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Neocosmospora perseae sp. nov., causing trunk cankers on avocado in Italy

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Abstract: Trunk and branch cankers are among the most important diseases compromising avocado production worldwide. A novel species, *Neocosmospora perseae* sp. nov. is described isolated from trunk lesions on *Persea americana* in the main avocado producing area of Sicily, Italy. The new species is characterised using a polyphasic approach including morphological characters and a multilocus molecular phylogenetic analysis based on partial sequences of the translation elongation factor-1α, the internal transcribed spacer regions plus the large subunit of the rDNA cistron, and the RNA polymerase II second largest subunit. Pathogenicity tests and the fulfilment of Koch's postulates confirm *N. perseae* as a novel canker pathogen of *Persea americana*.

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INTRODUCTION

Fusaria are omnipresent fungi belonging to *Nectriaceae*, commonly found in soil, water, air, dead or living plant material, food, and many other substrates, where they are acting mainly as saprobes (Lombard *et al.* 2015). Nevertheless, some species are of great importance as mycotoxin producers which can affect human and animal health. The genus *Fusarium sensu lato* has recently been segregated into several fusarium-like genera, i.e. *Albonectria*, *Bisifusarium*, *Cyanonectria, Geejayessia, Neocosmospora* and *Rectifusarium* (Gräfenhan *et al.* 2011, Lombard *et al.* 2015). These taxa are among the most impactful human, animal and plant pathogens, affecting an extensive variety of hosts (O'Donnell *et al.* 2008, 2010, Lombard *et al.* 2015).

The agri-food production sector has been undergoing major changes over the last few decades in Italy. These changes especially concern the introduction of alternative crops such as avocado. In the 20th century, avocado (*Persea americana*) was introduced to Italy and cultivated for ornamental purposes. However, due to a decline in demand for lemon, and a global increasing demand for avocado, it took the place of lemon orchards in eastern Sicily, where it represents an important fruit industry and a viable alternative crop to citrus (Guarnaccia *et al*. 2016). Unfortunately, avocado production is compromised by several pathogens causing branch cankers (Menge & Ploetz 2003, Guarnaccia *et al*. 2016). Frost or mechanical injuries such as pruning wounds may represent the initial access wounds for these canker-causing pathogens. Moreover, species belonging to *Nectriaceae* are well-known as responsible for diseases on avocado plants (Vitale *et al*. 2012, Parkinson *et al*. 2017), including several members of *Fusarium* and fusarium-like genera, such as *Albonectria* and *Neocosmospora* (Farr & Rossman 2018).

In one of the most renowned cases, damage was inflicted to avocado trees in Israel in 2009, caused by the ambrosia beetle *Euwallacea fornicatus,* and a vectored symbiotic fungal species belonging to *Neocosmospora* (formerly the *Fusarium solani* species complex, FSSC; O'Donnell *et al*. 2008, Lombard *et al*. 2015, Aoki *et al*. 2018). The affected plants showed dieback, wilt, including sugar or gum exudates, and ultimately host tree mortality (Mendel *et al*. 2012). In 2012, the beetle was recorded on several tree species in southern California and Israel, playing a major role as serious threat to avocado production (Mendel *et al*. 2012, Freeman *et al*. 2013, Kasson *et al*. 2013). "*Fusarium" euwallaceae*, found associated with the beetle is closely related to *Neocosmospora ambrosia*, another obligate symbiont occurring in Sri Lanka and India causing damage to tea plantations (Lombard *et al*. 2015). Both fungal pathogens are nested in an exclusive lineage (the Ambrosia clade) within Clade 3 of *Neocosmospora,* together with at least another eight unnamed phylogenetic species, all symbionts of the fungus-farming *Euwallacea ambrosia* beetles and one of the best examples of host-fungus co-evolution (Freeman *et al*. 2013, O'Donnell *et al*. 2016, Aoki *et al*. 2018). The fulfilment of Koch's postulates (Mendel *et al*. 2012) demonstrated the ability of "*Fusarium" euwallaceae* to cause wilt and dieback on avocado in Israel and California with no beetle-association (Freeman *et al*. 2013).

After the observation of prominent trunk cankers on avocado trees in an orchard located in the Catania province (eastern Sicily) during 2015, efforts were made to identify the causal agent.

In this study, a new fungal pathogen of avocado belonging to the genus *Neocosmospora* is proposed. The fungus is described on the basis of morphological and cultural characteristics as well as phylogenetic analyses of combined DNA sequences. Moreover, the pathogenicity on the host from which the fungus was isolated, is evaluated.

MATERIALS AND METHODS

Field sampling and isolation

During 2015, trunk canker symptoms were observed in a 14-yr-old avocado (Hass cultivar) orchard, located in the avocado plant-production region in eastern Sicily. The disease incidence (DI) was

recorded based on the number of symptomatic plants compared to the total number present. Branch canker samples were taken from 10 plants. Fragments (5 × 5 mm) of symptomatic tissues were cut from the lesion margins, 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 between sterilised filter papers, placed on 2 % potato dextrose agar (PDA; Difco, Leeuwarden, The Netherlands) amended with 100 µg/mL penicillin and 100 µg/mL streptomycin (PDA-PS) and incubated at 25 °C until characteristic fungal colonies were observed. Pure cultures were obtained by transferring germinating single conidia to fresh PDA plates with the aid of a Nikon SMZ1000 dissecting microscope.

Fungal isolates and morphological characterization

The cultural and micromorphological features of all the isolates included in this study were evaluated following the procedures of Aoki *et al*. (2003) with some modification as described previously (Sandoval-Denis *et al*. 2018). Colour notation followed the mycological colour charts of Rayner (1970). Micromorphological characteristics were examined and photographed using 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 camera. Photographs and measurements were taken using the Nikon software NIS-elements D software v. 4.50.

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for 4–7 d at room temperature, under a natural day/night photoperiod. Total genomic DNA was extracted from fresh mycelium scraped from the colony surface using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, WI, USA). Fragments of four nuclear loci including the translation elongation factor 1-alpha (*EF-1α*), the internal transcribed spacer region of the rDNA (ITS), the large subunit of the rDNA (LSU) and the RNA polymerase second largest subunit (*RPB2*) were PCR amplified as described previously (O'Donnell *et al*. 2009, 2010, Sandoval-Denis *et al.* 2018) and sequenced using the following primer pairs: EF-1/EF-2 for *EF-1α* (O'Donnell *et al*. 2008), ITS4/ITS5 for ITS (White *et al*. 1990), LR0R/LR5 for LSU (Vilgalys & Hester 1990, Vilgalys & Sun 1994) and 5f2/7cr and 7cf/11ar for *RPB2* (Liu *et al*. 1999, Sung *et al*. 2007). Sequences generated in this study were uploaded to GenBank and the European Nucleotide Archive (ENA) databases (Table 1).

Phylogenetic analyses and molecular identification

Sequence alignments were performed individually for each locus using MAFFT on the European Bioinformatics Institute (EMBL-EBI) portal (http://www.ebi.ac.uk/Tools/msa/mafft/). BLASTn searches on GenBank and pairwise sequence alignments on the *Fusarium* MLST database of the Westerdijk Fungal Biodiversity Institute (http://www.westerdijkinstitute.nl/fusarium/) were performed using *EF-1α* and *RPB2* sequences in order to preliminary identify the fungal isolates to generic level. Following this initial identification, a combination of DNA sequences from four loci (*EF-1α*, ITS, LSU and *RPB2*) was used for the final molecular identification and phylogenetic analyses (O'Donnell *et al*. 2008).

The different gene datasets were analysed independently and combined using RAxML (ML) and Bayesian methods (BI) as described previously (Sandoval-Denis *et al*. 2018). Evolutionary models for the four loci (GTR+I+G for ITS, LSU and *RPB2*; GTR+G for *EF-1*α) were calculated using MrModelTest v. 2.3 (Nylander 2004) selecting the best-fit model for each data partition according to the Akaike criterion.

Pathogenicity tests

Pathogenicity tests were performed on potted, healthy avocado seedlings (6-mo-old) with a subset of two representative isolates. Each experiment was conducted twice. For each experiment three replicates per isolate were used with 10 plants per replicate. Twigs were superficially wounded between two nodes forming a slit using a sterile blade. Inoculations were conducted by placing a 1-wk-old, 6-mm-diam colonised agar plug from each fungal isolate on a wound. Wounds were then wrapped with Parafilm® (American National Can, Chicago, IL, USA). Ten twigs were inoculated as described above with 6-mm-diam non-colonised MEA plugs as negative controls. The same number of wounds/plants were inoculated with sterile MEA plugs and served as controls. After inoculation, plants were covered with a plastic bag for 48 h and maintained at 25 ± 1 °C and 95 % relative humidity (RH) under a 12-h fluorescent light/dark regime. All plants were irrigated 2–3 times per week and examined weekly for disease symptom development. Disease incidence (DI) was recorded as described above.

RESULTS

Field sampling and fungal isolation

Symptoms referable to fusaria species were detected in an avocado orchard in the main avocado-producing region of Eastern Sicily, Italy (GPS coordinates: 37.687247, 15.175479). The disease was observed on established plants (14-yr-old) in an open field. Disease incidence was ascertained at 10 %. The symptoms observed on avocado plants consisted of trunk cankers. Bark appeared cracked, darkly discoloured and/or slightly sunken. Occasionally, a sugar exudate was present on the surface. Cankers were internally reddish brown in colour and variable in shape. Transverse cuts revealed a characteristic wedge-shaped canker extending deep into the xylem (Fig. 1). Only fusarium-like isolates growing in pure culture were obtained from the symptomatic avocado trees, from which five monosporic strains were retained.

Phylogenetic analyses and species identification

Pairwise sequence alignments on the *Fusarium* MLST database and GenBank BLASTn searches demonstrated that the five fungal isolates belonged to the genus *Neocosmospora*.

Subsequently, more inclusive multilocus phylogenetic analyses were performed based on *EF-1α*, ITS, LSU, and *RPB2* sequences. A first analysis spanned the currently known phylogenetic diversity of the genus *Neocosmospora*, and included sequences from a total of 365 strains, based on the alignments published by O'Donnell *et al*. (2008). According to this analysis, the five strains from avocado formed an exclusive new linage in the genus *Neocosmospora* (data not shown, alignments, trees and statistics all available at TreeBASE). A second analysis was run based on a selected subset of DNA data representing most of the species of *Neocosmospora* currently assigned with Latin binomials, plus several yet unnamed phylogenetic clades phylogenetically related to the new lineage (Fig. 2). This final analysis included sequences from 80 strains, representing 48 taxa and a total of 2 917 character sites, of which 2 203 were conserved (*EF-1*α 212, ITS 372, LSU 441 and *RPB2* 1178), and 555 were variable and phylogenetically informative (*EF-1*α 69, ITS 101, LSU 35 and *RPB2* 350). The BI analyses identified a total of 774 unique sites (*EF-1*α 134, ITS 179, LSU 43 and *RPB2* 418) and sampled a total 315 000 trees, from which 236 250 were used to calculate the 50 % consensus tree and posterior probability (PP) values, after discarding 25 % of trees as burn-in fraction. Results from ML and BI methods showed that the

Table 1. Collection details and GenBank accession numbers of isolates included in this study.

Species	Clade number[a]	Strain number[b]	Country and substrate	GenBank/EBI accession number[c]			
				EF-1α	ITS	LSU	*RPB2*
Fusarium brasiliense		NRRL 22743	Brazil, *Glycine max*	EF408407	FJ919502	FJ919502	EU329525
Fusarium cuneirostrum		NRRL 31104	Japan, *Phaseolus vulgaris*	EF408413	FJ919509	FJ919509	EU329558
Fusarium ensiforme	FSSC 15	NRRL 28009	USA, human eye	DQ246869	DQ094351	DQ236393	EF470136
	FSSC 15	NRRL 32792	Japan, human	DQ247101	DQ094561	DQ236603	EU329621
Fusarium euwallaceae		CBS 135855 = NRRL 54723	Israel, Beetle from Avocado Tree	JQ038008	JQ038015	JQ038015	JQ038029
		CBS 135856 = NRRL 54724	Israel, Beetle from Avocado Tree	JQ038009	JQ038016	JQ038016	JQ038030
Fusarium keratoplasticum	FSSC 2	CBS 490.63^{T} = NRRL 22661	Japan, human eye	DQ246846	DQ094331	DQ236373	EU329524
	FSSC 2	NRRL 28561	USA, human	DQ246902	DQ094375	DQ236417	EU329552
Fusarium lichenicola	FSSC 16	NRRL 34123	India, human eye	DQ247192	DQ094645	DQ236687	EU329635
Fusarium paranaense		CML 1830^{T}	Brazil, Soybean root	KF597797			KF680011
		CML 1833	Brazil, Soybean root	KF597798			KF680012
Fusarium petroliphilum	FSSC 1	NRRL 22141	New Zealand, cucurbit	AF178329	DQ094307	DQ236349	EU329491
	FSSC 1	NRRL 43812	USA, contact lens solution	EF453054	EF453205	EF453205	EF470093
Fusarium solani f. sp. *pisi*	FSSC 11	NRRL 22820	USA, *Glycine max*	AF178355	DQ094310	DQ236352	EU329532
	FSSC 11	NRRL 45880	USA, Lab cross T10 (pea) and T219 (soil)	FJ240352	EU329689	EU329689	EU329640
Fusarium solani f. sp. *batatas*	FSSC 23	NRRL 22400	USA, *Ipomoea batatas*	AF178343	AF178407	DQ236345	EU329509
Fusarium solani f. sp. *xanthoxyli*	FSSC 22	NRRL 22163	Japan, *Xanthoxylum* sp.	AF178336	AF178401	AF178370	FJ240380
Fusarium striatum	FSSC 21	NRRL 22101	Panama, cotton cloth	AF178333	AF178398	AF178367	EU329490
Neocosmospora ambrosia	FSSC 19	NRRL 20438	India, *Camellia sinensis*	AF178332	AF178397	DQ236357	JX171584
	FSSC 19	NRRL 22346	India, *Camellia sinensis*	FJ240350	EU329669	EU329669	EU329503
Neocosmospora croci		CBS 142423^{T} = CPC 27186	Italy, *Citrus sinensis*	LT746216	LT746264	LT746264	LT746329
Neocosmospora croci		CPC 27187	Italy, *Citrus sinensis*	LT746217	LT746265	LT746265	LT746330
Neocosmospora cyanescens	FSSC 27	CBS 518.82^{T} = NRRL 37625	Netherlands, human foot	FJ240353	EU329684	EU329684	EU329637
Neocosmospora falciformis	FSSC 3+4	NRRL 32757	USA, sand	DQ247075	DQ094536	DQ236578	EU329614
	FSSC 3+4	NRRL 32828	USA, human	DQ247135	DQ094594	DQ236636	EU329626
Neocosmospora illudens		NRRL 22090	New Zealand, *Beilschmiedia tawa*	AF178326	AF178393	AF178362	JX171601
Neocosmospora macrospora		CBS 142424^{T} = CPC 28191	Italy, *Citrus sinensis*	LT746218	LT746266	LT746281	LT746331
		CPC 28192	Italy, *Citrus sinensis*	LT746219	LT746267	LT746282	LT746332

Table 1. (Continued).

Species	Clade number[a]	Strain number[b]	Country and substrate	GenBank/EBI accession number[c]			
				EF-1α	ITS	LSU	*RPB2*
		CPC 28193	Italy, *Citrus sinensis*	LT746220	LT746268	LT746283	LT746333
Neocosmospora perseae		CBS 144142T[#] = CPC 26829	Italy, *Persea americana*	**LT991902**	**LT991940**	**LT991947**	**LT991909**
		CBS 144143[#] = CPC 26830	Italy, *Persea americana*	**LT991903**	**LT991941**	**LT991948**	**LT991910**
		CBS 144144 = CPC 26831	Italy, *Persea americana*	**LT991904**	**LT991942**	**LT991949**	**LT991911**
		CBS 144145 = CPC 26832	Italy, *Persea americana*	**LT991905**	**LT991943**	**LT991950**	**LT991912**
		CBS 144146 = CPC 26833	Italy, *Persea americana*	**LT991906**	**LT991944**	**LT991951**	**LT991913**
Neocosmospora plagianthi		NRRL 22632	New Zealand, *Hoheria glabrata*	AF178354	AF178417	AF178386	JX171614
Neocosmospora pseudensiformis	FSSC 33	NRRL 22354	French Guiana, bark	AF178338	AF178402	DQ236358	EU329504
Neocosmospora solani	FSSC 5	CBS 140079[ET] = NRRL 66304	Slovenia, *Solanum tuberosum*	KT313611	KT313633	KT313633	KT313623
	FSSC 5	CPC 27736	Italy, *Ficus carica*	**LT991907**	**LT991945**	**LT991952**	**LT991914**
	FSSC 5	CPC 27737	Italy, *Ficus carica*	**LT991908**	**LT991946**	**LT991953**	**LT991915**
	FSSC 5	NRRL 32741	USA, human eye	DQ247061	DQ094522	DQ236564	EU329608
Neocosmospora sp.	FSSC 6	CBS 143194 = NRRL 22782	Spain, human corneal ulcer	DQ246850	EU329670	EU329670	EU329528
	FSSC 6	CBS 143210 = NRRL 32785	USA, human toenail cancer	DQ247094	*	FJ240371	EU329618
	FSSC 7	CBS 130181 = NRRL 43502	USA, human eye	DQ790488	DQ790532	DQ790532	DQ790576
	FSSC 7	CBS 143209 = NRRL 32770	USA, human eye	DQ247083	DQ094544	DQ236586	EU329615
	FSSC 9	CBS 143208 = NRRL 32755	USA, turtle head lesion	DQ247073	DQ094534	DQ236576	EU329613
	FSSC 10	NRRL 22098	USA, cucurbit	DQ247073	DQ094534	DQ236576	EU329613
	FSSC 10	NRRL 22153	Panama, cucurbit	AF178346	DQ094302	DQ236344	EU329492
	FSSC 12	CBS 143212 = NRRL 32821	USA, turtle eggs	DQ247128	DQ094587	DQ236629	EU329625
	FSSC 12	NRRL 22642	Japan, *Penaceous japonicus*	DQ246844	DQ094329	DQ236371	EU329522
	FSSC 13	NRRL 22161	Japan, *Robinia pseudoacacia*	AF178330	DQ094311	DQ236353	EU329494
	FSSC 13	NRRL 22586	Japan, *Robinia pseudoacacia*	AF178353	AF178416	AF178385	EU329516
	FSSC 14	NRRL 32705	USA, human skin	DQ247025	DQ094488	DQ236530	EU329594
	FSSC 14	NRRL 32736	USA, human eye	DQ247056	DQ094517	DQ236559	EU329605
	FSSC 17	NRRL 22157	Japan, *Morus alba*	AF178359	DQ094306	DQ236348	EU329493
	FSSC 17	NRRL 22230	Japan, *Morus alba*	AF178358	DQ094305	DQ236347	EU329499
	FSSC 18	NRRL 31158	USA, human	DQ246916	DQ094389	DQ236431	EU329559
	FSSC 18	NRRL 32301	USA, human eye	DQ246929	EU329677	EU329677	EU329567

Table 1. (Continued).

Species	Clade number[a]	Strain number[b]	Country and substrate	GenBank/EBI accession number[c]			
				EF-1α	ITS	LSU	*RPB2*
	FSSC 20	CBS 143214 = NRRL 32858	USA, human wound	DQ247163	DQ094617	DQ236659	EU329630
	FSSC 20	NRRL 28001	USA, human skin	DQ246866	DQ094348	DQ236390	EF470129
	FSSC 24	CBS 117481 = NRRL 22389	USA, *Liriodendron tulipifera*	AF178340	AF178404	DQ236356	EU329506
	FSSC 25	CBS 130328 = NRRL 31169	USA, human oral wound	DQ246923	DQ094396	DQ236438	KR673999
	FSSC 26	NRRL 28541	USA, human synovial fluid	DQ246882	EU329674	EU329674	EU329542
	FSSC 28	CBS 109028 = NRRL 32437	Switzerland, human subcutaneous nodule	DQ246979	DQ094446	DQ236488	EU329581
	FSSC 29	NRRL 28008	USA, human	DQ246868	DQ094350	DQ236392	EF470135
	FSSC 30	NRRL 22579	Indonesia, tree bark	AF178352	AF178415	AF178384	EU329515
	FSSC 31	NRRL 22570	Brazil, *Piper nigrum*	AF178360	AF178422	AF178391	EU329513
	FSSC 32	NRRL 22178	Venezuela, dicot tree	AF178334	AF178399	AF178368	EU329498
	FSSC 34	NRRL 46703	Spain, nematode	HM347126	EU329712	EU329712	EU329661
	FSSC 35	NRRL 46707	Brazil, human	HM347127	EU329716	EU329716	EU329665
	FSSC 37	NRRL 25137	New Guinea, diseased cocoa pods	JF740757	JF740899	JF740899	JF741084
	FSSC 37	NRRL 25138	New Guinea, diseased cocoa pods	DQ247537	JF740900	JF740900	JF741085
	FSSC 38	NRRL 52781	Benin, *Hypothenemus hampei* adult	JF740849	*	*	JF741175
	FSSC 38	NRRL 52782	Benin, *Hypothenemus hampei* adult	*	JF740850	JF740850	JF741176
	FSSC 38	NRRL 52783	Benin, *Hypothenemus hampei* adult	JF740851	*	*	JF741177
	FSSC 39	FRC S-2432	USA, building	JN235756	JN235326	JN235326	JN235941
	FSSC 43	NRRL 54992	USA, Zebra shark multiple tissues	KC808213	KC808255		KC808354
	FSSC 43	NRRL 54993	USA, Zebra shark multiple tissues	KC808214	KC808256		KC808355
	FSSC 45	NRRL 62797	USA, *Xylosandrus compactus*	KF906129	KF906130	KF906130	KF906132
Neocosmospora vasinfecta	FSSC 8	CBS 130182 = NRRL 43467	USA, human eye	EF452940	EF453092	EF453092	EF469979
	FSSC 8	NRRL 22436	South Africa, soil	AF178348	AF178412	DQ236359	JX171610

[a] Clade nomenclature follows O'Donnell *et al.* (2008, 2016).

[b] CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CPC: Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute; CML: Coleção Micológica de Lavras, Universidade Federal de Lavras, Minas Gerais, Brazil; F: College of Forestry, Northwest A&F University, Taicheng Road, Yangling, Shaanxi China; FRC: Fusarium Research Center, University Park, PA, USA; NRRL: Agricultural Research Service, Peoria, IL, USA. Ex- and ex-epitype strains are indicated with [T], and [ET], respectively. [#] Strains used in the pathogenicity tests.

[c] *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; *RPB2*: RNA polymerase II largest subunit. * Sequences not publicly available, provided as DNA datasets by Kerry O'Donnell.

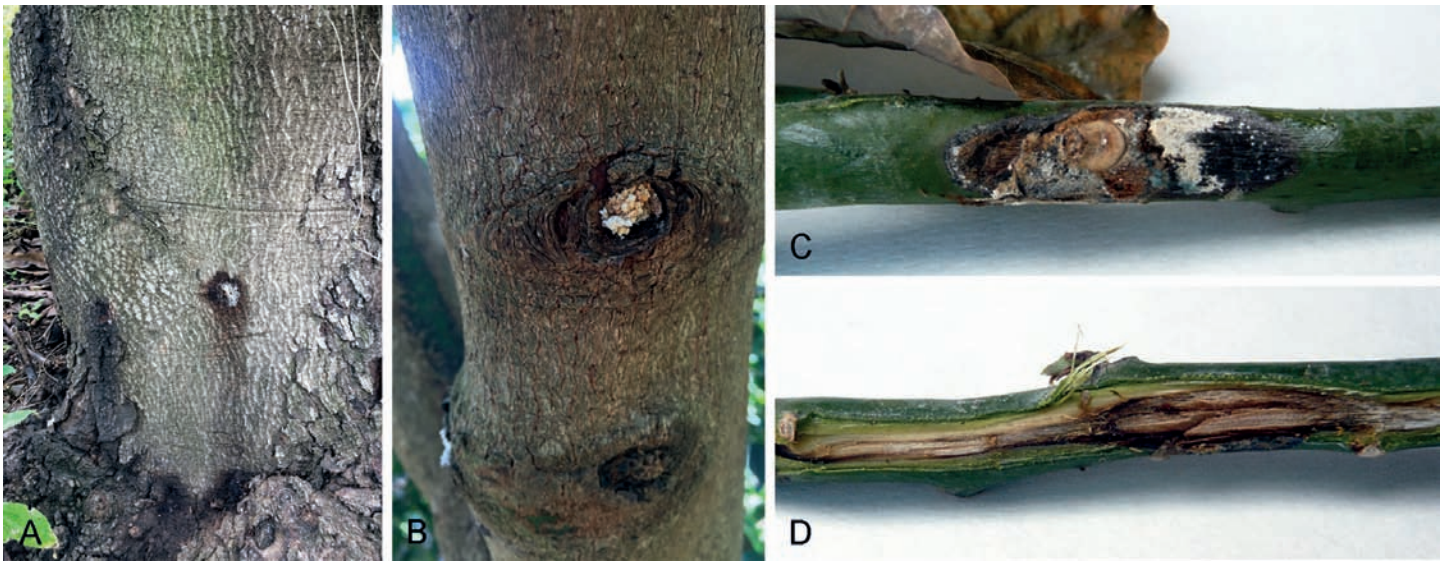


Fig. 1. Natural and artificial symptoms referable to *Neocosmospora perseae.* **A, B**. Sugar exudation from avocado trunk cankers. **C, D**. External and internal canker caused by *N. perseae* inoculation.

clade encompassing the five strains from cankers on *P. americana* (CPC 29829 to 26833) correspond to a new linage in *Neocosmospora* (BS 96 / PP 1), closely related to the unnamed phylogenetic species FSSC 37 and 38, and clearly unrelated with the common *Persea* pathogens in the Ambrosia clade of *Neocosmospora* (clade nomenclature according to O'Donnell *et al*. 2008, 2016). The new lineage is proposed here as the new species *Neocosmospora perseae*.

Pathogenicity tests

Two *Neocosmospora* isolates tested were pathogenic to the *Persea americana* seedlings inoculated, and produced symptoms similar to those observed on diseased plants in the avocado orchard. Canker and internal discolouration symptoms were observed corresponding to inoculation points on avocado plants. Initial symptoms were observed after 1 mo. High DI (100 %) was observed after 3 mo with serious symptoms leading to plant death (Fig. 1). Similar results were obtained in both tests performed.

The pathogen was re-isolated from the artificially inoculated plants and identified as previously described, completing Koch's postulates. No symptoms were observed on control plants.

TAXONOMY

Neocosmospora perseae Sandoval-Denis & Guarnaccia, ***sp. nov.***
MycoBank MB824587. Fig. 3.

Etymology: Named after the host genus *Persea*.

Sporulation abundant from conidiophores formed directly on the substrate and aerial mycelium, and from sporodochia. *Conidiophores* straight to slightly flexuous, up to 350 µm tall, solitary and simple or branched one to several times irregularly and laterally, verticillately or sympodially, each branch bearing a single terminal monophialide; *phialides* subulate to subcylindrical, smooth- and thin-walled, (40.5–)45–66.5(–90.5) µm long, (2–)2.5–3(–3.5) µm wide at the base, tapering to (1–)1.5–2(–2.5) µm wide at the apex, often with conspicuous periclinal thickening and a minute, discrete collarette; *conidia* formed on aerial conidiophores, hyaline, obovoid, ellipsoidal, short clavate to cylindrical, symmetrical or gently bent dorsoventrally, smooth- and thin-walled, 0(–1)-septate, (4.5–)6–10.5(–13.5) × (1.5–)2.5–4(–6) µm, clustering in false heads at the tip of monophialides. *Sporodochia* at first white to cream-coloured, becoming pale luteous, green to dark blue-green when mature, formed abundantly on the surface of carnation leaves and lately on and under the agar surface. *Conidiophores* in sporodochia 26–54 µm tall, densely packed in a cushion-like structure, irregularly or verticillately branched, with terminal branches bearing verticills of 1–3 monophialides; *sporodochial phialides* doliiform, subulate to subcylindrical, (13.5–)14.5–18.5(–20.5) × 2.5–3.5(–4.5) µm, smooth- and thin-walled, with periclinal thickening and an inconspicuous apical collarette. *Sporodochial conidia* falcate, wedge-shaped, tapering toward the basal part, robust; smaller sized conidia often conspicuously curved; large sized conidia somewhat straight on its ventral line with a moderate dorsal curvature; apical cell blunt, more or less equally sized than the adjacent cell; basal cell distinctly notched, (3–)4–5(–6)-septate, hyaline, thick- and smooth-walled. Three-septate conidia: 30.5–32.5 × 5–5.5 µm; four-septate conidia: (39–)40.5–47(–49) × 5–5.5(–6.5) µm; five-septate conidia: (39.5–)45.5–51.5(–56) × (4.5–)5.5–6(–6.5) µm; six-septate conidia: 49–53.5(–55) × (5–)6–7 µm; overall (30.5–)43.5–52(–55.5) × (4.5–)5.5–6(–7) µm. *Chlamydospores* abundant and rapidly formed on agar media (approx. 7 d), hyaline to pale brown, spherical to subspherical (4.5–)6–8(–9) µm diam, solitary or in chains, terminal, intercalary or borne on short lateral pegs, smooth- and thick-walled.

Cardinal temperatures for growth: Minimum 9 °C, maximum 36 °C, optimum 27–30 °C.

Culture characteristics: *Colonies* on PDA showing radial growth rates of 4.4–7.2 mm/d at 27 °C and 4.1–6.8 mm/d at 30 °C in the dark, reaching a diameter of 72–74 mm after 7 d at 24 °C. Colony surface straw to pale luteous, flat, felty to floccose, aerial mycelium and sporulation abundant, white, becoming pale luteous to sulphur yellow; colony margins regular and filiform. Reverse amber to sulphur yellow, becoming bright red to scarlet with the production of abundant diffusible pigment. Colonies on OA showing a diameter of 62–66 mm after 7 d at 24 °C. Colony colour white with sienna to umber patches, flat to slightly umbonate and radiate, felty to floccose, aerial mycelium and sporulation abundant; margins filiform and slightly undulate. Reverse pale luteous with slight production of a scarlet to sienna coloured diffusible pigment.

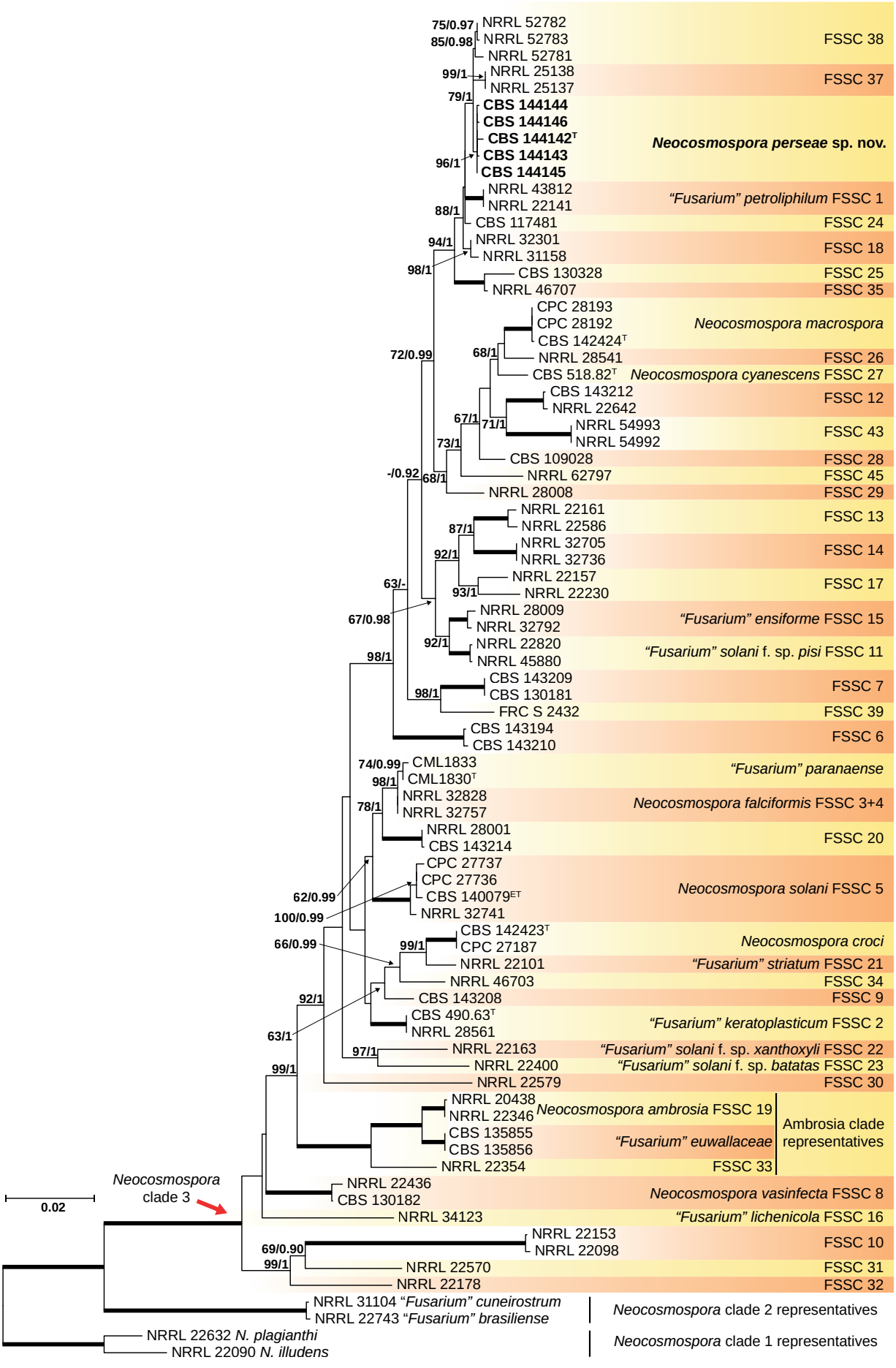
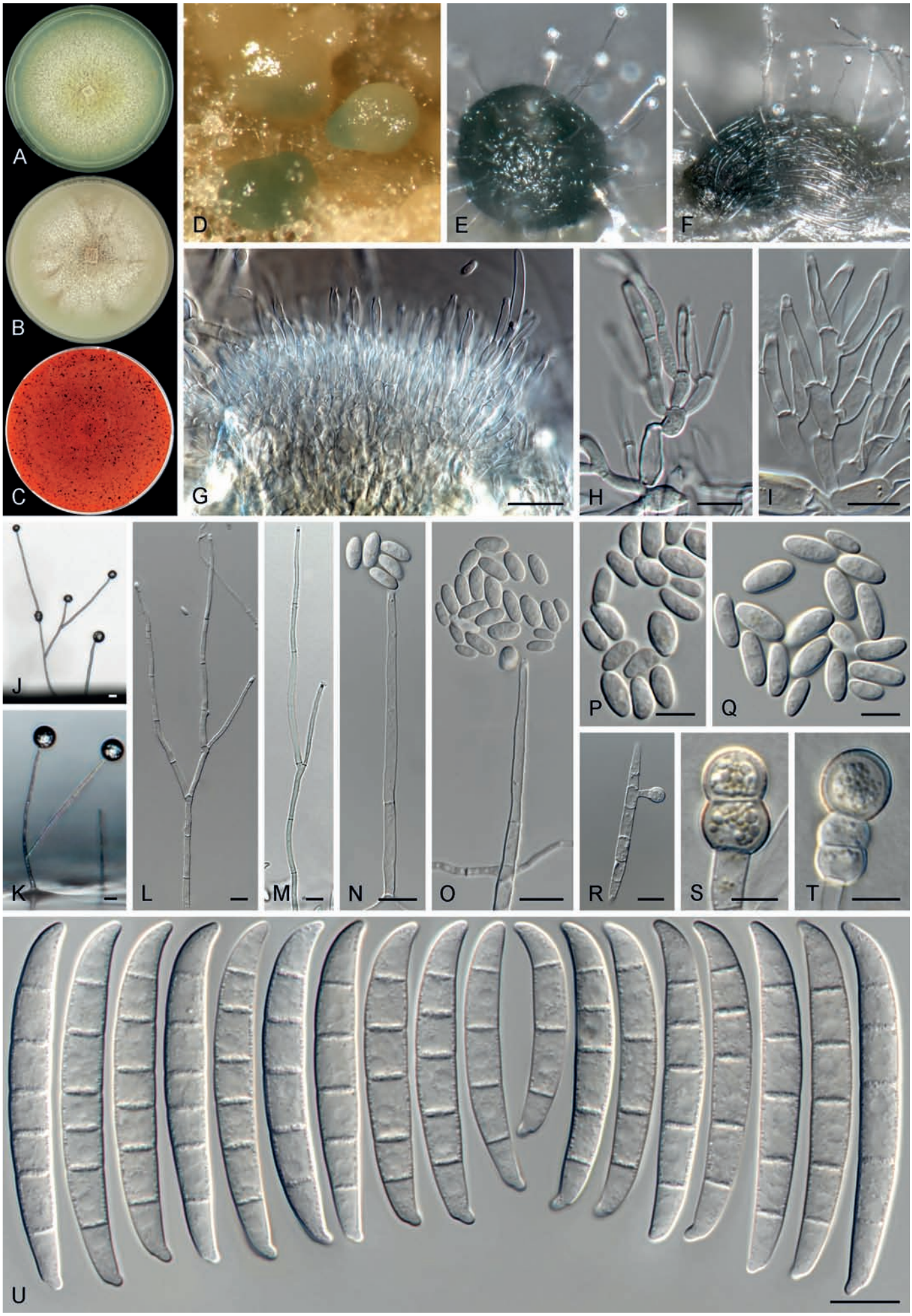


Fig. 2. Maximum-likelihood (ML) phylogram of the genus *Neocosmospora* obtained from combined *EF-1α*, ITS, LSU and *RPB2* sequences. Branch lengths are proportional to distance. Numbers on the nodes are ML bootstrap values (BS) above 55 %; and Bayesian posterior probability values (PP) above 0.95. Full supported branches (BS = 100 and PP = 1) and isolates obtained from *Persea americana* are indicated in bold. Ex-type and ex-epitype strains are indicated with ^{T}, and ^{ET}, respectively.



Typification: **Italy**, Catania, San Leonardello, from trunk canker lesions on *Persea americana*, 25 Mar. 2015, *G. Polizzi* (holotype CBS H-23433, culture ex-type CBS 144142 = CPC 26829).

Additional isolates examined: **Italy**, Catania, San Leonardello, from trunk canker lesions on *Persea americana*, 25 Mar. 2015, *G. Polizzi* (CBS 144143 = CPC 26830; CBS 144144 = CPC 26831; CBS 144145 = CPC 26832; CBS 144146 = CPC 26833).

DISCUSSION

In this study, five *Neocosmospora* isolates were recovered from *Persea americana* trees showing trunk canker symptoms in Sicily (Southern Italy) during 2015, and identified based on single and multilocus phylogenetic analyses of four loci (*EF-1α*, ITS, LSU and *RPB2*), as well as morphological characters. These analyses revealed that the five isolates belonged to a novel species, described here *N. perseae*.

The robust four-loci based analysis allowed to distinguish *N. perseae* from "*Fusarium*" *euwallaceae* and *N. ambrosia*, already known as canker-causing species associated with symbiotic *Euwallacea* beetles. In spite of the recent detection of similar cankers caused by other fungal species in the same area (Guarnaccia *et al*. 2016), *N. perseae* was found as the only fungus associated with the disease. Because cankers developed in the absence of *Euwallacea* beetles, the fungus is clearly able to cause wood cankers independently. Furthermore, pathogenicity tests confirmed that *N. perseae* causes a high disease incidence on *Persea americana*, thereby fulfilling Koch's postulates.

Neocosmospora perseae was clearly not related phylogenetically or morphologically with the most significant *Neocosmospora* canker pathogens affecting *Persea*, known to belong to the Ambrosia clade (Aoki *et al*. 2018). Moreover, while the new species exhibits the typical hyaline, falcate and multiseptate macroconidia and short clavate to cylindrical microconidia commonly attributed to this genus, the *Persea* pathogens in the Ambrosia clade of *Neocosmospora* are characterised by their irregularly clavate, somewhat swollen conidia, a putative evolutionary adaptation to its host (Freeman *et al.* 2013). Additionally, all currently known members of the Ambrosia clade exhibit a symbiotic lifestyle, associated with species of the shot hole borer beetle genus *Euwallacea* (Coleoptera, Xyleborini) (Mendel *et al.* 2012, Freeman *et al.* 2013, Kasson *et al.* 2013). In contrast, *N. perseae* showed no evidence of association with any vector, as demonstrated by the absence of wood galleries or any other sign of insect infestation in the trees. Its transmission is therefore more likely to respond to soil contamination and plant-associated reservoirs. Furthermore, the new species proved to be genetically closely related to two undescribed lineages (FSSC 37 and FSSC 38), yet, being phylogenetically and ecologically distinct. So far, phylogenetic species FSSC 37 is only known from diseased cocoa pods in New Guinea. However, FSSC 38, known from Benin & Uganda, has been isolated from the coffee borer beetle *Hypothenemus hampei* (Coleoptera, Scotylini) (O'Donnell *et al.* 2012), a relative to *Euwallacea* beetles. Similarly, the unrelated phylogenetic species FSSC 45 is known to inhabit the abdomen and external surfaces of *Xylosandrus compactus* (Coleoptera, Xyleborini) and its galleries (Bateman *et al*. 2016), which could suggest either that a similar insect-fungus mutualism or opportunism could also exist in other *Neocosmospora* lineages. However, no clear indication exists of FSSC 38 or FSSC 45 having either a pathogenic or symbiotic lifestyle with their insect hosts.

This study has revealed and characterised a new pathogenic fungal species, *N. perseae,* associated with trunk cankers on avocado in Italy, and includes information on its pathogenicity. As no epidemiological data are yet available it is not possible to suggest any control strategies to avoid *N. perseae* infections. Previous studies in the same geographical area have revealed a diversity of soil-borne fungal species (Polizzi *et al*. 2012, Vitale *et al*. 2012), including species pathogenic to avocado trees (Dann *et al*. 2012). Thus, these and other diseases might threaten avocado production, and could become a major limiting factor for future production.

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Fig. 3. *Neocosmospora perseae* (from ex-type CBS 144142). **A, B.** Colonies on PDA and OA, respectively, after 7 d at 24 °C in the dark. **C.** Colony on PDA after 20 d at 24 °C under continuous white light. **D–F.** Sporodochia formed on the surface of carnation leaves. **G–I.** Sporodochial conidiophores. **J–O.** Aerial conidiophores and phialides. **P, Q.** Aerial conidia (microconidia). **R–T.** Chlamydospores. **U.** Sporodochial conidia (macroconidia). Scale bars: P, Q, S, T = 5 µm, G = 20 µm, all others = 10 µm.

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