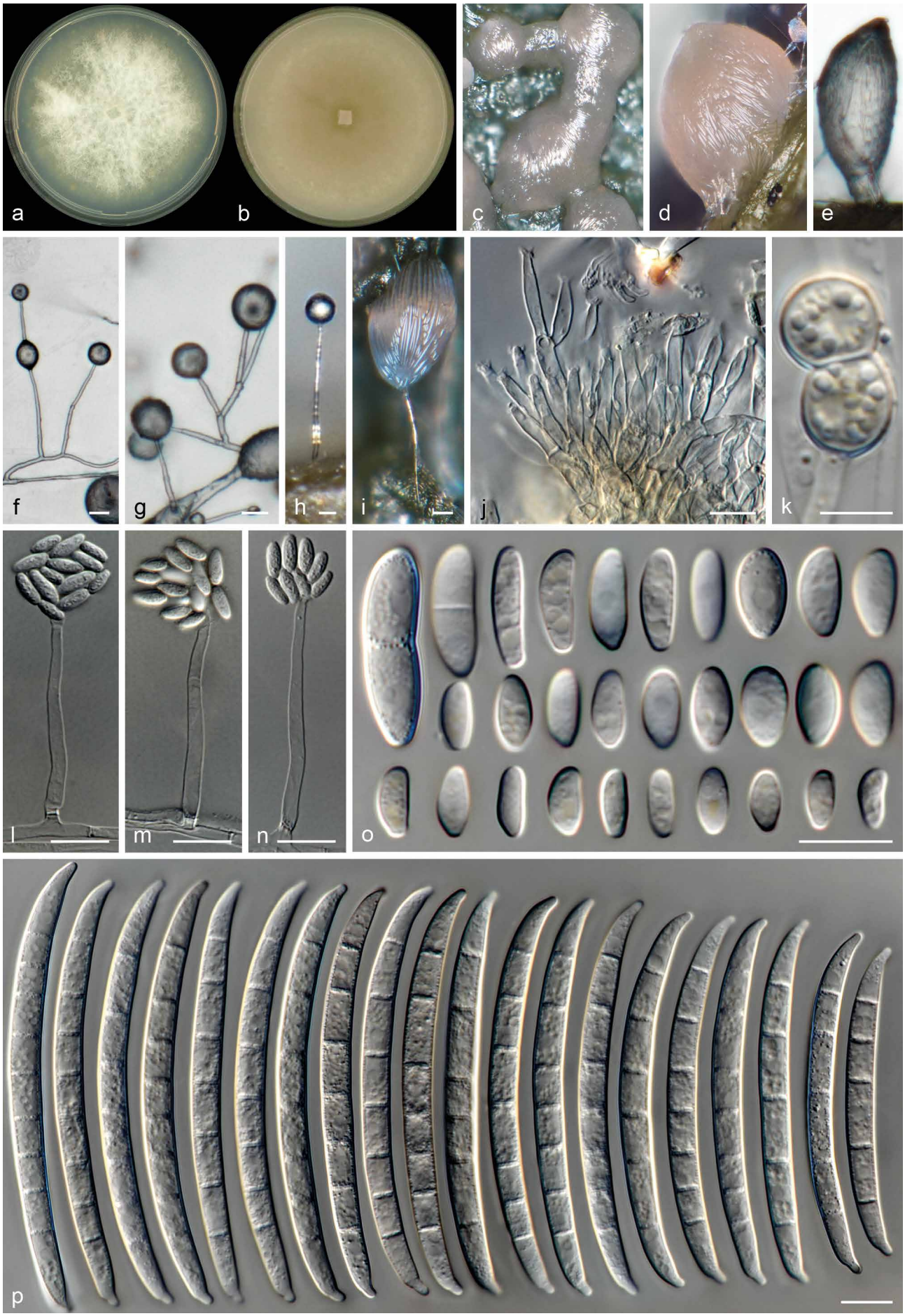

Fig. 10 *Neocosmospora macrospora* CBS 142424. a–b. Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark; c–e. sporodochia formed on the surface of carnation leaves; f–i. aerial conidiophores; j. sporodochial conidiophores and phialides; k. chlamydospores; l–n. aerial phialides and conidia; o. aerial conidia (microconidia); p. sporodochial conidia (macroconidia). — Scale bars: k = 5 µm, all others = 10 µm.

conidia: (32.7–)33.4–43.8(–52.6) × (5.3–)5.4–6(–6.2) µm; four-septate conidia: (42.9–)46.9–53.7(–56.2) × (5.3–)5.6–6.2(–6.8) µm; five-septate conidia: (47.8–)51.7–60.5(–65.3) × (5–)5.7–6.3(–6.6) µm.

Cardinal temperatures for growth — Minimum 9 °C, maximum 36 °C, optimal 24–30 °C.

Specimens examined. Italy, Sicily, Catania, Paternó, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CBS H-23022, holotype, dried culture on SNA with carnation leaves, culture ex-type CBS 142423 = CPC 27186); Sicily, Catania, Paternó, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CPC 27187).

Notes — *Neocosmospora croci* belongs to clade 3 of *Neocosmospora*, a group including important plant pathogens and human and animal opportunistic parasites (O'Donnell et al. 2008, Schroers et al. 2016). It matches in all aspects with the morphological characteristics of the *Neocosmospora* (*Fusarium*) *solani* species complex, known to include several cryptic species with overlapping morphological traits (Schroers et al. 2016). However, *N. croci* can be distinguished from *N. solani* s.str. by the slower growth rates on artificial media, the presence of a saffron diffusible pigment when incubated on PDA at 36 °C and its somewhat reduced conidiophores (54.5–94 × 3.5–5.5 µm vs (27–)67–123(–230) × (2–)3.5–5(–7) µm in *N. solani*) (Schroers et al. 2016).

Neocosmospora macrospora Sandoval-Denis, Guarnaccia & Polizzi, *sp. nov.* — MycoBank MB820253; Fig. 10

Etymology. Refers to the large macroconidia produced by this species.

Colonies on PDA growing in the dark with an average radial growth rate of 2.5–5 and 3–6.1 mm/d at 21 and 24 °C, respectively (reaching 66–70 mm diam in 7 d at 24 °C). Colony colour at first white, becoming pale grey to pale buff with scarce interleaved red coloured hyphae; flat to slightly umbonate, felty to cottony. Aerial mycelium abundant, loose to densely floccose; margins regular and fimbriate; odour absent or mouldy. Reverse white, pale yellow, straw, peach to pale saffron coloured at the centre, a luteous to saffron coloured diffusible pigment can be present when incubated at temperatures equal or above 30 °C. Colonies on OA incubated at 24 °C in the dark reaching a maximum of 60–68 mm diam at 7 d. Colony surface pale luteous, at first flat, membranous and glabrous becoming felty to cottony with the formation of an elevated marginal ring composed of white loose and floccose aerial mycelium; margins regular, fimbriate to crenate. Reverse pale luteous. On SNA, hyphae hyaline, smooth-walled, 1–10 µm wide. *Chlamydospores* can be formed in the hyphae, globose, subglobose to oval, subhyaline, smooth-walled, terminal or intercalary, solitary, in pairs or catenate, 5–8.5 × 4.5–8 µm. Sporulation scant from erect conidiophores or aggregated in sporodochia. *Conidiophores* in aerial mycelium 56.5–96.5 × 3–4.5 µm, mostly unbranched or sparingly and irregularly branched, forming terminal phialides; *phialides* subulate to subcylindrical, straight to flexuous, monophialidic, smooth- and thin-walled, 19–67 × 2–5 µm, with a minute flared apical collarette; *conidia* short obovate, clavate to cylindrical, straight or gently curved, hyaline or showing pale yellow intracellular inclusions, smooth- and thin-walled, 0(–1)-septate, (5.6–)6.6–9.9(–13.2) × (2.2–)2.7–6.3(–9.7) µm, arranged in slimy heads at the tip of monophialides. *Sporodochia* cream to pale pink coloured, produced on the surface of carnation leaves. *Conidiophores* in sporodochia 28–123 µm tall, densely and irregularly or verticillately branched, bearing 1–2 apical monophialides; *sporodochial phialides* short lageniform, subcylindrical to doliiform, 10–23 × 2–4.5 µm, often with periclinal thickening at the tip and a small flared collarette; *sporodochial conidia* cylindrical to falcate and curved with nearly symmetrical dorsal and ventral lines or finely tapering towards the basal and apical part, with a blunt to slightly papillate apical cell and a well-developed foot-shaped basal cell, 3–9-septate (commonly 7-septate), hyaline, thick- and smooth-walled. Three-septate conidia: (68–)72.1–77.1(–75.7) × 5.7–6 µm; four-septate conidia: (73.5–)74–83.9(–84.5) × 5.9–6.3 µm; five-septate conidia: (59.3–)61–76.6(–85.3) × (5.2–)5.5–6(–6.2) µm; six-septate conidia: (73.8–)74.5–81.4(–84) × (5.3–)5.6–6.3(–6.5) µm; seven-septate conidia: (72–)75.2–84.1(–89.2) × (5.7–)5.9–6.4(–6.7) µm; eight-septate conidia: (79.4–)81.9–86.3(–87) × (5.8–)5.9–6.4(–6.6) µm; nine-septate conidia: (86–)86.3–89.7(–90) × 5.4–6.1(–6.2) µm.

Cardinal temperatures for growth — Minimum 9 °C, maximum 36 °C, optimal 21–30 °C.

Specimens examined. Italy, Sicily, Catania, Guardia, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CBS H-23023, holotype, dried culture on SNA with carnation leaves, culture ex-type CBS 142424 = CPC 28191); Sicily, Catania, Guardia, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CPC 28192); Sicily, Catania, Guardia, from *Citrus sinensis* crown, 9 Mar. 2015, *V. Guarnaccia* (CPC 28193).

Notes — *Neocosmospora macrospora* was isolated from *Citrus sinensis* in Catania province, Italy. The new species is totally divergent from the traditional morphological concept of *N. solani* s.lat. (Wollenweber 1913, Wollenweber & Reinking 1935 Snyder & Hansen 1940), differing from most currently accepted taxa in *Neocosmospora* by the presence of large 3–9-septate (commonly 7-septate) sporodochial conidia. Other taxa of this complex producing long multiseptate sporodochial conidia are two species not yet formally transferred to *Neocosmospora*, '*Fusarium' ensiforme* and '*F'. eumartii*; and *N. pseudensiformis* (Carpenter 1915, Wollenweber & Reinking 1925, Nalim et al. 2011). However, '*F'. ensiforme* and *N. pseudensiformis* produce macroconidia with up to seven and eight septa, respectively, while those in '*F'. eumartii* are commonly 5–7-septate, but rarely 8–9-septate (Gerlach & Nirenberg 1982, Domsch et al. 2007). In contrast, nine-septate macroconidia are a commonly observed feature of *N. macrospora*, being also longer (up to 90 µm long vs up to 81 µm long in *'F'. ensiforme*; and up to 85 µm long in *'F'. eumartii* and *N. pseudensiformis*).

Neocosmospora macrospora is also reminiscent of '*Fusarium' decemcellulare*, particularly in the macroconidial features; however, the latter species produces aseptate microconidia arranged in long chains and an *Albonectria* sexual morph (*A. rigidiuscula*), being also phylogenetically distant (Gräfenhan et al. 2011, Schroers et al. 2011, O'Donnell et al. 2013).

Pathogenicity

The four tested isolates of *F. citricola* and *F. salinense* were pathogenic to the three *Citrus* hosts used. Monosporic isolations of the causal agent from the lesions had identical *RPB2* sequences to those of the ex-type strains of *F. citricola* and *F. salinense* (CBS 142421 and CBS 142420, respectively). The inoculated twigs developed identical cankers to those detected in the orchards, thus fulfilling Koch's postulates (Fig. 11). Canker and internal discolouration symptoms were observed corresponding to inoculation points. On the contrary, no symptoms were observed on control plants and on plants inoculated with isolates of *F. sarcochroum*. No evident difference in aggressiveness was observed among the isolates.

DISCUSSION

Molecular phylogenetic and morphological analyses were used to evaluate the diversity of *Fusarium* and fusarium-like species from *Citrus* in the Mediterranean basin, focusing especially on Southern Italy.

Fig. 11 Natural (a–c) and artificial symptoms (d–g) on citrus with *F. citricola* species complex spp. associated. a. Trunk canker; b. injured crown of orange tree sampled; c. canker on lemon twigs with gum exudation; d–e. external and internal canker caused by *F. salinense* inoculation; f–g. internal discoloration of twigs inoculated with *F. citricola*.

These fungi are well established in the Mediterranean environment in association with significant agricultural crop diseases (Wong & Jeffries 2006, Vitale et al. 2014). In Europe, different *Fusarium* species are reported as pathogens of citrus, i.e., *F. oxysporum*, *F. proliferatum*, *F. sambucinum* and *F. solani* s.lat. (Malikoutsaki-Mathioudi et al. 1987, Polizzi et al. 1992, Yaseen & D'Onghia 2012). *Citrus* is the most important agricultural crop in Southern Italy, and is already compromised by a range of other fungal pathogens (Aiello et al. 2015), and fusaria represent a further serious threat to this crop.

Six *Fusarium* and five *Neocosmospora* species were isolated from symptomatic trees in three Mediterranean countries, all isolated from symptomatic *Citrus* tissues. However, considering the narrow geographic area studied, it is likely that many other species would also be isolated if a wider sampling area was surveyed.

Three of the species newly described here (*F. siculi*, *N. croci* and *N. macrospora*) and five known species (*F. ensiforme*, *F. oxysporum*, *N. solani*, and the unnamed phylogenetic species *Neocosmospora* sp. FSSC 9 and *Neocosmospora* sp. FSSC 28) were associated with dry root rot of orange trees in our survey. Of these, only *F. oxysporum*, *F. proliferatum* and *N. solani* s.str. were considered pathogens associated with this disease prior to the present study (Menge 1988, Adesemoye et al. 2011). Our results reveal a large diversity of *Fusarium* species spanning several species complexes, associated with dry root rot in a restricted area of Southern Italy, and major and minor Italian islands. Considering the uncertainty of a well-established method to artificially reproduce this disease (Graham et al. 1985, Dandurand & Menge 1993), the pathogenicity of these eight fusaria could not be tested in the present study. Nevertheless, we demonstrated their ability to produce cankers on *Citrus sinensis* stem tissues. Further surveys in other citrus-producing areas of the globe, more *Fusarium* isolations and studies on pathogenicity in association with abiotic factors, should be performed.

Fusarium sarcochroum was isolated from lemon and mandarin twigs showing dieback, being found on citrus for the first time in Italy and Spain in the present study; though, it was already reported from Greece (Pantidou 1973). We confirm the ability of this species to colonise several *Citrus* spp. as endophyte. However, even though *F. sarcochroum*, *F. citricola* and *F. salinense* were recovered from citrus cankers, we were able to confirm pathogenicity on multiple hosts only for the latter two species. *Fusarium salinense* is described in the present study as causing cankers on twigs of *C. sinensis* in Sicily and the

Aeolian Islands, while *F. citricola* was recovered in other southern regions of Italy, on multiple *Citrus* spp., causing cankers on different woody organs of these plant hosts. These results suggest a geographical distinction between the species. However, more surveys are needed to clarify their host specificity. Furthermore, these species can be added to other citrus canker causing pathogens reported worldwide (Adesemoye et al. 2014, Mayorquin et al. 2016).

The results of our molecular analyses indicate that the two new species, *F. citricola* and *F. salinense*, not only represent new taxa but constitute a novel lineage in *Fusarium*, closely related to the FTSC, here designated as FCCSC. The reduced production of aerial microconidia on short phialides or phialidic pegs, the abundant bright orange sporodochia and the shape of its sporodochial conidia are characters that compare FCCSC morphologically with other species complexes in *Fusarium* such as the FCSC, the *F. graminearum* species complex (FGSC) or the *Fusarium sambucinum* species complex (FSASC). However, clear differences do exist, particularly in the robustness, degree of septation and curvature of the macroconidia, while microconidia are always lacking in FGSC and are an uncommon feature in FSASC. Species in FTSC, the closest phylogenetic relatives, share similar cultural characteristics with FCCSC like the production of red pigments on PDA; nevertheless, the newly proposed species do not produce pyriform conidia or chlamydospores as many of the currently described species in FTSC, which also with the exception of *F. torulosum*, are characterised by the production of strongly curved to lunate conidia with pointed ends, differing from the gently curved conidia in FCCSC. In addition to the morphological traits, species in the new lineage show considerable ecological differences allowing for its clear delimitation. Both species in this complex seemed to be confined to particular geographical regions in Italy. *Fusarium salinense* was isolated from two different locations in Sicily and Salina (Aeolian Islands), from the same host in two independent collections, and was demonstrated to be pathogenic to *Citrus*, as supported by our pathogenicity tests. *Fusarium citricola*, however, was isolated from two regions in southern continental Italy, also appearing to be a prominent canker pathogen on many different *Citrus* species. In contrast, species in FTSC are common in temperate areas where they are mostly weak pathogens causing foot and root rot of cereals (Yli-Mattila et al. 2002, Leslie & Summerell 2006). Some species in FTSC have been reported previously from *Citrus* in Asia and USA, like *F. acuminatum* and *F. avenaceum* (Gerlach & Ershad 1970, Tai 1979, French 1987, 1989); however, there is no certainty about their true pathogenicity to this host, while the identity of the isolates has been confirmed by DNA sequencing for only a limited number of cases (Nalim et al. 2009).

Although *F. siculi* was isolated from symptomatic crowns of *Citrus sinensis*, we were unable to confirm its pathogenicity to this host given the difficulties in replicating disease symptoms. *Fusarium siculi* is nested within the FFSC, a species-rich complex that includes many species of economic significance, mycotoxigenic species and agent of plant disease mostly related to graminicolous plants and soil, but also includes important tree pathogenic species affecting woody organs, such as *Fusarium circinatum*, agent of pitch canker of *Pinus* spp. (Nirenberg & O'Donnell 1998, Herron et al. 2015). Reports from *Citrus* spp. are scarce with only *F. proliferatum* reported from fruit rot in Asia and associated with dry root rot (Hyun et al. 2000, Adesemoye et al. 2011, Farr & Rossman 2017). Further testing is needed to confirm the ecological relevance of the new species.

The recent works by Gräfenhan et al. (2011) and Lombard et al. (2015) and the resulting segregation of *Fusarium* has been controversial in the sense that it excludes many agricultural and medically important species from *Fusarium*, particularly those belonging to the *F. solani* and *F. dimerum* species complexes, a move which could bring confusion to the *Fusarium* research community (Geiser et al. 2013, Aoki et al. 2014). However, despite the practical considerations, splitting the genus seem justified phylogenetically and morphologically (Gräfenhan et al. 2011, Geiser et al. 2013, O'Donnell et al. 2013, Aoki et al. 2014, Lombard et al. 2015). Here, two new saprophytic species are described in *Neocosmospora*. *Neocosmospora croci*, although phylogenetically well defined, is difficult to distinguish morphologically from *N. solani* s.str. (Schroers et al. 2016). This reflects the limitations of the morphological species recognition criteria in this genus, known to include at least 60 narrowly defined phylogenetic species, distributed into three main clades, for which distinct morphological traits are minimal or absent (O'Donnell et al. 2008, Geiser et al. 2013).

The present study introduces new insights into the biodiversity of *Fusarium* and *Neocosmospora* species associated with *Citrus* in Europe. Surprisingly, a remarkable diversity of *Fusarium* and *Neocosmospora* species was found in a somewhat reduced sampling area. Furthermore, five new species were described, two of them belonging to a new, undescribed lineage in *Fusarium*, with demonstrated pathogenicity to *Citrus*. This shows that despite the worldwide distribution of *Citrus*, and previous knowledge about its associated microbes, the fungal species-richness in *Citrus* spp. is still underestimated. More studies are therefore needed on these new taxa in order to elucidate their host range, specificity, and global distribution, as well as their potential impact on the *Citrus* industry.

Acknowledgements We thank Kerry O'Donnell (Mycotoxin Prevention and Applied Microbiology Research Unit, Agricultural Research Service, US Department of Agriculture, Peoria, Illinois) for kindly providing sequence datasets used in this article, including unpublished sequences. We also thank Ewald Groenewald for his assistance constructing the phylogenies. Keith A. Seifert and Brett A. Summerell are thanked for their valuable comments and corrections on the manuscript.

REFERENCES

Adesemoye AO, Eskalen A, Faber B, et al. 2011. Current knowledge on Fusarium dry root rot of citrus. Citrograph 2: 29–33.

Adesemoye AO, Mayorquin JS, Wang DH, et al. 2014. Identification of species of Botryosphaeriaceae causing bot gummosis in citrus in California. Plant Disease 98: 55–61.

Aiello D, Carrieri R, Guarnaccia V, et al. 2015. Characterization and pathogenicity of Colletotrichum gloeosporioides and C. karstii causing preharvest disease on Citrus sinensis in Italy. Journal of Phytopathology 163: 168–177.

Aoki T, Nirenberg HI. 1999. Fusarium globosum from subtropical Japan and the effect of different light conditions on its conidiogenesis. Mycoscience 40: 1–9.

Aoki T, O'Donnell K, Geiser DM. 2014. Systematics of key phytopathogenic Fusarium species: current status and future challenges. Journal of General Plant Pathology 80: 189–201.

Aoki T, O'Donnell K, Homma Y, et al. 2003. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the Fusarium solani species complex - F. virguliforme in North America and F. tucumaniae in South America. Mycologia 95: 660–684.

Aoki T, O'Donnell K, Scandiani MM. 2005. Sudden death syndrome of soybean in South America is caused by four species of Fusarium: Fusarium brasiliense sp. nov., F. cuneirostrum sp. nov., F. tucumaniae and F. virguliforme. Mycologia 46: 162–183.

Aoki T, Smith JA, Mount LL, et al. 2013. Fusarium torreyae sp. nov., a pathogen causing canker disease of Florida torreya (Torreya taxifolia), a critically endangered conifer restricted to northern Florida and southwestern Georgia. Mycologia 105: 312–319.

Booth C. 1971. The genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England.

Bruen TC, Philippe H, Bryant D. 2006. A simple and robust statistical test for detecting the presence of recombination. Genetics 172: 2665–2681.

Burgess LW, Lidell CM, Summerell BA. 1988. Laboratory manual for Fusarium research, 2nd ed. University of Sydney, Sydney, Australia.

Carpenter CW. 1915. Some potato tuber-rots caused by species of Fusarium. Journal of Agricultural Research 5: 183–209.
Chitrampalam P, Nelson Jr B. 2016. Multilocus phylogeny reveals an association of agriculturally important Fusarium solani species complex (FSSC) 11, and clinically important FSSC 5 and FSSC 3 + 4 with soybean roots in the north central United States. Antonie van Leeuwenhoek 109: 335–347.
Crous PW, Verkley GJM, Groenewald JZ, et al. (eds). 2009. Fungal Biodiversity. CBS Laboratory Manual Series 1. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.
Dandurand LM, Menge JA. 1993. Influence of Fusarium solani on citrus root growth and population dynamics of Phytophthora parasitica and Phytophthora citrophthora. Phytopathology 83: 767–771.
Dao HT, Beattie GAC, Rossman AY, et al. 2015. Systematics and biology of two species of Microcera associated with armoured scales on citrus in Australia. Mycological Progress 14: 1–14.
Dean R, Van Kan JAL, Pretorius ZA, et al. 2012. The top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology 13: 414–430.
Derrick KS, Timmer LW. 2000. Citrus Blight and other diseases of recalcitrant etiology. Annual Review of Phytopathology 38: 181–205.
Domsch KH, Gams W, Anderson TH. 2007. Compendium of soil fungi. 2nd edn. IHW Verlag, Eching, Germany.
Edwards J, Auer D, De Alwis SK, et al. 2016. Fusarium agapanthi sp. nov., a novel bikaverin and fusarubin-producing leaf and stem spot pathogen of Agapanthus praecox (African lily) from Australia and Italy. Mycologia 108: 981–992.
FAO - Food and Agricultural Organization of the United Nations, Rome. 2016. Citrus fruits fresh and processed: annual statistics. http://www.fao.org/3/a-i5558e.pdf.
Farr DF, Rossman AY. 2017. Fungal databases, systematic mycology and microbiology laboratory, ARS, USDA. Retrieved January 26.
Fisher NL, Burguess LW, Toussoun TA, et al. 1982. Carnation leaves as a substrate and for preserving cultures of Fusarium species. Phytopathology 72: 151–153.
French AM. 1987. California plant disease host index. Part 1: Fruit and nuts. California Department of Food and Agriculture, Sacramento.
French AM. 1989. California plant disease host index. California Department of Food and Agriculture, Sacramento.
Gams W, Nirenberg HI, Seifert KA, et al. 1997. Proposal to conserve the name Fusarium sambucinum (Hyphomycetes). Taxon 46: 111–113.
Geiser DM, Aoki T, Bacon CW, et al. 2013. One fungus, one name: defining the genus Fusarium in a scientifically robust way that preserves longstanding use. Phytopathology 103: 400–408.
Geiser DM, Jiménez-Gasco MdD, Kang S, et al. 2004. FUSARIUM-ID version 1.0: a DNA sequence database for identifying Fusarium. European Journal of Plant Pathology 110: 473–479.
Gerlach W, Ershad D. 1970. Beitrag zur Kenntnis der Fusarium – und Cylindrocarpon-Arten in Iran. Nova Hedwigia 20: 725–784.
Gerlach W, Nirenberg HI. 1982. The genus Fusarium – a pictorial atlas. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 209: 1–406.
Gräfenhan T, Schroers HJ, Nirenberg HI, et al. 2011. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in Cosmospora, Acremonium, Fusarium, Stilbella, and Volutella. Studies in Mycology 68: 79–113.
Graham JH, Brlansky RH, Timmer LW, et al. 1985. Comparison of citrus tree declines with necrosis of major roots and their association with Fusarium solani. Plant Disease 69: 1055–1058.
Hannachi I, Rezgui S, Cherif M. 2014. First report of mature citrus trees being affected by Fusarium wilt in Tunisia. Plant Disease 98: 566.
Herron DA, Wingfield MJ, Wingfield BD, et al. 2015. Novel taxa in the Fusarium fujikuroi species complex from Pinus spp. Studies in Mycology 80: 131–150.
Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23: 254–267.
Hyun JW, Lee SC, Kim DH, et al. 2000. Fusarium fruit rot of Citrus in Jeju Island. Mycobiology 28: 158–162.
Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.
Leslie JF, Summerell BA. 2006. The Fusarium laboratory manual. Blackwell Publishing, Ames.
Leslie JF, Summerell BA. 2011. In search of new Fusarium species. Plant Breeding and Seed Science 63: 94–101.
Li W, Cowley A, Uludag M, et al. 2015. The EMBL-EBI bioinformatics web and programmatic tools framework. Nucleic Acids Research 43: W580–584.
Link HF. 1809. Observationes in ordines plantarum naturales. Dissertatio I. Magazin der Gesellschaft Naturforschenden Freunde Berlin 3: 3–42.
Lombard L, Van der Merwe NA, Groenewald JZ, et al. 2015. Generic concepts in Nectriaceae. Studies in Mycology 80: 189–245.
Malikoutsaki-Mathioudi M, Bourbos VA, Skoudridakis MT. 1987. La pourriture sèche des racines – une maladie très grave des agrumes en Grèce. EPPO Bulletin 17: 335–340.
Mason-Gamer R, Kellogg E. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Systematic Biology 45: 524–545.
Mayorquin JS, Wang DH, Twizeyimana M, et al. 2016. Identification, distribution, and pathogenicity of Diatrypaceae and Botryosphaeriaceae associated with citrus branch canker in the Southern California Desert. Plant Disease 100: 2402–2413.
McCoy CW, Samson RA, Boucias DG, et al. 2009. Pathogens infecting insects and mites of citrus. LLC Friends of Microbes, USA.
Menge JA. 1988. Dry root rot. In: Whiteside JO, Garnsey SM, Timmer LW (eds), Compendium of Citrus diseases: 14–15. APS Press, USA.
Miller MA, Pfeiffer W, Schwartz T. 2012. The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond: 1–8. Association for Computing Machinery, USA.
Moore SD, Duncan LW. 2016. Microbial control of insect and mite pests of citrus. In: Lacey LA (ed), Microbial control of insect and mite pests: from theory to practice: 283–298. Academic Press, UK.
Nalim FA, Elmer WH, McGovern RJ, et al. 2009. Multilocus phylogenetic diversity of Fusarium avenaceum pathogenic on Lisianthus. Phytopathology 99: 462–468.
Nalim FA, Samuels GJ, Wijesundera RL, et al. 2011. New species from the Fusarium solani species complex derived from perithecia and soil in the old World tropics. Mycologia 103: 1302–1330.
Nelson PE, Toussoun TA, Marasas FO. 1983. Fusarium species: an illustrated manual for identification. Pennsylvania State University Press, University Park.
Nirenberg HI. 1976. Untersuchungen 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, O'Donnell K. 1998. New Fusarium species and combinations within the Gibberella fujikuroi species complex. Mycologia 90: 434–458.
Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
O'Donnell K, Cigelnik E, Nirenberg H. 1998a. Molecular systematics and phylogeography of the Gibberella fujikuroi species complex. Mycologia 90: 465–493.
O'Donnell K, Gueidan C, Sink S, et al. 2009a. 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, Kistler HC, Cigelnik E, et al. 1998b. 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, Nirenberg HI, Aoki T, et al. 2000. A multigene phylogeny of the Gibberella fujikuroi species complex: Detection of additional phylogenetically distinct species. Mycoscience 1: 61–78.
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, 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, Sutton DA, Fothergill A, et al. 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the Fusarium solani species complex. Journal of Clinical Microbiology 46: 2477–2490.
O'Donnell K, Sutton DA, Rinaldi MG, et al. 2009b. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the Fusarium incarnatum - F. 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, Sutton DA, Wiederhold N, et al. 2016. Veterinary fusarioses within the United States. Journal of Clinical Microbiology 54: 2813–2819.
Pantidou ME. 1973. Fungus-host index for Greece. Benaki Phytopathological Institute, Kiphissia, Athens, Greece.

Polizzi G, Magnano di San Lio G, Catara A. 1992. Dry root rot of citranges in Italy. Proceedings of the International Society of Citriculture. VII International Citrus Congress, Acireale 1992: 890–893.

Quaedvlieg W, Binder M, Groenewald JZ, et al. 2014. Introducing the Consolidated Species Concept to resolve species in the Teratosphaeriaceae. Persoonia 33: 1–40.

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

Rheeder JP, Marasas WFO, Nelson PE. 1996. Fusarium globosum, a new species from corn in southern Africa. Mycologia 88: 509–513.

Schroers HJ, Gräfenhan T, Nirenberg HI, et al. 2011. A revision of Cyanonectria and Geejayessia gen. nov., and related species with Fusarium-like anamorphs. Studies in Mycology 68: 115–138.

Schroers HJ, O'Donnell K, Lamprecht SC, et al. 2009. Taxonomy and phylogeny of the Fusarium dimerum species group. Mycologia 101: 44–70.

Schroers HJ, Samuels GJ, Zhang N, et al. 2016. Epitypification of Fusisporium (Fusarium) solani and its assignment to a common phylogenetic species in the Fusarium solani species complex. Mycologia 108: 806–819.

Smith IM, Dunez J, Phillips DH, et al. 1988. European handbook of plant diseases. Blackwell Scientific Publications, UK.

Snyder WC, Hansen HN. 1940. The species concept in Fusarium. American Journal of Botany 27: 64–67.

Snyder WC, Hansen HN. 1947. Advantages of natural media and environments in the culture of fungi. Phytopathology 37: 420–421.

Spina S, Coco V, Gentile A, et al. 2008. Association of Fusarium solani with rolabc and wild type Troyer Citrange. Journal of Plant Pathology 90: 479–486.

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.

Tai FL. 1979. Sylloge Fungorum Sinicorum. Science Press, Academia Sinica, Peking (Beijing).

Tamura K, Stecher G, Peterson D, et al. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Timmer LW. 1982. Host range and host colonization, temperature effects, and dispersal of Fusarium oxysporum f. sp. citri. Phytopathology 72: 698–702.

Timmer LW, Garnsey SM, Grimm GR, et al. 1979. Wilt and dieback of Mexican lime caused by Fusarium oxysporum. Phytopathology 69: 730–734.

Toussoun TA, Nelson PE. 1976. Fusarium: A pictorial guide to the identification of Fusarium species according to the taxonomic system of Snyder and Hansen. 2nd edn. Pennsylvania State University Press, USA.

Van Diepeningen AD, Al-Hatmi AMS, Brankovics B, et al. 2014. Taxonomy and clinical spectra of Fusarium species: where do we stand in 2014? Current Clinical Microbiology Reports 1: 10–18.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.

Vilgalys R, Sun BL. 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom Pleurotus revealed by phylogenetic analysis of ribosomal DNA sequences. Proceedings of the National Academy of Sciences of the United States of America 91: 4599–4603.

Vitale A, Rocco M, Arena S, et al. 2014. Tomato susceptibility to Fusarium crown and root rot: Effect of grafting combination and proteomic analysis of tolerance expression in the rootstock. Plant Physiology and Biochemistry 83: 207–216.

White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innes MA, Gelfand DH, Sninsky et al. (eds), PCR protocols: a guide to methods and applications: 315–322. Academic Press, USA.

Wiens JJ. 1998. Testing phylogenetic methods with tree congruence: phylogenetic analysis of polymorphic morphological characters in phrynosomatid lizards. Systematic Biology 47: 427–444.

Wollenweber HW. 1913. Studies on the Fusarium problem. Phytopathology 3: 24–50.

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 Bekämpfung. Paul Parey, Berlin.

Wong JY, Jeffries P. 2006. Diversity of pathogenic Fusarium populations associated with asparagus roots in decline soils in Spain and the UK. Plant Pathology 55: 331–342.

Woudenberg JHC, Aveskamp MM, De Gruyter J, et al. 2009. Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. Persoonia 22: 56–62.

Yaseen T, D'Onghia AM. 2012. Fusarium spp. associated to citrus dry root rot: An emerging issue for Mediterranean citriculture. Acta Horticulturae 940: 647–655.

Yli-Mattila T, Paavanen-Huhtala S, Bulat SA, et al. 2002. Molecular, morphological and phylogenetic analysis of the Fusarium avenaceum / F. arthrosporioides / F. tricinctum species complex – a polyphasic approach. Mycological Research 106: 655–669.

Zhang N, O'Donnell K, Sutton DA, et al. 2006. Members of the Fusarium solani species complex that cause infections in both humans and plants are common in the environment. Journal of Clinical Microbiology 44: 2186–2190.