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## Molecular phylogeny of *Phoma* and allied anamorph genera: Towards a reclassification of the *Phoma* complex

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### ABSTRACT

The present generic concept of *Phoma* is broadly defined, with nine sections being recognised based on morphological characters. Teleomorph states of *Phoma* have been described in the genera *Didymella*, *Leptosphaeria*, *Pleospora* and *Mycosphaerella*, indicating that *Phoma* anamorphs represent a polyphyletic group. In an attempt to delineate generic boundaries, representative strains of the various *Phoma* sections and allied coelomycetous genera were included for study. Sequence data of the 18S nrDNA (SSU) and the 28S nrDNA (LSU) regions of 18 *Phoma* strains included were compared with those of representative strains of 39 allied anamorph genera, including *Ascochyta*, *Coniothyrium*, *Deuterophoma*, *Microsphaeropsis*, *Pleurophoma*, *Pyrenochaeta*, and 11 teleomorph genera. The type species of the *Phoma* sections *Phoma*, *Phyllostictoides*, *Sclerophomella*, *Macrospora* and *Peyronellaea* grouped in a subclade in the *Pleosporales* with the type species of *Ascochyta* and *Microsphaeropsis*. The new family *Didymellaceae* is proposed to accommodate these *Phoma* sections and related anamorph genera. The present study demonstrated that *Phoma radicina*, the type species of *Phoma* sect. *Paraphoma* and *Phoma heteromorphospora*, the type species of *Phoma* sect. *Heterospora* can be assigned to the *Phaeosphaeriaceae* and *Leptosphaeriaceae* respectively.

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### Introduction

Species belonging to the genus *Phoma* and related coelomycetes are often encountered as serious plant pathogens. For appropriate morphological identifications in these genera *in vitro* studies are essential and the use of conidiogenesis as a taxonomic criterion is a main feature for the present generic delimitation of coelomycetes (Hughes 1953; Sutton 1964, 1977, 1980). Numerous pycnidial-producing species in the genus *Phoma* were reclassified and many synonyms were found after

comparing herbarium material with *in vitro* characters of fresh isolates in the last decades. In these studies, other morphologically related anamorphic genera such as *Pyrenochaeta*, *Asteromella* and *Phomopsis* were also involved. In spite of a concerted effort, however, various *Phoma* names still need to be revised (van der Aa *et al.* 1990).

Results of an extensive study of *Phoma* taxonomy based on *in vitro* characters over the past 40 y were summarised in the recently published *Phoma* Identification Manual (Boerema *et al.* 2004). A total of 223 specific and infra-specific taxa of

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*Phoma* were classified in nine *Phoma* sections, namely *Phoma*, *Heterospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Sclerophomella*, *Plenodomus*, *Macrospora* and *Pilosa* (Boerema 1997; Boerema et al. 2004). Furthermore, these *Phoma* sections have teleomorph relations described in the genera *Didymella*, *Mycosphaerella*, *Leptosphaeria* and *Pleospora* (Boerema 1997), indicating that *Phoma* anamorphs represent a polyphyletic group. Several additional teleomorphs have over the years been linked to *Phoma*, but in general, these lack any conclusive evidence (Aveskamp et al. 2008).

The classification of species in *Phoma* and allied genera is still controversial. Confusing characters are reported among several *Phoma* sections and related genera such as *Ascochyta*, *Asteromella*, *Microsphaeropsis*, *Phomopsis*, *Phyllosticta*, *Pleurophoma*, *Pyrenochaeta* and *Stagonospora*. In some instances, the *Phoma* sections have been considered ambiguous, because of shared characters among the various sections, and a rejection of the hypothesis of convergent development (Punithalingam 2004). Furthermore, it has been suggested that the *Phoma* classification system would be improved by adding DNA phylogenetic data, and delineating more natural groups (Grondona et al. 1997; Torres et al. 2005).

To circumscribe *Phoma* and allied genera by means of a molecular phylogenetic study, genera classified in the suborder *Phialoptycniidiineae* (*Phialidales*, *Enteroblastomycetidae*) (Sutton 1980) should be reevaluated. The *Phialoptycniidiineae* is characterised by simple, thin-walled pycnidia, aseptate conidia and ampulliform phialides or separate conidiophores. However, Sutton (1980) applied a wider concept, including thick-walled pycnidia, septate, pigmented conidia and filiform conidiophores. The suborder *Phialoptycniidiineae* includes 55 genera, such as *Phoma* and most genera that have often been confused with it, including *Ascochyta*, *Asteromella*, *Microsphaeropsis*, *Pleurophoma* and *Pyrenochaeta*. Representative strains of 26 genera of this suborder were available for examination and thus included, as well as species of other misinterpreted genera such as *Coniothyrium*, *Stagonospora* and *Phyllosticta*. In the present study, the status of the genus *Phoma* and related genera, as well as teleomorph associations are studied by means of DNA phylogenetic analyses. Sequence data of the 18S (SSU) and the 28S (LSU) nrDNA regions of 15 representative strains of the type species of the *Phoma* sections (Boerema et al. 2004) and three reference strains of *Phoma* were compared with those of 93 strains representing 39 anamorph and 11 teleomorph genera. Type species of the genera and ex-type strains were selected as far as possible. These results were compared with related molecular data, as well as morphological and ecological information. Finally, the anamorph–teleomorph relations within this complex are discussed, and a new family in the *Pleosporales* is proposed.

## Material and methods

### Strain selection, cultural studies and DNA extraction

Freeze-dried strains were obtained from the culture collections of Centraalbureau voor Schimmelcultures (CBS) and the Dutch National Reference Laboratory of the Plant Protection Service (PD). These included 15 strains representing the *Phoma*

sections, and 84 strains of related species (Table 1). Strains were revived overnight in 2 ml malt/pepton (50%/50%) liquid medium. Subsequently, the cultures were transferred and maintained on oatmeal agar (OA) (Gams et al. 2007). Morphological studies of the strains were performed on OA, malt agar and cherry-decoction agar as described in Boerema et al. (2004). DNA extraction was done using the Ultraclean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, California), according to the instructions of the manufacturer. All DNA extracts were diluted 10 times in milliQ water and stored at 4 °C before use.

### PCR and sequencing

The SSU region was amplified with the primers NS1 and NS4 (White et al. 1990) and the LSU region was amplified with the primers LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990). The PCRs were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) in a total volume of 12.5 µl. The PCR mixture contained 0.5 µl diluted genomic DNA, 0.2 µM of each primer, 0.5 Unit *Taq* polymerase E (Genaxxon Bioscience, Biberach, Germany), 0.04 µM (SSU) or 0.06 µM (LSU) dNTP's, 2 µM MgCl<sub>2</sub> and 1 × PCR buffer E incomplete (Genaxxon Bioscience). Conditions for amplification for both regions were an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of denaturation, annealing and elongation and a final elongation step of 7 min at 72 °C. For the SSU amplification, the 35 cycles consisted of 30 s at 94 °C, 50 s at 48 °C and 90 s at 72 °C; for the LSU 45 s at 94 °C, 45 s at 48 °C and 2 min at 72 °C. The PCR products were analysed by electrophoresis on a 1% (w/v) agarose gel containing 0.1 µg ml<sup>-1</sup> ethidium bromide in 1 × TAE buffer (0.4 M Tris, 0.05 M NaAc, 0.01 M EDTA, pH 7.85) and visualised under UV light. Hyperladder I (Bioline, Luckenwalde, Germany) was applied as size standard.

The PCR products were sequenced in both directions using the PCR primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's recommendations. The primer LR5 was used as an additional sequencing primer for the LSU (Vilgalys & Hester 1990). The sequence products were purified using Sephadex G-50 Superfine (Amersham Biosciences, Roosendaal, Netherlands) and analysed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the manufacturer's instructions. Consensus sequences were computed from forward and reverse sequences using the BioNumerics v4.60 software package (Applied Maths, Sint-Martens-Latem, Belgium).

### Phylogenetic analyses

The obtained sequence data were aligned with 13 reference sequences that were obtained from public databases (Table 1) using the same BioNumerics software. Where necessary, manual adjustments for improvement were made by eye. The phylogeny was rooted to *Saccharomyces cerevisiae*, strain S228C. The phylogenetic analyses were done for each data set separately, as well as with a combined alignment consisting of both the SSU and LSU regions.

A Neighbour-Joining (NJ) distance analysis was conducted using PAUP (Phylogenetic Analysis Using Parsimony) v4b10

Table 1 – Fungal isolates included for SSU and LSU sequences analysis (alphabetic order of anamorph names)

Species name (anamorph/teleomorph)	Strain Nr	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
<i>Allantophoma endogenospora</i>	CBS 178.79		EU754026	EU754125	<i>Cedrus atlantica</i> (Pinaceae)	Netherlands
	CBS 600.76		EU754027	EU754126	<i>Vriesea</i> sp. (Bromeliaceae)	Netherlands
<i>Ampelomyces quercinus</i>	CBS 633.92, ATCC 36786		EU754028	EU754127	Unknown	Unknown
<i>Ampelomyces quisqualis</i> (TS*)	CBS 129.79		EU754029	EU754128	mildew on <i>Cucumis sativus</i>	Canada
	CBS 131.79		EU754030	EU754129	mildew on <i>Cucumis sativus</i>	Canada
<i>Aposphaeria populina</i>	CBS 543.70		EU754031	EU754130	<i>Populus canadensis</i> (Salicaceae)	Netherlands
<i>Ascochyta caulina</i> , teleom. <i>Pleospora calvescens</i>	CBS 246.79, PD 77/655		EU754032	EU754131	<i>Atriplex hastata</i> (Chenopodiaceae)	Germany
	CBS 344.78, PD 68/682		EU754033	EU754132	<i>Atriplex hastata</i> (Chenopodiaceae)	Netherlands
<i>Ascochyta fabae</i> , teleom. <i>Didymella fabae</i>	CBS 524.77		EU754034	EU754133	<i>Phaseolus vulgaris</i> (Leguminosae)	Belgium
<i>Ascochyta hordei</i> var. <i>hordei</i>	CBS 544.74		EU754035	EU754134	<i>Triticum aestivum</i> (Gramineae)	South Africa
<i>Ascochyta pinodes</i> , teleom. <i>Didymella pinodes</i>	CBS 374.84, PD 79/674		EU754036	EU754135	<i>Pisum sativum</i> (Leguminosae)	Netherlands
	CBS 525.77		EU754037	EU754136	<i>Pisum sativum</i> (Leguminosae)	Belgium
<i>Ascochyta pisi</i> (TS)	CBS 126.54		EU754038	EU754137	<i>Pisum sativum</i> (Leguminosae)	Netherlands
	CBS 122785, PD 78/517		EU754039	EU754138	<i>Pisum sativum</i> (Leguminosae)	Netherlands
<i>Asteromella tiliae</i>	CBS 265.94		EU754040	EU754139	<i>Tilia platyphyllos</i> (Tiliaceae)	Austria
<i>Chaetasbolisia erysiphoides</i> (TS)	CBS 148.94		EU754041	EU754140	Unknown	Unknown
<i>Chaetoconis polygoni</i> (TS)	CBS 405.95		EU754042	EU754141	<i>Polygonum sachalinense</i> (Polygonaceae)	Netherlands
<i>Chaetodiplodia</i>	CBS 568.88		EU754043	EU754142	Rock	Israel
<i>Chaetophoma</i>	CBS 119963		EU754044	EU754143	Man	Brasil
<i>Chaetosphaeronema hispidulum</i> (TS)	CBS 216.75		EU754045	EU754144	<i>Anthyllis vulneraria</i> (Leguminosae)	Germany
	CBS 826.88		EU754046	EU754145	Soil	Israel
<i>Cochliobolus sativus</i> (teleom.)	DAOM 226212 (R*)	1271	DQ677995	DQ678045	(Graminae)	Unknown
<i>Coleophoma crateriformis</i> (TS)	CBS 473.69		EU754047	EU754146	<i>Phillyrea angustifolia</i> (Oleaceae)	Spain
<i>Coleophoma maculans</i>	CBS 896.69		EU754048	EU754147	<i>Populus balsamifera</i> (Salicaceae)	Netherlands
<i>Coleophoma oleae</i>	CBS 615.72, ATCC 24520, DSM 62123		EU754049	EU754148	<i>Olea europaea</i> (Oleaceae)	Greece
<i>Coniella fragariae</i>	CBS 167.84		EU754050	EU754149	<i>Vitis vinifera</i> (Vitaceae)	Unknown
	CBS 198.82		EU754051	EU754150	Soil sample, vine orchard	France
<i>Coniothyrium cerealis</i>	CBS 122787, PD 0703486691		EU754052	EU754151	Unknown	Germany
<i>Coniothyrium concentricum</i>	CBS 589.79		EU754053	EU754152	<i>Yucca</i> (Agavaceae)	Netherlands
<i>Coniothyrium palmarum</i> (TS)	CBS 400.71		EU754054	EU754153	<i>Chamaerops humilis</i> (Palmae)	Italy
	CBS 758.73		EU754055	EU754154	<i>Phoenix dactylifera</i> (Palmae)	Israel
<i>Didymella exigua</i> (teleom.)	CBS 183.55		EU754056	EU754155	<i>Rumex arifolius</i> (Polygonaceae)	France
<i>Diplodia pinea</i>	CBS 109726		EU754057	EU754156	<i>Pinus patula</i> (Pinaceae)	South Africa
	CBS 393.84		EU754058	EU754157	<i>Pinus nigra</i> (Pinaceae)	Netherlands
<i>Diplodina coloradensis</i>	CBS 138.25		EU754059	EU754158	<i>Senecio</i> sp. (Compositae)	Unknown
<i>Diplodina microsperma</i> teleom. <i>Cryptodiaporthe salicella</i>	CBS 110159		EU754060	EU754159	<i>Salix</i> sp. (Salicaceae)	Netherlands
<i>Dothidea insculpta</i> (teleom.)	CBS 189.58 (R)	921	DQ247810	DQ247802	<i>Clematis vitalba</i> (Ranunculaceae)	France
<i>Dothiora cannabinae</i> (teleom.)	CBS 737.71 (T* R)	1359	DQ479933	DQ470984	<i>Daphne cannabina</i> (Thymelaeaceae)	India
<i>Dothiorella ulmi</i>	CBS 172.34, ATCC 22376, IMI 045826		EU754061	EU754160	<i>Ulmus</i> sp. (Ulmaceae)	USA
<i>Eleutheromyces subulatus</i> (TS)	CBS 139.90		EU754062	EU754161	(Russulaceae)	Canada
	CBS 458.88		EU754063	EU754162	<i>Lactarius scrobiculatus</i> (Russulaceae)	Germany
<i>Godronia urceolus</i> (teleom.)	CBS 110435		EU754064	EU754163	<i>Betula pendula</i> (Betulaceae)	UK, Scotland
	CBS 215.58		EU754065	EU754164	<i>Betula</i> (Betulaceae)	Norway

<i>Guignardia citricarpa</i> (teleom.)	CBS 102373		EU754066	EU754165	<i>Citrus aurantium</i> (Rutaceae)	Netherlands
<i>Leptosphaerulina australis</i> (teleom.) (TS)	CBS 317.83		EU754067	EU754166	<i>Eugenia aromatica</i> (Myrtaceae)	Indonesia
	CBS 939.69		EU754068	EU754167	Soil	Netherlands
<i>Macrophomina phaseolina</i>	CBS 121.82		EU754069	EU754168	<i>Sesamum indicum</i> (Pedaliaceae)	Sweden
	CBS 460.70, IMI 147232		EU754070	EU754169	<i>Glycine max</i> (Leguminosae)	Denmark
<i>Microsphaeropsis olivacea</i> (TS)	CBS 116669		EU754071	EU754170	<i>Sarothamnus scoparius</i> (Leguminosae)	Netherlands
	CBS 442.83		EU754072	EU754171	<i>Taxus baccata</i> (Taxaceae)	Netherlands
<i>Mycosphaerella punctiformis</i> (teleom.)	CBS 113265 (T R)	942	AY490775	DQ470920	<i>Quercus robur</i> (Fagaceae)	Netherlands
<i>Neottiosporina paspali</i>	CBS 331.37		EU754073	EU754172	<i>Paspalum notatum</i> (Gramineae)	USA, Florida
<i>Ophiosphaerella herpotricha</i>	CBS 240.31, ATCC 12279 (R)	1595	DQ767650	DQ767656	Unknown	France
<i>Paraconiothyrium minitans</i>	CBS 122788, PD 07 03486739		EU754074	EU754173	Unknown	United Kingdom
	CBS 122786, PD 99/1064-1		EU754075	EU754174	Unknown	Unknown
<i>Phaeosphaeria nodorum</i> (teleom.)	CBS 110109		EU754076	EU754175	<i>Lolium perenne</i> (Gramineae)	Denmark
<i>Phialophorophoma litoralis</i> (TS)	CBS 234.92		EU754077	EU754176	<i>Olea europaea</i> (Oleaceae)	Italy
	CBS 297.74		EU754078	EU754177	Sea water	Serbia and Montenegro
<i>Phoma betae</i> , teleom. <i>Pleospora betae</i>	CBS 109410, PD 77/113		EU754079	EU754178	<i>Beta vulgaris</i> (Chenopodiaceae)	Netherlands
	CBS 523.66, PD 66/270		EU754080	EU754179	<i>Beta vulgaris</i> (Chenopodiaceae)	Netherlands
<i>Phoma complanata</i>	CBS 268.92, PD75/3		EU754081	EU754180	<i>Anglica sylvestris</i> (Umbelliferae)	Netherlands
	CBS 100311		EU754082	EU754181	<i>Heracleum sphondylium</i> (Umbelliferae)	Netherlands
<i>Phoma cucurbitacearum</i> , teleom. <i>Didymella bryoniae</i>	IMI 373225 (R)		AY293779	AY293792	<i>Cucumis sativus</i> (Cucurbitaceae)	USA
<i>Phoma exigua</i> var. <i>exigua</i>	CBS 101150, PD 79/118		EU754083	EU754182	<i>Cichorium entybus</i> (Compositae)	Netherlands
	CBS 431.74, PD 74/2447		EU754084	EU754183	<i>Solanum tuberosum</i> (Solanaceae)	Netherlands
<i>Phoma glomerata</i>	CBS 528.66, PD 63/590		EU754085	EU754184	<i>Chrysanthemum</i> (Compositae)	Netherlands
	CBS 464.97		EU754086	EU754185	Sample bathroom	Netherlands
<i>Phoma herbarum</i> (TS)	CBS 615.75, PD 73/665, IMI 199779		EU754087	EU754186	<i>Rosa multiflora</i> (Rosaceae)	Netherlands
	ATCC 12569, IMI 049948 (R)		AY293778	AY293791	White lead paint	United Kingdom
<i>Phoma heteromorphospora</i>	CBS 448.68		EU754088	EU754187	<i>Chenopodium album</i> (Chenopodiaceae)	Netherlands
	CBS 115.96, PD 94/1576		EU754089	EU754188	<i>Chenopodium album</i> (Chenopodiaceae)	Netherlands
<i>Phoma lingam</i> , teleom. <i>Leptosphaeria maculans</i>	CBS 532.66, PD 65/911		EU754090	EU754189	<i>Brassica</i> sp. (Brassicaceae)	Netherlands
	DAOM 229267 (R)	277	DQ470993	DQ470946	<i>Brassica</i> sp. (Brassicaceae)	Unknown
<i>Phoma radicina</i>	CBS 102875, PD 78/1097		EU754091	EU754190	<i>Lycopersicon esculentum</i> (Solanaceae)	Germany
	CBS 111.79, PD 76/437, IMI 386094		EU754092	EU754191	<i>Malus sylvestris</i> (Rosaceae)	Netherlands
<i>Phoma zeae-maydis</i> , teleom. <i>Didymella zeae-maydis</i>	CBS 588.69 (T)		EU754093	EU754192	<i>Zea mays</i> (Gramineae)	USA, Wisconsin
<i>Phyllosticta abietis</i>	CBS 112067		EU754094	EU754193	<i>Abies concolor</i> (Pinaceae)	Canada
<i>Phyllosticta minima</i>	CBS 111635		EU754095	EU754194	<i>Acer rubrum</i> (Aceraceae)	USA
<i>Plectophomella visci</i>	CBS 122783, PD 74/1021		EU754096	EU754195	<i>Viscum album</i> (Viscaceae)	France
<i>Plenodomus fuscomaculans</i>	CBS 559.78, PD 78/241		EU754097	EU754196	<i>Malus sylvestris</i> (Rosaceae)	Japan
	CBS 116.16		EU754098	EU754197	<i>Malus</i> sp.	USA
<i>Pleurophoma cava</i>	CBS 115979		EU754099	EU754198	Unknown	Netherlands
	CBS 257.68, IMI 331911		EU754100	EU754199	Wheat-field soil	Germany
<i>Pleurophoma pleurospora</i> (TS)	CBS 101461		EU754101	EU754200	Man, cutaneous lesions	USA, Texas
<i>Pseudodiplodia</i> sp.	CBS 255.86		EU754102	EU754201	<i>Vitis vinifera</i> (Vitaceae)	Italy
<i>Pseudorobillarda phragmitis</i> (TS)	CBS 842.84		EU754103	EU754202	<i>Lolium perenne</i> (Gramineae)	Netherlands
	CBS 398.61, IMI 070678 (T)		EU754104	EU754203	<i>Phragmitis australis</i> (Gramineae)	United Kingdom
<i>Pyrenochaeta acicola</i>	CBS 122789, PD 0703486800		EU754105	EU754204	<i>Hordeum vulgare</i> (Gramineae)	Unknown
<i>Pyrenochaeta lycopersici</i>	CBS 306.65, DSM 62931 (T)		EU754106	EU754205	<i>Lycopersicon esculentum</i> (Solanaceae)	Germany
<i>Pyrenochaeta nobilis</i> (TS)	CBS 407.76 (T)		EU754107	EU754206	<i>Laurus nobilis</i> (Lauraceae)	Italy

(continued on next page)

Table 1 – (continued)						
Species name (anamorph/teleomorph)	Strain Nr	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
<i>Pyrenochaeta romeroi</i>	CBS 252.60, ATCC 13735 (T)		EU754108	EU754207	Maduromycosis in man	Venezuela
	CBS 122784, PD 84/1022		EU754109	EU754208	<i>Hordeum vulgare</i> (Gramineae)	Unknown
<i>Pyrenophora tritici-repentis</i> (teleom.)	OSC 100066 (R)	173	AY544716	AY544672	(Gramineae)	Italy
<i>Readeriella mirabilis</i> (TS)	CBS 116293, CPC 10506		EU754110	EU754209	<i>Eucalyptus fastigata</i> (Myrtaceae)	New Zealand
	CBS 358.64, IMI 108602		EU754111	EU754210	<i>Eucalyptus regnans</i> (Myrtaceae)	Australia
<i>Rhizosphaera pini</i> (TS)	CBS 189.26		EU754112	EU754211	Unknown	Netherlands
<i>Saccharomyces cerevisiae</i>	S 288C, ATCC 204508 (R)				Unknown	Unknown
<i>Selenophoma linicola</i>	CBS 468.48		EU754113	EU754212	<i>Linum usitatissimum</i> (Linaceae)	Canada
<i>Selenophoma mahoniae</i>	CBS 388.92		EU754114	EU754213	<i>Mahonia repens</i> (Berberidaceae)	USA, Colorado
<i>Sirococcus conigenus</i>	CBS 113.75		EU754115	EU754214	<i>Picea pungens</i> var. <i>glauca</i> (Pinaceae)	Germany
<i>Sphaeropsis visci</i> (TS)	CBS 100163		EU754116	EU754215	<i>Viscum album</i> (Viscaceae)	Luxembourg
	CBS 186.97		EU754117	EU754216	<i>Viscum album</i> (Viscaceae)	Germany
<i>Sporormiella minima</i> (teleom.)	CBS 524.50 (R)	1256	DQ678003	DQ678056	Dung of goat	Panama
<i>Stagonospora foliicola</i>	CBS 110111		EU754118	EU754217	<i>Phalaris arundinacea</i> (Gramineae)	USA, Maryland
<i>Stagonospora neglecta</i> var. <i>colorata</i>	CBS 343.86		EU754119	EU754218	<i>Phragmites australis</i> (Gramineae)	France
<i>Stenocarpella macrospora</i> (TS)	CBS 117560 (T)		EU754120	EU754219	<i>Zea mays</i> (Gramineae)	South Africa
	CBS 164.31		EU754121	EU754220	<i>Zea mays</i> (Gramineae)	Unknown
<i>Trematophoma</i> sp.	CBS 157.86		EU754122	EU754221	Soil	USA
<i>Trematosphaeria pertusa</i> (teleom.)	CBS 400.97 (R)	1589	DQ678020	DQ678072	<i>Fagus</i> sp. (Fagaceae)	Belgium
<i>Wojnowicia hirta</i> (TS)	CBS 160.73		EU754123	EU754222	<i>Triticum aestivum</i> (Gramineae)	Germany
	CBS 295.69		EU754124	EU754223	<i>Lolium multiflorum</i> (Gramineae)	Germany

T: ex-type strain; TS: type species of the genus; R: Sequence data reference strain.

(Swofford, 2003) with the uncorrected “p”, Jukes-Cantor and Kimura 2-parameter substitution models. The robustness of the trees obtained was evaluated by 1000 bootstrap replications. A Bayesian analysis was conducted with the MrBayes v3.1.2 programme (Huelsenbeck & Ronqvist 2001) using the default settings but with the following adjustments: GTR model with gamma-distributed rate variation in two parallel runs, model selected using Findmodel (<http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html>) for each data partition, and an MCMC heated chain with a “temperature” value of 0.05. The number of generations, sample frequencies and burn-in ratio were set at 5 M, 10 and 0.1 respectively and the run was automatically stopped as soon as the average standard deviation of split frequencies equalled 0.05. The resulting trees were printed with TreeView v1.6.6 (Page 1996) and alignments and trees are lodged in TreeBASE ([www.treebase.org](http://www.treebase.org)).

## Results

### DNA phylogeny

The aligned sequence length obtained for the SSU and LSU regions was 1545 (positions 1–1545 in the TreeBASE alignment) and 1634 (positions 1546–3180 in the TreeBASE alignment) nucleotide characters, respectively.

In the alignment, insertions in SSU at the positions 445–1028 were only observed for the species *Neottiosporina paspali* (syn. *Stagonospora paspali*) (CBS 331.37), *Chaetosphaeroma hispidulum* (CBS 216.75), *Plenodomus fuscomaculans* (CBS 559.78), *Wojnowicia hirta* (CBS 295.69), *Phialophorophoma litoralis* (CBS 234.92), *Ophiosphaerella herpotricha* (= *Ophiobolus herpotrichus*) (CBS 240.31), *Pseudodiplodia* sp. (CBS 255.86) and *Stenocarpella macrospora* (CBS 117560), and these insertions were excluded from the phylogenetic analyses. The insertions were comparable, except that of *S. macrospora*, showing a longer, poorly alignable fragment. Also an insertion in the LSU region at positions 2399–2764 – that was only observed in *Godronia urceolus* (CBS 215.58) – was excluded from the analyses. The combined data set used for the analyses contained 2230 characters, which consisted of 111 taxa including the outgroup taxon. Of these 2230 characters, 212 and 423 unique site patterns were present for SSU and LSU respectively.

The analysis run in MrBayes resulted in 7920 trees after 881000 generations, from which the consensus tree and posterior probabilities were calculated. The PAUP NJ analyses with the three substitution models showed similar tree topologies, and were congruent with those obtained with the Bayesian analysis. For the individual SSU and LSU alignments, the obtained trees were compared by eye and the tree topology of the individual data sets was similar to each other and to the tree obtained from the combined alignment. The phylogenetic tree based on the combined LSU and SSU sequence data calculated with MrBayes (Fig 1) showed the highest branch support values and branched into three main clades and multiple subclades, which are named A (1–11), B (1–2) and C (1–2). These clades and subclades are discussed below.

### The Pleosporales and Botryosphaerales

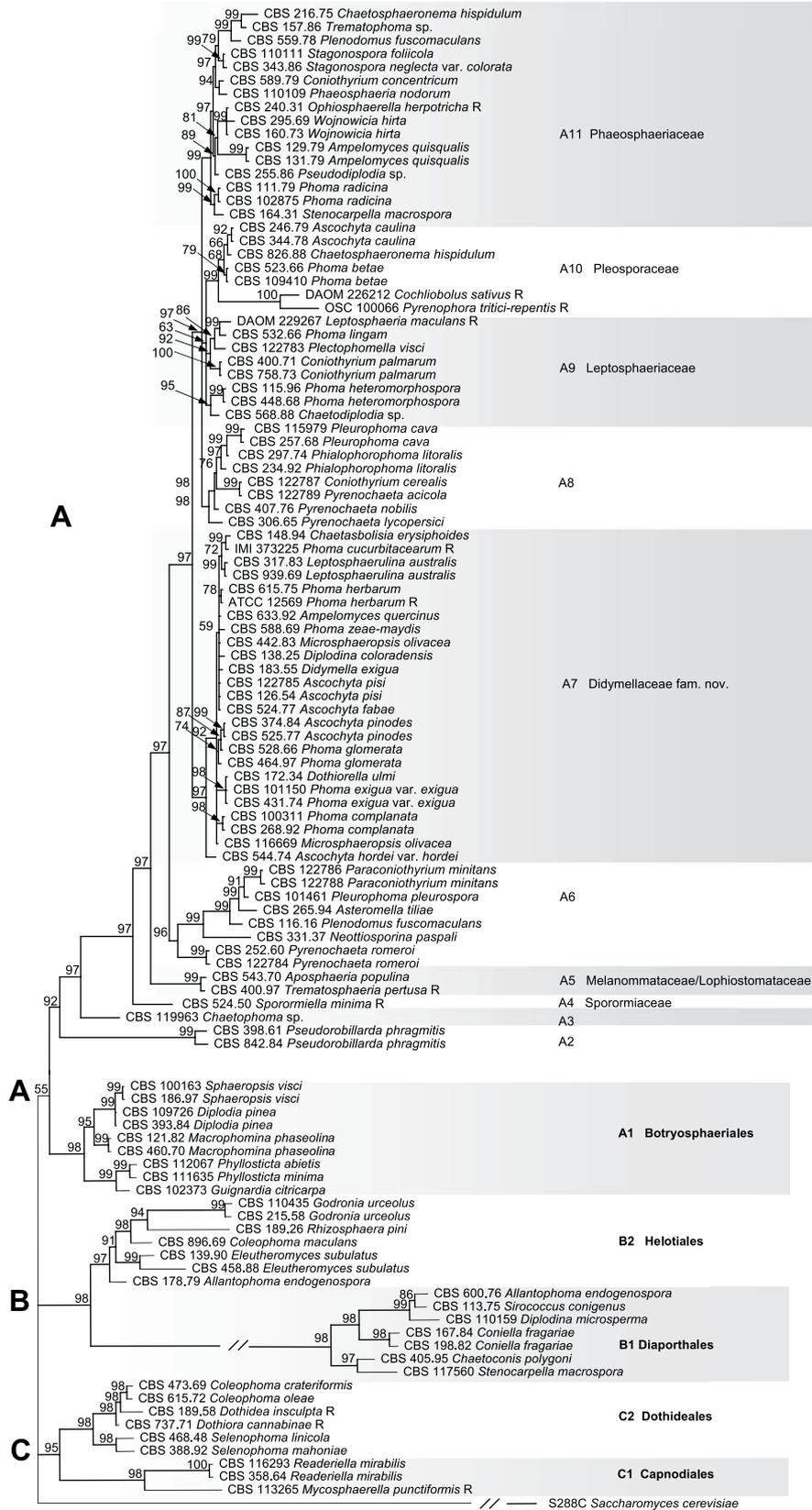
The representative strains of the type species of the nine *Phoma* sections clustered all in clade A. This clade could be mainly assigned to the Pleosporales, subclades A4–A11, characterised by the reference strains *Cochliobolus sativus* (DAOM 226212, AFToL 271), *Sporormiella* (= *Preussia*) *minima* (CBS 524.50, AFToL 1256), *Leptosphaeria maculans* (DAOM 229267, AFToL 277), *Ophiosphaerella herpotricha* (CBS 240.31, AFToL 1595), *Pyrenophora tritici-repentis* (OSC 100066, AFToL 173) and *Trematosphaeria pertusa* (CBS 400.97, AFToL 1589) (Schoch et al. 2006). Subclade A1 represents the order Botryosphaerales, including three strains of the genus *Guignardia* and its anamorph *Phyllosticta* (CBS 102373, 112067, 111635), and two strains of *Macrophomina phaseolina* (CBS 460.70, 121.80). These results agreed with the clade recognised as the Botryosphaerales (Schoch et al. 2006) based on *Guignardia bidwellii* (CBS 237.48, AFToL 1618), *Guignardia gaultheriae* (CBS 447.70, AFToL 1784) and *M. phaseolina* (CBS 227.33, AFToL 1783). Both strains of *Sphaeropsis visci* (TS)<sup>1</sup> (CBS 186.97, 100163) and *Diplodia pinea* (CBS 393.84, 109726) were also found to cluster in this subclade, the latter in agreement with a study among Botryosphaeria species based on ITS and 5.8S rDNA sequences (Zhou & Stanosz 2001) and LSU sequences (Crous et al. 2006). Both strains of *Pseudorbillarda phragmitis* (TS) (CBS 398.61 (T)<sup>2</sup>, CBS 243.78) resided in subclade A2. No teleomorph connections are known in this genus (Vujanovic & St-Arnaud 2003). Strain CBS 119963, identified as *Chaetophoma* sp. (ex-man), was found as single strain in subclade A3. The coprophilous species *Sporormiella minima* (CBS 524.50) Sporormiaceae, subclade A4, belongs to the Pleosporales (Barr 2002), and is related with *Westerdykella cylindrica* isolated from cow dung (Schoch et al. 2006). The close relation of both genera agrees with a report of Krays et al. (2006).

Subclade A5 consisted of the reference strain *T. pertusa* (CBS 400.97), neotype species of the genus *Trematosphaeria*, and *Aposphaeria populina* (CBS 543.70), both isolated from woody hosts. The *in vitro* characters of both strains were similar. These results are in congruence with the report of an aposphaeria-like anamorph of *T. pertusa* obtained in culture (Boise 1985). Both species belong to the Pleosporales. *T. pertusa* was included in a molecular phylogenetic study and has been classified in the Lophiostomataceae (Schoch et al. 2006).

In subclade A6 four well-supported groups could be recognised. Both strains of *Pyrenochaeta romeroi* (CBS 252.60 (T), CBS 122784) represented a distinct subclade, only distantly related with *Pyrenochaeta nobilis* (TS) (CBS 407.76, subclade A8). This finding agrees with an earlier interpretation of *P. romeroi* as surely not belonging to *Pyrenochaeta* (Schneider 1979). *Paraconiothyrium minitans* (CBS 122786, 122788) represented the *Paraconiothyrium/Paraphaeosphaeria* cluster (Verkley et al. 2004a; Damm et al. 2008). Strain CBS 101461, identified as representing *Pleurophoma pleurospora* (TS), was also found in this subclade A6. It is possible, however, that this strain, obtained from human cutaneous lesions (Dooley et al. 1989), does not represent the genus *Pleurophoma* s.str. The typical conspicuous filiform septate conidiophores, with conidial formation just

<sup>1</sup> (TS): Type species of the genus.

<sup>2</sup> (T): Ex-type strain.



**Fig 1** – The phylogenetic relationships of *Phoma* and allied genera based on the strict consensus tree from a Bayesian analysis of 111 LSU/SSU sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted with *Saccharomyces cerevisiae* S288C). Clades and subclades are indicated to the left and the right of the tree, respectively. R: Sequence data reference strain.

below the transverse septa were not observed, and the conidiogenous cells were more or less globose to occasionally elongated. It is possible that this strain could have degenerated over time. However, the initially hyaline, smooth-walled conidia, quickly discolouring to olivaceous, resemble those observed in the genus *Paraconiothyrium* (Verkley et al. 2004a). Moreover, the genus *Pleurophoma* mostly includes (opportunistic) plant pathogens on mainly woody hosts, represented here by *Pleurophoma cava*, which is embedded in subclade A8.

Remarkable was the finding of *Asteromella tiliae* (CBS 265.94) in subclade A6 in the *Pleosporales*. The genus *Asteromella* is generally considered as a spermatial state of *Mycosphaerellaceae* (*Capnodiales*) (Vanev & van der Aa, 1998). The connection of *A. tiliae* with the teleomorph *Didymosphaeria petrakiana*, *Didymosphaeriaceae*, *Dothideales* has been demonstrated (Butin & Kehr 1995). Two other sterile strains, *Neottiosporina paspali* (CBS 331.37) and *Plenodomus fuscomaculans* (CBS 116.16), which both originated from the USA, could also be found in this subclade. A teleomorph relation of the genus *Neottiosporina* is unknown. It is likely that *P. fuscomaculans* does not belong to the genus *Plenodomus* if compared with the clustering of *Phoma lingam*, the type species of *Phoma* sect. *Plenodomus* in subclade A9.

Subclade A7 included the type species of five out of the nine *Phoma* sections: *P. herbarum* (sect. *Phoma*), *P. exigua* var. *exigua* (*Phyllostictoides*), *P. glomerata* (*Peyronellaea*), *P. complanata* (*Sclerophomella*) and *P. zaeae-maydis* (*Macrospora*). Within this subclade, also two *Didymella* strains, *Didymella exigua* (CBS 183.55), type species of the genus *Didymella* and the reference strain *Didymella bryoniae* (IMI 373225) anam. *Phoma cucurbitacearum* could be found, the latter in congruence with observations made by Reddy et al. (1998). *Ascochyta pisi* (TS) (CBS 126.54, 122786), *Microsphaeropsis olivacea* (TS) (CBS 442.83, 116.669) and *Leptosphaerulina australis* (TS) (CBS 939.69, 317.83) also clustered in this subclade, which can be characterised as a '*Didymella* clade'. The finding of the teleomorph *Leptosphaerulina* grouping with *Didymella* supports the earlier report of *D. bryoniae* as being closely related with *Leptosphaerulina chartarum* and *Leptosphaerulina crassiasca* (Silva-Hanlin & Hanlin 1999). Furthermore, *Ampelomyces quercinus* (CBS 633.92) fitted in this subclade A7, consistent with data previously reported based on rDNA ITS studies (Szentiványi et al. 2005). *A. quercinus* represents faster-growing pycnidial isolates obtained from powdery mildew colonies, and does not belong to *Ampelomyces* (Szentiványi et al. 2005). Both strains of *Ampelomyces quisqualis* (TS) (CBS 129.79, 131.79) were located in subclade A11. The molecular data and morphological features of isolate *Dothiorella ulmi* (CBS 172.34) showed similarity with *Phoma exigua* var. *exigua*. *Chaetosporia erysiphoides* (TS), strain CBS 148.94, also belonged to this subclade A7.

In subclade A8, *Pleurophoma cava* (CBS 257.68, 115979) proved to be closely related with *Phialophorophoma litoralis* (CBS 297.74, 234.92), the type species of the monotypic genus *Phialophorophoma*. The teleomorphs of both genera are unknown. Two closely related isolates, maintained at the PD as *Coniothyrium cerealis* (CBS 122787; sterile) and *Pyrenochaeta acicola* (CBS 122789) grouped in this subclade. *P. acicola* is considered to be a synonym of *Phoma leveillei*. However, this isolate showed characteristics resembling the genus *Pyrenochaeta*, producing pycnidia with setae (as well as mycelial

hairs), and branched, filiform, septate conidiogenous cells. These characters supported its close relation with *P. nobilis* (TS) (CBS 407.76, ex-neotype) and *Pyrenochaeta lycopersici* (CBS 306.65, ex-isotype).

Subclade A9 included *Phoma lingam* (CBS 532.66 and reference strain DAOM 229267) (Schoch et al. 2006), type species of *Phoma* sect. *Plenodomus*, teleom. *L. maculans*, and *Coniothyrium palmarum* (TS) (CBS 400.71, 758.73). Also the genus *Coniothyrium* is linked to *Leptosphaeria* (Kirk et al. 2008). Other species in this subclade with unknown teleomorphs were a strain identified as *Chaetodiplodia* sp. (CBS 568.88) and *Plectophomella visci* (TS) (CBS 122783). The finding of *Phoma heteromorphospora* (CBS 448.68, 115.96), the type species of *Phoma* sect. *Heterospora* in this subclade was remarkable.

Subclade A10 included two important pathogens on *Chenopodiaceae*, viz. *Phoma betae* (CBS 523.66, 109410) type species of *Phoma* sect. *Pilosa*, teleom. *Pleospora betae*, and *Ascochyta caulina* (CBS 344.78, 246.79), teleom. *Pleospora calvescens*, a mycoherbicide against *Chenopodium album*. Both species proved to be closely related as earlier suggested (Boerema 1984). Strain CBS 826.88, isolated from soil and identified as *Chaetosphaeronema hispidulum*, proved to be different from the typical strain of this necrotroph, CBS 216.75 (subclade A11), collected by R. Schneider from *Anthyllis vulneraria*. Moreover, this strain showed the typical pilose pycnidia, characteristic of *P. betae* and *A. caulina*. The reference strains *C. sativus* (DAOM 226212) and *P. tritici-repentis* (AY 545672) (Schoch et al. 2006) showed to be more distantly related in this subclade.

*Phoma radicina* (CBS 111.79, 102875) type species of *Phoma* sect. *Paraphoma*, and often associated with monocotyledonous plants as a saprobe, fitted in a large subclade A11, including species that are classified in the *Phaeosphaeriaceae*. This subclade included several pathogens on *Gramineae*, such as *O. herpotricha* (CBS 240.31), *Phaeosphaeria nodorum* (CBS 110109), *Stagonospora foliicola* (CBS 110111), *Stagonospora neglecta* var. *colorata* (CBS 343.86) and *Wojnowicia hirta* (TS) (CBS 295.69, 160.73). Other species involved in subclade A11 were *A. quisqualis* (TS) (CBS 129.79, 131.79) isolated from mildew on *Cucumis sativus* and *Coniothyrium concentricum* (CBS 589.79), a specific pathogen on *Yucca* spp. The general culture characteristics of a sterile strain CBS 559.78, *P. fuscomaculans*, differed from those of the representative strain CBS 116.16, subclade A6. Another sterile strain CBS 164.31, *Stenocarpella macrospora*, showed different cultural characters and sequence data of those of the epitype strain CBS 117560, subclade B1. Therefore, the original identification of both sterile strains is not correct and should be revised.

### The Diaporthales and Helotiales

Clade B included two subclades, subclade B1 represented the *Diaporthales*, analogous with literature data, such as *Coniella fragariae* (CBS 167.84, 198.82) (Castlebury et al. 2002; van Niekerk et al. 2004), *Stenocarpella macrospora* (CBS 117560, TS) (Crous et al. 2006), *Sirococcus conigenus* (Konrad et al. 2007; Rossman et al. 2008) and *Diplodina microsperma* (teleom. *Cryptodiaporthe salicella*) (Green & Castlebury 2007). *Chaetoconis polygoni*, anamorph of the genus *Ceriospora*, has been classified in the *Xylariales*, but has unclear affinities (Kirk et al. 2008). The

data presented here suggest this species has to be placed in the *Diaporthales*. Subclade B2 represented the *Helotiales*, characterised by *Godronia urceolus* (CBS 215.58, 110435) in congruence with the placement of *Godronia cassandrae* in the *Helotiales* (Konrad et al. 2007). Species in this subclade with unknown teleomorphs were *Eleutheromyces subulatus* (CBS 139.90, 458.88) and *Coleophoma maculans* (CBS 896.69). The latter proved to be unrelated to *Coleophoma crateriformis* (TS) (clade C). A sterile isolate identified as *Rhizosphaera pini* also belonged to this subclade. However, this identity is uncertain, because *Rhizosphaera* is closely related to *Phaeocryptopus nudus* (TS) classified in the *Dothideales* (Winton et al. 2007). Both strains of *Allantophoma endogenospora* (CBS 600.76, 178.79) clustered in clades B1 and B2 respectively.

### The Capnodiales and Dothideales

Clade C included the two subclades C1 and C2. The reference strain *Mycosphaerella punctiformis* (TS) (CBS 113265, ex-lectotype strain), and *Readeriella mirabilis* (TS) (CBS 358.64, 116293) clustered in subclade C1, representing the *Capnodiales* (Verkley et al. 2004b). This agreed with data concerning *Readeriella novaezealandiae* CBS 114357, which was found to be related to *Teratosphaeria* (Crous et al. 2004, 2007).

The reference strains *Dothiora cannabinae* (CBS 737.71 (T), AFToL 1359) and *Dothidea insculpta* (CBS 189.58, AFToL 921) (Schoch et al. 2006) were found in subclade C2 representing the *Dothideaceae*, *Dothideales*. The genera *Selenophoma* and *Coleophoma*, represented by *S. linicola* (CBS 468.48), *S. mahoniae* (CBS 388.92) and *C. crateriformis* (TS) (CBS 473.69), *C. oleae* (CBS 615.72) respectively, also clustered in this subclade. *C. crateriformis* is closely related with *C. oleae*, the latter was reassigned to the genus *Coleonaema* based on conidiomatal development (Duan et al. 2007).

## Taxonomy

Five out of nine *Phoma* sections proved to be related with the teleomorph *Didymella*. The name *Didymella* has been discussed by several authors without a satisfactorily solution (Holm 1975), and therefore considered as not validly published. *Didymella* was first mentioned as *Didymosphaeria* (*Didymella*) *culmigena* (Saccardo 1878), with *Didymella* in parenthesis indicating that Saccardo did not accept the genus *Didymella*, but still listed the species under *Didymosphaeria* (Holm 1975). Later, Saccardo used *Didymella* for the first time at generic level with the description of *Didymella exigua* (Saccardo 1880). However, in our opinion the genus *Didymella* was later validated by Saccardo (1882), when a Latin diagnosis was provided.

*D. exigua*, as the first species described in the genus *Didymella* (Saccardo 1880), has been accepted as the type or lectotype species of the genus *Didymella* by several authors (Corlett 1981). In a detailed study of the genus *Didymella* (Corbaz 1957), *D. exigua* has been described *in vitro* and *in vivo* on *Rumex arifolius*, sampled at Memise sur Thollon, Savoie, France, by M.Ch. Terrier, 27-09-1953. This is also the origin of strain CBS 183.55, *D. exigua* ex *Rumex arifolius* (Polygonaceae) France, deposited by E. Müller. Blasting ITS sequence data of CBS 183.55 did not find any match in GenBank, nor with any

species in our database. Two other strains preserved as *D. exigua* in the CBS collection, CBS 629.76 ex packing material, and CBS 282.76 ex *Brassica* (*Cruciferae*) showed a different ITS profile corresponding with a *Phoma* species, and will be published elsewhere. According to these data, the genus *Didymella* is circumscribed as follows:

*Didymella* Sacc. ex Sacc. Syll. Fung. 1: 545 (1882).

=*Didymella* Sacc., *Michelia* 2: 57 (1880).

=*Didymosphaeria* (*Didymella*) Sacc., *Michelia* 1: 377 (1878).

Type species: *Didymella exigua* (Niessl) Sacc., *Michelia* 2: 57 (1880).

=*Didymosphaeria exigua* Niessl, *Oesterr. Bot. Z.* 25: 165 (1875).

The herbarium material of the holotype *Didymosphaeria exigua* Niessl was not present in M or BRNU, where the main original collections of Niessl von Mayendorf are preserved, and apparently have been lost. The neotype is therefore designated here:

Neotype: dried culture CBS H-20123, culture ex-neotype CBS 183.55, ex *Rumex arifolius* (Polygonaceae) France.

The genera *Didymella* and *Mycosphaerella* were originally described in the *Mycosphaerellaceae*. Later, *Didymella* was placed in the *Pleosporales* (*Pleosporaceae*) (Sivanesan 1984), *Phaeosphaeriaceae* (Barr 1979; Silva-Hanlin & Hanlin 1999), *Venturiaceae* (Reddy et al. 1998), or considered as a genus *incertae sedis* (Lumbsch & Huhndorf 2007). The data obtained in this study support the description of the obtained '*Didymella* subclade' on family level. Therefore, the following family name is introduced:

*Didymellaceae* Gruyter, Aveskamp & Verkley, fam. nov.

Mycobank no.: MB 508292.

*Pseudothecia immersa*, raro superficialia, separata vel gregaria, globosa ad complanata, ostiolata, 80–450 µm, 2–5(–8) stratis cellularum pseudoparenchymatarum. Asci bitunicati, cylindracei, clavati vel saccati, octospori, ex hymenio lato inter pseudoparaphyses oriundi. Ascospores saepe hyalinae vel brunneolae, didymosporae vel pluriseptate dictyosporae.

Typus: *Didymella* Sacc. ex Sacc., Syll. Fung. 1: 545 (1882).

*Pseudothecia* immersed, rarely superficial, separate or gregarious, globose to flattened, ostiolate, 80–450 µm, with 2–5(–8) layers of pseudoparenchymatal cells. Asci bitunicate, cylindrical to clavate or saccate, 8-spored; asci arising from a broad hymenium among pseudoparaphyses. Ascospores mostly hyaline, or brownish, 1-septate spores (didymosporae) or multi-septate dictyosporae.

The obtained phylogenetic results showed that the teleomorphs *Didymella* and *Leptosphaerulina*, producing dictyosporae and 1-septate phragmosporae, respectively, both are classified in the *Didymellaceae*. This finding resembled the close relation of the genera *Mycosphaerella* and *Sphaerulina* in the *Dothideales* (Crous et al. 2003). As a result, the suggested value of ascospore septation as an important taxonomic character in the *Dothideales* (Silva-Hanlin & Hanlin 1999) was not supported.

## Discussion

The combined SSU/LSU nrDNA phylogeny presented here revealed the suborder *Phialopecynidiineae* to be artificial. Species of 14 coelomycetous genera described in this suborder could be assigned to the *Pleosporales*, while eight genera clustered in the *Botryosphaeriales*, *Dothideales*, *Helotiales*, *Diaporthales* or *Capnodiales*.

The representative species of all sections of *Phoma* and *Ascochyta* are placed in the *Pleosporales* (clade A). However, the genus *Phoma* proved to be polyphyletic in this order, as the representative species are present in four different subclades. Also the allied anamorph genera *Ascochyta*, *Coniothyrium* and *Pyrenochaeta* proved to be polyphyletic in the *Pleosporales*.

The type species of the *Phoma* sections *Phoma*, *Phyllostictoides*, *Sclerophomella*, *Macrospora* and *Peyronellaea* clustered in subclade A7. In addition, the type species of the anamorphs *Ascochyta* and *Microsphaeropsis* also belonged to this subclade. As far as teleomorphs have been described for anamorphs in this clade, they belong to the genus *Didymella*. *Phoma radicina*, type species of *Phoma* sect. *Paraphoma*, proved to be closely related with species classified in the *Phaeosphaeriaceae*. This subclade includes especially pathogens on monocotyledonous plants. Based on the setose pycnidia of *Phoma* sect. *Paraphoma*, a phylogenetic relation with the genus *Pyrenochaeta* was expected. However, the type species of *Phoma* sect. *Paraphoma* proved to be more distantly related. Typical species of the genera *Pyrenochaeta* and *Pleurophoma* were found in one subclade, including the type species of the monotypic genus *Phialophorophoma*. These genera all produce typical elongated, filiform, multiseptate conidiophores, and their teleomorph relationships remain unclear.

*Phoma lingam* (teleom. *Leptosphaeria maculans*), type species of *Phoma* sect. *Plenodomus*, clustered in the *Leptosphaeriaceae* as well as *Coniothyrium palmarum* and *Plectophomella visci*. Species classified in the *Leptosphaeriaceae* and *Phaeosphaeriaceae* grouped in separate subclades in this study. *Phoma heteromorphospora*, type species of *Phoma* sect. *Heterospora*, was also found in this subclade. *P. heteromorphospora* may produce relatively thick-walled pycnidia. However, a scleroplectenchymatous pycnidial cell wall, characteristic for *Phoma* sect. *Plenodomus*, has not been observed thus far. *Phoma betae* (teleom. *Pleospora betae*), type species of *Phoma* sect. *Pilosa*, clustered with *Ascochyta caulina* (teleom. *Pleospora calvescens*), which both produce typical pilose pycnidia. These pilose pycnidia were also found in strain CBS 826.88, preserved as *Chaetosphaeronema hispidulum*. This subclade is the representative of the *Pleosporaceae*.

The delimitation of the genera *Phoma* and *Phyllosticta* that often have been confused in the past (van der Aa et al. 1990) is clearly demonstrated, as the included species of *Phyllosticta* and its teleomorph *Guignardia* clustered in the *Botryosphaeriales* (Crous et al. 2006; Phillips et al. 2008). Morphologically *Phyllosticta* can also be distinguished from *Phoma* by its aseptate, hyaline conidia surrounded by a mucous layer, with a typical conidial appendage (Punithalingam & Woodhams 1982; van der Aa & Vanev 2002). For a long time the genera *Phoma* and *Ascochyta*, both classified in the *Pleosporales*, have

already been considered as closely related. The conidiogenesis of the type species of both genera has been studied in detail. In *Phoma*, it was described as blastic, phialidic (Boerema & Bollen 1975), while in *Ascochyta*, it was seen as holoblastic, annellidic (Boerema & Bollen 1975) or phialidic (Buchanan 1987; Punithalingam 1979). The practical criterion for delimitation of species in these genera is the ratio of septate conidia produced on artificial medium. *Phoma* species produce mainly aseptate conidia *in vitro*, whereas *Ascochyta* strains produce predominantly septate conidia both *in vivo* and *in vitro* (Boerema & Bollen 1975; Onfroy et al. 1999; Rai 2000). Currently *Ascochyta* has teleomorphs described in both *Mycosphaerella* and *Didymella* (Corlett 1981; Peever et al. 2007). The type strain of the genus, *A. pisi*, grouped in the *Didymella* clade as described above, as well as *A. fabae* (teleom. *D. fabae*), and *A. pinodes* (teleom. *D. pinodes*), whereas *A. hordei* var. *hordei* is closely related. These results indicate that the teleomorph *Didymella* is the only genus correctly linked to species of *Ascochyta* s.str. These findings are in congruence with a recent study based on ITS sequences and three protein-coding genes of *Ascochyta* species on Leguminosae, in which a monophyletic *Didymella* clade was found including non-leguminous *Didymella* and *Phoma* species (Peever et al. 2007). Similar results with *Ascochyta* and *Phoma* pathogens on Leguminosae were obtained based on mitochondrial RFLP data, conidial size and host preference (Fatehi et al. 2003). The three species *A. pinodes*, *Phoma pinodella* and *Ascochyta phaseolorum* that were involved in this study were considered as host-adapted populations of a single taxon.

The data obtained by Peever et al. (2007) supported *A. pisi*, *A. fabae* and *A. lentis* as a distinct species complex in the *Didymella* clade. A detailed phylogenetic study including all *Phoma* species of the *Phoma* sections and *Ascochyta* species involved in the *Didymellaceae* will elucidate the taxonomic status of *Ascochyta* (these studies are currently in progress). Compared to this *Ascochyta* species complex, *Ascochyta rabiei* is more distantly related (Peever et al. 2007), and could be better placed in *Phoma* based on its conidiogenesis (Singh et al. 1997), and therefore we prefer the use of the name *Phoma rabiei*. *Phoma zae-maydis*, type species of *Phoma* sect. *Macrospora* also fits in this *Didymella* clade, and therefore the name *Didymella zae-maydis* (syn. *Mycosphaerella zae-maydis*) should be used.

In future studies a further delimitation in the *Didymellaceae* will be made, including the *Phoma* species of the five *Phoma* sections found in this group, and additional species of the other genera involved. Sequence data of protein-coding genes provide reliable data for delimitation below genus level. Besides *Ascochyta*, the position of the genus *Microsphaeropsis* has to be elucidated. In this study, *Microsphaeropsis olivacea* proved to be closely related with *Phoma herbarum*, the type species of the genus *Phoma*. The brown discolouring of the conidia, characteristic for *Microsphaeropsis*, has also been observed in old pycnidia of members of *Phoma* sect. *Peyronellaea* (Boerema et al. 2004). Also the classification of *Ampelomyces quercinus*, representing fast-growing pycnidial fungi growing on plant pathogenic powdery mildew species being different from the type species of the genus, has to be clarified.

A reclassification of *Phoma* sections outside the *Didymellaceae* is ongoing to establish monotypic teleomorph–anamorph relations. *Phoma lingam* (teleom. *Leptosphaeria maculans*), fits in

a subclade to be assigned as the *Leptosphaeriaceae*. *P. lingam* and allied *Phoma* species of sect. *Plenodomus* can be better placed in the genus *Plenodomus* as previously suggested (Reddy et al. 1998). This can be achieved as part of a molecular study on all species of *Phoma* sect. *Plenodomus* described in vitro. Similar studies are in progress in *Phoma* sect. *Paraphoma*, including the genera *Pyrenochaeta* and *Pleurophoma*, and in the *Phoma* sections *Heterospora* and *Pilosa*.

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## REFERENCES

- van der Aa HA, Noordeloos ME, de Gruyter J, 1990. Species concepts in some larger genera of the coelomycetes. *Studies in Mycology* **32**: 3–19.
- van der Aa HA, Vanev S, 2002. *A Revision of the Species Described in Phyllosticta*. Centraalbureau voor Schimmelcultures, Utrecht, 510 pp.
- Aveskamp MM, de Gruyter J, Crous PW, 2008. Biology and systematics of *Phoma*: a complex genus of major quarantine significance. *Fungal Diversity* **31**: 1–18.
- Barr ME, 1979. A classification of the *Loculoascomycetes*. *Mycologia* **71**: 935–957.
- Barr ME, 2002. *Teichosporaceae*, another family in the *Pleosporales*. *Mycotaxon* **82**: 373–389.
- Boerema GH, 1984. Mycologisch–taxonomisch onderzoek. *Ascochyta's met lichtbruine conidien die pathogen zijn voor Chenopodiaceae*. *Verslagen en Mededelingen Plantenziektenkundige Dienst Wageningen* **162**: 31–34 Jaarb. 1983.
- Boerema GH, 1997. Contributions towards a monograph of *Phoma* (coelomycetes) – V. Subdivision of the genus in sections. *Mycotaxon* **64**: 321–333.
- Boerema GH, Bollen GJ, 1975. Conidiogenesis and conidial septation as differentiating criteria between *Phoma* and *Ascochyta*. *Persoonia* **8**: 111–144.
- Boerema GH, de Gruyter J, Noordeloos ME, Hamers MEC, 2004. *Phoma Identification Manual. Differentiation of specific and infra-specific taxa in culture*. Cabi Publishing, pp. 470.
- Boise J, 1985. An amended description of *Trematosphaeria*. *Mycologia* **77**: 230–237.
- Buchanan PK, 1987. A reappraisal of *Ascochyta* and *Ascochyella* (coelomycetes). *Mycological Papers* **156**: 1–83.
- Butin H, Kehr R, 1995. Leaf blotch of lime associated with *Asteromella tiliae* comb. nov. and the latter's connection to *Didymosphaeria petrakiana*. *Mycological Research* **99**: 1191–1194.
- Castlebury LA, Rossman AY, Jaklitsch WJ, LN Vasilyeva, 2002. A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.
- Corbaz R, 1957. Recherches sur le genre *Didymella* Sacc. *Phytopathologische Zeitschrift* **28**: 375–414.
- Corlett M, 1981. A taxonomic survey of some species of *Didymella* and *Didymella*-like species. *Canadian Journal of Botany* **59**: 2016–2042.
- Crous PW, Braun U, Groenewald JZ, 2007. *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**: 1–32.
- Crous PW, Groenewald JZ, Mansilla JP, Hunter GC, MJ Wingfield, 2004. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. *Studies in Mycology* **50**: 195–214.
- Crous PW, Groenewald JZ, Wingfield MJ, Aptroot A, 2003. The value of ascospore septation in separating *Mycosphaerella* from *Sphaerulina* in the *Dothideales*: a Saccardoan myth? *Sydowia* **55**: 136–152.
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WOF, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ, 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**: 235–253.
- Damm U, Verkley GJM, Crous PW, Fourie PH, Haegi A, Riccioni L, 2008. Novel *Paraconiothyrium* species on stone fruit trees and other woody hosts. *Persoonia* **20**: 9–17.
- Dooley DP, Beckius ML, Jeffery BS, McAllister CK, Radentz WH, Feldman AR, Rinaldi MG, Bailey SR, Keeling JH, 1989. Phaeohyphomycotic cutaneous disease caused by *Pleurophoma* in a cardiac transplant patient. *Journal of Infectious Diseases* **159**: 503–507.
- Duan JX, Wu WP, Liu XZ, 2007. Reinstatement of *Coleonaema* for *Coleophoma oleae* and notes on *Coleophoma*. *Fungal Diversity* **26**: 187–204.
- Fatehi J, Bridge PD, Punithalingam E, 2003. Molecular relatedness within the "Ascochyta pinodes-complex". *Mycopathologia* **156**: 317–327.
- Gams W, Verkley GJM, Crous PW, 2007. *CBS Course of Mycology*, fifth edn. Centraalbureau voor Schimmelcultures, Utrecht, pp. 242.
- Green S, Castlebury LA, 2007. Connection of *Gnomonia intermedia* to *Discula betulina* and its relationship to other taxa in *Gnomoniaceae*. *Mycological Research* **111**: 62–69.
- Grondona I, Monte E, Garcia-Acha I, Sutton BC, 1997. *Pyrenochaeta dolichi*: an example of a confusing species. *Mycological Research* **101**: 1405–1408.
- Holm L, 1975. Nomenclatorial notes on *Pyrenomyces*. *Taxon* **24**: 475–488.
- Huelsenbeck JP, Ronquist F, 2001. MRBAYES: Bayesian inference of Phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Hughes SJ, 1953. Conidiophores, conidia and classification. *Canadian Journal of Botany* **31**: 577–659.
- Kirk PM, Cannon PF, Minter DW, Stalpers JA, 2008. *Ainsworth & Bisby's Dictionary of the Fungi*, 10th edn. CABI Bioscience, p. 771.
- Konrad H, Stauffer C, Kirisits T, Halmschlager E, 2007. Phylogeographic variation among isolates of the *Sirococcus conigenus* P group. *Forest Pathology* **37**: 22–39.
- Kruys Å, Eriksson OE, Wedin M, 2006. Phylogenetic relationships of coprophilous *Pleosporales* (*Dothideomycetes*, *Ascomycota*), and the classification of some bitunicate taxa of unknown position. *Mycological Research* **110**: 527–536.
- Lumbsch HT, Huhndorf SM (eds), 2007. *Outline of the Ascomycota* – 2007. *Myconet* **13**: 1–58.
- van Niekerk JM, Groenewald JZ, Verkley GJM, Fourie PH, Wingfield MJ, Crous PW, 2004. Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. *Mycological Research* **108**: 283–303.
- Onfroy C, Tivoli B, Corbière R, Bouznad Z, 1999. Cultural, molecular and pathogenic variability of *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella* isolates from dried pea (*Pisum sativum*) in France. *Plant Pathology* **48**: 218–229.
- Page RDM, 1996. Treeview: an application to display phylogenetic trees on personal computers. *Computer Application in the Biosciences* **12**: 357–358.
- Peever TL, Barve MP, Stone LJ, Kaiser WJ, 2007. Evolutionary relationships among *Ascochyta* species infecting wild and culti-

- vated hosts in the legume tribes *Cicereae* and *Vicieae*. *Mycologia* **99**: 59–77.
- Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A, Akulov A, Crous PW, 2008. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* **21**: 29–55.
- Punithalingam E, 1979. Graminicolous *Ascochyta* species. *Mycological Papers* **142**: 1–214.
- Punithalingam E, 2004. Book review: conidial fungi, *Phoma* Identification Manual: differentiation of specific and infra-specific taxa in culture. *Mycotaxon* **90**: 487–493.
- Punithalingam E, Woodhams JE, 1982. The conidial appendage in *Phyllosticta* spp. *Nova Hedwigia* **36**: 151–198.
- Rai MK, 2000. *The genus Phoma (Identity and Taxonomy)*. International Book Distributors, Dehra Dun, India, pp. 159.
- Reddy PV, Patel R, White Jr JF, 1998. Phylogenetic and developmental evidence supporting reclassification of cruciferous pathogens *Phoma lingam* and *Phoma wasabiae* in *Plenodomus*. *Canadian Journal of Botany* **76**: 1916–1922.
- Rehner SA, Samuels GJ, 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.
- Rossman AY, Castlebury LA, Farr DF, Stanosz GR, 2008. *Sirococcus conigenus*, *Sirococcus piceicola* sp. nov. and *Sirococcus tsugae* sp. nov. on conifers: anamorphic fungi in the *Gnomoniaceae*, *Diaporthales*. *Forest Pathology* **38**: 47–60.
- Saccardo PA, 1878. *Fungi veneti novi vel critici vel mycologiae venetae addendi ser. IX. Michelia* **1**: 361–445.
- Saccardo PA, 1880. *Fungi Gallici ser. II. Michelia* **2**: 39–135.
- Saccardo PA, 1882. *Sylloge Fungorum* **1**. Patavii.
- Schneider R, 1979. Die Gattung *Pyrenochaeta* De Notaris. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft* **189**: 1–73.
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW, 2006. A multigene phylogeny of *Dothideomycetes* using four nuclear loci. *Mycologia* **98**: 1041–1052.
- Silva-Hanlin DMW, Hanlin RT, 1999. Small subunit ribosomal RNA gene phylogeny of several *Loculoascomycetes* and its taxonomic implications. *Mycological Research* **103**: 153–160.
- Singh PJ, Pal M, Prakash N, 1997. Ultrastructural studies of conidiogenesis of *Ascochyta rabiei*, the causal organism of chickpea blight. *Phytoparasitica* **25**: 291–304.
- Sivanesan A, 1984. *The Bitunicate Ascomycetes and their Anamorphs*. J. Cramer, Vaduz, Liechtenstein, pp. 701.
- Sutton BC, 1964. *Phoma* and related genera. *Transactions of the British Mycological Society* **47**: 497–509.
- Sutton BC, 1977. Coelomycetes VI. Nomenclature of generic names proposed for coelomycetes. *Mycological Papers* **141**: 1–253.
- Sutton BC, 1980. *The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. CMI, Kew, pp. 696.
- Swofford DL, 2003. *PAUP\*: phylogenetic analysis using parsimony (\*and other methods) version 4.0b 10*. Sinauer Associates, Sunderland, MA.
- Szentiványi O, Kiss L, Russell JC, Kovács GM, Varga K, Jankovics T, Lesemann S, Xu XM, Jeffries P, 2005. *Ampelomyces* mycoparasites from apple powdery mildew identified as a distinct group based on single-stranded conformation polymorphism analysis of the rDNA ITS region. *Mycological Research* **109**: 429–438.
- Torres MS, White JF, Cazares G, Bergen M, Bischoff JF, Sullivan RF, 2005. A new species and its phylogenetic placement in the *Didymella/Phoma* complex (*Phaeosphaeriaceae*, *Pleosporales*). *Mycotaxon* **93**: 297–308.
- Vanev SG, van der Aa HA, 1998. An annotated list of the published names in *Asteromella*. *Persoonia* **17**: 47–67.
- Verkley GJM, da Silva M, Wicklow DT, Crous PW, 2004a. *Paraconiothyrium*, a new genus to accommodate the mycoparasite *Coniothyrium minitans*, anamorphs of *Paraphaeosphaeria*, and four new species. *Studies in Mycology* **50**: 323–335.
- Verkley GJM, Crous PW, Groenewald JZ, Braun U, Aptroot A, 2004b. *Mycosphaerella punctiformis* revisited: morphology, phylogeny, and epitypification of the type species of the genus *Mycosphaerella* (*Dothideales*, *Ascomycota*). *Mycological Research* **108**: 1271–1282.
- Vilgalys R, Hester M, 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
- Vujanovic V, St-Arnaud M, 2003. A new species of *Pseudorobillarda*, an endophyte from *Thuja occidentalis* in Canada, and a key to the species. *Mycologia* **95**: 955–958.
- White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego, CA, pp. 315–322.
- Winton LM, Stone JK, Hansen EM, Shoemaker RA, 2007. The systematic position of *Phaeocryptopus gaemannii*. *Mycologia* **99**: 240–252.
- Zhou S, Stanosz GR, 2001. Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia* **93**: 516–527.