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. pistacina, 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 conidialmatal 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.

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 rots, blossom and shoot blight, canker and rust, and other problems (Michailides 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.

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 pistacinae 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, Kirgizistan, 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 2006). Septoria pistacinae is known from Greece (Chitzanidis 1956), Syria, Turkey and Iran (Aghajani et al. 2009), and appears to have a more limited distribution.

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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 LSU1Ff (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. 1990) 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 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 RP2B-5F (Liu et al. 1999) and RP2B-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](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 Z1 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 hyphe surrounding pycnidia). Conidia hyaline, smooth, cylindrical, mostly straight, rarely slightly curved, apex 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
A Bayesian 50% majority rule RPB2/LSU consensus tree containing representative isolates belonging to *Pseudocercospora* and related genera (*Myocosphaerellaceae*). 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 verkleij* (CBS 133618). The scalebar indicates 0.1 expected changes per site.
| Species                     | Isolate no. | Host                | Location | Collected by | GenBank accession no. |
|-----------------------------|-------------|---------------------|----------|--------------|-----------------------|
|                             |             |                     |          |              | 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 carpophyllus | Netherlands | Schouten | KF252301 | KF251799 | –   | –    | –    |
| Cercospora apii              | CBS 118712  | –                   | Fiji     | P. Tyler     | KF252302 | KF251800 | –   | –    | –    |
| Cercospora ariminensis       | CBS 137.56  | Hedeyeum coronarium | Italy    | M. Ribaldi   | KF252303 | KF251801 | –   | –    | –    |
| Cercospora beticola          | CBS 124.31  | Beta vulgaris       | Romania  | –            | KF252155 | KF251650 | –   | –    | –    |
| Cercospora zebrina           | CBS 118790  | Trifolium subteraneum | Austria  | M.J. Barbetti | KF252156 | KF251651 | –   | –    | –    |
| Cylindroseptoria pistacae    | CBS 471.69  | Pistacia lentiscus  | Spain    | H.A. van der Aa | KF252161 | KF251656 | –   | –    | –    |
| Pallidocercospora acaciaigena| 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  | Leucospemum         | 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. Afnenas | KF442694 | KF442660 | –   | –    | –    |
|                             | CPC 11716   | –                   | Brazil   | A.C. Afnenas | 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 irregularifrons | CBS 112111 | 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 | –   | –    | –    |
| 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    | *Solanum nigrum*           | South Korea  | H.D. Shin                  | JX902006                | JX901883 | –   | –    | –    |
| *Pseudocercospora basiramifera*        | CBS 111072   | *Eucalyptus pellita*       | Thailand     | M.J. Wingfield             | KF442706                | GU253709 | –   | –    | –    |
| *Pseudocercospora basitruncata*        | CBS 114664   | *Eucalyptus grandis*       | Colombia     | M.J. Wingfield             | KF442708                | GU253710 | –   | –    | –    |
| *Pseudocercospora cecidio-chinesis*    | CPC 14481    | *Cercis chinensis*         | South Korea  | H.D. Shin                  | JX902007                | JX901884 | –   | –    | –    |
| *Pseudocercospora chiangmaiensis*      | CBS 123244   | *Eucalyptus camaldulensis*  | 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 | –   | –    | –    |
| *Pseudocercospora fori*                | CBS 124990   | *Eucalyptus camaldulensis*  | Thailand     | W. Himaman                 | JX902028                | JX901905 | –   | –    | –    |
| *Pseudocercospora gracilis*            | CPC 113285   | *Eucalyptus grandis*       | South Africa | G.C. Hunter                | KF442713                | GU253824 | –   | –    | –    |
| *Pseudocercospora humul-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 pisticina*           | CPC 21874    | *Pistacia vera*            | Turkey       | K. Sarpkaya                | KF442719                | KF442675 | KF442648 | KF442734 | KF442638 |
|                                        | CPC 23117; 27N1080 | *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; 45Shn005 | *Pistacia vera*            | Turkey       | K. Sarpkaya                | KF442720                | KF442676 | KF442649 | KF442735 | KF442639 |
|                                        | CBS 135841; 63br043 | *Pistacia vera*            | Turkey       | K. Sarpkaya                | KF442721                | KF442677 | KF442650 | KF442736 | KF442640 |

¹ Species collection number.

² GenBank accession numbers for the different loci.
| Species                        | Isolate no. | Host                  | Location      | Collected by     | GenBank accession no. | 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 11175   | *Eucalyptus robusta*  | Malaysia      | M.J. Wingfield   | JX902027              | JX901904 | –   | –   | –   | –     |
| *Pseudocercospora sphaeruliniae* | CBS 112621  | *Eucalyptus sp.*      | –             | P.W. Crous       | JX902029              | JX901906 | –   | –   | –   | –     |
| *Pseudocercospora subulata*   | CBS 118489  | *Eucalyptus botryoides* | New Zealand   | M. Dick          | JX902030              | JX901907 | –   | –   | –   | –     |
| *Pseudocercospora tereticomis* | CPC 13315   | *Eucalyptus nitens*   | Australia     | P.W. Crous       | JX902032              | JX901909 | –   | –   | –   | –     |
| *Pseudocercospora vitis*      | CPC 11959   | *Vitis vinifera*      | South Korea   | H.D. Shin        | JX902035              | JX901912 | –   | –   | –   | –     |
| *Ramulispora sorghi*          | CBS 110578  | *Sorghum bicolor*     | South Africa  | D. Nowell        | KF442722              | KF442678 | –   | –   | –   | –     |
| *Septoria astralagi*          | CBS 109117  | *Astragalus glycphylos* | Austria       | G. Verkley       | KF252350              | KF251853 | –   | –   | –   | –     |
| *Septoria cythi*              | 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 lamicola*           | CBS 109113  | *lamium album*        | Austria       | G. Verkley       | KF252443              | KF251950 | –   | –   | –   | –     |
| *Septoria pistaciae*          | CBS 420.51  | *Pistacia vera*       | Italy         | G. Goldanich     | KF442724              | KF252025 | –   | –   | –   | –     |
| *Septoria pistachiurum*       | CPC 23116; 5DMR032 | *Pistacia vera*     | Turkey        | K. Sarpkaya      | KF442725              | KF442680 | KF442651 | KF442737 | KF442635 |
| *Septoria protearium*         | CBS 101013  | *Masdevallia sp.*     | Netherlands   | W. Veenbaas-Rijks | KF252504              | KF252013 | –   | –   | –   | –     |
| *Septoria rumicum*            | CBS 503.76  | *Grevia avellana*     | New Zealand   | S. Ganev         | KF252505              | KF252014 | –   | –   | –   | –     |
| *Septoria stellariae*         | CBS 102376  | *Stellaria media*     | Netherlands   | G. Verkley       | KF252567              | KF252079 | –   | –   | –   | –     |
Pseudocercospora pistacina (Allesch.) Crous, Quaedvlieg & Sarpkaya, comb. nov.  
*Basionym:* *Septoria pistacina* Allesch., *Rabenh.* *Krypt.-Fl.* 1(6): 830 (1900) [*1899*].  
*Synonyms:* *Dothidea pistacina* Lév., in Démidoff, *Voy. Russ. Mér.* 2: 108 (1842).  
*Non* *Pseudocercospora pistacina* (Chupp) Crous & U. Braun, *Mycotaxon* 78: 338 (2001).  
*Septoria pistacae* (Lév.) Cooke, *Grevillea* 13 (66): 45 (1884); *nom. illegit.*, *non* *S. pistacæ* Desm. 1842.  
*Mycosphaerella pistacina* Chitazan., *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, 0–3-septate, branched or not, 10–30 × 3–5 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, ascii 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 *Pistacia vera*, 2010, K. Sarpkaya (CPC 45sln005 = CBS 135840). *Gaziantep*: Nizip, on *P. vera*, 2010, K. Sarpkaya (CPC 27NZ2080 = CPC 23117). *Sanliurfa*: Birecik, on *P. vera*, 2010, K. Sarpkaya (CPC 63br043 = CBS 135841). *Aydır*: 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 *Kirrmyces* (syn. *Teratosphaeria*; Crous *et al.* 2009a, b), though it is phylogenetically unrelated to these genera. *Pseudocercospora pistacina* clusters basally within *Pseudocercospora*, but based

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### Table 1. (Continued.)

| Species | Isolate no. | Host | Location | GenBank accession no. | EF-α | LSU | ITS | Btub |
|---------|-------------|------|----------|-----------------------|------|-----|-----|------|
| *Candida sativa* | CBS 115368 | Castanea sativa | Netherlands | EF-1α: KF442730, ITS: KF442685, Btub: KF252110, EF-1α: KF252124, LSU: KF252125 | | | | |
| *Eutypa globulina* | CBS 116724 | Eucalyptus globulus | Spain | EF-1α: KF442730, ITS: KF442685, Btub: KF252110, EF-1α: KF252124, LSU: KF252125 | | | | |
| *Betula platypila* | CBS 128600 | Betula pubescens | South Korea | EF-1α: KF442730, ITS: KF442685, Btub: KF252110, EF-1α: KF252124, LSU: KF252125 | | | | |
| *P. annua* | CBS 133618 | Poa annua | Netherlands | EF-1α: KF442730, ITS: KF442685, Btub: KF252110, EF-1α: KF252124, LSU: KF252125 | | | | |
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. 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.

**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.
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 \textit{P. pistacina} is rather atypical within \textit{Pseudocercospora s. str.}

\textit{Septoria pistaciae} Desm., \textit{Annls Sci. Nat., Bot., sér. 2} \textbf{17}: 112 (1842).

\textbf{Synonyms:} \textit{Phloeospora pistaciae} (Desm.) Petr., \textit{Annls mycol.} \textbf{20}: 18 (1922).

\textit{Cylindrosporium pistaciae} (Desm.) Vassil., \textit{Fungi Imp. Paras.} \textbf{2}: 510 (1950).

\textbf{Description:} \textit{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. \textit{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 \textit{textura angularis}. \textit{Conidiophores} reduced to conidiogenous cells, or up to 4-septate, subcylindrical with lateral and terminal conidiogenous cells, 5–25 × 3–4 μm. \textit{Conidiogenous cells} hyaline, smooth, ampulliform to subcylindrical, 5–10 × 3–4 μm, lining inner layer of conidiomatal cavity, proliferating sympodially, rarely percurrently. \textit{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. \textit{Spermatial state} occurring in conidiomata along with conidia. \textit{Spermogogenous cells} hyaline, smooth, ampulliform, 4–6 × 3–5 μm. \textit{Spermatia} hyaline, smooth, ellipsoid to subcylindrical, with obtuse ends, 2.5–3.5 × 1.5 μm.

\textbf{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).

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

\textbf{Notes:} \textit{Septoria pistaciae} is part of the species complex for which Verkley \textit{et al.} (2013) adopted the oldest name, \textit{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 \textit{et al.} (2013), awaits further study and inoculation trials.

Andrianova & Minter (2004) described conidia of \textit{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 \textit{S. protearum} has conidia that are (0–)1–3(–4)-septate, (6–)12–22(–30) × 1.5–2 μm, obclavate to narrowly subcylindrical (Swart \textit{et al.} (2009c)).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{\textit{Septoria pistaciae} (PC 0142143). \textbf{A}. Herbarium specimen. \textbf{B–F}. Conidiogenous cells giving rise to conidia. \textbf{G}. Conidia. Bars = 10 μm, \textbf{D} applies to \textbf{E–G}.}
\end{figure}
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–)65–65 (–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. pistaccae* 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 Hawkesworth 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 conidial fascicles, is *Ciferriella*, which has also been reduced to synonymy with *Pseudocercospora* (Quaedvlieg et al. 2013). These findings support the view that conidial 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. pistaccae*, and *S. pistaciarum* on *Pistacia vera* is well-documented (Michailides 2005), nothing is known about that of *Cylindroseptoria pistaccae*, 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 pistaccae* |
|   | 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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### 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 4(6): 961–1016.

Andrianova TV, Minter DW (2004) *Septoria* pistaciae. *IML Descriptions of Fungi and Bacteria* 1586: 1–2.

Bremer H, Karel G, Býrokojlu K, Göksel 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 Kültürleri Hastalıkları. *Türkiye Fitotopolojisi* 295: 109–110.

Caracciolo F (1934) Una grave septoria del pistacchio. *Bolle Di Studi ed Informazione del R. Giardino di Palermo* 13: 66–73.
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.