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Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean

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Abstract: Angular leaf spot of *Phaseolus vulgaris* is a serious disease caused by *Phaeoisariopsis griseola*, in which two major gene pools occur, namely Andean and Middle-American. Sequence analysis of the SSU region of nrDNA revealed the genus *Phaeoisariopsis* to be indistinguishable from other hyphomycete anamorph genera associated with *Mycosphaerella*, namely *Pseudocercospora* and *Stigmina*. A new combination is therefore proposed in the genus *Pseudocercospora*, a name to be conserved over *Phaeoisariopsis* and *Stigmina*. Further comparisons by means of morphology, cultural characteristics, and DNA sequence analysis of the ITS, calmodulin, and actin gene regions delineated two groups within *P. griseola*, which are recognised as two formae, namely f. *griseola* and f. *mesoamericana*.

Taxonomic novelties: *Pseudocercospora griseola* (Sacc.) Crous & U. Braun comb. nov., *P. griseola* f. *mesoamericana* Crous & U. Braun f. nov.
Key words: *Ascomycetes*, DNA sequence comparisons, *Mycosphaerella*, *Phaeoisariopsis*, *Phaseolus vulgaris*, *Pseudocercospora*, systematics.

INTRODUCTION

Angular leaf spot (ALS) of beans (*Phaseolus vulgaris*) is caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris. The disease is of major importance in tropical and subtropical areas, causing yield losses of up to 80 % (Schwartz *et al*. 1981, Saettler 1991, Liebenberg & Pretorius 1997). The disease affects pods and foliage, and is particularly destructive in warm, humid areas (Saettler 1991). Pod symptoms consist of circular to elliptical red-brown lesions, while leaf lesions start as small, brown or grey spots that become angular and necrotic, being confined by leaf veins. Leaf spots eventually coalesce, causing premature defoliation (Correa-Victoria *et al.* 1989, Saettler 1991). Furthermore, the disease also affects the quality and marketability of seed across bean-producing areas of the world (Pastor-Corrales *et al*. 1998).

In the Great Lakes Region of Africa, losses attributed to ALS have been estimated to be around 374 800 t (Wortmann *et al*. 1998). Disease control is best achieved via the selection of resistant varieties. Breeding for resistance against ALS is complicated, as the pathogen is highly variable with regard to pathogenicity, which means that durable resistance is difficult to achieve (Pastor-Corrales *et al.* 1998). High levels of pathogenic and genetic variation have been reported in *P. griseola* by various authors (Guzmán *et al*. 1995, Boshoff *et al*. 1996, Busogoro *et al*. 1999, Mahuku *et al*. 2002, Wagara *et al.* 2004).

There are indications of at least two main, morphologically distinguishable domestication events for the common bean, which in turn gave rise to two main gene pools, namely large-seeded beans of Andean origin, and small to medium-sized beans of Middle-American origin (Brown *et al*. 1982, Gepts & Bliss 1985, 1986, Gepts *et al*. 1986, Koenig & Gepts 1989, Sprecher & Isleib 1989, Koenig *et al*. 1990, Singh *et al*. 1991a, b, Miklas & Kelly 1992, Skroch *et al*. 1992, Chacón *et al*. 2005).

Several fungal pathogens of *P. vulgaris*, in particular *Phaeoisariopsis griseola*, causal organism of ALS, *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, the causal organism of anthracnose, and *Uromyces appendiculatus* (Pers. : Pers.) Unger var. *appendiculatus*, the causal organism of bean rust, have undergone parallel micro-evolution with the host. Although there is considerable variation within gene pools, differences are particularly evident when the reactions of isolates to differential lines of known Andean and Middle-American origin are compared. Isolates originating from the Andes are virulent only on large-seeded lines, whereas those originating from countries such as Central America, Mexico, Bolivia and Brazil are generally virulent on lines from both groups (Steadman 1995, Liebenberg 1996, Pastor-Corrales 1996, Chacón *et al*. 1997, Araya & Steadman 1998, Sandlin *et al*. 1999, Araya *et al*. 2004). Using isozyme analysis, Correa-Victoria (1987) could distinguish two groups in 55 *P. griseola* isolates from Africa, the U.S.A. and Latin America. All 26 isolates from Africa clustered in one group, whereas Latin American isolates clustered in both groups. However, recently the presence of both groups was reported from Africa (Liebenberg 1996, Wagara *et al*. 2004), which was also supported by data derived from isozyme analysis (Boshoff *et al*. 1996). Guzmán *et al.* (1995) used RAPD analysis to divide 62 *P. griseola* isolates from Brazil, Wisconsin (U.S.A.) and Malawi into two broad groups. Isolates in the Andean group, collected predominantly from Andean bean host genotypes, were more pathogenic on Andean genotypes, whereas those from the second group,

originating predominantly from Middle-American bean genotypes, were more pathogenic on Middle-American bean genotypes. The 11 Brazilian isolates fell in the second group, whereas 39 of the 42 Malawian isolates belonged to the Andean group. This grouping reflects the preference for small-seeded beans in Brazil, and large-seeded beans in Malawi. A third, more virulent group reported in Africa (CIAT 1996, Liebenberg 1996) appears to be a variation of the Andean group (Mahuku *et al.* 2002).

Buruchara (1983) observed differences in conidial size and amount of septation between isolates. However, he concluded that, due to the extent of variation within groups, these characteristics could not be used for grouping isolates. Several authors have attempted to associate lesion size with pathogenicity differences. Verma & Sharma (1984) observed two types of lesions in the field that differed in size, but found no significant differences in the number and size of lesions caused by the two groups of isolates, or in their radial growth in culture. Lesion size can vary considerably, but Correa-Victoria (1987) found no significant correlation between disease severity and lesion size, and no correlation between spore production and lesion size, but reported it to be highly dependent on the host cultivar (Correa-Victoria 1987). Lesion size may be affected by the interaction between host gene pool and pathogen origin (Liebenberg *et al*. 1996). These phenomena gave rise to questions as to the extent of differences between the Andean and Middle-American groups.

Ferraris (1909) erected the genus *Phaeoisariopsis* Ferraris for four *Isariopsis*-like species, including *Isariopsis griseola* Sacc. (Saccardo 1878), the type species, characterised by having synnematous conidiophore fascicles and pigmented conidiophores and conidia. In subsequent years several diverse elements were included in the genus (Ellis 1971, 1976, von Arx 1983). Chupp (1954) described a bean pathogen in his monograph under *Cercospora columnaris* Ellis & Everh., but cited the older name *Phaeoisariopsis griseola* as synonym. In his notes he stressed to favour the retention of *Phaeoisariopsis*. Deighton (1990) reassessed the genus, and considered the synnematous arrangement of conidiophores to be unsuitable as sole character for generic differentiation. Subsequently he confined *Phaeoisariopsis* to a few species similar to *P. griseola,* having non-geniculate conidiogenous cells with flattened, but conspicuous scars. Deighton placed species with conspicuously geniculate conidiogenous cells and thickened, darkened scars in *Passalora* Fr., whereas taxa with quite inconspicuous conidiogenous loci were reallocated to *Pseudocercospora* Speg. Von Arx (1983) and Braun (1992, 1995a, b) preferred to maintain *Phaeoisariopsis*, based on synnematous conidiomata, but confined it to species with conspicuous (slightly thickened, not darkened) conidiogenous loci.

The primary aim of the present study was to resolve the generic status of *Phaeoisariopsis* within *Mycosphaerella* Johanson, for which a subset of isolates were subjected to DNA sequence analysis of the SSU region. A further aim was to compare isolates of the Andean and Middle-American groups to address the question if they represent two groups or species. For this purpose isolates were compared by means of morphology, cultural characteristics, and DNA sequence analysis of their internal transcribed spacer region (ITS-1, ITS-2 and 5.8S), calmodulin, and actin regions.

MATERIALS AND METHODS

Isolates

Phaseolus leaves exhibiting ALS symptoms, collected in Africa and South America, were studied (Table 1). Single-conidial cultures were established on 2 % malt extract agar (MEA) (Biolab, Midrand, South Africa) as outlined by Crous (1998). Colonies were subcultured onto 2 % potato-dextrose agar (PDA; Gams *et al***.** 1998) and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation.

DNA phylogeny

Genomic DNA was isolated from fungal mycelium grown on MEA in Petri dishes and the ITS, actin (ACT) and calmodulin (CAL) regions were amplified and sequenced using the protocols and primers as described by Crous *et al*. (2004). The 5' end of the 18S rRNA gene (SSU) was amplified and sequenced as described by Braun *et al*. (2003).

The nucleotide sequences generated in this study were added to other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) and the alignment was assembled using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002) with manual improvement of the alignment where necessary. Sequence data were analysed as explained in Braun *et al*. (2003) using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002) with both neighbour-joining and parsimony algorithms. Neighbour-joining analyses were conducted with the uncorrected ("p"), the Kimura 2-parameter and the HKY85 substitution models in PAUP. When they were encountered, ties were broken randomly. For parsimony analysis, alignment gaps were treated as new character states and all characters were unordered and of equal weight. Heuristic searches were performed with 10 random taxon additions. A partition homogeneity test (Farris *et al.* 1994) was conducted in PAUP to consider the feasibility of combining the ITS, actin and calmodulin data sets Sequence data were deposited in GenBank (Table 1) and the alignments in TreeBASE (S1507, M2709-10).

Determination of virulence phenotypes

The monoconidial isolates studied (Table 1) have previously been subjected to virulence phenotype characterisation on ALS differential lines from both the large- and small-seeded gene pools, as published previously (Liebenberg 1996, Mahuku *et al*. 2002).

Morphology and cultural characteristics

Wherever possible, thirty measurements (× 1000 magnification) were made of structures mounted in

lactic acid, and the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 14 d on PDA at 25 °C in the dark, using the colour charts of Rayner (1970). Cardinal temperatures for growth (from 9–33 °C, in 3° intervals) were determined on PDA plates as explained in Crous (1998). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands (Table 1).

RESULTS

DNA phylogeny

The manually adjusted SSU sequence alignment contains 29 isolates (including the two outgroups) and 1029 characters including alignment gaps; of these characters 38 are parsimony-informative, 57 are variable and parsimony-uninformative, and 934 are constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with identical topologies (data not shown). The same overall topology was also obtained with the parsimony analysis, which yielded 13 most parsimonious trees (TL = 135 steps; CI = 0.807; RI = 0.809; RC = 0.653), one of which is shown in Fig. 1. In this tree, species of *Pseudocercospora* and *Stigmina* form a well-defined clade (bootstrap support value of 83 %) within *Mycosphaerella*.

The ITS region was sequenced to provide better resolution of the order of the species within the *Pseudocercospora* clade. The manually adjusted ITS sequence alignment contains 45 isolates (including the two outgroups) and 499 characters including alignment gaps; of these characters 168 are parsimony-informative, 25 are variable and parsimony-uninformative, and 306

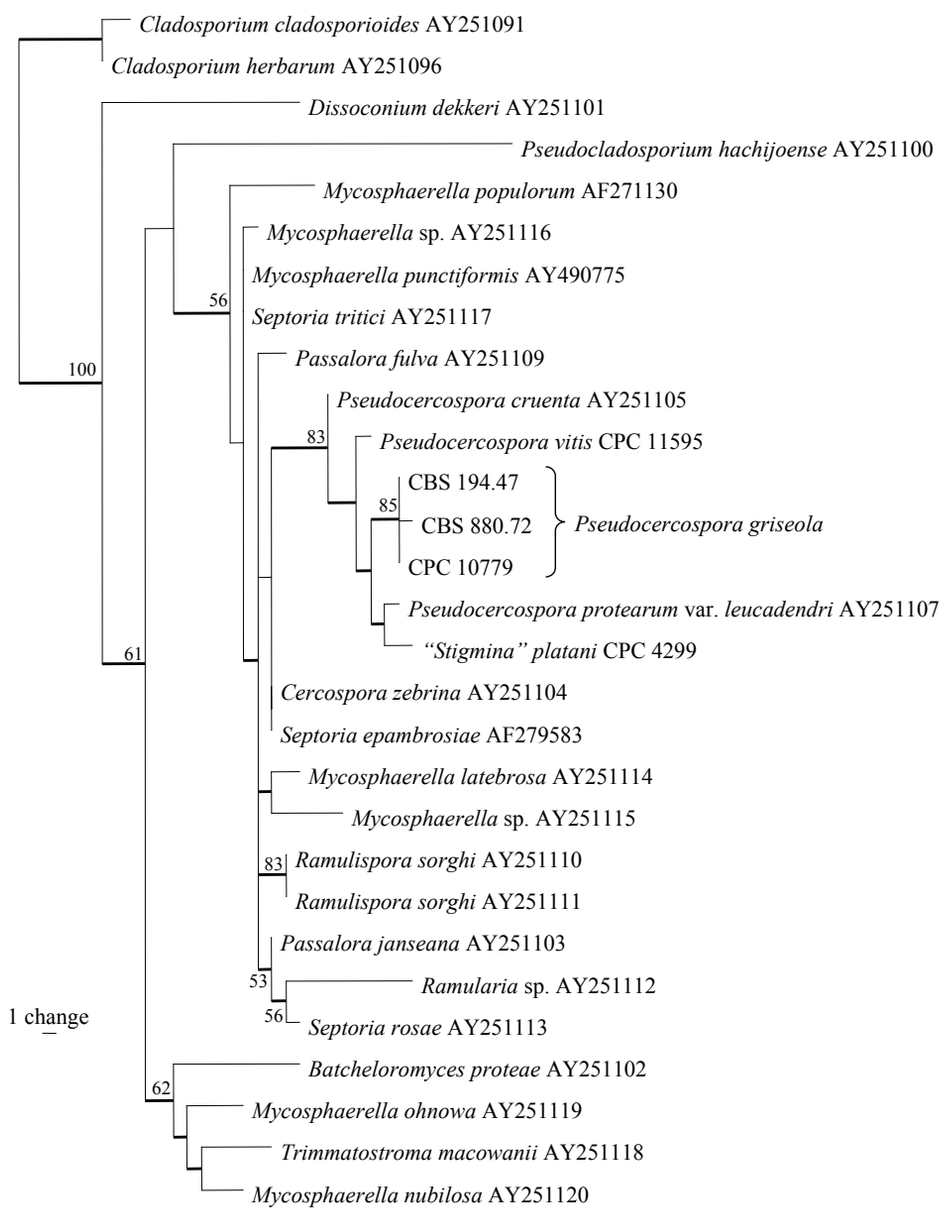

Fig. 1. One of 15 most parsimonious trees obtained from a heuristic search with 10 random taxon additions of the 18S rRNA gene sequence alignment. The scale bar shows a single change and bootstrap support values from 1000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and the tree was rooted to two *Cladosporium* species.

Table 1. Isolates used for sequence analysis.

Species	Accession number[1]	Host	Virulence type	Origin	Collector	GenBank numbers[2] (ITS, CAL, SSU, ACT)
Cladosporium herbarum	CBS 572.78	*Polyporus radiatus*	—	Russia	—	DQ289799, DQ289831, —, DQ289866
Davidiella tassiana	CPC 11600	*Delphinium barbeyi*	—	U.S.A.	A. Ramalay	DQ289800, DQ289832, —, DQ289867
Pseudocercospora griseola f. *griseola*	CBS 194.47; ATCC 22393	*Phaseolus vulgaris*	—	Portugal	—	DQ289801, DQ289833, DQ289861, DQ289868
	CBS 880.72	*Phaseolus vulgaris*	—	Netherlands	H. A. v. Kesteren	DQ289802, DQ289834, DQ289862, DQ289869
	CPC 5592; Pg97MZ41	*Phaseolus vulgaris*	Andes	Zambia	R. Buruchara	DQ289803, DQ289835, —, DQ289870
	CPC 5594; Pg97LB48	*Phaseolus vulgaris*	Andes	South Africa	M.M. Liebenberg	DQ289804, DQ289836, —, DQ289871
	CPC 10457; Pg97MZ64	*Phaseolus vulgaris*	Andes	Zambia	R. Buruchara	DQ289805, DQ289837, —, DQ289872
	CPC 10458; Pg96CE7	*Phaseolus vulgaris*	Andes	South Africa	M.M. Liebenberg	DQ289806, DQ289838, —, DQ289873
	CPC 10459; Pg97CE78	*Phaseolus vulgaris*	Andes	South Africa	M.M. Liebenberg	DQ289807, DQ289839, —, DQ289874
	CPC 10460; Pg97AT101	*Phaseolus vulgaris*	Andes	Tanzania	F.S. Ngulu; C. Mushi	DQ289808, DQ289840, —, DQ289875
	CPC 10464; Pg97CE105	*Phaseolus vulgaris*	Andes	—	—	DQ289809, DQ289841, —, DQ289876
	CPC 10465; Pg97CE106	*Phaseolus vulgaris*	Andes	—	—	DQ289810, DQ289842, —, DQ289877
	CPC 10467; Pg97MZ42	*Phaseolus vulgaris*	Andes	Zambia	R. Buruchara	DQ289811, DQ289843, —, DQ289878
	CPC 10468; Pg97AT95	*Phaseolus vulgaris*	Andes	Tanzania	F.S. Ngulu; C. Mushi	DQ289812, DQ289844, —, DQ289879
	CPC 10469; Pg97KZ44	*Phaseolus vulgaris*	Andes	Zambia	R. Buruchara	DQ289813, DQ289845, —, DQ289880
	CPC 10477; Pg97CE23	*Phaseolus vulgaris*	Andes	South Africa	M.M. Liebenberg	DQ289814, DQ289846, —, DQ289881
	CPC 10480; Pg96VI90	*Phaseolus vulgaris*	Andes	South Africa	M.M. Liebenberg	DQ289815, DQ289847, —, DQ289882
	CPC 10481; Pg95GT5	*Phaseolus vulgaris*	Andes	South Africa	A.J. Liebenberg	DQ289816, DQ289848, —, DQ289883
	CPC 10484; Pg95CE7	*Phaseolus vulgaris*	Andes	South Africa	M.M. Liebenberg	DQ289817, DQ289849, —, DQ289884
	CPC 10779	*Phaseolus vulgaris*	—	Korea	H.D. Shin	DQ289818, DQ289850, DQ289863, DQ289885
	CPC 12238; Pg350	*Phaseolus vulgaris*	Andes	Colombia	G. Mahuku	DQ289819, DQ289851, —, DQ289886
	CPC 12239; Pg3	*Phaseolus vulgaris*	Andes	Colombia	G. Mahuku	DQ289820, DQ289852, —, DQ289887
	CPC 12240; Pg266	*Phaseolus vulgaris*	Andes	Colombia	G. Mahuku	DQ289821, DQ289853, —, DQ289888
Ps. griseola f. *mesoamericana*	CPC 5596; Pg99GT4	*Phaseolus vulgaris*	Middel-Amerikaans	South Africa	A.J. Liebenberg	DQ289822, DQ289854, —, DQ289889
	CPC 5597; Pg97TM109	*Phaseolus vulgaris*	Middel-Amerikaans	Malawi	A.J. Liebenberg	DQ289823, DQ289855, —, DQ289890
	CPC 10463; Pg96GT35	*Phaseolus vulgaris*	Middel-Amerikaans	South Africa	M.M. Liebenberg	DQ289824, DQ289856, —, DQ289891
	CPC 10474; Pg96GT32	*Phaseolus vulgaris*	Middel-Amerikaans	South Africa	M.M. Liebenberg	DQ289825, DQ289857, —, DQ289892
	CPC 10479; Pg99CE5	*Phaseolus vulgaris*	Middel-Amerikaans	South Africa	M.M. Liebenberg	DQ289826, DQ289858, —, DQ289893
	CPC 12241; Pg8	*Phaseolus vulgaris*	Middel-Amerikaans	Honduras	G. Mahuku	DQ289827, DQ289859, —, DQ289894
	CPC 12242; Pg32	*Phaseolus vulgaris*	Middel-Amerikaans	Colombia	G. Mahuku	DQ289828, DQ289860, —, DQ289895
Pseudocercospora vitis	CPC 11595	*Vitis vinifera*	—	Korea	H.D. Shin	DQ289829, —, DQ289864, —
	CPC 11660	*Vitis flexuosa*	—	Korea	H.D. Shin	DQ289830, —, —, —
"*Stigmina*" *platani*	CBS 110755; CPC 4299; IMI 136770	*Platanus orientalis*	—	India	—	AY260090, —, DQ289865, —

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, U.K.

[2]ITS: internal transcribed spacer region, CAL: partial calmodulin gene, SSU: partial 18S rRNA gene, ACT: partial actin gene. All DQ numbers refer to newly generated sequences.

are constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with identical topologies (data not shown). Only the order and grouping of the deeper nodes differed between the neighbour-joining and parsimony analyses (data not shown). Parsimony analysis yielded 13 most parsimonious trees (TL = 293 steps; CI = 0.816; RI = 0.918; RC = 0.749), one of which is shown in Fig. 2. In this tree, isolates of *Ps. griseola* are grouped together with a bootstrap support value of 100 %, with the Middle-American isolates (*Ps. griseola* f. *mesoamericana*) grouping together with a bootstrap support value of 84 %. Also in the tree are other *Pseudocercospora* species (89 % bootstrap support), two strains of *Ps. vitis* (type species of *Pseudocercospora*, 95 % bootstrap support) and a basal well-defined clade (bootstrap support value of 100 %) of two GenBank sequences of *Stigmina platani*.

To determine whether *Ps. griseola* isolates from Middle-American and Andean origin can be distinguished phylogenetically, the ACT (235 characters) and CAL (316 characters) sequences were combined with the ITS sequences. The partition homogeneity test showed that the three loci were combinable into a single analysis (P = 0.6550). The manually adjusted combined alignment consists of 1050 bases (including alignment gaps) and

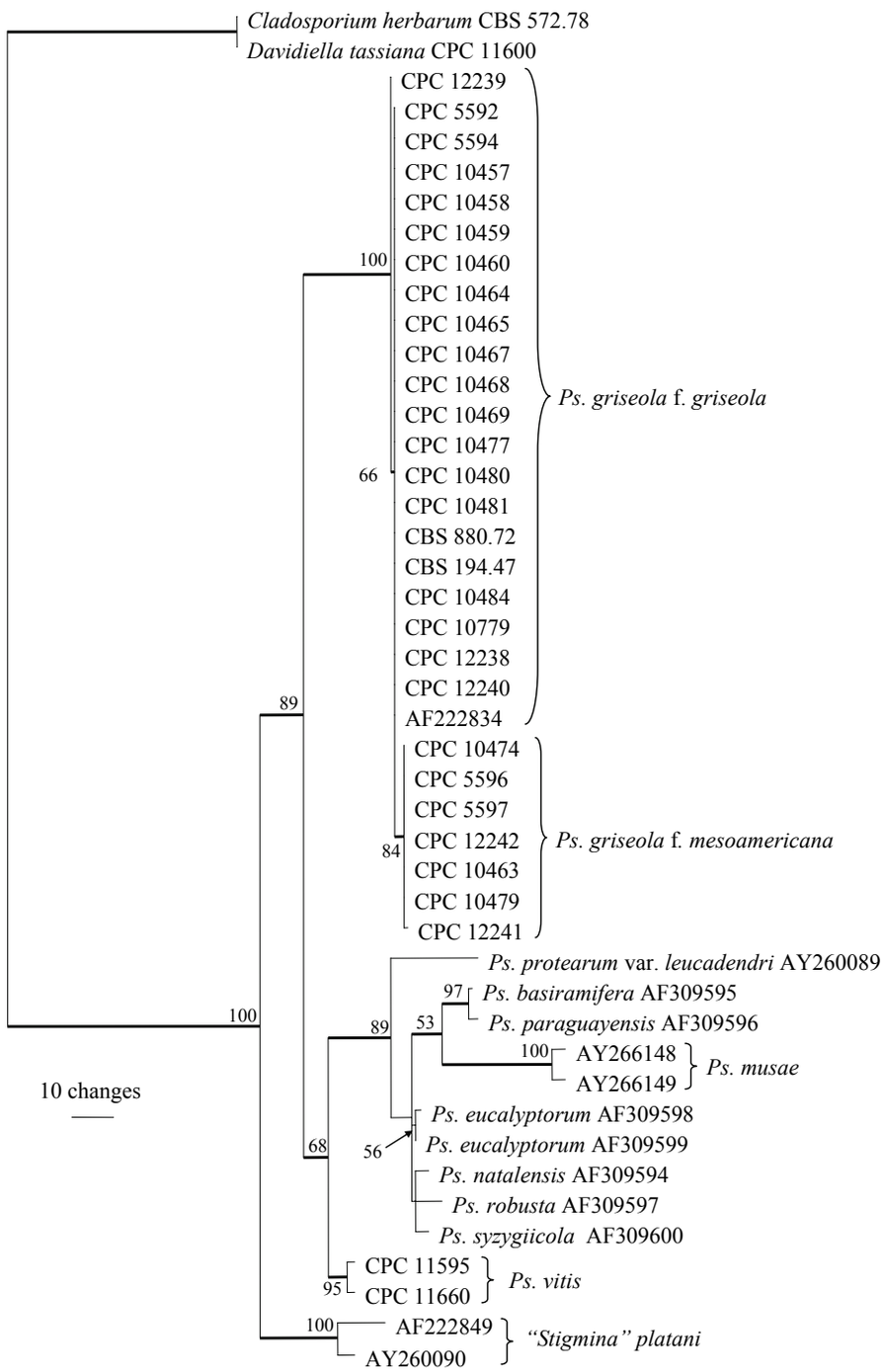

Fig. 2. One of 13 most parsimonious trees obtained from a heuristic search with 10 random taxon additions of the ITS sequence alignment. The scale bar shows 10 changes and bootstrap support values from 1000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and the tree was rooted to *Cladosporium* herbarum and *Davidiella tassiana*.

30 isolates (including the two outgroups). Of the 1050 characters, 288 are parsimony-informative, 42 were variable and parsimony-uninformative, and 720 were constant. The topologies of the trees obtained from the neighbour-joining analyses were identical to each other and also to that obtained from the parsimony analysis (data not shown). Parsimony analysis of the combined data yielded three most parsimonious trees (TL = 353 steps; CI = 0.994; RI = 0.994; RC = 0.988), one of which is shown in Fig. 3. The tree shows two distinct clades, namely *Ps. griseola* f. *griseola* and what we call here the *Ps. griseola* f. *mesoamericana* clade. Bootstrapping using parsimony results in support values of 53 % and 71 % for each clade, respectively. These values increase to 62 % and 98 %, respectively, if neighbour-joining with the HKY85 substitution model is used for bootstrapping. The *Ps. griseola* f. *griseola* clade is further split into two groups (62 / 95 % and 52 / 71 % bootstrap support, respectively), which is the result of three characters that changed in the CAL sequence of isolates CPC 12238 and CPC 12239 (99.04 % sequence similarity to the other *Ps. griseola* f. *griseola* isolates).

Taxonomy

Pseudocercospora griseola (Sacc.) Crous & U. Braun, **comb. nov.** MycoBank MB500855. Fig. 4.

Basionum: *Isariopsis griseola* Sacc., Michelia 1: 273. 1878.

≡ *Phaeoisariopsis griseola* (Sacc.) Ferraris, Ann. Mycol. 7: 273. 1909.

≡ *Lindaumyces griseolus* (Sacc.) Gonz. Frag. (as "*g riseola*"), Mem. R. Acad. Ci. Exact. Madrid, Ser. 2, 6: 339. 1927.

≡ *Cercospora griseola* (Sacc.) Ragunath. & K. Ramakr., J. Madras Univ. 35–36: 11. (1965–1966) 1968.

= *Cylindrosporium phaseoli* (*Cylindrospora*) Rabenh., Klotzschii Herbarium vivum mycologicum, Editio nova, Series Prima, Centuria 4, No. 327, Dresden 1856, *nom. nud.*, also Bot. Zeitung 15(6): 94. 1857, *nom. nud*. and Flora 15(9): 134. 1857, *nom. nud.*

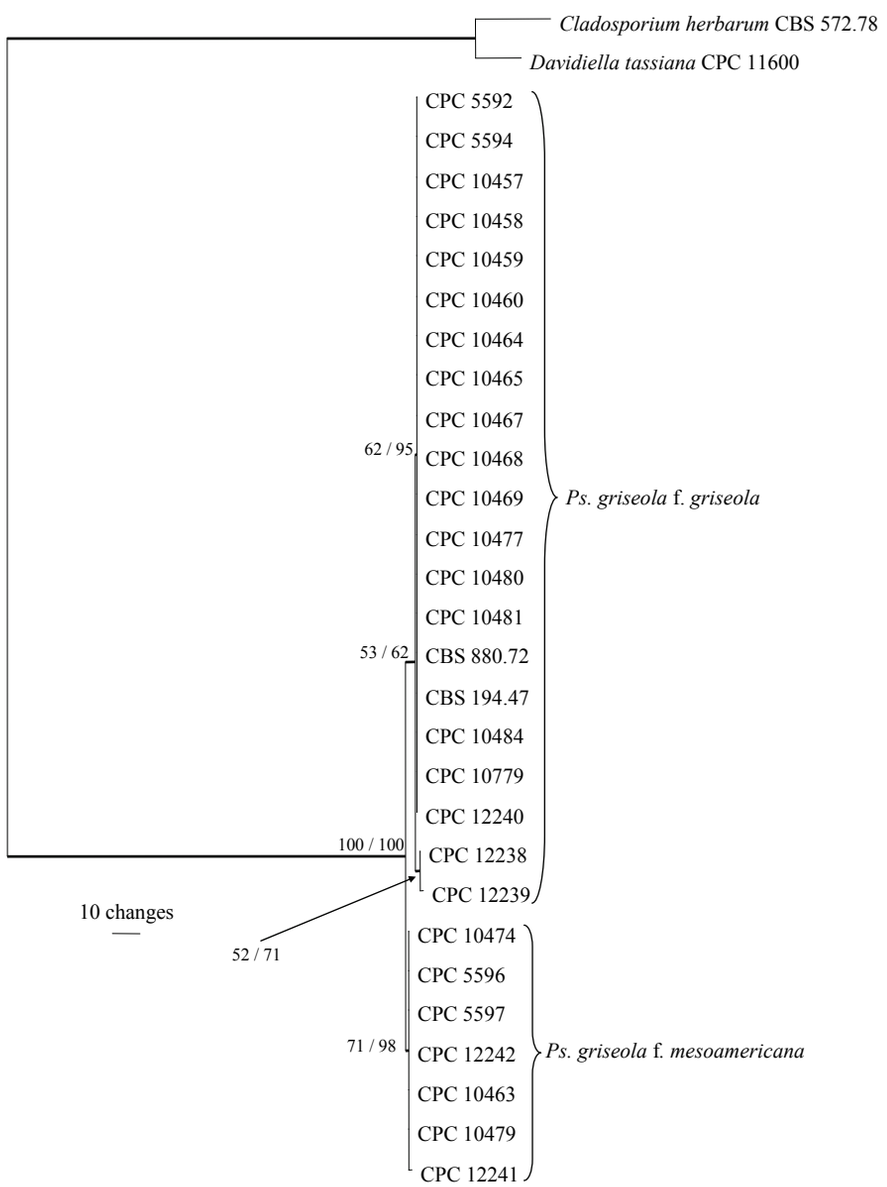

Fig. 3. One of three most parsimonious trees obtained from a heuristic search with 10 random taxon additions of a combined ITS, actin and calmodulin sequence alignment. The scale bar shows 10 changes and bootstrap support values from 1000 replicates are shown at the nodes (values from parsimony before the slash and neighbour-joining with the HKY85 substitution model after the slash). Thickened lines indicate branches found in the strict consensus parsimony tree and the tree was rooted to *Cladosporium herbarum* and *Davidiella tassiana*.

= *Graphium laxum* Ellis, Bull. Torrey Bot. Club 8: 64. 1881.
≡ *Isariopsis laxa* (Ellis) Sacc., Syll. Fung. 4: 631. 1886.
≡ *Phaeoisariopsis laxa* (Ellis) S.C. Jong & E.F. Morris, Mycopathol. Mycol. Appl. 34: 269. 1968.
= *Cercospora solimanii* Speg. (*solimani*), Anales Soc. Ci. Argent. 16: 167. 1883.
= *Cercospora columnaris* Ellis & Everh. (as "*columnare*"), Proc. Acad. Nat. Sci. Philadelphia 46: 380. 1894.
≡ *Pseudocercospora columnaris* (Ellis & Everh.) J.M. Yen, in Yen & Lim, Gard. Bull., Singapore 33: 172. 1980.
= *Arthrobotryum puttemansii* Henn., Hedwigia 41: 309. 1902.
= *Cercospora stuhlmannii* Henn., Bot. Jahrb. Syst. 33: 40. 1904.

Syntypes: on *Phaseolus vulgaris*, Italy, Selva, Aug. 1877, Saccardo, Mycotheca Veneta 1247 (e.g., B, HAL, PAD).

Formae novae:
Pseudocercospora griseola (Sacc.) Crous & U. Braun, f. *griseola*

Specimen examined: **Tanzania**, on *Phaseolus vulgaris*, F.S. Ngulu & C. Mushi, CBS H-19683, **epitype designated here**, CBS 119906 = CPC 10468. culture ex-epitype. The epithet "*griseola*" was based on European material, and from our analysis, it appears that European material is representative of *P. griseola* f. *griseola*.

Pseudocercospora griseola (Sacc.) Crous & U. Braun, **f. *mesoamericana*** Crous & U. Braun **f. nov.** MycoBank MB500856.

Differt a f. griseola variatione virulentiae majore, culturis crescentibus ad ≥ 30 °C.

Morphologically similar to *P. griseola* f. *griseola*, but distinct by having a broader range of virulence on different bean types, and being able to grow at or above 30 °C, which is not the case for f. *griseola*.

Specimen examined: **South Africa**, on *Phaseolus vulgaris*, M.M. Liebenberg, CBS H-19684, **holotype**, culture ex-type CBS 119113 = CPC 10463.

Descriptions (*selection*): Gonzáles Fragoso (1927: 339), Chupp (1954: 295, as *Cercospora columnaris*), Ellis (1971: 269), Shin & Kim (2001: 151–153).

Illustrations (*selection*): Saccardo, Fungi italici, Pl. 838, Padova 1881; Briosi & Cavara, Funghi parassiti delle piante coltivate od utili, Fasc. I, No. 17, figs 1–2, Pavia 1888; Gonzáles Fragoso (1927: 340, fig. 79); Ellis (1971: 269, fig. 183); Deighton (1990: 1098, figs 2–3); Shin & Kim (2001: 153, fig. 65).

Description in vivo: On leaves, petioles, stems and pods; *leaf spots* amphigenous, angular–irregular, rarely subcircular–elliptical, mostly vein-limited, 1–8 mm wide, finally sometimes confluent, forming larger patches, brown, ranging from pale olivaceous, olivaceous-brown, yellowish brown, greyish brown to dark brown, on pods often reddish brown and more regular, subcircular–elliptical, margin indefinite, only delimited by veins, or surrounded by a narrow, dark brown border or marginal line. *Caespituli* on petioles, pods, stems and leaves, amphigenous, mostly hypophyllous, usually scattered, occasionally aggregated, conspicuous, punctiform, dark brown to blackish grey. *Mycelium* internal. *Stromata* almost lacking to well-developed, subglobose, depressed to lacrimoid, up to 70 µm diam, brown. *Conidiophores* numerous, up to approx. 40, in dense fascicles, often forming synnematous conidiomata, erumpent, 100–500 × 20–70 µm, rarely longer, olivaceous-brown, composed of a more or less firm stipe of closely appressed conidiophores and a terminal, loose capitulum, i.e. conidiophores splaying out at the end of the conidiomata, free ends usually up to 100 µm long, individual conidiophores filiform, appressed threads 2–5 µm wide, up to 7 µm wide towards the apex, pluriseptate, subhyaline to olivaceous-brown, thin-walled, occasionally becoming rough-walled with age. *Conidiogenous cells* integrated, terminal, 20–100 µm long, subcylindrical to subclavate, usually not or only barely geniculate, but moderately geniculate in some collections; conidiogenous loci terminal and lateral, quite inconspicuous to subconspicuous, i.e. unthickened or almost so, but slightly darkened-refractive, in surface view visible as minute circles, 1.5–2.5 µm diam, usually flat, non-protruding. *Conidia* solitary, obclavate-cylindrical, broadly subfusiform, short conidia sometimes ellipsoid-ovoid to short cylindrical, straight to curved, 20–75(–85) × 4–9 µm, (0–)1–5(–6)-septate, usually not constricted at the septa, rarely with slight constrictions, subhyaline to pale olivaceous or olivaceous-brown, thin-walled, smooth, sometimes rough-walled, with obtuse apex, and obconically truncate to rounded base, 1.5–2.5(–3) µm wide, hila unthickened or almost so, at most somewhat refractive.

Cultural characteristics: Forma *griseola*; on OA colonies flat to slightly erumpent, spreading with moderate aerial mycelium; margins smooth, regular, surface with patches of olivaceous-grey and smoke-grey to dirty-white; on PDA erumpent with moderate aerial mycelium, surface pale olivaceous-grey to olivaceous-grey in the central part; margin iron-grey, and also iron-grey in reverse. Cardinal temperature requirements for growth: minimum 6 > °C, optimum = 24 °C, maximum < 30 °C. Forma *mesoamericana*; on OA flat to slightly erumpent, spreading, with moderate aerial mycelium; margins irregular, feathery to smooth, even; surface with the central part dirty-white to pale or darker olivaceous-grey, outer region iron-grey; on PDA spreading, erumpent, with moderate aerial mycelium; surface olivaceous-grey in the central part; outer region and reverse iron-grey, margins feathery, irregular. Cardinal temperature requirements for growth: minimum 6 > °C, optimum 24 °C, maximum > 30 °C.

Herbarium specimens examined: On *Lablab niger*, Japan, Tokyo, Toyoda, Itino-machi, Minamitama-gun, 9 Aug. 1962, S. Takamoto (IMI 96372). On *Phaseolus vulgaris*, Italy, Selva, Aug. 1877, Sacc., Mycoth. Ven. 1247 (HAL), type of *Isariopsis griseola*; Italy, Pavia, Casatisma e Albaredo Arnaboldi, 1888, Briosi & Cavara, Funghi parass. 17 (HAL); Russia, Czernigov, Borzova, Aug. 1914, G. Nevodovsky, Petr. Mycoth. gen. 249 (B); South Korea, Chunchon, 7 Oct. 2003, H.D. Shin (HAL). On *Phaseolus* sp., Brazil, São Paulo, Botanical Garden, 26 Dec. 1901, Puttemans, No. 413 (B), type of *Arthrobotryum puttemansii*; Italy, Bugellae et Vercellis, Cesati, Rabenh., Herb. mycol., Ed. 2, No. 327 (HAL), type of *Cylindrosporium phaseoli*; USA, N.J., Newfield, 27 Sep. 1894, J.B. Ellis (NY), type of *Cercospora columnaris*. Unidentified host (*Phaseolus* sp.?), Paraguay, Caá-guazú, Jan. 1882, B. Balansa, No. 3492 (LSP 918), type of *Cercospora solimanii*.

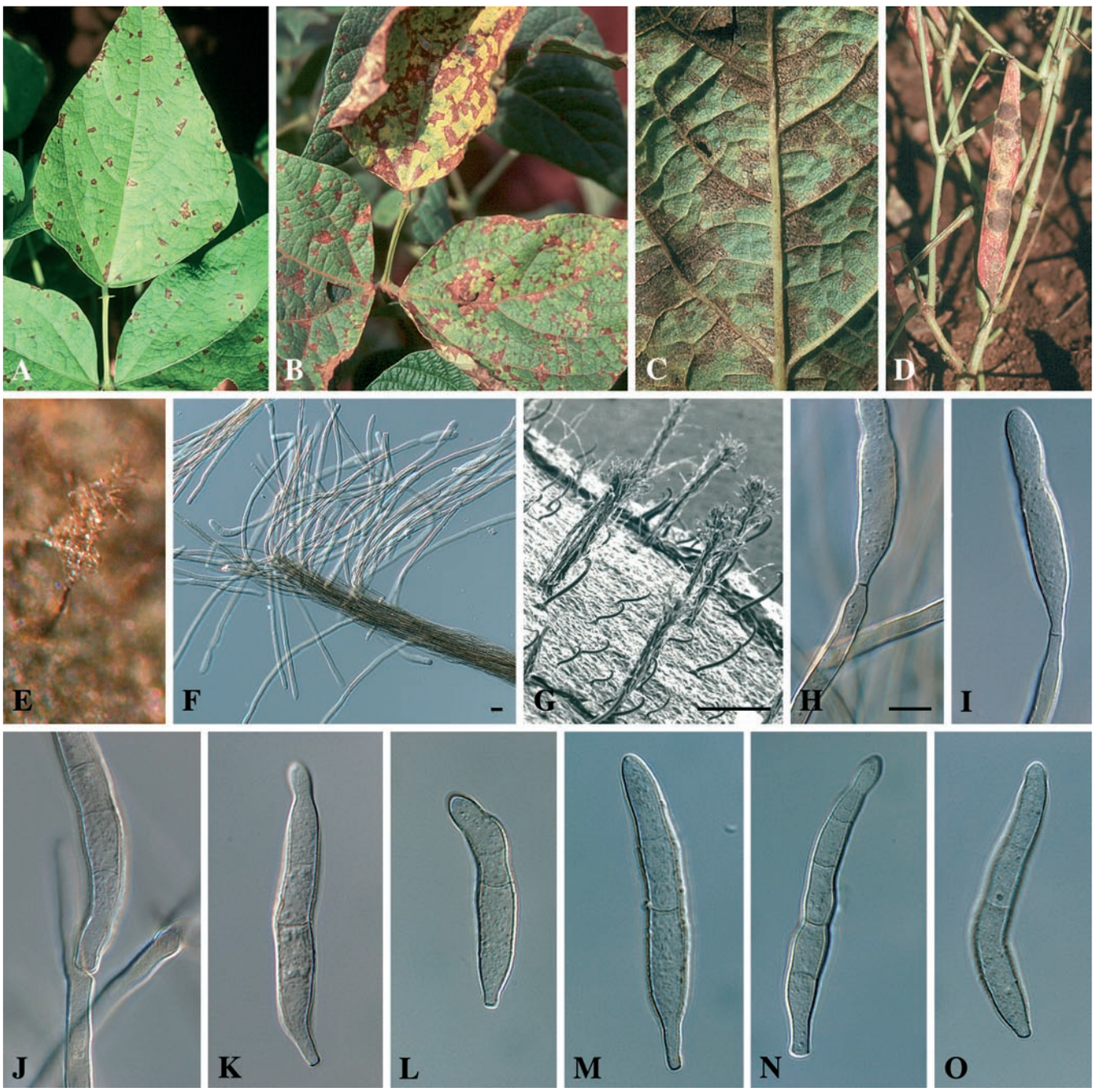

Fig. 4. *Pseudocercospora griseola*. A–C. Leaf disease symptoms. D. Lesions on bean pod. E–G. Fasciculate conidiophores. H–J. Conidiogenous cells giving rise to conidia. K–O. Conidia. Scale bars: F = 8 µm, G = 200 µm, H = 10 µm.

Hosts and distribution: *Lablab niger, ?L. purpureus, ?Lathyrus odoratus, ?Macroptilium atropurpureum, Phaseolus acutifolius, P. aureus, P. coccineus, P. lunatus, P. pubescens, P. vulgaris, Vigna angularis, V. mungo, V. radiata, V. sinensis, V. unguiculata* (*Leguminosae*), worldwide, including Angola, Argentina, Armenia, Australia, Austria, Bhutan, Brazil, Bulgaria, Burundi, Cameroon, Canada, China, Colombia, Congo, Costa Rica, Croatia, Cuba, Dominican Republ., Ecuador, El Salvador, Ethiopia, Fiji, France, Georgia, Germany, Ghana, Great Britain, Greece, Guatemala, Haiti, Hungary, Jamaica, Japan, India, Indonesia, Iran, Ireland, Israel, Italy, Ivory Coast, Jamaica, Japan, Kenya, Korea, Laos, Latvia, Malawi, Madagascar, Malaysia, Mauritius, Mexico, Mozambique, Nepal, Netherlands, Netherlands Antilles, New Caledonia, New Zealand, Nicaragua, Nigeria, Norfolk Island, Panama, Papua New Guinea, Paraguay, Peru, Philippines, Poland, Portugal, Puerto Rico, Réunion, Romania, Russia, Rwanda, Saint Helena, Senegal, Sierra Leone, Singapore, Slovenia, Solomon Islands, Somalia, South Africa, Spain, Sudan, Suriname, Swaziland, Switzerland, Taiwan, Tanzania, Thailand, Trinidad and Tobago, Turkey, Uganda, Ukraine, U.S.A. (CT, DE, Eastern states, FL, HI, IN, MA, MD, ME, MI, MS, NC, NH, NJ, NY, OK, PA, SC, TX, VA, WI), Vanuatu, Venezuela, Virgin Islands, Yugoslavia, Zambia, Zimbabwe (Crous & Braun 2003).

Notes: As a consequence of molecular sequence analyses (Figs 1–3), and re-examination and reassessments of the synnematous conidiomata and scar and hilum structures (Fig. 4, see Discussion),

Phaeoisariopsis griseola proved to be congeneric with *Pseudocercospora*. The proposed assignment of this species to *Pseudocercospora* presupposes acceptance of a formal proposal to conserve the latter genus against the older names *Phaeoisariopsis* and *Stigmina* (Braun & Crous 2006). All other taxa formerly placed in *Phaeoisariopsis* have already been treated and reallocated elsewhere (Crous & Braun 2003).

Cylindrosporium phaseoli Rabenh. is the oldest name coined for this species, which appeared first on the printed label of 'Rabenh., Herb. mycol. 327, 1856'. This name was repeated in Fürnrohr (1857), Schlechtendal (1857) and Saccardo (1884), but in all cases without any description (*nom. nud*.). Gonzáles Fragoso (1927: 339) was the first author who correctly cited this name as synonym of *Phaeoisariopsis griseola*, which we confirm after having re-examined Rabenhorst's original material.

Deighton (1990) reduced *Cercospora solimanii* Speg. to synonymy with *Ph. griseola*, but without any comments and references. Braun (2000) examined type material of this species and confirmed Deighton's (1990) synonymy.

Although there are two clear entities associated with the angular leaf spot disease of bean on pathological or molecular grounds, we were unable to find enough morphological, cultural or phylogenetic support to separate these as two species. Because isolates can readily be classed as either one or the other type based on their host reaction on differential cultivars, we have chosen to designate them as *formae* of the same species.

DISCUSSION

A primary aim of the present study was to determine the species status of the Andean and Middle-American groups of the angular leaf spot pathogen of beans. Because we have been unable to obtain good morphological differences between the two groups (other than cardinal temperatures for growth), nor clear phylogenetic support for the separation based on various gene loci, we have chosen to recognise these two operational units as *formae* of the same species, namely f. *griseola* and f. *mesoamericana***.**

Two basic characters have in the past been used for the discrimination of *Phaeoisariopsis* and *Pseudocercospora*, namely the structure of the conidiomata and the type of conidiogenous loci and conidial hila. In molecular studies, the conidiomatal structures were shown to be unreliable at the genus level for anamorphs of *Mycosphaerella*. This is aptly illustrated by the examples of *Septoria* Sacc. (pycnidia) and *Phloeospora* Wallr. (acervuli) (Verkley *et al***.** 2004), *Colletogloeopsis* Crous & M.J. Wingf. (acervuli) and *Phaeophleospora*-like species with aseptate conidia and pycnidia (Cortinas *et al*. 2005), *Ramularia* Unger (normal fascicles) and *Phacellium* Bonord. (synnemata) (Crous *et al.*, unpubl. data), which are all irregularly scattered among the cladograms. The coelomycete genus *Septoria* (pycnidia) always clusters basal to *Cercospora* Fresen. (fasciculate hyphomycete) (Crous *et al*. 2000, 2001). The presence of synnemata is thus insufficient to separate *Phaeoisariopsis* from *Pseudocercospora* (Crous *et al*. 2001, Crous & Braun 2003). Furthermore, *Pseudocercospora* already includes some synnematous species [e.g. the type species, *P. vitis* (Lév.) Speg.]. Several species originally placed in *Phaeoisariopsis*, but with inconspicuous conidial scars, have already been reallocated in *Pseudocercospora* (Deighton 1990). There are also some other genera of hyphomycetes with synnematous as well as non-synnematous species, e.g., *Spiropes* Cif. (Ellis 1971).

The structure of the conidiogenous loci and conidial hila represent another important character used for the distinction of *Phaeoisariopsis* and *Pseudocercospora*. Prior to the introduction of the scar structure as basic feature in the taxonomy of cercosporoid genera (Deighton 1967, 1973, 1974, 1976), *Phaeoisariopsis* was mainly or even solely based on the synnematous arrangement of the conidiophores, combined with pigmented conidia formed singly. Therefore, it was hardly surprising that Sawada (1922) transferred *Septonema vitis* Lév., the type species of *Pseudocercospora*, to *Phaeoisariopsis***,** and thus reduced *Pseudocercospora* to synonymy with *Phaeoisariopsis*. The heterogeneity of *Phaeoisariopsis* is also reflected by the exclusion of all species, except for the type species, *I. griseola*, originally placed in this genus by Ferraris (1909): *Isariopsis grayiana* Ellis (≡ *Fusicladium grayianum* (Ellis) Deighton & M.B. Ellis), *I. mexicana* Ellis & Everh. (≡ *Exosporium mexicanum* (Ellis & Everh.) M.B. Ellis) and *I. pilosa* Earle (= *Morrisographium persicae* (Schwein.) Deighton) (see Deighton 1990). Von Arx (1983), Deighton (1990) and Braun (1992, 1995, 1998) considered the conidiogenous loci and conidial hila in *Phaeoisariopsis* to be conspicuous or at least subconspicuous, i.e., barely to slightly thickened and darkened. However, Yen (Yen & Lim 1980) already placed the ALS pathogen in *Pseudocercospora* (conidiogenous loci inconspicuous), although the wrong combination [*Pseudocercospora columnaris* (Ellis & Everh.) J.M. Yen] was introduced, and the correct basionym, *Isariopsis griseola*, cited as synonym. The inclusion of *Phaeoisariopsis griseola* in *Pseudocercospora* (Sawada 1922) thus reduces *Pseudocercospora* to synonymy with *Phaeoisariopsis***.** We have re-examined the scars and hila in *Ph. griseola* in detail, based on a wide range of samples *in vivo* and *in vitro*, including type material of *Isariopsis griseola***,** *Cylindrosporium phaseoli***,** *Cercospora columnaris* and *C. solimanii*. The conidiogenous cells are usually not or barely geniculate, the conidiogenous cells are terminal to lateral, non-protruding, quite inconspicuous to subconspicuous, i.e. unthickened or almost so, but slightly darkened-refractive. There are collections with completely inconspicuous conidiogenous loci, e.g. the types of *Isariopsis griseola* and *Cercospora solimanii*. In other samples, the loci range from being quite inconspicuous to subconspicuous. The African collection illustrated by Deighton (1990) is an example of subconspicuous loci. However, as demonstrated earlier by molecular examinations, taxa

with subconspicuous loci and hila (unthicked or almost so, but slightly darkened-refractive or only the ultimate rim slightly thickened and darkened) clustered together with *Pseudocercospora* species, so that further segregate-genera like *Paracercospora* Deighton and *Pseudophaeoramularia* U. Braun had to be reduced to synonymy with *Pseudocercospora* (Crous *et al*. 2000, 2001; Crous & Braun 2003).

Based on the molecular data presented here, the type species of *Pseudocercospora* (*P. vitis*) clusters with the type of *Phaeoisariopsis* (*P. griseola*), and the type of *Stigmina* Sacc. [*S. platani* (Fuckel) Sacc.]. The close affinity of these three genera underlines earlier suspicions of mycologists that criteria such as 1) slightly thickened conidial hila and scars, 2) synnematous to fasciculate to sporodochial conidiomata, 3) transverse to muriformly septate conidia, 4) euseptate to distoseptate conidia, 5) smooth percurrent proliferations and sympodial proliferation, versus irregular, rough percurrent proliferations on conidiogenous cells, are an insufficient basis to separate anamorph genera in *Mycosphaerella*.

Given the fact that these three genera represent anamorph forms of *Mycosphaerella*, and that they phylogenetically reside in the same clade, the next predicament arises as to what name should be applied: *Pseudocercospora* (1910; 1171 names), *Phaeoisariopsis* (1909, 65 names), or *Stigmina* (1880, 161 names). Although *Stigmina* is the oldest name, *Pseudocercospora* is the most commonly used, and many species of *Stigmina* in fact represent other fungi. *Phaeoisariopsis*, which also is older than *Pseudocercospora*, has been reduced to its type species, with most other species being placed in either *Passalora* or *Pseudocercospora***.** *Stigmina* predates *Phaeoisariopsis*. If the Code of Botanical Nomenclature were to be strictly applied, all species in this complex should be transferred to *Stigmina*. As the latter is a poorly resolved, still heterogeneous genus, we choose to avoid this upheaval, and support conservation of the commonly used and accepted generic name, *Pseudocercospora* (Braun & Crous 2006). The latter genus should be used for the whole complex of hyphomycetes formerly placed in *Phaeoisariopsis* and some of *Stigmina*. A formal conservation proposal to this extent has been prepared for Taxon (Braun & Crous 2006).

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