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

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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, Bisfusarium, 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 & Rossmann 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 plantation 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 S12/Tcr 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/maa/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 preliminarily 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+H+G for ITS, LSU and RPB2; GTR+G for EF-1α) 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 lineage 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-1α 212, ITS 372, LSU 441 and RPB2 1178), and 555 were variable and phylogenetically informative (EF-1α 69, ITS 101, LSU 35 and RPB2 350). The BI analyses identified a total of 774 unique sites (EF-1α 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
| Species                  | Clade numbera | Strain numberb | Country and substrate                          | GenBank/EBI accession numberc |
|-------------------------|---------------|----------------|-----------------------------------------------|------------------------------|
| **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 |
| **Fusarium euwallaceae**|               | CBS 135855 = NRRL 54723 | Israel, Beetle from Avocado Tree           | JQ038008 JQ038015 JQ038015 JQ038029 |
| **Fusarium keratoplasticum**| FSSC 2      | CBS 490.63 = NRRL 22661 | Japan, human eye                           | DQ246846 DQ094331 DQ236373 EU329524 |
| **Fusarium lichenicola**| FSSC 16       | NRRL 34123     | India, human eye                              | DQ247192 DQ094645 DQ236687 EU329635 |
| **Fusarium paraenaense**|               | CML 1830       | Brazil, Soybean root                          | KF597797 KF680011 KF680012 |
| **Fusarium petroliphilum**| FSSC 1       | NRRL 22141     | New Zealand, cucurbit                         | AF178329 DQ094307 DQ236349 EU329491 |
| **Fusarium solani f. sp. pisi**| FSSC 11     | NRRL 22820     | USA, Glycine max                              | AF178355 DQ094310 DQ236352 EU329532 |
| **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 FI240380 |
| **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 |
| **Neocosmospora croci**  | FSSC 19       | NRRL 22346     | India, Camellia sinensis                     | AF178332 AF178397 DQ236357 JX171584 |
| **Neocosmospora cyanescens**| FSSC 27      | CBS 518.82 = NRRL 37625 | Netherlands, human foot                | FI240353 EU329684 EU329684 EU329637 |
| **Neocosmospora falciformis**| FSSC 3+4    | NRRL 32757     | USA, sand                                    | DQ247075 DQ094536 DQ236578 EU329614 |
| **Neocosmospora illudens**| FSSC 3+4     | NRRL 32828     | USA, human                                   | DQ247135 DQ094594 DQ236636 EU329626 |
| **Neocosmospora macrospora**| FSSC 3+4    | NRRL 22090     | New Zealand, Beilschmiedia tawa             | AF178326 AF178393 AF178362 JX171601 |
| **Neocosmospora peresae**| FSSC 3+4     | CBS 142424 = CPC 28191 | Italy, Citrus sinensis           | LT746219 LT746264 LT746264 LT746329 |
| **Neocosmospora solani**  | FSSC 21       | CPC 27187      | Italy, Citrus sinensis                        | LT746217 LT746265 LT746265 LT746330 |
| **Neocosmospora solani**  | FSSC 27       | CPC 27187      | Italy, Citrus sinensis                        | LT746217 LT746265 LT746265 LT746330 |

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

| Species | Strain number | GenBank/EBI accession number | Country and substrate | Clade number | GenBank/EBI accession number |
|---------|---------------|-----------------------------|----------------------|--------------|-----------------------------|
| Neocosmospora perseae | CPC 28193 | LT741068 LT741069 LT741070 LT741071 | Italy, Citrus sinensis | FSCC 193 | LT741068 LT741069 LT741070 LT741071 |
| Neocosmospora solani | CBS 144146 = CPC 26831 | LT791903 LT791904 LT791905 LT791906 | Italy, Persea americana | FSCC 5 = FSCC 6 = FSCC 7 | LT791903 LT791904 LT791905 LT791906 |
| Neocosmospora plagiata | CBS 144145 = CPC 26832 | LT791907 LT791908 LT791909 LT791910 | Italy, Persea americana | FSCC 9 = FSCC 10 | LT791907 LT791908 LT791909 LT791910 |
| Neocosmospora pseudensiformis | CBS 144146 = CPC 26833 | LT791911 LT791912 LT791913 LT791914 | Italy, Persea americana | FSCC 11 = FSCC 12 | LT791911 LT791912 LT791913 LT791914 |
| Neocosmospora pseudensiformis | NRRL 22632 | New Zealand, Hoheria glabrata | New Zealand, Hoheria glabrata | FSCC 13 | New Zealand, Hoheria glabrata |
| Neocosmospora solani | FSSC 5 | Italy, Ficus carica | Italy, Ficus carica | FSCC 14 | Italy, Ficus carica |
| Neocosmospora solani | FSSC 6 | USA, human corneal ulcer | USA, human corneal ulcer | FSCC 15 | USA, human corneal ulcer |
| Neocosmospora solani | FSSC 7 | Spain, human corneal ulcer | Spain, human corneal ulcer | FSCC 16 | Spain, human corneal ulcer |
| Neocosmospora solani | FSSC 8 | USA, human corneal ulcer | USA, human corneal ulcer | FSCC 17 | USA, human corneal ulcer |
| Neocosmospora solani | FSSC 9 | USA, human tumor | USA, human tumor | FSCC 18 | USA, human tumor |
| Neocosmospora solani | FSSC 10 | USA, human tumor | USA, human tumor | FSCC 19 | USA, human tumor |
| Neocosmospora solani | FSSC 11 | USA, human tumor | USA, human tumor | FSCC 20 | USA, human tumor |
| Neocosmospora solani | FSSC 12 | USA, human tumor | USA, human tumor | FSCC 21 | USA, human tumor |
| Neocosmospora solani | FSSC 13 | USA, human tumor | USA, human tumor | FSCC 22 | USA, human tumor |
| Neocosmospora solani | FSSC 14 | USA, human tumor | USA, human tumor | FSCC 23 | USA, human tumor |
| Neocosmospora solani | FSSC 15 | USA, human tumor | USA, human tumor | FSCC 24 | USA, human tumor |
| Neocosmospora solani | FSSC 16 | USA, human tumor | USA, human tumor | FSCC 25 | USA, human tumor |
| Neocosmospora solani | FSSC 17 | USA, human tumor | USA, human tumor | FSCC 26 | USA, human tumor |
| Neocosmospora solani | FSSC 18 | USA, human tumor | USA, human tumor | FSCC 27 | USA, human tumor |
Table 1. (Continued).

| Species                  | Clade number | Strain number | Country and substrate                  | GenBank/EBI accession number |
|--------------------------|--------------|---------------|----------------------------------------|------------------------------|
| Neocosmospora perseae   | FSSC 20      | CBS: 142134 = NRRRL 22358 | USA, human wound                      | DQ246163                     |
| Neocosmospora perseae   | FSSC 20      | NRRL 28001    | USA, human skin                        | DQ246866                     |
| Neocosmospora perseae   | FSSC 24      | CBS: 117481 = NRRRL 22388 | USA, Uridendron tulipifera            | DQ246866                     |
| Neocosmospora perseae   | FSSC 25      | CBS: 130328 = NRRRL 31169 | USA, human oral wound                  | DQ246936                     |
| Neocosmospora perseae   | FSSC 26      | CBS: 285141   | USA, human synovial fluid              | DQ246882                     |
| Neocosmospora perseae   | FSSC 27      | CBS: 285651   | Switzerland, human subumbilical nodule | DQ246898                     |
| Neocosmospora perseae   | FSSC 30      | CBS: 108028 = NRRRL 32437 | USA, human                              | DQ246968                     |
| Neocosmospora perseae   | FSSC 29      | NRRL 28018    | USA, human                             | DQ246968                     |
| Neocosmospora perseae   | FSSC 31      | NRRRL 22579   | Indonesia, tree bark                   | DQ246896                     |
| Neocosmospora perseae   | FSSC 32      | NRRRL 22977   | Brazil, P. nigricans                   | DQ246896                     |
| Neocosmospora perseae   | FSSC 33      | NRRRL 22278   | Switzerland, human synovial fluid      | DQ246896                     |
| Neocosmospora perseae   | FSSC 34      | CBS: 130182 = NRRRL 43467 | USA, human eye                         | DQ246896                     |
| Neocosmospora vasinfecta| FSSC 8       | NRRL 22436    | South Africa, soil                     | DQ246944                     |

Clade nomenclature follows O’Donnell et al. (2008, 2016). *CBS: Westerndijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CPC: Culture collection of P.W. Crous, housed at Westerndijk Fungal Biodiversity Institute; CML: Colección Micológica de Lavras, Unversidade Federal de Lavras, Minas Gerais, Brazil; F: College of Forestry, Northwest A&F University, 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. 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.
clade encompassing the five strains from cankers on *P. americana* (CPC 29829 to 26833) correspond to a new lineage 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 those observed on diseased plants in the avocado orchard. Canker and seedlings inoculated, and produced symptoms similar to *Neocosmospora perseae* inoculation.

**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 verticils 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- to five-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: 45–53.5(–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.

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![Image](image_url)
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 T100 and PP = 1) and isolates obtained from *Persea americana* are indicated in bold. Ex-type and ex-epitype strains are indicated with T100 and PP = 1) and isolates obtained from *Persea americana* are indicated in bold. Ex-type and ex-epitype strains are indicated with T.
**Neocosmospora perseae** sp. nov. in Italy

**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 multisepitate 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 cacao pods in New Guinea. However, FSSC 38, known from Benin & Uganda, has been isolated from the coffee borer beetle *Hypothenemus hampei* (Coleoptera, Scytolophini) (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, Xylenebrini) 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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