Title

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Journal

IMA fungus, 9(2)

ISSN

2210-6340

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Publication Date

2018-07-01

DOI

10.5598/imafungus.2018.09.02.07

Peer reviewed

doi:10.5598/imafungus.2018.09.02.07 IMA FUNGUS · 9(2): 333–370 (2018)

ARTICLE

Molecular phylogeny of *Cytospora* species associated with canker diseases of fruit and nut crops in California, with the descriptions of ten new species and one new combination

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Abstract: *Cytospora* species are destructive canker and dieback pathogens of woody hosts in natural and agroecosystems around the world. In this genus, molecular identification has been limited due to the paucity of multi-locus sequence typing studies and the lack of sequence data from type specimens in public repositories, stalling robust phylogenetic reconstructions. In most cases a morphological species concept could not be applied due to the plasticity of characters and significant overlap of morphological features such as spore dimensions and fruiting body characters. In this study, we employed a molecular phylogenetic framework with the inclusion of four nuclear loci (ITS, translation elongation factor 1-alpha, actin, and beta-tubulin) to unveil the biodiversity and taxonomy of this understudied important genus of plant pathogens. Phylogenetic inferences based on 150 Californian isolates revealed 15 *Cytospora* species associated with branch and twig cankers and dieback of almond, apricot, cherry, cottonwood, olive, peach, pistachio, plum, pomegranate, and walnut trees in California. Of the 15 species recovered in this study, 10 are newly described and typified, in addition to one new combination. The pathogenic status of the newly described *Cytospora* species requires further investigation as most species were associated with severe dieback and decline of diverse and economically important fruit and nut crops in California.

Key words:
Cytosporaceae
Cytospora canker
Diaporthales
multigene phylogeny
new taxa
taxonomy

Article info: Submitted: 30 March 2018; Accepted: 12 September 2018; Published: 26 September 2018.

INTRODUCTION

The generic name *Cytospora* (*Sordariomycetes*, *Diaporthales*, *Cytosporaceae*) was introduced in 1818 and includes seemingly innocuous endophytes isolated from the bark, xylem, and leaves of asymptomatic woody plants (Spielman 1983, Bills 1996), saprobes that colonize and degrade the wood of dead or dying trees (Christensen 1940), and destructive canker pathogens (known as Cytospora-, Leucostoma-, Valsa-, or perennial canker) that cause dieback of more than 85 woody plant species (Sinclair *et al.* 1987, Adams *et al.* 2005, 2006). The chronic wood infections caused by *Cytospora* species can be devastating to stone fruit, pome fruit, and nut crops such as *Prunus persica*, *P. armeniaca*, *P. avium*, *Malus* spp., and *Juglans* spp. (Biggs & Grove 2005, Wang *et al.* 2011, Fan *et al.* 2015a). *Cytospora* species mainly impact branches, but they can cause more destructive infections in the trunks and larger scaffolds, severely limiting the longevity and productivity of orchards (Biggs 1989, Chang *et al.* 1991).

To date, approximately 612 *Cytospora* species have been described according to Index Fungorum. Kirk *et al.* (2008) listed approximately 110 accepted *Cytospora* species, while all other species names were considered synonyms of previously described taxa or treated as non-*Cytospora* species before the one fungus = one name rule came into force in July 2011 (Hawksworth 2011). Therefore, all taxa including the former sexual and asexual morphs that no longer have nomenclatural priority should be considered in order to resolve nomenclatural issues in this group of challenging fungi. The asexual morph is commonly encountered in nature. The pycnidia arise in a stroma embedded in host tissues (Grove 1923), and possess either a single locule or a complex of invaginated walls producing labyrinthine locules with filamentous conidiophores which may be reduced to conidiogenous cells that bear hyaline, allantoid conidia (Adams *et al.* 2006). Pycnidia exude conidia in a yellow, orange to red polysaccharide matrix, a cirrus, *via* an ostiole (Adams *et al.* 2005, 2006). Conidia oozing from pycnidia embedded in dead or dying host cortical tissues during humid or wet conditions are considered the infectious propagules potentially initiating new infections; the role of ascospores has not been determined. Conidia are dispersed to new plant tissues by rain-splash, where they germinate and infect the host plant *via* cracks and wounds to the bark created by pruning wounds, leaf scars, insect injuries,

winter-injured buds, twigs and bark, and breakage of shade-weakened twigs and branches (Tekauz & Patrick 1974, Biggs 1989). Bertrand & English (1976b) showed that *Cytospora* conidia were routinely trapped up to 76.8 m from the primary inoculum source after wind-blown rain in California, thus providing compelling evidence for *Cytospora* spore dispersal across large areas within orchards during times of inclement weather.

Species diagnosis in *Cytospora* has traditionally relied on morphological characters of pycnidia/perithecia (Grove 1923), including locule shape/organization and spore dimensions (Spielman 1985), as well as the arrangement of stromatic tissues (Adams *et al.* 2002). This morphological species approach is confounded by many examples of morphological character overlap among species and by the morphological plasticity of pycnidial locules which are affected by the host bark and cambium characteristics (Adams *et al.* 2002, Wang *et al.* 2011). Species diagnosis based on host association has also proven unreliable as several species of *Cytospora* have been recovered from multiple distantly related hosts, while a single host species can harbour more than one species of *Cytospora* (Adams *et al.* 2005, 2006, Wang *et al.* 2011, Fan *et al.* 2015a, b).

Défago (1935) questioned the utility of morphological characters in delimiting *Cytospora* species. Spielman (1985) reported that the asexual morph of *Cytospora leucosperma* was indistinguishable from that of many other species of *Cytospora*. Traditionally, sexual morphs of *Cytospora* were classified within several genera including *Leucostoma*, *Valsa*, *Valsella*, and *Valseutypella*. Tulasne & Tulasne (1863) postulated that *Cytospora* and *Valsa* were the asexual and sexual morphs of the same fungus. All these studies have highlighted the difficulty to properly disentangle taxa that share similar morphologies. Species identification based on molecular data could overcome these difficulties, which has been illustrated using ITS rDNA phylogenies (Adams *et al.* 2002, 2005, 2006). Recently, the use of the generic name *Cytospora* has been recommended for protection and use over *Leucocytospora*, *Leucostoma*, *Valsa*, *Valsella*, and *Valseutypella* (Rossman *et al.* 2015).

According to Norphanphoun *et al.* (2017) there are currently only 23 ex-type *Cytospora* species sequences deposited in GenBank. The majority of these sequences correspond to a single nuclear ribosomal gene region covering the ITS or the partial nuclear large ribosomal RNA subunit (nrLSU). Molecular data from type specimens are thus limited in public repositories and hamper abilities to properly circumscribe or identify taxa to the species-level in *Cytospora*. Recently, the utility of additional protein-coding loci, such as beta-tubulin, actin, and translation elongation factor 1-alpha, has been demonstrated for *Cytospora* sequence-based identification: more *Cytospora* species were recognized when using analyses including multiple protein-coding loci, relative to analyses relying on ITS only or combined ITS and nrLSU (Lawrence *et al.* 2017a).

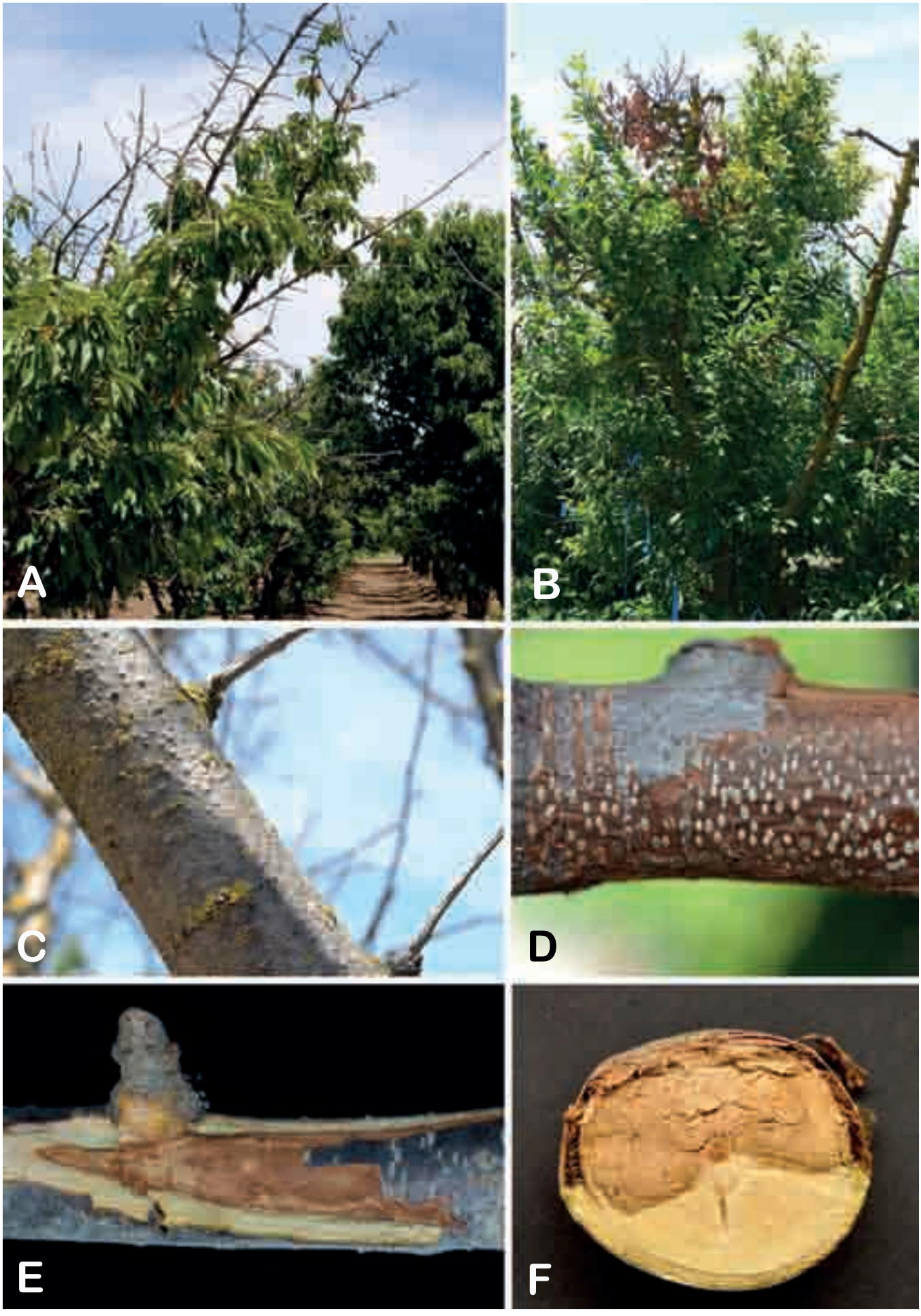
Although *Cytospora* species are known pathogens of stone fruits and nut crops worldwide, the taxonomy and host distribution of *Cytospora* species occurring in California orchards are still elusive, with only *C. leucostoma* and/or *C. cincta* known to affect French prune (Bertrand & English 1976a), peach and nectarine (French 1989), and sweet cherry (Trouillas *et al.* 2012). California is the largest and most productive perennial agricultural area in North America, producing diverse fruit and nut crops which constitute potential hosts for *Cytospora* species. The objectives of this study were to examine the phylogenetic diversity of *Cytospora* species isolated from orchards exhibiting dieback and canker diseases in California. Our hypotheses were that new *Cytospora* species would be identified from a region and crops that have been under-examined, especially given the recent advances in molecular identification of fungi (Hibbett *et al.* 2016). We hypothesized also that distinct species of *Cytospora* would infect distinct crop species, as expected if host specificity would favour pathogen speciation (Giraud *et al.* 2006). Morphological characters in conjunction with multi-locus phylogenetic analyses will afford us the first glimpse into the biodiversity of this important genus of canker pathogens.

MATERIALS AND METHODS

Fruit and nut crop sampling and fungal isolation

Between 2010 and 2017, putative *Cytospora* species were isolated periodically from declining fruit and nut trees throughout the Central Valley region of California as part of the diagnosis activity of the co-operative extension laboratories at the Kearney Agricultural Research and Extension Centre, in the centre of major agricultural industries. Sampled trees expressed general symptoms and signs of canker diseases including branch dieback, leaf wilting, dead and split bark, sunken lesions on branches, internal wood discoloration, gumming on trunks and scaffold limbs, cracked bark revealing blackened tissues, and presence of pinhead-sized dark pycnidia erupting through the bark or exposed upon peeling the outer layer of the bark (Figs 1–3). Mass-hyphal isolates were recovered using 10–12 wood pieces (4 × 4 × 2 mm) per sample, cut from the margins of necrotic and apparently healthy wood, surface disinfested in 0.6 % sodium hypochlorite for 60 s, rinsed in two serial baths of sterile deionized water for 30 s, and plated on potato dextrose agar (PDA, Difco, Detroit, MI) dishes amended with tetracycline (1 mg L^{-1}). A number of isolates were also collected directly from conidial masses exuding from freshly exposed pycnidia on declining branches. Masses of conidia were collected using a sterilized needle, placed into 1.5 mL tubes containing sterile water, and spread onto the surface of PDA Petri dishes. Petri dishes were incubated at 25 °C in the dark for up to 28 d. Isolates with morphological characters of *Cytospora*, namely

Fig. 1. Signs and symptoms of Cytospora canker/dieback in various fruit and nut crops in California. **A.** Twig dieback in sweet cherry. **B.** Twig and scaffold branch dieback in French prune. **C.** Pimpled-bark indicating underlying asexual fruiting bodies in a sweet cherry branch affected with Cytospora canker. **D.** Below bark, asexual fruiting bodies associated with Cytospora canker of French prune. **E–F.** Cankers and wood discoloration associated with Cytospora canker of sweet cherry.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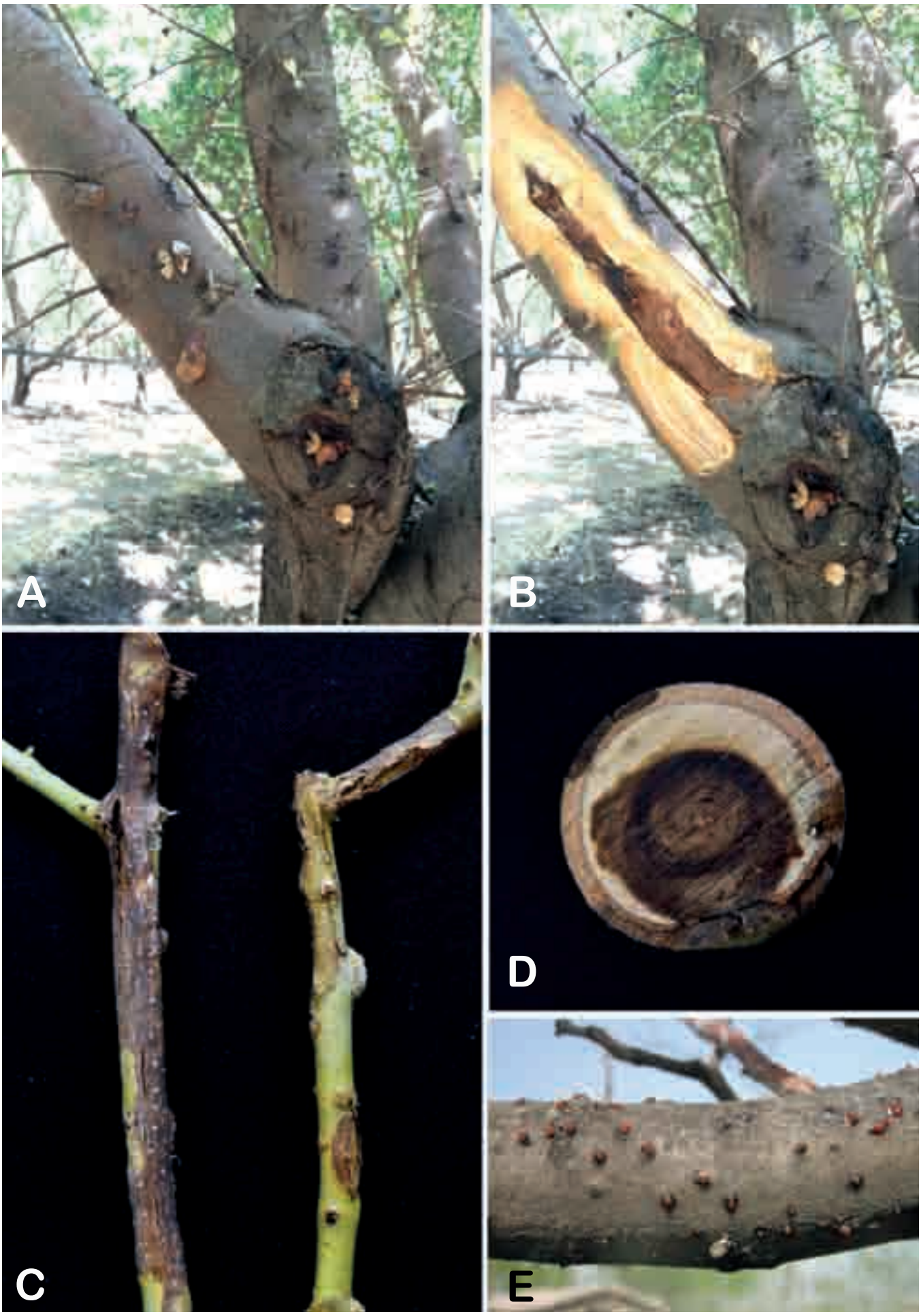
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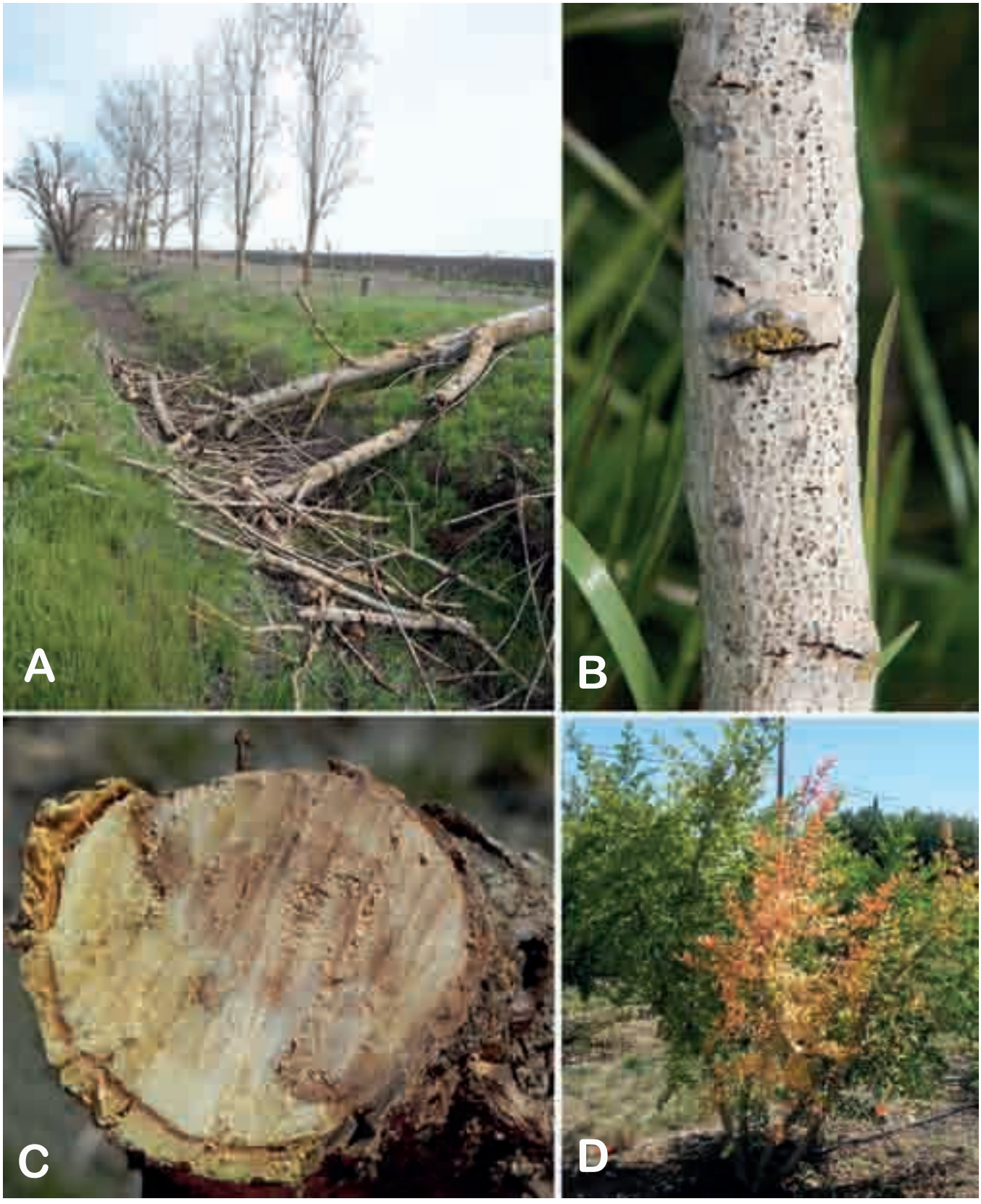

Fig. 3. Signs and symptoms of Cytospora canker/dieback in cottonwood and pomegranate hosts in California. **A–B.** Dead cottonwood tree parts on a roadside surrounding orchards and associated *Cytospora* asexual fruiting bodies erupting through the bark. **C–D.** Cytospora canker, wood discoloration and associated branch dieback in pomegranate.

Fig. 2. Signs and symptoms of Cytospora canker/dieback in various fruit and nut crops in California. **A–B.** Gumming and underlying elongated canker associated with Cytospora canker in almond. **C.** *Cytospora* associated cankers in olive twigs. **D.** Cankers and wood discoloration associated with *Cytospora* in pistachio. **E.** Conidial masses exuding from *Cytospora* asexual fruiting bodies in walnut.

colonies with uneven to highly uneven growth margins and thus lobate to highly lobate colony morphology, were hyphal-tip purified to fresh PDA dishes. In total, 150 isolates from symptomatic orchards and adjacent ornamental trees throughout the Central Valley of California were recovered in pure culture and used for phylogenetic and morphological analyses (Table 1). Representative cultures used in this study are permanently preserved in the collections of the Department of Plant Pathology at the Kearney Agricultural Research and Extension Centre of the University of California, Parlier, CA. The holotypes of the newly described species are preserved as dried cultures in BPI, with ex-type cultures deposited in CBS.

DNA extraction, sequencing, and phylogenetic analyses

Total genomic DNA was isolated from mycelium scraped with a sterile scalpel from the surface of 14-day-old cultures using the DNeasy Plant kit (Qiagen, Valencia, CA), following the manufacturer's instructions. All PCR reactions utilized AccuPower™ PCR Premix (Bioneer, Alameda, CA), following the manufacturer's instructions. Amplification of rDNA, including the intervening ITS regions and 5.8S rDNA (ITS1–5.8S–ITS2), using the primer set ITS5 and ITS4 followed the protocol of White *et al.* (1990). Amplification of translation elongation factor 1-α (*TEF1*) fragments utilized the primer set EF1-688F and EF1-1251R (Alves *et al.* 2008), beta-tubulin (*TUB2*) utilized primers Bt1a and Bt1b (Glass & Donaldson 1995), and actin (*ACT1*) utilized primers ACT-512F and ACT-783R (Carbone & Kohn 1999), with a slightly modified PCR program for *TUB2* and *ACT1* [initial denaturation (94 °C, 5 min) followed by 35 cycles of denaturation (94 °C, 30 s), annealing (58 °C for *TUB2* and 63 °C for *ACT1*, 30 s), extension (72 °C, 60 s), and a final extension (72 °C, 10 min)]. PCR amplification of the *TUB2* locus for some Californian *Cytospora* isolates (described below) was attempted at different annealing temperatures (50–60 °C). PCR products were visualized on a 1.5 % agarose gel (120 V for 25 min) stained with GelRed® (Biotium, Fremont, CA), following the manufacturer's instructions, to confirm presence and size of amplicons, purified via Exonuclease I and recombinant Shrimp Alkaline Phosphatase (Affymetrix, Santa Clara, CA), and sequenced bidirectionally *via* BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Thermo Fischer Scientific, Waltham, MA) on an ABI 3730 Capillary Electrophoresis Genetic Analyzer (College of Biological Sciences Sequencing Facility, University of California, Davis).

Forward and reverse nucleotide sequences were assembled, proofread, and edited in Sequencher v. 5 (Gene Codes Corporation, Ann Arbor, MI) and deposited in GenBank (Table 1). Homologous sequences with high similarity from ex-type and non-type *Cytospora* isolates were included for phylogenetic reference utilizing the BLASTn function in NCBI and extensive literature review (Table 2). Multiple sequence alignments were performed in MEGA v. 6 (Tamura *et al.* 2013) and manually adjusted where necessary in Mesquite v. 3.10 (Maddison & Maddison 2016). Alignments were submitted to TreeBASE under accession number S22195. Phylogenetic analyses were performed for each individual locus and for a four-gene concatenated dataset. Each dataset was analyzed using two different optimality search criteria, maximum parsimony (MP) and maximum likelihood (ML), in MEGA v. 6 (Tamura *et al.* 2013). For MP analyses, heuristic searches with 1000 random sequence additions were implemented with the Tree-Bisection-Reconnection algorithm, gaps were treated as missing data. Bootstrap analyses with 1000 pseudoreplicates were used to estimate branch support. For ML analyses, MEGA was used to infer a model of nucleotide substitution for each dataset, using the Akaike Information Criterion (AIC). All ML analyses utilized the Nearest-Neighbor-Interchange heuristic method and branch support was determined by 1000 bootstrap pseudoreplicates. Sequences of *Diaporthe ampelina* isolate Wolf912 and *D. benedicti* isolate SBen914 (*Diaporthales*, *Diaporthaceae*) (Lawrence *et al.* 2015) served as the outgroup taxa in all analyses.

Morphology

Mycelial plugs (5 mm diam) were taken from the margin of selected, actively growing cultures based on preliminary phylogenetic results and transferred to triplicate 90 mm diam Petri dishes containing 2 % PDA and incubated in the dark at 25 °C for 14 d. Radial growth was measured after 7 d by taking two measurements perpendicular to each other. Assessments of colony colour (Rayner 1970) and morphology were made after 14 d. Pycnidia were induced on corticated cherry wood embedded in PDA medium. Cherry cuttings (approx. 1 cm diam) were collected and cut into 5 cm sections. Sections were placed in glass Petri dishes and autoclaved twice, 24 h apart, at 122 °C for 25 min. Autoclaved wood sections were placed in 90 mm diam plastic Petri dishes, two sections per dish, and PDA was poured to embed them. A mycelial plug from an actively growing culture was placed between the two wood sections in each dish, one isolate per dish. Petri dishes were incubated at room temperature under natural photoperiod in August 2017, and pycnidial formation was monitored weekly for four weeks. Morphological characterization of the pycnidia (n = 20) included the diameter, presence/absence of a conceptacle, and colour using a binocular Leica MZ95 dissecting microscope (Leica microsystems CMS, Wetzlar, Germany). Pycnidial locular arrangements were assessed by transversely sectioning pycnidia by hand with a razor blade and observing as above. Conidial dimensions (n = 30) and conidiogenous cells (n = 20) were measured at ×1000 from approximately 28-day-old cultures by producing a pycnidial squash mount that was crushed in a sterile 50 % glycerol solution (no stain was applied, thus the natural pigments of each species was preserved) and observed with a Leica DM500B microscope (Leica microsystems CMS, Wetzlar, Germany). Morphological measurements are represented by the mean as a range depicting the standard deviation in the centre with minima and maxima in parentheses, respectively, in the species descriptions and taxonomy section below.

RESULTS

Disease symptoms, hosts, and distribution

In total, 92 samples were obtained from symptomatic trees in 70 orchards of various fruit and nut crops including almond (*Prunus dulcis*), apricot (*Prunus armeniaca*), cherry (*Prunus*

Table 1. *Cytospora* species recovered from symptomatic hosts in California.

Species	Isolate[a]	Host	Geographic origin	GenBank Accession No.			
				ITS	*ACT1*	*TEF1*	*TUB2*
Cytospora amydgali	LH356	*Prunus dulcis*	Yolo Co., California, USA	MG971852	MG972001	MG971658	MG971717
C. amydgali	**LH357/ CBS 144233**	*Prunus dulcis*	Yolo Co., California, USA	MG971853	MG972002	MG971659	MG971718
C. californica	**9C-24/ CBS 144234**	*Juglans regia*	Lake Co., California, USA	MG971935	MG972083	MG971645	—
C. californica	KARE264	*Pistacia vera*	Kern Co., California, USA	MG971920	MG972069	MG971630	MG971780
C. californica	KARE265	*Pistacia vera*	Kern Co., California, USA	MG971914	MG972064	MG971624	MG971776
C. californica	KARE303	*Pistacia vera*	Kern Co., California, USA	MG971913	MG972063	MG971623	MG971775
C. californica	KARE324	*Pistacia vera*	Kern Co., California, USA	MG971911	MG972061	MG971621	MG971773
C. californica	KARE325	*Pistacia vera*	Kern Co., California, USA	MG971918	MG972067	MG971628	—
C. californica	KARE326	*Pistacia vera*	Kern Co., California, USA	MG971919	MG972068	MG971629	—
C. californica	KARE1091	*Pistacia vera*	Kern Co., California, USA	MG971946	MG972096	MG971662	MG971790
C. californica	KARE1104	*Prunus dulcis*	Fresno Co., California, USA	MG971928	MG972077	MG971638	MG971783
C. californica	KARE1107	*Prunus dulcis*	Fresno Co., California, USA	MG971929	MG972078	MG971639	—
C. californica	KARE166	*Prunus dulcis*	Fresno Co., California, USA	MG971916	MG972093	MG971626	MG971778
C. californica	KARE197	*Prunus dulcis*	Fresno Co., California, USA	MG971932	MG972081	MG971642	MG971786
C. californica	KARE198	*Prunus dulcis*	Fresno Co., California, USA	MG971915	MG972065	MG971625	MG971777
C. californica	KARE1105	*Prunus dulcis*	Fresno Co., California, USA	MG971947	MG972097	MG971663	MG971791
C. californica	KARE1106	*Prunus dulcis*	Fresno Co., California, USA	MG971948	MG972094	MG971647	MG971788
C. californica	KARE1377	*Prunus dulcis*	Glenn Co., California, USA	MG971933	MG972057	MG971643	MG971787
C. californica	KARE1191	*Prunus dulcis*	Glenn Co., California, USA	MG971945	MG972095	MG971661	MG971789
C. californica	KARE884	*Prunus dulcis*	San Joaquin Co., California, USA	MG971925	MG972074	MG971635	—
C. californica	KARE894	*Prunus dulcis*	San Joaquin Co., California, USA	MG971927	MG972076	MG971637	—
C. californica	KARE895	*Prunus dulcis*	San Joaquin Co., California, USA	MG971926	MG972075	MG971636	—
C. californica	KARE896	*Prunus dulcis*	San Joaquin Co., California, USA	MG971936	MG972084	MG971646	—
C. californica	KARE902	*Prunus dulcis*	San Joaquin Co., California, USA	MG971924	MG972073	MG971634	MG971782
C. californica	KARE903	*Prunus dulcis*	San Joaquin Co., California, USA	MG971922	MG972071	MG971632	MG971781
C. californica	KARE904	*Prunus dulcis*	San Joaquin Co., California, USA	MG971923	MG972072	MG971633	—
C. californica	KARE905	*Prunus dulcis*	San Joaquin Co., California, USA	MG971921	MG972070	MG971631	—
C. californica	KARE62	*Prunus dulcis*	Stanislaus Co., California, USA	MG971912	MG972062	MG971622	MG971774
C. californica	KARE883	*Prunus dulcis*	Stanislaus Co., California, USA	MG971934	MG972082	MG971644	—
C. californica	KARE93	*Prunus dulcis*	Stanislaus Co., California, USA	MG971930	MG972079	MG971640	MG971784
C. californica	KARE94	*Prunus dulcis*	Stanislaus Co., California, USA	MG971931	MG972080	MG971641	MG971785
C. californica	KARE99	*Prunus dulcis*	Stanislaus Co., California, USA	MG971917	MG972066	MG971627	MG971779
C. chrysosperma	9E-33/ CBS 144242	*Camellia*	Fresno Co., California, USA	MG971892	MG972041	MG971602	MG971758

Table 1. (Continued).

Species	Isolate[a]	Host	Geographic origin	GenBank Accession No.			
				ITS	*ACT1*	*TEF1*	*TUB2*
C. eucalypti	KARE1585/ CBS 144241	*Prunus dulcis*	Merced Co., California, USA	MG971907	MG972056	MG971617	MG971772
C. eucalypti	KARE888	*Prunus dulcis*	San Joaquin Co., California, USA	MG971909	MG972059	MG971619	—
C. eucalypti	KARE889	*Prunus dulcis*	San Joaquin Co., California, USA	MG971908	MG972058	MG971618	—
C. eucalypti	KARE890	*Prunus dulcis*	San Joaquin Co., California, USA	MG971906	MG972055	MG971616	—
C. eucalypti	7G-62	*Sequoiadendron giganteum*	Fresno Co., California, USA	MG971910	MG972060	MG971620	—
C. granati	**6F-45/ CBS 144237**	*Punica granatum*	Tulare Co., California, USA	MG971799	MG971949	MG971514	MG971664
C. joaquinensis	9E-95	*Juglans regia*	Tulare Co., California, USA	MG971896	MG972045	MG971606	MG971762
C. joaquinensis	9E-44	*Pistacia vera*	Fresno Co., California, USA	MG971897	MG972046	MG971607	MG971763
C. joaquinensis	KARE195	*Pistacia vera*	Kern Co., California, USA	MG971894	MG972043	MG971604	MG971760
C. joaquinensis	KARE231	*Pistacia vera*	Kern Co., California, USA	MG971893	MG972042	MG971603	MG971759
C. joaquinensis	**KARE975/ CBS 144235**	*Populus deltoides*	San Joaquin Co., California, USA	MG971895	MG972044	MG971605	MG971761
C. longispora	**10F-57/ CBS 144236**	*Prunus domestica*	Glenn Co., California, USA	MG971905	MG972054	MG971615	MG971764
C. oleicola	**KARE1021/ CBS 144248**	*Olea europaea*	San Joaquin Co., California, USA	MG971944	MG972098	MG971660	MG971752
C. parakantschavelii	KARE974/ CBS 144243	*Populus deltoides*	San Joaquin Co., California, USA	MG971898	MG972047	MG971608	MG971765
C. parakantschavelii	KARE966	*Populus fremontii*	Yolo Co., California, USA	MG971903	MG972052	MG971613	MG971770
C. parakantschavelii	KARE967	*Populus fremontii*	Yolo Co., California, USA	MG971901	MG972050	MG971611	MG971768
C. parakantschavelii	KARE968	*Populus fremontii*	Yolo Co., California, USA	MG971900	MG972049	MG971610	MG971767
C. parakantschavelii	KARE969	*Populus fremontii*	Yolo Co., California, USA	MG971904	MG972053	MG971614	MG971771
C. parakantschavelii	KARE970	*Populus fremontii*	Yolo Co., California, USA	MG971902	MG972051	MG971612	MG971769
C. parakantschavelii	KARE971	*Populus fremontii*	Yolo Co., California, USA	MG971899	MG972048	MG971609	MG971766
C. parapistaciae	KARE232	*Pistacia vera*	Kern Co., California, USA	MG971807	MG971957	MG971522	MG971672
C. parapistaciae	KARE268	*Pistacia vera*	Kern Co., California, USA	MG971806	MG971956	MG971521	MG971671
C. parapistaciae	KARE269	*Pistacia vera*	Kern Co., California, USA	MG971805	MG971955	MG971520	MG971670
C. parapistaciae	**KARE270/ CBS 144506**	*Pistacia vera*	Kern Co., California, USA	MG971804	MG971954	MG971519	MG971669
C. pistaciae	KARE441	*Pistacia vera*	Merced Co., California, USA	MG971800	MG971950	MG971515	MG971665
C. pistaciae	KARE442	*Pistacia vera*	Merced Co., California, USA	MG971803	MG971953	MG971518	MG971668
C. pistaciae	**KARE443/ CBS 144238**	*Pistacia vera*	Merced Co., California, USA	MG971802	MG971952	MG971517	MG971667
C. pistaciae	KARE444	*Pistacia vera*	Merced Co., California, USA	MG971801	MG971951	MG971516	MG971666
C. plurivora	8C-55	*Juglans regia*	Butte Co., California, USA	MG971871	MG972020	MG971582	MG971736
C. plurivora	9F-01	*Juglans regia*	Glenn Co., California, USA	MG971873	MG972022	MG971584	MG971738
C. plurivora	9F-03	*Juglans regia*	Glenn Co., California, USA	MG971865	MG972014	MG971576	MG971730
C. plurivora	11I-89	*Juglans regia*	Sutter Co., California, USA	MG971855	MG972004	MG971566	MG971720
C. plurivora	9F-08	*Juglans regia*	Tehama Co., California, USA	MG971884	MG972033	MG971594	MG971749

Table 1. (Continued).

Species	Isolate[a]	Host	Geographic origin	GenBank Accession No.			
				ITS	*ACT1*	*TEF1*	*TUB2*
C. plurivora	**KARE1452/ CBS 144239**	*Olea europaea*	San Joaquin Co., California, USA	MG971861	MG972010	MG971572	MG971726
C. plurivora	9E-42	*Pistacia vera*	Colusa Co., California, USA	MG971870	MG972019	MG971581	MG971735
C. plurivora	9E-86	*Prunus domestica*	Sutter Co., California, USA	MG971869	MG972018	MG971580	MG971734
C. plurivora	11I-87	*Prunus domestica*	Sutter Co., California, USA	MG971872	MG972021	MG971583	MG971737
C. plurivora	8J-57	*Prunus domestica*	Tehama Co., California, USA	MG971854	MG972003	MG971565	MG971719
C. plurivora	11I-19	*Prunus domestica*	Tulare Co., California, USA	MG971866	MG972015	MG971577	MG971731
C. plurivora	11I-20	*Prunus domestica*	Tulare Co., California, USA	MG971868	MG972017	MG971579	MG971733
C. plurivora	11I-21	*Prunus domestica*	Tulare Co., California, USA	MG971867	MG972016	MG971578	MG971732
C. plurivora	KARE486	*Prunus domestica*	Tulare Co., California, USA	MG971879	MG972028	MG971655	MG971744
C. plurivora	KARE487	*Prunus domestica*	Tulare Co., California, USA	MG971878	MG972027	MG971589	MG971743
C. plurivora	9D-71	*Prunus domestica*	Yuba Co., California, USA	MG971858	MG972007	MG971569	MG971723
C. plurivora	9D-72	*Prunus domestica*	Yuba Co., California, USA	MG971857	MG972006	MG971568	MG971722
C. plurivora	KARE1518	*Prunus dulcis*	Kern Co., California, USA	MG971875	MG972024	MG971586	MG971740
C. plurivora	KARE1519	*Prunus dulcis*	Kern Co., California, USA	MG971876	MG972025	MG971587	MG971741
C. plurivora	KARE50	*Prunus dulcis*	Fresno Co., California, USA	MG971877	MG972026	MG971588	MG971742
C. plurivora	KARE1449	*Prunus dulcis*	Kern Co., California, USA	MG971859	MG972008	MG971570	MG971724
C. plurivora	KARE1450	*Prunus dulcis*	Kern Co., California, USA	MG971860	MG972009	MG971571	MG971725
C. plurivora	KARE91	*Prunus dulcis*	Stanislaus Co., California, USA	MG971862	MG972011	MG971573	MG971727
C. plurivora	6F-18	*Prunus persica*	Contra Costa Co., California, USA	MG971874	MG972023	MG971585	MG971739
C. plurivora	KARE79	*Prunus persica*	Fresno Co., California, USA	MG971882	MG972031	MG971592	MG971747
C. plurivora	KARE80	*Prunus persica*	Fresno Co., California, USA	MG971883	MG972032	MG971593	MG971748
C. plurivora	KARE81	*Prunus persica*	Fresno Co., California, USA	MG971881	MG972030	MG971591	MG971746
C. plurivora	KARE82	*Prunus persica*	Fresno Co., California, USA	MG971880	MG972029	MG971590	MG971745
C. plurivora	5L-29	*Prunus domestica*	Fresno Co., California, USA	MG971856	MG972005	MG971567	MG971721
C. plurivora	KARE1536	*Prunus domestica*	Glenn Co., California, USA	MG971886	MG972035	MG971596	MG971751
C. plurivora	KARE1537	*Prunus domestica*	Glenn Co., California, USA	MG971864	MG972013	MG971575	MG971729
C. plurivora	KARE1538	*Prunus domestica*	Glenn Co., California, USA	MG971863	MG972012	MG971574	MG971728
C. plurivora	KARE1539	*Prunus domestica*	Glenn Co., California, USA	MG971885	MG972034	MG971595	MG971750
C. populicola	**KARE973/ CBS 144240**	*Populus deltoides*	San Joaquin Co., California, USA	MG971891	MG972040	MG971601	MG971757
C. punicae	7C-09	*Punica granatum*	Fresno Co., California, USA	MG971939	MG972087	MG971650	MG971794
C. punicae	7C-10	*Punica granatum*	Fresno Co., California, USA	MG971937	MG972085	MG971648	MG971792
C. punicae	7C-11	*Punica granatum*	Fresno Co., California, USA	MG971942	MG972090	MG971653	MG971797
C. punicae	5A-80/ CBS 144244	*Punica granatum*	Madera Co., California, USA	MG971943	MG972091	MG971654	MG971798

Table 1. (Continued).

Species	Isolate[a]	Host	Geographic origin	GenBank Accession No.			
				ITS	*ACT1*	*TEF1*	*TUB2*
C. punicae	5A-81	*Punica granatum*	Madera Co., California, USA	MG971938	MG972086	MG971649	MG971793
C. punicae	5A-82	*Punica granatum*	Madera Co., California, USA	MG971941	MG972089	MG971652	MG971796
C. punicae	7C-33	*Punica granatum*	Stanislaus Co., California, USA	MG971940	MG972088	MG971651	MG971795
C. sorbicola	KARE1451	*Olea europaea*	Kings Co., California, USA	MG971850	MG971999	MG971563	MG971715
C. sorbicola	5D-48	*Prunus armeniaca*	Fresno Co., California, USA	MG971817	MG971967	MG971532	MG971682
C. sorbicola	KARE626	*Prunus avium*	Contra Costa Co., California, USA	MG971829	MG971979	MG971544	MG971694
C. sorbicola	KARE876	*Prunus avium*	Contra Costa Co., California, USA	MG971826	MG971976	MG971541	MG971691
C. sorbicola	KARE158	*Prunus avium*	Fresno Co., California, USA	MG971847	MG971996	MG971560	MG971712
C. sorbicola	KARE162	*Prunus avium*	Fresno Co., California, USA	MG971846	MG971995	MG971559	MG971711
C. sorbicola	3G-09	*Prunus avium*	Kern Co., California, USA	MG971838	MG971988	MG971656	MG971703
C. sorbicola	KARE1241	*Prunus avium*	Kings Co., California, USA	MG971851	MG972000	MG971564	MG971716
C. sorbicola	KARE612	*Prunus avium*	Sacramento Co., California, USA	MG971822	MG971972	MG971537	MG971687
C. sorbicola	KARE623	*Prunus avium*	Sacramento Co., California, USA	MG971809	MG971959	MG971524	MG971674
C. sorbicola	KARE882	*Prunus avium*	Sacramento Co., California, USA	MG971836	MG971986	MG971551	MG971701
C. sorbicola	5D-42	*Prunus avium*	San Benito Co., California, USA	MG971841	MG971991	MG971555	MG971706
C. sorbicola	5D-44	*Prunus avium*	San Benito Co., California, USA	MG971840	MG971990	MG971554	MG971705
C. sorbicola	KARE615	*Prunus avium*	San Joaquin Co., California, USA	MG971819	MG971969	MG971534	MG971684
C. sorbicola	KARE617	*Prunus avium*	San Joaquin Co., California, USA	MG971815	MG971965	MG971530	MG971680
C. sorbicola	KARE618	*Prunus avium*	San Joaquin Co., California, USA	MG971814	MG971964	MG971529	MG971679
C. sorbicola	KARE619	*Prunus avium*	San Joaquin Co., California, USA	MG971813	MG971963	MG971528	MG971678
C. sorbicola	KARE621	*Prunus avium*	San Joaquin Co., California, USA	MG971811	MG971961	MG971526	MG971676
C. sorbicola	KARE622	*Prunus avium*	San Joaquin Co., California, USA	MG971810	MG971960	MG971525	MG971675
C. sorbicola	KARE624	*Prunus avium*	San Joaquin Co., California, USA	MG971808	MG971958	MG971523	MG971673
C. sorbicola	KARE625	*Prunus avium*	San Joaquin Co., California, USA	MG971830	MG971980	MG971545	MG971695
C. sorbicola	KARE877	*Prunus avium*	San Joaquin Co., California, USA	MG971825	MG971975	MG971540	MG971690
C. sorbicola	KARE879	*Prunus avium*	San Joaquin Co., California, USA	MG971823	MG971973	MG971538	MG971688
C. sorbicola	KARE881	*Prunus avium*	San Joaquin Co., California, USA	MG971837	MG971987	MG971552	MG971702
C. sorbicola	KARE589	*Prunus avium*	Yolo Co., California, USA	MG971848	MG971997	MG971561	MG971713
C. sorbicola	KARE590	*Prunus avium*	Yolo Co., California, USA	MG971845	MG971994	MG971558	MG971710
C. sorbicola	KARE613	*Prunus avium*	Yolo Co., California, USA	MG971821	MG971971	MG971536	MG971686
C. sorbicola	KARE614	*Prunus avium*	Yolo Co., California, USA	MG971820	MG971970	MG971535	MG971685
C. sorbicola	KARE616	*Prunus avium*	Yolo Co., California, USA	MG971816	MG971966	MG971531	MG971681
C. sorbicola	KARE620	*Prunus avium*	Yolo Co., California, USA	MG971812	MG971962	MG971527	MG971677
C. sorbicola	KARE874	*Prunus avium*	Yolo Co., California, USA	MG971828	MG971978	MG971543	MG971693

Table 1. (Continued).

Species	Isolate[a]	Host	Geographic origin	GenBank Accession No.			
				ITS	*ACT1*	*TEF1*	*TUB2*
C. sorbicola	KARE875	*Prunus avium*	Yolo Co., California, USA	MG971827	MG971977	MG971542	MG971692
C. sorbicola	KARE878	*Prunus avium*	Yolo Co., California, USA	MG971824	MG971974	MG971539	MG971689
C. sorbicola	4L-58	*Prunus domestica*	Yuba Co., California, USA	MG971839	MG971989	MG971553	MG971704
C. sorbicola	KARE59	*Prunus dulcis*	Fresno Co., California, USA	MG971849	MG971998	MG971562	MG971714
C. sorbicola	KARE78	*Prunus dulcis*	Fresno Co., California, USA	MG971844	MG971993	MG971557	MG971709
C. sorbicola	KARE226	*Prunus dulcis*	Stanislaus Co., California, USA	MG971835	MG971985	MG971550	MG971700
C. sorbicola	KARE227	*Prunus dulcis*	Stanislaus Co., California, USA	MG971834	MG971984	MG971549	MG971699
C. sorbicola	KARE228/ CBS 144245	*Prunus dulcis*	Stanislaus Co., California, USA	MG971833	MG971983	MG971548	MG971698
C. sorbicola	KARE249	*Prunus dulcis*	Stanislaus Co., California, USA	MG971832	MG971982	MG971547	MG971697
C. sorbicola	KARE251	*Prunus dulcis*	Stanislaus Co., California, USA	MG971831	MG971981	MG971546	MG971696
C. sorbicola	KARE92	*Prunus dulcis*	Stanislaus Co., California, USA	MG971843	MG972092	MG971657	MG971708
C. sorbicola	KARE83	*Prunus persica*	Fresno Co., California, USA	MG971842	MG971992	MG971556	MG971707
C. sorbicola	9C-89	*Prunus persica*	Merced Co., California, USA	MG971818	MG971968	MG971533	MG971683

[a]Isolates in bold represent type specimens.

Table 2. Fungal isolates used in this study and GenBank accession numbers.

Species	Isolate[a]	Host	Geographic origin	GenBank Accession			
				ITS	*ACT1*[b]	*TEF1*	*TUB2*[b]
Cytospora abyssinica	**CMW 10181**	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347353	—	—	—
C. ampulliformis	**MFLUCC 16-0629**	*Acer platanoides*	Russia	KY417727	KY417693	—	—
C. atrocirrhata	**CFCC 89615**	*Jugulans regia*	Xining, Qinghai, China	KF225610	—	—	—
C. austromontana	**CMW 6735**	*Eucalyptus pauciflora*	NSW, Australia	AY347361	—	—	—
C. berberidis	**CFCC 89927**	*Berberis dasystachyum*	Qinghai Province, China	KP340985	—	—	—
C. berkeleyi	**StanfordT3**	*Eucalyptus globulus*	Palo Alto, California, USA	AY347350	—	—	—
C. brevispora	**CBS 116811**	*Eucalyptus grandis ×tereticornis*	Tchittanga, Republic of Congo	AF192315	—	—	—
C. carbonacea	**CFCC 50055**	*Ulmus pumila*	Qiqihar, Heilongjiang, China	KP281262	—	KP310851	—
C. cedri	CBS 196.50	Unknown host	Italy	AF192311	—	JX438575	—
C. centrivillosa	**MFLUCC 16-1206**	*Sorbus domestica*	Italy	MF190122	—	—	—
C. chrysosperma	**CFCC 89619**	*Juglans regia*	Yinchuan, Ningxia, China	KF225614	KF498677	—	—
C. cincta	LP47	*Prunus armeniaca*	Michigan, USA	AF191169	—	—	—
C. cinereostroma	**CMW 5700**	*Eucalyptus globulus*	Chile	AY347377	—	—	—
C. cotini	MFLUCC 14-1050	*Cotinus coggygria*	Russia	KX430142	—	—	—
C. curvata	**MFLUCC 15-0865**	*Salix alba*	Russia	KY417728	KY417694	—	—

Table 2. (Continued).

Species	Isolate[a]	Host	Geographic origin	GenBank Accession			
				ITS	*ACT1*[b]	*TEF1*	*TUB2*[b]
C. davidiana	**CXY1350**	*Populus davidiana*	China	KM034870	—	—	—
C. diatrypelloidea	**CMW 8549**	*Eucalyptus globulus*	Orbost, Victoria, Australia	AY347368	—	—	—
C. disciformis	**CMW 6509**	*Eucalyptus grandis*	Uruguay	AY347374	—	—	—
C. donetzica	**MFLUCC 16-0574**	*Rosa* sp.	Russia	KY417731	KY417697	—	—
C. elaeagni	**CFCC 89632**	*Elaeagnus angustifolia*	Guyuan, Ningxia, China	KF765676	—	—	—
C. eriobotryae	**IMI 136523**	*Eriobotrya japonica*	Saharanpur, India	AY347327	—	—	—
C. erumpens	**MFLUCC 16-0580**	*Salix ×fragilis*	Russia	KY417733	KY417699	—	—
C. eucalypticola	**ATCC 96150**	*Eucalyptus nitens*	Tasmainia, Australia	AY347358	—	—	—
C. eucalyptina	**CMW5882**	*Eucalyptus grandis*	Cali, Colombia	AY347375	—	—	—
C. eugeniae	CBS 118569	*Eugenia* sp.	Tanzania	AY347344	—	—	—
C. fraxinigena	**MFLUCC 14-0868**	*Fraxinus ornus*	Italy	MF190133	—	—	—
C. fugax	**CBS 203.42**	*Salix* sp.	Switzerland	AY347323	—	—	—
C. gigalocus	**HMBF155**	*Juglans regia*	Xining, Qinghai, China	KF225609	—	—	—
C. gigaspora	**CFCC 89634**	*Salix psammophila*	China	KF765671	KU711000	—	—
C. hippophaes	**CFCC 89639**	*Hippophae rhamnoides*	Gannan, Gansu, China	KF765681	—	—	—
C. junipericola	**MFLUCC 17-0882**	*Juniperus communis*	Italy	MF190125	—	—	—
C. kantschavelii	287-2	*Populus deltoides*	Iran	EF447367	—	—	—
C. leucosperma	CBS 191.42	*Taxus baccata*	Switzerland	AY347330	—	—	—
C. longiostiolata	**MFLUCC 16-0628**	*Salix ×fragilis*	Russia	KY417734	—	—	—
C. melnikii	**MFLUCC 15-0851**	*Malus domestica*	Russia	KY417735	KY417701	—	—
C. multicollis	**CBS 105.89**	*Quercus ilex* subsp. *rotundifolia*	Spain	DQ243803	—	—	—
C. myrtagena	**HiloTib1**	*Tibouchina urvilleana*	Hilo, Hawaii, USA	AY347363	—	—	—
C. nitschkii	**CMW 10180**	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347356	—	—	—
C. nivea	**CFCC 89642**	*Salix psammophila*	Yulin, Shaanxi, China	KF765684	KU711006	—	—
C. notastroma	**Cottonwood16**	*Populus tremuloides*	Colorado, USA	JX438631	—	—	—
C. palmoides	**CXY1276**	*Cotinus coggygria*	Beijing, Xiangshan, China	JN402990	—	—	—
C. parakantschavelii	**MFLUCC 15-0857**	*Populus ×sibirica*	Russia	KY417738	KY417704	—	—
C. parasitica	MFLUCC 15-0507	*Malus domestica*	Russia	KY417740	KY417706	—	—
C. paratranslucens	**MFLUCC 15-0506**	*Populus alba* var. *bolleana*	Russia	KY417741	KY417707	—	—
C. personata	CBS 117.67	*Rhododendron ponticum*	Netherlands	AY347331	—	—	—
C. pinastri	CBS 194.42	*Abies alba*	Switzerland	AY347328	—	—	—
C. pini	CBS 197.42	*Pinus sylvestris*	Switzerland	AY347332	—	—	—
C. populina	**CFCC 89644**	*Salix psammophila*	Yulin, Shaanxi, China	KF765686	—	—	—
C. pruinopsis	**CFCC 50034**	*Ulmus pumila*	Harbin, Heilongjiang, China	KP281259	—	—	—

Table 2. (Continued).

Species	Isolate[a]	Host	Geographic origin	GenBank Accession			
				ITS	*ACT1*[b]	*TEF1*	*TUB2*[b]
C. pruinosa	CBS 118555	*Olea europaea* v. *africana*	South Africa	DQ243790	—	—	—
C. punicae	CBS 199.50	*Punica granatum*	Turkey	JX438622	—	JX438568	—
C. quercicola	**MFLUCC 14-0867**	*Quercus* sp.	Italy	MF190129	—	—	—
C. ribis	**CFCC 50026**	*Ulmus pumila*	Yulin, Shaanxi, China	KP281267	—	—	—
C. rosae	**MFLUCC 14-0845**	*Rosa canina*	Italy	MF190131	—	—	—
C. rosarum	218	*Rosa canina*	Iran	EF447387			
C. rostrata	**Ls251**	*Salix cupularis*	Gansu, China	KC313890	—	JX438568	—
C. rusanovii	**MFLUCC 15-0854**	*Salix babylonica*	Russia	KY417744	KY417710	—	—
C. sacculus	CFCC 89624	*Juglans regia*	Gannan, Gansu, China	KF225615	—	KP310860	—
C. salicacearum	**MFLUCC 15-0509**	*Salix alba*	Russia	KY417745	KY417711	—	—
C. salicicola	MFLUCC 15-0866	*Salix alba*	Russia	KU982635	KU982637	—	—
C. salicina	**MFLUCC 15-0862**	*Salix alba*	Russia	KY417750	KY417716	—	—
C. schulzeri	CBS 118570	*Malus domestica*	Michigan, USA	DQ243802	—	—	—
C. sequioae	CBS 116815	*Sequoia sempervirens*	California, USA	AY347340	—	—	—
C. sibiraeae	**CFCC 50045**	*Sibiraea angustata*	Gannan, Gansu, China	KP340987	—	—	—
C. sophorae	**CFCC 89598**	*Sophora japonica*	China	KR045654	KU711018	KU710941	KR045695
C. sophoricola	CFCC 89595	*Sophora japonica* var. *pendula*	Gannan, Gansu, China	KC880148	—	—	—
C. sorbi	**MFLUCC 16-0631**	*Sorbus aucuparia*	Russia	KY417752	KY417718	—	—
C. sorbicola	**MFLUCC 16-0584**	*Acer pseudoplatanus*	Russia	KY417755	KY417721	—	—
C. spiraeae	**CFCC 50049**	*Spiraea salicirolia*	Gansu, China	MG707859	MG708196		
C. tanaitica	MFLUCC 14-1057	*Betula pubescens*	Russia	KT459411	KT459413	—	—
C. translucens	CBS 152.42	*Salix* sp.	St. Moritz, Switzerland	AF191182	—	—	—
C. ulmi	**MFLUCC 15-0863**	*Ulmus minor*	Russia	KY417759	KY417725	—	—
C. valsoidea	**CMW 4309**	*Eucalyptus grandis*	Sumatra, Indonesia	AF192312	—	—	—
C. variostromatica	**CMW 6766**	*Eucalyptus globulus*	Orbost, Victoria, Australia	AY347366	—	—	—
C. vinacea	**CBS 141585**	*Vitis* interspecific hybrid 'Vidal'	New Hampshire, USA	KX256256	—	KX256277	—
C. viticola	**CBS 141586**	*Vitis vinifera* 'Cabernet Franc'	Connecticut, USA	KX256239	—	KX256260	—
Diaporthe ampelina	Wolf912	*Vitis vinifera* 'Thompson seedless'	Solano Co., California, USA	KM669964	JGI	KM669820	JGI
Diaporthe benedicti	**SBen914**	*Salix* sp.	San Benito Co., California, USA	KM669929	—	KM669785	—
Leucostoma parapersoonii	**CBS 116845**	*Pyrus serotina*	Michigan, USA	AF191181			
Valsa sordida	CBS 197.50	*Populus tremula*	United Kingdom	AY347322	—	—	—

[a]Isolates in bold represent type specimens.

[b]JGI Represents sequences that were retreived from the JGI Mycocosm genome portal.

avium), olive (*Olea europaea*), peach (*Prunus persica*), pistachio (*Pistacia vera*), pomegranate (*Punica granatum*), prune (*Prunus domestica*), walnut (*Juglans regia*), and woody ornamentals such cottonwoods (*Populus deltoides* and *P. fremontii*), camellia (*Camellia* sp.) and sequoia (*Sequoiadendron giganteum*). Cankers and accompanying branch and twig dieback were the most common symptoms associated with *Cytospora* species. Trees expressing Cytospora cankers were observed in Butte, Colusa, Contra Costa, Fresno, Glenn, Kern, Kings, Lake, Madera, Merced, Sacramento, San Benito, San Joaquin, Stanislaus, Sutter, Tehama, Tulare, Yolo, and Yuba counties in California. Dieback symptoms were most obvious during the warm summer months, although putative infections might have occurred during the rainy winter and early spring seasons in California. Symptoms of Cytospora canker includes bark lesions with dead phloem and cambium, discoloration of the xylem, wood necrosis and gumming occurring at the canker margin. Cankers were often depressed or sunken, eventually causing splitting of the bark or girdling of branches. Cankers were most commonly associated with pruning wounds, sunburn, and oil injuries. A single French prune orchard in Yuba County, where the grower re-planted trees to fill the gaps from trees killed by Cytospora canker, showed 92 % *Cytospora* infection of pruning cuts made to select the main scaffolds of the newly planted trees. Wood cankers expressed as wedge shaped to irregularly shaped vascular discolorations of the xylem tissue below the affected bark area. Eventually, pycnidia occurred just beneath the periderm giving the bark a pimpled appearance diagnostic of *Cytospora* infection. Removing the periderm generally exposed numerous, solitary and scattered pycnidia. Erumpent pycnidia eventually ruptured the bark outermost layers exposing white (characteristic in branches of French prune) apical discs above the cankered area or in the dead branches and twigs. Spore tendrils consisting of conidial masses (cirrus) exuding from pycnidia generally were visible in the orchards following spring rains. Signs and symptoms of *Cytospora* associated cankers in various fruit and nut host plants are illustrated in Figs 1–3.

Phylogenetic analyses

For ML analyses, the best-fit model of nucleotide evolution was deduced based on the AIC (K2+G+I for both *ACT1* and *TEF1*, HKY+G for *TUB2*, and ITS and combined analyses both utilized GTR+G+I). PCR amplification of the ITS region generated 497–527 bp fragments and the alignment of 229 ITS sequences resulted in a 604-character dataset (350 characters were constant, 74 characters were parsimony-uninformative, and 180 characters were parsimony informative (30 %)). MP analyses produced a single most parsimonious tree of 973 steps and a consistency index (CI), retention index (RI), and rescaled consistency index (RC) of 0.4193, 0.8813, and 0.3692, respectively. PCR amplification of the *TEF1* locus generated 588–664 bp fragments and the alignment of 161 *TEF1* sequences resulted in a 799-character dataset (313 characters were constant, 124 characters were parsimony-uninformative, and 362 characters were parsimony informative (45 %)). MP analyses produced four equally most parsimonious trees of 1411 steps and a CI, RI, and RC of 0.5506, 0.9470, and 0.5227, respectively. PCR amplification of the *TUB2* locus was problematic for 14 isolates, which reside in sister clades as described below, nevertheless PCR amplification of the *TUB2* locus generated 527–554 bp fragments and the alignment of 136 *TUB2* sequences resulted in a 575-character dataset (428 characters were constant, 28 characters were parsimony-uninformative, and 119 characters were parsimony informative (21 %)). MP analyses produced four equally most parsimonious trees of 350 steps and a CI, RI, and RC of 0.6171, 0.9485, and 0.5834, respectively. PCR amplification of the *ACT1* locus generated 280–298 bp fragments and the alignment of 184 *ACT1* sequences resulted in a 365-character dataset (149 characters were constant, 74 characters were parsimony-uninformative, and 142 characters were parsimony informative (39 %)). MP analyses produced a single most parsimonious tree of 585 steps and a CI, RI, and RC of 0.4825, 0.9308, and 0.4836, respectively. The analysis of individual datasets yielded similar trees that only differed in the order of species divergences and varying levels of clade support (ITS, Fig. S1; *TEF1*, Fig. S2; *TUB2*, Fig. S3; and *ACT1*, Fig. S4).

The multi-locus dataset consisted of 2334 characters (1242 characters were constant, 293 characters were parsimony-uninformative, and 799 characters were parsimony informative (34 %)). MP analysis produced a single most parsimonious tree of 3434 steps and a CI, RI, and RC of 0.4947, 0.9253, and 0.4589, respectively. MP and ML analyses revealed that 150 Californian *Cytospora* isolates were divided into 15 species, five of which have been described previously (*C. chrysosperma*, *C. parakantschavelli*, *C. punicae*, *C. sorbicola*, and *Valsa eucalypti*) and 10 of which are not associated with a type or non-type isolate with DNA sequence data and thus represent novel phylogenetic species (Fig. 4). Descriptions of all species and taxonomic proposals are provided in the species descriptions and taxonomy section below.

TAXONOMY

Morphological comparisons coupled with multi-locus phylogenetic analyses (MP and ML) of the combined four-gene dataset identified 10 distinct and strongly supported lineages for which no apparent species names exist. Thus, we propose the following new species names and a new combination to properly circumscribe these unique taxa. Additionally, two previously described species are described from North America for the first time.

Fig. 4. The single most parsimonious tree generated from maximum parsimony analysis of the four-gene (ITS, *TEF1*, *TUB2*, and *ACT1*) combined dataset. Numbers in front and after the slash represent parsimony and likelihood bootstrap values from 1000 replicates, respectively. Values represented by an asterisk were less than 70 % for the bootstrap analyses. Ex-type isolates are indicated in **bold**. Bar indicates the number of nucleotide changes.


Cytospora sorbicola
Cytospora amygdali sp. nov.
Cytospora pruinora sp. nov.

Fig. 4. (Continued).

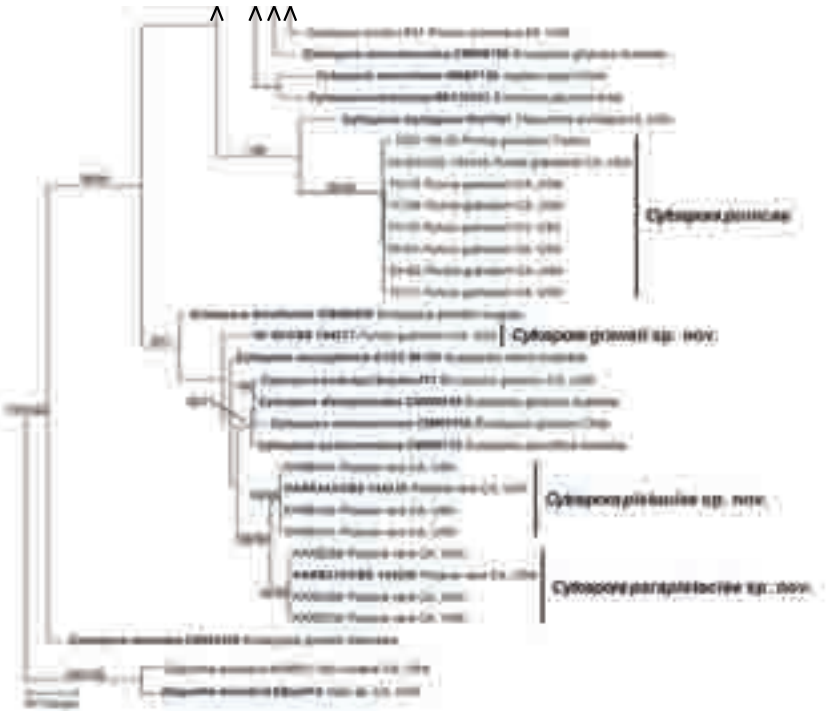

Fig. 4. (Continued).

Cytospora amygdali D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824274
(Figs 4–5)

Etymology: The name refers to the host, almond.

Diagnosis: *Cytospora amygdali* can be distinguished from the phylogenetically closely related *C. plurivora* by the production of large robust conidia and solitary pycnidia in culture.

Type: **USA**: *California*: Yolo County, isolated from wood canker of *Prunus dulcis*, 3 Mar. 2016, *L.A. Holland LH357* (BPI 910650 [dried culture] – holotype; CBS 144233 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, solitary, globose to subglobose, without conceptacle, mouse-grey in centre with white to off-white surface hyphae, (455–)570–690(–850) µm diam (*n* = 20), with 1–2 internal locules. *Conidiophores* hyaline, smooth-walled, reduced to single monoblastic straight filamentous conidiogenous cells (5.5–)5.9–7.1(–7.5) × (1.0–)0.9–1.1(–1.0) µm (*n* = 20), that are wider at the base and taper towards apex. *Conidia* abundant, relatively large with wide girth, single, hyaline, eguttulate, aseptate, allantoid, (6.0–)6.2–7.0(–7.0) × (1.5–)1.6–1.8(–2.0) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 57 mm, medium-growing, slightly dentate, off-white outer margin, and cinnamon-colored inner margin with centre of the colony becoming dark mouse-grey with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Yolo County (California, USA).

Host: *Prunus dulcis*.

Notes: Based on the phylogenetic inference obtained in this study, *C. plurivora* is the closest relative to *C. amygdali*, albeit without significant bootstrap support. *Cytospora amygdali* produces larger conidia, (6.0–)6.2–7.0(–7.0) × (1.5–)1.6–1.8(–2.0) µm, in terms of both length and width and pycnidia are always solitary, contrary to smaller conidia, (3.5–)3.8–4.4(–4.5) × (1.0–)0.9–1.1(–1.5) µm, and aggregated pycnidia produced by *C. plurivora*.

Cytospora californica D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824275
(Figs 4 and 6)

Etymology: The name refers to the geographical region, California, from where this fungus was first isolated.

Diagnosis: *Cytospora californica* can be distinguished from the species *C. eucalypti* by the former producing, on average, shorter conidia (*C. californica* (4.0–)4.5–5.5(–6.0) × (1.0–)1.2–1.6(–1.5) µm *vs*. *C. eucalypti* (5.0–)5.4–6.5(–7.5) × (1.0–)1.2–1.6(–2.0) µm) and slower growth rate (*C. californica* 58.8 mm in 7 d *vs*. *C. eucalypti* 85 mm in 7 d) and pattern in culture (*C. californica* produces two distinct margins in culture, with the internal margin darker than the peripheral margin, while *C. eucalypti* generally produces a homogenous pattern in culture).

Type: **USA**: *California*: Lake County, isolated from wood canker of *Juglans regia*, 14 Mar. 2014, *T.J. Michailides 9C-24* (BPI 910651 [dried culture] – holotype; CBS 144234 – ex-holotype culture).

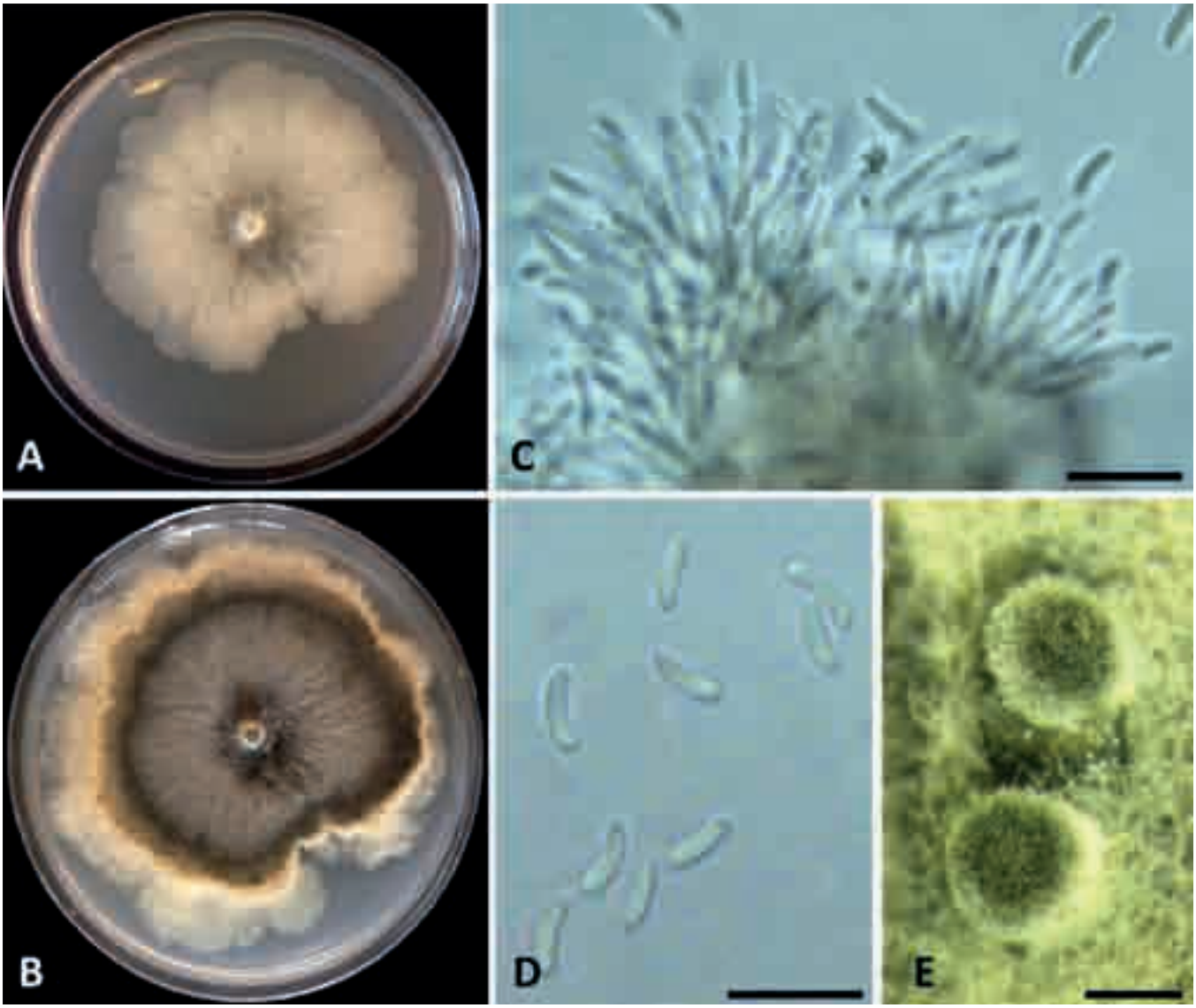

Fig. 5. *Cytospora amygdali* (ex-holotype culture CBS 144233). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Conidiophores and filamentous conidiogenous cells. **D.** Conidia. **E.** Pycnidia. Bars C–D = 10 µm; E = 500 µm.

Description: *Conidiomata* on PDA pycnidial, mostly solitary, rarely aggregated, globose to subglobose, without conceptacle, white, (1255–)1356–1834(–2100) µm diam (*n* = 20), with multiple internal locules with shared invaginated walls. *Conidiophores* hyaline, smooth-walled, reduced to 3–4 monoblastic branching filamentous conidiogenous cells (5.0–)5.9–7.9(–9.0) × (1.0–)1.1–1.5(–1.5) µm (*n* = 20) that taper towards the apex. *Conidia* abundant, single, hyaline to brown, eguttulate, aseptate, allantoid, (4.0–)4.5–5.5(–6.5) × (1.0–)1.2–1.6(–2.0) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 58.8 mm, medium-growing, margin mostly smooth with some unevenness, with short aerial tufts giving a cottony appearance, margin white to off-white with buff centre. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Glenn, Fresno, Kern, Lake, San Joaquin, and Stanislaus Counties (California, USA).

Hosts: *Juglans regia*, *Pistacia vera*, and *Prunus dulcis*.

Notes: Based on the phylogenetic inference obtained in this study, *C. eucalypti* (syn. *Valsa eucalypti*) is the closest relative to *C. californica*. Most micro-morphological observations between the two species overlap, however the colony growth rate of *C. californica* is much slower (58.8 mm in 7 d) than that of *C. eucalypti* (85 mm in 7 d), and *C. californica* produces, on average, shorter conidia (4.0–)4.5–5.5(–6.5) than *C. eucalypti* (5.0–)5.4–6.5(–7.5). Amplification of the *TUB2* locus using the primers Bt1a/Bt1b was problematic for this taxon. Several different annealing temperatures were attempted (annealing temperature ranging from 50–60 °C) with marginal success as only 19 out of 30 *C. californica* isolates produced a reliable *TUB2* PCR amplicon.

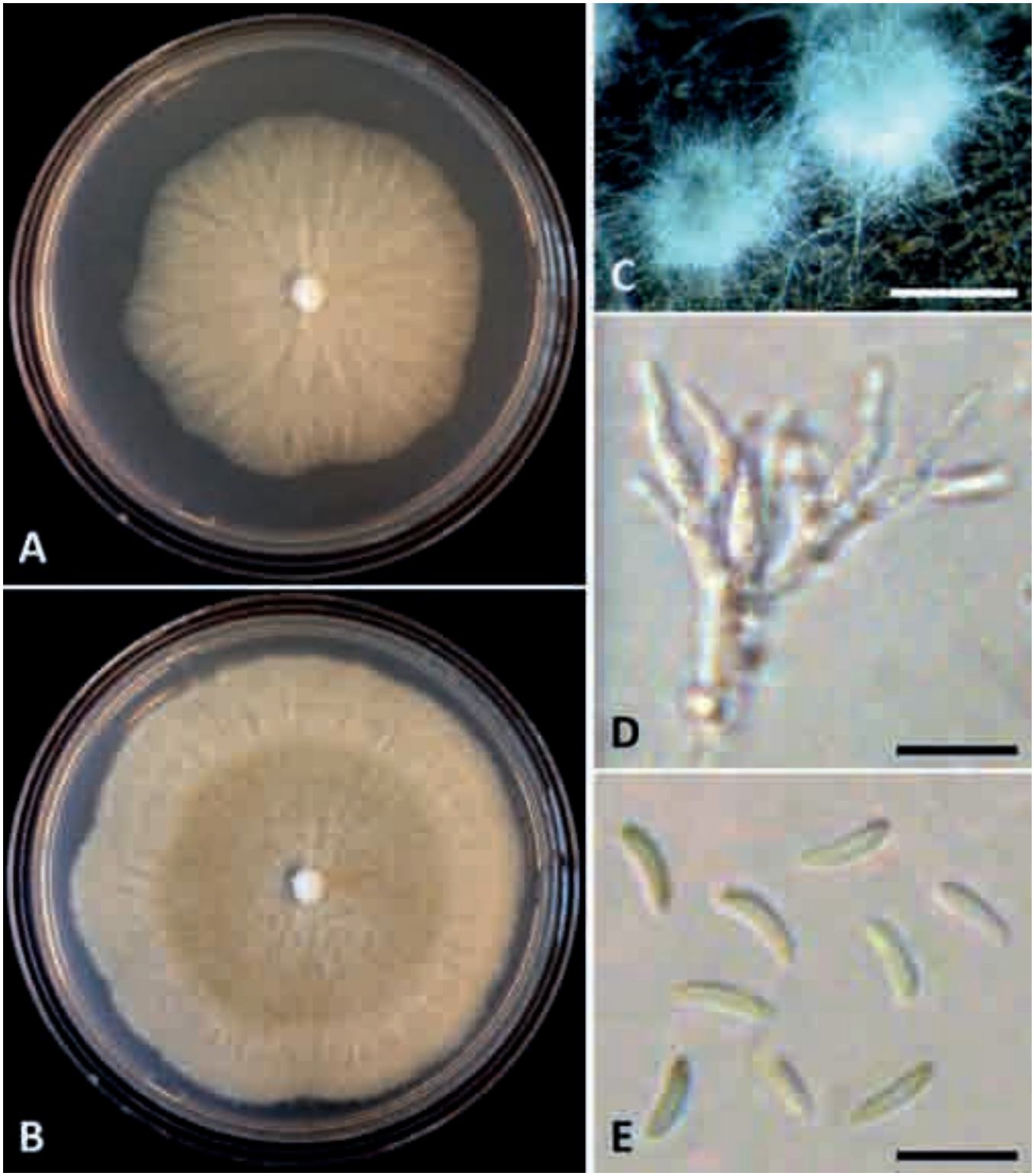

Fig. 6. *Cytospora californica* (ex-holotype culture CBS 144234). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 1 mm; D = 10 µm; E = 5 µm.

Cytospora chrysosperma (Pers.) Fr., *Syst. Mycol.* **2**(2): 542 (1823); nom. sanct.

Basionym: *Sphaeria chrysosperma* Pers., *Neues Mag. Bot.* **1**: 82 (1794).

Synonyms: *Naemaspora chrysosperma* (Pers.) Pers., *Obs. Mycol.* **1**: 80 (1796). *Naemaspora populina* Spreng., *Fl. Hal.*: 354 (1806).

(Figs 4 and 7)

Description: *Conidiomata* on PDA pycnidial, mostly solitary, sometimes aggregated, globose to subglobose, without conceptacle, grey with off-white surface hyphae, (960–)1119–1681(–2070) µm diam (*n* = 20), with multiple internal locules with shared invaginated walls. *Conidiophores* some straight, some reduced to branching filamentous conidiogenous cells that taper towards the apex (7.0–)7.2–8.8(–10.0) × (1.0–)1.1–1.3(–1.5) µm (*n* = 20). *Conidia* abundant, single,

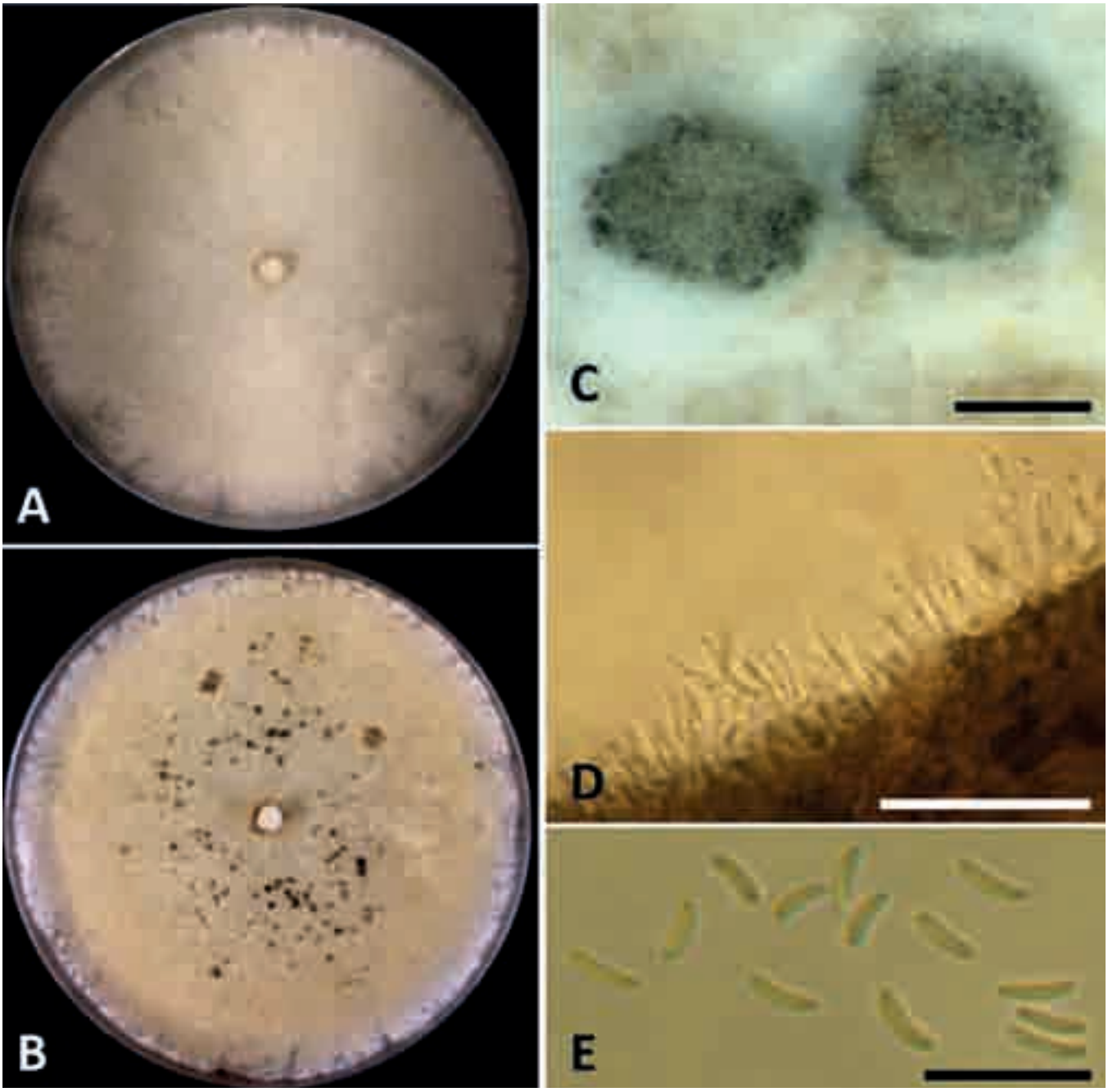

Fig. 7. *Cytospora chrysosperma* (CBS 144242). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 1 mm; D = 20 µm; E = 10 µm.

hyaline to light brown, eguttulate, aseptate, allantoid, small, (3.0–)3.0–3.6(–4.0) × (1.0–)0.9–1.1(–1.0) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colony of *C. chrysosperma* isolate 9E-33, 90 mm diam in 7 d at 25 °C on PDA, fast-growing, off-white to cream with short aerial tufts giving a cottony appearance, aerial hyphae becoming darker with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: China, Germany, Iran, The Netherlands, South Africa, Switzerland, the UK, and USA (Fresno County, California).

Hosts: The USDA Fungus-Host Distribution Database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm lists more than 260 host records for *C. chrysosperma*, therefore a limited list is provided here: *Crataegus azarolus*, *Ficus carica*, *Juglans regia*, *Ligustrum latifolium*, *Malus pumila*, *Morus alba*, *Olea sativa*, *Persica vulgaris*, *Prunus armeniaca*, *Prunus domestica*, *Robinia pseudoacacia*, *Salicaceae*, *Sophora japonica*, *Thuja orientalis*, *Triticum*, *Ulmus*, and *Vitis vinifera*.

Notes: Based on the phylogenetic inference obtained in this study, *C. chrysosperma* is sister to the clade that contains *C. joaquinensis*, *C. longiostiolata*, *C. melnikii*, *C. populicola*, *C. rostrata*, *C. salicacearum*, and *C. salicina*. *Cytospora chrysosperma* is the type species of the genus, and CFCC

89600 is an ex-type strain of the species (Fan *et al.* 2015) and our isolate 9E-33 clusters strongly with that strain.

Specimen examined: **USA**, *California*: Fresno County, isolated from shoot of *Camellia* sp., 21 May 2014, *T.J. Michailides 9E-33* (BPI 910652 [dried culture]; CBS 144242).

Cytospora eucalypti (Cooke & Harkn.) D.P. Lawr., L.A. Holland & Trouillas, **comb. nov.**
MycoBank MB824284
(Figs 4 and 8)

Basionym: *Valsa eucalypti* Cooke & Harkn., *Grevillea* **9**: 51 (1881).

Synonyms: *Engizostoma eucalypti* (Cooke & Harkn.) Kuntze, *Rev. Gen. Plant.* **3**(2): 474 (1884).

Valsa eucalypti var. *myrti* Rolland, *Bull. Soc. Mycol. France* **21**: 22 (1905).

Leucostoma sequoiae Bonar, *Mycologia* **20**: 295 (1928).

Type: **USA**: *California*: on dead branches of *Eucalyptus globulus* 1880, *Cooke & Harkness* (UM 15128, MSC 11471 – isotypes).

Description: *Conidiomata* on PDA pycnidial, mostly solitary, rarely aggregated, globose, without conceptacle, dark black-grey, appearing dry, (990–)1268–1742(–2060) µm diam (*n* = 20), with multiple internal locules with shared invaginated walls. *Conidiophores* short, reduced to branching filamentous conidiogenous cells tapering toward apices (5.5–)8.1–11.1(–11.5) × (1.0–)1.3–2.1(–2.5) µm (*n* = 20). *Conidia* abundant, relatively large, single, hyaline, eguttulate, aseptate, allantoid, (5.0–)5.4–6.5(–7.5) × (1.0–)1.2–1.6(–2.0) µm (*n* = 50). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 85 mm, fast-growing, buff to honey with short aerial tufts giving a cottony appearance, aerial hyphae becoming darker with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Fresno, Marin, Merced, San Joaquin, and Santa Clara Counties (California, USA).

Hosts: *Eucalyptus globulus*, *Eucalyptus paniculata*, *Eucalyptus* sp., *Prunus dulcis*, *Sequoia sempervirens*, and *Sequoiadendron gigateum*.

Notes: The species name *Cytospora eucalypti* has been applied in the past (Sharma *et al*. 1985), however no type was indicated and this appeared in a research report that

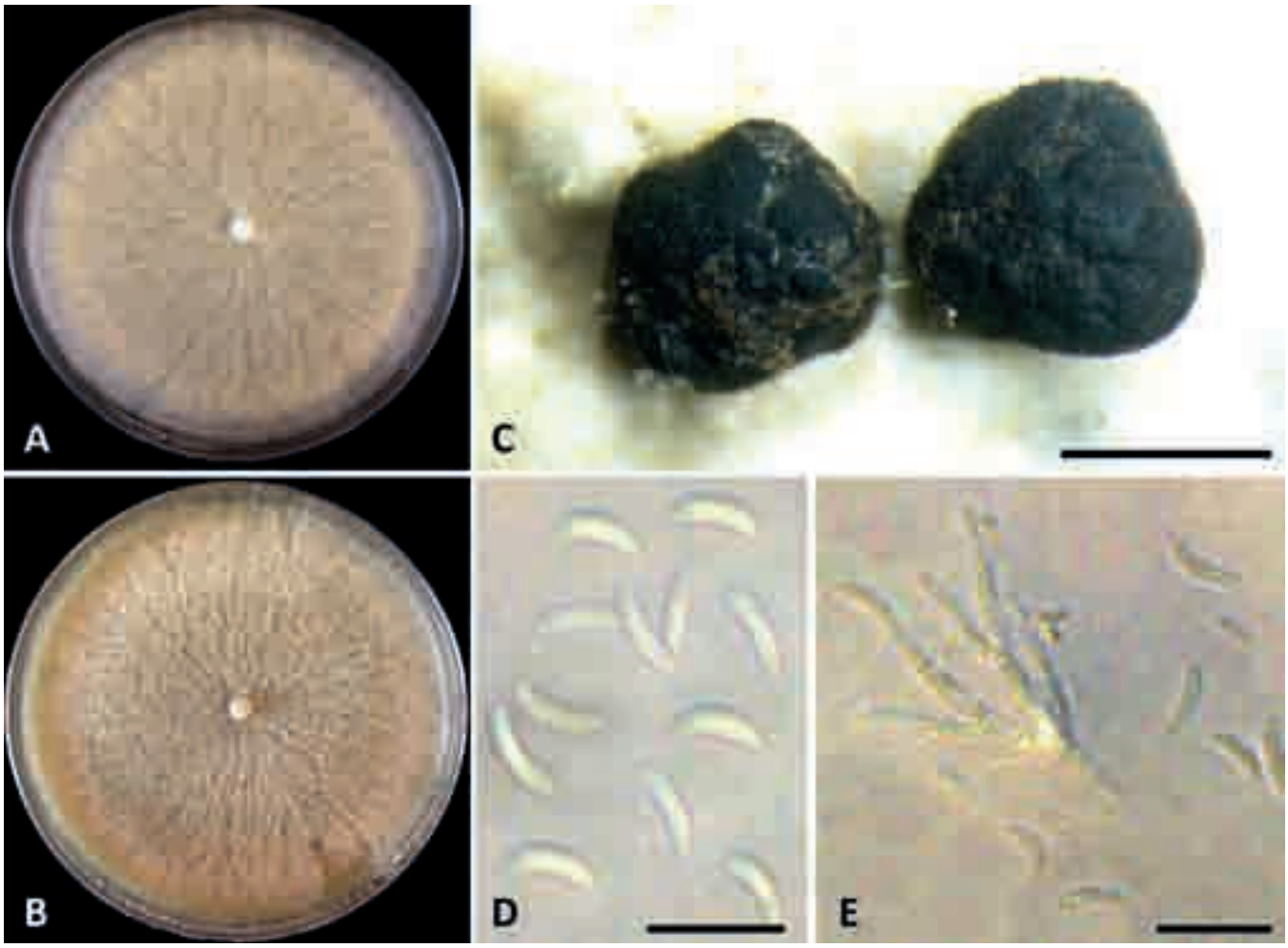

Fig. 8. *Cytospora eucalypti* (CBS 144241). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidia. **E.** Conidiophores and filamentous conidiogenous cells. Bars C = 1 mm; D–E = 10 µm.

was not effectively published, so the name was not validly published (Adams *et al*. 2005). The Californian isolates cluster strongly with an isolate named as *Valsa eucalypti* (CBS 116815 from *Sequoia sempervirens*) which also clusters with isolates collected from *Eucalyptus* in California (Adams *et al.* 2005, 2006). Based on the phylogenetic inference obtained in this study, *C*. *eucalypti* is sister to *C. californica*. Most morphological observations between the two species overlap, however, the colony growth rate of *C*. *eucalypti* is much faster (85 mm in 7 d) than that of *C*. *californica* (58.8 mm in 7 d), and *C*. *eucalypti* produces, on average, longer conidia than *C*. *californica* (4.0–)4.5–5.5(–6.0). Amplification of the *TUB2* locus using the primers Bt1a/Bt1b was problematic. Several different annealing temperatures were attempted with little success as only one out of five isolates produced a reliable *TUB2* amplicon. Similar *TUB2* PCR failures were encountered with the sister species *C*. *californica*, suggesting apomorphic nucleotide substitution(s) in these primer site(s).

Specimen examined: **USA**: *California*: Merced County, isolated from wood canker of *Prunus dulcis*, 28 Sep. 2016, *F.P. Trouillas KARE1585* (BPI 910653 [dried culture]; CBS 144241).

Cytospora granati D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824278
(Figs 4 and 9)

Etymology: The name refers to the host, *Punica granatum*, from which this fungus was first isolated.

Diagnosis: *Cytospora granati* can be distinguished from *C*. *eucalypticola* by the former producing, on average, longer and wider conidia.

Type: **USA**: *California*: Tulare County, isolated from wood canker of *Punica granatum*, 29 Jul. 2011, *T.J. Michailides 6F-45* (BPI 910654 [dried culture] – holotype; CBS 144237 – ex-holotype culture).

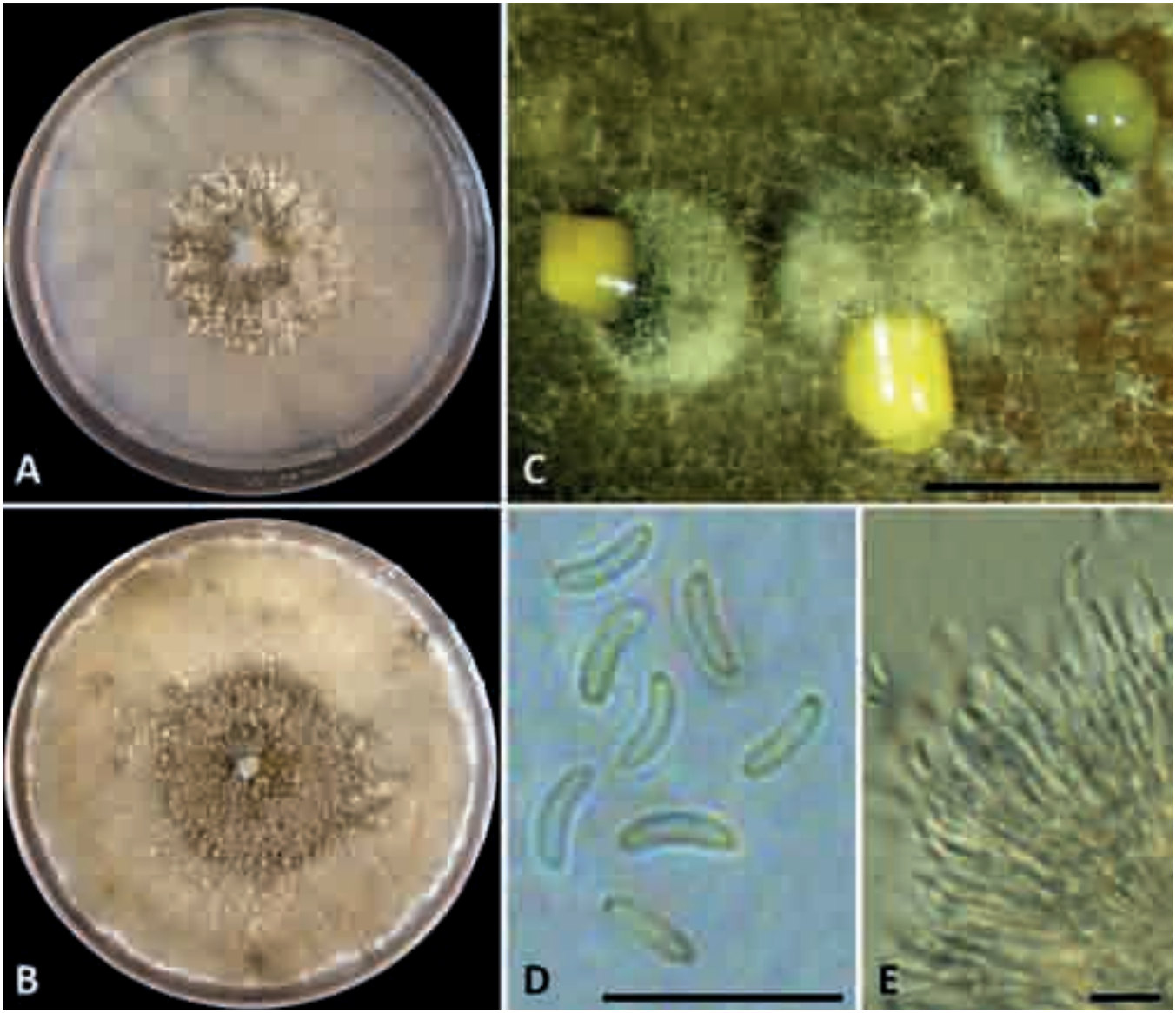

Fig. 9. *Cytospora granati* (ex-holotype culture CBS 144237). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidia. **E.** Conidiophores and filamentous conidiogenous cells. Bars C = 1 mm; D–E = 10 µm.

Description: *Conidiomata* on PDA pycnidial, mostly solitary, sometimes aggregated, globose, conical to discoid, with yellow-coloured conidial exudate, without conceptacle, off-white to light-grey, (610–)673–897(–975) µm diam (*n* = 20), with a single internal locule. *Conidiophores* reduced to straight filamentous conidiogenous cells (16.0–)19.3–23.5(–26.5) × (2.0–)3.7–4.1(–5.0) µm (*n* = 20). *Conidia* copious, single, hyaline to light brown, aseptate, allantoid (4.0–)4.1–4.5(–5.0) × (1.0–)1.1–1.3(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 87.3 mm, fast-growing, white to buff, raised mixed olivaceous white colony centre with flat colony expansion throughout with buff margin in mature colonies. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Tulare County (California, USA).

Host: *Punica granatum*.

Notes: Based on the phylogenetic inference obtained in this study, *C. granati* resides in a clade that contains *Cytospora* species isolated from *Eucalyptus* in Australia (*C. austromontana*, *C. diatrypelloidea*, and *C. eucalypticola*), California (*C. berkeleyi*), Chile (*C. cinereostroma*), and Uruguay (*C. disciformis*), and from *Pistacia vera* in California (*C. parapistaciae* and *C. pistaciae*). This study identified two distantly related *Cytospora* species recovered from symptomatic pomegranate trees. *Cytospora granati* is easily distinguished from *C. punicae* by differences in pycnidial sizes (*C. granati* pycnidia (610–)673–897(–975) µm are almost twice as large, on average, as compared to *C. punicae* (210–)237–383(–490) µm)), the much faster colony growth rate (*C. granati* (87.3 mm in 7 d) than *C. punicae* (64.7 mm in 7 d)), and colony colour/morphology (*C. granati* produces a white to buff colony while *C. punicae* produces a characteristic dark red colony).

Cytospora joaquinensis D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824276
(Figs 4 and 10)

Etymology: The name refers to the San Joaquin Valley of California where the species was found.

Diagnosis: *Cytospora joaquinensis* can be distinguished from the related *C. melnikii*, *C. salicacearum*, and *C. salicina* as *C. joaquinensis* produces, on average, longer conidia.

Type: **USA**: *California*: San Joaquin County, isolated from wood canker of *Populus deltoides*, 21 Apr. 2016, *F.P. Trouillas KARE975* (BPI 910655 [dried culture] – holotype; CBS 144235 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, mostly solitary, rarely aggregated, most with yellow conidial exudate, globose, no conceptacle, black-grey with off-white surface hyphae, (970–)1097–1533(–1760) µm diam (*n* = 20), with multiple internal locules with shared invaginated walls. *Conidiophores* reduced to mostly straight unbranched filamentous conidiogenous cells (6.5–)7.7–10.1(–13.5) × (1.0–)1.1–1.3(–1.5) µm (*n* = 20). *Conidia* abundant, single, hyaline to light brown, eguttulate, aseptate, allantoid, (5.0–)5.1–5.7(–6.0) × (1.0–)1.1–1.3(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 86.7 mm, fast-growing, buff-coloured with short aerial tufts giving a cottony appearance, aerial hyphae becoming darker with age, centre becoming honey-coloured that extends to a white margin. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Fresno, Kern, San Joaquin, and Tulare Counties (California, USA).

Hosts: *Juglans regia*, *Pistacia vera*, and *Populus deltoides*.

Notes: Based on the phylogenetic inference obtained in this study, *C. melnikii*, *C. salicacearum*, and *C. salicina* are the closest relatives to *C. joaquinensis*. Conidia of *C. joaquinensis* (5.0–)5.1–5.7(–6.0) × (1.0–)1.1–1.3(–1.5), on average, are longer than *C. melnikii* (3.1–)4.5–5 × 1–1.2(–1.3) µm, *C. salicacearum* (3.6–)4.9–6.4 × 0.9–1(–1.3) µm, and *C. salicina* (3.6–)4.2–4.7 × 1–1.1(–1.3) µm (Norphanphoun *et al.* 2017).

Cytospora longispora D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824277
(Figs 4 and 11)

Etymology: The name refers to the exceptionally long conidia of this species.

Diagnosis: Unique mosaic colony morphology and conidia that are relatively long (6.0–)6.6–7.4(–7.5) × (1.0–)1.1–1.4(–1.5) µm as compared to most other *Cytospora* species.

Type: **USA**: *California*: Glenn County, isolated from wood canker of *Prunus domestica*, 22 Oct. 2014, *T.J. Michailides 10F-57* (BPI 910656 [dried culture] – holotype; CBS 144236 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, solitary, sometimes aggregated, many with cream-coloured conidial exudate, globose, no conceptacle, (805–)827–1393(–1635) µm (*n* = 20), with a single internal locule. *Conidiophores* smooth-walled, straight, reduced to filamentous conidiogenous cells (6.5–)7.9–10.9(–11.5) × (1.0–)1.0–1.4(–1.5) µm (*n* = 20). *Conidia* long, abundant, single, hyaline, eguttulate, aseptate, allantoid, (6.0–)6.6–7.4(–7.5) × (1.0–)1.1–1.4(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 67.3 mm, medium-growing, white to buff with short aerial tufts giving a cottony appearance in the centre, radially growing hyphae submerged, hyphae becoming darker with age. Outer margin a mosaic of sienna and amber with dark patches and a buff margin. *Hyphae* hyaline, smooth, straight, branched, and septate.

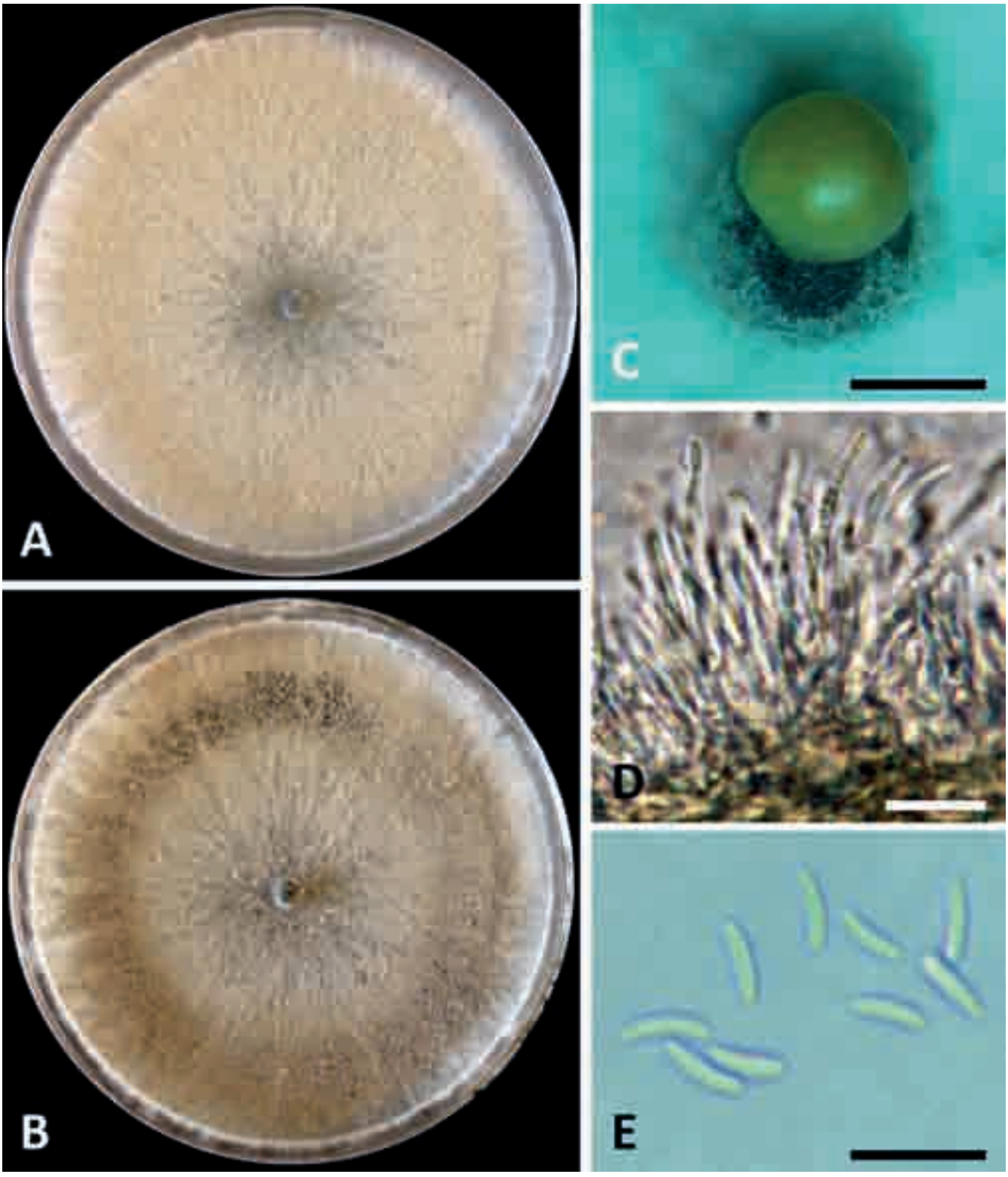

Fig. 10. *Cytospora joaquinensis* (ex-holotype culture CBS 144235). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidium. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 1 mm; D = 20 µm; E = 10 µm.

Distribution: Glenn County (California, USA).

Host: *Prunus domestica*.

Notes: Based on the phylogenetic inference obtained in this study, *C. longispora* clusters in a strongly supported clade that contains *C. ampulliformis*, *C. cotini*, *C. personata*, *C. ribis*, *C. rosarum*, *C. tanaitica*, and *C. ulmi*. Conidia of all relatives are, on average, much shorter than *C. longispora*, with the exception of the recently described *C. ampulliformis* which produces larger conidia to 9 µm in length (Norphanphoun *et al.* 2017).

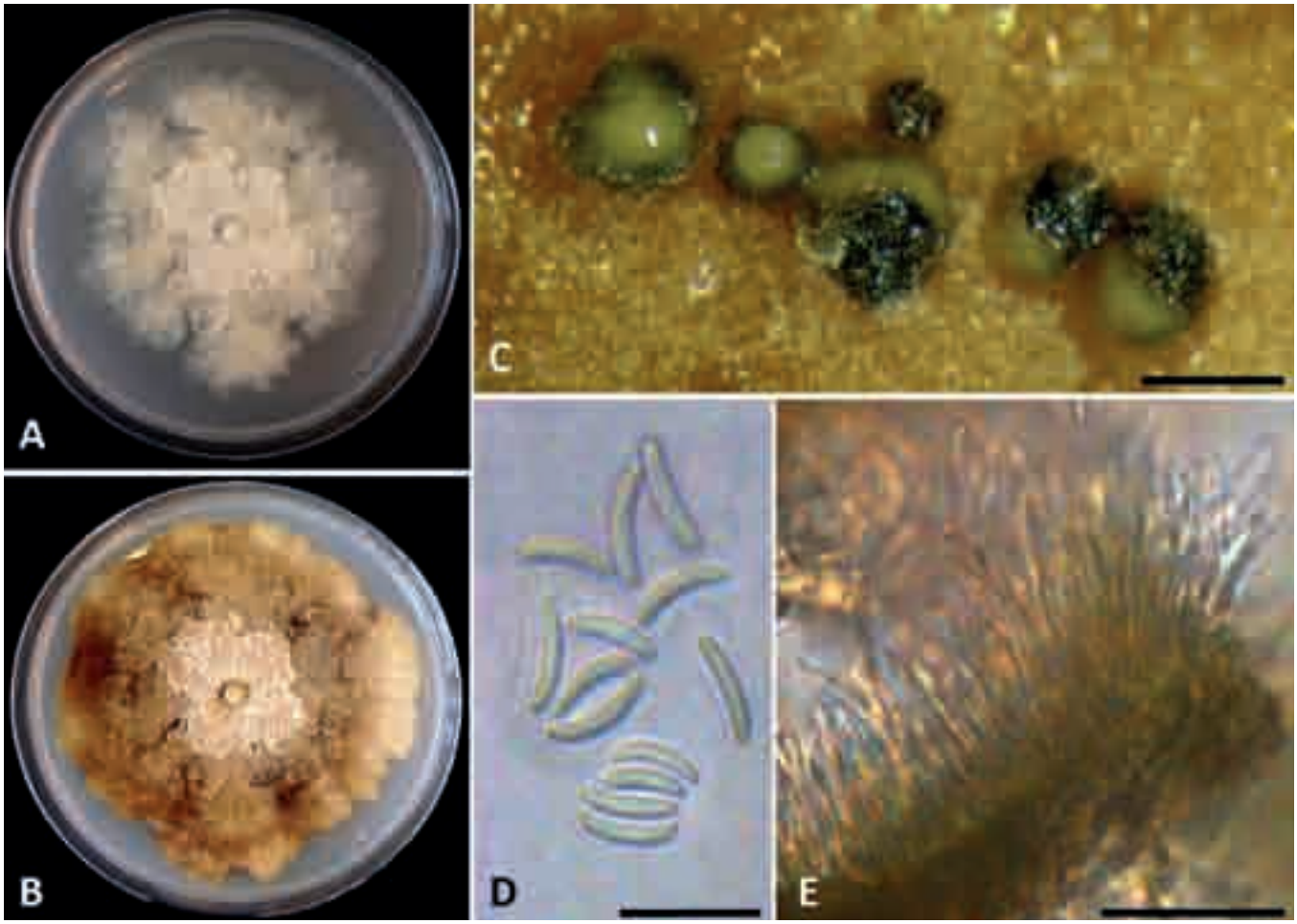

Fig. 11. *Cytospora longispora* (ex-holotype culture CBS 144236). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidia. **E.** Conidiophores and filamentous conidiogenous cells. Bars C = 1 mm; D = 10 µm; E = 20 µm.

Cytospora oleicola D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824279
(Figs 4 and 12)

Etymology: The name refers to the host *Olea* and *-cola* for inhabitor.

Diagnosis: Conidia of *C. oleicola* are wider and longer, on average, as compared to the closely related *C. pruinosa*.

Type: **USA**: *California*: San Joaquin County, isolated from twig canker of *Olea europaea*, 19 Apr. 2016, *F.P. Trouillas KARE1021* (BPI 910657 [dried culture] – holotype; CBS 144248 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, mostly solitary, rarely aggregated, globose, light mouse-grey to almost black (640–)715–1185(–1545) µm diam (*n* = 20), with a single internal locule. *Conidiophores* straight, reduced to branching filamentous conidiogenous cells (6.5–)7.5–9.3(–12.5) × (1.0–)1.0–1.6(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline to light brown, eguttulate, aseptate, allantoid, relatively large (5.5–)5.9–6.5(–7.0) × (1.5–)1.5–1.7(–2.0) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 63.7 mm, medium-growing, white to off-white with sparse aerial tufts, peripheral hyphae submerged, hyphae becoming buff with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: San Joaquin County (California, USA).

Host: *Olea europaea*.

Notes: Based on the phylogenetic inference obtained in this study, *C. pruinosa* (isolated from *Olea europaea* var. *africana* in South Africa) is the closest relative to *C. oleicola*. Conidia of *C. oleicola* (5.5–)5.9–6.5(–7.0) × (1.5–)1.5–1.7(–2.0) µm are, on average, larger in terms of both length and width than conidia of *C. pruinosa* (5–6 × 1.2 µm; Adams *et al*. 2006).

Cytospora parakantschavelli Norphanph. *et al.*, *Mycosphere* **8**: 1 (2017).
(Figs 4 and 13)

Type: **Russia:** on branches and twigs of *Populus* ×*sibirica* 12 May 2015, *T. Bulgakov* (MFLUCC 15-2094 – holotype).

Description: *Conidiomata* in PDA pycnidial, mostly solitary, rarely aggregated, globose, without conceptacle, black-grey

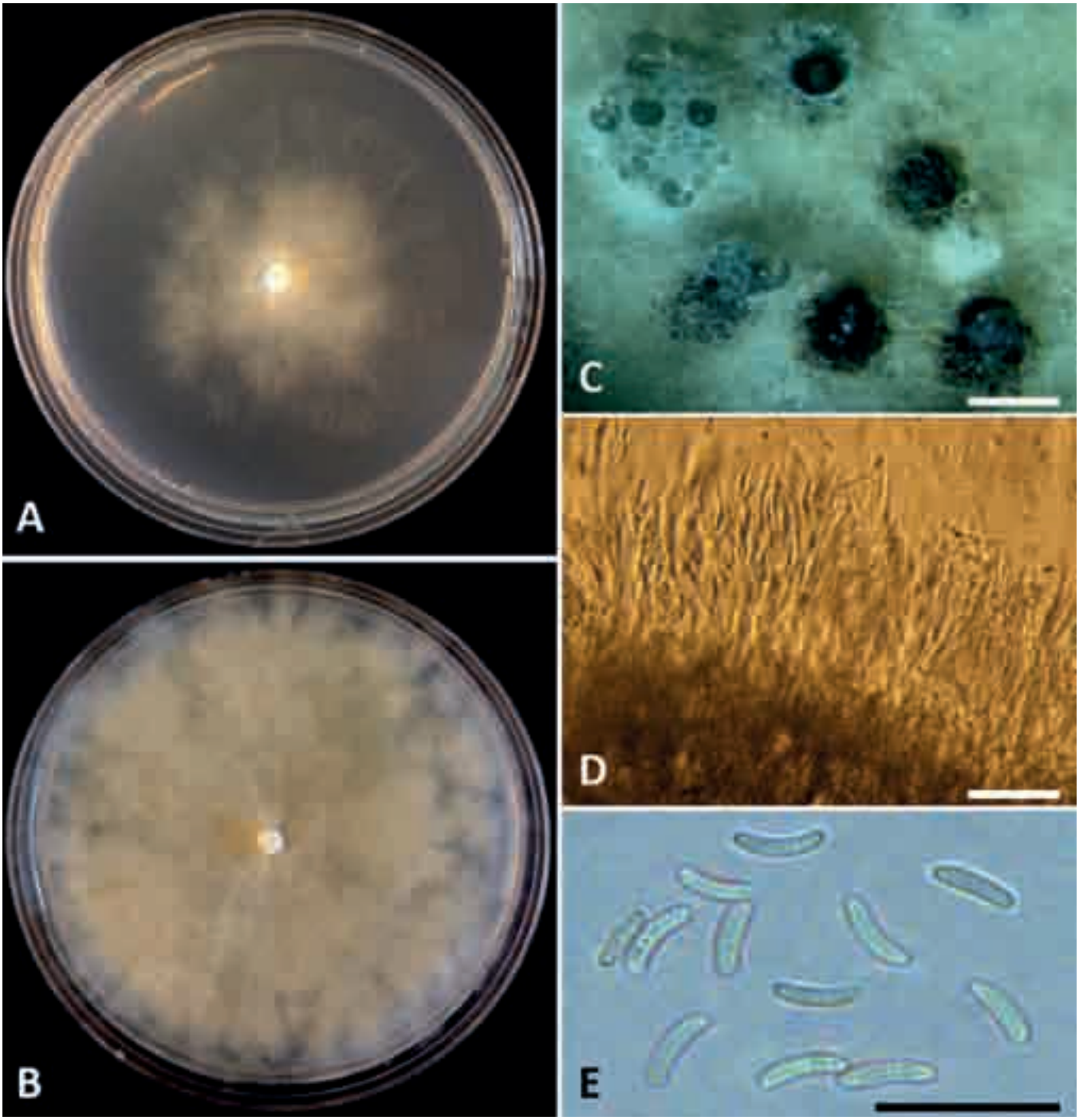

Fig. 12. *Cytospora oleicola* (ex-holotype culture CBS 144248). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 500 µm; D = 20 µm; E = 10 µm.

with off-white surface hyphae, (1215–)1381–2099(–2600) µm diam (*n* = 20), with a single internal locule. *Conidiophores* straight, slender, then branching into 3–4 conidiogenous cells (6.0–)6.9–9.5(–9.5) × (1.0–)1.1–1.5(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline to light brown, eguttulate, aseptate, allantoid, (5.5–)6.0–7.0(–7.5) × (1.0–)1.2–1.6(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colony of *C. parakantschavelli* isolate KARE974 70 mm diam in 7 d at 25 °C on PDA, fast-growing, off-white with cream centre with short aerial tufts giving a cottony appearance, peripheral hyphae submerged, aerial hyphae becoming darker with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Rostov Region, Russia and San Joaquin and Yolo Counties (California, USA).

Hosts: *Populus deltoides*, *Populus freemontii*, *Populus ×sibirica*, and *Pyrus pyraster*.

Notes: Based on the phylogenetic inference obtained in this study, *C. salicicola* and *C. kantschavelli* are the closest relatives to *C. parakantschavellii*. The name *C.*

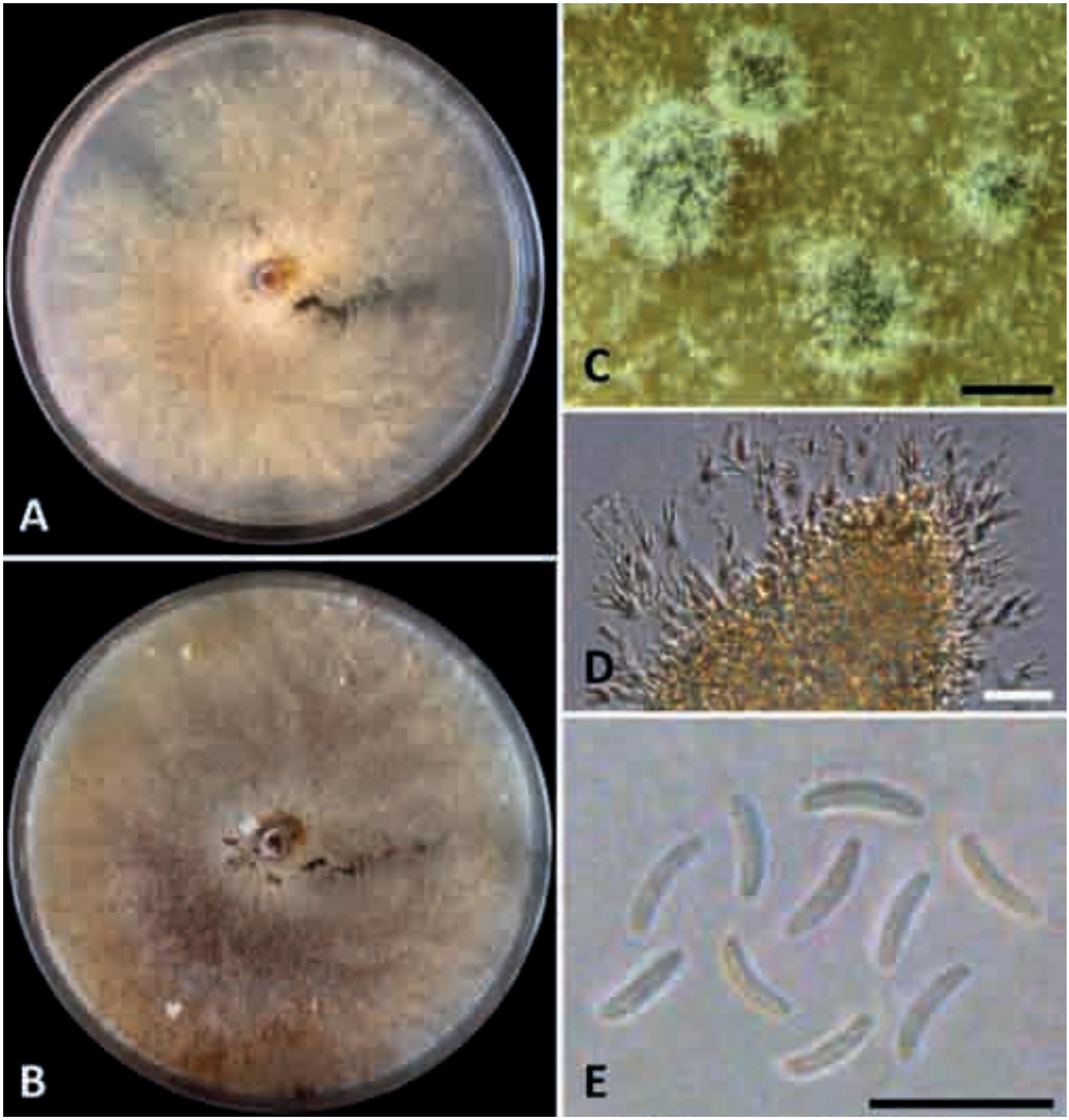

Fig. 13. *Cytospora parakantschavelii* (CBS 144243). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Conidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Pycnidia. Bars C = 20 µm; D = 10 µm; E = 1 mm.

parakantschavellii was recently introduced by Norphanphoun *et al*. (2017) from *Populus* and *Pyrus* in Russia.

Additional specimen examined: **USA**, *California*: San Joaquin County, isolated from wood canker of *Prunus dulcis*, 21 Apr. 2016, *F.P. Trouillas KARE974* (BPI 910658 [dried culture]; CBS 144243).

Cytospora parapistaciae D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824280
(Figs 4 and 14)

Etymology: The name refers to the phylogenetic position of this fungus in relation to the sister taxon *C. pistaciae*.

Diagnosis: *Cytospora parapistaciae* is readily distinguished from *C. pistaciae* based on pycnidial shape (mostly solitary submerged *vs*. globose aggregated) and conidiogenous cells (single straight cells *vs*. 3–4 branching cells).

Type: **USA**: *California*: Kern County, isolated from wood canker of *Pistacia vera*, 26 June 2015, *M.T. Nouri KARE270* (BPI 910659 [dried culture] – holotype; CBS 144506 – ex-holotype culture).

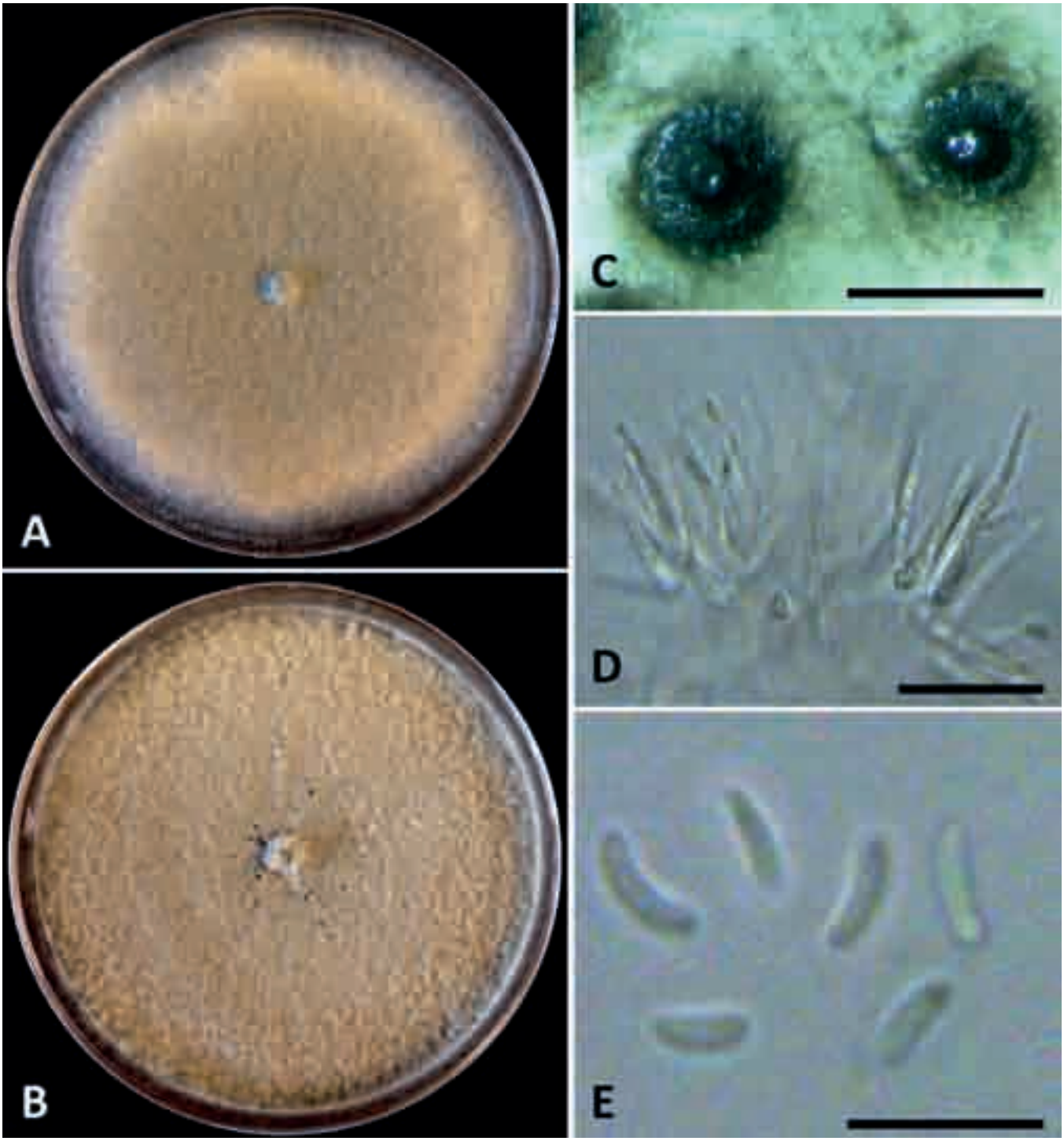

Fig. 14. *Cytospora parapistaciae* (ex-holotype culture CBS 144506). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 500 µm; D–E = 10 µm.

Description: *Conidiomata* on PDA pycnidial, mostly solitary, rarely aggregated, submerged to partially submerged, without conceptacle, black-grey, (335–)390–550(–590) µm diam (*n* = 20), with a single internal locule. *Conidiophores* hyaline, reduced to straight, slender, filamentous conidiogenous cells (7.0–)7.6–9.6(–11.0) × (1.0–)1.2–1.6(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline to light brown, eguttulate, aseptate, allantoid, small, (3.0–)3.5–4.3(–4.5) × (1.0–)0.9–1.1(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 87.3 mm, fast-growing, buff to honey with short aerial tufts giving a cottony appearance, aerial hyphae very dense becoming darker buff to honey with white margin with age. *Hyphae* hyaline to light brown, smooth, straight, branched, and septate.

Distribution: Kern County (California, USA).

Host: *Pistacia vera*.

Notes: Based on the phylogenetic inference obtained in this study, *C. pistaciae* is the closest relative of *C. parapistaciae*, both of which originated from pistachio cankers in two separate counties in California.

Cytospora pistaciae D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824281
(Figs 4 and 15)

Etymology: The name refers to the host, *Pistacia vera*, from which this fungus was first isolated.

Diagnosis: *Cytospora pistaciae* is readily distinguished from *C. parapistaciae* based on pycnidial shape (globose aggregated *vs*. mostly solitary submerged) and conidiogenous cells (3–4 branching cells *vs*. single straight cells).

Type: **USA**: *California*: Merced County, isolated from wood canker of *Pistacia vera*, 14 Oct. 2015, *F.P. Trouillas KARE443*

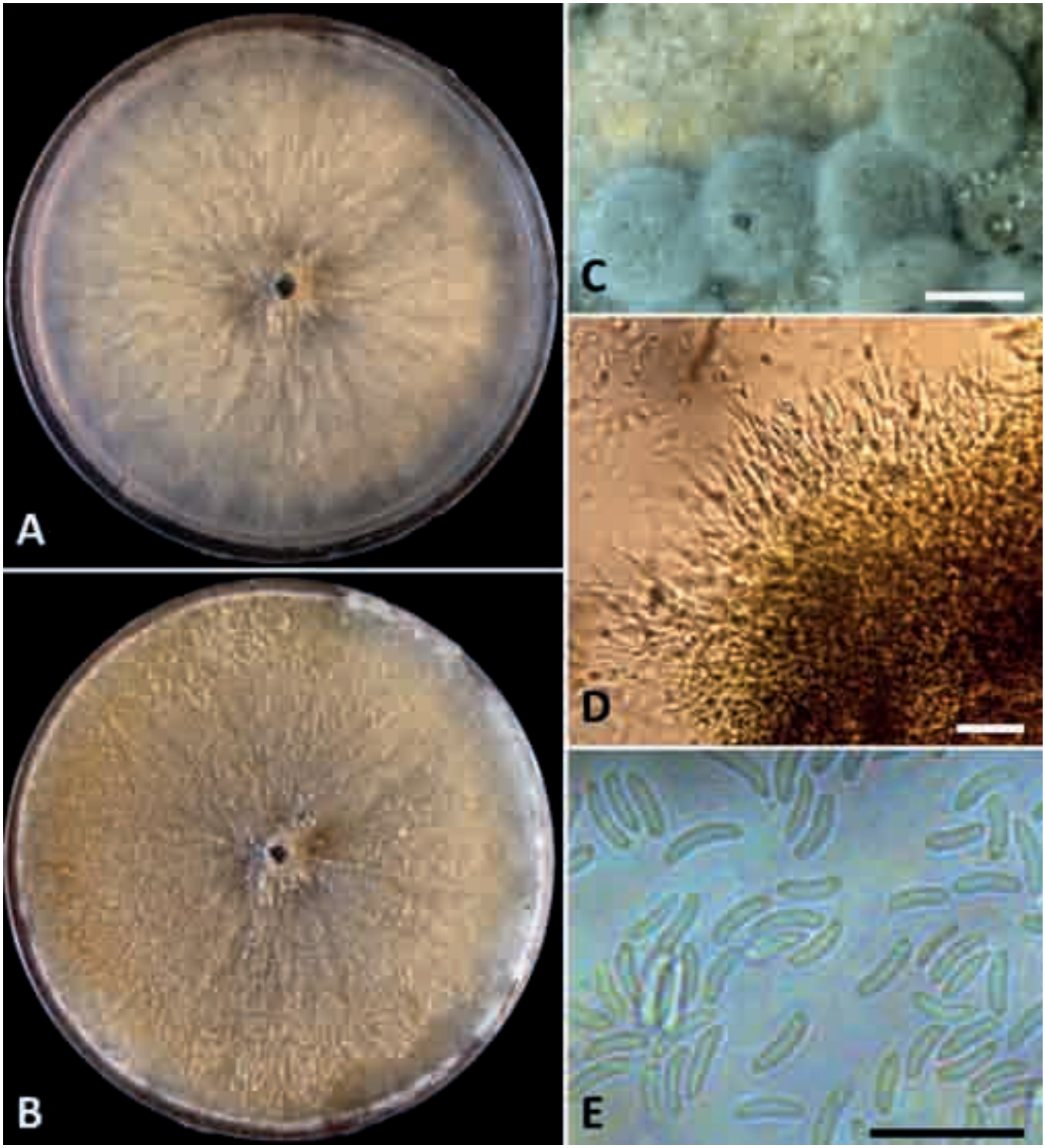

Fig. 15. *Cytospora pistaciae* (ex-holotype culture CBS 144238). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 1 mm; D = 10 µm; E = 20 µm.

(BPI 910660 [dried culture] – holotype; CBS 144238 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, solitary to regularly aggregated, globose, without conceptacle, light mouse-grey, (975–)1196–2184(–2655) µm diam (*n* = 20), with a single internal locule. *Conidiophores* straight, reduced to 3–4 branching filamentous conidiogenous cells (5.5–)7.1–8.9(–10.0) × (1.0–)1.1–1.5(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline, eguttulate, aseptate, allantoid, (3.5–)4.0–4.8(–5.5) × (1.0–)1.1–1.3(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 87.3 mm, fast-growing, buff becoming honey with short aerial tufts giving a cottony appearance, peripheral hyphae submerged, aerial hyphae becoming darker with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Merced County (California, USA).

Host: *Pistacia vera*.

Notes: Based on the phylogenetic inference obtained in this study, *C. parapistaciae* is the closest relative of *C. pistaciae*.

Cytospora plurivora D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824282
(Figs 4 and 16)

Etymology: The name refers to the plethora of hosts this fungus was routinely isolated from.

Diagnosis: *Cytospora plurivora* is distinguished from *C. amygdali* and *C. erumpens* in the smaller conidia in terms of length and width.

Type: **USA**: *California*: San Joaquin County, isolated from twig lesions of *Olea europaea*, 24 June 2016, *F.P. Trouillas KARE1452* (BPI 910661 [dried culture] – holotype; CBS 144239 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, large, some solitary, many gregarious, globose to extended globose,

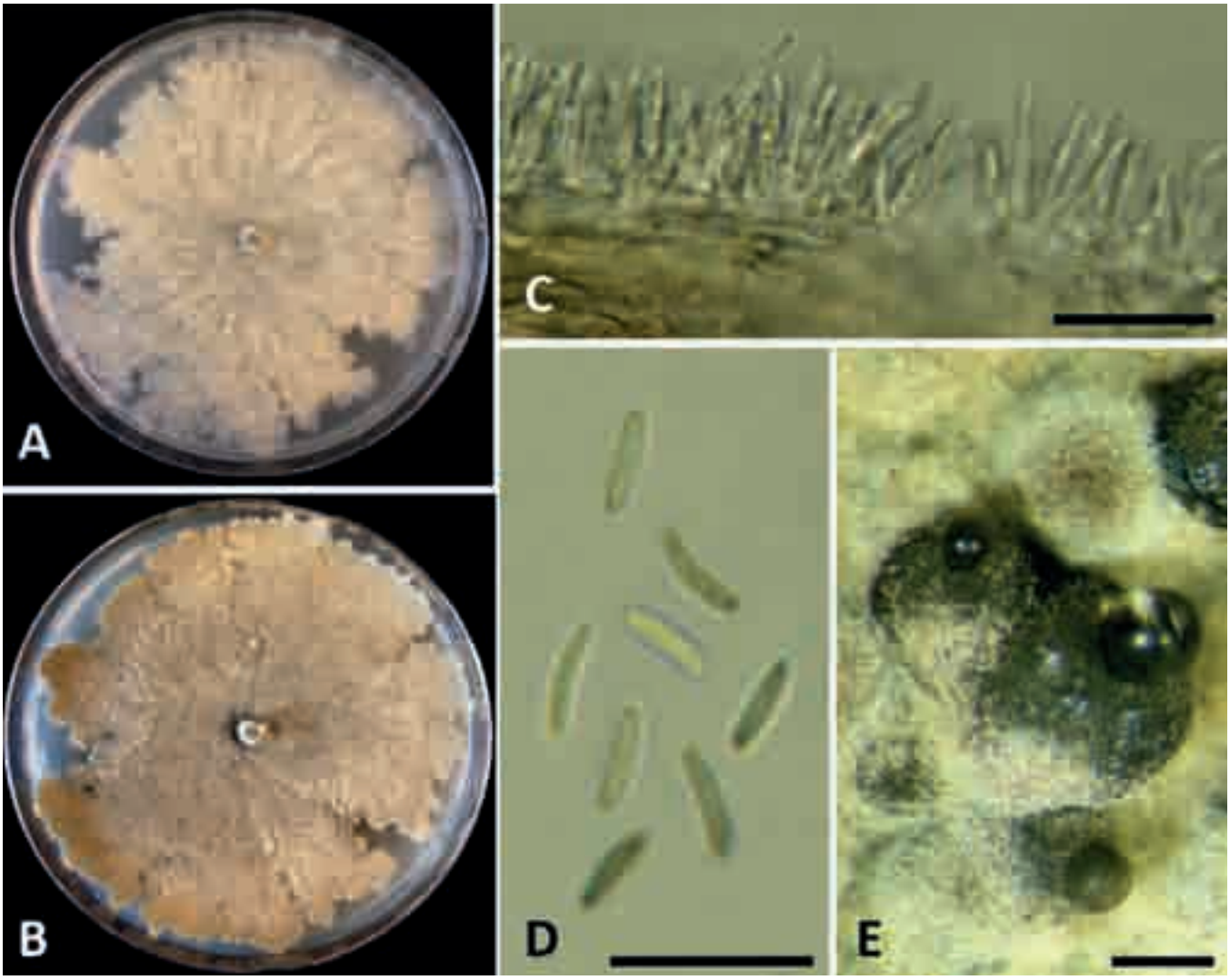

Fig. 16. *Cytospora plurivora* (ex-holotype culture CBS 144239). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Conidiophores and filamentous conidiogenous cells. **D.** Conidia. **E.** Pycnidia. Bars C = 20 µm; D = 10 µm; E = 1 mm.

no conceptacle, black-grey with off-white surface hyphae, (1110–)1152–1968(–2745) µm diam (*n* = 20), with a single internal locule. *Conidiophores* reduced to single, straight, slender, filamentous conidiogenous cells (7.0–)7.7–10.0(–11.0) × (1.0–)1.0–1.4(–1.5) µm (*n* = 20). *Conidia* abundant, single, hyaline to dark brown, eguttulate, aseptate, allantoid, (3.5–)3.8–4.4(–4.5) × (1.0–)0.9–1.1(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 82 mm, fast-growing, uneven lobate growth margin, off-white to cream with short aerial tufts giving a cottony appearance, aerial hyphae becoming light brown with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Butte, Colusa, Contra Costa, Fresno, Glenn, Kern, San Joaquin, Stanislaus, Sutter, Tehama, Tulare, and Yuba Counties (California, USA).

Hosts: *Juglans regia*, *Olea europaea*, *Pistacia vera*, *Prunus domestica*, *Prunus dulcis*, and *Prunus persica*.

Notes: Based on the phylogenetic inference obtained in this study, *C. amygdali* is the closest species to *C. plurivora*, albeit with no statistical support. *Cytospora plurivora* is the most genetically diverse clade identified in this study which in part is likely due to its incidence on many different fruit and nut crop hosts throughout California.

Cytospora populicola D.P. Lawr., L.A. Holland & Trouillas, **sp. nov.**
MycoBank MB824283
(Figs 4 and 17)

Etymology: The name refers to the host *Populus* and *-cola* for inhabitor.

Diagnosis: *Cytospora populicola* is distinguished from *C. longiostiolata* and *C. rostrata* in the shorter conidia than *C. longiostiolata* and larger conidia than *C. rostrata*, respectively.

Type: **USA**: *California*: San Joaquin County, isolated from wood canker of *Populus deltoides*, 21 Apr. 2016, *F.P. Trouillas KARE973* (BPI 910662 [dried culture] – holotype; CBS 144240 – ex-holotype culture).

Description: *Conidiomata* on PDA pycnidial, mostly solitary, rarely aggregated, some with yellow conidial exudate, globose to conical, without conceptacle, black-grey, (1015–)1210–2210(–2735) µm diam (*n* = 20), with a single internal locule. *Conidiophores* reduced to 3–4 filamentous branching conidiogenous cells tapering toward apices (5.5–)6.1–8.1(–10.0) × (1.0–)1.5–1.9(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline, eguttulate, aseptate, allantoid, (4.5–)4.7–5.3(–5.5) × (1.0–)1.1–1.4(–1.5) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colonies after 7 d at 25 °C on PDA average 87.3 mm, medium-growing with uneven margin expansion, off-white with short aerial tufts giving a cottony appearance, aerial hyphae becoming cream-coloured with age. *Hyphae* hyaline, smooth, straight, branched, and septate. *Distribution*: San Joaquin County (California, USA).

Host: *Populus deltoides*.

Notes: Based on the phylogenetic inference obtained in this study, *C. longiostiolata* and *C. rostrata*, both isolated from *Salix*, are the closest species to *C. populicola*. Conidia of *C. populicola* are, on average, larger than those of *C. rostrata* 3.6–4.8 × 1.0–1.6 µm (av. 4.1 × 1.4 µm) and smaller than those of *C. longiostiolata* (3.9)5.4–6.6 × 1.0–1.2(–1.5) (av. 5.5 × 1.3 µm).

Cytospora punicae Sacc., *Michelia* **1**: 367 (1878) ; as '*punica*'.
Figs 4 and 18.

Description: *Conidiomata* on PDA pycnidial, gregarious, globose to subglobose, no conceptacle, black-grey with off-white surface hyphae, (210–)237–383(–490) µm diam (*n* = 20), with multiple internal locules with shared invaginated walls. *Conidiophores* single, straight, filamentous conidiogenous cells (5.5–)5.8–8.6(–9.5) × (1.0–)1.1–1.4(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline to light brown, eguttulate, aseptate, allantoid, (3.5–)3.8–4.6(–5.0) × (0.5–)0.8–1.0(–1.0) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colony of *C. punicae* isolate 5A-80 64.7 mm diam in 7 d at 25 °C on PDA. Medium-growing, dark red becoming lighter with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Fresno, Madera, and Stanislaus Counties (California, USA), Cyprus, Greece, Iran, South Africa, and Turkey.

Host: *Punica granatum*.

Notes: Based on the phylogenetic inference obtained in this study, *C. myrtagena* is the closest species to *C. punicae*. Only two species of *Cytospora* are known from pomegranate (*C. granati* and *C. punicae*) and these can be distinguished by the diagnostic red hyphae/colony of *C. punicae* in culture. The colony growth of *Cytospora punicae* is also much slower (64.7 mm in 7 d) compared to *C. granati* (87.3 mm in 7 d).

Specimen examined: **USA**: *California*: Madera County, isolated from wood canker of *Punica granatum*, 21 July 2010, *T.J. Michailides 5A-80* (BPI 910663 [dried culture]; CBS 144244).

Cytospora sorbicola Norphanph. *et al.*, *Mycosphere* **8**: 1 (2017).
Figs 4 and 19.

Type: **Russia:** on dead and dying branches of *Acer pseudoplatanus* 18 June 2015, *T. Bulgakov* (MFLUCC 15-2203 – holotype).

Description: *Conidiomata* on PDA pycnidial, mostly solitary, sometimes aggregated, globose, without conceptacle,

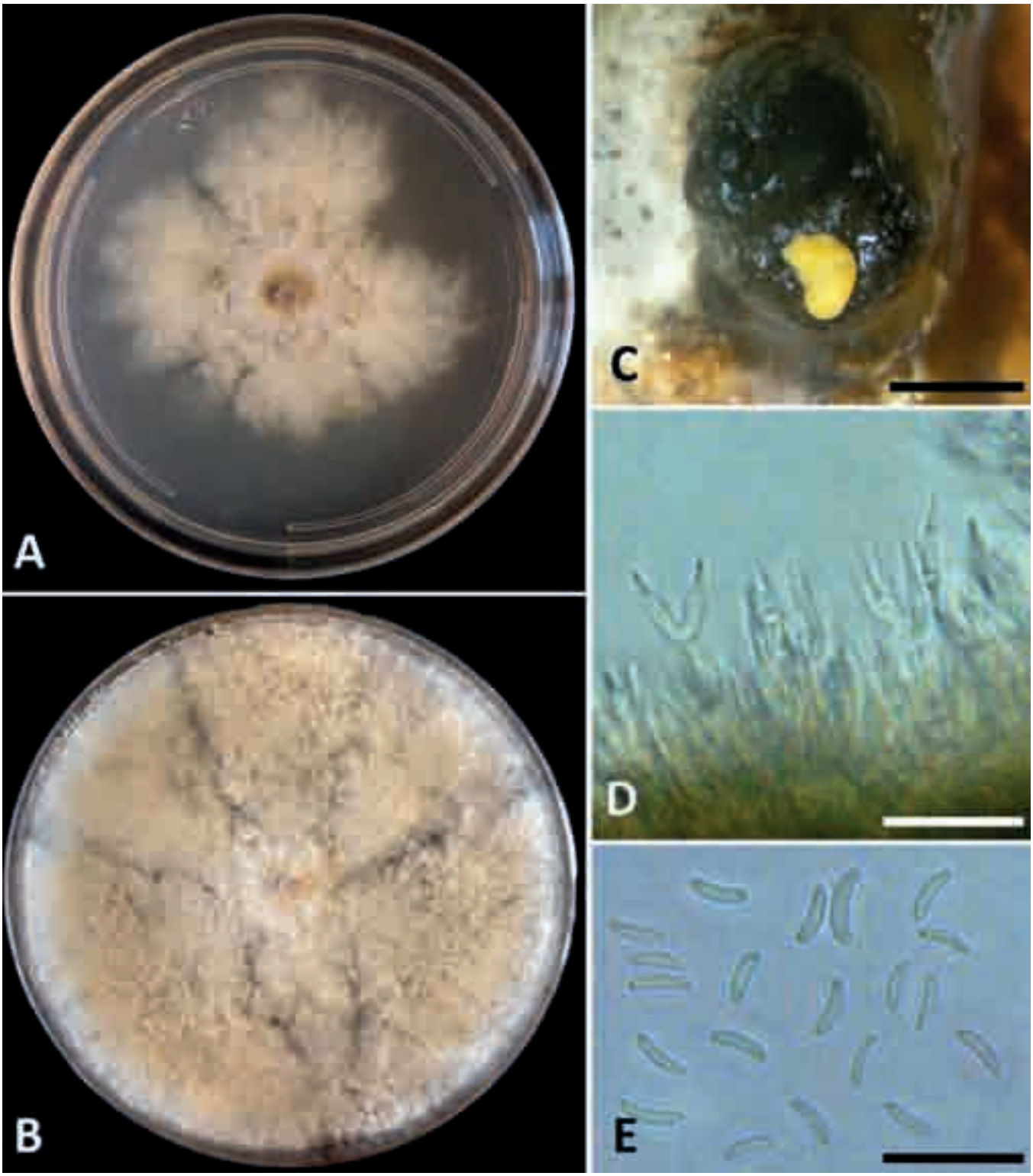

Fig. 17. *Cytospora populicola* (ex-holotype culture CBS 144240). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 500 µm; D–E = 10 µm.

mouse-grey, (1020–)1220–1900(–2420) µm diam (*n* = 20) with 1–2 locules. *Conidiophores* branched, reduced to filamentous conidiogenous cells that taper towards the apices (4.5–)6.4–9.6(–10.0) × (1.0–)1.0–1.4(–2.0) µm (*n* = 20). *Conidia* abundant, single, hyaline to light brown, eguttulate, aseptate, allantoid, (3.5–)4.0–4.6(–4.5) × (1.0–)0.9–1.1(–1.0) µm (*n* = 30). No sexual morph observed.

Culture characteristics: Colony of *C. sorbicola* isolate KARE228 81.7 mm diam in 7 d at 25 °C on PDA, fast-growing, off-white to cream with general lack of aerial hyphae, colony darkens with age. *Hyphae* hyaline, smooth, straight, branched, and septate.

Distribution: Contra Costa, Fresno, Kings, Merced, Sacramento, San Benito, San Joaquin, Stanislaus, Yolo, and

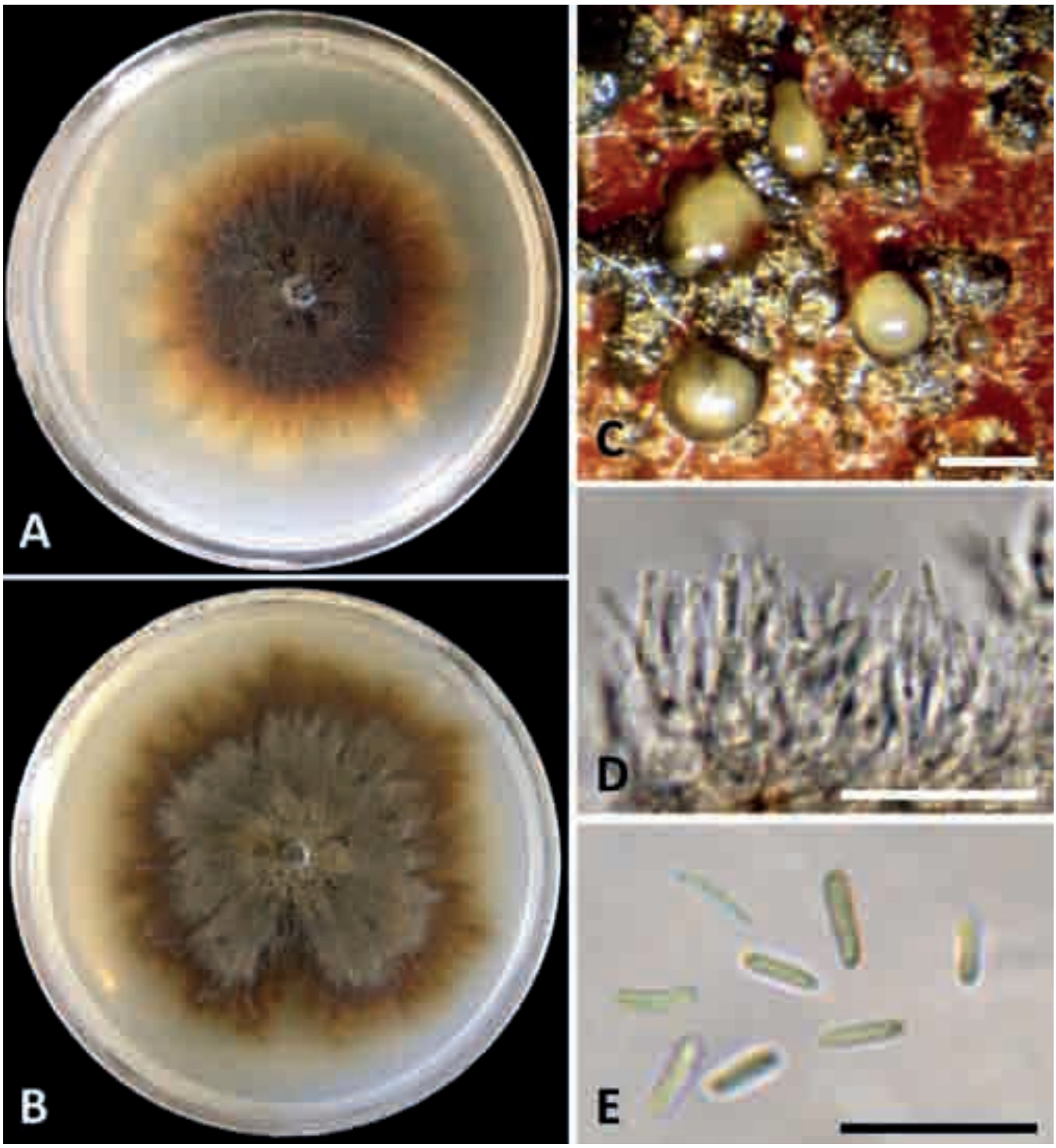

Fig. 18. *Cytospora punicae* (CBS 144244). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 500 µm; D = 20 µm ; E = 10 µm.

Yuba Counties (California, USA), and Rostov Region, Russia.

Hosts: *Acer pseudoplatanus*, *Cotonoeaster melanocarpus*, *Prunus armeniaca*, *P. avium*, *P. cerasus*, *P. domestica*, *P. dulcis*, *P. persica*, and *Sorbaronia mitschurinii*.

Notes: Based on the phylogenetic inference obtained in this study, *C. donetzica* is the closest species to *C. sorbicola*. The *C. sorbicola* isolates collected in this study display some host affiliation with cherry, clustering strongly in the MP analysis and no support in the ML analysis. The level of support for the California-only *C. sorbicola* isolates and differences in morphology suggests that they may represent a distinct lineage sister to *C. sorbicola* collected in Russia. Additional data such as *TEF1* and *TUB2* from the Russian type of *C. sorbicola* will help answer this question.

Additional specimen examined: **USA**, *California*: Stanislaus County, isolated from bark canker of *Prunus dulcis*, 15 July 2015, *M.T. Nouri KARE228* (BPI 910664 [dried culture]; CBS 144245).

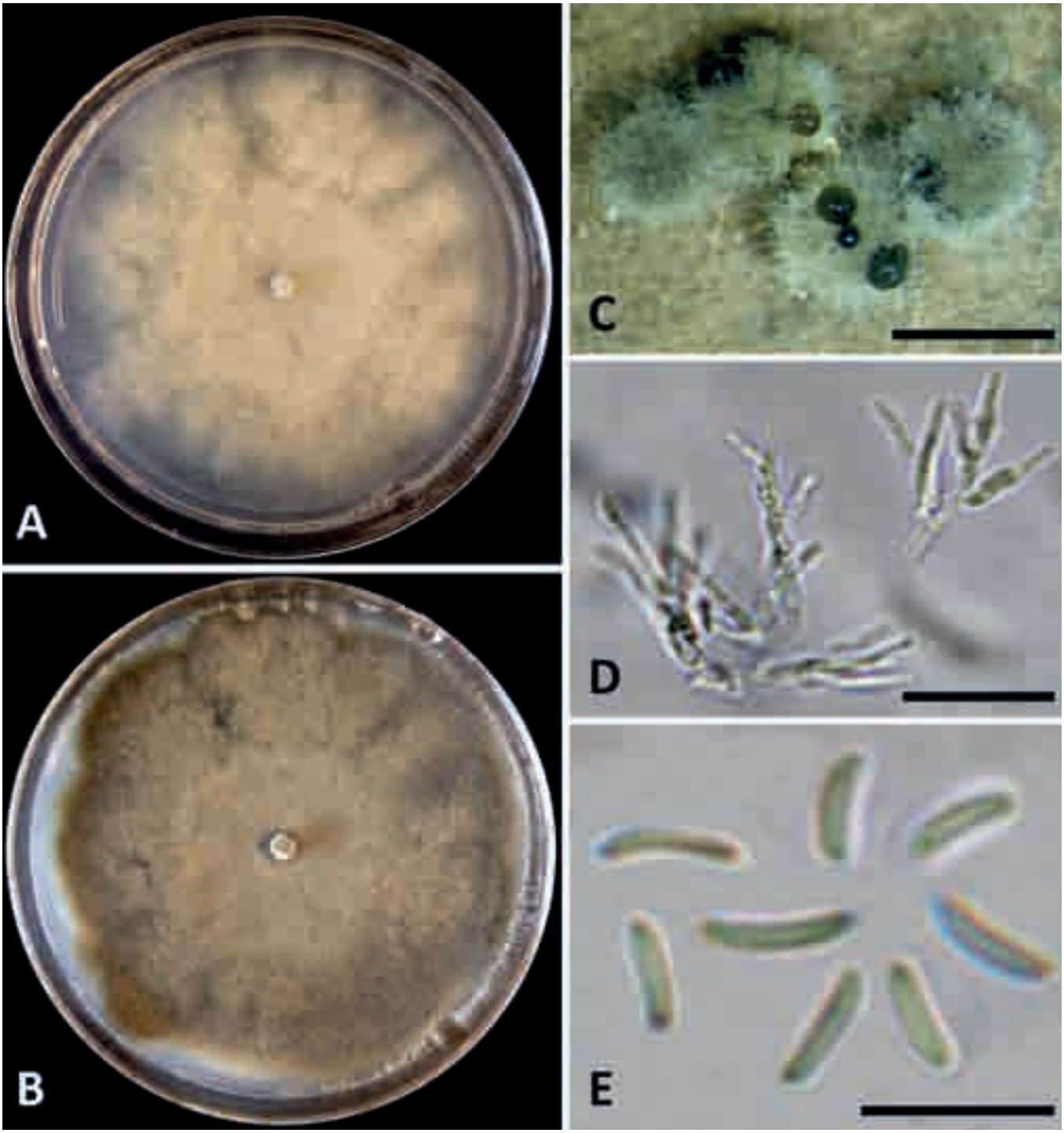

Fig. 19. *Cytospora sorbicola* (CBS 144245). **A.** Seven-day-old PDA culture. **B.** Fourteen-day-old PDA culture. **C.** Pycnidia. **D.** Conidiophores and filamentous conidiogenous cells. **E.** Conidia. Bars C = 1 mm; D = 20 µm; E = 5 µm.

DISCUSSION

This manuscript presents a comprehensive molecular phylogenetic overview of *Cytospora* species currently known from culture, which was initiated due to a high incidence of *Cytospora* species associated with canker symptoms across diverse orchard crops in California. All *Cytospora* species known from culture and linked to publicly-available molecular data were considered for phylogenetic analyses in this study. The lack of ex-type cultures or sequence data for many species names makes it difficult to assess many older species names, especially for those described only by morphology. We deposited ten new ex-type specimens with two different public fungal biodiversity repositories, the Mycology and Nematology Genetic Diversity and Biology Laboratory in Beltsville, MD (BPI), and the Westerdijk Fungal Biodiversity Institute in The Netherlands (CBS), in conjunction with molecular data in GenBank, in order to strengthen and stabilize the taxonomy of *Cytospora* and to aid in the identification of *Cytospora* species *via* DNA sequence data in future studies by other mycologists and plant pathologists.

Cytospora species are ubiquitous, important pathogens of many woody hosts causing cankers, dieback and mortality of forest and urban trees (Adams *et al.* 2005, 2006, Worrall *et al.* 2010) and of many economically important crops including *Juglans*, *Malus*, *Prunus*, and *Vitis* (Biggs & Grove 2005, Wang *et al.* 2011, Fan *et al.* 2015a, Lawrence *et al.* 2017a). Results from this study unveiled 15 species of *Cytospora* from infected orchard crops and adjacent ornamentals in the Central Valley of California. These species include the previously described taxa *C. chrysosperma*, *C. parakantschavelli*, *C. punicae*, and *C. sorbicola* and 10 previously undescribed taxa names which are newly introduced: *C. amygdali*, *C. californica*, *C. granati*, *C. joaquinensis*, *C. longispora*, *C. oleicola*, *C. parapistaciae*, *C. pistaciae*, *C. plurivora*, and *C. populicola*, and a new combination, *C. eucalypti*. All species were strongly supported by both DNA sequence data and morphological observations. This study reports *C. parakantschavelii* and *C. sorbicola* for the first time in North America, including new host records for each species, *Populus deltoides* and *P. freemontii* for *C. parakantschavelii* and *Olea europaea*, *Prunus avium*, *P. domestica*, *P. dulcis*, and *P. persica* for *C. sorbicola*. Our Californian *Cytospora eucalypti* (syn. *Valsa eucalypti*) isolates cluster strongly with an isolate from the coastal redwoods (*Sequoia sempervirens*) reported in Adams *et al*. (2005), which also clusters strongly with isolates previously referred to as *Valsa eucalypti*, isolated from four species of *Eucalyptus* in California (Adams *et al.* 2006). This study expanded the known host range of *C. eucalypti* to include *Prunus dulcis* and *Sequoiadendron gigateum* (giant sequoia) in California.

The utility of asexual morph characters for species recognition has been questioned in *Cytospora*. Locule morphology seems to be influenced by the depth in the bark at which the pycnidia form, with variations from unilocular cytosporoid when formed deep in the bark to rosette cytosporoid when formed near the bark surface (Adams *et al.* 2005). Also, asexual morphs that form in nature can vary considerably from those forming in culture, and these morphological characters are not necessarily taxonomically informative (Adams *et al.* 2005). Considering that sexual morphs are rarely found in nature, the use of sexual morph morphology in species diagnosis has been limited. Furthermore, both ascospores and conidia of many *Cytospora* species are of similar shapes (single, allantoid, and aseptate) and sizes (4–8 × 1–2 µm) thus complicating morphological separation of distinct lineages (Adams *et al.* 2002, 2005, Wang *et al.* 2011). In this study, we found the morphological characteristics of the conidia were indistinguishable among most species, with similar dimensions among the examined species; most asexual morph characters were not taxonomically informative.

The genus *Cytospora* includes both generalist pathogens (i.e. *C. chrysosperma* with 265 host records; USDA Fungus-Host Distribution Database, https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm) and specialist pathogens (i.e. *C. punicae* with only one host record in the same USDA Database). As such, host associations do not appear to constitute an appropriate criterion for species recognition, as previously discussed (Adams *et al.* 2005, 2006). In this study, host association was not found to be taxonomically informative as many *Cytospora* species were recovered from multiple hosts. However, our work highlighted a few instances of close host associations. Prior to this study, *C. punicae* had been reported causing wood canker on pomegranate trees in California, Cyprus, and Iran (Peduto Hand *et al.* 2014, Samouel & Kanetis 2016, Mahdikhani & Davoodi 2017), pomegranate collar rot in Greece (Palavouzis *et al.* 2015), and pomegranate fruit rot in South Africa (Venter *et al.* 2017). *Cytospora punicae* was only recovered from pomegranate trees in this study, supporting this species as host specific despite a wide geographical distribution. Pomegranate trees harboured a second species, *C. granati*, which was only recovered from this host. Both *C. punicae* and *C. granati* have similar conidial shapes and dimensions, but the species have distinct pycnidial shapes and sizes and colony morphologies. Thus, host association paired with morphological observations may have utility when examining *Cytospora* species on pomegranate. In contrast, *C. sorbicola* was isolated from six hosts (almond, apricot, cherry, olive, peach, and plum) and these hosts typically harboured more than one *Cytospora* species. Within the *C. sorbicola* clade, a subclade strongly supported by parsimony analysis (86 %) but showing low support by likelihood analysis (<70 %) contained isolates that originated almost exclusively from cherry. These findings suggest some level of genetic divergence for *C. sorbicola* isolates from cherry, which could indicate some host specialization in these isolates; a preliminary step towards reproductive isolation and ecological speciation (Giraud *et al.* 2010).

Given the variability, plasticity, and complexity of morphological characters in the genus (e.g. stromatal arrangement in the host tissues, locular arrangement within pycnidia, locule division into chambers, independent or shared locular walls), previous studies have advocated the use of molecular data to accurately identify *Cytospora* species (Adams *et al.* 2002, 2005, 2006). In this study, we used molecular phylogenetic analyses of four loci (ITS+*TUB2*+*TEF1*+*ACT1*), not only to identify species but also to provide reference data for future phylogenetic studies. Before this study, most *Cytospora* sequences deposited in GenBank consisted of ITS. While ITS is the primary marker for fungal barcoding (Schoch *et al.* 2012), in some fungal groups, ITS has insufficient power for species recognition whereas protein-coding genes can be more informative sequence regions for species delineation (O'Donnell *et al.* 2015, Lawrence *et al.* 2017b). For instance, analyses of *TEF1* sequence data provided more discriminatory power than ITS in delineating two recently described *Cytospora* species occurring on grapevine, *C. vinacea* and *C. viticola* (Lawrence *et al.* 2017a). In other xylophilous fungi, 'secondary barcodes' such as *TUB2*, *TEF1*, and histone 3 (*HIS*) can also be preferable based on their ability to delineate closely related or cryptic species and on the availability of sequence data for ex-type specimens. For example *TUB2* is the preferred marker for identification of fungi in the *Togniniaceae* (i.e. *Phaeoacremonium minimum*) and *TEF1* is the preferred marker for the *Botryosphaeriaceae* (i.e. *Neofusicoccum parvum*) and *Diaporthales* (which includes *Cytosporaceae*) (Lawrence *et al.* 2017b), especially for closely related or cryptic species. In agreement with previous studies (Adams

et al. 2002, 2005), our findings revealed that ITS has sufficient power to discriminate the 15 *Cytospora* species reported from orchard crops in California. However, based on comparisons of clade support values of each locus used in this study, it appears that *TEF1* is the preferential locus to use for *Cytospora* identification as it was able to strongly support all 15 lineages in this study. Moreover, in our study, 362/799 (45 %) of the aligned nucleotide positions in *TEF1* and 142/365 (39 %) in *ACT1* were parsimony informative, whereas only 119/575 (21 %) and 180/604 (30 %) were parsimony informative in *TUB2* and ITS, respectively. Therefore, a DNA-based approach utilizing several gene regions (in order of priority: *TEF1*, *ACT1*, ITS, and *TUB2* using the primer pairs in this study) would be the best method to resolve *Cytospora* species concepts, especially when morphological characters and host occurrences may be misleading due to significant overlap.

Until the present study, the diversity of *Cytospora* species affecting perennial crops in California has been largely overlooked and underestimated. Historically, two species, *C. cincta* and *C. leucostoma*, have been associated with Cytospora canker of stone fruits and pome fruits in North America (Bertrand & English 1976b, Biggs 1989, Biggs & Grove 2005). Surprisingly, we did not isolate either species in this study, suggesting that *C. cincta* and *C. leucostoma* were originally misidentified as the causal agents of Cytospora canker of stone fruits and pome fruits in California. Our findings suggest that many species of *Cytospora* are involved in the decline of fruit and nut crops in California, and they do not include either *C. cincta* nor *C. leucostoma*. The main putative causal agents of Cytospora canker of stone fruits (apricot, cherry, peach, and prune) in California included *C. plurivora* and *C. sorbicola*. Similarly, the main putative causal agents of Cytospora canker of nut crops (almond, pistachio, and walnut) in California included *C. amygdali*, *C. californica*, *C. eucalypti*, *C. joaquinensis*, *C. parapistaciae*, *C. pistaciae*, *C. plurivora*, and *C. sorbicola*. Three species were associated with Cytospora canker of *Populus* trees, *C. joaquinensis*, *C. parakantschvelii*, and *C. populicola*. *Cytospora joaquinensis* was also associated with cankers in pistachio and walnut, suggesting that cross infections occur between orchards and adjacent ornamentals and *vice versa*. Three species were associated with Cytospora canker of olive (*C. oleicola*, *C. plurivora*, and *C. sorbicola*) with the two latter species also collected from other hosts. Two species were exclusively associated with Cytospora canker of pomegranate (*C. granati* and *C. punicae*). These results strongly suggest the need for additional research concerning the epidemiology of *Cytospora* species that cause Cytospora canker in fruit and nut crops and proximal ornamentals in the diverse agricultural areas of the Central Valley of California.

Research on Cytospora canker of stone fruits had received broad attention before the advent of molecular identification of fungi, focusing on seasonal activities of pathogenic species (Bertrand & English 1976a), spore production (Bertrand & English 1976b), etiology, epidemiology and host resistance (Biggs 1989). According to our findings, pathogenicity studies should now be conducted to elucidate the role of the newly described *Cytospora* species in the fruit and nut crops in California. The large diversity of species revealed in this study also suggests that management of Cytospora canker needs to be re-evaluated following accurate molecular identification to determine the main pathogenic species involved within each crop. Control of *Cytospora* diseases is difficult and focusing management efforts against the most aggressive encountered *Cytospora* species will be essential. The genus *Cytospora* represents a good example of a fungal group where morphological features are extremely complex and not necessarily informative from a taxonomic standpoint, which could in part explain why in North America only two species were previously considered the main causal agents of Cytospora canker of perennial crops. This study constitutes a further step towards a sequence-based description of fungal species in an important group of plant pathogens, revealing a large species richness, providing type specimens associated with molecular data for new taxa, detailed morphological descriptions, and some evidence for appropriate selection of loci for molecular typing. Furthermore, this study provides a firm foundation for future pathogenicity, ecological, and epidemiological studies to better help manage canker diseases in perennial crops infected by *Cytospora* species.

ACKNOWLEGEMENTS

This manuscript is dedicated to the 200-year-old generic name *Cytospora*. We thank the California Cherry Board, the California Pistachio Research Board and the Almond Board of California for financial support. We thank also Francesca Peduto-Hand for supplying images of Cytospora canker of pomegranate.

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Supplementary files can be found on the IMA Fungus website, http://www.imafungus.org/:

Fig. S1. The single most parsimonious tree generated from maximum parsimony analysis of the ITS locus. Numbers in front and after the slash represent parsimony and likelihood bootstrap values from 1000 replicates, respectively. Values represented by an asterisk were less than 70 % for the bootstrap analyses. Bar indicates the number of nucleotide changes.

Fig. S2. One of four equally most parsimonious trees generated from maximum parsimony analysis of the *TEF1* locus. Numbers in front and after the slash represent parsimony and likelihood bootstrap values from 1000 replicates, respectively. Values represented by an asterisk were less than 70 % for the bootstrap analyses. Bar indicates the number of nucleotide changes.

Fig. S3. One of four equally most parsimonious trees generated from maximum parsimony analysis of the *TUB2* locus. Numbers in front and after the slash represent parsimony and likelihood bootstrap values from 1000 replicates, respectively. Values represented by an asterisk were less than 70 % for the bootstrap analyses. Bar indicates the number of nucleotide changes.

Fig. S4. Single most parsimonious tree generated from maximum parsimony analysis of the *ACT1* locus. Numbers in front and after the slash represent parsimony and likelihood bootstrap values from 1000 replicates, respectively. Values represented by an asterisk were less than 70 % for the bootstrap analyses. Bar indicates the number of nucleotide changes.

Figure S1

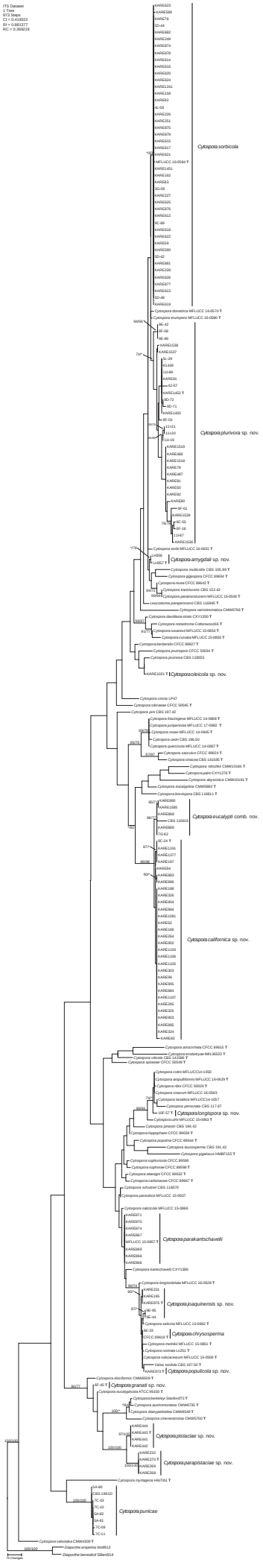

ITS Dataset
1 Tree
973 Steps
CI = 0.410322
RI = 0.881277
RC = 0.360018
Cytospora sorbicola
Cytospora plurivora sp. nov.
Cytospora amygdali sp. nov.
Cytospora oleicola sp. nov.
Cytospora eucalypti comb. nov.
Cytospora californica sp. nov.
Cytospora longispora sp. nov.
Cytospora parakantschavelii
Cytospora joaquinensis sp. nov.
Cytospora chrysosperma
Cytospora populicola sp. nov.
Cytospora granati sp. nov.
Cytospora pistaciae sp. nov.
Cytospora parapistaciae sp. nov.
Cytospora punicae
0.1 Changes

Figure S2

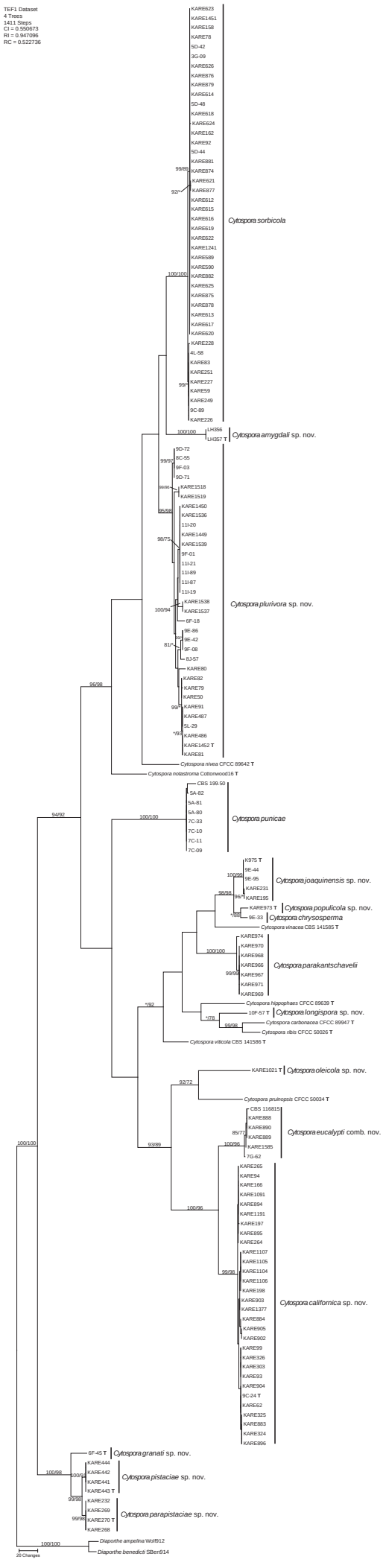
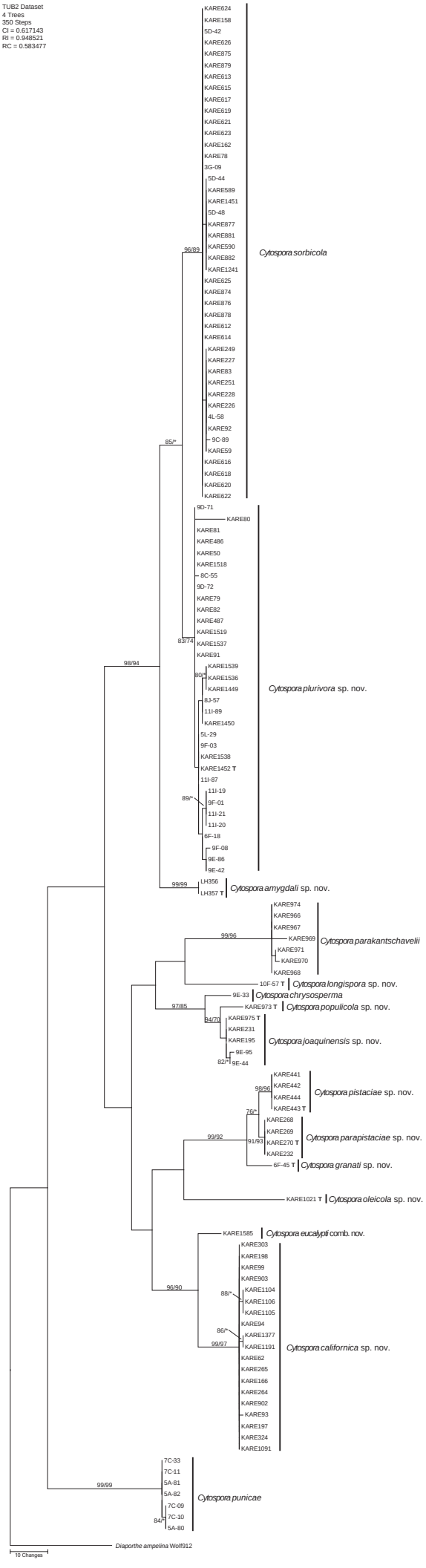

TEF1 Dataset
4 Trees
1411 Steps
CI = 0.550673
RI = 0.947096
RC = 0.522736
Cytospora sorbicola
Cytospora amygdali sp. nov.
Cytospora plurivora sp. nov.
Cytospora nivea CFCC 89642 T
Cytospora notastroma Cottonwood16 T
Cytospora punicae
Cytospora joaquinensis sp. nov.
Cytospora populicola sp. nov.
Cytospora chrysosperma
Cytospora vinacea CBS 141585 T
Cytospora parakantschavelii
Cytospora hippophaes CFCC 89639 T
Cytospora longispora sp. nov.
Cytospora carbonacea CFCC 89947 T
Cytospora ribis CFCC 50026 T
Cytospora viticola CBS 141586 T
Cytospora oleicola sp. nov.
Cytospora pruinopsis CFCC 50034 T
Cytospora eucalypti comb. nov.
Cytospora californica sp. nov.
Cytospora granati sp. nov.
Cytospora pistaciae sp. nov.
Cytospora parapistaciae sp. nov.
Diaporthe ampelina Wolf912
Diaporthe benedicti SBen914
20 Changes

Figure S3


TUB2 Dataset
4 Trees
350 Steps
CI = 0.617143
RI = 0.948521
RC = 0.583477
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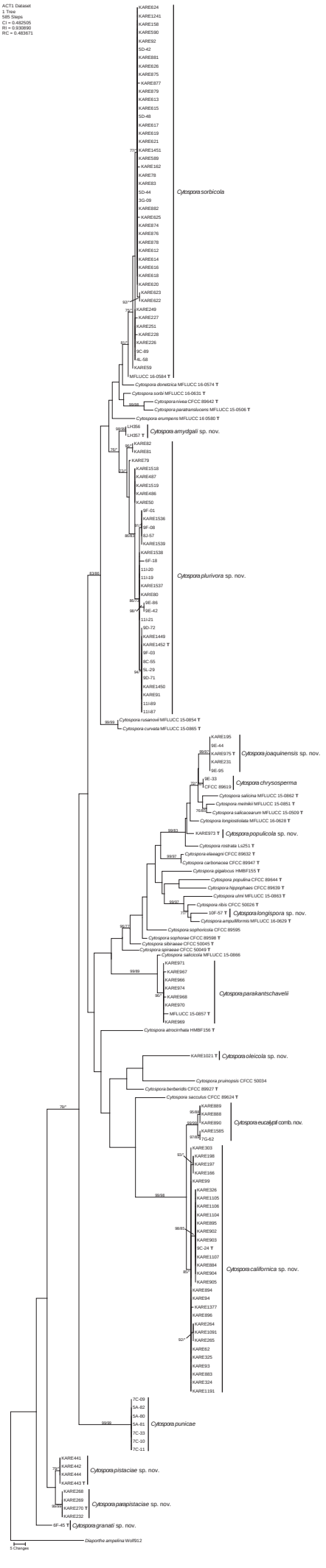
KARE158
5D-42
KARE626
KARE875
KARE879
KARE613
KARE615
KARE617
KARE619
KARE621
KARE623
KARE162
KARE78
3G-09
5D-44
KARE589
KARE1451
5D-48
KARE877
KARE881
KARE590
KARE882
KARE1241
KARE625
KARE874
KARE876
KARE878
KARE612
KARE614
KARE249
KARE227
KARE83
KARE251
KARE228
KARE226
4L-58
KARE92
9C-89
KARE59
KARE616
KARE618
KARE620
KARE622
Cytospora sorbicola
96/89
85/*
9D-71
KARE80
KARE81
KARE486
KARE50
KARE1518
8C-55
9D-72
KARE79
KARE82
KARE487
KARE1519
KARE1537
KARE91
KARE1539
KARE1536
KARE1449
8J-57
11I-89
KARE1450
5L-29
9F-03
KARE1538
KARE1452 T
11I-87
11I-19
9F-01
11I-21
11I-20
6F-18
9F-08
9E-86
9E-42
Cytospora plurivora sp. nov.
83/74
80
89/*
98/94
99/99
LH356
LH357 T
Cytospora amygdali sp. nov.
KARE974
KARE966
KARE967
KARE969
KARE971
KARE970
KARE968
Cytospora parakantschavelii
99/96
10F-57 T
Cytospora longispora sp. nov.
9E-33
Cytospora chrysosperma
KARE973 T
Cytospora populicola sp. nov.
97/85
94/70
KARE975 T
KARE231
KARE195
9E-95
9E-44
Cytospora joaquinensis sp. nov.
82/*
KARE441
KARE442
KARE444
KARE443 T
Cytospora pistaciae sp. nov.
98/96
76/*
KARE268
KARE269
KARE270 T
KARE232
Cytospora parapistaciae sp. nov.
99/92
91/93
6F-45 T
Cytospora granati sp. nov.
KARE1021 T
Cytospora oleicola sp. nov.
KARE1585
Cytospora eucalypti comb. nov.
KARE303
KARE198
KARE99
KARE903
KARE1104
KARE1106
KARE1105
KARE94
KARE1377
KARE1191
KARE62
KARE265
KARE166
KARE264
KARE902
KARE93
KARE197
KARE324
KARE1091
Cytospora californica sp. nov.
96/90
88/*
86/*
99/97
7C-33
7C-11
5A-81
5A-82
7C-09
7C-10
5A-80
Cytospora punicae
99/99
84/*
Diaporthe ampelina Wolf912
10 Changes

Figure S4


ACT1 Dataset
1 Tree
585 Steps
CI = 0.482505
RI = 0.930690
RC = 0.483671
Cytospora sorbicola
Cytospora amygdali sp. nov.
Cytospora plurivora sp. nov.
Cytospora joaquinensis sp. nov.
Cytospora chrysosperma
Cytospora populicola sp. nov.
Cytospora longispora sp. nov.
Cytospora parakantschavelii
Cytospora oleicola sp. nov.
Cytospora eucalypti comb. nov.
Cytospora californica sp. nov.
Cytospora punicae
Cytospora pistaciae sp. nov.
Cytospora parapistaciae sp. nov.
Cytospora granati sp. nov.
Diaporthe ampelina Wolf912
5 Changes