

## Septoria-like pathogens causing leaf and fruit spot of pistachio

Pedro W. Crous<sup>1,2,3</sup>, William Quaedvlieg<sup>1,2</sup>, Kamil Sarpkaya<sup>4</sup>, Canan Can<sup>5</sup>, and Ali Erkiç<sup>6</sup>

<sup>1</sup>CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl

<sup>2</sup>Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

<sup>3</sup>Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

<sup>4</sup>Pistachio Research Station, Üniversite Bulvari 27060 Şahinbey/Gaziantep, Turkey

<sup>5</sup>Gaziantep University, Faculty of Art and Science, Department of Biology, Gaziantep, Turkey

<sup>6</sup>Cukurova University, Faculty of Agriculture, Department of Plant Protection, Adana, Turkey

**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-1 $\alpha$ , 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

**Article info:** Submitted: 22 August 2013; Accepted: 15 October 2013; Published: 28 October 2013.

## INTRODUCTION

The genus *Pistacia* (*Anacardiaceae*), which is believed to have originated in Central Asia, consists of at least 11 species (Parfitt & Badenies 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/fungal-databases/>). Of these, *Septoria* leaf spot is one of the more important diseases associated with fruit and leaf spot.

Desmazières (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 Desmazières' 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 (California), 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.

© 2013 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

**Attribution:** You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

**Non-commercial:** You may not use this work for commercial purposes.

**No derivative works:** You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

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 UltraClean™ 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  $\beta$ -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-1 $\alpha$  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 were unique, the RPB2 sequence contained 317 base pairs, of which 157 were 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 were 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  $\mu$ m 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  $\times$  3–4  $\mu$ m, 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)  $\times$  2.5–3(–3.5)  $\mu$ m (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

**Mycosphaerellaceae**



**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. <sup>1</sup>	Host	Location	Collected by	GenBank accession no. <sup>2</sup>				
					RPB2	LSU	ITS	Btub	EF-1 $\alpha$
<i>Caryophylloseptoria lychnidis</i>	CBS 109098	<i>Silene pratensis</i>	Austria	G. Verkley	KF252292	KF251790	–	–	–
<i>Caryophylloseptoria silenes</i>	CBS 109100	<i>Silene nutans</i>	Austria	G. Verkley	KF252298	KF251796	–	–	–
	CBS 109103	<i>Silene pratensis</i>	Austria	G. Verkley	KF252299	KF251797	–	–	–
<i>Caryophylloseptoria spergulae</i>	CBS 109010	<i>Spergula morisonii</i>	Netherlands	A. Aptroot	KF252487	KF251995	–	–	–
	CBS 397.52	<i>Dianthus caryophyllus</i>	Netherlands	Schouten	KF252301	KF251799	–	–	–
<i>Cercospora apii</i>	CBS 118712	–	Fiji	P. Tyler	KF252302	KF251800	–	–	–
<i>Cercospora ariminensis</i>	CBS 137.56	<i>Hedysarum coronarium</i>	Italy	M. Ribaldi	KF252303	KF251801	–	–	–
<i>Cercospora beticola</i>	CBS 124.31	<i>Beta vulgaris</i>	Romania	–	KF252155	KF251650	–	–	–
<i>Cercospora zebrina</i>	CBS 118790	<i>Trifolium subterraneum</i>	Australia	M.J. Barbetti	KF252156	KF251651	–	–	–
<i>Cylindroseptoria pistaciae</i>	CBS 471.69	<i>Pistacia lentiscus</i>	Spain	H.A. van der Aa	KF252161	KF251656	–	–	–
<i>Pallidocercospora acaciigena</i>	CBS 112515	<i>Acacia mangium</i>	Venezuela	M.J. Wingfield	KF442687	KF442656	–	–	–
	CBS 112516	<i>Acacia mangium</i>	Venezuela	M.J. Wingfield	KF442688	GU253697	–	–	–
<i>Pallidocercospora colombiensis</i>	CBS 110968	<i>Eucalyptus urophylla</i>	Colombia	M.J. Wingfield	KF442689	AY752148	–	–	–
	CBS 110969	<i>Eucalyptus urophylla</i>	Colombia	M.J. Wingfield	KF442690	KF442657	–	–	–
<i>Pallidocercospora crystallina</i>	CBS 110699	<i>Leucospermum</i>	USA	P.W. Crous	KF442691	KF442658	–	–	–
	CBS 111045	<i>Eucalyptus grandis</i>	South Africa	M.J. Wingfield	KF442692	KF442659	–	–	–
	CBS 681.95	<i>Eucalyptus bicostata</i>	South Africa	M.J. Wingfield	KF442693	EU167579	–	–	–
<i>Pallidocercospora heimii</i>	CPC 11441	–	Brazil	A.C. Alfenas	KF442694	KF442660	–	–	–
	CPC 11716	–	Brazil	A.C. Alfenas	KF442695	KF442661	–	–	–
	CPC 11926	<i>Acacia auriculiformis</i>	Thailand	W. Himaman	KF442696	KF442662	–	–	–
	CPC 13099	<i>Eucalyptus dunnii</i>	Australia	A.J. Carnegie	KF442697	GO852606	–	–	–
	CBS 110682	<i>Eucalyptus sp.</i>	Madagascar	P.W. Crous	KF442698	GO852604	–	–	–
<i>Pallidocercospora heimioidea</i>	CBS 111190	<i>Eucalyptus sp.</i>	Indonesia	M.J. Wingfield	KF442699	GU214439	–	–	–
	CBS 111364	–	Indonesia	M.J. Wingfield	KF442700	KF442663	–	–	–
<i>Pallidocercospora irregulariramosa</i>	CBS 111211	<i>Eucalyptus saligna</i>	South Africa	M.J. Wingfield	KF442701	KF442664	–	–	–
<i>Pallidocercospora konae</i>	CPC 10992	<i>Eucalyptus sp.</i>	Colombia	M.J. Wingfield	KF442702	KF442665	–	–	–
<i>Pallidocercospora sp.</i>	CPC 21817	<i>Ventilago denticulata</i>	Thailand	P.W. Crous	KF442703	KF442666	KF442645	–	–
<i>Pallidocercospora thailandica</i>	CBS 120723	<i>Eucalyptus camaldulensis</i>	Thailand	W. Himaman	KF442704	KF442667	–	–	–
<i>Phaeophleospora stonei</i>	CBS 120830	<i>Eucalyptus sp.</i>	Australia	P.W. Crous	KF442705	KF442668	–	–	–
<i>Pseudocercospora angolensis</i>	CBS 112748	<i>Citrus sp.</i>	Zimbabwe	P.W. Crous	JX902001	JX901878	–	–	–
	CBS 112933	<i>Citrus sp.</i>	Zimbabwe	M.C. Pretorius	JX902002	JX901879	–	–	–
	CBS 115645	<i>Citrus sp.</i>	Zimbabwe	P.W. Crous	JX902003	JX901880	–	–	–
	CBS 149.53	<i>Citrus sinensis</i>	Angola	T. de Carvalho & O. Mendes	JX902004	JX901881	–	–	–

Table 1. (Continued).

Species	Isolate no. <sup>1</sup>	Host	Location	Collected by	GenBank accession no. <sup>2</sup>				
					RPB2	LSU	ITS	Btub	EF-1α
	CBS 244.94	Citrus sp.	Zimbabwe	P.W. Crous	JX902000	JX901877	-	-	-
<i>Pseudocercospora assamensis</i>	CBS 122467	Musa sp.	India	I.W. Buddenhagen	JX902005	JX901882	-	-	-
<i>Pseudocercospora atomarginalis</i>	CPC 11372	Solanum nigrum	South Korea	H.D. Shin	JX902006	JX901883	-	-	-
<i>Pseudocercospora basiramifera</i>	CBS 111072	<i>Eucalyptus pellita</i>	Thailand	M.J. Wingfield	KF442706	GU253709	-	-	-
	CBS 114757	<i>Eucalyptus pellita</i>	Thailand	M.J. Wingfield	KF442707	GU253802	-	-	-
<i>Pseudocercospora basitruncata</i>	CBS 114664	<i>Eucalyptus grandis</i>	Colombia	M.J. Wingfield	KF442708	GU253710	-	-	-
<i>Pseudocercospora cercidis-chinensis</i>	CPC 14481	<i>Cercis chinensis</i>	South Korea	H.D. Shin	JX902007	JX901884	-	-	-
<i>Pseudocercospora chiangmaiensis</i>	CBS 123244	<i>Eucalyptus camaldulensis</i>	Thailand	R. Cheewangkoon	JX902008	JX901885	-	-	-
<i>Pseudocercospora clematidis</i>	CPC 11657	<i>Clematis</i> sp.	USA	M. Palm	JX902009	JX901886	-	-	-
<i>Pseudocercospora crousii</i>	CBS 119487	<i>Eucalyptus</i> sp.	New Zealand	C.F. Hill	KF442709	GO852631	-	-	-
<i>Pseudocercospora eucalyptorum</i>	CBS 110776	<i>Eucalyptus nitens</i>	South Africa	P.W. Crous	KF442710	KF442669	-	-	-
	CBS 110903	<i>Eucalyptus nitens</i>	South Africa	P.W. Crous	KF442711	KF442670	-	-	-
	CBS 111268	<i>Eucalyptus nitens</i>	South Africa	P.W. Crous	KF442712	KF442671	-	-	-
<i>Pseudocercospora flavomarginata</i>	CBS 118824	<i>Eucalyptus grandis</i>	Kenya	M.J. Wingfield	KF442712	KF442671	-	-	-
	CBS 118824	<i>Eucalyptus camaldulensis</i>	China	M.J. Wingfield	JX902010	JX901887	-	-	-
	CBS 124990	<i>Eucalyptus camaldulensis</i>	Thailand	W. Himaman	JX902028	JX901905	-	-	-
<i>Pseudocercospora fori</i>	CBS 113285	<i>Eucalyptus grandis</i>	South Africa	G.C. Hunter	KF442713	GU253824	-	-	-
	CBS 113286	<i>Eucalyptus</i> sp.	South Africa	J. Roux	KF442714	KF442672	-	-	-
<i>Pseudocercospora gracilis</i>	CPC 11144	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	JX902011	JX901888	-	-	-
	CPC 11181	<i>Eucalyptus</i> sp.	Indonesia	M.J. Wingfield	JX902012	JX901889	-	-	-
	CBS 111189	<i>Eucalyptus urophylla</i>	-	M.J. Wingfield	JX902013	JX901890	-	-	-
<i>Pseudocercospora humuli-japonici</i>	CPC 11315	<i>Humulus japonicus</i>	South Korea	H.D. Shin	JX902014	JX901891	-	-	-
<i>Pseudocercospora madagascariensis</i>	CBS 124155	<i>Eucalyptus camaldulensis</i>	Madagascar	M.J. Wingfield	JX902016	JX901893	-	-	-
<i>Pseudocercospora marginalis</i>	CBS 131582	<i>Fraxinus rhynchophylla</i>	South Korea	H.D. Shin	KF442715	GU253812	-	-	-
<i>Pseudocercospora natalensis</i>	CBS 111069	<i>Eucalyptus nitens</i>	South Africa	T. Coutinho	KF442716	KF302405	-	-	-
<i>Pseudocercospora norchiensis</i>	CBS 120738	<i>Eucalyptus</i> sp.	Italy	W. Gams	JX902017	JX901894	-	-	-
<i>Pseudocercospora paraguayensis</i>	CBS 111286	<i>Eucalyptus nitens</i>	Brazil	P.W. Crous	JX902018	JX901895	-	-	-
<i>Pseudocercospora pini-densiflorae</i>	CBS 125138	<i>Pinus</i> sp.	Japan	Sung-Oui Suh	JX902021	JX901898	-	-	-
	CBS 125140	<i>Pinus kesiya</i>	Japan	Sung-Oui Suh	JX902020	JX901897	-	-	-
<i>Pseudocercospora pistacina</i>	CPC 21874	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442719	KF442675	KF442648	KF442734	KF442638
	CPC 23117; 27NT080	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442717	KF442673	KF442646	KF442732	KF442636
	CPC 23118; 09mrk010	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442718	KF442674	KF442647	KF442733	KF442637
	CBS 135840; 45sln005	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442720	KF442676	KF442649	KF442735	KF442639
	CBS 135841; 63br043	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442721	KF442677	KF442650	KF442736	KF442640

Table 1. (Continued).

Species	Isolate no. <sup>1</sup>	Host	Location	Collected by	GenBank accession no. <sup>2</sup>				
					RPB2	LSU	ITS	Btub	EF-1 $\alpha$
<i>Pseudocercospora plechranthi</i>	CPC 11462	<i>Plectranthus</i>	South Korea	H.D. Shin	JX902015	JX901892	–	–	–
<i>Pseudocercospora pyracanthigena</i>	CPC 10808	<i>Pyracantha angustifolia</i>	South Korea	H.D. Shin	KF252323	KF251823	–	–	–
<i>Pseudocercospora rhoisa</i>	CPC 11464	<i>Rhus chinensis</i>	South Korea	H.D. Shin	JX902026	JX901903	–	–	–
<i>Pseudocercospora robusta</i>	CBS 111175	<i>Eucalyptus robur</i>	Malaysia	M.J. Wingfield	JX902027	JX901904	–	–	–
<i>Pseudocercospora sphaerulinae</i>	CBS 112621	<i>Eucalyptus</i> sp.	–	P.W. Crous	JX902029	JX901906	–	–	–
<i>Pseudocercospora subulata</i>	CBS 118489	<i>Eucalyptus botryoides</i>	New Zealand	M. Dick	JX902030	JX901907	–	–	–
<i>Pseudocercospora tereticornis</i>	CPC 13315	<i>Eucalyptus nitens</i>	Australia	P.W. Crous	JX902032	JX901909	–	–	–
<i>Pseudocercospora vitis</i>	CBS 124996	<i>Eucalyptus nitens</i>	Australia	A.J. Carnegie	JX902033	JX901910	–	–	–
<i>Ramulispora sorghi</i>	CPC 11595	<i>Vitis vinifera</i>	South Korea	H.D. Shin	JX902035	JX901912	–	–	–
<i>Septoria astragali</i>	CBS 110578	<i>Sorghum bicolor</i>	South Africa	D. Nowell	KF442722	KF442678	–	–	–
	CBS 110579	<i>Sorghum bicolor</i>	South Africa	D. Nowell	KF442723	KF442679	–	–	–
	CBS 109117	<i>Astragalus glycyphyllos</i>	Austria	G. Verkley	KF252350	KF251853	–	–	–
	CBS 123878	<i>Astragalus glycyphyllos</i>	Czech Republic	G. Verkley	KF252351	KF251854	–	–	–
<i>Septoria cytisi</i>	USO 378994	<i>Laburnum anagyroides</i>	Czech Republic	J. A. Baumler	–	JF700954	–	–	–
<i>Septoria hibiscicola</i>	CBS 128611	<i>Hibiscus syriacus</i>	South Korea	H.D. Shin	KF252430	KF251937	–	–	–
<i>Septoria hippocastani</i>	CBS 411.61	<i>Aesculus hippocastanum</i>	Germany	W. Gerlach	KF252432	KF251939	–	–	–
<i>Septoria justiciae</i>	CBS 128610	<i>Justicia procumbens</i>	South Korea	H.D. Shin	KF252433	KF251940	–	–	–
<i>Septoria lamicola</i>	CBS 109113	<i>lamium album</i>	Austria	G. Verkley	KF252443	KF251950	–	–	–
<i>Septoria pistaciae</i>	CBS 420.51	<i>Pistacia vera</i>	Italy	G. Goidánich	KF442724	KF252025	–	–	–
	CPC 23116; 5DMR032	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442725	KF442680	KF442651	KF442737	KF442635
	CPC 23114; 003c	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442726	KF442681	KF442652	KF442738	KF442641
	CPC 23115; 002B	<i>Pistacia terebinthus</i>	Turkey	K. Sarpkaya	KF442727	KF442682	KF442653	KF442739	KF442642
	CBS 135838; 45sIn034	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442728	KF442683	KF442654	KF442740	KF442643
	CBS 135839; 001A	<i>Pistacia vera</i>	Turkey	K. Sarpkaya	KF442729	KF442684	KF442655	KF442741	KF442644
<i>Septoria protearum</i>	CBS 101013	<i>Masdevallia</i> sp.	Netherlands	W. Veenbaas-Rijks	KF252504	KF252013	–	–	–
	CBS 101354	<i>Gevuina avellana</i>	New Zealand	S. Ganey	KF252505	KF252014	–	–	–
	CBS 113392	<i>Lobelia erinus</i>	–	S. Wolcon	KF252507	KF252016	–	–	–
	CBS 410.61	<i>Gerbera jamesonii</i>	Italy	W. Gerlach	KF252514	KF252024	–	–	–
	CBS 566.88	<i>Hedera helix</i>	France	H.A. van der Aa	KF252515	KF442693	–	–	–
<i>Septoria rumicum</i>	CBS 503.76	<i>Rumex acetosa</i>	France	H. A. van der Aa	KF252523	KF252034	–	–	–
<i>Septoria stellariae</i>	CBS 102376	<i>Stellaria media</i>	Netherlands	G. Verkley	KF252567	KF252079	–	–	–

Table 1. (Continued).

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

<sup>1</sup>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)

<sup>2</sup>Btub:  $\beta$ -tubulin; EF-1 $\alpha$ : Translation elongation factor 1- $\alpha$ ; ITS: internal transcribed spacers and intervening 5.8S rDNA; LSU: 28S large subunit of the rRNA 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)

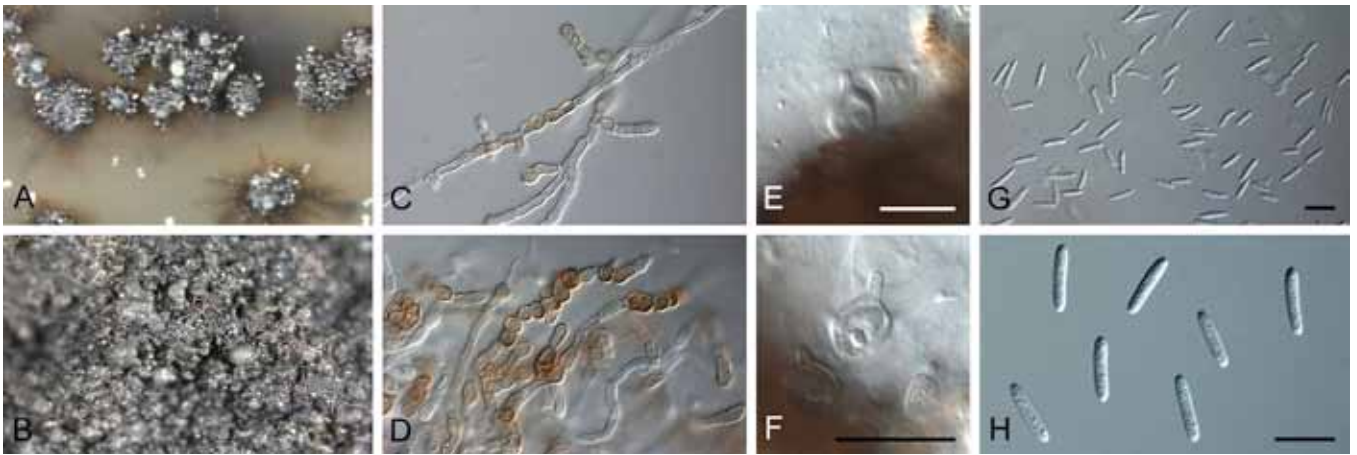
*Description:* *Leaf spots* numerous, brown, amphigenous, angular, confined by leaf veins, to 30 mm long, 3–6 mm diam, containing numerous small, aggregated, immersed conidiomata. *Fruit spots* grey to pale brown, 1–4 mm diam, coalescing to form larger spots, surrounded by a distinct, reddish margin. *Conidiomata* subepidermal, globose to depressed, to 300  $\mu$ m diam with a wide central ostiole, to 100  $\mu$ m diam; wall 10–20  $\mu$ m thick, of 3–6 layers of brown *textura angularis*. *Conidiophores* subcylindrical, pale brown, smooth, 0–3-septate, branched or not, 10–30  $\times$  3–5  $\mu$ m. *Conidiogenous cells* terminal and sublateral, pale brown, smooth, subcylindrical to doliiform, 6–15  $\times$  2.5–4  $\mu$ m; 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  $\mu$ m diam, (32–) 35–42(–50)  $\times$  (3–)3.5–4(–5)  $\mu$ m.

Chitzanidis (1956) reports ascomata as 90–110  $\times$  80–110  $\mu$ m, asci as 44.5–54.5  $\times$  13–14.5  $\mu$ m, and ascospores as 26–40  $\times$  3–5  $\mu$ m.

*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 45sn005 = 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 chlamyospore-like cells. **E–F.** Conidiogenous cells. **G–H.** Conidia. Bars = 10 µm, H applies to C and D.

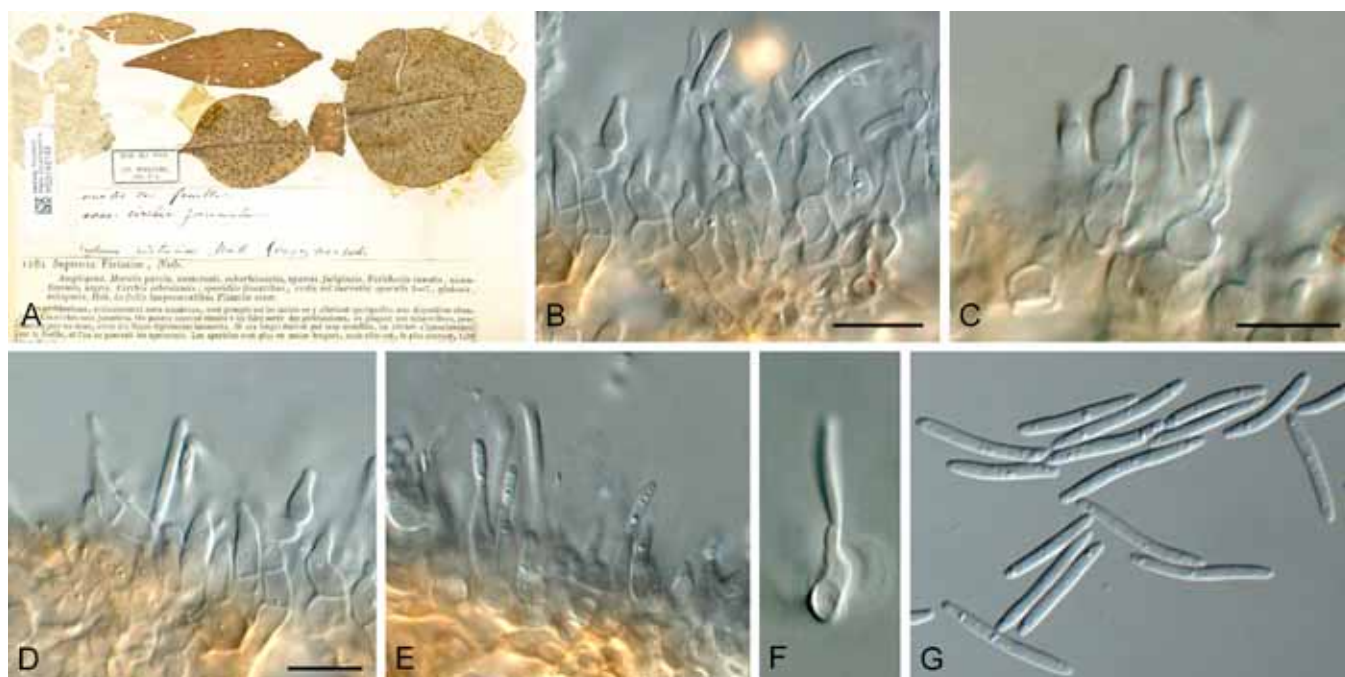
on the genes studied here, could not be recognised as a separate genus. The genus *Pseudocercospora* was recently circumscribed as having species with conidiophores that are solitary, fasciculate, synnematal, or arranged in sporodochia,

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  $\mu\text{m}$ , 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)  $\mu\text{m}$  diam, with central ostiole, 15–20  $\mu\text{m}$  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  $\times$  3–4  $\mu\text{m}$ . *Conidiogenous cells* hyaline, smooth, ampulliform to subcylindrical, 5–10  $\times$  3–4  $\mu\text{m}$ , lining inner layer of conidiomatal cavity, proliferating sympodially, rarely percurrently. *Conidia* hyaline, smooth, 0–3-septate, (9–)13–22(–25)  $\times$  (1.5–)2(–3)  $\mu\text{m}$ , 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  $\times$  3–5  $\mu\text{m}$ . *Spermatia*

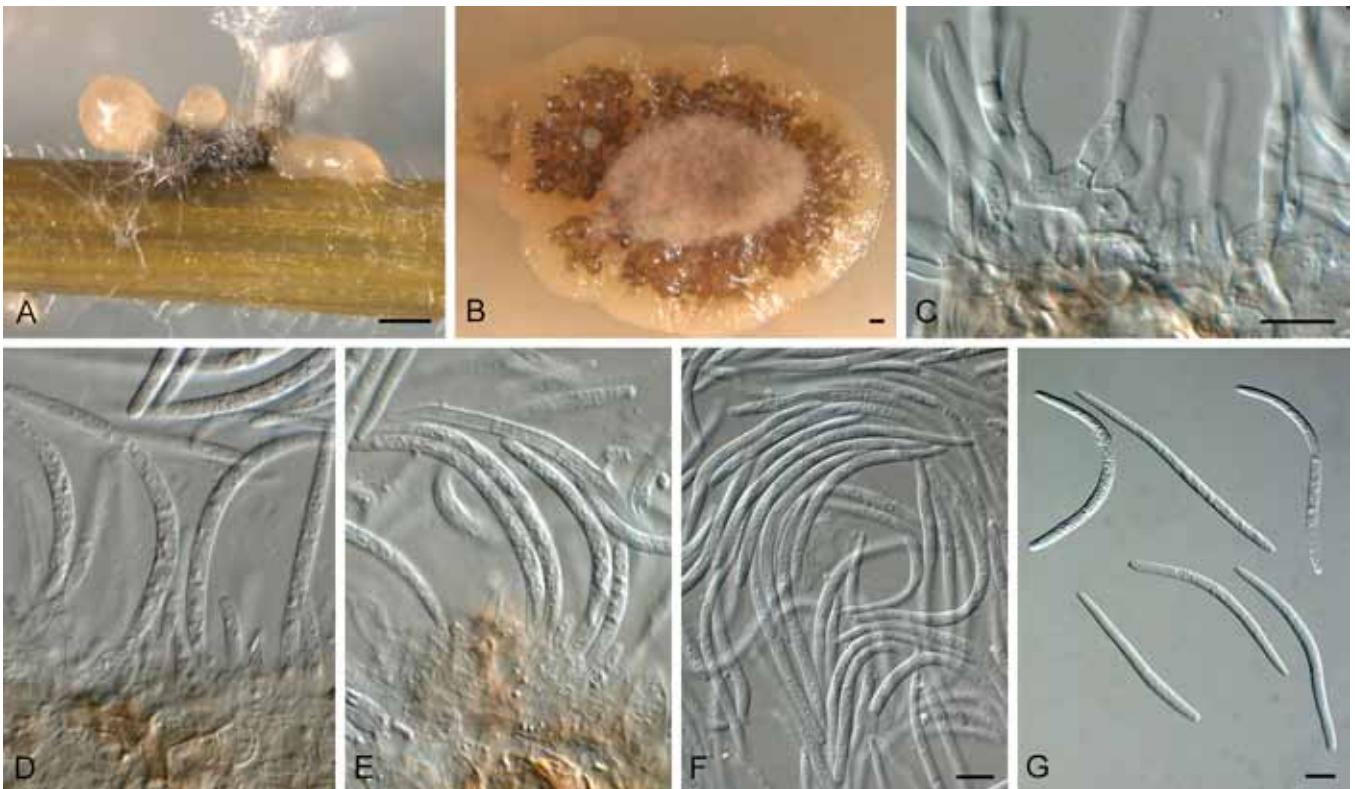
hyaline, smooth, ellipsoid to subcylindrical, with obtuse ends, 2.5–3.5  $\times$  1.5  $\mu\text{m}$ .

**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)  $\times$  1.5(–2)  $\mu\text{m}$ , 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)  $\times$  (1.5–)2(–3)  $\mu\text{m}$  (PC 0142144). Type material of *S. protearum* has conidia that are (0–)1–3(–4)-septate, (6–)12–22(–30)  $\times$  1.5–2  $\mu\text{m}$ , 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 cirrhous 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 distinguished 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 controversial, 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 surprising. 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 phytopathological 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***

### ACKNOWLEDGEMENTS

We thank the technical staff, Arien van Iperen (cultures), Marjan Vermaas (photographic plates), and Mieke Starink-Willems (DNA isolation, amplification and sequencing) for their invaluable assistance.

### REFERENCES

Aghajani MA, Aghapour B, Michailides TJ (2009) First report of *Septoria* leaf spot of pistachio in Iran. *Australasian Plant Disease Notes* **4**: 29–31.

Allescher A (1901) Fungi Imperfecti: Hyalin-sporige Sphaerioideen. *Dr L. Rabenhorst's Kryptogamen-Flora von Deutschland, Oestereich und der Schweiz* **1**(6): 961–1016.  
 Andrianova TV, Minter DW (2004) *Septoria pistaciae*. *IMI Descriptions of Fungi and Bacteria* **1586**: 1–2.  
 Bremer H, Karel G, Bıyıkođlu K, Göksele N, Petrak F (1952) Beiträge zur Kenntnis der parasitischen Pilze der Türkei. VI. *Revue de la Faculté des Sciences de l'Université d'Istanbul, Série B*, **17**: 260–261.  
 Bremer H (1954) Bahçe Kùltürleri Hastalıkları. *Türkiye Fitopatolojisi* **295**: 109–110.  
 Caracciolo F (1934) Una grave septoriosi del pistacchio. *Bolle Di Studi ed Informazione del R. Giardino di Palermo* **13**: 66–73.

- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.
- Chitzanidis A (1956) Species of *Septoria* on the leaves of *Pistacia vera* and their perfect states. *Annales de l'Institut Phytopathologique Benaki* **10**: 29–44.
- Cooke MC (1884) Synopsis Pyrenomycetum (continued). *Grevillea* **13**: 41–45.
- Crous PW, Braun U, Groenewald JZ (2007) *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**: 1–32.
- Crous PW, Braun U, Hunter GC, Wingfield MJ, Verkley GJM, Shin H-D, Nakashima C, Groenewald JZ (2013a) Phylogenetic lineages in *Pseudocercospora*. *Studies in Mycology* **75**: 37–114.
- Crous PW, Denman S, Taylor JE, Swart L, Bezuidenhout CM, Hoffman L, Palm ME, Groenewald JZ (2013b) *Cultivation and diseases of Proteaceae: Leucadendron, Leucospermum and Protea*. 2<sup>nd</sup> edn. [CBS Biodiversity Series no. 13.] Utrecht: CBS-KNAW Fungal Biodiversity Centre.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.
- Crous PW, Groenewald JZ (2005) Hosts, species and genotypes: opinions versus data. *Australasian Plant Pathology* **34**: 463–470.
- Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, et al. (2009a) Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology* **64**: 17–47.
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, et al. (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**: 235–253.
- Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, et al. (2009b) Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* **23**: 99–118.
- Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds) (2009c) *Fungal Biodiversity*. [CBS Laboratory Manual Series no.1.] Utrecht: CBS-KNAW Fungal Biodiversity Centre.
- Crous PW, Wingfield MJ, Park RF (1991) *Mycosphaerella nubilosa* a synonym of *M. molleriana*. *Mycological Research* **95**: 628–632.
- Desmazières JB (1842) Neuvième notice sur quelques plantes cryptogames. *Annales des Sciences Naturelles, Botanique, série 2* **17**: 91–118.
- Diñç N (1983) Antepfıstıđı Hastalıkları ve Mücadele Usulleri. *Mesleki Eserler Serisi* **2**: 19–34.
- Diñç N, Göksedef O, Turan K (1979) Gaziantep ili antepfıstıklarında zarar yapan Karazenk hastalığı (*Septoria pistacina* All.) nın Bio-ekolojisi ve korunma olanakları üzerine arařtırmalar. *Bitki Koruma Bülteni* **19**: 16–38.
- Dudka IO, Heluta VP, Tykhonenko YY, Andrianova TV, Hayova VP, et al. (2004) *Fungi of the Crimean Peninsula*. Kiev: M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine.
- Haggag WM, Abou Rayya MSM, Kasim NE (2006) First report of *Septoria pistaciae* causing leaf spot of pistachio in Egypt. *Plant Disease* **90**: 1553.
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, et al. (2011) The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112.
- Hoog GS de, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous *Basidiomycetes*. *Mycoses* **41**: 183–189.
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.
- Koike SK, Baameur A, Groenewald JZ, Crous PW. (2011). Cercosporoid leaf pathogens from whorled milkweed and spineless safflower in California. *IMA Fungus* **2**: 7–12.
- Lombard L, Crous PW, Wingfield BD, Wingfield MJ (2010) Species concepts in *Calonectria* (*Cylindrocladium*). *Studies in Mycology* **66**: 1–13.
- Leveillé JH (1842) Observations médicales et énumérations des plantes recueillies en Tauride. In: *Voyage dans la Russie meridionale et la Crimée* (Démidoff A, ed.) **2**: 80–135. Paris: Ernes Boudier.
- Liu Y, Whelen S, Hall B (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* **16**: 1799–1808.
- Mason-Gamer RJ, Kellogg EA (1996) Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae* (*Gramineae*). *Systematic Biology* **45**: 524–545.
- Michailides TJ (2005) Pest, disease, and physiological disorders management: above ground fungal diseases. In: *Pistachio Production Manual* (Beede RH, Freeman MW, Haviland DR, Holtz BA, Kallsen CE, eds): 214–232. Davis, CA: Fruit and Nut Research and Information Center, Department of Plant Sciences, University of California Davis.
- Michailides TJ, Morgan DP, Doster MA (1995) Diseases of pistachio in California and their significance. *Acta Horticulturae* **419**: 337–343.
- Nag Raj TR (1993) *Coelomycetous anamorphs with appendage-bearing conidia*. Waterloo, ON: Mycologue Publications.
- Nylander JAA (2004) *MrModeltest*. Version 2. Uppsala: Distributed by the author. Evolutionary Biology Centre, Uppsala University.
- O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.
- O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences, USA* **95**: 2044–2049.
- Pantidou ML (1973) *Fungus-Host Index for Greece*. Athens: Benaki Phytopathological Institute.
- Parfitt DE, Badenies ML (1997) Phylogeny of the genus *Pistacia* as determined from analysis of the chloroplast genome. *Proceedings of the National Academy of Sciences, USA* **94**: 7987–7992.
- Quaedvlieg W, Kema GHJ, Groenewald JZ, Verkley GJM, Seifbarghi S, Razavi M, Gohari AM, Mehrabi R, Crous PW (2011) *Zymoseptoria* gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. *Persoonia* **26**: 57–69.
- Quaedvlieg W, Verkley GJM, Shin H-D, Barreto RW, Alfenas AC, et al. (2013) Sizing up *Septoria*. *Studies in Mycology* **75**: 307–390.
- Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.

- Stukenbrock EH, Quaedvlieg W, Javan-Nikhah M, Zala M, Crous PW, McDonald BA (2012) *Zymoseptoria ardabilia* and *Z. pseudotritici*, two progenitor species of the septoria tritici leaf blotch fungus *Z. tritici* (synonym: *Mycosphaerella graminicola*). *Mycologia* **104**: 1397–1407.
- Sutton BC (1980) *The Coelomycetes: fungi imperfecti with pycnidia, acervuli, and stromata*. Kew: Commonwealth Mycological Institute.
- Swart L, Crous PW, Denman S, Palm ME (1998) Fungi occurring on *Proteaceae*. I. *South African Journal of Botany* **64**: 137–145.
- Teviotdale BL, Michailides TJ, MacDonald J (2001) *Diseases of Pistachio (Pistacia vera L.)*. *Common Names of Plant Diseases*. St Paul, MN: American Phytopathological Society Press.
- Tous J, Ferguson L (1996) Mediterranean fruits. In: *Progress in New Crops* (Janick J ed.): 416–430. Arlington, VA: American Society for Horticultural Science Press.
- Vassiljevsky NI, Karakulin BP (1950) *Parazitnye Nesovershennye Griby*. Vol. 2. *Melanconiales*. Moscow: Izdatel'stvo, AN SSSR.
- Verkley GJM, Crous PW, Groenewald JZ, Braun U, Aptroot A (2004) *Mycosphaerella punctiformis* revisited: morphology, phylogeny, and epitypification of the type species of the genus *Mycosphaerella* (Dothideales, Ascomycota). *Mycological Research* **108**: 1271–1282.
- Verkley GJM, Quaedvlieg W, Shin HD, Crous PW (2013) A new approach to species delimitation in *Septoria*. *Studies in Mycology* **75**: 213–305.
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
- White TJ, Bruns T, Lee J, Taylor SB (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ eds): 315–322. San Diego: Academic Press.
- Wingfield MJ, De Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, *et al.* (2012) One fungus, one name promotes progressive plant pathology. *Molecular Plant Pathology* **13**: 604–613.
- Young DJ, Michailides TJ (1989) First report of *Septoria* leaf spot of pistachio in Arizona. *Plant Disease* **73**: 775.