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

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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-tu) 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 dieback of citrus trees. The most commonly isolated species were Fusarium sambucinum, F. oxysporum and Neocosmospora solani. Three new Fusarium species are described, i.e., F. cicitola and F. salinense belonging to the newly described F. cicitola 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.

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 mycotoxicogenic 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 specialae based on pathogenicity and host ranges (Wollenweber & Rein- king 1935, Snyder & Hansen 1940, Tousson & 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.

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 (perithelial ascocoma white, yellow, orange to dark purple-black coloured with warty superficial peridium cells, forming (0–)1–3-septate, smooth, ellipsoidoid ascospores) and in this new circumscription it includes at least 16 species complexes and numerous monotypic linages (O’Donnell et al. 2013). Neocosmospora now includes 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 warded, large and angular superficial peridial cells, producing aseptate or 1-septate, globose to ellipsoidoid, 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. verticilloides 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 abscession 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 coccophila) 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 after 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; conidioaphore length and branching patterns, nature of the conidiogenous cells and presence or absence of chlamydoospores 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.
| Species name¹ | Strain number² | Country and region | Source | Associated symptoms | GenBank accession number³ | CAM | EF-1α | IGS | ITS | LSU | RPB1 | RPB2 | TUB |
|----------------|----------------|--------------------|--------|---------------------|--------------------------|-----|------|-----|-----|-----|------|------|------|
| F. citriola | CPC 27067 | Italy, Cosenza | Citrus limon | Twigs canker | LT746194 LT746242 LT746242 LT746287 LT746307 | LT746194 LT746242 LT746242 LT746287 LT746307 | LT746194 LT746242 LT746242 LT746287 LT746307 |
| F. ensiforme | CPC 27190 | Italy, Catania | Citrus sinensis | Dry root rot | LT746199 LT746247 LT746247 LT746312 | LT746199 LT746247 LT746247 LT746312 | LT746199 LT746247 LT746247 LT746312 |
| F. oxysporum | CPC 27194 | Italy, Siracusa | Citrus sinensis | Dry root rot | LT746201 LT746233 LT746234 LT746314 | LT746201 LT746233 LT746234 LT746314 | LT746201 LT746233 LT746234 LT746314 |
| F. salsense | CPC 26403 | Italy, Catania | Citrus sinensis | Twigs canker | LT746239 LT746239 LT746294 LT746304 | LT746239 LT746239 LT746294 LT746304 | LT746239 LT746239 LT746294 LT746304 |
| F. sarothronium | CPC 26369 | Italy, Catania | Citrus limon | Twigs dieback | LT746207 LT746255 LT746255 LT746320 | LT746207 LT746255 LT746255 LT746320 | LT746207 LT746255 LT746255 LT746320 |
| F. siculi | CPC 27188 = CBS 14242² | Italy, Catania | Citrus sinensis | Dry root rot | LT746214 LT746262 LT746262 LT746327 LT746346 | LT746214 LT746262 LT746262 LT746327 LT746346 | LT746214 LT746262 LT746262 LT746327 LT746346 |
| N. croci | CPC 27186 = CBS 14243² | Italy, Catania | Citrus sinensis | Dry root rot | LT746215 LT746263 LT746263 LT746328 LT746347 | LT746215 LT746263 LT746263 LT746328 LT746347 | LT746215 LT746263 LT746263 LT746328 LT746347 |
| N. macrospora | CPC 28191 = CBS 14244² | Italy, Catania | Citrus sinensis | Dry root rot | LT746218 LT746266 LT746266 LT746331 | LT746218 LT746266 LT746266 LT746331 | LT746218 LT746266 LT746266 LT746331 |
| N. solani | CPC 27192 | Italy, Siracusa | Citrus sinensis | Dry root rot | LT746221 LT746269 LT746269 LT746334 | LT746221 LT746269 LT746269 LT746334 | LT746221 LT746269 LT746269 LT746334 |
| Neocosmospora sp. FSSC 9 | CPC 27195 | Italy, Siracusa | Citrus sinensis | Dry root rot | LT746227 LT746273 LT746273 LT746338 | LT746227 LT746273 LT746273 LT746338 | LT746227 LT746273 LT746273 LT746338 |
| Neocosmospora sp. FSSC 28 | CPC 28194 | Italy, Siracusa | Citrus sinensis | Dry root rot | LT746228 LT746276 LT746276 LT746341 | LT746228 LT746276 LT746276 LT746341 | LT746228 LT746276 LT746276 LT746341 |

¹ F. Fusarium; N. Neocosmospora.
² Ex-type strains.
³ CAL: 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.
| Species name | Strain number | Country and source | GenBank accession number |
|--------------|---------------|--------------------|-------------------------|
| F. acuminatum | NRRL 36147 = CBS 109232 | Unknown, human bronchial secretion | GQ505642, GQ505452, GQ505452, HM347174, GQ505448 |
| F. agapanthi | NRRL 54483 | Australia, Agapanthus sp. | KU0900611, KU0900630, KU0900620, KU0900625, KU0900635 |
| F. ananatum | NRRL 22945 = CBS 184.29 | England, Ananas comosus | KR071762, U34562, JX171505 |
| F. andrysts | NRRL 53131 | Italy, human | HM347128, HM347198, HM347213 |
| F. angiosclera | NRRL 25385 | South Africa, Sorghum bicolor soil debris | KR071718, KR071651, KT154004, KP662894 |
| F. anglicoles | NRRL 13602 = ATCC 6737.97 | Germany, Hippophaera strum | AF160292, –, –, U61541 |
| F. armeniacum | NRRL 6227 = ATCC 36781 | USA, fescue hay | –, –, –, JX171560 |
| F. asiaticum | NRRL 13818 | Japan, barley | –, –, –, JX171573 |
| F. avenaeaceum | FRC R-0095 | USA, Lisanthes sp. | GQ915502, GQ915486 |
| F. babinda | NRRL 25128 | Poland, Hymenoptera ichneumonidae | JF740751, JF740894, JF740894, JF740962, JF741079 |
| F. begoniae | NRRL 13607 = CBS 403.97 | Germany, Begonia elatior hybrid plant | AF160293, –, –, –, U61543 |
| F. beciforme | NRRL 25174 = CBS 740.97 | New Caledonia, soil | –, –, –, JX171619 |
| F. brasiliensis | NRRL 22743 | Brazil, Glycine max | EF408407, FJ919502, FJ919502, EU3925 |
| F. buharicum | NRRL 13371 = CBS 796.70 | Iran, Hibiscus cannabinus | –, –, –, JX171449, JX171563 |
| F. bulbicola | NRRL 13618 = CBS 220.76 | Germany, Nerine bowderrii | KF466327, AF160294, U61676, KF466394, KF466404, KF466437 |
| F. burgessii | CBS 125837T = RBG 5315 | Australia, soil | –, –, –, HO646393 |
| F. cirratum | NRRL 25331 = CBS 405.97 | USA, Monterey pine tree | AF158348, AF160295, NR120263, JX171510, JX171623, KM232080 |
| F. coicis | NRRL 66233 | Australia, Coli gastrieti | –, –, –, –, KX083274 |
| F. concentricum | NRRL 25181 = CBS 450.97 | Costa Rica, Musa acanthum | AF160282, NR111886, –, –, JX171569 |
| F. color | NRRL 13491 = CBS 961.87 | South Africa, plant debris | –, –, –, JX171569 |
| F. colurnus | NRRL 25475 | Denmark, barley kernel | –, –, –, JX171628 |
| F. cuneiforme | NRRL 31104 | Japan, Phaseolus vulgaris | –, –, –, EU329588 |
| F. denticulatum | NRRL 25302 = CBS 735.97 | USA, Ipomoea batatas | AF160269, –, –, U61559 |
| F. dambus | NRRL 43665 | USA, contact lens | –, –, –, EF407035 |
| F. ensiforme | NRRL 28009 = CDC B-5543 | USA, human eye | DQ246869, DQ094351, DQ236393, EF407036 |
| F. equiseti | NRRL 20697 = CBS 245.61 | Chile, Beta vulgaris | GQ505594, GQ505483, GQ505483, JX171481, JX171595 |
| F. euwolosioae | NRRL 54723 = CBS 135855 | Israel, beet from avocado tree | JQ080008, JQ080150, JQ080150, JQ080150, JQ080150 |
| F. fujikuroi | NRRL 54724 = CBS 135856 | Israel, beet from avocado tree | JQ080009, JQ080168, JQ080168, JQ080168, JQ080168 |
| F. flocciferum | NRRL 25473 = CBS 831.85 | Germany, Triticum aestivum | –, –, –, JX171514, JX171627 |
| F. globosum | NRRL 45999 | USA, UHSOC 5-8644 | –, –, –, GQ505433, GQ505465, GQ505465, HM347195, GQ505497 |
| F. fraxtillexum | NRRL 28852 | Japan, Cymbidium sp. | AF158341, AF160288, AF158304, –, –, –, –, – |
| F. fujikuroi | NRRL 13586 = ATCC 38941 | China, Oryza sativa | AF160279, U34557, JX171456, JX171570 |
| F. gattii | NRRL 45417 = FRC M-8754 | Australia, Heteropogon triticeus | –, –, –, JX171570 |
| F. globosum | CBS 429.97 = NRRL 26132 | South Africa, Zea mays seed | LT746320, LT746278, LT746301, LT746343, LT746348 |
| F. globosum | CBS 430.97 = NRRL 26133 | South Africa, Zea mays seed | LT746231, LT746279, LT746302, LT746344, LT746349 |
| F. globosum | CBS 431.97 = NRRL 26134 | South Africa, Zea mays seed | LT746232, LT746280, LT746303, LT746345, LT746350 |
| F. globosum | NRRL 26131 = CBS 428.97 | South Africa, corn seed | KF466329, AF160285, –, KF466396, KF466404, KF466439 |
| F. granamheanum | NRRL 31084 | USA, corn | –, –, –, JX171570 |
| F. heterosporum | NRRL 20692 = CBS 737.79 | Ethiopia, Cynodon dactylon | –, –, –, JX171479, JX171593 |
| F. heterosporum | NRRL 20693 = CBS 720.79 | Netherlands, Claviceps purpurea on Lolium perenne | –, –, –, JX171480, JX171584 |
| F. hostae | NRRL 29889 = FRC 0-2074 | USA, Hosta sp. | – | – | – | – | – | JX171640 |
| F. inflexum | NRRL 20433 = CBS 716.74 | Germany, Viola faba | AF158366 | AF008479 | U34577 | – | – | JX171649 | JX171583 |
| F. keratoplasticum | NRRL 22661 | Japan, human eye | – | – | – | – | – | – | – |
| F. korzum | NRRL 53387 | Brazil, Araucaria angustifolia | – | – | – | – | – | – | – |
| F. laceratum | NRRL 20423 = CBS 130185 | India, lizard skin | – | – | – | – | – | – |
| F. lactis | NRRL 25200 = CBS 411.97 | USA, Ficus carica | AF158325 | AF160272 | NR111887 | – | – | U61551 |
| F. lateritium | FRC L110 | Zimbabwe, Coffea arabica berries | – | – | – | – | – | – |
| F. hostae | NRRL 13622 | USA, elm tree | – | – | – | – | – | JX171457 | JX171571 |
| F. hostae | NRRL 25197 = CBS 748.79 | New Zealand, Citrus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 37021 | New Zealand, Citrus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 34123 | India, human eye | – | – | – | – | – | – |
| F. hostae | NRRL 54252 = CBS 125536 | Australia, Sorghum interjectum | – | – | – | – | – | – |
| F. hostae | NRRL 25226 = BBA 69962 | India, Mangifera indica | AF158334 | AF160281 | U61891 | – | – | JX171509 | JX171622 | U61561 |
| F. hostae | NRRL 47273 | Mexico, mango inflorescence | – | – | – | – | – | – |
| F. hostae | NRRL 36452 | USA, elm tree | – | – | – | – | – | – |
| F. hostae | NRRL 13338 | Australia, soil | – | – | – | – | – | – |
| F. hostae | NRRL 25179 = CBS 742.97 | New Zealand, Cupressus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 25485 = CBS 748.79 | New Zealand, Cupressus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 37021 | New Zealand, Cupressus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 34123 | India, human eye | – | – | – | – | – | – |
| F. hostae | NRRL 54252 = CBS 125536 | Australia, Sorghum interjectum | – | – | – | – | – | – |
| F. hostae | NRRL 25226 = BBA 69962 | India, Mangifera indica | AF158334 | AF160281 | U61891 | – | – | JX171509 | JX171622 | U61561 |
| F. hostae | NRRL 47273 | Mexico, mango inflorescence | – | – | – | – | – | – |
| F. hostae | NRRL 36452 | USA, elm tree | – | – | – | – | – | – |
| F. hostae | NRRL 13338 | Australia, soil | – | – | – | – | – | – |
| F. hostae | NRRL 25179 = CBS 742.97 | New Zealand, Cupressus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 25485 = CBS 748.79 | New Zealand, Cupressus sp. | – | – | – | – | – | – |
| F. hostae | NRRL 37021 | New Zealand, Cupressus sp. | – | – | – | – | – | – |
| 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 | – | – | – | – | – | – | JX171586 |
| **F. scipi** | NRRL 13402 | Australia, pine nursery soil | – | GQ505592 | GQ505681 | GQ505681 | JX171452 | JX171566 |
| **Fusarium sp.** | F201237 | China, Zanthoxylum beauverum | – | KM527105 | – | – | – | KM520371 |
| **F. f. scirpi** | NRRL 13444 | Australia, pine nursery soil | – | – | – | – | – | – | JX171566 |
| **F. f. sp.** | NRRL 20429 = ATCC 15662 | Nyasaland, coffee bark | – | – | – | – | – | – | JX171468 |
| **F. f. subglutinans** | NRRL 13613 = CBS 219.76 | Germany, Succisa pratensis | – | AF178340 | AF178404 | AF178373 | – | – | EU329506 |
| **F. f. sublunatum** | NRRL 22090 | New Zealand, Beilschmiedia tawa | – | – | – | – | – | – | JX171601 |
| **F. f. udum** | NRRL 23346 | USA, human eye | – | – | – | – | – | – | EU329903 |
| **F. f. venenatum** | NRRL 22389 = BBA 67587 | USA, Eurygaster sp. | – | JF740804 | JF740915 | JF740915 | JF740980 | JF741130 |
| **F. f. verrucosum** | NRRL 20438 = IMI 297027 | India, Camellia sinensis | – | AF178332 | AF178397 | AF178367 | – | – | EU329903 |
| **F. f. verticillioides** | NRRL 22364 | USA, human eye | – | – | – | – | – | – | EU329903 |
| **F. f. illudens** | NRRL 32741 | USA, human eye | – | – | – | – | – | – | EU329903 |
| **F. f. sp.** | NRRL 32741 | USA, human eye | – | – | – | – | – | – | EU329903 |

Table 2 (cont.)
| Strain | Source | GenBank Accession Numbers |
|--------|--------|---------------------------|
| FRC S 2432 | USA, university building | JN23576 JN235326 JN235526 |
| LEMM 110739 | Colombia, human toenail | LN827969 LN828118 |
| LEMM 111347 | Colombia, human toenail | LN827970 LN828119 |
| NRRL 22098 | USA, cucurbit | AF178327 DQ094301 DQ236343 |
| NRRL 22153 | USA, cucumber | AF178346 DQ094302 DQ236344 |
| NRRL 22157 = ATCC 18689 | Japan, Morus alba | AF178359 DQ094306 DQ236348 |
| NRRL 22161 = ATCC 18692 | Japan, Robinia pseudoacacia | AF178330 DQ094311 DQ236353 |
| NRRL 22163 | Japan, Xanthoxylum piperitum | AF178328 AF178394 AF178363 |
| NRRL 22218 | Venezuela, diosoy tree | AF178334 AF178399 AF178368 |
| NRRL 22230 = ATCC 44934 | Japan, Morus alba | AF178358 DQ094305 DQ236347 |
| NRRL 22240 | USA, human | AF178338 AF178402 AF178367 |
| NRRL 22257 | Brazil, Piper nigrum | AF178360 AF178422 AF178391 |
| NRRL 22266 = BBA 67586 | USA, Robinia pseudoacacia | AF178353 DQ094312 DQ236354 |
| NRRL 22262 = ATCC 38341 | Japan, gill of Penaeus japonicus | DQ246844 DQ094329 DQ236371 |
| NRRL 22268 | Spain, human | AF178355 DQ094310 DQ236352 |
| NRRL 22280 | USA, Gynaie max | AF178360 AF178422 AF178391 |
| NRRL 22537 | Papua New Guinea, diseased cocoa pods | JF740757 JF740899 JF741084 |
| NRRL 22801 | USA, human | DQ246866 DQ094348 DQ236390 |
| NRRL 22808 = CDC B-4701 | USA, unknown | DQ246868 DQ094350 DQ236392 |
| NRRL 22851 = UTHSC 98-1305 | USA, synovial fluid | DQ246882 EU329674 EU329674 |
| NRRL 31198 | USA, human wound | DQ246816 DQ094389 DQ236431 |
| NRRL 31189 | USA, human oral wound | DQ246816 EU329677 EU329677 |
| NRRL 32301 = UTHSC 01-595 | USA, human eye | DQ246929 EU329677 EU329677 |
| NRRL 32347 = CBS 109028 | Switzerland, human subcutaneous nodule | DQ246979 DQ094446 DQ236488 |
| NRRL 32705 | USA, human | DQ247025 DQ094448 DQ236530 |
| NRRL 32736 | USA, human eye | DQ247056 DQ094517 DQ236559 |
| NRRL 32755 | USA, turfee | DQ247073 DQ094534 DQ236576 |
| NRRL 32770 | USA, human eye | DQ247083 DQ094544 DQ236586 |
| NRRL 32785 | USA, human | DQ247094 FJ240371 FJ240371 |
| NRRL 32821 = FRC S-1230 | USA, turkey egg | DQ247128 DQ094587 DQ236629 |
| NRRL 32858 | USA, human | DQ247163 EU329684 EU329684 |
| NRRL 37625 | Netherlands, human | FJ24035 EU329684 EU329684 |
| NRRL 43502 | USA, human eye | DQ247048 DQ094557 DQ236592 |
| NRRL 45880 | USA, Pisum sativum | DQ247142 DQ094565 DQ236623 |
| NRRL 46703 | Spain, nematode | DQ247128 DQ094587 DQ236629 |
| NRRL 46707 = FMR 8030 | USA, human eye | DQ247037 FJ240371 FJ240371 |
| NRRL 52781 | Benin, Hypoxenenum hampei | JF415849 JF415849 JF415849 |
| NRRL 54982 = UTHSC 09-1008 | USA, Zebra shark | KC808213 KC808255 KC808255 |
| NRRL 54983 = UTHSC 09-1009 | USA, Zebra shark | KC808214 KC808256 KC808256 |
| NRRL 62797 | USA, unknown | KF906129 KF906130 KF906130 |
| NRRL 22246 | South Africa, soil | AF178348 AF178402 AF178367 |
| NRRL 43467 = CBS 130182 | USA, human eye | EF452920 EF453092 EF453092 |

**N. vasinfecta**

1. F: Fusarium, N: Neocosmospora.
2. **T**: Ex-type, **ET**: Ex-epitype, **NT**: Ex-neotype. ATCC: American Type Culture Collection, Manassas, 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; IIM: CABF 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, Texas, 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.
4. 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), iNL1/iNCS1 and the internal sequencing primers NLa/CNSa for IGS (O’Donnell et al. 2009a), ITS4/ITSS 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 the web server of the European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/Tools/msa/mafft/) (Katoh & Standley 2013). A manually corrected if necessary using MEGA v. 6.06 (Tamura et al. 2013). Alignments were checked and manually corrected if necessary using MEGA v. 6.06 (Tamura et al. 2013). 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). 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).

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

| Genus/species complex (SC) | Locus | Number of sites | Evolutionary model |
|----------------------------|-------|----------------|-------------------|
|                            |       | 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+H                    |
|                            | RPB2  | 1640  | 1305     | 335       | 216       | GTR+H+G                  |
|                            | TUB   | 507   | 387      | 119       | 59        | SYM+G                    |
| F. oxyssporum 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        | GTR+G                    |
|                            | RPB2  | 696   | 540      | 156       | 77        | GTR+H+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     | 346       | 361       | GTR+H+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.
The names of known species complexes are shown in bold. 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 homology 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 (Huelsen & 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](image-url)  One of five Maximum parsimony (MP) best-tree phylograms obtained from combined CAM, EF-1a, 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 $^\dagger$ and $^{\ddagger}$, 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).
Siracuso 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 fulfill 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 Fusicoilla 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 clad 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, Fusarium sarcochroum.

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),...
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 ($\Phi_W = 0.8$ 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 ($\Phi_W = 1.0$, 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 frilliform. 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 60–80 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, frilliform. Reverse sulphur to pure yellow, without diffusible pigments. On SNA, hyphae hyaline, smooth-walled,
Clade 3 representatives. 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 red font. Ex-type and ex-epitype strains are indicated in bold. Ex-type and ex-epitype strains are indicated in bold.

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 red font. Ex-type and ex-epitype strains are indicated in bold.
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 carpelation leaves or the surface of the agar. Conidiophores in sporodochia 20–62.5 μm tall, verticillately branched and densely packed, bearing apical whors 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. 1× ν, Cosenza, Rocca Imperiale, from Citrus limon twigs, 9 June 2015, V. Guarnaccia (CBS 27067); Taranto, Massafra, from Citrus sinensis twigs, 9 June 2015, V. Guarnaccia (CBS 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 (CBS 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 feltly 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 doliform, 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(–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 spor, 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(–8.1) 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. 1× ν, Sicily, Catania, Riposto, from Citrus sinensis ‘Valencia’ twigs, 2 Mar. 2015, V. Guarnaccia (CBS 28403); Sicily, Catania, Riposto, from Citrus sinensis ‘Valencia’ twigs, 2 Mar. 2015. V. Guarnaccia (CBS 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 conidio- phores 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 Bis fusarium which, however, differs in the absence of macroconidia 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 macroconidia and chlamydospores. The shape and size of the macroconidia and the characteristics of the sporodochia also aligns F. salin- ense 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 papil-
litate 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 MB8820248; 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 scantily 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 μm wide. *Chlamydosporales* 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 branch-
ing verticillate or less common sympodially, bearing terminal mono-
phialides, 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 collarates, 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, ar-
anged 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 collarate; *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–)3.3–3.8–(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, maxi-
mum 36 °C, optimal 21–27 °C.

*Specimens examined.* Istvr, Sicily, Catania, Paternò, 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, Paternò, from *Citrus sinensis* crown, 9 Mar. 2015, V. Guarnaccia (CPC 28189).

Notes — *Fusarium siculi* is phylogenetically related to *F. glo-
bosum*, a species known from maize and wheat from Africa and Asia (Rheedet al. 1996, Aoki & Nirenberg 1999). However, the two species are morphologically clearly differentiated by the presence of clavate and globose microconidia in *F. globo-
sum*. 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 2008). 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 incuba-
tion 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. globo-
sum*). *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. fujiku-
roii* 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* dif-
ers in its host association, with none of the species mentioned above yet reported from *Citrus* (Fair & 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, res-
pectively (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 felly with scant aerial mycelium; mar-
gins 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, mem-
branous 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. *Chlamydosporales* scarcely produced in hyphae, sub-
globe 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. *Conidia-
phores* 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, 16–83.5 × 2–5 μm, with slight periclinal thickening at the tip and a short flocculent apical collarate; *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–)17.2–17.2(–33.9) × (2.4–)3.2–4.8(–6.5) μm, arranged in slims heads at the tip of phialides; and b) cylindrical to falcate, formed on the agar surface and morphologically indistinguish-
able from sporodochial conidia. *Sporodochia* cream coloured, scantily produced on the surface of carnation leaves. *Conidia-
phores* 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, fiared collarate; *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.
**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, Paternò, from Citrus sinensis crown, 9 Mar. 2015, V. Guarriacca (CBS H-23022, holotype, dried culture on SNA with carnation leaves, culture ex-type CBS 142423 = CPC 27186); Sicily, Catania, Paternò, from Citrus sinensis crown, 9 Mar. 2015, V. Guarriacca (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, Guarriacca & 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 inter-leafed red coloured hyphae; flat to slightly umbonate, fleshy to cottony. Aerial mycelium abundant, loose to densely floccose; margins regular and fimbriate; odour absent or mousy. Reverse white, pale yellow, straw, peach to pale saffron coloured at the margins regular and leaved red coloured hyphae; flat to slightly umbonate, felty to creamy in the Mediterranean basin, focusing especially on Southern Italy.

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 RB2 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 discoloration 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. sarcochrum. 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.
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. macrospera) 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 sarcocrochrum 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. sarcocrochrum, 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

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.
Aeolian Islands, while *F. citrulina* 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, Majorquin et al. 2016).

The results of our molecular analyses indicate that the two new species, *F. citrulina* 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 characterized 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 citrulina*, 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 (Našim 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 seems 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. (Schoerens 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.

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