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RESEARCH PAPERS

Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot

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Summary. *Macrophomina phaseolina* (*Botryosphaeriaceae*) is an important soil- and seed-borne pathogen. This pathogen has a broad geographic distribution, and a large host range. The aim of the present study was to determine the genetic variation among a global set of 189 isolates of *M. phaseolina*, isolated from 23 hosts and 30 soil samples in 15 countries. To achieve this goal a multi-gene DNA analysis was conducted for the following five loci, ITS, TEF, ACT, CAL and TUB. Based on these results two well-defined clusters could be delineated, one corresponding to *M. phaseolina s. str.*, for which a suitable epitype is designated. The second clade corresponds to *M. pseudophaseolina*, a novel species occurring on *Abelmoschus esculentus*, *Arachis hypogaea*, *Hibiscus sabdarifa* and *Vigna unguiculata* in Senegal. No consistent correlation was found among genotype, host and geographic location, and both species could even occur on the same host at the same location. Although *M. pseudophaseolina* is presently only known from Senegal, further research is required to determine its virulence compared to *M. phaseolina*, and its geographic distribution.

Key words: genetic diversity, Senegal, soilborne pathogen, systematics, *Tiarosporella phaseolina*.

Introduction

Macrophomina phaseolina, the causal agent of charcoal rot, is a soil- and seed-borne polyphagous pathogen. It causes diseases of more than 500 crop and non-crop species, including economically important hosts such as soybean, common bean, corn, sorghum, cowpea, peanut and cotton (Dhingra and Sinclair, 1977; Ndiaye *et al*., 2010) (Figure 1). The fungus has a worldwide distribution, but is regarded as economically more important in subtropical and tropical countries with semi-arid climates (Wrather *et al*., 1997, 2001).

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Macrophomina phaseolina induces diseases on a range of crops, ranging from seedling blight, root and stem rot, wilt, and pre- to post-emergent damping off, which result in decreased stem height, girth, root and head weight, or death, of affected plants (Raut, 1983). The abundant production of minute black sclerotia of the fungus cause the rotted tissues to become blackened, and for this reason the various diseases are known as charcoal rot. Wrather and Koenning (2010) stated that average yield losses due to charcoal rot in the USA were estimated at about 27 million bushels of soybeans per year from 1996 to 2009. In the Sahelian zone of West Africa (including Burkina Faso, Niger and Senegal), charcoal rot causes an average yield loss of 10%, which is equivalent to 30,000 t of cowpea with an estimated value of $US 146 million for Niger and Senegal (Ndiaye,

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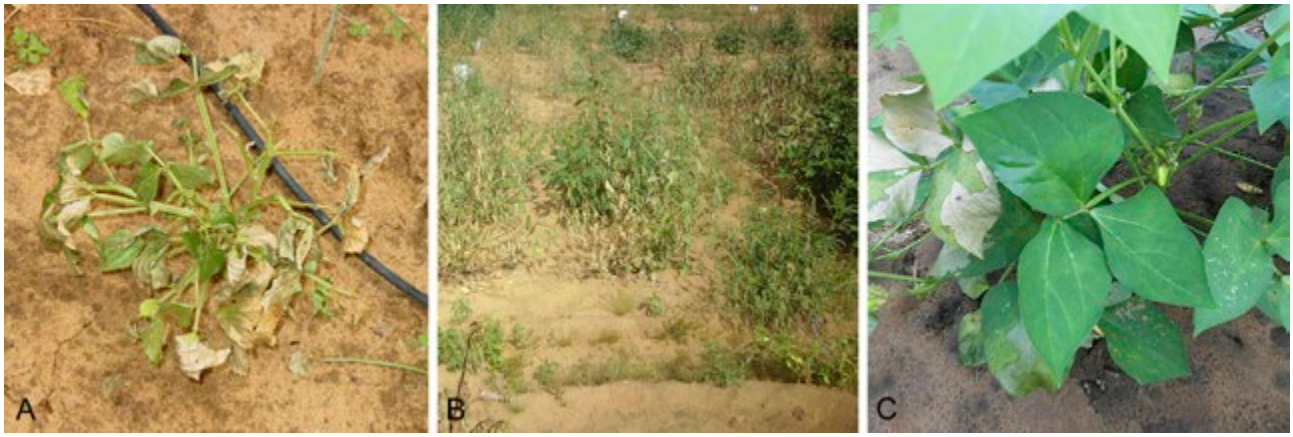

Figure 1. Disease symptoms of *Macrophomina* (charcoal rot) in infested fields. A. Advanced level of infestation on cowpea (*Vigna unguiculata*) in an experimental field with a drip irrigation system (Agrhymet, Niger). B. Charcoal rot symptoms in a sorrel (*Hibiscus sabdarifa*) field during the rainy season (Agrhymet, Niger). C. Early infection in a young cowpea (*Vigna unguiculata*) plant, in an experimental field during the rainy season (Bambey, Senegal).

2007). However, when conditions are favourable for the growth and development of *M. phaseolina*, infections can result in total crop failures (Orellana, 1971; Tikhonov *et al*., 1976; Jimenez *et al*., 1983). Although it is an important plant pathogen, several case reports are known of *M. phaseolina* also acting as an opportunistic human pathogen (Tan *et al*., 2008; Srinivasan *et al*., 2009), especially in immunosuppressed patients, including those receiving prophylactic antifungal therapy (Arora *et al*., 2012).

Macrophomina phaseolina produces asexual structures, microsclerotia and pycnidia, which can be detected in soil and host tissue using quantitative real-time PCR assays (Babu *et al*., 2011). Microsclerotia can survive in soil for 2–15 y, or in root debris for longer periods (Cook *et al*., 1973; Papavizas, 1977; Short *et al*., 1980; Baird *et al*., 2003). Once the host tissues start to decompose, microsclerotia are released into the soil (Hartman *et al*., 1999). Microsclerotia enable the fungus to survive adverse environmental conditions in the field (Short *et al*., 1980). Pycnidia develop readily on plant tissues, and range from being immersed to erumpent, each opening via a central ostiole, through which hyaline, aseptate, ellipsoid to ovoid conidia are discharged (Dhingra and Sinclair, 1977).

Macrophomina phaseolina (= *Tiarosporella phaseolina*) is the type species of *Macrophomina*, which is a distinct genus in the *Botryosphaeriaceae* (Crous *et al*., 2006; Slippers *et al*., 2013). Although five species have previously been described in *Macrophomina* (MycoBank, accessed November 2013), recent phylogenetic studies suggest that the genus could be monotypic (Phillips *et al*., 2013). Morphologically, *Macrophomina* resembles species of *Tiarosporella*, as members of both genera have conidia with hyaline apical appendages. However, *Tiarosporella* lacks microsclerotia, the conidia do not turn brown with age, and its conidiogenous cells do not have conspicuous percurrent proliferations (Crous *et al*., 2006).

Several recent studies have been devoted to characterising the genetic and pathogenic variability of *M. phaseolina*. Advances in molecular techniques and refined PCR-based technology, such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP), have contributed to a better understanding of the genetic and pathogenic variability within populations of this pathogen (Fuhlbohm, 1997; Su *et al*., 2001; Jimenez, 2011). In studying Brazilian populations, Almeida *et al*. (2003) reported that although a single root could be infected by more than one genotype, the overall diversity in populations was low, and that the pathogen probably lacked a sexual cycle. Several studies have focused on developing sets of microsatellite markers to study population variation in *M. phaesolina* (Baird *et al*., 2009, 2010; Arias *et al*., 2011). Although these studies distinguished phylogenetic clades in their analyses, the clades generally did not

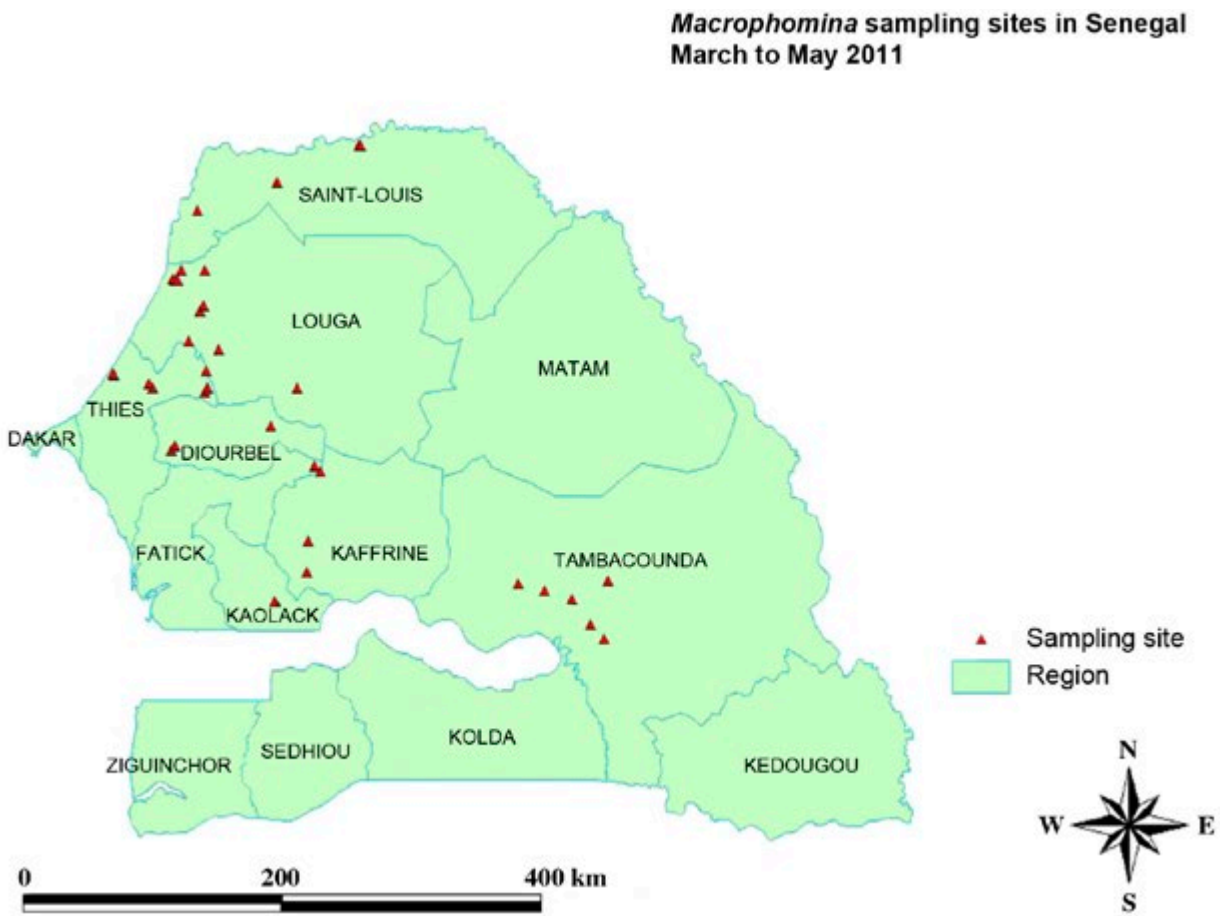

Figure 2. Senegalese cities where samples of different hosts of *Macrophomina* strains were collected.

correlate with geography or host, nor addressed speciation. The aim of the present study was, therefore, to employ multi-gene sequence analyses to examine a large sample of isolates representing different hosts and continents, to determine if all isolates represented a single species, or if more than one taxon were involved as the causes of charcoal rot.

Materials and methods

Fungal isolates

All strains of *M. phaseolina* available at the CBS-KNAW Fungal Biodiversity Centre (CBS) in the Netherlands, and from the working collection of Pedro Crous (CPC), maintained at CBS, were included in this study. Furthermore, from September to November 2011, charcoal rot-affected stems or roots of cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*), sorrel (*Hibiscus sabdarifa*), sorghum (*Shorghum bicolor*) and okra (*Abelmoschus esculentus*) were collected from different cropping regions of Senegal. Depending on the number of diseased foci, three to five plants were sampled per field, from the major crop production areas of Senegal. Details regarding the geographical and host origin of all the isolates are provided in Figures 2 and 3, and Table 1.

Soil isolations. Isolates were obtained from soil using a soil assay technique based on the method of Alabouvette (1976). Soil samples were dried in an oven at 37°C, crushed through a 1 mm mesh sieve, mixed thoroughly, and subsamples of 5 g were taken. Each subsample was submerged in 0.52% NaOCl for 10 min. The soil-NaOCl mixture was washed with distilled water through two sieves of, respectively,

180 and 45 μm mesh sizes. The residue retained on the 45 μm mesh sieve was incorporated into 100 mL of a semi-selective medium (SS medium) for *M. phaseolina.* This consisted of potato dextrose agar (PDA; 39 g L^{-1}) maintained at 55°C in a water bath, with the following ingredients added: 1.5 mL 0.52% NaOCl, 1 mL 0.5% choramphenicol dissolved in 95% alcohol, and 10 mL 2.25% quintozene (PCNB). The medium was poured into 9 cm Petri dishes to solidify, and then incubated at 25ºC.

*Host tissue isolations.*Uprooted plants were rinsed under running tap water, and blotted dry with a sterile paper towel. Separate subsamples of roots and stems of each plant were treated by immersion in 0.5% NaOCl for 10 min to eliminate secondary invaders, followed by rinsing for 30 min in sterile distilled water. Tissues excised from dying rootlets, necrotic taproots and stems were cut into 2–3 mm long fragments, and 15 of these were plated on three Petri dishes containing the above-described SS-medium and incubated at 30°C for 5-8 d.

Colonies of *M. phaseolina* each appeared as a ring of fluffy white mycelium surrounding a central area with black microsclerotia. Each colony was observed under the microscope for the presence of microsclerotia. Typical colonies from soil and host tissue isolations were propagated further onto PDA, and stored at 5°C (Table 1).

DNA isolation, amplification and analyses

Fungal colonies of 104 isolates obtained from host tissues and another 85 strains of *Macrophomina* from the CBS and CPC culture collections were established on 2% malt extract agar (MEA) plates for DNA isolation. For each isolate, total genomic DNA was extracted using UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc.) according to the manufacturer's protocol. The pure quantified DNA samples were stored in 2 mL tubes at 4°C for further use.

Five loci were amplified and sequenced, namely: the Internal Transcribed Spacer regions of the nuclear rDNA operon with the primers ITS 5 and ITS 4 (White *et al*., 1990), part of the TEF-1α gene region using primers EF1-728F (Carbone and Kohn, 1999) and EF2 (O'Donnell *et al*., 1998); part of the TUB gene region using primers T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous *et al.*, 2004b); part of the actin (ACT) gene region using primers ACT-512F and ACT-783R (Carbone and Kohn, 1999); and part of CAL gene region using primers CAL-228F and CAL-737R (Carbone and Kohn, 1999). However, some of these primer pairs failed to amplify with some of the isolates, so additional combinations were used. The primers EF1-728F and EF 986R were used for amplification of part of the TEF-1α gene region, ACT-512F and ACT-2RD for part of the actin (ACT) gene region and T1-T22 for the partial beta-tubulin gene (O'Donnell and Cigelnik 1997).

The basic PCR protocol described by Lombard *et al*. (2010) was used and consisted of 2.5 units FastStart Taq polymerase (Roche Applied Science), 10 × PCR buffer, 0.25 mM of each dNTP, 0.5 μM of each primer and ±30 ng of fungal genomic DNA, made up to a total reaction volume of 12.5 μL.

Amplification cycles were carried out for 2 min at 94°C as initial denaturation, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 48–55°C for 30 s, extension at 72°C for 1 min with a final extension step at 72°C for 10 min and a hold step at 10°C. Amplification conditions were the same for all loci, except for optimised $MgCl_2$ concentrations (1.26 mM for ITS and CAL, 1 mM for TEF-1α and ACT, 0.75 mM for TUB) and for optimised annealing temperatures (48°C for ITS, 52°C for TEF-1α, 55°C for CAL, ACT and TUB). Following PCR amplification, amplicons were visualised on 1% agarose gels stained with GelRed™ (Biotium Inc.) and viewed under ultra-violet light, and sizes of amplicons were determined against a HyperLadder™ I molecular marker (BIOLINE).

To ensure high quality sequences, amplified fragments were sequenced in both directions using the same primer pairs used for amplification. For this purpose, the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems) and a 3730xl DNA Analyzer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG). The resulting sequences were analysed for homologies to sequences deposited in the NCBI's GenBank nucleotide databases using megablast searches. The genotypic differences were confirmed by manual inspection of the raw sequence data and the subsequent alignments were performed using the online interface of MAFFT v. 6 (Katoh and Toh, 2010), and manually corrected where necessary.

Congruency of the sequence datasets for the separate loci were determined using tree topolo-

Table 1. Details of *Macrophomina* strains included in the molecular and morphological analyses.

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
M. phaseolina								
CBS 121.82	*Sesamum indicum*, seed	—		KF951621	—	—	KF951995	—
CBS 162.25	*Eucalyptus* sp.	Uganda		—	KF951803	—	KF951996	—
CBS 205.47	*Phaseolus vulgaris*	Italy		KF951622	KF951804	—	KF951997	—
CBS 215.35	*Derris elliptica*, root	Malaysia		KF951623	—	—	—	—
CBS 224.33	*Sesamum indicum*	Uganda		KF951624	KF951805	—	KF951998	—
CBS 225.33	*Brassica rapa*, leaf	Sierra Leone		KF951625	KF951806	—	KF951999	—
CBS 226.33	*Nicotiana tabacum*	Palestine		KF951626	—	—	—	—
CBS 227.33	*Zea mays*	Palestine		KF951627	KF951807	—	KF952000	—
CBS 228.33	*Cajanus indicus*	Ceylon		KF951628	KF951808	—	KF952001	—
CBS 229.33	*Cajanus indicus*	Ceylon		KF951629	KF951809	—	KF952002	—
CBS 230.33	*Gossypium herbaceum*, seedling	Sudan		KF951630	KF951810	—	KF952003	—
CBS 231.33	*Saccharum officinarum*	India		KF951631	KF951811	—	KF952004	—
CBS 270.34	*Vigna sinensis*, root	USA: Missouri		KF951632	KF951812	—	KF952005	—
CBS 271.34	*Chrysanthemum* sp., stem	USA: Missouri		KF951633	KF951813	—	KF952006	—
CBS 313.51	*Sorghum* sp.	Venezuela		KF951634	—	—	KF952007	—
CBS 416.62	*Arachis hypogaea*	Portugal		KF951635	KF951814	—	KF952008	—
CBS 457.70 = IMI 147229	*Phaseolus aureus*, seed	Denmark		KF951636	KF951815	—	KF952009	—
CBS 458.70 = IMI 147230	*Phaseolus mungo*, seed	Denmark		KF951637	—	—	KF952010	—
CBS 459.70	*Abelmoschus esculentus*, seed	—		KF951638	—	—	KF952011	—
CBS 460.70 = IMI 147232	*Glycine max*, seed	Denmark		KF951639	—	—	KF952012	—
CBS 461.70	*Phaseolus vulgaris*, seed	Denmark		KF951640	KF951816	—	KF952013	—
CPC 11052 = BaraTN2	*Vigna unguiculata*	Niger		KF951641	KF951817	—	KF952014	—
CPC 11053 = GGRMN6	*Vigna unguiculata*	Niger		KF951642	—	—	KF952015	—
CPC 11054	*Vigna unguiculata*	Niger		KF951643	KF951818	—	KF952016	—

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 11055 = GGTMN4	*Vigna unguiculata*	Niger		KF951644	KF951819	—	KF952017	—
CPC 11056 = Bara TN7	*Vigna unguiculata*	Niger		KF951645	KF951820	—	KF952018	—
CPC 11057 = Bara RMN2	*Vigna unguiculata*	Niger		KF951646	KF951821	—	KF952019	—
CPC 11058 = Bara RMN3	*Vigna unguiculata*	Niger		KF951647	—	—	KF952020	—
CPC 11059 = PiTN2	*Vigna unguiculata*	Niger		KF951648	—	—	KF952021	—
CPC 11060 = BeRMN 1	*Vigna unguiculata*	Niger		KF951649	—	—	KF952022	—
CPC 11061 = BeRMN 2	*Vigna unguiculata*	Niger		KF951650	KF951822	—	KF952023	—
CPC 11062 = BeRMN 3	*Vigna unguiculata*	Niger		KF951651	KF951823	—	—	—
CPC 11063 = CATMN1	*Vigna unguiculata*	Niger		KF951652	—	—	KF952024	—
CPC 11064 = CATMN2	*Vigna unguiculata*	Niger		KF951653	—	—	—	—
CPC 11065 = CATMN3	*Vigna unguiculata*	Niger		KF951654	—	—	KF952025	—
CPC 11066 = CATN5	*Vigna unguiculata*	Niger		KF951655	KF951824	—	KF952026	—
CPC 11067 = CATMN5	*Vigna unguiculata*	Niger		KF951656	—	—	KF952027	—
CPC 11068 = CATMN6	*Vigna unguiculata*	Niger		KF951657	—	—	KF952028	—
CPC 11069 = Bara RN1	*Vigna unguiculata*	Niger		KF951658	—	—	KF952029	—
CPC 11070 = Bara TN4	*Vigna unguiculata*	Niger		KF951659	—	—	KF952030	—
CPC 11071 = Bara TN9	*Vigna unguiculata*	Niger		KF951660	—	—	KF952031	—
CPC 11072 = GGRMN2	*Vigna unguiculata*	Niger		KF951661	—	—	KF952032	—
CPC 11073 = Bam1	Soil	Senegal		KF951662	KF951825	—	KF952033	—
CPC 11074 = Bam2	Soil	Senegal		KF951663	—	—	KF952034	—
CPC 11075 = Bam3	Soil	Senegal		KF951664	—	—	—	—
CPC 11076 = Bam4	Soil	Senegal		KF951665	—	—	KF952035	—
CPC 11077 = Bam6	Soil	Senegal		KF951666	—	—	KF952036	—
CPC 11079 = Bam8	Soil	Senegal		KF951667	—	—	KF952037	—
CPC 11080 = Bam9	Soil	Senegal		KF951668	KF951826	—	KF952038	—
CPC 11082 = Bam11	Soil	Senegal		KF951669	—	—	KF952039	—

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 11083 = Bam12	Soil	Senegal		KF951670	—	—	KF952040	—
CPC 11085 = Bam 14	Soil	Senegal		—	KF951827	—	KF952041	—
CPC 11086 = Bam16	Soil	Senegal		KF951671	—	—	KF952042	—
CPC 11087 = Bam 17	Soil	Senegal		—	KF951828	—	—	—
CPC 11088 = Bam18	Soil	Senegal		KF951672	KF951829	—	KF952043	—
CPC 11089 = Bam19	Soil	Senegal		KF951673	KF951830	—	KF952044	—
CPC 11090 = Bam21	Soil	Senegal		KF951674	—	—	KF952045	—
CPC 11091 = Bam22	Soil	Senegal		KF951675	—	—	—	—
CPC 11093 = Bam24	Soil	Senegal		KF951676	KF951831	—	KF952046	—
CPC 11094 = Kebe4	Soil	Senegal		KF951677	KF951832	—	KF952047	—
CPC 11095 = Kebe5	Soil	Senegal		KF951678	—	—	KF952048	—
CPC 11096 = Kebe6	Soil	Senegal		KF951679	KF951833	—	KF952049	—
CPC 11098 = Kebe9	Soil	Senegal		KF951680	KF951834	—	KF952050	—
CPC 11099 = Kebe10	Soil	Senegal		KF951681	KF951835	—	KF952051	—
CPC 11100 = Kebe12	Soil	Senegal		KF951682	—	—	KF952052	—
CPC 11101 = Kebe13	Soil	Senegal		KF951683	—	—	KF952053	—
CPC 11102 = Kebe14	Soil	Senegal		KF951684	—	—	KF952054	—
CPC 11103 = Kebe15	Soil	Senegal		KF951685	—	—	KF952055	—
CPC 11104 = Kebe1	Millet	Senegal		KF951686	KF951836	—	KF952056	—
CPC 11105 = Kebe16	Soil	Senegal		KF951687	—	—	KF952057	—
CPC 11106 = Kebe 17	Soil	Senegal		—	—	—	KF952058	—
CPC 11107 = Kebe18	Soil	Senegal		KF951688	KF951837	—	KF952059	—
CPC 11109 = Kebe25	Soil	Senegal		KF951689	KF951838	—	KF952060	—
CPC 11110 = GGRM1	Millet	Niger		—	KF951839	—	KF952061	—
CPC 11111 = GGRM2	Millet	Niger		KF951690	KF951840	—	KF952062	—
CPC 11112 = GGRM3	Millet	Niger		KF951691	—	—	KF952063	—

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 11113 = GGRM5	Millet	Niger		KF951692	—	—	KF952064	—
CPC 11114 = GGRM6	Millet	Niger		KF951693	—	—	KF952065	—
CPC 11115 = GGRM7	Millet	Niger		KF951694	KF951841	—	KF952066	—
CPC 13080 = HMP 18 - 3	*Sesamum indicum*	Mexico		KF951695	—	—	KF952067	—
CPC 13081 = HMP 48 -1	*Glycine max*	Mexico		KF951696	—	—	KF952068	—
CPC 13082 = HMP 46- 1	*Sorghum bicolor*	Mexico		KF951697	—	—	KF952069	—
CPC 13084 = HMP 47- 1	*Phaseolus vulgaris*	Mexico		KF951698	—	—	KF952070	—
CPC 13085 = HMP 53	*Phaseolus vulgaris*	Mexico		KF951699	—	—	KF952071	—
CPC 13086 = HMP 21-1	*Solanum melongena*	Mexico		KF951700	—	—	—	—
CPC 21386	*Hibiscus sabdarifa*	Senegal: Louga	Feathery, grey	KF951701	—	—	KF952072	—
CPC 21387	*Hibiscus sabdarifa*	Senegal: Louga	Feathery, grey	KF951702	KF951842	—	KF952073	—
CPC 21388	*Hibiscus sabdarifa*	Senegal: Louga	Feathery, grey	KF951703	KF951843	KF951929	KF952074	KF952165
CPC 21391	*Vigna unguiculata*	Senegal		KF951704	—	KF951930	KF952075	KF952166
CPC 21392	*Vigna unguiculata*	Senegal: Thiès	Dense, black	KF951705	KF951844	KF951931	KF952076	KF952167
CPC 21393	*Vigna unguiculata*	Senegal		—	KF951845	—	—	—
CPC 21395	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951706	KF951846	KF951932	KF952077	KF952168
CPC 21399	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951707	KF951847	KF951933	KF952078	KF952169
CPC 21401	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951708	KF951848	—	KF952079	—
CPC 21405	*Vigna unguiculata*	Senegal: Louga	Restricted, white	KF951709	KF951849	KF951934	KF952080	KF952170
CPC 21406	*Vigna unguiculata*	Senegal: Louga	Restricted, grey	KF951710	KF951850	KF951935	KF952081	KF952171
CPC 21407	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951711	KF951851	—	KF952082	KF952172
CPC 21409	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951712	KF951852	KF951936	KF952083	KF952173
CPC 21410	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951713	KF951853	KF951937	KF952084	KF952174
CPC 21411	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951714	KF951854	—	KF952085	KF952175
CPC 21416	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951715	KF951855	KF951938	KF952086	KF952176
CPC 21419	*Arachis hypogaea*	Senegal: Thiès	Dense, black	KF951716	KF951856	KF951939	KF952087	KF952177

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 21420	*Vigna unguiculata*	Senegal: Saint-Louis	Dense, black	KF951717	KF951857	KF951940	KF952088	KF952178
CPC 21421	*Vigna unguiculata*	Senegal: Saint-Louis	Feathery, grey	KF951718	KF951858	KF951941	KF952089	KF952179
CPC 21423	*Vigna unguiculata*	Senegal: Saint-Louis	Feathery, grey	KF951719	KF951859	KF951942	KF952090	KF952180
CPC 21424	*Arachis hypogaea*	Senegal: Diourbel	Dense, black	KF951720	—	—	KF952091	KF952181
CPC 21425	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951721	KF951860	KF951943	KF952092	KF952182
CPC 21426	*Vigna unguiculata*	Senegal: Louga	Dense, black	KF951722	KF951861	KF951944	KF952093	KF952183
CPC 21427	*Vigna unguiculata*	Senegal: Louga	Dense, black	KF951723	—	KF951945	KF952094	KF952184
CPC 21428	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951724	KF951862	KF951946	KF952095	KF952185
CPC 21429	*Vigna unguiculata*	Senegal: Louga	Dense, black	KF951725	KF951863	KF951947	—	KF952186
CPC 21430	*Arachis hypogaea*	Senegal: Thiès	Feathery, grey	KF951726	KF951864	KF951948	KF952096	KF952187
CPC 21431	*Hibiscus sabdarifa*	Senegal: Thiès	Feathery, grey	KF951727	KF951865	KF951949	KF952097	KF952188
CPC 21433	*Arachis hypogaea*	Senegal: Diourbel	Feathery, grey	KF951728	KF951866	KF951950	KF952098	KF952189
CPC 21436	*Arachis hypogaea*	Senegal: Thiès	Dense, black	KF951729	KF951867	KF951951	KF952099	KF952190
CPC 21437	*Arachis hypogaea*	Senegal: Thiès	Dense , black	KF951730	KF951868	—	KF952100	—
CPC 21438	*Arachis hypogaea*	Senegal: Thiès	Dense, black	KF951731	KF951869	KF951952	KF952101	KF952191
CPC 21441	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951732	KF951870	—	KF952102	KF952192
CPC 21442	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951733	KF951871	KF951953	KF952103	KF952193
CPC 21443	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951734	KF951872	KF951954	KF952104	KF952194
CPC 21444	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951735	KF951873	KF951955	KF952105	KF952195
CPC 21445	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951736	KF951874	KF951956	KF952106	KF952196
CPC 21447	*Arachis hypogaea*	Senegal: Kaolack	Restricted, grey	KF951737	KF951875	KF951957	KF952107	KF952197
CPC 21448	*Arachis hypogaea*	Senegal: Kaolack	Feathery, grey	KF951738	KF951876	KF951958	KF952108	KF952198
CPC 21450	*Vigna unguiculata*	Senegal: Diourbel	Dense, black	KF951739	KF951877	—	KF952109	—
CPC 21451	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951740	KF951878	KF951959	KF952110	KF952199
CPC 21452	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951741	KF951879	KF951960	KF952111	—
CPC 21453	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951742	KF951880	KF951961	KF952112	KF952200

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 21454	*Vigna unguiculata*	Senegal: Diourbel	Dense, black	KF951743	KF951881	—	KF952113	KF952201
CPC 21455	*Vigna unguiculata*	Senegal: Diourbel	Dense, black	KF951744	KF951882	—	KF952114	KF952202
CPC 21456	*Vigna unguiculata*	Senegal: Louga	Dense, black	KF951745	KF951883	KF951962	KF952115	KF952203
CPC 21460	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951746	KF951884	KF951963	KF952116	KF952204
CPC 21461	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951747	KF951885	—	KF952117	—
CPC 21462	*Vigna unguiculata*	Senegal: Louga	Feathery, grey	KF951748	KF951886	—	KF952118	KF952205
CPC 21463	*Vigna unguiculata*	Senegal: Thiès	Dense, black	KF951749	KF951887	KF951964	KF952119	KF952206
CPC 21464	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951750	—	—	—	—
CPC 21468	*Abelmoschus esculentus*	Senegal		KF951751	—	—	—	—
CPC 21470	*Abelmoschus esculentus*	Senegal: Saint-Louis	Dense, black	KF951752	—	—	KF952120	—
CPC 21474	*Abelmoschus esculentus*	Senegal: Saint-Louis	Restricted, grey	KF951753	—	—	KF952121	—
CPC 21475	*Abelmoschus esculentus*	Senegal		KF951754	—	—	—	—
CPC 21476	*Abelmoschus esculentus*	Senegal: Saint-Louis	Feathery, grey	KF951755	KF951888	KF951965	KF952122	KF952207
CPC 21477	*Arachis hypogaea*	Senegal: Tambacounda	Dense, black	KF951756	KF951889	KF951966	KF952123	KF952208
CPC 21478	*Arachis hypogaea*	Senegal: Tambacounda	Feathery, grey	KF951757	KF951890	KF951967	KF952124	KF952209
CPC 21481	*Hibiscus sabdarifa*	Senegal: Tambacounda	Feathery, grey	KF951758	KF951891	KF951968	KF952125	KF952210
CPC 21483	*Arachis hypogaea*	Senegal: Tambacounda	Restricted, black	KF951759	—	—	—	—
CPC 21485	*Sorghum* sp.	Senegal: Saint-Louis	Dense, black	KF951760	KF951892	—	KF952126	—
CPC 21486	*Abelmoschus esculentus*	Senegal: Saint-Louis	Restricted, grey	KF951761	KF951893	KF951969	KF952127	KF952211
CPC 21490	*Arachis hypogaea*	Senegal: Tambacounda	Dense, black	KF951762	KF951894	KF951970	KF952128	KF952212
CPC 21491	*Vigna unguiculata*	Senegal: Tambacounda	Feathery, grey	KF951763	—	—	—	—
CPC 21492	*Vigna unguiculata*	Senegal: Tambacounda	Restricted, grey	KF951764	—	KF951971	KF952129	KF952213
CPC 21495	*Vigna unguiculata*	Senegal: Tambacounda	Feathery, grey	KF951765	KF951895	—	KF952130	—
CPC 21496	*Vigna unguiculata*	Senegal: Tambacounda	Feathery, grey	KF951766	KF951896	—	KF952131	—
CPC 21497	*Vigna unguiculata*	Senegal: Tambacounda	Feathery, grey	KF951767	KF951897	KF951972	KF952132	KF952214
CPC 21498	*Arachis hypogaea*	Senegal: Tambacounda	Feathery, grey	KF951768	KF951898	—	KF952133	KF952215

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 21504	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Feathery, grey	KF951769	KF951899	KF951973	KF952134	KF952216
CPC 21505	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Feathery, grey	KF951770	KF951900	—	KF952135	KF952217
CPC 21506	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951771	KF951901	KF951974	KF952136	KF952218
CPC 21508	*Abelmoschus esculentus*	Senegal		KF951772	—	—	—	—
CPC 21512	*Abelmoschus esculentus*	Senegal: Saint-Louis	Dense, black	KF951773	KF951902	KF951975	KF952137	KF952219
CPC 21513	*Vigna unguiculata*	Senegal: Diourbel	Dense, black	KF951774	KF951903	KF951976	KF952138	KF952220
CPC 21514	*Vigna unguiculata*	Senegal		KF951775	—	—	—	—
CPC 21515	*Vigna unguiculata*	Senegal		KF951776	KF951904	—	—	—
CPC 21516	*Vigna unguiculata*	Senegal: Diourbel	Feathery, grey	KF951777	KF951905	—	KF952139	KF952221
CPC 21517	*Vigna unguiculata*	Senegal		KF951778	—	—	KF952140	—
CPC 21518	*Vigna unguiculata*	Senegal: Diourbel	Feathery, black	KF951779	KF951906	KF951977	KF952141	—
CPC 21519	*Vigna unguiculata*	Senegal: Diourbel	Feathery, black	KF951780	KF951907	KF951978	KF952142	KF952222
CPC 21520	*Vigna unguiculata*	Senegal: Diourbel	Feathery, black	KF951781	KF951908	KF951979	KF952143	KF952223
CPC 21521	*Vigna unguiculata*	Senegal: Diourbel	Feathery, black	KF951782	KF951909	KF951980	KF952144	KF952224
CPC 21532	*Vigna unguiculata*	Senegal: Thiès	Dense, black	KF951783	KF951910	KF951981	KF952145	KF952225
CPC 21534	*Vigna unguiculata*	Senegal: Thiès	Restricted, black	KF951784	KF951911	KF951982	KF952146	KF952226
CPC 21535	*Vigna unguiculata*	Senegal: Thiès	Feathery, grey	KF951785	KF951912	—	KF952147	KF952227
M. pseudophaseolina								
CPC 21394 = CBS 137166	*Vigna unguiculata*	Senegal: Thiès	Dense, black	KF951786	KF951913	KF951983	KF952148	KF952228
CPC 21398	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951787	KF951914	—	KF952149	KF952229
CPC 21400	*Arachis hypogaea*	Senegal: Louga	Feathery, grey	KF951788	KF951915	—	KF952150	KF952230
CPC 21402	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951789	KF951916	KF951984	KF952151	KF952231
CPC 21403	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951790	KF951917	KF951985	KF952152	KF952232
CPC 21417 = CBS 137165	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951791	KF951918	KF951986	KF952153	KF952233
CPC 21422	*Vigna unguiculata*	Senegal: Saint-Louis	Feathery, grey	KF951792	KF951919	—	KF952154	KF952234

(Continued)

Table 1. (Continued)

Strain accession number[1]	Substrate of isolation	Origin	Structure and colour on PDA	GenBank accession numbers[2]				
				ITS	ACT	CAL	TEF-1α	TUB
CPC 21458	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951793	KF951920	KF951987	KF952155	KF952235
CPC 21459	*Arachis hypogaea*	Senegal: Louga	Dense, black	KF951794	KF951921	KF951988	KF952156	KF952236
CPC 21500 = CBS 137167	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Dense, black	KF951795	KF951922	KF951989	KF952157	KF952237
CPC 21501	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Feathery, black	KF951796	KF951923	KF951990	KF952158	KF952238
CPC 21502	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Feathery, grey	KF951797	KF951924	KF951991	KF952159	KF952239
CPC 21503	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Dense, black	—	—	—	KF952160	—
CPC 21511 = CBS 137168	*Abelmoschus esculentus*	Senegal		KF951798	—	—	—	—
CPC 21524	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Dense, black	KF951799	KF951925	KF951992	KF952161	KF952240
CPC 21525	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Dense, black	KF951800	KF951926	—	KF952162	KF952241
CPC 21527	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Dense, black	KF951801	KF951927	KF951993	KF952163	KF952242
CPC 21528	*Hibiscus sabdarifa*	Senegal: Saint-Louis	Dense, black	KF951802	KF951928	KF951994	KF952164	KF952243

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA; ACT: partial actin gene; CAL: partial calmodulin gene; TEF-1α: partial translation elongation factor 1-alpha gene; TUB: partial beta-tubulin gene.

gies of 70% reciprocal Neighbour-Joining bootstrap trees with Maximum Likelihood distances that were compared visually to identify conflicts between partitions (Mason-Gamer and Kellogg, 1996; Gueidan *et al*., 2007). A maximum parsimony analysis was performed on the combined alignments with Phylogenetic Analysis Using Parsimony (PAUP) v. 4.0b10 (Swofford, 2003), using the heuristic search option with 100 random sequence additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and the first 1,000 multiple, equally most parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1,000 bootstrap replications (Hillis and Bull, 1993). All characters were weighted equally and alignment gaps were treated as new state data. Tree length, consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy indices (HI) were calculated for the resulting trees. The resulting phylogenetic tree (Figure 3) was printed with Geneious v. 5.5.4 (Drummond *et al*., 2011), and the layout of the tree for publication was carried out using in Adobe Illustrator v. CS5.1. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al*., 2004a).

Morphology

Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Colony characters and pigment production were noted after 3 d of growth on PDA at 25ºC. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Morphological descriptions were based on colonies sporulating on sterile pine needles on water agar (PNA; Smith *et al*., 1996).

Results

Phylogeny

One hundred and four *Macrophomina* isolates were obtained from five plant species and six regions in Senegal (Figure 2, Table 1). The majority of these isolates were sequenced for all five loci, and these data were combined with the sequence data from additional strains from different hosts and continents available from the CBS and CPC collections. Two phylogenies were generated. The first was based on 118 isolates (60 isolates from six regions in Senegal and 58 CBS strains) and the *Botryosphaeria dothidea* outgroup sequence (ITS, TEF-1α and ACT; Figure 3). The second phylogeny was based on 60 isolates (60 isolates from six regions in Senegal) and the *Botryosphaeria dothidea* outgroup sequence (all five loci; data not shown, alignment and tree available in TreeBASE).

The first analysis (combined ITS, TEF-1α and ACT alignment) is based on 119 isolates (including the outgroup sequence) and the resulting dataset of 1047 characters, including alignment gaps which are treated as fifth base, consisted of 879 constant characters, 123 variable parsimony-uninformative characters and 45 parsimony-informative characters. The maximum of 1,000 equally most parsimonious trees were obtained (TL = 189; CI = 0.931; RI = 0.979; RC = 0.911; HI = 0.069, of which the first tree is presented in Figure 3. The second analysis (combined 5-gene alignment) is based on 61 isolates (including the outgroup sequence) and the resulting dataset of 2,171 characters, including alignment gaps which are treated as fifth base, consisted of 1,842 constant characters, 262 variable parsimony-uninformative characters and 67 parsimony-informative characters. Ninety equally most parsimonious trees (TL = 341; CI = 0.988; RI = 0.995; RC = 0.983; HI = 0.012) were obtained and the resulting phylogenetic tree confirms the topology obtained from the 3-gene phylogeny (data not shown, available from TreeBASE).

In the phylogenetic tree (Figure 3), *Macrophomina* isolates are divided into two main clades that are treated below as two distinct species. The first main clade has a bootstrap support value (BS) of 98% and contains the majority of *Macrophomina* strains, including all the CBS and older CPC strains used in this study. Although some clustering of strains occurs in this main clade, e.g. the two strains from USA (CBS 270.34 and CBS 271.34), bootstrap support values are generally low and not significant at species level. The strain from Italy (CBS 205.47), designated below as ex-epitype culture of *M. phaseolina*, clusters in this clade and therefore all the strains in this main clade constitute the real *M. phaseolina*. The second main clade (BS = 100%) contains 16 isolates, all of

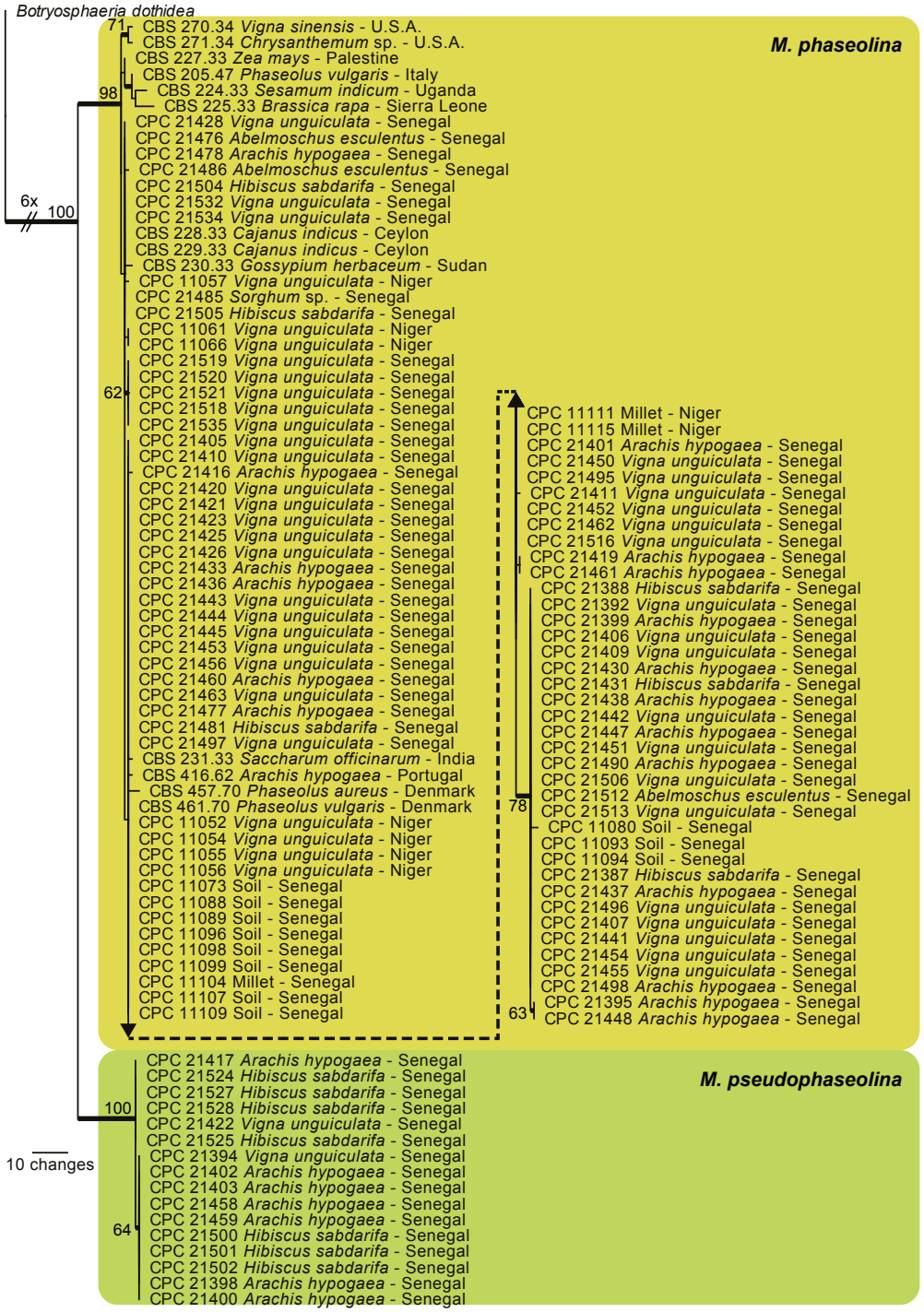

Figure 3. The first of 1,000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined three-gene (ITS, TEF-1α, ACT) sequence alignment. The scale bar shows ten changes, and bootstrap support values from 1,000 replicates are shown at the nodes. Culture collection numbers, substrate and country of origin are shown for each sequence, and coloured blocks indicate the two Macrophomina species. Branches present in the strict consensus tree are thickened and the tree was rooted to sequences of *Botryosphaeria dothidea* (obtained from the genome data available at: http://genome.jgi.doe.gov/Botdo1/Botdo1.home.html).

which were recently collected from diverse hosts from Senegal. This clade is phylogenetically distinct from the *M. phaseolina* clade in all analyses. A novel species is therefore introduced below for these distinct isolates.

Taxonomy

Macrophomina phaseolina (Tassi) Goid., *Annali Sper. agr. N.S.* **1**: 457 (1947)
Basionym: *Macrophoma phaseolina* Tassi, *Bull. Lab. Ort bot. Siena* **4**: 9 (1901)
= *Tiarosporella phaseoli* (Maubl.) Aa, *Verh. Kon. Ned. Akad. Wetensch., Sectie 2*, **68**: 4 (1977)
(Figure 4)
Additional synonyms listed by Holliday and Punithalingam (1988).

Sclerotia developing on PNA or in the water agar, black, smooth, hard, 100–400 µm diam. *Conidiomata* pycnidial, dark brown to black, solitary or gregarious, up to 300 µm diam., each opening by a central ostiole; wall multilayered, cells dark brown, thick-walled. *Conidiogenous cells* lining the inner surface of the conidioma, hyaline, short obpyriform to subcylindrical, proliferating several times percurrently near the apex, 6–12 × 4–6 µm; young conidiogenous cells covered by a mucous layer that extends over the apex of the developing conidium. *Conidia* ellipsoid to obovoid, (19–)22–26(–30) × (6–)8(–9) µm (av. 24 × 8 µm); immature conidia hyaline, enclosed in a mucous sheath, that upon dehiscence encloses the top half of the conidium, transformed into two lateral tentaculiform, apical mucoid appendages (type C; Nag Raj, 1993); mature conidia becoming medium to dark brown, each with a granular outer layer that in some cases appears pitted, without any mucoid appendages; conidial hilum frequently with marginal frills. *Microconidia* aseptate, hyaline, smooth, guttulate to granular, straight to curved, ellipsoid to subcylindrical to irregular, 5–8(–10) × 3–5 µm.

Culture characteristics. Colonies with even margins, and abundant, fluffy aerial mycelium. On PDA buff, turning vinaceous buff to pale olivaceous grey with dense, black sclerotial masses. Colonies after 2 d

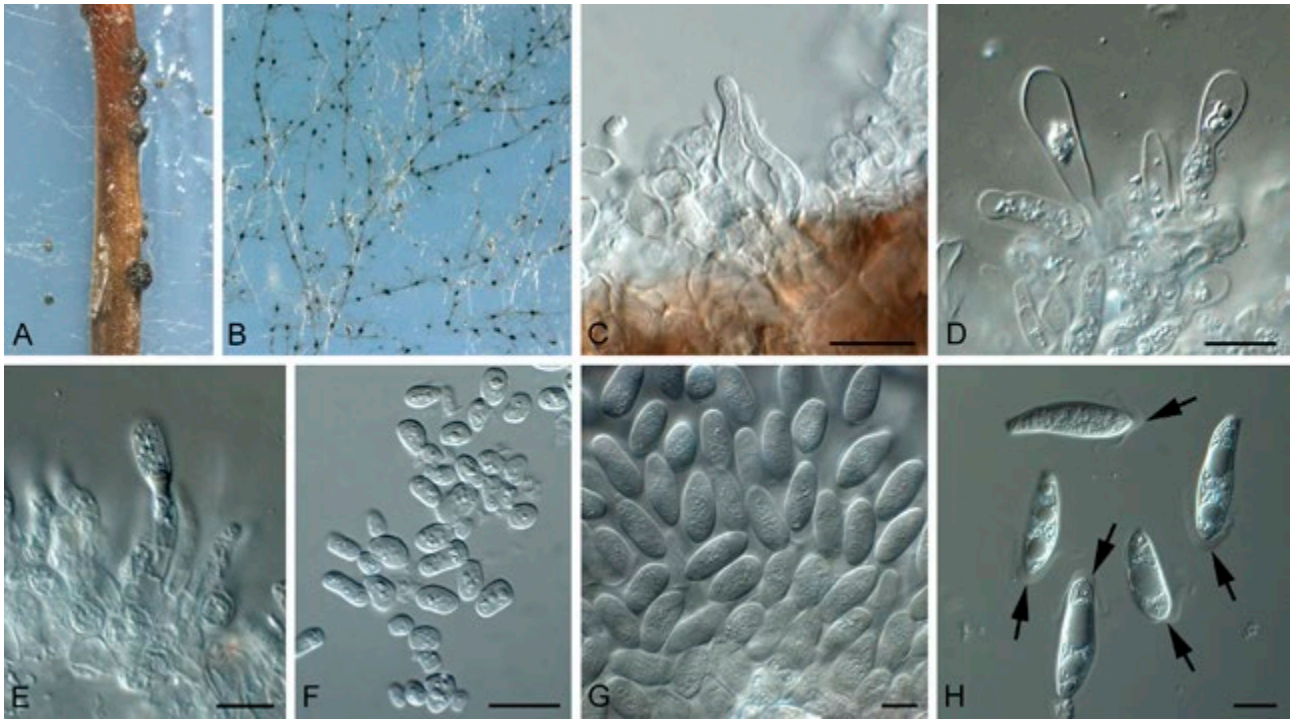

Figure 4. *Macrophomina phaseolina* (CBS 205.47). Pycnidia forming on pine needle agar. B. Sclerotia on tap water agar. C–E. Conidiogenous cells. F. Microconidia. G, H. Macroconidia with apical appendages (arrows). Scale bars = 10 µm.

not growing at 6 or 9ºC, growing 9–10 mm diam. at 12ºC, optimal growth at 30–36ºC (80 mm diam.), and still growing at 40ºC (25–40 mm diam.). On average growing faster than isolates of *M. pseudophaseolina*.

Specimen examined: **Italy**: Siena Botanical Garden, on leaves of *Phaseolus* sp., Sept. 1901, holotype Siena. Italy, on *Phaseolus vulgaris*, Mar. 1947, *G. Goidánich*, epitype designated here CBS H-21519, "MBT176946", culture ex-epitype CBS 205.47.

Macrophomina pseudophaseolina Crous, Sarr & Ndiaye, **sp. nov.**
MycoBank MB807468
(Figure 5)

Etymology: Named for morphological similarity to *M. phaseolina*.

Diagnosis. *Conidia* hyaline, smooth, granular to guttulate, ellipsoid to obovoid, (19–)20–24(–27) × (7.5–)8(–9) µm (av. 22 × 8 µm).

Sclerotia developing on PNA or in the water agar, black, smooth, hard, 100–400 µm diam. *Conidiomata* pycnidial, dark brown to black, glabrous, solitary or gregarious, globose to slightly elongated, up to 300 µm diam., each opening by a central ostiole; wall of five to six layers of dark brown, thick-walled *textura angularis*. *Conidiophores* reduced to conidiogenous cells or each with a supporting cell, hyaline, ampulliform, branched at base or not, 15–20 × 5–8 µm. *Conidiogenous cells* lining the inner surface of the conidioma, hyaline, subcylindrical, each proliferating several times percurrently near the apex, 8–15 × 3–4 µm; young conidiogenous cells each covered by a mucous layer that extends over the apex of the developing conidium. *Conidia* hyaline, smooth, granular to guttulate, ellipsoid to obovoid, widest in upper third, apices subobtuse, bases truncate, 2.5–3 µm diam., with minute marginal frills, (19–)20–24(–27) × (7.5–)8(–9) µm (av. 22 × 8 µm); immature conidia hyaline, each enclosed in a mucous sheath, that upon dehiscence encloses the top half of the conidium, transformed into two lateral tentaculiform, apical mucoid appendages (type C; Nag Raj, 1993); mature

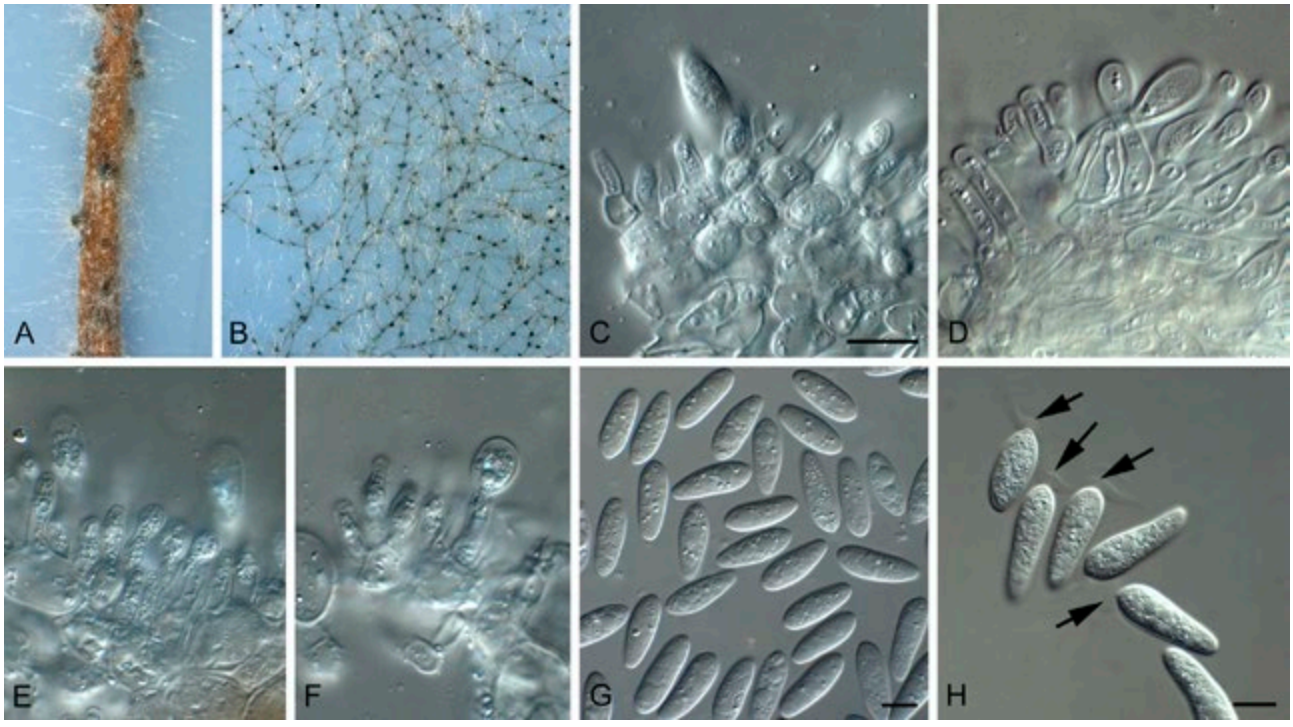

Figure 5. *Macrophomina pseudophaseolina* (CPC 21417). Pycnidia forming on pine needle agar. B. Sclerotia on tap water agar. C–F. Conidiogenous cells. G, H. Macroconidia with apical appendages (arrows). Scale bars = 10 µm.

conidia becoming medium to dark brown, without any mucoid appendages.

Culture characteristics. Colonies with even margins, and abundant, fluffy aerial mycelium. On PDA buff, turning vinaceous buff to pale olivaceous grey, with dense, black sclerotial masses. Colonies after 2 d not growing at 6 or 9ºC, growing 4–5 mm diam. at 12ºC, optimal growth at 30–36ºC (80 mm diam.), and still growing at 40ºC (10–15 mm diam.).

Specimen examined. **Senegal**: Louga, on *Arachis hypogaea*, 2011, *M.P. Sarr*, holotype CBS H-21518, culture ex-type CPC 21417 = CBS 137165.

Notes. Morphologically *M. phaseolina* is very similar to *M. pseudophaseolina*, except that conidia of the latter are somewhat shorter. These two species occur in the same regions and on the same hosts in Senegal. *Macrophomina pseudophaseolina,* however, is only known from Senegal, occurring on *Abelmoschus*, *Arachis*, *Hibiscus* and *Vigna*. ITS analysis indicated that the two species can be distinguished on three nucleotide duplications in the first internal transcribed spacers. In ACT there are two fixed nucleotide differences, and on CAL 15, TEF-1α 22, and TUB 15 fixed nucleotide differences (see 5-gene alignment in TreeBASE). However, the TEF-1α was problematic for obtaining a good consensus sequence, possibly due to the presence of an extra copy or pseudogene copy of the gene in the genome. All TEF-1α trace files used in this study were therefore carefully evaluated and judged against the data from the other loci. A frequent problem would be that the sequence in the one orientation would not match the one from the other direction, i.e. the one direction would be from *M. phaseolina* and the other from *M. pseudophaseolina*. Whether this is a remnant of the speciation event requires further investigation, preferably using whole genome sequence data from both species.

Discussion

The genus *Macrophomina* is based on the type species, *M. phaseolina*, which was originally described from *Phaseolus* collected in Italy. During the course of this study, *M. phaseolina* was resolved as a widely distributed pathogen occurring on a broad host range. Furthermore, one isolate in the collection, originating from *Phaseolus* in Italy (CBS 205.47), proved suitable as the epitype, thus helping to fix the genetic application of the name (Cannon *et al*., 2012). The concept of *Macrophomina* as employed by Phillips *et al.* (2013) in their recent treatment of the *Botryosphaeriaceae*, was correct, representing a genus distinct from *Tiarosporella*, a morphologically similar genus in the family (Crous *et al.*, 2006).

Macrophomina phaseolina has previously been considered as heterogeneous with regard to morphology, physiology, ecology and generic characteristics (Indera *et al*., 1986; Babu *et al*., 2010; Ndiaye *et al*., 2010). In the present study, a multigene analysis (ITS, TEF, ACT, CAL and TUB) representing a large sample of *Macrophomina* isolates from many hosts and geographical locations provided robust support for the identification of a new species from Senegal, named *M. pseudophaseolina*. The phylogenetic analysis clusters 16 strains from three hosts (*Arachis hypogaea*, *Hibiscus sabdarifa* and *Vigna unguiculata*) and four locations in Senegal (Podor, Saint-louis, Louga and Thiès) in the *M. pseudophaseolina* clade, and 102 strains from 12 countries and 17 hosts in the *M. phaseolina* clade. Very little is known about the distribution of *M. pseudophaseolina*, and although all isolates available to us originate from Senegal, it is possible that this fungus also has a wider distribution.

Based on the resulting three-gene tree (ITS, TEF, ACT) (Figure 3), isolates could not be allocated to specific groups according to host or geographic origins. Some isolates from the same host or location tended to group together (e.g. CPC 21527 and CPC 21528; CPC 21500, CPC 21501 and CPC 21502; CPC 21425 and CPC 21426). However, some strains from the same host or location separated into the two respective species, e.g. *M. phaseolina* (CPC 21399) and *M. pseudophaseolina* (CPC 21400). Therefore, no correlation was observed between hosts and geographic locations. Our results supported those of Vandemark *et al*. (2000), who concluded that based on AFLP analysis, it was not possible to correlate DNA polymorphisms with geographic location or host. Most other studies have also failed to find any associations in populations between DNA genotypes and host origins (Almeida *et al.*, 2003; Reyes-Franco *et al*., 2006). Jana *et al.* (2003), however, used one specific RAPD primer (OPA-13), with which it was possible to distinguish *M. phaseolina* isolates from soybean, sesame, groundnut, chickpea, cotton, common bean, and other hosts. These differences were at population level, however, and not indicative of species.

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