**Plectosphaerella** species associated with root and collar roots of horticultural crops in southern Italy

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Abstract *Plectosphaerella cucumerina*, most frequently encountered in its *Plecostosporium* state, is well known as a pathogen of several plant species causing fruit, root and collar rot, and collapse. It is considered to pose a serious threat to melon (*Cucumis melo*) production in Italy. In the present study, an intensive sampling of diseased cucurbits as well as tomato and bell pepper was done and the fungal pathogens present on them were isolated. Phylogenetic relationships of the isolates were determined through a study of ribosomal RNA gene sequences (ITS cluster and D1/D2 domain of the 28S rRNA gene). Combining morphological, culture and molecular data, six species were distinguished. One of these (*Pa. cucumerina*) is already known. Four new species are described as *Plectosphaerella citrulli*, *Pa. pauciseptata*, *Pa. plurivora* and *Pa. ramiseptata*. *Acremonium curcitaeaceum* is shown to be a synonym of *Nodulisporium melonis* and is transferred to *Plectosphaerella* as *Plectosphaerella melonis* comb. nov. A further three known species of *Plecostosporium* are recombined in *Plectosphaerella*.

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INTRODUCTION

Melon (*Cucumis melo*) is an important horticultural crop in Southern Italy (Apulia), which annually produces approximately 647 370 t on 2 498 ha (Anonymous 2010). In the last 15 yr this crop has suffered significant losses due to root and collar roots, wilt and collapse of the vines (Gennari et al. 1999, Buizi et al. 2002, 2004, Infantino et al. 2002, 2004, Montuschi 2002). Symptoms of the disease are similar to those described for vine decline of melons (Watanabe 1979, Bruton 1998) including the development of brown lesions, corky and decayed areas on roots, yellowing of older leaves and general wilting and death of plants during fruit ripening. Several fungi have been isolated from root, collar and fruit of melon plants with symptoms of vine decline or collapse. Thus, *Fusarium oxysporum* f. sp. melonis and *Verticillium dahliae* have been implicated as causes of melon wilt (Buizi et al. 2002, Infantino et al. 2004), while *Rhizoctonia solani* AG 4 and *Pyrenochaeta lycopersici* have been reported as causing corky rot and root rot respectively (Corazza et al. 1992, Infantino et al. 2004).

Since the 1980s a disease known as melon collapse has been reported from Japan (Watanabe 1979), Israel (Reuveni et al. 1983, Cohen et al. 2000, Pevonia et al. 2002), Spain (Ruano 1990, 1991, Garcia-Jimenez et al. 1993, 2000, Sales 2001), and the USA (Hansen 2000, Boucher & Wick 2004) including California (Bruton et al. 1995, Stanghellini et al. 2004) and Texas (Mertelely et al. 1993, Martyn & Miller 1996, Bruton 2000). Subsequently, it was reported from Italy (Stravato et al. 2002, Infantino et al. 2002, 2004, Buizi et al. 2004, Chilosi et al. 2008). The main causes have been attributed to *Monosporascus cannonballus* and *Acremonium cucurbitacearum* (Armengol et al. 1998, Bruton 2000). Other putative fungal pathogens frequently isolated from cucurbits and associated with the disease are *Plectosphaerella cucumerina (= Plectosporium tabacinum)* (Bost & Mullins 1992, Palm et al. 1995) and *Rhizopycnis vagum* (Farr et al. 1998, Montuschi 2002, Armengol et al. 2003). In Japan, Sato et al. (1995) and Watanabe & Sato (1995) reported *Nodulisporium melonis* as the causal pathogen of cucurbit decline. In New England, Hansen (2000) and Boucher & Wick (2004) reported *Pa. cucumerina* (as *Pm. tabacinum*) as the causal agent of *Plecostosporium* Blight causing large losses of pumpkin and zucchini. In Italy *Pa. cucumerina* has been reported by Carlucci et al. (2006) as one of the fungi associated with cucurbit collapse. This species is known as a ubiquitous and polyphagous fungus frequently isolated from several different plant hosts (Pascoe et al. 1984, Palm et al. 1995).

*Plecostosporium* was introduced by Palm et al. (1995) for *Fusarium* tabacinum, the anamorph of *Plectosphaerella cucumerina*. Palm et al. (1995) noted considerable morphological variation between isolates of *Pa. cucumerina* and considered that this could indicate a complex of species. Asexual states of *Plecostosphaerella* are differentiated based on the proportion of septate conidia (Pitt et al. 2004), presence or absence of chlamydospores (Pitt et al. 2004), conidial shape (Antignani et al. 2008) and conidial dimensions (Duc et al. 2009), together with ITS sequence data. Since the introduction of *Plecostosporium* for *Plecostosphaerella* anamorphs, three further species have been described in this genus. Pitt et al. (2004) transferred *Rhynchosporium atkinsonii* to *Plecostosporium*, Antignani et al. (2008) described *Plecostosporium delsorboi* and Duc et al. (2009) described *Plecostosporium oratosquillae* infecting mantis shrimp in Japan.

*Plecostosphaerella* was introduced by Klebahn (1929) who described *Plecostosphaerella cucumeris* from young cucumber plants in Germany. According to Uecker (1993), Elbakayan (1970) regarded Klebahn’s fungus as conspecific with *Venturia cucumerina* (Lindfors 1919). The combination *Plecostosphaerella cucumerina* was introduced by Gams (Domsh & Gams 1972). It has been regarded as a member of the *Hypocreaceae* (Barr...
1990, Gams & Gerlach 1968), while Uecker (1993) suggested that based on centrum development type it is closer to the Sor­
daraceae. More recently Zare et al. (2007) proposed the family Plectosphaerellaceae to accommodate Acrostalagmus, Gibel­
lulopsis, Musicilium, Plectosphaera (as Plectosporium) and Verticillium.

In the present work a collection of isolates tentatively identified as Pa. cucumerina, mainly from melon and watermelon, but also from other cucurbits, tomato, bell pepper, asparagus and parsley was studied. Phylogenetic relationships of the isolates together with other examples of the Plectosphaerellaceae were determined through a study of ribosomal RNA gene sequences (ITS cluster and D1/D2 domain of the 28S rRNA gene).

MATERIALS AND METHODS

Isolates and isolations

Isolations were made by directly plating out pieces of symp­
tomatic collar and root from melon, watermelon, tomato, bell pepper, parsley and asparagus plants on PDA amended with 400 ppm streptomycin sulphate, after surface sterilization in 5 % NaOCl for 1 min. After 5–7 d of incubation at 21 ± 2 °C, conidia were spread over plates of PDA and after incubating overnight, single germinating conidia were transferred to fresh PDA plates. Single conidial isolates were stored on PDA slopes at 3 ± 2 °C at the Department DiSACD, University of Foggia. References isolates and specimens were deposited in the public culture collection at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Isolates studied are listed in Table 1.

Morphology

Growth rates were determined after 14 d of incubation on PDA at 23 ± 2 °C in the dark. Colony characters were determined on cultures grown under the same conditions. Cardinal tempera­tures for growth were determined on PDA plates incubated in the dark at temperatures ranging from 3 to 40 °C in 3 °C intervals. Microscopic characters were determined from slide cultures prepared according to the method described by Palm et al. (1995), except that 100 % lactic acid was used as mountant. For observations of conidiogenesis, a small block of the agar (about 2 mm³) from a young fungal colony was placed in the centre of clean and sterile glass microscope slide, which was kept in a moist chamber consisting of a sterile Petri plate lined with filter-paper soaked in distilled water. After 7–10 d of incubation at 21 ± 2 °C in the dark, the block of agar was removed and mycelium, conidiogenous hyphae and conidia were mounted in 100 % lactic acid. Dimensions of conidiogenous cells, hyphal coils and conidia was measured with the Leica IM500 measure­ment module (Leica Microsystems GmbH, Wetzlar, Germany) from images recorded on a Leica DFC 320 digital camera on a Leica DMR microscope fitted with Nomarski differential interference contrast optics. From measurements of at least 25 conidia, the mean, standard deviation and 95 % confidence intervals were calculated. Dimensions of other structures are given as the range of at least 20 measurements.

DNA isolation and amplification

Genomic DNA was extract by E.Z.N.A. Plant Kit (Omega, Bio­tek), and part of the nuclear rRNA cluster comprising the ITS and D1/D2 regions of the ribosomal LSU gene was amplified with the primers ITS1 and ITS4 (White et al. 1990) and NL1 and NL4 (O’Donnell & Gray 1993), respectively. PCR reac­tions were carried out with Taq polymerase, nucleotides and buffers supplied by MBI Fermentas 144 (Vilnius, Lithuania) and PCR reaction mixtures were prepared according to Alves et al. (2004), with the addition of 5 % DMSO to improve the amplification. The amplified PCR fragments were purified with the JETQUICK PCR Purification Spin Kit (GENOMED, Löhne, Germany). Both strands of the PCR products were sequenced by STAB Vida Lda (Portugal). The nucleotide sequences were read and edited using BioEdit. All sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences. GenBank accession numbers of published sequences are shown in the phylogenetic trees, while accession numbers of sequences obtained in this study are presented in Table 1.

Phylogenetic analyses

For the LSU dataset, sequences of representatives of the Plec­tosphaerellaceae (Zare et al. 2007) were downloaded from Gen­Bank together with representatives of closely related families. For the ITS dataset, sequences of closely related species of Plectosporium and other closely related genera were selected in BLAST searches. Sequences for both datasets were aligned with ClustalX v. 1.83 (Thompson et al. 1997). Phylogenetic information contained in indels in the ITS and dataset was incorporated into the phylogenetic analysis using simple indel coding as implemented by GapCoder (Young & Healy 2003). Maximum likelihood analyses were done using RAxML (Stamatakis 2006) on the webserver (Stamatakis, 2008) at http://phylo­bench.vital-it.ch/raxml-bb/index.php with the gamma model of rate heterogeneity in effect and maximum likelihood search. Bayesian analyses were done with MrBayes v. 3.0b (Ronquist & Huelsenbeck 2003) employing a Markov Chain Monte Carlo (MCMC) method. The general time-reversible model of evolu­tion (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories was used. Four MCMC chains were run simultane­ously, starting from random trees, for 10⁶ generations. Trees were sampled every 100th generation for a total of 10⁴ trees. The first 10⁴ trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala & Yang 1996) were determined from a 50 % majority-rule consensus tree generated from the remaining 9 000 trees. The analysis was repeated three times starting from different random trees to ensure trees from the same tree space were being sampled during each analysis. Maximum parsimony genealogies were estimated in PAUP using heuristic searches based on 1 000 random taxon addition sequences and the best trees were saved. Sequences derived in this study were lodged at GenBank, alignments and trees in TreeBASE (www.treebase.org) and taxonomic novelties in MycoBank (www.mycobank.org; Crous et al. 2004).

RESULTS

Phylogenetic analyses

The LSU sequences generated for 46 of the isolates studied (Table 1) were aligned with 56 sequences retrieved from Gen­Bank, representing a selection of families and genera in the Hypocreales. After alignment the LSU dataset consisted of 458 characters including alignment gaps, and 102 taxa including the outgroup taxon Leptogriphum procerum (AY789163). ML and Bayesian analyses resulted in trees with the same topol­ogy (TreeBASE S12518). The Plectosphaerellaceae was well­supported in both methods (100/1.00), but support for branches within the family was generally low, except for Acrostalagmus, Musicilium and Verticillium (Fig. 1). The isolates sequenced in this study and tentatively assigned to Plectosphaerella lay within three clades each supported by moderate bootstrap values. Internal support for branches leading to these clades received low support effectively resulting in a polytomy with Verticillium and Gibellulopsis.

ITS sequences were generated for 58 isolates and these were aligned with 38 sequences retrieved from GenBank. The data-
set consisted of 96 taxa, including two outgroup taxa (**Gibellulopsis nigrescens**, **Cephalosporium serrae** var. **fuscum**). After alignment the dataset consisted of 490 characters including the coded gap matrix appended to the sequences. ML, MP and Bayesian analyses resulted in trees with similar topologies (TreeBASE S12166). The isolates sequenced in this work clustered in six clades (Fig. 2). Most of the isolates clustered in a single clade considered to be **Pa. cucumerina**, including **CBS 137.37**, ex-holotype of **Cephalosporium ciferri**. Four isolates clustered with the ex-type isolate of **A. cucurbitacearum** (CBS 525.93, GenBank AJ621754) and the ex-type isolate of **N. melonis** (CBS 489.96, GenBank AJ621770) in a clad sister to **P. delsorboi** and **P. alismatis**. The remaining isolates formed a cluster of four clades supported by moderate to high bootstrap support in the ML tree.
Fig. 1 Maximum Likelihood tree obtained from LSU sequence data with bootstrap support values in Maximum Likelihood/Bayesian Posterior Probability scores.
Fig. 2  Maximum Likelihood tree obtained from ITS sequence data with bootstrap support values from Maximum Parsimony/Bayesian Posterior Probability/Maximum Likelihood. Ex-type isolates are in **bold** face red.
TAXONOMY

Ten species were resolved in *Plectosphaerella*. Four of them are presently known (*Pm. alismatis*, *Pa. cucumerina*, *Pm. delsorboi* and *Pm. oratosquillae*) while one has been described as *A. cucurbitacearum*. No names are available for four of the clades revealed in this work and on account of the phylogenetic and morphological distinctions they are described as new species. *Acremonium cucurbitacearum* and *Nodulisporium melonis* are confirmed to be a species of *Plectosphaerella* and a new combination (*Pa. melonis*) is made here. Isolate Plect 148 formed a branch sister to *Pa. melonis* and may represent another species. However, since only one isolate was available no name was proposed. Two sequences from GenBank (AB264781, EF495236) formed a clade sister to *Pa. plurivora* and *Pa. citrullae*. Although these appear to represent another species of *Plectosphaerella*, no cultures were available for study. *Plectosporium alismatis*, *Pm. delsorboi* and *Pm. oratosquillae* were confirmed to be species in *Plectosphaerella* and new combinations are introduced.

*Plectosphaerella alismatis* (Oudem.) A.J.L. Phillips, A. Carlucci & M.L. Raimondo, comb. nov. — MycoBank MB564575

Basionym. *Septoria alismatis* Oudem., Ned. Kruidk. Arch., Ser. 2, 2: 100. 1875.
≡ *Rhynchosporium alismatis* (Oudem.) Davis, Trans. Wisconsin Acad. Sci. 20: 420. 1922.
≡ *Didymaria alismatis* (Oudem.) Davis, Parasitic Fungi of Wisconsin: 103. 1942.
≡ *Spermosporina alismatis* (Oudem.) U. Braun, Cryptog. Bot. 4: 111. 1993.
≡ *Ascochyta alismatis* Ellis & Everh., J. Mycol. 5: 148. 1889.
≡ *Ramularia alismatis* Fautrey, Rev. Mycol. (Toulouse) 12: 125. 1890.
≡ *Ovularia alismatis* Pass., Diagnosi di Funghi nuovi IV: 13, Roma 1890.
≡ *Didymaria aquatica* Starbäck, Bot. Centralbl. 64: 383. 1895.
≡ *Ramularia sagittariae* Bres., Hedwigia 36: 200. 1896.
≡ *Spermosporina sagittariae* (Bres.) U. Braun, Cryptog. Bot. 4: 113. 1993.
≡ *Cylindrosporium baudysianum* Sacc., Ann. Mycol. 12: 296. 1914.

Fig. 3 *Plectosphaerella citrullae*. a, b. Colonies on PDA after 14 d at 23 ± 2 °C; c–e. conidiophores and phialides; f–h. phialides; i, j. hyphal coils; k. conidia.
— Scale bars: c–e, i = 10 μm; f–h, j, k = 5 μm.
Plectosphaerella citrullae A.J.L. Phillips, A. Carlucci & M.L. Raimondo, sp. nov. — MycoBank MB564523; Fig. 3

Etymology. Named after Citrullus (watermelon) from which it was first isolated.

Colonies on PDA pale pink, mycelium appressed, slimy, aerial mycelium sparse or absent, reaching a diameter of 8 cm after 14 d at 23 ± 2 °C. Minimum temperature for growth 9 °C, optimum 25 °C, maximum 29 °C. Mycelium hyaline, branched, septate, forming hyphal coils on PDA, with phialides produced on the coils. Conidiophores solitary, unbranched or rarely irregularly branched, hyaline, smooth, thin-walled. Conidiogenous cells phialidic, determinate, discrete, hyaline, smooth, solitary, with a single basal septum, phialide apex straight, sometimes

Fig. 4 Plectosphaerella cucumerina. a–e. Colonies on PDA after 14 d at 23 ± 2 °C; f–h. asci and ascospores; i. hyphal coil with phialides; j–p. phialides; q. aseptate conidia; r. 1-septate conidia; s. conidiophore. — Scale bars: f–i = 10 μm; j–s = 5 μm.
crooked or sinuous, widest at the base, gradually tapering to the apex (15–)19–39(–60) × (1.5–)2–4(–6) μm, periclinal wall thickened, collarette cylindrical, 1.5–2 μm deep. Conidia aggregating in slimy heads, ellipsoid, tapering gradually to broadly rounded apex and base, hyaline, smooth, thin-walled, 1- or 2-guttulate, aseptate (5.5–)6.5–9(–10.5) × (2.5–)3–4 μm, mean ± S.D. of 101 conidia = 7.9 ± 0.9 × 3.5 ± 0.3 μm, 95 % confidence limits of 7.8–8.1 × 3.4–3.6 μm, L/W ratio = 2.3 ± 0.3. Chlamydospores absent.

Specimen examined. **ITALY,** Apulia, Foggia, on root of watermelon (**Citrullus lanatus**), 2005, A. Carlucci, holotype CBS H-20898, culture ex-type CBS 131741.

Notes — This species was isolated from diseased roots of **C. lanatus** in Apulia province of Italy, but its role in root rot has not been proved. Although similar to **Pa. cucumerina,** the longer conidiophores and conidiogenous cells of **Pa. citrullae** distinguish the two species and septate conidia have not been seen in **Pa. citrullae.**

**Plectosphaerella cucumerina** (Lindf.) W. Gams, in Domsch & Gams, Fungi in agricultural soils: 160. 1972. — Fig. 4, 5

**Basionym.** **Venturia cucumerina** Lindf., Meddn. CentAnst. FörsVäs. JordbrÖmnad., Stockholm 197/17: 7. 1919.

≡ **Monographella cucumerina** (Lindf.) Arx, Trans Brit. Mycol. Soc. 82: 374. 1984.

≡ **Plectosphaerella cucumeris** Kleb., Phytopathol. Z. 1: 43. 1930.

≡ **Micronectriella cucumeris** (Kleb.) C. Booth, The genus Fusarium: 39. 1971.

≡ **Cephalosporium tabacinum** J.F.H. Beyma, Zentralbl. Bakteriol., 2 Abt. 89: 240. 1933.

≡ **Fusarium tabacinum** (J.F.H. Beyma) W. Gams, in Gams & Gerlagh, Persoonia 5: 179. 1968.

≡ **Microdochium tabacinum** (J.F.H. Beyma) Arx, Trans. Brit. Mycol. Soc. 83: 374. 1984.

≡ **Plectosporium tabacinum** (J.F.H. Beyma) M.E. Palm, W. Gams & Nirenberg, Mycologia 87: 399. 1995.

≡ **Cephalosporium ciferrii** Verona, Studio sulle cause microbiche che danneggiano la carta ed i libri, Roma: 30. 1939.

≡ **Cephalosporiopsis imperfecta** Moreau & V. Moreau, Rev. Mycol. 6: 67. 1941. [nom. inval.].

Colonies on PDA various shades of buff to salmon pink, mycelium appressed, slimy, aerial mycelium sparse or absent (Fig. 5),

Fig. 5 Variation in culture morphology in **Plectosphaerella cucumerina.** All cultures were grown at 23 °C on PDA for 14 d.
reaching a diameter of 7.8 cm after 14 d at 23 ± 2 °C. Minimum temperature for growth 6 °C, optimum 25 °C, maximum 31 °C. Ascomata globose to pyriform, thin-walled, pale brown, 90–130 μm wide. Asci unitunicate, cylindrical apical apparatus absent, 50–80 × 6–9 μm. Ascospores hyaline, smooth, thin-walled, ellipsoid, both ends rounded, 1-septate, (9–)10.5–14(–15) × 2.5–3(–4) μm. Mycelium hyaline, branched, septate, forming hyphal coils on PDA, with phialides produced from the coils. Conidiophores solitary, unbranched or rarely irregularly branched, hyaline, smooth, thin-walled. Conidiogenous cells phialidic, determinate, discrete, hyaline, smooth, solitary, occasionally 1-septate near the base, phialide apex straight, sometimes crooked or sinuous, sometimes forming a branch just below the apex, cylindrical, widest at base, tapering gradually to the apex, (6–)10–35(–69) μm, periclinal wall thickened, collarette cylindrical 1.5–2 μm deep. Conidia aggregating in slimy heads, ellipsoidal, tapering gradually to rounded apex and base, widest in the middle, hyaline, smooth, thin-walled, septate or aseptate (varies between isolates), guttulate; aseptate conidia (4.5–)6–8.5(–9.5) × (1.7–)2.3–3.6(–3.9) μm, mean ± S.D. of 278 conidia = 6.8 ± 1.1 × 2.7 ± 0.4 μm, 95 % confidence limits = 6.7–7 × 2.7–2.8 μm, L/W ratio = 2.6 ± 0.4; septate conidia (5.2–)7–10.5(–11.8) × (1.9–)2.5–3.5(–4.4) μm, mean ± S.D. of 322 conidia = 8.8 ± 1.3 × 2.8 ± 0.4 μm, 95 % confidence limits = 8.7–8.9 × 2.7–2.9 μm.

**Fig. 6** *Plectosphaerella melonis*. a. Colony on PDA after 14 d at 23 ± 2 °C; b–d. conidiophores with phialides; e–g. phialides; h, i. conidia; j. hyphal coil; k–m. chlamydospores. — Scale bars: b–e = 10 μm; f–m = 5 μm.
limits = 8.6–8.9 × 2.8–2.9 μm, L/W ratio = 3.1 ± 0.6. Chlamydospores absent.

**Plectosphaerella delsorboi** (Antignani & W. Gams) A.J.L. Phillips, A. Carlucci & M.L. Raimondo, **comb. nov.** — MycoBank MB564576

*Basionym*. Plectosporium delsorboi Antignani & W. Gams, *Nova Hedwigia* 86: 212. 2008.

**Plectosphaerella melonis** (Ts. Watan. & Mas. Sato) A.J.L. Phillips, A. Carlucci & M.L. Raimondo, **comb. nov.** — MycoBank MB564527; Fig. 6

*Basionym*. Nodulisporium melonis Ts. Watan. & Mas. Sato, *Ann. Phytopathol. Soc. Japan* 61: 330. 1995. = Acremonium cucurbitacearum Alfaro-García, W. Gams & J. García-Jim., *Mycologia* 88: 805. 1996.

Colonies on PDA white, with abundant fluffy or cottony aerial mycelium, reaching a diameter of 8 cm after 14 d at 23 ± 2 °C. Minimum temperature for growth 9 °C, optimum 25 °C, maximum 31 °C. Mycelium hyaline, branched, septate, occasionally forming loose hyphal coils. Conidiophores solitary, sparingly branched, hyaline, smooth, thin-walled. Conidiogenous cells phialidic, determinate, discrete, hyaline, smooth, thin-walled, with single basal septum, widest at base, straight, gradually tapering to the apex, phialide apex straight or sometimes sinuous (12–)15–70(–84) × 1.5–2.5(–4) μm, periclinal wall thickened, collarette minute, cylindrical, 0.5–1 μm deep. Conidia aggregating in slimy heads, ellipsoid, tapered to rounded apex and base, hyaline, smooth, thin-walled, with a minute apiculus at either end, mostly aseptate (80 %) or 1-septate; aseptate conidia (4.5–)5.5–8.5(–12) × (2–)2.5–3.5(–4) μm, mean ± S.D. of 91 conidia = 6.7 ± 1.2 × 2.8 ± 0.5 μm, 95 % confidence limits = 6.4–6.9 × 2.7–2.8 μm, L/W ratio = 2.5 ± 0.5; septate conidia (7.5–)8–9(–10) × 2–3(–3.5) μm, mean ± S.D. of 23 conidia = 8.5 ± 0.6 × 2.8 ± 0.3 μm, 95 % confidence limits = 8.2–8.7 × 2.7–2.9 μm, L/W ratio = 3 ± 0.4, constricted at septum. Chlamydospores intercalary, hyaline, thick-walled, 9–22 × 15–25 μm.

*Notes* — The abundant hyaline chlamydospores differentiate *Pa. cucurbitacearum* and *Pa. alismatis* from all other species of *Plectosphaerella*, while the smaller conidia of *Pa. cucurbitacearum* distinguish it from *Pa. alismatis*.

**Plectosphaerella oratosquillae** (P.M. Duc, Yaguchi & Udagawa) A.J.L. Phillips, A. Carlucci & M.L. Raimondo, **comb. nov.** — MycoBank MB564577

*Basionym*. Plectosporium oratosquillae P.M. Duc, Yaguchi & Udagawa, *Mycopathologia* 167: 237. 2009.

**Plectosphaerella pauciseptata** A.J.L. Phillips, A. Carlucci & M.L. Raimondo, **sp. nov.** — MycoBank MB564524; Fig. 7

*Etymology*. Named for the scarcity of septate conidia.

Colonies on PDA pink or buff, mycelium appressed, slimy but sometimes with aerial mycelium at centre of the colony, reaching a diameter of 8 cm after 14 d at 23 ± 2 °C. Minimum temperature for growth 6 °C, optimum 25 °C, maximum 29 °C. Mycelium hyaline, branched, septate, forming coils on PDA with phialides produced on the coils. Conidiophores solitary, unbranched or rarely irregularly branched, hyaline, smooth, thin-walled. Conidiogenous cells phialidic, sometimes polyphialidic, determinate, discrete, hyaline, smooth, 0-septate, rarely 1-septate, apex straight, widest at the base, gradually tapering to the apex, (8–)11–23(–40) × 1.5–3.2 μm, periclinal wall thick-
ened, collarette minute. Conidia aggregating in slimy heads, ellipsoid to ovoid, apex rounded, base sub-acute, hyaline, smooth, thin-walled, eguttulate, mostly aseptate, sometimes 1-septate (<25% septate); aseptate conidia (4.5–)5.5–7(–7.5) × 2–3 μm, mean ± S.D. of 50 conidia = 6.5 ± 0.7 × 2.5 ± 0.3 μm, 95% confidence limits = 6.3–6.6 × 2.4–2.6 μm, L/W ratio = 2.6 ± 0.3; seaptate conidia (7–)7.5–9(–9.5) × 2–3 μm, mean ± S.D. of 18 conidia = 8.2 ± 0.7 × 3 ± 0.3 μm, 95% confidence limits = 7.9–8.5 × 2.8–3.1 μm, L/W ratio = 2.8 ± 0.4. Chlamy-
dospores absent.

Specimen examined. ITALY, Apulia, Rignano Garganico, on root of tomato (Lycopersicon esculentum), 2005, A. Carlucci, holotype CBS H-20901, culture ex-type CBS 131745.

Notes — This species is morphologically and phylogenetically close to Pa. plurivora and Pa. ramiseptata. The main differentiating feature is that in Pa. pauciseptata most of the conidia are aseptate, while in Pa. plurivora and Pa. ramiseptata septate and aseptate conidia occur in roughly equal proportions. The rarely septate conidiogenous cells with polyphialides further differentiate Pa. pauciseptata from Pa. ramiseptata.

Plectosphaerella plurivora A.J.L. Phillips, A. Carlucci & M.L. Raimondo, sp. nov. — MycoBank MB564525; Fig. 8

Etymology. Named for its wide host range.

Colonies on PDA various shades of buff or pink, mycelium appressed, slimy, little or no aerial mycelium, reaching a diameter of 7 cm after 14 d at 23 ± 2 °C. Minimum temperature for growth 6 °C, optimum 21 °C, maximum 29 °C. Mycelium hyaline, branched, septate, forming hyphal coils on PDA with phialides produced on the coils. Conidiophores solitary, unbranched, hyaline, smooth, thin-walled. Conidiogenous cells phialidic, determinate, discrete, hyaline, smooth, solitary, commonly with a basal septum, phialide apex straight, sometimes crooked or sinuous, widest at the base or lower third, gradually tapering to the apex (4–)7–19(–31.5) × 1.5–2 μm deep. Conidia aggregating in slimy heads, ellipsoid, tapering gradually to broadly rounded apex and base, hyaline, smooth, thin-walled, with a minute apiculus at either end, biguttulate, mostly aseptate (60%); aseptate conidia (4.5–5.5–8(–9) × 2–3.5(–5.5) μm, mean ± S.D. of 142 conidia = 7 ± 0.8 × 2.6 ± 0.5 μm, 95% confidence limits = 6.9–7.2 × 2.5–2.7 μm, L/W ratio = 2.7 ± 0.5; 1-septate conidia (6.5–)7.5–9.5(–10.5) × 2–3(–4) μm, mean ± S.D. of 90 conidia = 8.8 ± 0.7 × 2.7 ± 0.3 μm, 95% confidence limits = 8.6–8.9 × 2.6–2.8 μm, L/W ratio = 3.3 ± 0.4. Chlamy-
dospores absent.

Specimen examined. ITALY, Apulia, Borgo Cervaro, on asparagus apex turion, 2006, A. Carlucci, holotype CBS H-20899, culture ex-type CBS 131742.

Notes — This species was isolated from a variety of hosts affected by root and collar rots, but pathogenicity has not yet

![Fig. 8 Plectosphaerella plurivora. a–d. Colonies on PDA after 14 d at 23 ± 2 °C; e–i. phialides; j, k. conidia; l, m. hyphal coils; n, o. swollen conidia becoming chlamydospore-like. — Scale bars: e–k, n, o = 5 μm; m, n = 10 μm.](image-url)
been proven and thus its role in disease is not known. In terms of the wide host range it is similar to *Pa. cucumerina* whereas other species of *Plectosphaerella* have thus far been reported from narrower ranges of hosts. Morphologically it is similar to *Pa. cucumerina*, but the conidiogenous cells are shorter and the conidia are smaller than in *Pa. cucumerina*.

**Plectosphaerella ramiseptata** A.J.L. Phillips, A. Carlucci & M.L. Raimondo, sp. nov. — MycoBank MB564526; Fig. 9

**Etymology.** Refers to the branched and septate conidiogenous cells.

**Colonies** on PDA various shades of buff or pink, mycelium appressed, slimy but sometimes with aerial mycelium, reaching a diameter of 7.8 cm after 14 d at 23 ± 2 °C. Minimum temperature for growth 6 °C, optimum 21 °C, maximum 29 °C. *Mycelium* hyaline, branched, septate, forming coils on PDA with phialides produced on the coils. *Conidiophores* solitary, unbranched or rarely irregularly branched, hyaline, smooth, thin-walled. *Conidiogenous cells* phialidic, determinate, discrete, hyaline, smooth, solitary, mostly with a basal septum, 0–3-septate, apex straight, sometimes crooked or sinuous, widest at the base, gradually tapering to the apex, (11–)14.5–32.5(–40.5) × (2.5–)3–4.5(–6) μm, occasionally branched at the tip or with conidiogenous loci at the sides of the tip, periclinal wall thickened, collarette cylindrical 1.5–2 μm deep. *Conidia* aggregating in slimy heads, ellipsoid to ovoid, apex rounded, base sub-acute, hyaline, smooth, thin-walled, mostly eguttulate, rarely 2-guttulate, aseptate or 1-septate (50 % aseptate); aseptate conidia (4.5–)5.5–6.5(–7) × 2–3 μm, mean ± S.D. of 50 conidia = 2.4 ± 0.3 × 5.9 ± 0.8 μm, 95 % confidence limits = 5.5–6.2 × 2.3–2.6 μm, L/W ratio = 2.4 ± 0.3; septate conidia (6–)6.5–8(–8.5) × 2–3 μm, mean ± S.D. of 50 conidia = 7.1 ± 0.5 × 2.8 ± 0.3 μm, 95 % confidence limits = 6.9–7.3 × 2.7–2.9 μm, L/W ratio = 2.5 ± 0.3. *Chlamydospores* absent.

**Specimen examined.** ITALY. Apulia, Foggia, on root of tomato (*Lycopersicon esculentum*), 2007, A. Carlucci, holotype CBS H-20900, culture ex-type CBS 131861.

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**Fig. 9** *Plectosphaerella ramiseptata*. a, b. Colonies on PDA after 14 d at 23 ± 2 °C; c–k. phialides; d. hyphal coil with phialides; l, m. conidia. — Scale bars: c–m = 5 μm.
Notes — This species is phylogenetically close to Pa. pauci-septata but they differ both phylogenetically and morphologically. Conidiogenous cells of Pa. ramiseptata are often septate with up to 3 septa, and the conidiogenous cells frequently branch at the tip giving rise to lateral phialides.

KEY TO THE SPECIES OF PLECTOSPHAERELLA

1. On plants ..................................... Pa. oratosquillae
2. Chlamydospores present ......................... 8
3. Chlamydospores absent ......................... 3
4. Phialides branched at tip ........................... Pa. ramiseptata
5. Phialides not branched at tip ......................... 6
6. Most phialides less than 20 μm long . . . . . . . . . . Pa. plurivora
7. Conidia aseptate, 6.5–9 × 3–4 μm .......... Pa. citrullae
8. Conidia aseptate or septate, septate conidia 7–11 × 3–3.5 μm .......... Pa. delsorboi
9. Conidia mostly septate 13–19.5 × 2.5–3 μm Pa. alismatis
10. Conidia mostly aseptate 5.5–8.5 × 1.5–3.5 μm . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Pa. melonis

DISCUSSION

The present study aimed to resolve the taxonomy of the Plectosphaerella species that are associated with root and collar rots of cucurbits and other horticultural crops in southern Italy. In the partial LSU phylogeny all isolates grouped in Plectosphaerella within the Plectosphaerellaceae. ITS sequence data revealed that six species of Plectosphaerella are associated with diseased roots and collars of melon, watermelon and other horticultural crops in southern Italy. The species were clearly distinguished on morphology and phylogenetic inference based on ITS and included Plectosphaerella cucumerina, four undescribed species and another species that is recombinated in Plectosphaerella.

The genus Plectosporium was introduced by Palm et al. (1995) for the species previously known as Fusarium tabacinum (=Cephalosporium tabacinum), the anamorph of Plectosphaerella cucumerina. Considering the change towards one fungus one name, and applying the normal rules of priority the teleomorph genus name (Plectosphaerella 1930) should take priority over Plectosporium 1995 (Wingfield et al. 2012). Since Pm. alismatis, Pm. delsorboi and Pm. oratosquillae clustered within Plectosphaerella these three species were recombinated in Plectosphaerella.

In this study Pa. cucumerina was the most frequently isolated species. This species is widely distributed and well known as a root pathogen on a wide range of hosts (Matta 1978, Odunfa 1979, Pascoe et al. 1984, Zazzerini & Tosi 1987). In addition to the wide host range, the fungus is known to be morphologically variable. Palm et al. (1995) suggested that this wide morphological variation may represent a complex of species. In the present study we have shown that isolates previously identified as Pa. cucumerina represent distinct species, partially explaining the variability that has been attributed to this species. We also reveal a certain amount of phylogenetic variation within the more strictly circumscribed Pa. cucumerina. However, we used only ITS and more gene loci need to be investigated to determine whether this is a single taxon or a complex of species. One of the subclades within Pa. cucumerina includes isolates that commonly form the telemorph in culture (isolates Plect 4, Plect 7, Plect 10 and Plect 11) while none of the other isolates in that clade formed the telemorph.

The four new species that we introduce in this paper all formed distinct clades in the ITS phylogeny, and all were supported by morphological differences that separated the species. Plectosphaerella citrullae has thus far been isolated only from Citrullus, but since only three isolates were studied it is not clear if this species is host specific, or if it is pathogenic. Although pathogenicity of the four species described here has not yet been confirmed, such studies are presently underway.

Pathogenicity of Pa. melonis is well established and it is known to be the primary cause of muskmelon collapse in California, Japan, Spain and Texas (Watanabe & Sato 1995, Alfaro-Garcia et al. 1996). The host range was determined by Armengol et al. (1998) who showed that it can cause disease on 31 cucurbits, 18 crop plant species and 15 weed species. The correct genus for this pathogen has been the subject of some debate. García-Jiménez et al. (1993) reported a disease of muskmelon in Spain caused by an Acremonium species. Watanabe & Sato (1995) described Nodulisporium melonis as the cause of a similar disease in Japan, and later Alfaro-Garcia et al. (1996) described Acremonium cucbitacearum as the cause of muskmelon collapse in Spain, California and Texas. In a phylogenetic study based on analysis of ITS sequences of A. cucbitacearum, Martínez-Culebras et al. (2004) showed that A. cucbitacearum had greater affinity to Plectosporium than to Acremonium. They further showed that N. melonis had identical ITS sequence to A. cucbitacearum. However, they did not make any taxonomic changes and preferred to wait until more data has been amassed before doing so. In the ITS phylogeny presented here the ex-type isolate of A. cucbitacearum (CBS 525.93, AJ621754) clustered with the ex-type isolate of N. melonis (CBS 489.96, AJ621770) indicating that they represent the same species. Since N. melonis is the older name A. cucbitacearum becomes a later synonym. The ITS dendrogram of Zare et al. (2007) showed A. cucbitacearum as sister to the Acremonium nepalense/Gliocladium ciboti clade and a small group of Plectosporium species. In the phylogenies constructed in the present work we show that N. melonis and A. cucbitacearum clearly fall within Plectosphaerella and for this reason we transfer N. melonis to Plectosphaerella as Pa. melonis comb. nov. One of our isolates (Plect 148) formed a branch sister to Pa. melonis and probably represents a separate species.

Watanabe & Sato (1995) did not report chlamydospores in any of their isolates of N. melonis. Alfaro-Garcia et al. (1996) observed a few hyaline, thick-walled chlamydospores in old cultures of one of their isolates of A. cucbitacearum (CBS 410.95). However, we found chlamydospores to be common in all of the isolates that we studied. Nevertheless, the isolates that we studied were otherwise morphologically indistinguishable from N. melonis and A. cucbitacearum. In addition, apart from isolate Plect 148, ITS sequences of the isolates from Italy were identical to the ex-type isolates of N. melonis and A. cucbitacearum.

Based on this initial phylogenetic study of Plectosphaerella it is clear that there are still further species to be described. Within this work at least two more species were apparent from the single locus phylogeny, but no names were applied because either no cultures were available or only a single isolate was available and thus intraspecies variation could not be assessed.
It is also likely that information gained from more loci will help resolve the variability within Pa. cucumerina and reveal further species in this genus. Work is presently underway to address these issues and to determine pathogenicity of the species that are already known.

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