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RESEARCH ARTICLE

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

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Key words

D1/D2
ITS
LSU
phylogeny
Plectosporium
rDNA
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Abstract *Plectosphaerella cucumerina*, most frequently encountered in its *Plectosporium* 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 citrullae*, *Pa. pauciseptata*, *Pa. plurivora* and *Pa. ramiseptata*. *Acremonium cucurbitacearum* 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 *Plectosporium* 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 rots, wilt and collapse of the vines (Gennari et al. 1999, Buzi 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 (Buzi et al. 2002, Infantino et al. 2004), while *Rhizoctonia solani* AG4 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, Pivonia 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 (Merteley et al. 1993, Martyn & Miller 1996, Bruton 2000). Subsequently, it was reported from Italy (Stravato et al. 2002, Infantino et al. 2002, 2004, Buzi 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 Plectosporium 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).

Plectosporium 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 *Plectosphaerella* 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 *Plectosporium* for *Plectosphaerella* anamorphs, three further species have been described in this genus. Pitt et al. (2004) transferred *Rhynchosporium alismatis* to *Plectosporium*, Antignani et al. (2008) described *Plectosporium delsorboi* and Duc et al. (2009) described *Plectosporium oratosquillae* infecting mantis shrimps in Japan.

Plectosphaerella was introduced by Klebahn (1929) who described *Plectosphaerella 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 *Plectosphaerella cucumerina* was introduced by Gams (Domsch & Gams 1972). It has been regarded as a member of the *Hypocreaceae* (Barr

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1990, Gams & Gerlach 1968), while Uecker (1993) suggested that based on centrum development type it is closer to the *Sordariaceae*. More recently Zare et al. (2007) proposed the family *Plectosphaerellaceae* to accommodate *Acrostalagmus*, *Gibellulopsis*, *Musicillium*, *Plectosphaerella* (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 symptomatic 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° 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 mm^3) 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 were 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 with 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 10^6 generations. Trees were sampled every 100th generation for a total of 10^4 trees. The first 10^3 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 *Leptographium procerum* (AY789163). ML and Bayesian analyses resulted in trees with the same topology (TreeBASE S12518). The *Plectosphaerellaceae* was well-supported in both methods (100/1.00), but support for branches within the family was generally low, except for *Acrostalagmus*, *Musicillium* 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-

Table 1 Isolates of *Plectosphaerella* species used in this study.

Species	Isolate number*	Host	Locality	Collector	GenBank D1/D2	GenBank ITS
Pa. alismatis	**CBS 113362**	*Alismata plantago-aquatica*	Pijnenburg, The Netherlands	W. Gams	JF780521	JF780523
Pa. citrullae	Plect 151; CBS 131740	Melon root	Torre Bianca, Foggia, Italy	A. Carlucci	HQ239047	HQ238961
	Plect 157; CBS 131741	Water melon root	Foggia, Italy	A. Carlucci	HQ239048	HQ238962
	Plect 189	Water melon root	Foggia, Italy	A. Carlucci	HQ239050	HQ238964
Pa. cucumerina	Plect 4	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239016	HQ238977
	Plect 7	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239019	HQ238978
	Plect 10	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239020	HQ238979
	Plect 11; CBS 131739	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239021	HQ238980
	Plect 22	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239022	HQ238981
	Plect 25	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239023	HQ238982
	Plect 28	Melon collar	Borgo Cervaro, Foggia, Italy	M.L. Raimondo	HQ239024	HQ238983
	Plect 75	Melon collar	Nardò, Lecce, Italy	A. Carlucci	HQ239025	HQ238984
	Plect 77	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239026	HQ238985
	Plect 133	Tomato collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239027	HQ238986
	Plect 143	Pepper collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239028	HQ238987
	Plect 144	Pepper collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239029	HQ238988
	Plect 167	Tomato collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239030	HQ238989
	Plect 168	Pepper collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239031	HQ238990
	Plect 170	Tomato graft	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239032	HQ238991
	Plect 190	Melon collar	Borgo Cervaro, Foggia, Italy	M.L. Raimondo	HQ239033	HQ239005
	Plect 216	Melon root	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239034	HQ238992
	Plect 225	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	–	HQ238993
	Plect 234	Melon root	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239035	HQ238994
	Plect 272	Water melon root	San Ferdinando, Foggia, Italy	A. Carlucci	HQ239036	HQ238995
	Plect 275	Water melon collar	Brindisi, Italy	A. Carlucci	HQ239037	HQ238996
	Plect 288	Melon collar	San Chitico Fracagnano, Lecce, Italy	A. Carlucci	HQ239038	HQ238997
	Plect 290	Melon root	Lecce, Italy	A. Carlucci	HQ239039	HQ238998
	Plect 292	Melon root	Lecce, Italy	A. Carlucci	HQ239040	HQ238999
	Plect 328	Melon hybrid	Almenara, Spain	J. Armengol	HQ239046	HQ239000
	Plect 334	Tomato root	Borgo Cervaro, Foggia, Italy	M.L. Raimondo	HQ239045	HQ239001
	Plect 341	Tomato root	Borgo Cervaro, Foggia, Italy	M.L. Raimondo	HQ239041	HQ239002
	Plect 342	Tomato root	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239042	HQ239003
	Plect 368	Asparagus base turion	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239043	HQ239004
	Plect 373	Tomato collar	Potenza, Italy	A. Carlucci	HQ239044	HQ239006
	CBS 137.37	Paper	Italy	O. Verona	JF780520	JF780522
Pa. delsorboi	**CBS 116708**	*Curcuma alismatifolia*	Portici, Italy	V. Antignani	EF543843	EF543847
Pa. melonis	Plect 148	Melon root	Nardò, Lecce, Italy	A. Carlucci	HQ239007	HQ238968
	Plect 211; CBS 131858	Melon collar	Lecce, Italy	A. Carlucci	HQ239008	HQ238965
	Plect 212	Melon collar	Nardò, Lecce, Italy	A. Carlucci	–	HQ238966
	Plect 228; CBS 131859	Melon root	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239009	HQ238967
Pa. pauciseptata	Plect 135	Tomato collar	Rignano Garganico, Foggia, Italy	A. Carlucci	–	JQ246958
	Plect 152; CBS 131744	Melon collar	Foggia, Italy	A. Carlucci	–	JQ246956
	Plect 186; CBS 131745	Tomato root	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239012	HQ238971
	Plect 279	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	–	JQ246959
	Plect 301	Melon collar	Borgo Cervaro, Foggia, Italy	A. Carlucci	–	JQ246957
	Plect 321	Cucumber root	Foggia, Italy	A. Carlucci	–	JQ246960
	Plect 459	Water melon root	Lecce, Italy	M.L. Raimondo	–	JQ246962
	Plect 466	Tomato root	Foggia, Italy	M.L. Raimondo	–	JQ246961
	Plect 470	Tomato root	Foggia, Italy	M.L. Raimondo	–	JQ246963
Pa. plurivora	Plect 32	Tomato collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239010	HQ238969
	Plect 63; CBS 131860	Tomato collar	Rignano Garganico, Foggia, Italy	A. Carlucci	HQ239011	HQ238970
	Plect 329	Water melon	Alboaria, Spain	J. Armengol	HQ239017	HQ238972
	Plect 361	Parsley root	Bari, Italy	A. Carlucci	HQ239013	HQ238973
	Plect 363	Parsley root	Bari, Italy	A. Carlucci	HQ239014	HQ238974
	Plect 365; CBS 131742	Asparagus apex turion	Borgo Cervaro, Foggia, Italy	A. Carlucci	HQ239015	HQ238975
	Plect 372	Asparagus base turion	Borgo Cervaro, Foggia, Italy	M.L. Raimondo	HQ239016	HQ238976
Pa. ramiseptata	Plect 158; CBS 131743	Water melon collar	Foggia, Italy	A. Carlucci	HQ239049	HQ238963
	Plect 403; CBS 131861	Tomato root	Borgo Cervaro, Foggia, Italy	A. Carlucci	–	JQ246953
	Plect 464	Tomato root	Foggia, Italy	M.L. Raimondo	–	JQ246955
	Plect 469	Tomato root	Foggia, Italy	M.L. Raimondo	–	JQ246954

* Ex-type isolates are shown in **bold**.

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 clade 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.

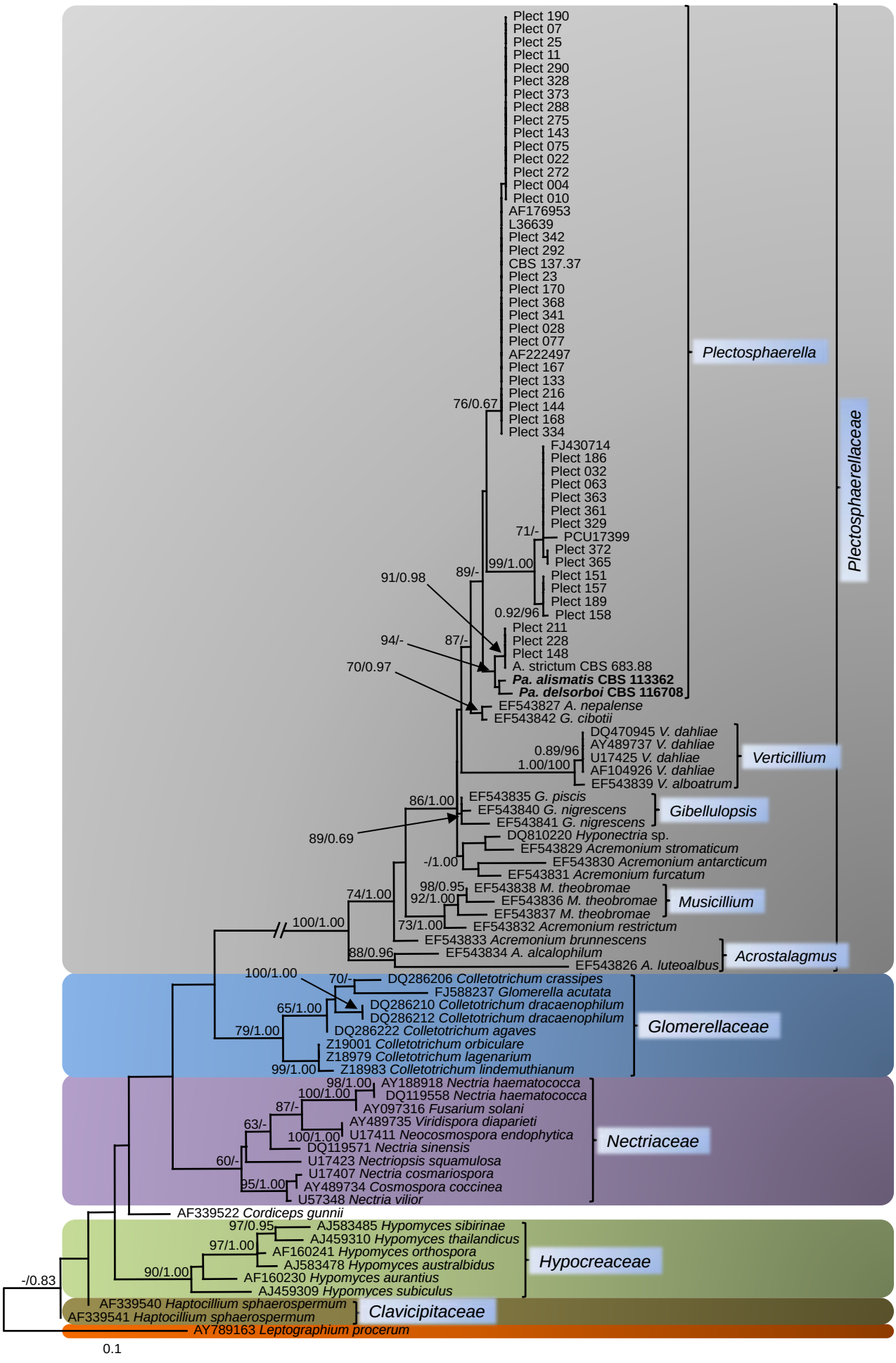

Fig. 1 Maximum Likelihood tree obtained from LSU sequence data with bootstrap support values in Maximum Likelihood/Bayesian Posterior Probability scores.

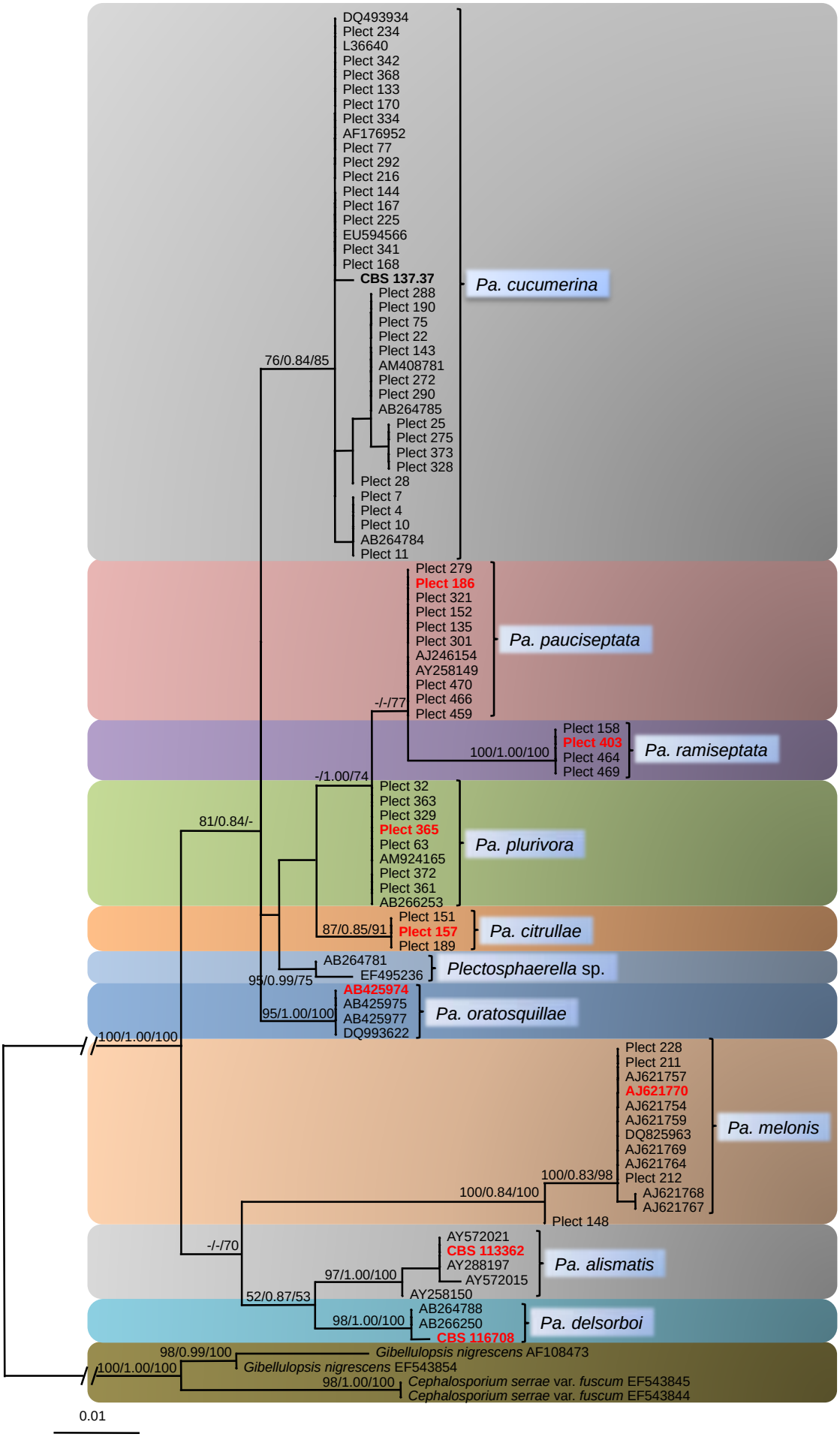

Fig. 2 Maximum Likelihood tree obtained from ITS sequence data with bootstrap support values from Maximum Parsimony/Bayesian Posterior Probability/Maximum Likelihood. Ex-type isolates are in **bold** face red.

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.

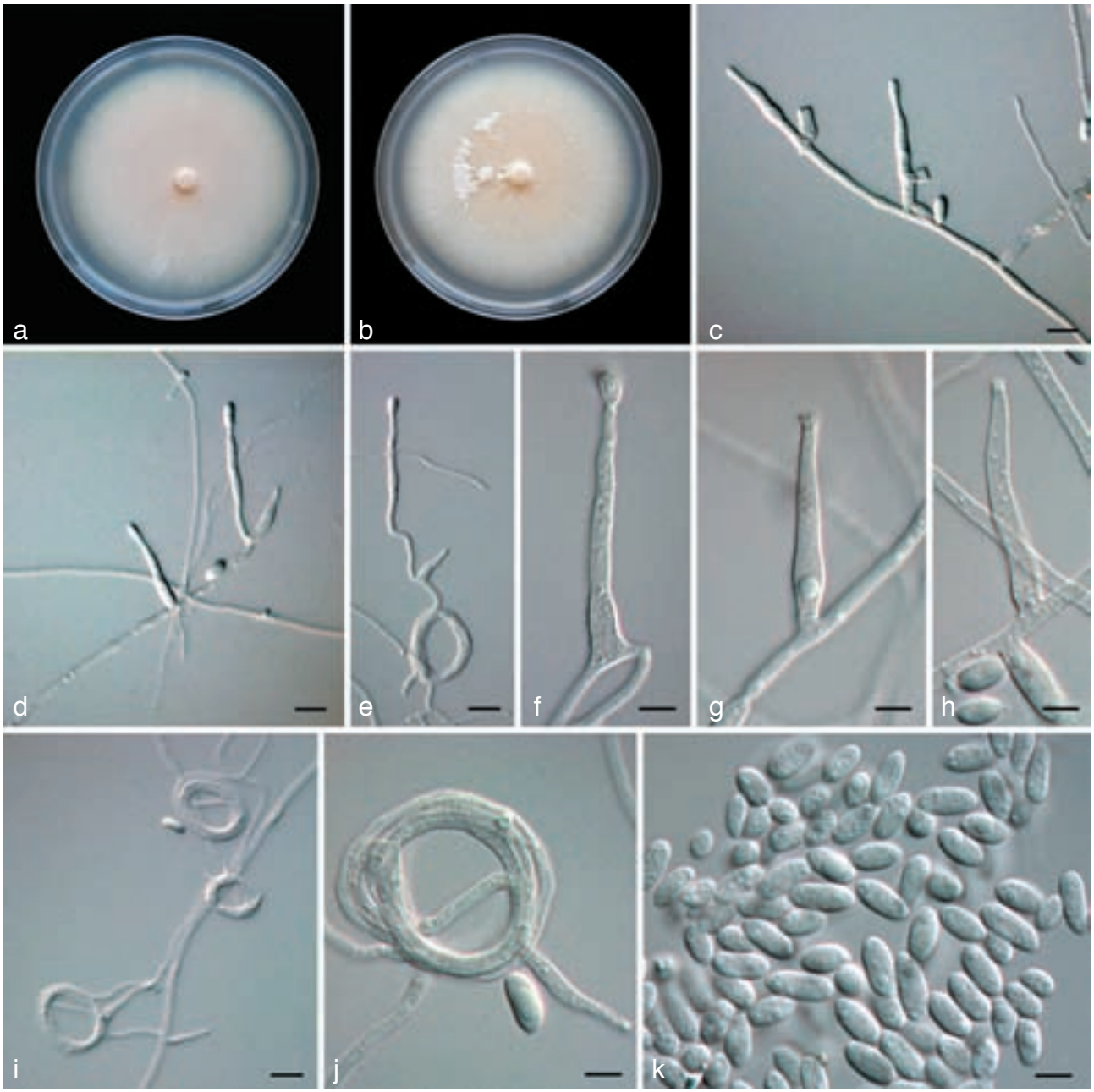

Fig. 3 *Plectosphaerella citrullae*. a, b. Colonies on PDA after 14 d at 23 ± 2 °C; c–e. conidiophores and phialides; f–h. phialides; i, j. hyphal coils; k. conidia. — Scale bars: c–e, i = 10 µm; f–h, j, k = 5 µm.

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

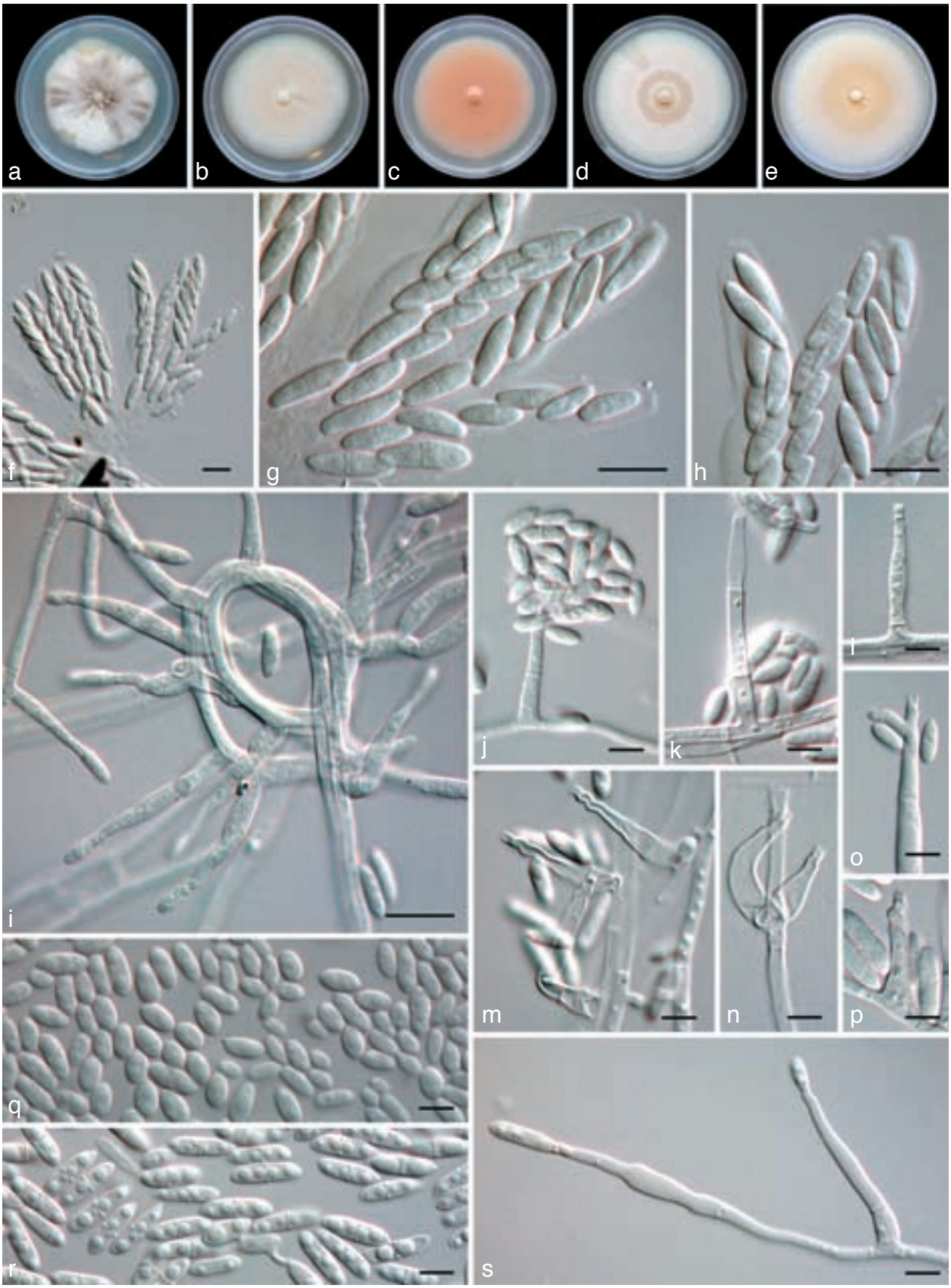

Fig. 4 *Plectosphaerella cucumerina*. a–e. Colonies on PDA after 14 d at 23 ± 2 °C; f–h. asci and ascospores; i. hyphal coil with phialides; j–p. phialides; q. aseptate conidia; r. 1-septate conidia; s. conidiophore. — Scale bars: f–i = 10 µm; j–s = 5 µm.

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. JordbrOmrad., 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 dannegiano la carte 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),

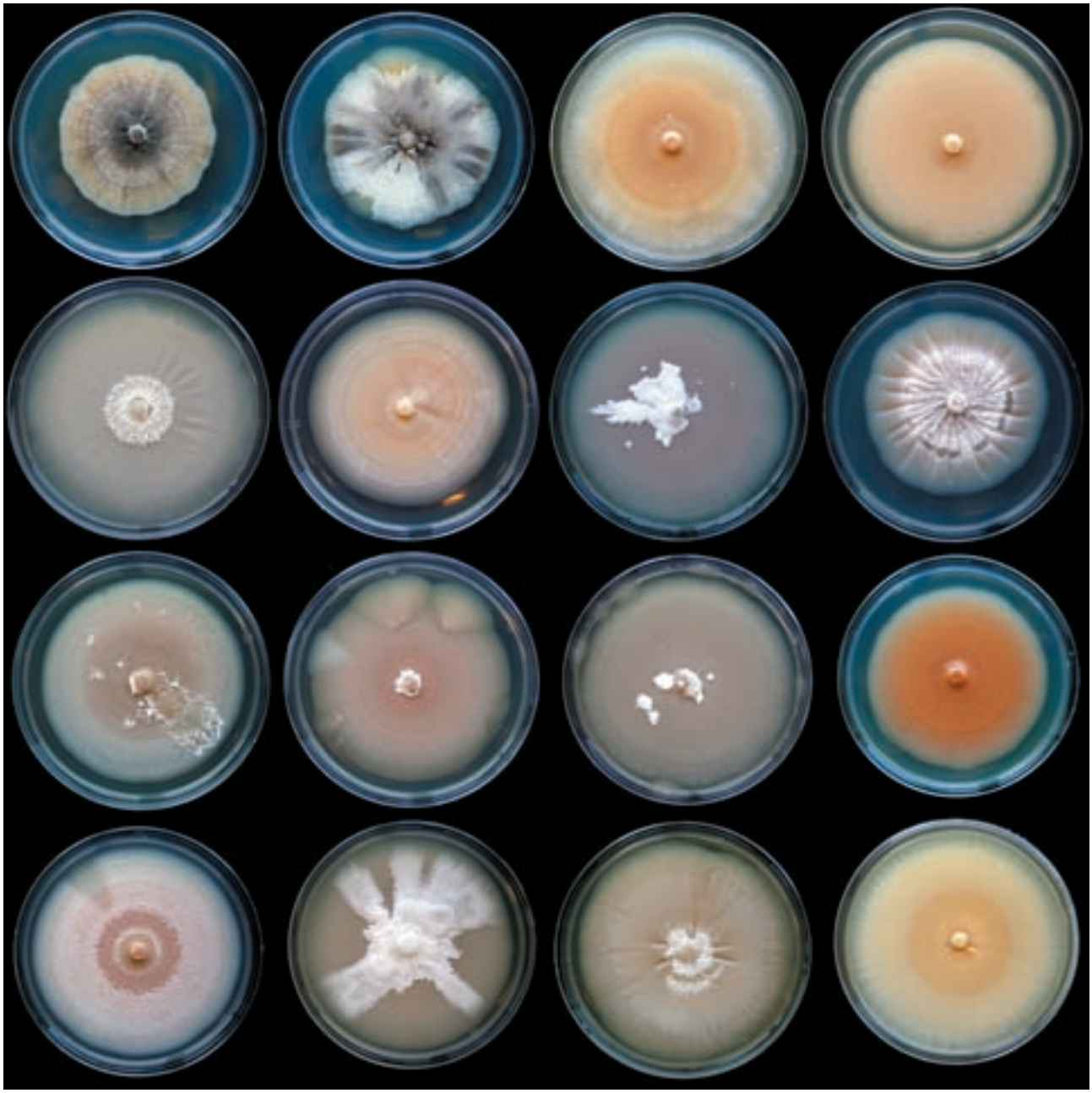

Fig. 5 Variation in culture morphology in *Plectosphaerella cucumerina*. All cultures were grown at 23 °C on PDA for 14 d.

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

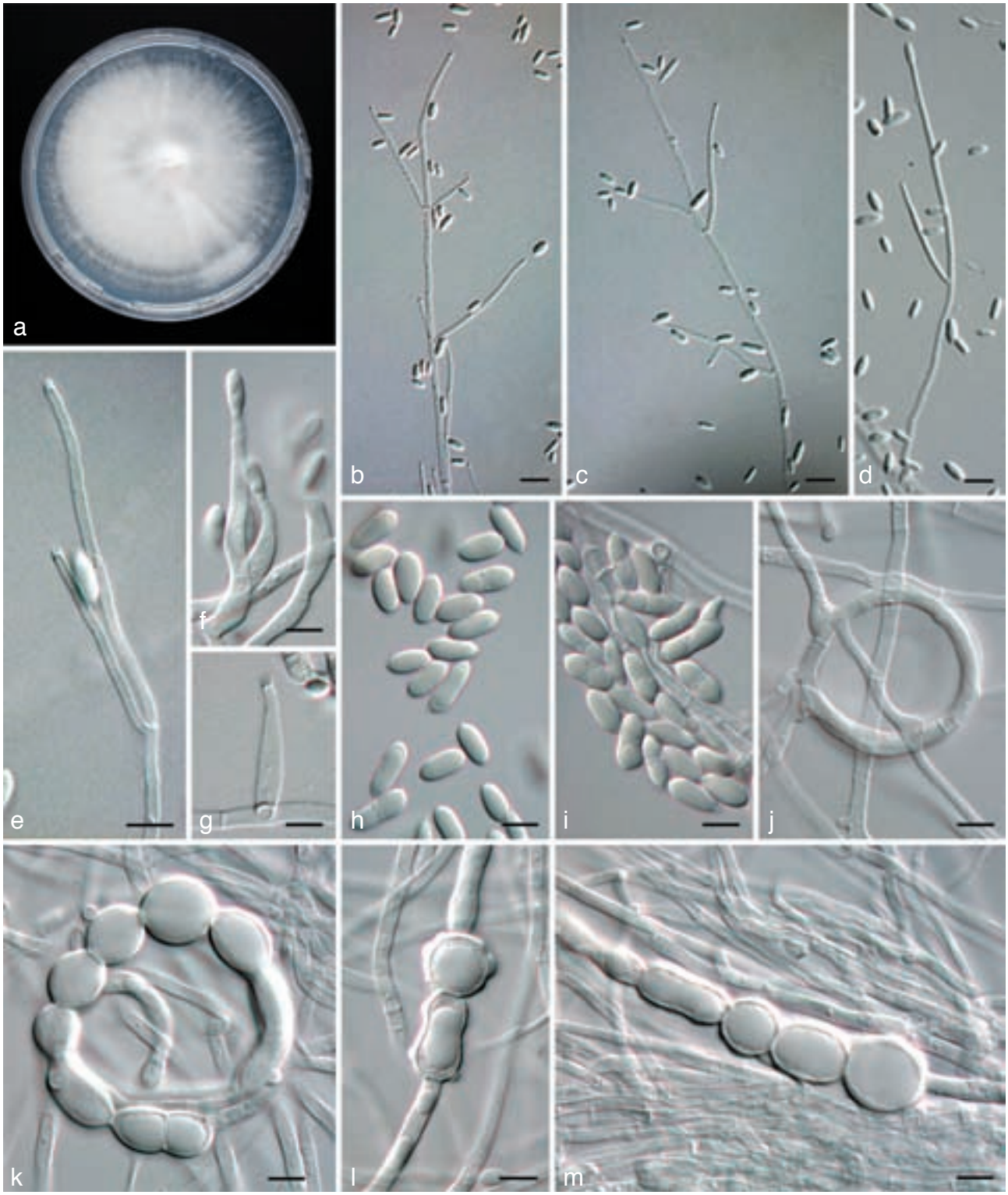

Fig. 6 *Plectosphaerella melonis*. a. Colony on PDA after 14 d at 23 ± 2 °C; b–d. conidiophores with phialides; e–g. phialides; h, i. conidia; j. hyphal coil; k–m. chlamydospores. — Scale bars: b–e = 10 µm; f–m = 5 µm.

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, tapering 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.

Specimen examined. Italy, Apulia, Borgo Cervaro, on root of melon (*Cucumis melo*), 2005, *A. Carlucci*, CBS H-20897, culture CBS 131858.

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, solitary, 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-

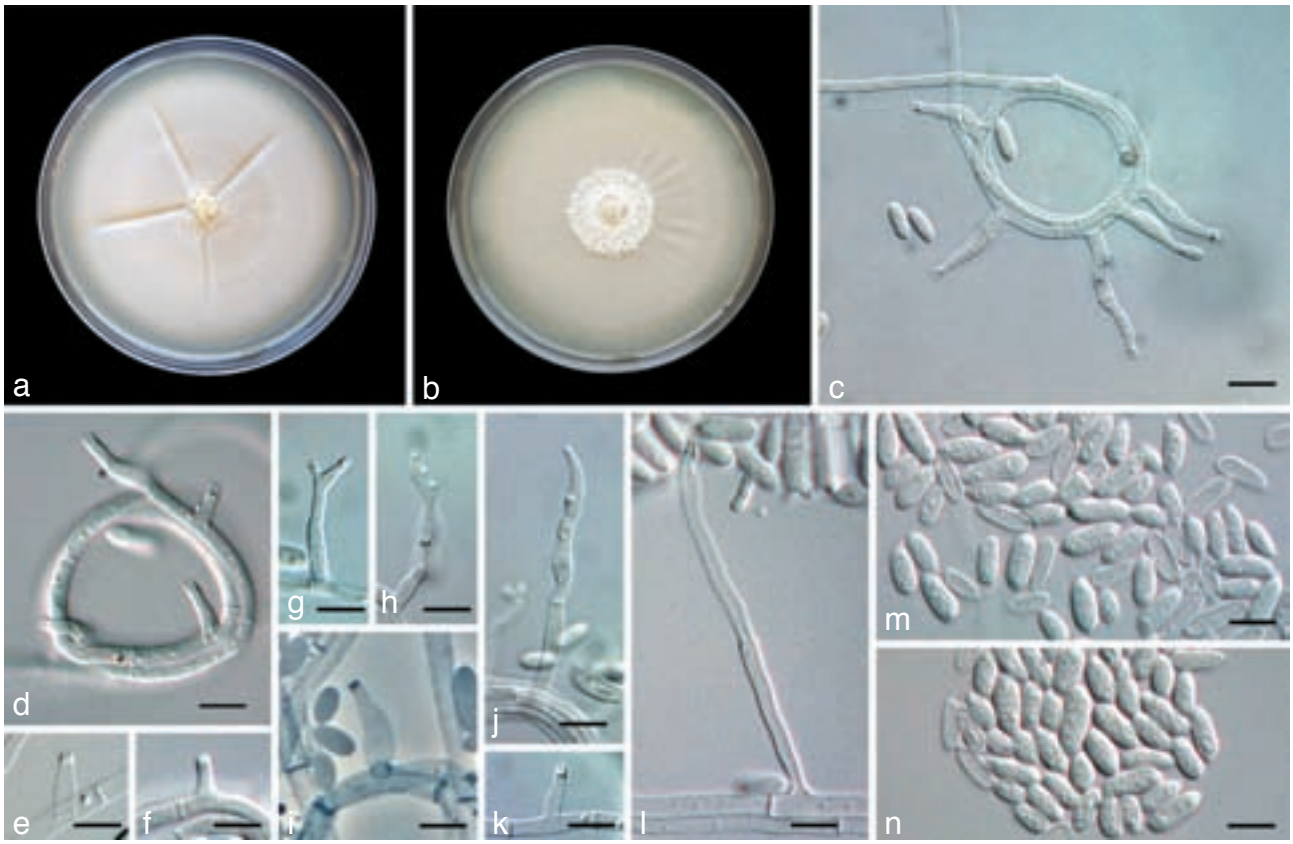

Fig. 7 *Plectosphaerella pauciseptata*. a, b. Colonies on PDA after 14 d at 23 ± 2 °C; c, d. hyphal coils; e–l. phialides; g, h, j. polyphialides; m, n. conidia. — Scale bars: c = 10 µm; d–n = 5 µm.

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; *septate 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. *Chlamydospores* 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) × 1.5–3(–4) 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, 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. *Chlamydospores* 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.

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.

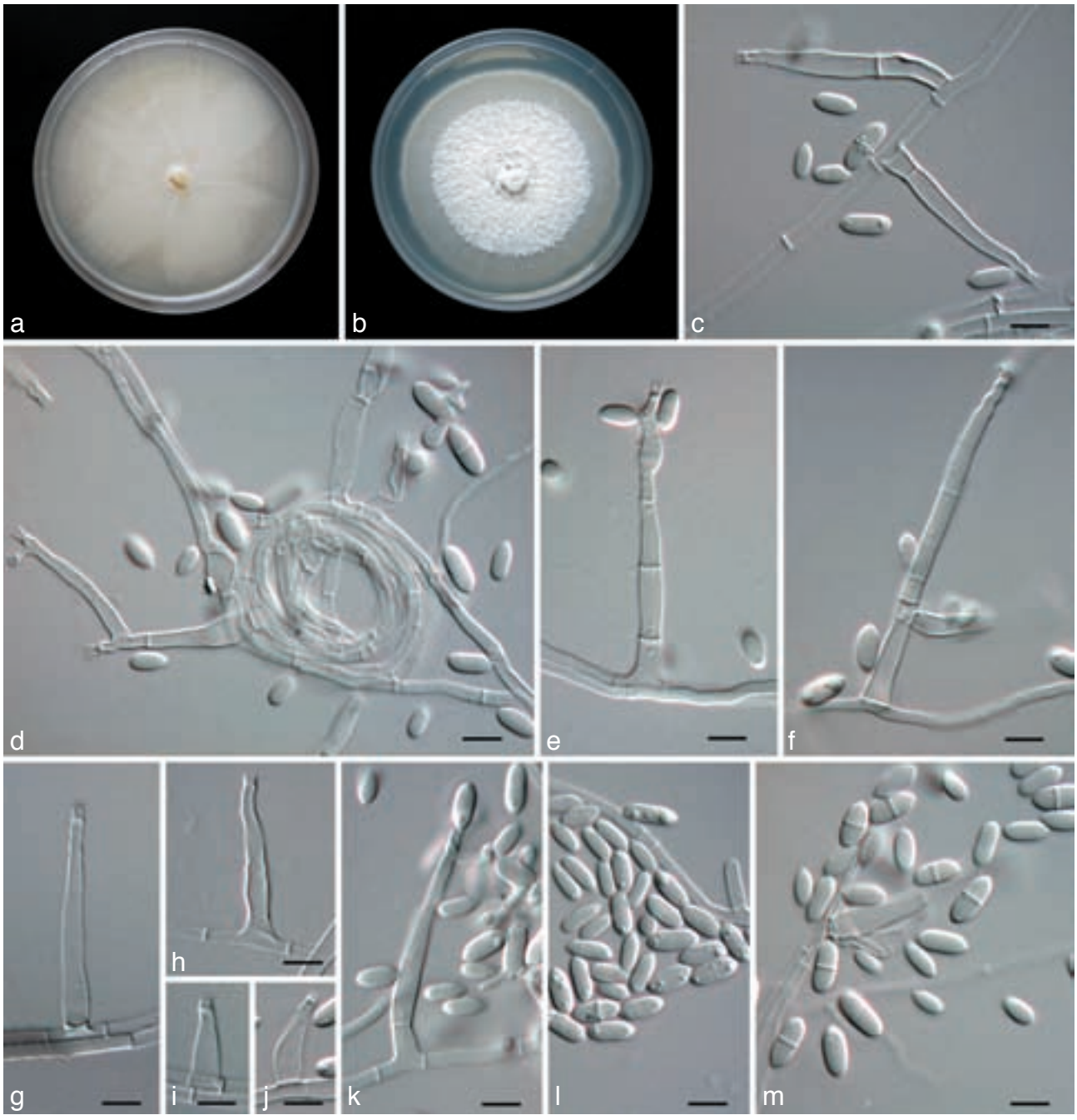

Fig. 9 *Plectosphaerella ramiseptata*. a, b. Colonies on PDA after 14 d at 23 ± 2 °C; c–k, phialides; d. hyphal coil with phialides; l, m. conidia. — Scale bars: c–m = 5 µm.

Notes — This species is phylogenetically close to *Pa. pauciseptata* 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 crustaceans . . . . . *Pa. oratosquillae*
1. On plants 2
2. Chlamydospores present 8
2. Chlamydospores absent 3
3. Phialides branched at tip 4
3. Phialides not branched at tip 6
4. Phialides often 3-septate . . . . . *Pa. ramiseptata*
4. Phialides 0–1-septate 5
5. < 25 % of conidia septate, polyphialides frequently seen . . . . . *Pa. pauciseptata*
5. Conidia septate or aseptate, polyphialides infrequent . . . . . *Pa. cucumerina*
6. Most phialides less than 20 µm long . . . . . *Pa. plurivora*
6. Phialides frequently more than 20 µm long 7
7. Conidia aseptate, 6.5–9 × 3–4 µm . . . . . *Pa. citrullae*
7. Conidia aseptate or septate, septate conidia 7–11 × 3–3.5 µm . . . . . *Pa. delsorboi*
8. Conidia mostly septate 13–19.5 × 2.5–3 µm *Pa. alismatis*
8. 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 recombined 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 recombined 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 teleomorph in culture (isolates Plect 4, Plect 7, Plect 10 and Plect 11) while none of the other isolates in that clade formed the teleomorph.

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-García 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-García et al. (1996) described *Acremonium cucurbitacearum* as the cause of muskmelon collapse in Spain, California and Texas. In a phylogenetic study based on analysis of ITS sequences of *A. cucurbitacearum*, Martínez-Culebras et al. (2004) showed that *A. cucurbitacearum* had greater affinity to *Plectosporium* than to *Acremonium*. They further showed that *N. melonis* had identical ITS sequence to *A. cucurbitacearum*. 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. cucurbitacearum* (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. cucurbitacearum* becomes a later synonym. The ITS dendrogram of Zare et al. (2007) showed *A. cucurbitacearum* as sister to the *Acremonium nepalense*/*Gliocladium cibotiii* clade and a small group of *Plectosporium* species. In the phylogenies constructed in the present work we show that *N. melonis* and *A. cucurbitacearum* clearly fall within *Plectosphaerella* and for this reason we transfer *N. melonis* to *Plectosphaerella* as *Pa. melonis* comb. nov. One of our isolates (Plecto 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-García et al. (1996) observed a few hyaline, thick-walled chlamydospores in old cultures of one of their isolates of *A. cucurbitacearum* (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. cucurbitacearum*. 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. cucurbitacearum*.

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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