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ARTICLE

Septoria-like pathogens causing leaf and fruit spot of pistachio

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Abstract: Several species of *Septoria* are associated with leaf and fruit spot of pistachio (*Pistacia vera*), though their identity has always been confused, making identification problematic. The present study elucidates the taxonomy of the *Septoria* spp. associated with pistachio, and distinguishes four species associated with this host genus. Partial nucleotide sequence data for five gene loci, ITS, LSU, EF-1α, RPB2 and Btub were generated for a subset of isolates. *Cylindroseptoria pistaciae*, which is associated with leaf spots of *Pistacia lentiscus* in Spain, is characterised by pycnidial conidiomata that give rise to cylindrical, aseptate conidia. Two species of *Septoria s. str.* are also recognised on pistachio, *S. pistaciarum*, and *S. pistaciae*. The latter is part of the *S. protearum* species complex, and appears to be a wide host range pathogen occurring on hosts in several different plant families. *Septoria pistacina*, a major pathogen of pistachio in Turkey, is shown to belong to *Pseudocercospora*, and not *Septoria* as earlier suspected. Other than for its pycnidial conidiomata, it is a typical species of *Pseudocercospora* based on its smooth, pigmented conidiogenous cells and septate conidia. This phenomenon has also been observed in *Pallidocercospora*, and seriously questions the value of conidiomatal structure at generic level, which has traditionally been used to separate hyphomycetous from coelomycetous ascomycetes. Other than DNA barcodes to facilitate the molecular identification of these taxa occurring on pistachio, a key is also provided to distinguish species based on morphology.

Key words:
Capnodiales
coelomycete
hyphomycete
ITS
LSU
Mycosphaerellaceae
Pistachia
Pseudocercospora
RPB2
Septoria
systematics

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INTRODUCTION

The genus *Pistacia* (*Anacardiaceae*), which is believed to have originated in Central Asia, consists of at least 11 species (Parfitt & Badenes 1997). Among these, *Pistacia vera* (pistachio), which is native to Western Asia and parts of the Middle East, is the only cultivated and economically important species (Tous & Ferguson 1996). Several important plant pathogens have been recorded from pistachio, causing fruit and root rot, blossom and shoot blight, canker and rust, and other problems (Michailides *et al*. 1995, http://nt.ars-grin.gov/fungaldatabases/). Of these, Septoria leaf spot is one of the more important diseases associated with fruit and leaf spot.

Desmaziéres (1842) published the first description of a *Septoria* species causing a leaf spot of *Pistacia vera* in northern France, for which he introduced the name *S. pistaciae*. In the same year, Léveillé (1842) described and illustrated *Dothidea pistaciae* causing a leaf spot of a *Pistacia* sp. in Crimea. Cooke (1884), upon examination of the type material on which Léveillé based *D. pistaciae*, transferred it to *Septoria*. Apparently Cooke was unaware of the existence of Desmaziéres' name. Allescher (1901) proposed the binominal *S. pistacina* to replace *S. pistaciae* (Lév.) Cooke 1884 and to differentiate it from *S. pistaciae* Desm. 1842. Caracciolo (1934) reported a third species from pistachio as causing a serious leaf spot in Sicily, which he subsequently described as *S. pistaciarum*. Finally, Chitzanidis (1956) reported sexual morphs for two of the three species, namely *Mycosphaerella pistacina* (for *Septoria pistacina*) and *Mycosphaerella pistaciarum* (for *Septoria pistaciarum*) (Teviotdale *et al*. 2001).

Septoria pistaciarum is known from the USA, and the eastern Mediterranean and southeast Anatolian regions (Young & Michailides 1989). *Septoria pistaciae* is known from the USA (Califonia), Asia (Armenia, Republic of Georgia, India, Israel, Kazakhstan, Kirgizstan, Syria, Tadjikistan, Turkey, Turkmenistan and Uzbekistan), Europe (Albania, France, Greece, Italy, Portugal and Ukraine), and Africa (Egypt) (Pantidou 1973, Dudka *et al*. 2004, Andrianova & Minter 2004, Haggag *et al*. 2006). *Septoria pistacina* is known from Greece (Chitzanidis 1956), Syria, Turkey and Iran (Aghajani *et al*. 2009), and appears to have a more limited distribution.

The application of these *Septoria* names to the respective diseases that they are associated with has been plagued by confusion ever since they were first introduced. The aim of the present study is to elucidate the taxonomy of the *Septoria* species associated with fruit and leaf spot diseases of pistachio, and to place them in a phylogenetic context within *Mycosphaerellaceae*.

MATERIALS AND METHODS

Isolates

Isolations were made from leaf spots by placing leaves in damp chambers for 1–2 d to enhance sporulation. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA) as described earlier (Crous *et al*. 1991). Additional strains were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA), and MEA (Crous *et al*. 2009c), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains were deposited at the CBS (Table 1).

DNA isolation, amplification and analyses

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's protocol. The primers LSU1Fd (Crous *et al*. 2009a) and LR5 (Vilgalys & Hester 1990) were used to amplify the partial 28S rRNA gene (LSU), ITS5 and ITS4 (White *et al.* 1990) were used to amplify the ITS region T1 (O'Donnell & Cigelnik 1997) and b-Sandy-R (Stukenbrock *et al*. 2012) were used to amplify the partial β-tubulin locus (Btub), EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell *et al*. 1998) were used to amplify the partial translation elongation factor-1α locus (EF) and fRPB2-5F (Liu *et al*. 1999) and fRPB2-414R (Quaedvlieg *et al*. 2011) were used to amplify the partial RNA polymerase II second largest subunit locus (RPB2). A basic alignment of the obtained sequence data was first done using MAFFT v. 7 [(http://mafft.cbrc.jp/alignment /server/index. html) (Katoh *et al*. 2002)] and if necessary, manually improved in BioEdit v. 7.0.5.2 (Hall 1999). To check the congruency of the RPB2 and LSU datasets, a 70 % neighbour-joining (NJ) reciprocal bootstrap was performed (Mason-Gamer & Kellogg 1996, Lombard *et al*. 2010). A Bayesian analysis (critical value for the topological convergence diagnostic set to 0.01) was performed on the concatenated LSU/RPB2 loci using MrBayes v. 3.2.1 (Huelsenbeck & Ronquist 2001) as described by Crous *et al*. (2006) using nucleotide substitution models that were selected using MrModeltest v. 2.3 (Nylander 2004). Sequences derived from this study were lodged at GenBank, and the alignment was deposited in TreeBASE (www.treebase.org/treebase/index.html).

Morphology

Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and Zen software. Colony characters and pigment production were noted after 2 wk of growth on MEA, PDA and OA incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Morphological descriptions were based on cultures sporulating on PDA, and taxonomic novelties and metadata were deposited in MycoBank (www.MycoBank.org; Crous *et al.* 2004).

RESULTS

The RPB2 and LSU sequence datasets did not show any conflicts in their tree topology for the 70 % reciprocal bootstrap trees, allowing us to combine them in the multigene analyses. The LSU sequence contained 745 base pairs, of which 99 where unique, the RPB2 sequence contained 317 base pairs, of which 157 where unique. For both datasets, the GTR-I-gamma substitution model (as calculated by MrModeltest) was used during the MrBayes run. During the generation of the tree (Fig. 1), a total of 7 216 trees were generated, and 5 412 (75 %) of them where sampled for the final tree

TAXONOMY

Cylindroseptoria pistaciae Quaedvlieg *et al.*, *Stud. Mycol*. **75**: 359 (2013).
(Fig. 2)

Description: *Conidiomata* pycnidial, erumpent, globose, black, separate, with a black crusty outer layer of cells, to 200 µm diam, with a central ostiole; wall of 3–6 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic (mostly monophialidic, but a few observed to also be polyphialidic), lining the inner cavity, hyaline, smooth, ampulliform, 5–8 × 3–4 µm, proliferating percurrently (inconspicuous) or with periclinal thickening at apex (also occurring as solitary loci on superficial hyphae surrounding pycnidia). *Conidia* hyaline, smooth, cylindrical, mostly straight, rarely slightly curved, apex subobtuse, base truncate, guttulate, aseptate, (9–)11–13(–18) × 2.5–3(–3.5) µm (from Quaedvlieg *et al*. 2013)

Culture characteristics: Colonies on PDA flat, circular, lacking aerial mycelium, surface fuscous-black, reverse olivaceous-black, after 14 d at 24 °C, 3.5 cm diam; on MEA surface fuscous-black, reverse olivaceous-black, after 14 d, 4.5 cm diam; on OA similar to PDA.

Type: **Spain**: *Mallorca*: El Arenal, on leaves of *Pistacia lentiscus*, 25 May 1969, *H. A. van der Aa* (CBS H-21301 – holotype; culture ex-type CBS 471.69).

Notes: Quaedvlieg *et al.* (2013) established the genus *Cylindroseptoria* for taxa having cupulate to pycnidial conidiomata, and phialidic conidiogenous cells with periclinal thickening, that give rise to cylindrical, aseptate conidia. Although *Cylindroseptoria pistaciae* was introduced as

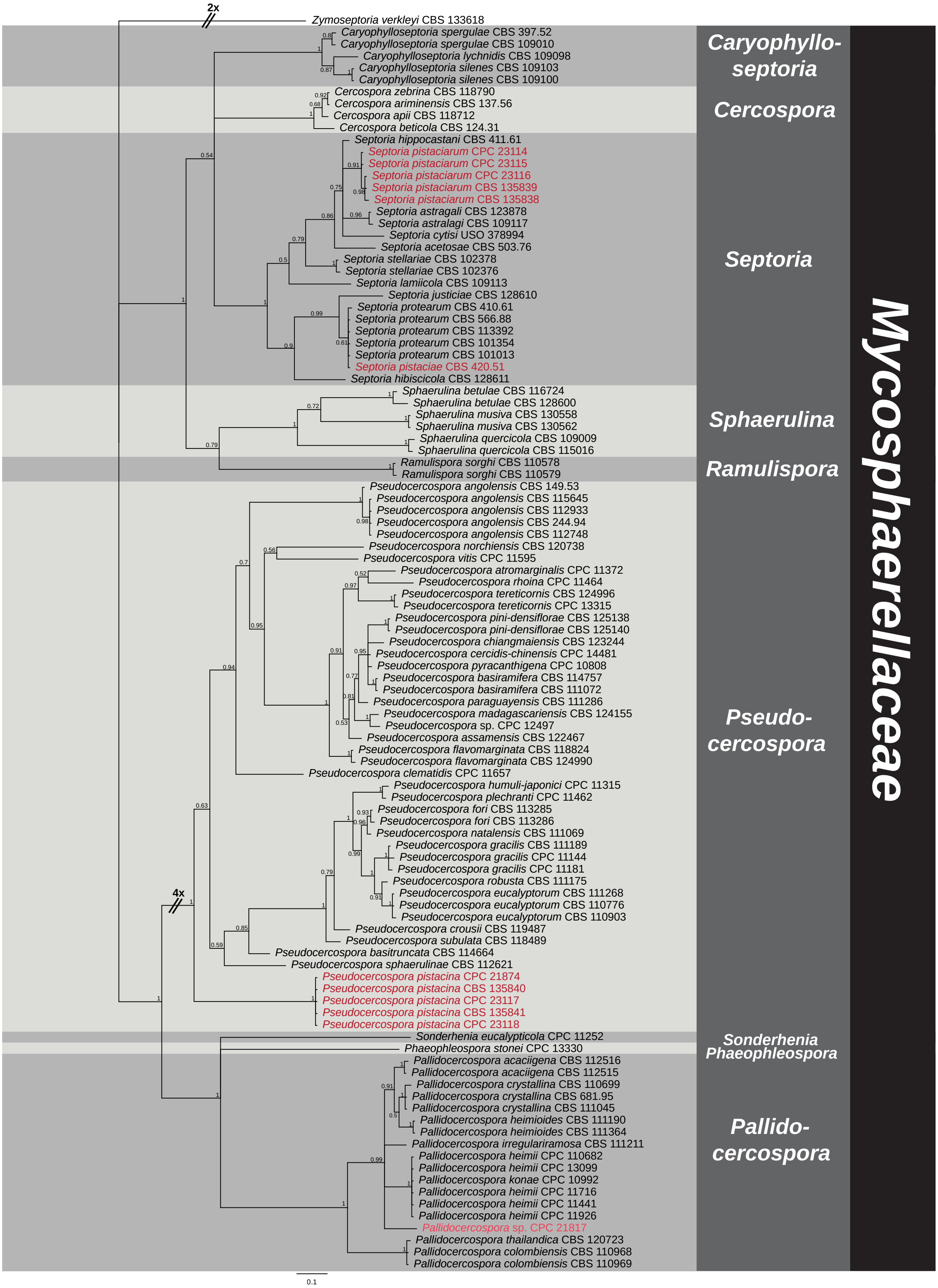

Fig. 1. A Bayesian 50 % majority rule RPB2/LSU consensus tree containing representative isolates belonging to *Pseudocercospora* and related genera (*Mycosphaerellaceae*). Bayesian posterior probabilities support values for the respective nodes are displayed in the tree. A stop rule (set to 0.01) for the critical value for the topological convergence diagnostic was used for the Bayesian analysis. The tree was rooted to *Zymoseptoria verkleyi* (CBS 133618). The scalebar indicates 0.1 expected changes per site.

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

Species	Isolate no.[1]	Host	Location	Collected by	GenBank accession no.[2]				
					RPB2	LSU	ITS	Btub	EF-1α
Caryophylloseptoria lychnidis	CBS 109098	*Silene pratensis*	Austria	G. Verkley	KF252292	KF251790	–	–	–
Caryophylloseptoria silenes	CBS 109100	*Silene nutans*	Austria	G. Verkley	KF252298	KF251796	–	–	–
	CBS 109103	*Silene pratensis*	Austria	G. Verkley	KF252299	KF251797	–	–	–
Caryophylloseptoria spergulae	CBS 109010	*Spergula morisonii*	Netherlands	A. Aptroot	KF252487	KF251995	–	–	–
	CBS 397.52	*Dianthus caryophyllus*	Netherlands	Schouten	KF252301	KF251799	–	–	–
Cercospora apii	CBS 118712	–	Fiji	P. Tyler	KF252302	KF251800	–	–	–
Cercospora ariminensis	CBS 137.56	*Hedysarum coronarium*	Italy	M. Ribaldi	KF252303	KF251801	–	–	–
Cercospora beticola	CBS 124.31	*Beta vulgaris*	Romania	–	KF252155	KF251650	–	–	–
Cercospora zebrina	CBS 118790	*Trifolium subterraneum*	Australia	M.J. Barbetti	KF252156	KF251651	–	–	–
Cylindroseptoria pistaciae	CBS 471.69	*Pistacia lentiscus*	Spain	H.A. van der Aa	KF252161	KF251656	–	–	–
Pallidocercospora acaciigena	CBS 112515	*Acacia mangium*	Venezuela	M.J. Wingfield	KF442687	KF442656	–	–	–
	CBS 112516	*Acacia mangium*	Venezuela	M.J. Wingfield	KF442688	GU253697	–	–	–
Pallidocercospora colombiensis	CBS 110968	*Eucalyptus urophylla*	Colombia	M.J. Wingfield	KF442689	AY752148	–	–	–
	CBS 110969	*Eucalyptus urophylla*	Colombia	M.J. Wingfield	KF442690	KF442657	–	–	–
Pallidocercospora crystallina	CBS 110699	*Leucospermum*	USA	P.W. Crous	KF442691	KF442658	–	–	–
	CBS 111045	*Eucalyptus grandis*	South Africa	M.J. Wingfield	KF442692	KF442659	–	–	–
	CBS 681.95	*Eucalyptus bicostata*	South Africa	M.J. Wingfield	KF442693	EU167579	–	–	–
Pallidocercospora heimii	CPC 11441	–	Brazil	A.C. Alfenas	KF442694	KF442660	–	–	–
	CPC 11716	–	Brazil	A.C. Alfenas	KF442695	KF442661	–	–	–
	CPC 11926	*Acacia auriculiformis*	Thailand	W. Himaman	KF442696	KF442662	–	–	–
	CPC 13099	*Eucalyptus dunnii*	Australia	A.J. Carnegie	KF442697	GQ852606	–	–	–
	CBS 110682	*Eucalyptus* sp.	Madagascar	P.W. Crous	KF442698	GQ852604	–	–	–
Pallidocercospora heimioides	CBS 111190	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	KF442699	GU214439	–	–	–
	CBS 111364	–	Indonesia	M.J. Wingfield	KF442700	KF442663	–	–	–
Pallidocercospora irregulariramosa	CBS 111211	*Eucalyptus saligna*	South Africa	M.J. Wingfield	KF442701	KF442664	–	–	–
Pallidocercospora konae	CPC 10992	*Eucalyptus* sp.	Colombia	M.J. Wingfield	KF442702	KF442665	–	–	–
Pallidocercospora sp.	CPC 21817	*Ventilago denticulata*	Thailand	P.W. Crous	KF442703	KF442666	KF442645	–	–
Pallidocercospora thailandica	CBS 120723	*Eucalyptus camaldulensis*	Thailand	W. Himaman	KF442704	KF442667	–	–	–
Phaeophleospora stonei	CBS 120830	*Eucalyptus* sp.	Australia	P.W. Crous	KF442705	KF442668	–	–	–
Pseudocercospora angolensis	CBS 112748	*Citrus* sp.	Zimbabwe	P.W. Crous	JX902001	JX901878	–	–	–
	CBS 112933	*Citrus* sp.	Zimbabwe	M.C. Pretorius	JX902002	JX901879	–	–	–
	CBS 115645	*Citrus* sp.	Zimbabwe	P.W. Crous	JX902003	JX901880	–	–	–
	CBS 149.53	*Citrus sinensis*	Angola	T. de Carvalho & O. Mendes	JX902004	JX901881	–	–	–

Table 1. (Continued).

Species	Isolate no.[1]	Host	Location	Collected by	GenBank accession no.[2]				
					RPB2	LSU	ITS	Btub	EF-1α
	CBS 244.94	*Citrus* sp.	Zimbabwe	P.W. Crous	JX902000	JX901877	–	–	–
Pseudocercospora assamensis	CBS 122467	*Musa* sp.	India	I.W. Buddenhagen	JX902005	JX901882	–	–	–
Pseudocercospora atromarginalis	CPC 11372	*Solanun nigrum*	South Korea	H.D. Shin	JX902006	JX901883	–	–	–
Pseudocercospora basiramifera	CBS 111072	*Eucalyptus pellita*	Thailand	M.J. Wingfield	KF442706	GU253709	–	–	–
	CBS 114757	*Eucalyptus pellita*	Thailand	M.J. Wingfield	KF442707	GU253802	–	–	–
Pseudocercospora basitruncata	CBS 114664	*Eucalyptus grandis*	Colombia	M.J. Wingfield	KF442708	GU253710	–	–	–
Pseudocercospora cercidis-chinensis	CPC 14481	*Cercis chinensis*	South Korea	H.D. Shin	JX902007	JX901884	–	–	–
Pseudocercospora chiangmaiensis	CBS 123244	*Eucalyptus camaldurensis*	Thailand	R. Cheewangkoon	JX902008	JX901885	–	–	–
Pseudocercospora clematidis	CPC 11657	*Clematis* sp.	USA	M. Palm	JX902009	JX901886	–	–	–
Pseudocercospora crousii	CBS 119487	*Eucalyptus* sp.	New Zealand	C.F. Hill	KF442709	GQ852631	–	–	–
Pseudocercospora eucalyptorum	CBS 110776	*Eucalyptus nitens*	South Africa	P.W. Crous	KF442710	KF442669	–	–	–
	CBS 110903	*Eucalyptus nitens*	South Africa	P.W. Crous	KF442711	KF442670	–	–	–
	CBS 111268	*Eucalyptus grandis*	Kenya	M.J. Wingfield	KF442712	KF442671	–	–	–
Pseudocercospora flavomarginata	CBS 118824	*Eucalyptus camaldulensis*	China	M.J. Wingfield	JX902010	JX901887	–	–	–
	CBS 124990	*Eucalyptus camaldulensis*	Thailand	W. Himaman	JX902028	JX901905	–	–	–
Pseudocercospora fori	CBS 113285	*Eucalyptus grandis*	South Africa	G.C. Hunter	KF442713	GU253824	–	–	–
	CBS 113286	*Eucalyptus* sp.	South Africa	J. Roux	KF442714	KF442672	–	–	–
Pseudocercospora gracilis	CPC 11144	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	JX902011	JX901888	–	–	–
	CPC 11181	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	JX902012	JX901889	–	–	–
	CBS 111189	*Eucalyptus urophylla*	–	M.J. Wingfield	JX902013	JX901890	–	–	–
Pseudocercospora humuli-japonici	CPC 11315	*Humulus japonicus*	South Korea	H.D. Shin	JX902014	JX901891	–	–	–
Pseudocercospora madagascariensis	CBS 124155	*Eucalyptus camaldulensis*	Madagascar	M.J. Wingfield	JX902016	JX901893	–	–	–
Pseudocercospora marginalis	CBS 131582	*Fraxinus rhynchophylla*	South Korea	H.D. Shin	KF442715	GU253812	–	–	–
Pseudocercospora natalensis	CBS 111069	*Eucalyptus nitens*	South Africa	T. Coutinho	KF442716	KF302405	–	–	–
Pseudocercospora norchiensis	CBS 120738	*Eucalyptus* sp.	Italy	W. Gams	JX902017	JX901894	–	–	–
Pseudocercospora paraguayensis	CBS 111286	*Eucalyptus nitens*	Brazil	P.W. Crous	JX902018	JX901895	–	–	–
Pseudocercospora pini-densiflorae	CBS 125138	*Pinus* sp.	Japan	Sung-Oui Suh	JX902021	JX901898	–	–	–
	CBS 125140	*Pinus kesiya*	Japan	Sung-Oui Suh	JX902020	JX901897	–	–	–
Pseudocercospora pistacina	CPC 21874	*Pistacia vera*	Turkey	K. Sarpkaya	KF442719	KF442675	KF442648	KF442734	KF442638
	CPC 23117; 27NT080	*Pistacia vera*	Turkey	K. Sarpkaya	KF442717	KF442673	KF442646	KF442732	KF442636
	CPC 23118; 09mrk010	*Pistacia vera*	Turkey	K. Sarpkaya	KF442718	KF442674	KF442647	KF442733	KF442637
	CBS 135840; 45sln005	*Pistacia vera*	Turkey	K. Sarpkaya	KF442720	KF442676	KF442649	KF442735	KF442639
	CBS 135841; 63br043	*Pistacia vera*	Turkey	K. Sarpkaya	KF442721	KF442677	KF442650	KF442736	KF442640

Table 1. (Continued).

Species	Isolate no.[1]	Host	Location	Collected by	GenBank accession no.[2]				
					RPB2	LSU	ITS	Btub	EF-1α
Pseudocercospora plechranti	CPC 11462	*Plectranthus*	South Korea	H.D. Shin	JX902015	JX901892	–	–	–
Pseudocercospora pyracanthigena	CPC 10808	*Pyracantha angustifolia*	South Korea	H.D. Shin	KF252323	KF251823	–	–	–
Pseudocercospora rhoina	CPC 11464	*Rhus chinensis*	South Korea	H.D. Shin	JX902026	JX901903	–	–	–
Pseudocercospora robusta	CBS 111175	*Eucalyptus robur*	Malaysia	M.J. Wingfield	JX902027	JX901904	–	–	–
Pseudocercospora sphaerulinae	CBS 112621	*Eucalyptus* sp.	–	P.W. Crous	JX902029	JX901906	–	–	–
Pseudocercospora subulata	CBS 118489	*Eucalyptus botryoides*	New Zealand	M. Dick	JX902030	JX901907	–	–	–
Pseudocercospora tereticornis	CPC 13315	*Eucalyptus nitens*	Australia	P.W. Crous	JX902032	JX901909	–	–	–
	CBS 124996	*Eucalyptus nitens*	Australia	A.J. Carnegie	JX902033	JX901910	–	–	–
Pseudocercospora vitis	CPC 11595	*Vitis vinifera*	South Korea	H.D. Shin	JX902035	JX901912	–	–	–
Ramulispora sorghi	CBS 110578	*Sorghum bicolor*	South Africa	D. Nowell	KF442722	KF442678	–	–	–
	CBS 110579	*Sorghum bicolor*	South Africa	D. Nowell	KF442723	KF442679	–	–	–
Septoria astragali	CBS 109117	*Astragalus glycyphyllos*	Austria	G. Verkley	KF252350	KF251853	–	–	–
	CBS 123878	*Astragalus glycyphyllos*	Czech Republic	G. Verkley	KF252351	KF251854	–	–	–
Septoria cytisi	USO 378994	*Laburnum anagyroides*	Czech Republic	J. A. Baumler	–	JF700954	–	–	–
Septoria hibiscicola	CBS 128611	*Hibiscus syriacus*	South Korea	H.D. Shin	KF252430	KF251937	–	–	–
Septoria hippocastani	CBS 411.61	*Aesculus hippocastanum*	Germany	W. Gerlach	KF252432	KF251939	–	–	–
Septoria justiciae	CBS 128610	*Justica procumbens*	South Korea	H.D. Shin	KF252433	KF251940	–	–	–
Septoria lamiicola	CBS 109113	*lamium album*	Austria	G. Verkley	KF252443	KF251950	–	–	–
Septoria pistaciae	CBS 420.51	*Pistacia vera*	Italy	G. Goidánich	KF442724	KF252025	–	–	–
Septoria pistaciarum	CPC 23116; 5DMR032	*Pistacia vera*	Turkey	K. Sarpkaya	KF442725	KF442680	KF442651	KF442737	KF442635
	CPC 23114; 003c	*Pistacia vera*	Turkey	K. Sarpkaya	KF442726	KF442681	KF442652	KF442738	KF442641
	CPC 23115; 002B	*Pistacia terebinthus*	Turkey	K. Sarpkaya	KF442727	KF442682	KF442653	KF442739	KF442642
	CBS 135838; 45sln034	*Pistacia vera*	Turkey	K. Sarpkaya	KF442728	KF442683	KF442654	KF442740	KF442643
	CBS 135839; 001A	*Pistacia vera*	Turkey	K. Sarpkaya	KF442729	KF442684	KF442655	KF442741	KF442644
Septoria protearum	CBS 101013	*Masdevallia* sp.	Netherlands	W. Veenbaas-Rijks	KF252504	KF252013	–	–	–
	CBS 101354	*Gevuina avellana*	New Zealand	S. Ganev	KF252505	KF252014	–	–	–
	CBS 113392	*Lobelia erinus*	–	S. Wolcon	KF252507	KF252016	–	–	–
	CBS 410.61	*Gerbera jamesonii*	Italy	W. Gerlach	KF252514	KF252024	–	–	–
	CBS 566.88	*Hedera helix*	France	H.A. van der Aa	KF252515	KF442693	–	–	–
Septoria rumicum	CBS 503.76	*Rumex acetosa*	France	H. A. van der Aa	KF252523	KF252034	–	–	–
Septoria stellariae	CBS 102376	*Stellaria media*	Netherlands	G. Verkley	KF252567	KF252079	–	–	–

Table 1. (Continued).

Species	Isolate no.[1]	Host	Location	Collected by	GenBank accession no.[2]				
					RPB2	LSU	ITS	Btub	EF-1α
	CBS 102378	*Castanea sativa*	Netherlands	G. Verkley	KF252568	KF252080	–	–	–
Sonderhenia eucalypticola	CPC 11252	*Eucalyptus globulus*	Spain	M.J. Wingfield	KF442730	KF442685	–	–	–
Sphaerulina betulae	CBS 116724	*Betula pubescens*	Scotland	S. Green	KF252595	KF252107	–	–	–
	CBS 128600	*Betula platyphylla*	South Korea	H.D. Shin	KF252598	KF252110	–	–	–
Sphaerulina musiva	CBS 130559	*Populus* sp.	Canada	J. LeBoldus	KF252611	KF252124	–	–	–
	CBS 130562	*Populus* sp.	Canada	J. LeBoldus	KF252612	KF252125	–	–	–
Sphaerulina quercicola	CBS 109009	*Quercus rubra*	Netherlands	G. Verkley	KF252619	KF252132	–	–	–
	CBS 115016	*Quercus robur*	Netherlands	G. Verkley	KF252620	KF252133	–	–	–
Zymoseptoria verkleyi	CBS 133618	*Poa annua*	Netherlands	S.I.R. Videira	KF442731	KF442686	–	–	–

[1]CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; USO: United States Department of Agriculture, National Fungus Collections (BPI)

[2]Btub: β-tubulin; EF-1α: Translation elongation factor 1-alpha; ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: 28S large subunit of the nrRNA gene; RPB2: RNA polymerase II second largest subunit.

a novel species (from symptomatic leaves of *Pistacia lentiscus*), no information is available about its potential role as plant pathogen.

Pseudocercospora pistacina (Allesch.) Crous, Quaedvlieg & Sarpkaya, **comb. nov.**

Basionym: *Septoria pistacina* Allesch., *Rabenh. Krypt.-Fl.* **1**(6): 830 (1900) ["1899"].

Synonyms: *Dothidea pistaciae* Lév., in Démidoff, *Voy. Russ. Mér*. **2**: 108 (1842).

Non *Pseudocercospora pistaciae* (Chupp) Crous & U. Braun, *Mycotaxon* **78**: 338 (2001).

Septoria pistaciae (Lév.) Cooke, *Grevillea* **13** (66): 45 (1884); *nom. illegit*., non *S. pistaciae* Desm. 1842.

Mycosphaerella pistacina Chitzan., *Ann. Inst. Phytopath. Benaki* **10**: 42 (1956).

MycoBank MB805893

(Fig. 3)

Description: *Leaf spots* numerous, brown, amphigenous, angular, confined by leaf veins, to 30 mm long, 3–6 mm diam, containing numerous small, aggregated, immersed conidiomata. *Fruit spots* grey to pale brown, 1–4 mm diam, coalescing to form larger spots, surrounded by a distinct, reddish margin. *Conidiomata* subepidermal, globose to depressed, to 300 µm diam with a wide central ostiole, to 100 µm diam; wall 10–20 µm thick, of 3–6 layers of brown *textura angularis*. *Conidiophores* subcylindrical, pale brown, smooth, 0–3-septate, branched or not, 10–30 × 3–5 µm. *Conidiogenous cells* terminal and sublateral, pale brown, smooth, subcylindrical to doliiform, 6–15 × 2.5–4 µm; proliferating several times percurrently at the apex. *Conidia* pale brown, smooth, guttulate, subcylindrical, curved, medianly 1-septate, constricted at the septum, apex obtuse, tapering at the base to a truncate hilum, 1.5–2 µm diam, (32–)35–42(–50) × (3–)3.5–4(–5) µm.

Chitzanidis (1956) reports ascomata as 90–110 × 80–110 µm, asci as 44.5–54.5 × 13–14.5 µm, and ascospores as 26–40 × 3–5 µm.

Culture characteristics: Colonies after 2 wk at 24 °C reaching 10 mm diam, erumpent with sparse aerial mycelium and even, lobate margins; on OA, MEA and PDA dirty white, remaining sterile; in reverse iron-grey.

Specimens examined: **Turkey**: *Manisa*: Selendi, on *Pistachio vera*, 2010, *K. Sarpkaya* (CPC 45sln005 = CBS 135840). *Gaziantep*: Nizip, on *P. vera*, 2010, *K. Sarpkaya* (CPC 27NZ080 = CPC 23117). *Sanliurfa*: Birecik, on *P. vera*, 2010, *K. Sarpkaya* (CPC 63br043 = CBS 135841). *Aydin*: Merkez, on *P. vera*, 2010, *K. Sarpkaya* (CPC 09mrk010 = CPC 23118); collection site unknown, on *P. vera*, 2010, *K. Sarpkaya* (CPC 21874).

Notes: Because of the pycnidial conidiomata and pigmented conidia, *Pseudocercospora pistacina* can be confused with *Phaeophloeospora* or *Kirramyces* (syn. *Teratosphaeria*; Crous *et al*. 2009a, b), though it is phylogenetically unrelated to these genera. *Pseudocercospora pistacina* clusters basally within *Pseudocercospora*, but based

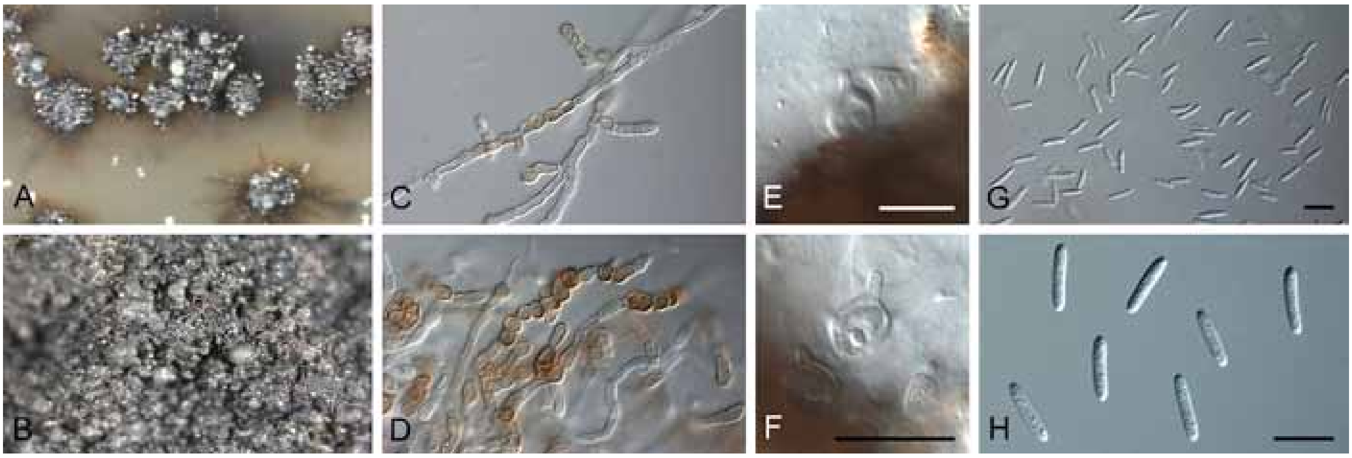

Fig. 2. *Cylindroseptoria pistaciae* (CBS 471.69). **A–B.** Conidiomata sporulating in culture. **C–D.** Intercalary chains of chlamydospore-like cells. **E–F.** Conidiogenous cells. **G–H.** Conidia. Bars = 10 µm, H applies to C and D.

on the genes studied here, could not be recognised as a separate genus. The genus *Pseudocercospora* was recently circumscribed as having species with conidiophores that are solitary, fasciculate, synnematal, or arranged in sporodochia, giving rise to conidia that are pigmented, have unthickened or slightly thickened and darkened scars (Crous *et al*. 2013a). By including *Septoria pistacina* in *Pseudocercospora*, we are expanding the generic circumscription of the latter to also

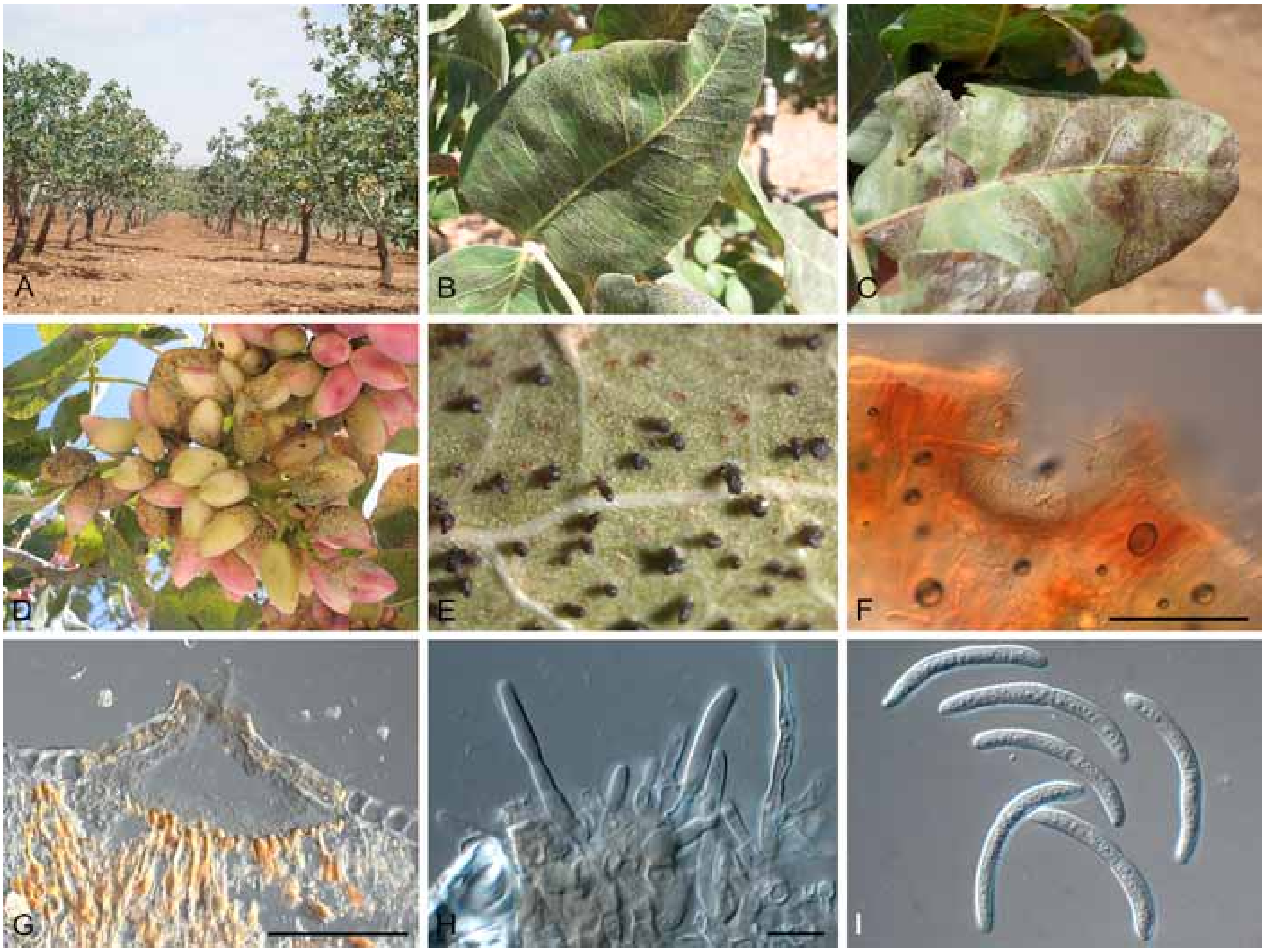

Fig. 3. *Pseudocercospora pistacina* (CBS 135840). **A.** Fruit tree orchard. **B–C.** Leaf spots. **D.** Disease symptoms on fruit. **E.** Conidia cirrhi oozing from immersed pycnidial conidiomata. **F–G.** Vertical section through pycnidia. **H.** Conidiogenous cells giving rise to conidia. **I.** Conidia. Bars: F = 300 µm, G = 150 µm, H–I = 10 µm.

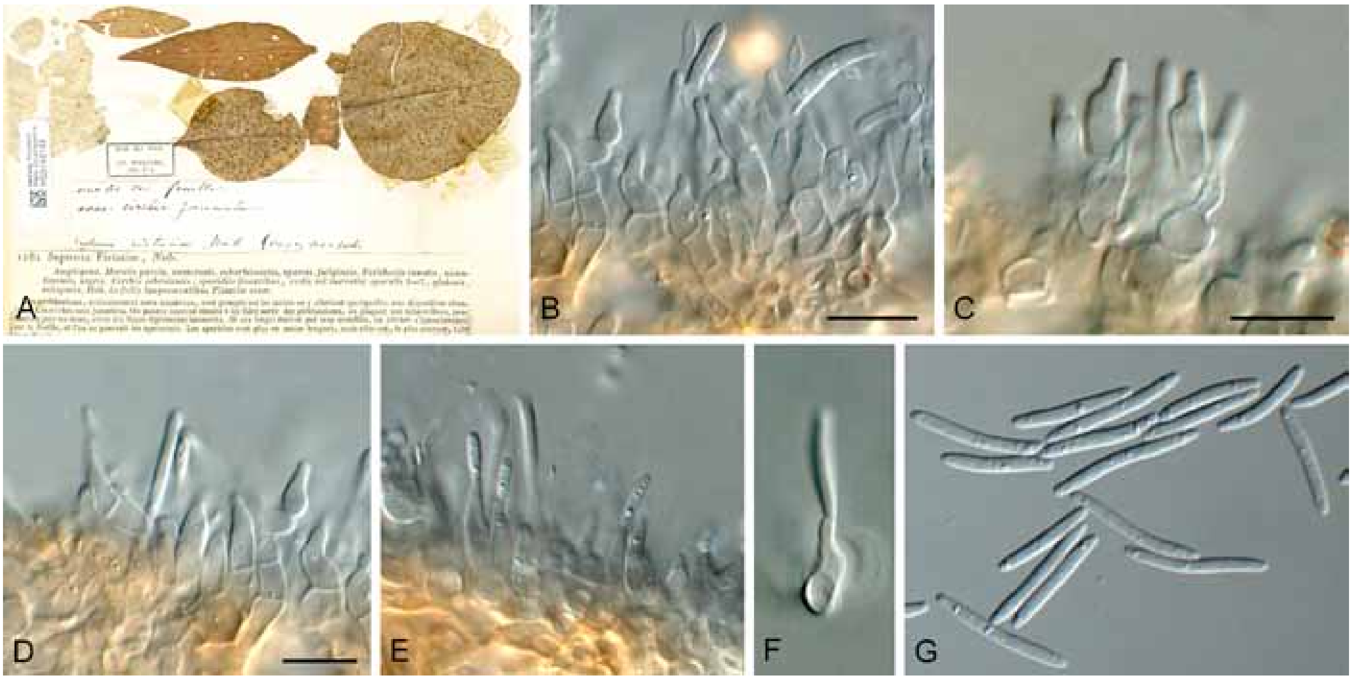

Fig. 4. *Septoria pistaciae* (PC 0142143). **A.** Herbarium specimen. **B–F.** Conidiogenous cells giving rise to conidia. **G.** Conidia. Bars = 10 µm, D applies to E–G.

include taxa with well-defined pycnidial conidiomata (on host and in culture). Conidiomatal structure has to date been paramount in identifying taxa with enclosed conidiomata (Sutton 1980, Nag Raj 1993), and thus *P. pistacina* is rather atypical within *Pseudocercospora s. str*.

Septoria pistaciae Desm., *Annls Sci. Nat.*, *Bot.*, *sér.* 2 **17**: 112 (1842).

Synonyms: *Phloeospora pistaciae* (Desm.) Petr., *Annls mycol.* **20**: 18 (1922).

Cylindrosporium pistaciae (Desm.) Vassil., *Fungi Imp. Paras.* **2**: 510 (1950).

(Fig. 4)

Description: *Leaf spots* initially small, orbicular or oblong, scattered, brown to dark brown, 1–2 mm diam, becoming irregular, 5–10 mm, covering large portions of leaf, becoming greyish brown with distinct, narrow brown margin. *Conidiomata* pycnidial, amphigenous, separate or densely aggregated in the centre of leaf spots, immersed, becoming erumpent, brown to dark brown, globose to pyriform, (40–) 70–90(–120) µm diam, with central ostiole, 15–20 µm diam; wall of 3–4 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells, or up to 4-septate, subcylindrical with lateral and terminal conidiogenous cells, 5–25 × 3–4 µm. *Conidiogenous cells* hyaline, smooth, ampulliform to subcylindrical, 5–10 × 3–4 µm, lining inner layer of conidiomatal cavity, proliferating sympodially, rarely percurrently. *Conidia* hyaline, smooth, 0–3-septate, (9–)13–22(–25) × (1.5–)2(–3) µm, obclavate to narrowly subcylindrical, apex subobtuse, base obconically truncate with flattened scar. *Spermatial state* occurring in conidiomata along with conidia. *Spermatogenous cells* hyaline, smooth, ampulliform, 4–6 × 3–5 µm. *Spermatia* hyaline, smooth, ellipsoid to subcylindrical, with obtuse ends, 2.5–3.5 × 1.5 µm.

Culture characteristics: Colonies after 2 wk at 25 °C reaching 40 mm diam on OA; surface sienna, smooth, with even margins, lacking aerial mycelium; culture sterile on OA, PDA, MEA and on barley leaves placed on synthetic nutrient-poor agar (Crous *et al.* 2009c).

Specimens examined: **France**: on leaves of *Pistacia vera*, 1842, *Desmazière* [*Pl. Crypt. Nord Fr.*, fasc. 24, no. 1181] (PC0142144 – holotype; authentic specimen from general herbarium (PC) no. 1181, PC0142143). – **Italy**: on leaves of *P. vera*, June 1951, *G. Goidánich* (CBS 420.51; culture sterile).

Notes: *Septoria pistaciae* is part of the species complex for which Verkley *et al*. (2013) adopted the oldest name, *S. protearum*, which has an ex-type culture. Isolates in this complex could not be robustly distinguished based on a seven-gene phylogeny, and represent collections with a range of hosts covering six different plant families. Whether this is one plurivorous taxon that can undergo host jumping (Crous & Groenewald 2005), or several closely related taxa that cannot be distinguished based on the set of genes employed by Verkley *et al.* (2013), awaits further study and inoculation trials.

Andrianova & Minter (2004) described conidia of *S. pistaciae* as 1–3-septate, (20–)22–25(–34) × 1.5(–2) µm, obclavate to narrowly subcylindrical (based on type material, LE 42353). Our measurements from type material are considerably smaller, namely 0–3-septate, (9–)13–22(–25) × (1.5–)2(–3) µm (PC 0142144). Type material of *S. protearum* has conidia that are (0–)1–3(–4)-septate, (6–)12–22(–30) × 1.5–2 µm, obclavate to narrowly subcylindrical (Swart *et al*.

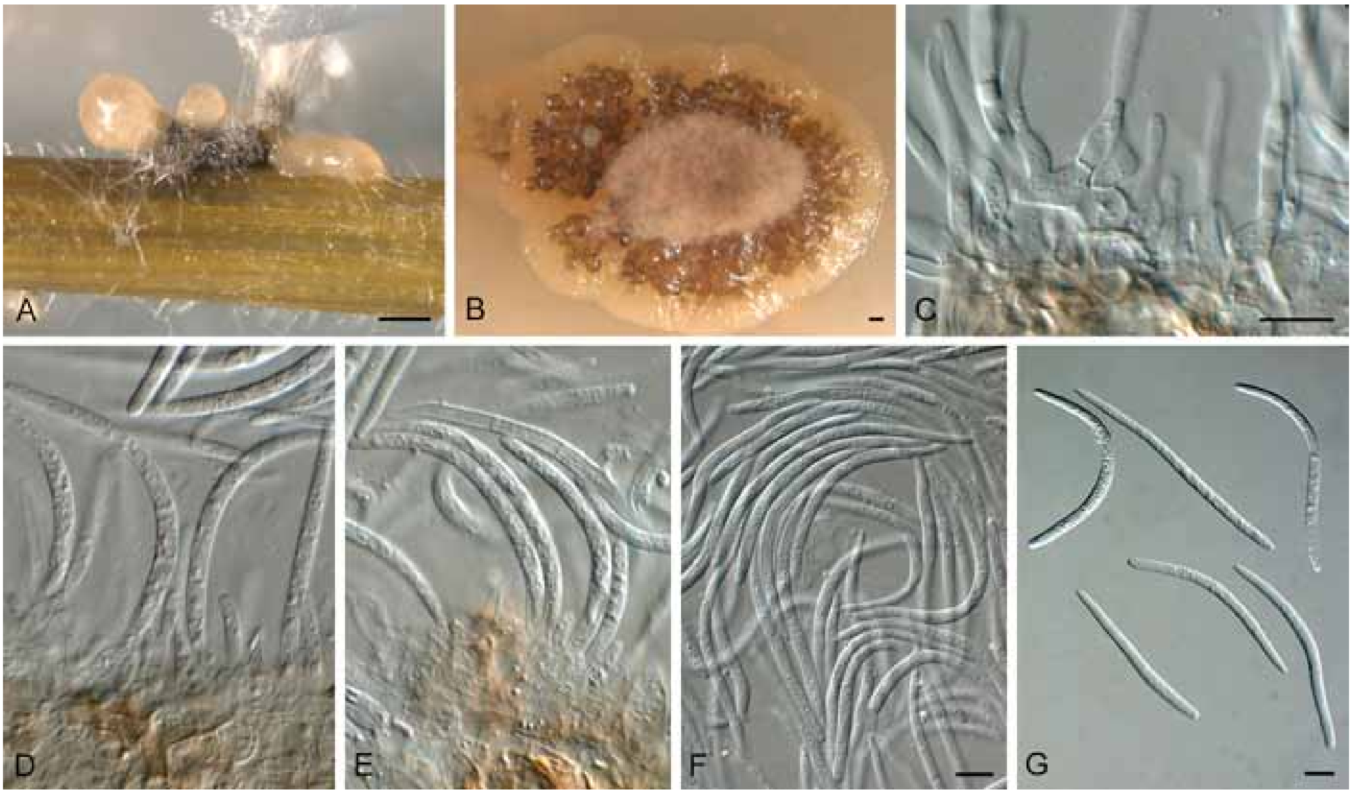

Fig. 5. *Septoria pistaciarum* (CBS 135838). **A.** Colony sporulating on SNA with sterile barley leaves. **B.** Colony on OA. **C–E.** Conidiogenous cells giving rise to conidia. **F–G.** Conidia. Bars: A–B = 200 µm, C, F–G = 10 µm, C applies to D and E.

1998, Crous *et al*. 2013b). If these two taxa are eventually shown to be synonymous, the name *S. pistaciae* (1842) predates that of *S. protearum* (1998), but even that may not be the oldest epithet for this taxon. The single isolate available to us for study (CBS 420.51) proved to be sterile, so its morphology could not be confirmed.

Septoria pistaciarum Caracc., *Boll. Stud. Inform. R. Giard Colon Palermo* **13**: 10 [extr.] (1934).

Synonym: *Mycosphaerella pistaciarum* Chitzan., *Ann. Inst. Phytopath. Benaki* **10**: 42 (1956).

(Fig. 5)

Description: *Leaf spots* angular, brown, amphigenous, 1–2 mm diam, coalescing to become larger leaf spots, confined by leaf veins. *Conidiomata* pycnidial, erumpent, brown, globose, to 200 µm diam, with central ostiole, exuding a crystalline cirrhus of conidia; wall of 3–6 layers of *brown textura angularis*. *Conidiophores* reduced to conidiogenous cells, or one supporting cell which can be branched at the base. *Conidiogenous cells* lining the inner cavity, hyaline, smooth, subcylindrical to ampulliform or doliiform, 5–15 × 2.5–4 µm, proliferating percurrently near apex, or sympodially. *Conidia* solitary, hyaline, smooth, guttulate, straight to curved, narrowly obclavate to subcylindrical, (1–)3-septate, apex subobtuse, base obconically truncate, 2 µm diam with minute marginal frill, (45–)55–65(–75) × (2.5–)3(–3.5) µm.

Chitzanidis (1956) reports ascomata as 95–130 × 85–120 µm, asci 47.5–60.5 × 8–12 µm, and ascospores 18–30 × 3–5 µm.

Culture characteristics: Colonies after 2 wk at 24 °C reaching 30 mm diam. Colonies erumpent, folded with feathery, lobate margins. On PDA surface olivaceous grey with patches of pale olivaceous grey and dirty white, reverse olivaceous grey. On OA surface greyish sepia with patches of dirty white, and an umber pigment diffusing into agar. On MEA surface pale olivaceous grey with patches of dirty white; olivaceous grey in reverse.

Specimens examined: **Turkey**: *Hatay*: Merkez, on *Pistacia terebinthus*, 2012, *K. Sarpkaya* (CPC 002B = CPC 23115). *Sakarya*: Geyve, on *P. vera*, 2012, *K. Sarpkaya* (CPC 003C = CPC 23114). *Kutahya*: Emet, on *P. vera*, 2012, *K. Sarpkaya* (CPC 001A = CBS 135839). *Manisa*: Selendi, on *P. vera*, 2012, *K. Sarpkaya* (CPC 45sln034 = CBS 135838); Demirci, on *P. vera*, 2012, *K. Sarpkaya* (CPC 5DMR032 = CPC 23116).

Notes: *Septoria pistaciarum* is morphologically distinct from the other species occurring on pistachio, in having much larger conidia (45–75 × 2.5–3.5 µm). In the field it can also be disnguished on diseased host plants in causing more angular leaf spots, confined by leaf veins.

DISCUSSION

The aim of the present study was to clarify which species of *Septoria* occur on pistachio, and to place them in a phylogenetic context within *Mycosphaerellaceae,* as recently circumscribed (Quaedvlieg *et al*. 2013, Verkley *et al*. 2013). From results obtained, it is clear that up to four septoria-like taxa occur on pistachio, of which two belong to other genera, namely *Cylindroseptoria pistaciae* and *Pseudocercospora pistacina*. The remaining two species represent true species of *Septoria*, namely *S. pistaciae* and *S. pistaciarum*. Because of discrepancies in previously published literature, much confusion arose regarding how to distinguish these taxa. In the present study we have been able to compile a key to facilitate identification of these taxa (*see below*). Sexual morphs have also been described for two of these taxa, namely *Septoria pistacina* (i.e. *Mycosphaerella pistacina*) and *Septoria pistaciarum* (i.e. *Mycosphaerella pistaciarum*) (Chitzanidis 1956, Teviotdale *et al*. 2001). However, because the genus *Mycosphaerella* is restricted to *Ramularia* (Verkley *et al*. 2004, Crous *et al*. 2009a, b, Koike *et al*. 2011), in moving towards a single nomenclature for fungi (see Hawksworth *et al.* 2011, Wingfield *et al*. 2012), the use of *Mycosphaerella* should be avoided for the mycosphaerella-like sexual morphs linked to *Septoria*.

The placement of *Septoria pistacina* in *Pseudocercospora* is somewhat controvertial, as it has a typical pycnidial conidioma, rather than superficial fascicles or synnemata encountered in *Pseudocercospora*. Phylogenetically, however, there is no support for recognising *S. pistacina* as a separate genus based on it being a "pigmented *Septoria*". Morphologically, the conidiogenous cells and conidia fit the circumscription of *Pseudocercospora*, but the conidiomatal anatomy does not. Although species of *Capnodiales* are known to have synasexual morphs with closed and open conidiomata (Crous *et al*. 2007, 2009a, b), this is the first example of a taxon with a pycnidial conidioma that clusters among species with fasciculate conidiomata. In addition to *S. pistacina*, we are also aware of a second as yet undescribed species of "pigmented *Septoria*" (Crous *et al*., unpubl.), which again clusters in *Pallidocercospora* (Crous *et al*. 2013a). Another example of a genus reported to have acervuli, but observed to have superficial conidiomatal fascicles, is *Ciferiella*, which has also been reduced to synonymy with *Pseudocercospora* (Quaedvlieg *et al*. 2013). These findings support the view that conidiomatal morphology in *Pseudocercospora* is a continuum from sporulating superficially (fascicles, synnemata, sporodochia), *via* acervuli, to sporulation in an enclosed structure (pycnidia).

In spite of being morphologically distinct, that two of the reported septoria-like taxa on pistachio represent different genera is rather suprising. Although the pathological relevance of *Pseudocercospora pistacina* (as *S. pistacina*), *S. pistaciae*, and *S. pistaciarum* on *Pistacia vera* is well-documented (Michailides 2005), nothing is known about that of *Cylindroseptoria pistaciae*, other than it was associated with leaf spots of *Pistacia lentiscus* in Spain. Furthermore, it also appears that the importance of certain diseases of pistachio differs between regions or countries. Approximately 85 % of the world's pistachio production presently comes from Iran, the USA, and Turkey (http://faostat.fao.org/site/339/default.aspx). However, pistachio is irrigated in Iran and the USA, where more rounded fruit bearing cultivars are grown. In contrast, irrigation is not practiced in Turkey, and more elongated fruit cultivars are commonly grown there. These differences in cultivation practices also lead to differences in phytopatological problems. In the USA, the main pathogens are reported to be *Botryosphaeria dothidea*, *Botrytis cinerea,* and *Alternaria alternata*. In Turkey, however, *Pseudocercospora pistacina* is the most common disease of pistachio (Dinç 1983, Michailides *et al*. 1995), leading to reports of 3–100 % yield loss in epidemic years by this pathogen (Dinç *et al.* 1979).

Key to septoria-like species occurring on pistachio

1	Conidia aseptate, 9–18 × 2.5–3.5 µm	***Cylindroseptoria pistaciae***
	Conidia septate	2
2 (1)	Conidia pale brown, medianly 1-septate, 32–50 × 3–5 µm	***Pseudocercospora pistacina***
	Conidia hyaline, 1–3-septate	3
3 (2)	Conidia 9–34 × 1.5–3 µm	***Septoria pistaciae***
	Conidia 45–75 × 2.5–3.5 µm	***Septoria pistaciarum***

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