Revised taxonomy of *Phoma* and allied genera

Johannes (Hans) de Gruyter

Thesis committee

Promoters

Prof. dr. P.W. Crous
Professor of Evolutionary Phytopathology,
Wageningen University

Prof. dr. ir. P.J.G.M. de Wit
Professor of Phytopathology,
Wageningen University

Other members

Dr. R.T.A. Cook, Consultant Plant Pathologist, York, UK
Prof. dr. T.W.M. Kuyper, Wageningen University
Dr. F.T. Bakker, Wageningen University
Dr. ir. A.J. Termorshuizen, BLGG AgroXpertus, Wageningen

This research was conducted under the auspices of the Research School Biodiversity

Revised taxonomy of *Phoma* and allied genera

Johannes (Hans) de Gruyter

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 12 November 2012
at 4 p.m. in the Aula.

Johannes (Hans) de Gruyter

Revised taxonomy of *Phoma* and allied genera,
181 pages.

PhD thesis Wageningen University, Wageningen, NL (2012)
With references, with summaries in English and Dutch

ISBN 978-94-6173-388-7

Dedicated to Gerhard Boerema †

CONTENTS

CHAPTER 1

Introduction

Chapter 1. This chapter gives a historic overview of the events leading to the initiative to write this dissertation. It discusses the changes in the taxonomic definition of *Phoma*, and highlights some of the important plant pathogenic species in the genus. The scope and outline of the thesis have been provided.

Phoma studies in the Netherlands

The anamorphic genus *Phoma* and morphologically similar coelomycetous genera have been studied intensively in the Netherlands during the past 50 years. *Phoma* became an important topic at the Plant Protection Service (Plantenziektenkundige Dienst, PD) in the sixties of the last century. A new fungal disease was found on seed potatoes, characterised by a dry rot that primarily developed during storage, leading to potentially severe economic losses. *Fusarium* species are commonly known as the cause of dry rot on potatoes (Secor & Salas 2001, Peters *et al.* 2008), but isolations made on artificial media at the Mycology Department of the PD revealed an unknown *Phoma* species as causal agent. Several *Phoma* species were described as soil inhabitants in literature and recorded on seed potatoes at that time, such as the plurivorous *Phoma exigua*, *Ph. glomerata*, *Ph. eupyrena* and *Ph. anserina*, but clear taxonomic characters for a proper identification of *Phoma* species were lacking.

Detailed *in vitro* studies that were undertaken at the PD by Boerema and co-workers clarified the taxonomy of these plurivorous *Phoma* species (Boerema & van Kesteren 1962, Dorenbosch 1970), which in turn revealed the disease symptoms to be caused by *Ph. foveata*, a pathogen first reported from Scotland (Foister 1940). *Phoma foveata* proved to be morphologically very similar to the plurivorous *Ph. exigua* (Boerema & Höweler 1967), which often appeared as a secondary invader on dry rot symptoms. *Phoma foveata* was considered as a pathogenic variety of the latter and reclassified as *Phoma exigua* var. *foveata*, the causal agent of potato gangrene (Boerema 1967). *Phoma exigua* var. *foveata* is identified in pure culture by the production of anthraquinones that crystallise into fine yellow-green needles. Application of a droplet of 1N NaOH on a culture on oatmeal agar, malt extract agar or cherry-decoction agar shows a violet to red discolouration of these pigments, whereas application of NaOH on a culture of *Ph. exigua* var. *exigua*, discolouration from initially green into red occurs, due to the presence of the metabolite 'substance E'; E^+ reaction (Boerema & Höweler 1967). *Phoma exigua* var. *foveata* was considered to pose a high risk to the potato industry and the pathogen was subsequently added to the list of quarantine organisms in Europe. A chromatographic detection method was developed to demonstrate the presence of anthraquinones (Mosch & Mooi 1975). To prevent the spread of the pathogen, all lots of seed potatoes were sampled and tested for several years for presence of the pathogen. In time the incidence of *Ph. exigua* var. *foveata* decreased, and the damage caused by the pathogen became less significant, possibly due to improved potato storage conditions. *Phoma exigua* var. *foveata* was eventually removed from the quarantine list. The pathogen was later accepted at species rank (Boerema, Loerakker & Hamers 1987) and has recently been accommodated in the newly established genus, *Boeremia* (Aveskamp *et al*. 2010). *Boeremia foveata* is still a quarantine organism in several countries outside Europe.

A second phytosanitary problem involving *Phoma* appeared some years later, with the discovery of *Phoma lingam*, the causal agent of blackleg on *Brassica* species and their seeds. This initated research to define the *in vitro* characteristics of the pathogen and those of morphologically related saprobic *Phoma* species that were simultaneously found on the seed coat (Boerema & van Kesteren 1964).

Fundamental studies of *Phoma* followed on the conidiogenesis of the generic type species *Phoma herbarum* (Boerema & Bollen 1975). The taxonomy of various *Phoma* species was

studied (Boerema & Dorenbosch 1973, Boerema 1976) by interpretation of previous *in vitro* studies by Wollenweber & Hochapfel (1936) and Dennis (1946). Metabolites observed and identified *in vitro*, such as pigments or crystals, proved to be reliable characters for species identification (Dorenbosch 1970, Monte *et al.* 1991, Noordeloos *et al.* 1993). Hundreds of isolates, mainly obtained from infested plant material submitted for diagnosis to the PD, provided the foundation for a thorough revision of the genus *Phoma*. The culture and morphological characters of the species were combined with ecological data, phytopathological studies and data obtained from herbarium specimens. *Phoma* species described in old literature could be interpreted, but also many new species were described. This work was performed in collaboration with the Centraalbureau voor Schimmelcultures (CBS) where the taxonomy of the coelomycetes *Ascochyta*, *Asteromella*, *Coniothyrium*, *Microsphaeropsis*, *Phyllosticta*, *Pleurophoma*, *Pyrenochaeta*, and *Stagonospora* was studied. These genera show morphological characters that are very similar to *Phoma* in its broadest generic concept.

A next step in *Phoma* taxonomy was the classification of the genus into nine sections (van der Aa *et al*. 1990, Boerema 1997). The *Phoma* sections *Phoma*, *Heterospora*, *Macrospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Pilosa*, *Plenodomus* and *Sclerophomella* were recognised based on the characteristics of the type species of each section. The sections include both specific pathogens of (cultivated) plants and plurivorous, saprophytic or opportunistic species. Standardised descriptions of the *Phoma* species per section followed in a series of "Contributions towards a monograph of *Phoma*", published in Persoonia in the period 1992–2003 (de Gruyter & Noordeloos 1992, Boerema 1993, 2003, de Gruyter *et al.* 1993, 1998, 2002, Boerema *et al*. 1994, 1996, 1997, Boerema & de Gruyter 1998, 1999, van der Aa *et al.* 2000, de Gruyter 2002, de Gruyter & Boerema 2002) including various new taxa. These contributions were the precursors of the "*Phoma* Identification Manual" (Boerema *et al*. 2004). The manual provides keys and descriptions of more than 200 specific and infra-specific *Phoma* taxa.

Our knowledge of the taxonomy of *Phoma* is still incomplete; the *Phoma* Identification Manual covers mainly *Phoma* species from European origin and the most common *Phoma* species known from other continents. Many *Phoma* species still remain to be discovered. There have been thousands of *Phoma*-like species described in literature, even including species from Europe, of which the true identity remains unknown.

Most of the *Phoma* species have an unknown sexual state. As far as has been recorded, the teleomorph of *Phoma* resides in *Didymella*, *Leptosphaeria*, *Mycosphaerella* and *Pleospora*. Several *Phoma* sections are linked to a specific teleomorph genus (Boerema *et al.* 2004). Synanamorphs have been described in the genera *Stagonosporopsis*, *Epicoccum*, *Phialophora*, *Sclerotium* and *Phaeomoniella* (Boerema and Bollen 1975, Sutton 1977, Boerema 1997, Boerema *et al.* 1993, 1994, 1997, Crous & Gams 2000).

It seemed that the taxonomic work on *Phoma* at the PD was completed with the publication of the *Phoma* Identification Manual in 2004. At that time, it could not be foreseen that already within one year a new project on the molecular phylogeny of *Phoma* would start. A joined research proposal was submitted by the PD and the Ministry of Agriculture Nature and Food Quality in the framework of the FES programme "Versterking Infrastructuur Plantgezondheid" and granted. The project comprised the setup of an online database providing biological, molecular and taxonomic information of important pests and diseases, as well as invasive plants. These data are related to reliable reference strains or voucher specimen maintained in official collections. *Phoma* was included as a priority genus in this project. The *Phoma* Identification Manual, with references to representative isolates preserved in the collections of CBS and PD,

formed an excellent platform for molecular phylogenetic studies on genus and species level. Species with shared morphological characteristics that are classified in other coelomycetous genera were also included in this project.

The morphological species concept of *Phoma*

Saccardo (1884) defined *Phoma* as an asexual genus comprising species that produce pycnidia with one-celled, hyaline conidia occurring on herbaceous stems. In the Saccardoan system, classification was not only according to the characters of the fungus, but the host plant and the infested part of the plant were also considered as criteria for genus and species delimitation. *Phoma*-like species found on leaves were described in *Phyllosticta*, or when septate conidia were observed, in *Ascochyta*. Species with setose pycnidia were attributed to *Pyrenochaeta*. Thousands of pycnidial fungi with hyaline conidia were published in the period until the first half of the 20th century following these criteria, including about 3 000 *Phoma* species. The true identities of many of these species remain unknown.

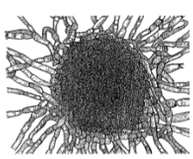
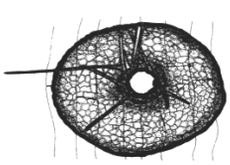
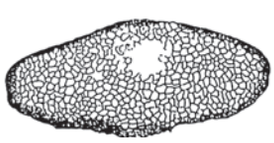
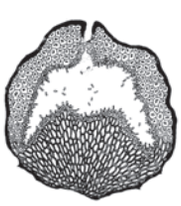
The introduction of studies of morphology of the fungi grown *in vitro* (Wollenweber & Hochapfel 1936, Dennis 1946) and of conidiogenesis as taxonomic criteria (Hughes 1953, Sutton 1964, 1977, 1980) resulted in an advanced and better defined delimitation of the asexual genera and species involved. It became clear that the Saccardoan system has led to many synonyms in *Phoma* and related coelomycetes. A glossary with taxonomic terms used in this thesis has been provided.

Phoma is characterised by the type species *Phoma herbarum* (Boerema 1964, 1970). The conidiogenesis of the species as taxonomic criterion was studied in detail and compared with that of the morphologically related *Ascochyta pisi*, type species of *Ascochyta* (Boerema 1965, Brewer & Boerema 1965, Boerema & Bollen 1975). *Phoma herbarum* is characterised by phialidic conidiogenesis, and ampulliform to doliiform conidiogenous cells. The pycnidia are mainly glabrous, sometimes semi-pilose, with a thin-walled (up to 5–7 layers of cells) pseudoparenchymatous cell wall and predetermined ostiole. The conidia produced are small, usually 5 × 2 µm, hyaline, and always unicellular both *in vitro* and *in vivo*. *Phoma herbarum* is a plurivorous species recorded on tissues of various herbaceous and woody plant species, but also on many other substrates, including humans, fish, soil and water (Boerema 1964, 1970, Aveskamp *et al.* 2008).

The morphological generic concept of *Phoma* has subsequently become more broadly defined. The genus now also includes species that produce septate conidia, a secondary step in conidial formation (Boerema & Bollen 1975). Pycnidia may be thick-walled, even with a scleroplectenchymatous wall structure (Boerema *et al.* 2004) and not only glabrous, but also pilose or setose. The taxonomic studies in *Phoma* finally revealed a new generic concept with a classification of *Phoma* into nine sections. The morphological key characters of the *Phoma* sections *Phoma*, *Heterospora*, *Macrospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Pilosa*, *Plenodomus* and *Sclerophomella* (Fig. 1.) were provided and the type species of the sections were defined (van der Aa *et al*. 1990, Boerema 1997).

The species that are classified in *Phoma* section *Phoma* show the fundamental characteristics of the generic type species *P. herbarum,* and produce unicellular conidia up to 14 µm in length. The species of *Phoma* section *Phyllostictoides* produce similar pycnidia with unicellular conidia,

Fig. 1. (p. 13). Morphological species concept of *Phoma* into nine sections with teleomorph relations, Boerema 1997.

Phoma sect./Type sp.	Pycnidia/ Conidia	Key characters	
Phoma *Ph. herbarum*		Pycnidia glabrous, with ostiole, thin-walled, pseudoparen-chymatous, conidia hyaline, unicellular	*Didymella*
Phyllostictoides *Ph. exigua var. exigua*		Unicellular and septate conidia	
Macrospora *Ph. zeae-maydis*		Distinctly large conidia	*Mycosphaerella*
Heterospora *Ph. heteromorphospora*		Two conidial types, small, phomoid and distinctly large	
Peyronellaea *Ph. glomerata*		Dictyochlamydospores	
Pilosa *Ph. betae*		Pycnidia covered by mycelial hairs, pore instead of ostiole	*Pleospora*
Paraphoma *Ph. radicina*		Pycnidia covered by setae	
Sclerophomella *Ph. complanata*		Pycnidia thick-walled, pore instead of ostiole	*Didymella*
Plenodomus *Ph. lingam*		Pycnidia thick-walled, scleroplecten-chymatous, pore instead of ostiole	*Leptosphaeria*

but also 1–2-septate conidia are produced (up to 5 %) *in vitro*. The percentage of septate conidia *in vivo* may be as high as 95 %, resembling species of *Ascochyta* producing predominantly septate conidia *in vitro* and *in vivo*. *Phoma* sections *Phoma* and *Phyllostictoides* include species with a teleomorph described in *Didymella*.

Phoma section *Heterospora* was introduced to accommodate the species that produce distinctly larger conidia up to 25 × 6 µm, or occasionally even larger, besides the relatively small phomoid conidia, which measure 3–11 × 1.5–5 µm. The larger conidia mainly occur *in vivo* and usually become septated due to secondary septation. This conidial state has been referred to as "ascochytoid-stagonosporoid" and various species of the section were described according to these large, septate conidia in the synanamorph *Stagonosporopsis* (Diedicke 1912). Conidial dimorphism proved to be a variable character, both in occurrence as well as in the size of the "ascochytoid-stagonosporoid" conidia produced. Its occurrence depends on the phase in the life cycle of the pathogen and the environmental conditions. Conidial dimorphism has led to many synonyms that have been described in the morphologically related genera *Ascochyta*, *Diplodina* and *Stagonospora*.

Species classified in *Phoma* section *Peyronellaea* produce *Alternaria*-like or *Stemphylium*-like dictyochlamydospores as an additional character. A sexual state of *Phoma* sections *Heterospora* and *Peyronellaea* is unknown.

Phoma section *Pilosa* is characterised by densely pilose, pseudoparenchymatous pycnidia producing unicellular conidia, with a teleomorph described in *Pleospora*. *Phoma* section *Paraphoma* with unknown teleomorph is characterised by setose pseudoparenchymatous pycnidia.

Phoma sections *Sclerophomella* and *Plenodomus* are characterised by thick-walled, pseudoparenchymatous or scleroplectenchymatous pycnidia, respectively. The pycnidia are initially closed, with an opening (pore) that develops at a later stage of the growing process. *Phoma* section *Sclerophomella* is associated with *Didymella*, whereas the teleomorph of many species of *Phoma* section *Plenodomus* has been described in *Leptosphaeria*.

Species of *Phoma* section *Macrospora* always produce remarkably larger, unicellular or septate conidia, up to 25 × 9 µm. Although somewhat controversial, a few teleomorph associations have been linked to *Didymella* (Müller & von Arx 1962, von Arx 1987) and *Mycosphaerella* (Corlett 1981, Punithalingam 1990, de Gruyter 2002).

The present delimitation of *Phoma* into sections is ambiguous, because the main character of a *Phoma* section can also be shared by some species classified in other sections (Boerema *et al*. 2004, Punithalingam 2004). In addition, morphological characters of certain *Phoma* sections can be shared by related coelomycetes including *Ascochyta*, *Asteromella*, *Microsphaeropsis*, *Phomopsis*, *Phyllosticta*, *Pleurophoma*, *Pyrenochaeta* and *Stagonospora*. Especially the delimitation of the genera *Pyrenochaeta*, *Pleurophoma* and *Phoma* section *Paraphoma* is still unclear (Grodona *et al.* 1997). It was therefore strongly recommended that the system of *Phoma* classification be improved by evaluating their phylogenetic placement in order to establish monophyletic groups (Torres *et al.* 2005).

Molecular studies may also determine the phylogeny of *Phoma* species with an unknown teleomorph and provide an answer where teleomorphs associated with *Phoma* species are disputed, e.g. *Didymella* or *Mycosphaerella* (Punithalingam, 2004). Finally, the phylogeny of *Phoma* spp. with *Epicoccum*, *Phaeomoniella*, *Phialophora*, *Sclerotium* and *Stagonosporopsis* synanamorphs (Boerema & Bollen 1975; Sutton 1977; Boerema 1993, 1997; Boerema *et al*. 1994, 1997, Crous & Gams 2000) still needs to be clarified.

Molecular phylogeny of *Phoma*, scope and outline of PhD thesis

It is questionable whether there is a genetic base for the present *Phoma* taxonomy and its delimitation from other coelomycetous genera. Molecular studies of *Phoma* and its teleomorphs published to date are limited, and concentrate on a small number of important plant pathogenic species. Examples are *Didymella bryoniae*, anam. *Ph. cucurbitacearum* (Somai *et al.* 2002), *Leptosphaeria maculans*, anam. *Ph. lingam* (Mendes-Pereira *et al.* 2003), *Ph. ligulicula* (Pethybridge *et al*. 2004) and *Ph. tracheiphila* (Balmas *et al.* 2005). In these studies, the number of reference *Phoma* species included, as well as the various genes studied, was limited. As a result, only few molecular sequences of *Phoma* species, usually ITS sequence data, were available at the onset of this project.

The detection and identification of *Phoma* pose difficulties to phytosanitary management. Identification of *Phoma* and related coelomycetes using morphological identification of isolates is time consuming and needs a high level of expertise. *Phoma* species may share morphological characteristics with related coelomycetes and within these genera pathogens and opportunists cannot always be distinguished solely based on morphological characters. *Phoma* actually comprises several cryptic taxa that cannot be discriminated *in vitro*, but only *in vivo* where they show differences in pathogenicity (Abeln *et al.* 2002). Molecular methods are helpful to speed up the diagnostic process related to consignments of plant material that is often perishable and therefore cannot be held in quarantine for long periods of time. A DNA-based identification system for identification of isolates, or preferably detection and identification directly from plant material, will rapidly enable identification of the risks of *Phoma*-like fungi associated with infected plant material.

The PhD project comprises the characterisation of *Phoma* species by DNA sequencing potentially informative loci of all reference strains available in the culture collections of CBS and PD. A literature review and DNA sequence analyses were performed to search for molecular data to aid in the delimitation of the genus *Phoma* and morphologically similar coelomycetous genera and related teleomorphs. Several potentially informative regions of the genome were sequenced in the first phase of the project. These loci included 18S nrDNA (SSU), Internal Transcribed Spacer (ITS) regions 1 & 2 together with the intervening 5.8S nrDNA, 28S nrDNA (LSU), β-tubulin (TUB), actin (ACT), and chitin synthase 1 (CHS-1). Additional sequence data of characterised strains of *Phoma* and related genera were retrieved from GenBank. The sequence data of the representative strains representing the *Phoma* sections formed the base for a thorough circumscription of *Phoma* and allied genera.

This thesis focuses on *Phoma*-like species that appeared related only distantly to the generic type species *Phoma herbarum*, and *Didymella* teleomorphs. Most of the species studied here are presently classified in *Phoma* sections *Paraphoma, Pleospora and Plenodomus*. Unnamed, often sterile *Phoma*-like strains in the collections, as well as new isolates obtained, are included.

In the second phase of the project, real-time PCR methods using actin sequence data were developed for detection and identification of the important plant pathogenic (quarantine) species *Stagonosporopsis andigena* (syn. *Ph. andigena*) and *S. crystalliniformis* (syn. *Ph. crystalliniformis*) in symptomatic leaves of potato and tomato.

Chapter 2. The morphological species concept of *Phoma* is polyphyletic. The classification of the genus in sections is useful for identification based on morphological characters that are clearly visible in cultures grown *in vitro*, but this classification is rather artificial because morphological characters may be shared among sections. Moreover, delimitation with related coelomycetes can be controversial, and there are multiple teleomorphs associated with *Phoma*,

including *Didymella, Leptosphaeria, Mycosphaerella and Pleospora*. Even these teleomorphs are shared with morphologically similar coelomycete genera.

To further clarify the molecular phylogeny of *Phoma* and its relatives, in this study sequence data of partial regions of SSU and LSU of the type species of the nine *Phoma* sections, morphologically similar genera including *Ascochyta, Coniothyrium, Deuterophoma, Microsphaeropsis, Pleurophoma, Pyrenochaeta* and related teleomorph genera are compared. The phylogenic relationship between the *Phoma*-like coelomycetes and allied teleomorphs in *Pleosporales* is also studied in more detail with molecular tools and the results are evaluated and placed in a broader context.

Chapter 3. This chapter continues with molecular phylogenic studies of *Phoma* sections that appeared to be related only distantly to *Didymellaceae* described in Chapter 2. *Phoma* section *Paraphoma*, characterised by setose pycnidia, resembles species of *Pyrenochaeta* (Schneider 1979) and *Pleurophoma*. Sequence data from the SSU and LSU regions of the species classified in *Phoma* section *Paraphoma* are compared with those of representative isolates of *Pyrenochaeta* and *Pleurophoma*, and of the type species of the *Phoma* sections *Phoma*, *Pilosa* and *Plenodomus*. The molecular phylogeny of *Phoma* sections *Paraphoma* and *Pyrenochaeta* appeared to be highly polyphyletic and a thorough reclassification of the genera is provided.

Chapter 4. The *Phoma* sections *Plenodomus, Pleospora* and *Heterospora* could not be maintained as was demonstrated in previous chapters. By employing a "one species = one name" approach to merge anamorph and teleomorph genera, the species described in *Phoma* sections *Plenodomus* and *Leptosphaeria* are reclassified in *Leptosphaeria, Plenodomus* and some newly proposed genera. Two species of *Phoma* section *Heterospora*, namely the type species *Phoma heteromorphospora* and its allied species *Ph. dimorphospora*, are reclassified. The classification of species of *Phoma* section *Pilosa* and allied *Ascochyta* species with pilose pycnidia in *Pleosporaceae* is demonstrated. The taxonomy of several *Phoma*-like species that grouped outside *Pleosporaceae* is redefined.

Chapter 5. Specific real-time (TaqMan) PCR assays are developed for the detection of the pathogens *Stagonosporopsis andigena* and *S. crystalliniformis* in leaves of potato or tomato. The molecular phylogeny of both species, using sequence polymorphisms in their actin gene, is demonstrated. The reliability of the assays for the detection of *S. andigena* and *S. crystalliniformis* in leaf material is tested in performance studies and the specificity, analytical sensitivity, reproducibility, repeatability and robustness of both DNA extraction and TaqMan PCR are described.

Chapter 6. All results obtained in this PhD study are summarised and the major implications arising from the data are discussed and placed in a broader context. The relation between morphology and the new classifications based on molecular tools are highlighted.

CHAPTER 2

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

J. de Gruyter[1–3], M.M. Aveskamp[1], J.H.C. Woudenberg[1], G.J.M. Verkley[1], J.Z. Groenewald[1], P.W. Crous[1, 3, 4]

[1]CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands;
[2]National Reference Centre, National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands;
[3]Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands;
[4]Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Mycological Research 113 (2009): 508–519.

Key words: *Ascochyta*, coelomycetes, *Coniothyrium*, *Didymella*, *Microsphaeropsis*, molecular phylogeny, *Pleosporales*, *Pleurophoma*, *Pyrenochaeta*.

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 *Phaeosphaeriaceae* and *Leptosphaeriaceae* respectively.

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 re-classified 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 years were summarised in the recently published *Phoma* Identification Manual (Boerema *et al.* 2004). A total of 223 specific and infra-specific taxa of *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 *Phialopycnidiineae* (*Phialidales, Enteroblastomycetidae*) (Sutton 1980) should be re-evaluated. The *Phialopycnidiineae* 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 *Phialopycnidiineae* 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* proposed.

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		
Allantophoma endogenospora	CBS 178.79		EU754026	EU754125	*Cedrus atlantica (Pinaceae)*	Netherlands
	CBS 600.76		EU754027	EU754126	*Vriesea* sp. *(Bromeliaceae)*	Netherlands
Ampelomyces quercinus	CBS 633.92, ATCC 36786		EU754028	EU754127	unknown	unknown
Ampelomyces quisqualis (TS*)	CBS 129.79		EU754029	EU754128	mildew on *Cucumis sativus*	Canada
	CBS 131.79		EU754030	EU754129	mildew on *Cucumis sativus*	Canada
Aposphaeria populina	CBS 543.70		EU754031	EU754130	*Populus canadensis (Salicaceae)*	Netherlands
Ascochyta caulina, teleom. *Pleospora calvescens*	CBS 246.79, PD 77/655		EU754032	EU754131	*Atriplex hastata (Chenopodiaceae)*	Germany
	CBS 344.78, PD 68/682		EU754033	EU754132	*Atriplex hastata (Chenopodiaceae)*	Netherlands
Ascochyta fabae, teleom. *Didymella fabae*	CBS 524.77		EU754034	EU754133	*Phaseolus vulgaris (Leguminosae)*	Belgium
Ascochyta hordei var. *hordei*	CBS 544.74		EU754035	EU754134	*Triticum aestivum (Gramineae)*	South Africa
Ascochyta pinodes, teleom. *Didymella pinodes*	CBS 374.84, PD 79/674		EU754036	EU754135	*Pisum sativum (Leguminosae)*	Netherlands
	CBS 525.77		EU754037	EU754136	*Pisum sativum (Leguminosae)*	Belgium
Ascochyta pisi (TS)	CBS 126.54		EU754038	EU754137	*Pisum sativum (Leguminosae)*	Netherlands
	CBS 122785, PD 78/517		EU754039	EU754138	*Pisum sativum (Leguminosae)*	Netherlands
Asteromella tiliae	CBS 265.94		EU754040	EU754139	*Tilia platyphyllos (Tiliaceae)*	Austria
Chaetasbolisia erysiphoides (TS)	CBS 148.94		EU754041	EU754140	unknown	unknown
Chaetoconis polygoni (TS)	CBS 405.95		EU754042	EU754141	*Polygonum sachalinense (Polygonaceae)*	Netherlands
Chaetodiplodia	CBS 568.88		EU754043	EU754142	rock	Israel
Chaetophoma	CBS 119963		EU754044	EU754143	man	Brasil

Table 1. (Continued).

Species name (anamorph/teleomorph)	Strain Nr.	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
Chaetosphaeronema hispidulum (TS)	CBS 216.75		EU754045	EU754144	*Anthyllis vulneraria (Leguminosae)*	Germany
	CBS 826.88		EU754046	EU754145	soil	Israel
Cochliobolus sativus (teleom.)	DAOM 226212 (R*)	1271	DQ677995	DQ678045	*(Graminae)*	unknown
Coleophoma crateriformis (TS)	CBS 473.69		EU754047	EU754146	*Phillyrea angustifolia (Oleaceae)*	Spain
Coleophoma maculans	CBS 896.69		EU754048	EU754147	*Populus balsamifera (Salicaceae)*	Netherlands
Coleophoma oleae	CBS 615.72, ATCC 24520, DSM 62123		EU754049	EU754148	*Olea europaea (Oleaceae)*	Greece
Coniella fragariae	CBS 167.84		EU754050	EU754149	*Vitis vinifera (Vitaceae)*	unknown
	CBS 198.82		EU754051	EU754150	soil sample, vine orchard	France
Coniothyrium cerealis	CBS 122787, PD 0703486691		EU754052	EU754151	unknown	Germany
Coniothyrium concentricum	CBS 589.79		EU754053	EU754152	*Yucca (Agavaceae)*	Netherlands
Coniothyrium palmarum (TS)	CBS 400.71		EU754054	EU754153	*Chamaerops humilis (Palmae)*	Italy
	CBS 758.73		EU754055	EU754154	*Phoenix dactylifera (Palmae)*	Israel
Didymella exigua (teleom.)	CBS 183.55		EU754056	EU754155	*Rumex arifolius (Polygonaceae)*	France
Diplodia pinea	CBS 109726		EU754057	EU754156	*Pinus patula (Pinaceae)*	South Africa
	CBS 393.84		EU754058	EU754157	*Pinus nigra (Pinaceae)*	Netherlands
Diplodina coloradensis	CBS 138.25		EU754059	EU754158	*Senecio* sp. *(Compositae)*	unknown
Diplodina microsperma teleom. *Cryptodiaporthe salicella*	CBS 110159		EU754060	EU754159	*Salix* sp. (*Salicaceae)*	Netherlands
Dothidea insculpta (teleom.)	CBS 189.58 (R)	921	DQ247810	DQ247802	*Clematis vitalba (Ranunculaceae)*	France

Table 1. (Continued).

Species name (anamorph/teleomorph)	Strain Nr.	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
Dothiora cannabinae (teleom.)	CBS 737.71 (T* R)	1359	DQ479933	DQ470984	*Daphne cannabina (Thymelaeaceae)*	India
Dothiorella ulmi	CBS 172.34, ATCC 22376, IMI 045826		EU754061	EU754160	*Ulmus* sp. *(Ulmaceae)*	USA
Eleutheromyces subulatus (TS)	CBS 139.90		EU754062	EU754161	*(Russulaceae)*	Canada
	CBS 458.88		EU754063	EU754162	*Lactarius scrobiculatus (Russulaceae)*	Germany
Godronia urceolus (teleom.)	CBS 110435		EU754064	EU754163	*Betula pendula (Betulaceae)*	UK
	CBS 215.58		EU754065	EU754164	*Betula (Betulaceae)*	Norway
Guignardia citricarpa (teleom.)	CBS 102373		EU754066	EU754165	*Citrus aurantium (Rutaceae)*	Netherlands
Leptosphaerulina australis (teleom.) (TS)	CBS 317.83		EU754067	EU754166	*Eugenia aromatica (Myrtaceae)*	Indonesia
	CBS 939.69		EU754068	EU754167	soil	Netherlands
Macrophomina phaseolina	CBS 121.82		EU754069	EU754168	*Sesamum indicum (Pedaliaceae)*	Sweden
	CBS 460.70, IMI 147232		EU754070	EU754169	*Glycine max (Leguminosae)*	Denmark
Microsphaeropsis olivacea (TS)	CBS 116669		EU754071	EU754170	*Sarothamnus scoparius (Leguminosae)*	Netherlands
	CBS 442.83		EU754072	EU754171	*Taxus baccata (Taxaceae)*	Netherlands
Mycosphaerella punctiformis (teleom.)	CBS 113265 (T R)	942	AY490775	DQ470920	*Quercus robur (Fagaceae)*	Netherlands
Neottiosporina paspali	CBS 331.37		EU754073	EU754172	*Paspalum notatum (Gramineae)*	USA
Ophiosphaerella herpotricha	CBS 240.31, ATCC 12279 (R)	1595	DQ767650	DQ767656	unknown	France

Table 1. (Continued).

Species name (anamorph/teleomorph)	Strain Nr.	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
Paraconiothyrium minitans	CBS 122788, PD 07 03486739		EU754074	EU754173	unknown	UK
	CBS 122786, PD 99/1064-1		EU754075	EU754174	unknown	unknown
Phaeosphaeria nodorum (teleom.)	CBS 110109		EU754076	EU754175	*Lolium perenne (Gramineae)*	Denmark
Phialophorophoma litoralis (TS)	CBS 234.92		EU754077	EU754176	*Olea europaea (Oleaceae)*	Italy
	CBS 297.74		EU754078	EU754177	sea water	Serbia and Montenegro
Phoma betae, teleom. *Pleospora betae*	CBS 109410, PD 77/113		EU754079	EU754178	*Beta vulgaris (Chenopodiaceae)*	Netherlands
	CBS 523.66, PD 66/270		EU754080	EU754179	*Beta vulgaris (Chenopodiaceae)*	Netherlands
Phoma complanata	CBS 268.92, PD75/3		EU754081	EU754180	*Anglica sylvestris (Umbelliferae)*	Netherlands
	CBS 100311		EU754082	EU754181	*Heracleum sphondylium (Umbelliferae)*	Netherlands
Phoma cucurbitacearum, teleom. *Didymella bryoniae*	IMI 373225 (R)		AY293779	AY293792	*Cucumis sativus (Cucurbitaceae)*	USA
Phoma exigua var. *exigua*	CBS 101150, PD 79/118		EU754083	EU754182	*Cichorium entybus (Compositae)*	Netherlands
	CBS 431.74, PD 74/2447		EU754084	EU754183	*Solanum tuberosum (Solanaceae)*	Netherlands
Phoma glomerata	CBS 528.66, PD 63/590		EU754085	EU754184	*Chrysanthemum (Compositae)*	Netherlands
	CBS 464.97		EU754086	EU754185	sample bathroom	Netherlands

Table 1. (Continued).

Species name (anamorph/teleomorph)	Strain Nr.	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
Phoma herbarum (TS)	CBS 615.75, PD 73/665, IMI 199779		EU754087	EU754186	*Rosa multiflora (Rosaceae)*	Netherlands
	ATCC 12569, IMI 049948 (R)		AY293778	AY293791	white lead paint	UK
Phoma heteromorphospora	CBS 448.68		EU754088	EU754187	*Chenopodium album (Chenopodiaceae)*	Netherlands
	CBS 115.96, PD 94/1576		EU754089	EU754188	*Chenopodium album (Chenopodiaceae)*	Netherlands
Phoma lingam, teleom. *Leptosphaeria maculans*	CBS 532.66, PD 65/911		EU754090	EU754189	*Brassica* sp. *(Brassicaceae)*	Netherlands
	DAOM 229267 (R)	277	DQ470993	DQ470946	*Brassica sp. (Brassicaceae)*	unknown
Phoma radicina	CBS 102875, PD 78/1097		EU754091	EU754190	*Lycopersicon esculentum (Solanaceae)*	Germany
	CBS 111.79, PD 76/437, IMI 386094		EU754092	EU754191	*Malus sylvestris (Rosaceae)*	Netherlands
Phoma zeae-maydis, teleom. *Didymella zeae-maydis*	CBS 588.69 (T)		EU754093	EU754192	*Zea mays (Gramineae)*	USA
Phyllosticta abietis	CBS 112067		EU754094	EU754193	*Abies concolor (Pinaceae)*	Canada
Phyllosticta minima	CBS 111635		EU754095	EU754194	*Acer rubrum (Aceraceae)*	USA
Plectophomella visci	CBS 122783, PD 74/1021		EU754096	EU754195	*Viscum album (Viscaceae)*	France
Plenodomus fuscomaculans	CBS 559.78, PD 78/241		EU754097	EU754196	*Malus sylvestris (Rosaceae)*	Japan
	CBS 116.16		EU754098	EU754197	*Malus* sp.	USA

Table 1. (Continued).

Species name (anamorph/teleomorph)	Strain Nr.	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
Pleurophoma cava	CBS 115979		EU754099	EU754198	unknown	Netherlands
	CBS 257.68, IMI 331911		EU754100	EU754199	wheat-field soil	Germany
Pleurophoma pleurospora (TS)	CBS 101461		EU754101	EU754200	man, cutaneous lesions	USA
Pseudodiplodia sp.	CBS 255.86		EU754102	EU754201	*Vitis vinifera (Vitaceae)*	Italy
Pseudorobillarda phragmitis (TS)	CBS 842.84		EU754103	EU754202	*Lolium perenne (Gramineae)*	Netherlands
	CBS 398.61, IMI 070678 (T)		EU754104	EU754203	*Phragmitis australis (Gramineae)*	UK
Pyrenochaeta acicola	CBS 122789, PD 0703486800		EU754105	EU754204	*Hordeum vulgare (Gramineae)*	unknown
Pyrenochaeta lycopersici	CBS 306.65, DSM 62931 (T)		EU754106	EU754205	*Lycopersicon esculentum (Solanaceae)*	Germany
Pyrenochaeta nobilis (TS)	CBS 407.76 (T)		EU754107	EU754206	*Laurus nobilis (Lauraceae)*	Italy
Pyrenochaeta romeroi	CBS 252.60, ATCC 13735 (T)		EU754108	EU754207	maduromycosis in man	Venezuela
	CBS 122784, PD 84/1022		EU754109	EU754208	*Hordeum vulgare (Gramineae)*	unknown
Pyrenophora tritici-repentis (teleom.)	OSC 100066 (R)	173	AY544716	AY544672	*(Gramineae)*	Italy
Readeriella mirabilis (TS)	CBS 116293, CPC 10506		EU754110	EU754209	*Eucalyptus fastigata (Myrtaceae)*	New Zealand
	CBS 358.64, IMI 108602		EU754111	EU754210	*Eucalyptus regnans (Myrtaceae)*	Australia

Table 1. (Continued).

Species name (anamorph/teleomorph)	Strain Nr.	AFToL	GenBank accession		Host, substrate	Country
			SSU	LSU		
Rhizosphaera pini (TS)	CBS 189.26		EU754112	EU754211	unknown	Netherlands
Saccharomyces cerevisiae	S 288C, ATCC 204508 (R)				unknown	unknown
Selenophoma linicola	CBS 468.48		EU754113	EU754212	*Linum usitatissimum (Linaceae)*	Canada
Selenophoma mahoniae	CBS 388.92		EU754114	EU754213	*Mahonia repens (Berberidaceae)*	USA
Sirococcus conigenus	CBS 113.75		EU754115	EU754214	*Picea pungens* var. *glauca (Pinaceae)*	Germany
Sphaeropsis visci (TS)	CBS 100163		EU754116	EU754215	*Viscum album (Viscaceae)*	Luxembourg
	CBS 186.97		EU754117	EU754216	*Viscum album (Viscaceae)*	Germany
Sporormiella minima (teleom.)	CBS 524.50 (R)	1256	DQ678003	DQ678056	dung of goat	Panama
Stagonospora foliicola	CBS 110111		EU754118	EU754217	*Phalaris arundinacea (Gramineae)*	USA
Stagonospora neglecta var. *colorata*	CBS 343.86		EU754119	EU754218	*Phragmites australis (Gramineae)*	France
Stenocarpella macrospora (TS)	CBS 117560 (T)		EU754120	EU754219	*Zea mays (Gramineae)*	South Africa
	CBS 164.31		EU754121	EU754220	*Zea mays (Gramineae)*	unknown
Trematophoma sp.	CBS 157.86		EU754122	EU754221	*soil*	USA
Trematosphaeria pertusa (teleom.)	CBS 400.97 (R)	1589	DQ678020	DQ678072	*Fagus* sp. *(Fagaceae)*	Belgium
Wojnowicia hirta (TS)	CBS 160.73		EU754123	EU754222	*Triticum aestivum (Gramineae)*	Germany
	CBS 295.69		EU754124	EU754223	*Lolium multiflorum (Gramineae)*	Germany

* T: ex-type strain, TS: type species of the genus, R: Sequence data reference strain

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 ºC 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 PCR's 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 mM (SSU) or 0.06 mM (LSU) dNTP's, 2 mM $MgCl_2$ and 1× PCR buffer E incomplete (Genaxxon Bioscience). Conditions for amplification for both regions were an initial denaturation step of 5 min at 94 ºC, followed by 35 cycles of denaturation, annealing and elongation and a final elongation step of 7 min at 72 ºC. For the SSU amplification, the 35 cycles consisted of 30 s at 94 ºC, 50 s at 48 ºC and 90 s at 72 ºC; for the LSU 45 s at 94 ºC, 45 s at 48 ºC and 2 min at 72 ºC. The PCR products were analysed by electrophoresis on a 1 % (w/v) agarose gel containing 0.1 µg/ml 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 dataset 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 (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 a MCMC heated chain with a "temperature" value of 0.05. The number of generations, sample frequencies and burn-in ratio were set at 5M, 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).

RESULTS

DNA phylogeny

The aligned sequence length obtained for the SSU and LSU regions was 1 545 (positions 1–1 545 in the TreeBASE alignment) and 1 634 (positions 1 546–3 180 in the TreeBASE alignment) nucleotide characters, respectively.

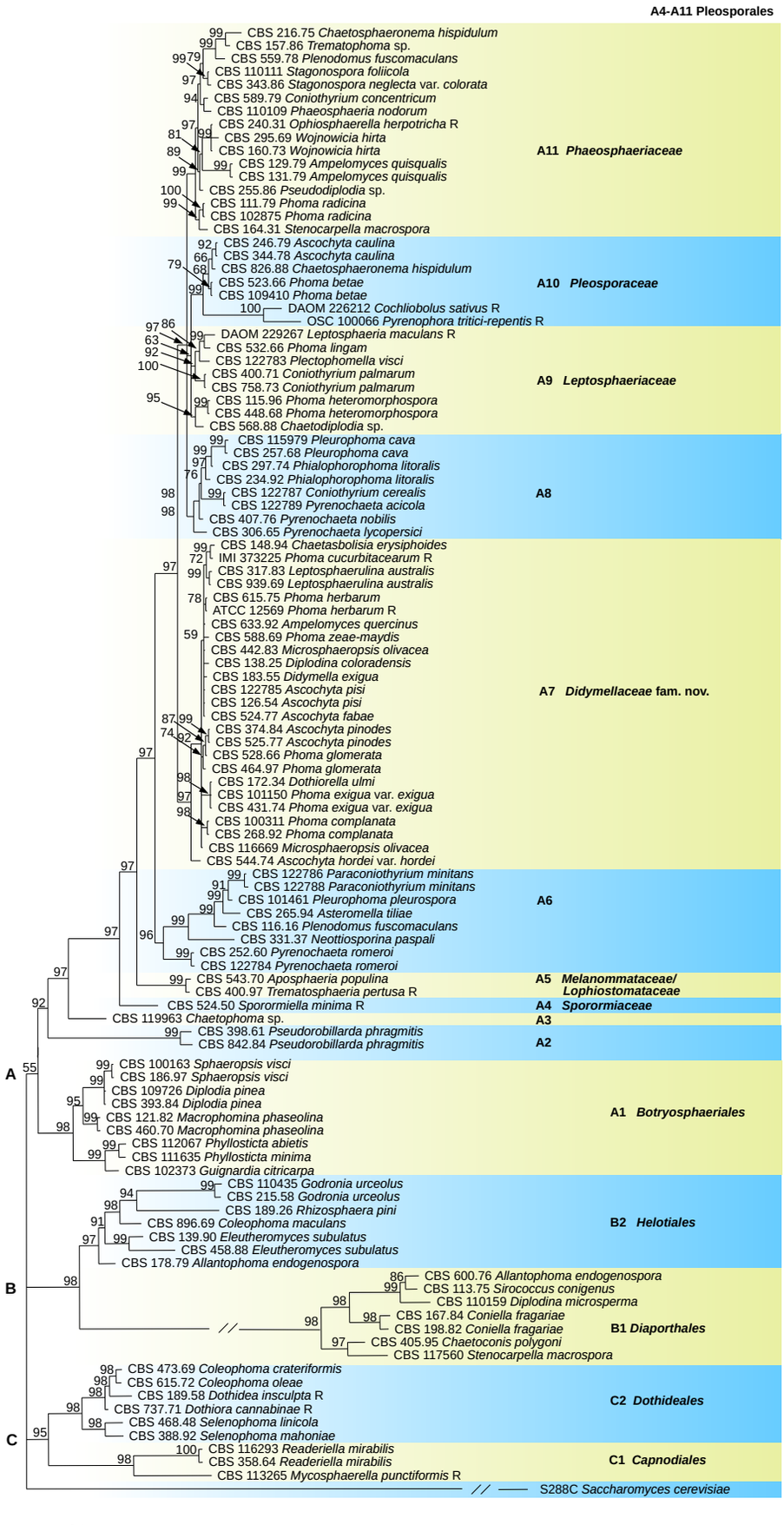
In the alignment, insertions in SSU at the positions 445–1 028 were only observed for the species *Neottiosporina paspali* (syn. *Stagonospora paspali*) (CBS 331.37), *Chaetosphaeronema hispidulum* (CBS 216.75), *Plenodomus fusco-maculans* (CBS 559.78), *Wojnowicia hirta* (CBS 295.69), *Phialophorophoma 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 2 399–2 764, that was only observed in *Godronia urceolus* (CBS 215.58), was excluded from the analyses. The combined dataset used for the analyses contained 2 230 characters, which consisted of 111 taxa including the outgroup taxon. Of these 2 230 characters, 212 and 423 unique site patterns were present for SSU and LSU respectively.

The analysis run in MrBayes resulted in 7 920 trees after 88 1000 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 datasets 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 *Botryosphaeriales*

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

Fig. 1. (p. 29). 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.


A4-A11 Pleosporales
CBS 216.75 Chaetosphaeronema hispidulum
CBS 157.86 Trematophoma sp.
CBS 559.78 Plenodomus fuscomaculans
CBS 110111 Stagonospora foliicola
CBS 343.86 Stagonospora neglecta var. colorata
CBS 589.79 Coniothyrium concentricum
CBS 110109 Phaeosphaeria nodorum
CBS 240.31 Ophiosphaerella herpotricha R
CBS 295.69 Wojnowicia hirta
CBS 160.73 Wojnowicia hirta
CBS 129.79 Ampelomyces quisqualis
CBS 131.79 Ampelomyces quisqualis
CBS 255.86 Pseudodiplodia sp.
CBS 111.79 Phoma radicina
CBS 102875 Phoma radicina
CBS 164.31 Stenocarpella macrospora
A11 Phaeosphaeriaceae
CBS 246.79 Ascochyta caulina
CBS 344.78 Ascochyta caulina
CBS 826.88 Chaetosphaeronema hispidulum
CBS 523.66 Phoma betae
CBS 109410 Phoma betae
DAOM 226212 Cochliobolus sativus R
OSC 100066 Pyrenophora tritici-repentis R
A10 Pleosporaceae
DAOM 229267 Leptosphaeria maculans R
CBS 532.66 Phoma lingam
CBS 122783 Plectophomella visci
CBS 400.71 Coniothyrium palmarum
CBS 758.73 Coniothyrium palmarum
CBS 115.96 Phoma heteromorphospora
CBS 448.68 Phoma heteromorphospora
CBS 568.88 Chaetodiplodia sp.
A9 Leptosphaeriaceae
CBS 115979 Pleurophoma cava
CBS 257.68 Pleurophoma cava
CBS 297.74 Phialophorophoma litoralis
CBS 234.92 Phialophorophoma litoralis
CBS 122787 Coniothyrium cerealis
CBS 122789 Pyrenochaeta acicola
CBS 407.76 Pyrenochaeta nobilis
CBS 306.65 Pyrenochaeta lycopersici
A8
CBS 148.94 Chaetasbolisia erysiphoides
IMI 373225 Phoma cucurbitacearum R
CBS 317.83 Leptosphaerulina australis
CBS 939.69 Leptosphaerulina australis
CBS 615.75 Phoma herbarum
ATCC 12569 Phoma herbarum R
CBS 633.92 Ampelomyces quercinus
CBS 588.69 Phoma zeae-maydis
CBS 442.83 Microsphaeropsis olivacea
CBS 138.25 Diplodina coloradensis
CBS 183.55 Didymella exigua
CBS 122785 Ascochyta pisi
CBS 126.54 Ascochyta pisi
CBS 524.77 Ascochyta fabae
CBS 374.84 Ascochyta pinodes
CBS 525.77 Ascochyta pinodes
CBS 528.66 Phoma glomerata
CBS 464.97 Phoma glomerata
CBS 172.34 Dothiorella ulmi
CBS 101150 Phoma exigua var. exigua
CBS 431.74 Phoma exigua var. exigua
CBS 100311 Phoma complanata
CBS 268.92 Phoma complanata
CBS 116669 Microsphaeropsis olivacea
CBS 544.74 Ascochyta hordei var. hordei
A7 Didymellaceae fam. nov.
CBS 122786 Paraconiothyrium minitans
CBS 122788 Paraconiothyrium minitans
CBS 101461 Pleurophoma pleurospora
CBS 265.94 Asteromella tiliae
CBS 116.16 Plenodomus fuscomaculans
CBS 331.37 Neottiosporina paspali
CBS 252.60 Pyrenochaeta romeroi
CBS 122784 Pyrenochaeta romeroi
A6
CBS 543.70 Aposphaeria populina
CBS 400.97 Trematosphaeria pertusa R
A5 Melanommataceae/ Lophiostomataceae
CBS 524.50 Sporormiella minima R
A4 Sporormiaceae
CBS 119963 Chaetophoma sp.
A3
CBS 398.61 Pseudorobillarda phragmitis
CBS 842.84 Pseudorobillarda phragmitis
A2
A
CBS 100163 Sphaeropsis visci
CBS 186.97 Sphaeropsis visci
CBS 109726 Diplodia pinea
CBS 393.84 Diplodia pinea
CBS 121.82 Macrophomina phaseolina
CBS 460.70 Macrophomina phaseolina
CBS 112067 Phyllosticta abietis
CBS 111635 Phyllosticta minima
CBS 102373 Guignardia citricarpa
A1 Botryosphaeriales
B
CBS 110435 Godronia urceolus
CBS 215.58 Godronia urceolus
CBS 189.26 Rhizosphaera pini
CBS 896.69 Coleophoma maculans
CBS 139.90 Eleutheromyces subulatus
CBS 458.88 Eleutheromyces subulatus
CBS 178.79 Allantophoma endogenospora
B2 Helotiales
CBS 600.76 Allantophoma endogenospora
CBS 113.75 Sirococcus conigenus
CBS 110159 Diplodina microsperma
CBS 167.84 Coniella fragariae
CBS 198.82 Coniella fragariae
CBS 405.95 Chaetoconis polygoni
CBS 117560 Stenocarpella macrospora
B1 Diaporthales
C
CBS 473.69 Coleophoma crateriformis
CBS 615.72 Coleophoma oleae
CBS 189.58 Dothidea insculpta R
CBS 737.71 Dothiora cannabinae R
CBS 468.48 Selenophoma linicola
CBS 388.92 Selenophoma mahoniae
C2 Dothideales
CBS 116293 Readeriella mirabilis
CBS 358.64 Readeriella mirabilis
CBS 113265 Mycosphaerella punctiformis R
C1 Capnodiales
S288C Saccharomyces cerevisiae
0.1

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 *Botryosphaeriales*, 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 *Botryosphaeriaceae* (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)[1] (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 *Pseudorobillarda phragmitis* (TS) (CBS 398.61 (T)[2], 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 Kruys *et al.* (2006).

Subclade A5 consisted of the reference strain *Trematosphaeria 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*. *Trematosphaeria pertusa* was included in a molecular phylogenetic study and has been classified in *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 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.

[1](TS): Type species of the genus
[2](T): Ex-type strain

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 fusco-maculans* (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. fusco-maculans* 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. zeae-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). *Ampelomyces 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*. *Chaetasbolisia 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. *Leptosphaeria 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 *et al.* 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 *Cochliobolus sativus* (DAOM 226212) and *Pyrenophora tritici-rependis* (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 *Phaeosphaeriaceae*. This subclade included several pathogens on Gramineae, such as *Ophiosphaerella 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 *Ampelomyces 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, *Plenodomus fusco-maculans*, 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 clade 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 novae-zelandiae* 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.

Didymella 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* (*Brassiciaceae*) 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, were 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 *Mycosphaerellaceae*. Later, *Didymella* was placed in *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 MB508292.

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. Ascosporae 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 (didymospores) or multiseptate dictyospores.

The obtained phylogenetic results showed that the teleomorphs *Didymella* and *Leptosphaerulina*, producing dictyospores and 1–septate phragmospores, respectively, both are classified in *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 *Phialopycnidiineae* 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 *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 *Leptosphaeriaceae* as well as *Coniothyrium palmarum* and *Plectophomella visci*. Species classified in *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 *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 *Ascochyta 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 *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 zeae-maydis*, type species of *Phoma* sect. *Macrospora* also fits in this *Didymella* clade, and therefore the name *Didymella zeae-maydis* (syn. *Mycosphaerella zeae-maydis*) should be used.

In future studies a further delimitation in *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 *Didymellaceae* is ongoing to establish monotypic teleomorph-anamorph relations. *Phoma lingam* (teleom. *Leptopshaeria maculans*), fits in a subclade to be assigned as *Leptosphaeriaceae*. *Phoma 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*.

ACKNOWLEDGEMENTS

The Dutch Ministry of Agriculture, Nature and Food Quality has supported this research through an endowment of the FES programme “Versterking infrastructuur Plantgezondheid”. We thank Karin Rosendahl for technical assistance and Machiel E. Noordeloos for critical reading the manuscript. Uwe Braun kindly provided the Latin diagnosis.

CHAPTER 3

Systematic reappraisal of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*

J. de Gruyter [1–3], J.H.C. Woudenberg[1], M.M. Aveskamp[1], G.J.M. Verkley[1], J.Z. Groenewald[1], P.W. Crous[1, 3, 4]

[1]CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands;
[2]National Reference Centre, National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands;
[3]Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands;
[4]Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Mycologia 102 (2010): 1066–1081.

Key words: coelomycetes, 18S nrDNA, molecular phylogeny, *Pleosporales*, *Pyrenochaetopsis* gen. nov, *Setophoma* gen. nov, 28S nrDNA, *Neosetophoma* gen. nov.

Abstract: Sequence data from the 18S nrDNA (SSU) and 28S nrDNA (LSU) regions of isolates of *Phoma* section *Paraphoma* were compared with those of representative cultures of the morphologically similar anamorph genera *Pleurophoma* and *Pyrenochaeta*, and of the type species of the *Phoma* sections *Phoma*, *Pilosa* and *Plenodomus*. *Phoma* section *Paraphoma* was found to be highly polyphyletic within the *Pleosporales*, and only distantly related to *Phoma* section *Phoma*. The genus *Paraphoma*, which is based on *Paraphoma radicina*, is reintroduced in *Phaeosphaeriaceae* with two additional taxa. The new genera *Setophoma* and *Neosetophoma*, type species *Setophoma terrestris* comb. nov. and *Neosetophoma samarorum* comb. nov., are introduced and represent species that are closely related to *Paraphoma*, but differ based on their morphological characters and molecular phylogeny. *Phoma coonsii* is transferred to the genus *Chaetosphaeronema* that also belongs to *Phaeosphaeriaceae*. *Pyrenochaetopsis* gen. nov. is introduced to accommodate the type species *Pyrenochaetopsis leptospora* comb. nov., as well as several other species formerly accommodated in *Phoma* and *Pyrenochaeta*. *Pyrenochaetopsis* is closely related to *Pyrenochaeta*, and classified in *Cucurbitariaceae*. *Pleurophoma cava* is transferred to the genus *Pyrenochaeta*. The new genera described elucidate the confusing taxonomy of species in the genera *Phoma*, *Pyrenochaeta* and *Pleurophoma* and recognise monophyletic genera with distinct teleomorph affinities.

INTRODUCTION

Asexual Ascomycetes producing ostiolate pycnidia with hyaline conidia have been classified in a number of coelomycetous genera. These genera are separated mainly on a combination of conidiomatal anatomy, conidiogenesis and conidial morphology. The genus *Phoma* takes a prominent position among these genera because it incorporates many important plant pathogenic species. The nearly 220 *Phoma* species that are recognised currently (Boerema *et al.* 2004), were classified in nine sections according to pycnidial, conidial and cultural characters. Some of these sections are highly artificial from an evolutionary perspective, and overlapping characters among the sections as well as with other genera of coelomycetes do occur (Boerema *et al.* 2004, Aveskamp *et al.* 2008).

The genus *Phoma* is characterised by hyaline, unicellular conidia which may becoming septate due to secondary septation, phialidic, ampulliform to doliiform conidiogenous cells and (sub-)globose, glabrous to pilose or setose, pseudoparenchymatous or scleroplectenchymatous pycnidia. Six *Phoma* sections include species with a known teleomorph in the genera *Didymella*, *Leptosphaeria* or *Pleospora*, indicating the genetic diversity in the genus *Phoma* (van der Aa *et al.* 1990, Boerema *et al.* 1997). A recent molecular study demonstrated that the type species of all *Phoma* sections grouped in the *Pleosporales*. The representatives of five sections clustered in the new family *Didymellaceae*, including *Phoma herbarum*, the type species of the genus *Phoma* (de Gruyter *et al.* 2009).

Phoma radicina, the type species of *Phoma* sect. *Paraphoma*, clustered with species classified in *Phaeosphaeriaceae* (de Gruyter *et al.* 2009). *Phoma* sect. *Paraphoma* is delimitated in the genus *Phoma* by its setose, pseudoparenchymatous pycnidia. The characteristics of the setae (viz. stiff or more hyphal-like) and their length and position on the pycnidial surface, are important for further species delimitation (de Gruyter & Boerema 2002). Species with setose pycnidia producing hyaline conidia were also classified in other genera, particularly *Pyrenochaeta*, for which the branched, filiform, septate, acropleurogenous conidiophores were considered the most important delimitating character (Schneider 1979). However, *Pyrenochaeta* species have been described with setose pycnidia but with a *Phoma*-like conidiogenesis such as *Pyrenochaeta romeroi*, which in rare cases causes human infections (Borelli 1959). Setose pycnidia also have been reported rarely in other *Phoma* sections, such as in the generic type species *Phoma herbarum* and in *Phoma glomerata* (Boerema *et al.* 2004). Furthermore *Phoma* species such as *Phoma clematidina* producing pycnidia with hyphal outgrowths resembling setose pycnidia have been described and classified in *Didymellaceae* (Woudenberg *et al.* 2009). In the related genus *Pleurophoma*, both types of conidiogenesis may be present, but species attributed to this genus produce glabrous pycnidia.

The classification of several species in the genera *Pyrenochaeta*, *Pleurophoma* and *Phoma* sect. *Paraphoma* has been disputed because of the overlapping characters of conidiogenesis and setose pycnidia (Schneider 1979, Grondona *et al.* 1997). The teleomorphs of *Phoma* section *Paraphoma* and *Pleurophoma* are still unknown, whereas in the genus *Pyrenochaeta* two species have been associated with a teleomorph, *Py. parasitica* (teleom. *Herpotrichia parasitica*, von Freyer & van der Aa 1975) and *Py. berberidis* (teleom. *Cucurbitaria berberidis* (Pers.) S.F. Gray, Sivanesan 1984). *Pyrenochaeta berberidis* was excluded from the genus by Schneider (1979) because the pycnidial characteristics were different, and setose pycnidia proved not to be a stable character. The multiple teleomorphs, and the overlapping morphological characters suggest that these anamorph genera are polyphyletic.

To clarify the systematics of these groups, DNA sequence data of the SSU and LSU regions of 61 isolates representing the species currently classified in *Phoma* section *Paraphoma*,

Table 1. Fungal isolates included in the SSU and LSU analyses. Newly generated sequences are indicated in bold.

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
Chaetodiplodia sp.		CBS 568.88	EU754043	EU754142	rock	Israel
Chaetopyrena penicillata		CBS 498.72	**GQ387512**	**GQ387573**	*Medicago sativa (Leguminosae-Papilionoideae)*	South Africa
		CBS 199.89	**GQ387513**	**GQ387574**	soil	Turkey
***Chaetosphaeronema coonsii* comb. nov.**	*Phoma coonsii*	CBS 141.84 (T), CECT 20047, PD 78/241, ATCC 56513	**GQ387514**	**GQ387575**	*Malus sylvestris (Rosaceae)*	Japan
Chaetosphaeronema hispidulum (TS)		CBS 216.75	EU754045	EU754144	*Anthyllis vulneraria (Leguminosae-Papilionoideae)*	Germany
Leptosphaeria doliolum subsp. doliolum (teleom.)		CBS 505.75	**GQ387515**	**GQ387576**	*Urtica dioica (Urticaceae)*	Netherlands
Neophaeosphaeria filamentosa (teleom.)		CBS 102202	**GQ387516**	**GQ387577**	*Yucca rostrata (Agavaceae)*	Mexico
***Neosetophoma samarorum* comb. nov.**	*Phoma samarorum*	CBS 568.94	**GQ387519**	**GQ387580**	*Urtica dioica (Urticaceae)*	Netherlands
	Phoma samarorum	CBS 138.96, PD 82/653	**GQ387517**	**GQ387578**	*Phlox paniculata (Polemoniaceae)*	Netherlands
	Phoma samarorum	CBS 139.96, PD 82/905	**GQ387518**	**GQ387579**	*(Graminae)*	Netherlands
Ophiosphaerella herpotricha (teleom.)		CBS 240.31, ATCC 12279	DQ767650	DQ767656	unknown	France
Paraconiothyrium minitans		CBS 122788, PD 07/03486739	EU754074	EU754173	unknown	UK

Table 1. (Continued).

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
Paraconiothyrium sp.	*Pleurophoma pleurospora*	CBS 101461	EU754101	EU754200	man, cutaneous lesions	USA
Paraphaeosphaeria michoti (teleom.)		CBS 652.86	**GQ387520**	**GQ387581**	*Typha latifolia (Typhaceae)*	Switzerland
***Paraphoma chrysanthemicola* comb. nov.**	*Phoma chrysanthemicola*	CBS 522.66 (NT)	**GQ387521**	**GQ387582**	*Chrysanthemum morifolium (Asteraceae)*	UK
	Phoma chrysanthemicola	CBS 172.70	**GQ387522**	**GQ387583**	*Chrysanthemum morifolium (Asteraceae)*	Germany
***Paraphoma fimeti* comb. nov.**	*Phoma fimeti*	CBS 170.70 (NT), IMI 163514, ATCC 22707	**GQ387523**	**GQ387584**	*Apium graveolens (Apiaceae)*	Netherlands
	Phoma fimeti	CBS 368.91, PD 78/1096	**GQ387524**	**GQ387585**	*Juniperus communis (Cupressaceae)*	Switzerland
Paraphoma radicina (TS)	*Phoma radicina*	CBS 111.79, IMI 386094, PD 76/437	EU754092	EU754191	*Malus sylvestris (Rosaceae)*	Netherlands
	Phoma radicina	CBS 102875, PD 78/1097	EU754091	EU754190	*Lycopersicum esculentum (Solanaceae)*	Germany
Phaeosphaeria caricicola (teleom.)		CBS 603.86	**GQ387529**	**GQ387590**	*Carex pendula (Cyperaceae)*	Switzerland
Phaeosphaeria nodorum (teleom.)		CBS 110109	EU754076	EU754175	*Lolium perenne (Gramineae)*	Denmark
Phaeosphaeria oryzae (teleom.)		CBS 110110	**GQ387530**	**GQ387591**	*Oryza sativa (Oryzeae)*	South Korea
Phaeosphaeriopsis glauco-punctata (teleom.)		CBS 653.86	**GQ387531**	**GQ387592**	*Ruscus aculeatus (Ruscaceae)*	Switzerland

Table 1. (Continued).

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
Phoma betae, teleom. *Pleospora betae*		CBS 109410, PD 77/113	EU754079	EU754178	*Beta vulgaris (Chenopodiaceae)*	Netherlands
Phoma carteri		CBS 101633, PD 84/74	**GQ387532**	**GQ387593**	*Quercus* sp. *Fagaceae)*	Netherlands
		CBS 105.91	**GQ387533**	**GQ387594**	*Quercus robur (Fagaceae)*	Germany
Phoma gardeniae		CBS 626.68, IMI 108771	**GQ387534**	**GQ387595**	*Gardenia jasminoides (Rubiaceae)*	India
		CBS 302.79, PD 79/1156	**GQ387535**	**GQ387596**	airborne	Netherlands Antilles
Phoma glycinicola		CBS 124455, IMI 294986	**GQ387536**	**GQ387597**	*Glycine max (Leguminosae-Papilionoideae)*	Zambia
		CBS 124141, PG-1	**GQ387537**	**GQ387598**	*Glycine max (Leguminosae-Papilionoideae)*	Zimbabwe
Phoma herbarum (TS)		CBS 615.75, IMI 199779	EU754087	EU754186	*Rosa multiflora (Rosaceae)*,	Netherlands
Phoma lingam, teleom. *Leptosphaeria maculans*		DAOM 229267	DQ470993	DQ470946	*Brassica* sp. *(Brassicaceae)*	unknown
Phoma septicidalis		CBS 188.71	**GQ387538**	**GQ387599**	air	Finland
		CBS 856.97	**GQ387539**	**GQ387600**	mineral wool	Finland
		CBS 101636, PD 86/1186	**GQ387540**	**GQ387601**	*Glycine max (Leguminosae-Papilionoideae)*	Zimbabwe
Pyrenochaeta acicola	*Phoma leveillei* var. *leveillei*	CBS 812.95	**GQ387541**	**GQ387602**	waterpipe	Netherlands

Table 1. (Continued).

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
	Phoma leveillei var. *leveillei*	CBS 101634, PD 76/416	**GQ387542**	**GQ387603**	*Fragaria (x) ananassa (Rosaceae)*	Netherlands
	Phoma leveillei var. *leveillei*	CBS 124142, PD 70/407	**GQ387543**	**GQ387604**	*Fragaria (x) ananassa (Rosaceae)*	Netherlands
Pyrenochaeta berberidis, teleom. Cucurbitaria berberidis		CBS 394.84	**GQ387544**	**GQ387605**	*Berberis julianae (Berberidaceae)*	Netherlands
		CBS 363.93	**GQ387545**	**GQ387606**	*Berberis vulgaris (Berberidaceae)*	Netherlands
***Pyrenochaeta cava* comb. nov.**	*Pleurophoma cava*	CBS 257.68, IMI 331911	EU754100	EU754199	wheat field soil	Germany
	Pleurophoma cava	CBS 115953	**GQ387546**	**GQ387607**	*Quercus cerris*	Italy
Pyrenochaeta corni		CBS 248.79	**GQ387547**	**GQ387608**	*Fraxinus excelsior (Oleaceae)*	Netherlands
	Phialophorophoma litoralis (TS)	CBS 234.92	EU754077	EU754176	*Olea europaea (Oleaceae)*	Italy
	Phialophorophoma litoralis	CBS 102828	**GQ387548**	**GQ387609**		Serbia
Pyrenochaeta dolichi		CBS 124143, IMI 217261	**GQ387549**	**GQ387610**	*Dolichos biforus (Leguminosae-Papilionoideae)*	India
		CBS 124140, IMI 217262	**GQ387550**	**GQ387611**	*Dolichos biforus (Leguminosae-Papilionoideae)*	India
Pyrenochaeta lycopersici		CBS 267.59	**GQ387551**	**GQ387612**	*Lycopersicon esculentum (Solanaceae)*	Netherlands

Table 1. (Continued).

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
		CBS 306.65	EU754106/ DQ898289	EU754205	*Lycopersicon esculentum (Solanaceae)*	Germany
Pyrenochaeta mackinnonii		CBS 674.75 (T)	**GQ387552**	**GQ387613**	black grain mycetoma, man	Venezuela
		CBS 110022	**GQ387553**	**GQ387614**	mycetoma of patient	Mexico
Pyrenochaeta nobilis var. ilicis		CBS 292.74	**GQ387554**	**GQ387615**	*Ilex aquifolium (Aquifoliaceae),*	Netherlands
Pyrenochaeta nobilis		CBS 566.75	**GQ387555**	**GQ387616**	*Buxus sempervirens (Buxaceae)*	Netherlands
		CBS 407.76 (NT)	EU754107/ DQ898287	EU754206	*Laurus nobilis (Lauraceae)*	Italy
Pyrenochaeta parasitica, teleom *Herpotrichia parasitica*		CBS 451.73 (T)	**GQ387556**	**GQ387617**	*Abies alba (Pinaceae)*	Germany
		CBS 218.77	**GQ387557**	**GQ387618**	*Abies alba (Pinaceae)*	Germany
Pyrenochaeta quercina		CBS 115095	**GQ387558**	**GQ387619**	*Quercus robur (Fagaceae)*	Italy
Pyrenochaeta quercina	*Phialophorophoma litoralis*	CBS 297.74	**GQ387559**	**GQ387620**	Sea water	Montenegro
Pyrenochaeta romeroi		CBS 252.60 (T), ATCC 13735	EU754108	EU754207	maduromycosis in man	Venezuela
		CBS 122784, PD 84/1022	EU754109	EU754208	*Hordeum vulgare (Gramineae)*	unknown
Pyrenochaeta unguis-hominis		CBS 378.92, IMI 227230	**GQ387560**	**GQ387621**	man, fingernail	UK
	Phoma septicidalis	CBS 112.79, IMI 386095, PD 74/1018	**GQ387561**	**GQ387622**	airborn	UK

Table 1. (Continued).

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
	Phoma leveillei var. *leveillei*	CBS 111112	**GQ387562**	**GQ387623**	*Agapornis* sp. (Aves), lung	Netherlands
***Pyrenochaetopsis decipiens* comb. nov.**	*Phoma terricola*	CBS 343.85, IMI 386097	**GQ387563**	**GQ387624**	*Globodera pallida*, cyst	Netherlands
	Phoma terricola	CBS 165.89	**GQ387564**	**GQ387625**	*Hordeum vulgare (Gramineae)*	Netherlands
***Pyrenochaetopsis indica* comb. nov.**	*Phoma indica*	CBS 124454, IMI 062569(b) (T)	**GQ387565**	**GQ387626**	*Saccharum officinalum (Poaceae)*	India
***Pyrenochaetopsis leptospora* comb. nov.**	*Phoma briardii*	CBS 101635 (T), PD 71/1027	**GQ387566**	**GQ387627**	*Secale cereale (Graminae)*	Europe
	Pyrenochaeta aciicola	CBS 122789, PD 07/03486800	EU754105	EU754204	*Hordeum vulgare (Gramineae)*	unknown
	Phoma leveillei var. *leveillei*	CBS 536.66	**GQ387567**	**GQ387628**	wheat field soil	Germany
	Phoma leveillei var. *leveillei*	CBS 131.69	**GQ387568**	**GQ387629**	dung of sheep	Netherlands
***Pyrenochaetopsis microspora* comb. nov.**	*Phoma leveillei* var. *microspora*	CBS 101333	**GQ387569**	**GQ387630**	*Buellia* (lichen, ascomata)	China
	Phoma leveillei var. *microspora*	CBS 102876 (T), PD 75/911	**GQ387570**	**GQ387631**	water	Montenegro
	Phoma leveillei var. *microspora*	CBS 119739	**GQ387571**	**GQ387632**	*Coffea arabica (Rubiaceae)*	Brazil
Setamelanomma holmii (teleom.)		CBS 110217	**GQ387572**	**GQ387633**	*Picea pungens (Pinaceae)*	USA

Table 1. (Continued).

Species name, final identtfication	Formerly identified	Strain Nr.	GenBank accession		Host, substrate	Country
			SSU	LSU		
***Setophoma sacchari*comb. nov.**	*Phoma setariae*	CBS 333.39	**GQ387525**	**GQ387586**	*Saccharum officinarum (Poaceae)*	Brazil
***Setophoma terrestris* comb. nov.**	*Phoma terrestris*	CBS 335.29	**GQ387526**	**GQ387587**	*Allium sativum (Alliaceae)*	USA
	Phoma terrestris	CBS 377.52, ATCC 11321	**GQ387527**	**GQ387588**	*Allium cepa (Alliaceae)*	Unknown
	Phoma terrestris	CBS 335.87	**GQ387528**	**GQ387589**	*Allium cepa (Alliaceae)*	Senegal
Sporormiella minima		CBS 524.50	DQ678003	DQ678056	dung of goat	Panama

T: ex-type strain

TS: type species of the genus

NT: ex-neotype strain

Pyrenochaeta and *Pleurophoma* were compared with those of 19 isolates representing related genera. The taxonomy of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma* is redefined, a classification at family level indicated and related teleomorph genera identified.

MATERIALS AND METHODS

Isolate selection, cultural studies and DNA extraction

The generic abbreviations used in this study include *Paraphoma* (*Pa*), *Phoma* (*Ph*), *Pleurophoma* (*Pl*), *Pyrenochaeta* (*Py*), *Pyrenochaetopsis* (*Pyr*) and *Setophoma* (*S*). Freeze-dried isolates were obtained from the culture collections of Centraalbureau voor Schimmelcultures (CBS) and the Dutch National Reference Laboratory of the Plant Protection Service (PD) (Table 1). Isolates were revived overnight in 2 mL malt/peptone (50 % / 50 %) liquid medium. Isolates were subsequently transferred and maintained on oatmeal agar (OA) (Crous *et al.* 2009). Cultures growing on OA, malt extract agar (MEA) and cherry-decoction agar (CHA) were studied morphologically as described in Boerema *et al.* (2004). DNA extraction was conducted 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 ºC 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 PCR's were performed and analysed as described by de Gruyter *et al.* (2009). Sequencing of the PCR amplicons was conducted with the same primer combinations, although 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, the Netherlands) and analyzed with an ABI Prism 3730xl Sequencer (Applied Biosystems) according to manufacturer instructions. Consensus sequences were computed from both forward and reverse sequences using the Bionumerics v4.61 software package (Applied Maths, Sint-Martens-Latem, Belgium) and were lodged with GenBank.

Phylogenetic analyses

The sequence data of 61 cultures generated in this study were aligned with sequences of 19 cultures that were obtained from GenBank (Table I). The alignment was automatically calculated by the BioNumerics software package, but manual adjustments for improvement were made by eye where necessary. The phylogeny was rooted to *Sporormiella minima* (CBS 524.50). The phylogenetic analyses were done for each dataset and based on similar tree topologies obtained, as well as with a combined alignment consisting of both SSU and LSU regions. A Neighbour-Joining (NJ) distance analysis was conducted using PAUP* v4b10 (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 MrBayes v3.1.2 program (Huelsenbeck & Ronqvist 2001) in two parallel runs, us,ing the default settings but with the following adjustments: the GTR model with gamma-distributed rate was selected for both partitions using the Findmodel freeware (http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html), and a MCMC heated chain was set with a "temperature" value of 0.05. The number of generations, sample frequencies and burn-

in ratio were set at 5M, 10 and 0.1 respectively and the run was stopped automatically as soon as the average standard deviation of split frequencies fell below 0.01. The resulting trees were printed with TreeView v1.6.6 (Page 1996) and alignments and trees are lodged with TreeBASE (www.treebase.org), including those trees that were obtained for the single SSU and LSU datasets.

RESULTS

The aligned sequence matrix obtained for the SSU and LSU regions had a total length of 2 633 nucleotide characters, 1332 (positions 1–1 332 in the TreeBASE alignment) and 1 301 (positions 1 333–2 633 in the TreeBASE alignment) respectively. In the alignment an insertion in the SSU at the positions 440–796 was observed for two cultures of *Phialophora litoralis, Phoma samarorum* and *Pyrenochaeta mackinnonii*, and a culture of *Ophiosphaerella herpotricha* (=*Ophiobolus herpotrichus*), *Phoma coonsii* and *Chaetosphaeronema hispidulum.* This insertion was excluded from further phylogenetic analyses. The combined dataset used in the analyses included 80 taxa and contained 2276 characters with 76 and 193 unique site patterns for SSU and LSU respectively. The PAUP* NJ analyses with the three substitution models showed similar tree topologies.

The analysis run in MrBayes resulted in 408 202 trees after 2 041 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 188 452 trees. For individual SSU and LSU alignments trees were compared by eye and the tree topology of the individual datasets was similar to each other and to the tree obtained from the combined alignment. The results of the PAUP NJ analyses were congruent with those obtained in the Bayesian analysis.

The phylogenetic tree based on the combined LSU and SSU sequence data calculated with MrBayes (Fig. 1) demonstrated that *Phoma* sect. *Paraphoma* was highly polyphyletic in that the taxa involved segregated into four distinct clades in the *Pleosporales*. The type species *Ph. radicina* grouped with *Ph. chrysanthemicola* and *Ph. fimeti* (support value > 70 %) in a main clade B that represents *Phaeosphaeriaceae.* In the same clade *Phoma terrestris* and *Ph. setariae,* two other species currently ascribed to *Phoma* sect. *Paraphoma,* clustered with *Phoma samarorum*, currently classified in *Phoma* section *Heterospora*. However, the support value (52 %) of this subclade in *Phaeosphaeriaceae* is low. *Phoma coonsii*, although being classified in *Phoma* section *Plenodomus*, also fits in *Phaeosphaeriaceae*, and is most related to *Chaetosphaeronema hispidulum* and the teleomorph *Phaeosphaeria carcicola.* The *Phoma* species that group in these different clades in *Phaeosphaeriaceae* are related only distantly to the *Phoma* species recently classified in *Didymellaceae* (de Gruyter *et al.* 2009) and therefore, *Phoma* sect. *Paraphoma* is elevated here to generic level by reinstatement of the genus *Paraphoma* previously described by Morgan-Jones & White (1983). The only member of *Phoma sect. Paraphoma* clustering with *Phoma herbarum* in *Didymellaceae* was *Phoma gardeniae*, which produces pycnidia with short setae. Also *Chaetopyrena penicillata*, characterised by setose pycnidia and *Phoma*-like conidiogenous cells, grouped with *Ph. herbarum* in *Didymellaceae*.

The other seven species currently classified in *Phoma* sect. *Paraphoma* clustered in clades that are not related to *Phaeosphaeriaceae* or to *Didymellaceae. Phoma carteri* clustered in clade C with an isolate identified as *Chaetodiplodia* sp. being the most related species. *Pyrenochaeta glycinicola* and *Py. dolichii*, both pathogens on Leguminosae and of tropical origin, and *Ph. septicidalis* also grouped in this clade.

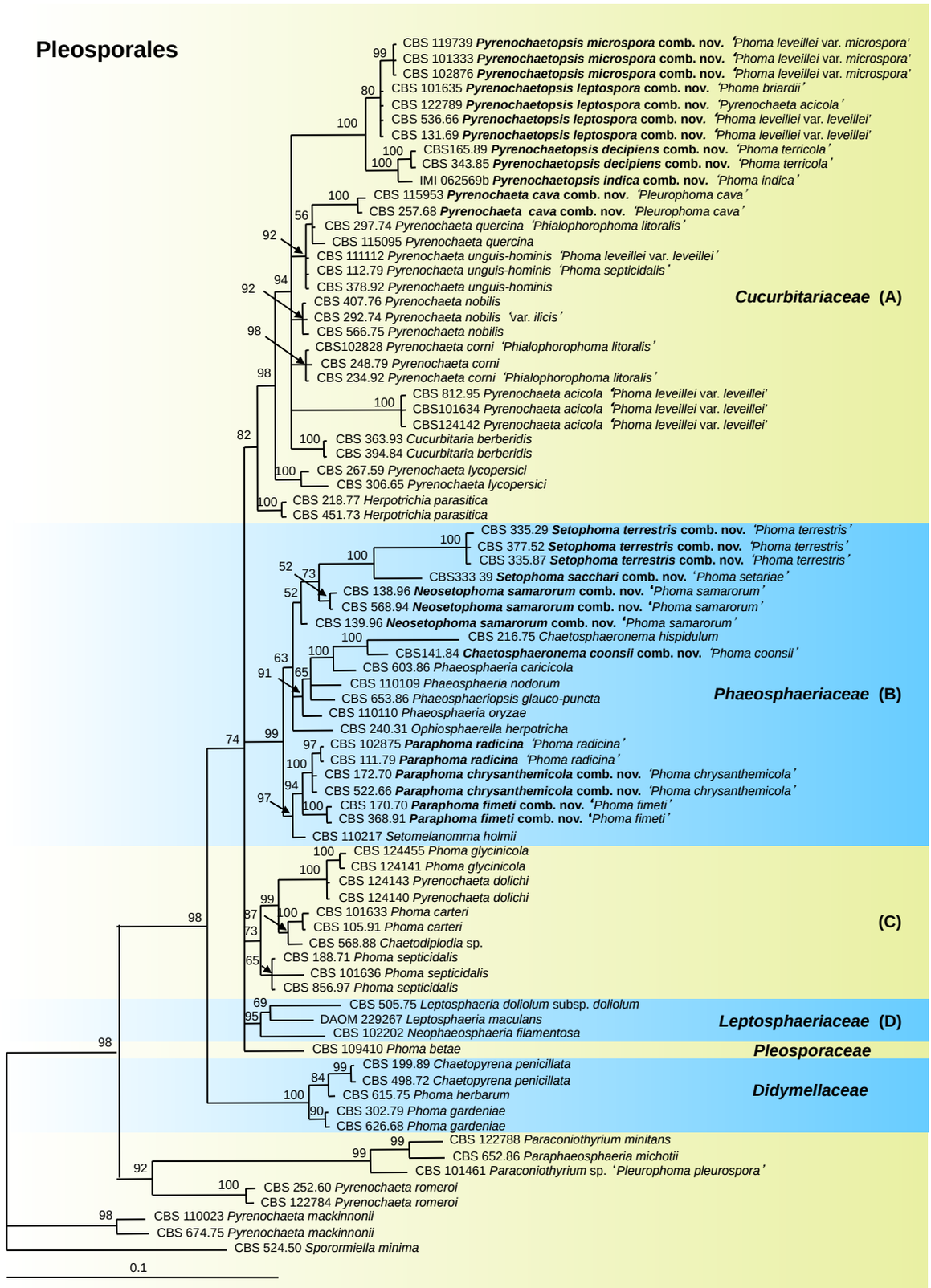
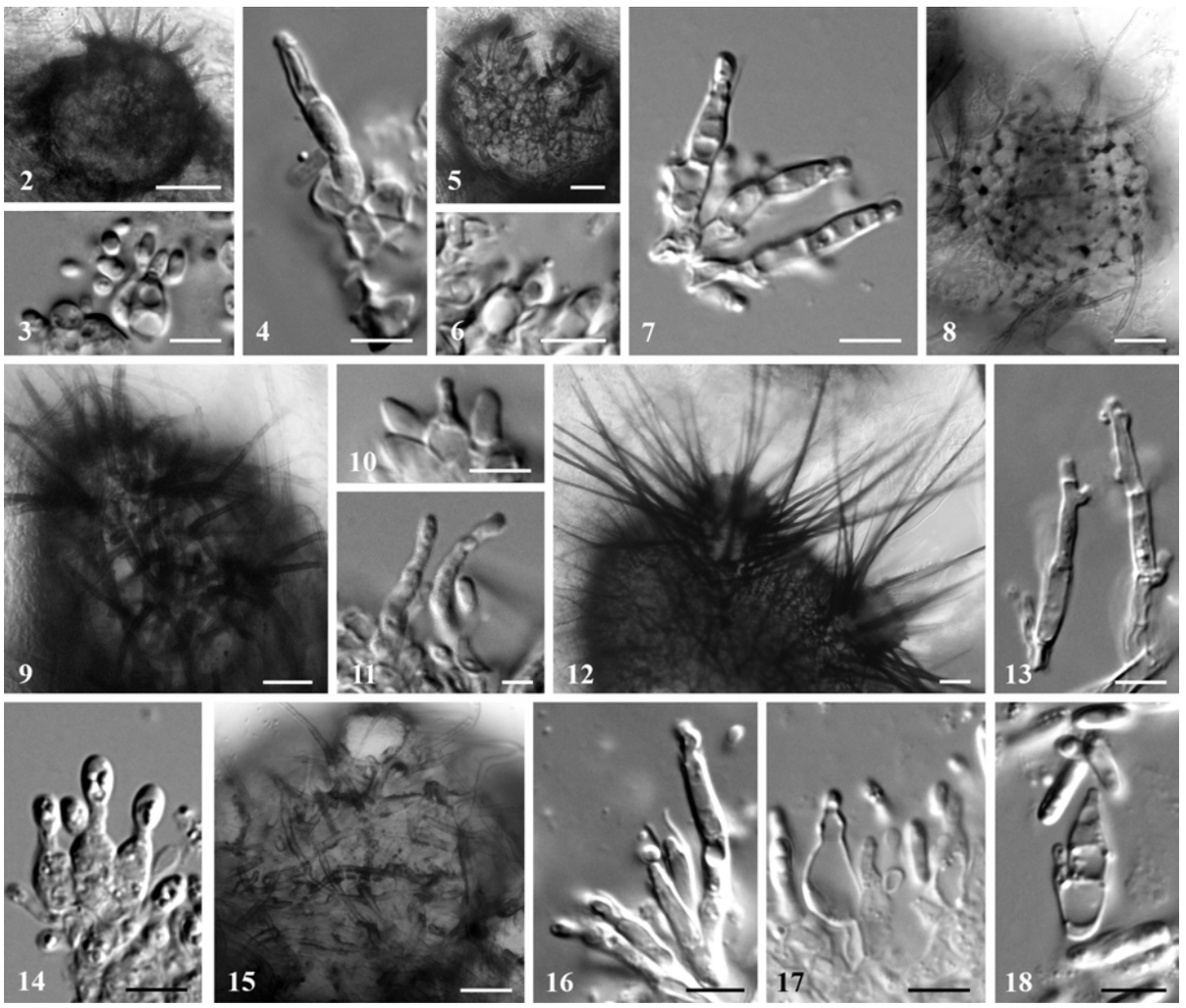


Fig. 1. The phylogenetic relationships of species in the genus *Paraphoma*, *Pyrenochaeta*, *Pyrenochaetopsis* gen. nov. and related genera based on the strict consensus tree from a Bayesian analysis of 80 SSU/LSU sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted with *Sporormiella minima* (CBS 524.50).



Figs 2–4. *Pyrenochaeta unguis-hominis* IMI 227230 = CBS 378.92. 2. Setose pycnidium. 3. *Phoma*-like conidiogenous cells. 4. Elongate, septate conidiophore. **Figs 5–7.** *Pyrenochaeta unguis-hominis* IMI 386095 = CBS 112.79, originally identified as *Phoma septicidalis*. 5. Setose pycnidium. 6. *Phoma*-like conidiogenous cells. 7. Elongate, septate conidiophores with acropleurogenous conidiogenesis and a discrete collarette. **Fig. 8.** *Pyrenochaeta cava* comb. nov., CBS 115953. Pycnidium with setae-like mycelial hairs. **Figs 9–11.** *Pyrenochaeta corni*. CBS 248.79. 9. Setose pycnidium. 10. *Phoma*-like conidiogenous cells. 11. Elongate conidiogenous cells. **Figs 12–13.** *Pyrenochaeta nobilis* CBS 292.74. 12. Setose pycnidium. 13. Elongate, septate conidiophores with acropleurogenous conidiogenesis. **Fig. 14.** *Pyrenochaeta acicola*, CBS 101634. *Phoma*-like conidiogenous cells. **Figs 15–16.** *Pyrenochaeta corni*, CBS 234.92, originally identified as *Phialophorophoma litoralis*. 15. Setose pycnidium. 16. Elongate, septate conidiophores, with acropleurogenous conidiogenesis, and discrete collarette. **Figs 17–18.** *Pyrenochaetopsis leptospora* comb. nov. CBS 101635. 17. *Phoma*-like conidiogenous cells. 18. Elongate conidiogenous cells. Bars: 2, 12 = 50 µm; 3–4, 6–7, 10–11, 13–14, 16–18 = 5 µm; 5, 8–9, 15 = 20 µm.

Phoma leveillei var. *leveillei* has been recorded as a cosmopolitan soil fungus, a heterogeneous species with much variability in morphological and physiological characters (Boerema *et al.* 2004). Our molecular data revealed that *Ph. leveillei* var. *leveillei* is indeed a species complex and moreover, included several isolates preserved under an incorrect name. Two isolates obtained respectively from wheat field soil and sheep dung (CBS 536.66, 131.69) were phylogenetically

identical to *Phoma briardii* (clade A), a morphologically similar species distinguished only by a minor difference in conidial length (de Gruyter & Boerema 2002). An isolate preserved as *Pyrenochaeta acicola* (CBS 122789), a synonym of *Ph. leveillei* var. *leveillei*, was similar to *Ph. briardii*.

Phoma leveillei var. *microspora*, *Ph. terricola* and *Ph. indica* could also be attributed to this group but both are slightly different morphologically and genetically. Most species in this group are soil-borne organisms and frequently found in association with members of the Gramineae. This group formed a distinct subclade in clade A, which mainly includes *Pyrenochaeta* species, among those the type species of the genus, *Py. nobilis*. Three other *Ph. leveillei* isolates (CBS 812.95, 101634, 124142), one isolated from a waterpipe and two from *Fragaria* (×) *ananassa*, respectively, could also be recognised in clade A, which further include the *Pyrenochaeta* species *Py. lycopersici*, *Py. parasitica* and *Py. berberidis.*

Isolate CBS 111112, preserved as *Phoma leveillei* var. *leveillei* and obtained from a lung of a lovebird (*Agapornis* sp.), was genetically similar to the human pathogen *Pyrenochaeta unguis-hominis.* Both species share *in vitro* characters but their conidiogenesis was considered to be significantly different and therefore, the species were classified respectively in *Phoma* (de Gruyter & Boerema 2002) and *Pyrenochaeta* (Punithalingam & English 1975). However, re-examination of this *Ph. leveillei* var. *leveillei* isolate revealed that in addition to the *Phoma*-like conidiogenous cells filiform, septate, acropleurogenous conidiophores are produced in pycnidia that are covered by relatively short setae.

Similar setose pycnidia with both types of conidiogenesis were also observed in the *Pyrenochaeta unguis-hominis* isolate IMI 227230 = CBS 378.92 (Figs 2–4). Branched, septate conidiophores have also been described as 'sometimes present' in *Phoma septicidalis* (Kinsey 2002). Isolate IMI 386095 = CBS 112.79, obtained from an air sample and originally identified as *Phoma septicidalis* (de Gruyter & Boerema 2002) was congruent with *Py. unguis-hominis* in the present study and indeed produced branched, septate, acropleurogenous conidiophores (Figs 5–7).

Pyrenochaeta unguis-hominis has been reported from human nails thus far, but these findings suggest that *Py. unguis-hominis* is a more common species that may be dispersed through air. *Pyrenochaeta quercina* and an isolate that originally was identified as *Phialophorophoma litoralis* (CBS 297.74) grouped in the same subclade, whereas both isolates of *Pleurophoma cava* clustered in a closely related subclade.

Pleurophoma cava has been described as producing glabrous pycnidia with two types of conidiogenesis similar to *Pyrenochaeta* or more *Phoma*-like (Boerema *et al*. 1996). However, in a recently obtained isolate, CBS 115953, the development of pycnidia with setae-like mycelial hairs has been observed *in vitro* (Fig. 8). The combined characters of two types of conidiogenesis produced in setose pycnidia also have been observed further in *Pyrenochaeta corni* (Figs 9–11). Based on these findings, *Pl. cava* is transferred to *Pyrenochaeta* in the taxonomy section of this paper.

Two isolates identified as *Phialophorophoma litoralis* (CBS 234.92, 102828) clustered with the reference isolate of *Pyrenochaeta corni* (CBS 248.79) in clade A. The human pathogens *Pyrenochaeta romeroi* and *Py. mackinnonii* clustered in separate clades only distantly related to the type species *Py. nobilis*, and therefore have to be excluded from the genus *Pyrenochaeta*, as was suggested already based on *in vitro* characters by Schneider (1979). Isolate CBS 101461, identified as *Pleurophoma pleurospora*, grouped in the *Paraconiothyrium/Paraphaeosphaeria* cluster as previously described (de Gruyter *et al.* 2009) and therefore is treated here as a species of *Paraconiothyrium*.

TAXONOMY

A modified classification of the *Phoma* species currently included in *Phoma* sect. *Paraphoma* is provided below. The genus *Paraphoma* is reintroduced, and two new related genera, *Setophoma* and *Neosetophoma*, are described. *Pyrenochaetopsis* gen. nov. is introduced here to accommodate the species closely related to *Pyrenochaeta* in *Cucurbitariaceae*.

Chaetosphaeronema coonsii (Boerema & Loer.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514647.
Basionym: *Phoma coonsii* Boerema & Loer., Trans. Brit. Mycol. Soc. 84: 289. 1985.

Specimens examined: **Japan**, Mazioka, on bark of *Malus pumila* 'Starking Delicious' (*Rosaceae*), 1978, H. Koganezana, **holotype** of *Phoma coonsii* Boerema & Loer. CBS-H 141.84, culture ex-holotype CBS 141.84 = PD 78/241 = ATCC 56513 = CECT 20047).

Notes: The ex-type culture was obtained from *Malus pumilae* 'Starking Delicious', an American apple tree variety, growing in Japan. The fungus was considered to be *Plenodomus fusco-maculans* (Sacc.) Coons (= *Phoma fusco-maculans* Sacc. = *Aposphaeria fusco-maculans* (Sacc.) Sacc. However, the type material of *A. fusco-maculans*, preserved in Saccardo's herbarium (PAD), is a species of *Aposphaeria* (Boerema & Loerakker, l.c.), and therefore, it was concluded that Coons' *Plenodomus fusco-maculans* was a misidentification. The name *Phoma coonsii* was introduced for '*Plenodomus fusco-maculans*' sensu Coons, J. Agric. Res. 5: 714. 1916, Rep. Mich. Acad. Sci. 17: 122. 1916. (Boerema & Loerakker, l.c.). LSU and SSU sequences of *Phoma coonsii* CBS 141.84 (= CBS 559.78, deposited as *Phoma fusco-maculans*) obtained in this study were different from those of isolate CBS 116.16 ex *Malus* sp. USA, deposited by Coons (de Gruyter *et al.* 2009), so the Japanese isolate is described as *Chaetosphaeronema coonsii*, a distinct species.

Neosetophoma Gruyter, Aveskamp & Verkley, **gen. nov.** MycoBank MB514648.
Etymology: *neo* Lat. = new. Refers to the similarity to *Setophoma*.

Pycnidia solitaria vel confluentia, superficialia in agaro, globosa vel irregularia, eminentiis hypharum vestita, ostiolis papillatis, interdum collis longis, mellea vel olivacea/olivaceo-nigra, parietes cellulis pseudoparenchymatis. Cellulae conidiogenae hyalinae, phialidicae, discretae. Conidia pallide lutea, continua vel septata, ellipsoidea vel cylindrica, plerumque una extremitate attenuata, saepe guttulata.

Pycnidia solitary to confluent, on upper surface of agar, globose to irregular, with mycelial outgrowths, with papillate ostioles, sometimes developing long necks, honey to olivaceous/olivaceous-black, the wall with pseudoparenchymatal cells. Conidiogenous cells hyaline, phialidic, discrete. Conidia slightly yellowish, aseptate, or septate, ellipsoidal to cylindrical, usually attenuate at one end, often guttulate.

Type species: *Neosetophoma samarorum* (Desm.) Gruyter, Aveskamp & Verkley.

Neosetophoma samarorum (Desm.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514649.

Basionym: *Phoma samarorum* Desm., Pl. Cryptog. N. France [ed. 1] Fasc. 7, No. 349. 1828.

For full synonymy see Boerema *et al.* (2004).

Specimens examined: **France**, *Fraxinus excelsior* (*Oleaceae*), 1828, Desmazières, Pl. Cryptog. N. France [ed. 1] Fasc. 7, No. 349 P, **holotype** of *Phoma samarorum* Desm. **The Netherlands**, Bladel, on *Phlox paniculata* (*Polemoniaceae*), 1982, G.H. Boerema, **epitype designated here** CBS H-20319, culture ex-epitype CBS 138.96.

Note: A pycnidial dimorph with large stagonosporoid conidia that are 1–3 septate, up to 25 µm long and 3.5 µm wide, has been described as *Stagonosporopsis fraxini* (Allesch.) Died.

Paraphoma Morgan-Jones & J.F. White, Mycotaxon 18: 58. 1983.
≡ *Phoma* sect. *Paraphoma* (Morgan-Jones & J.F. White) Boerema, van der Aa *et al.*, Stud. Mycol. 32: 7. 1990.

Type species: *Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White.

Paraphoma radicina (McAlpine) Morgan-Jones & J.F. White, Mycotaxon 18: 60. 1983.
Basionym: *Pyrenochaeta radicina* McAlpine, Fung. Dis. Stone-fruit-trees Melb. 127. 1902.
≡ *Phoma radicina* (McAlpine) Boerema, Boerema & Dorenbosch, Versl. Meded. Plantenziektenk.Dienst Wageningen 153 (Jaarb. 1978): 20. 1979.

Specimens examined: **Australia**, Shepparton, Victoria. On roots of *Prunus cerasus* (*Rosaceae*), 21 Oct 1901, Piscott, 2064.3 **Holotype** of *Pyrenochaeta radicina* McAlpine, VPRI. **The Netherlands**, Wanssum, on *Malus sylvestris* (*Rosaceae*), grafting base 'M 106', Jun 1976, G.H. Boerema, **epitype designated here** CBS H-16560, culture ex-epitype CBS 111.79 = IMI 386094 = PD 76/437). **Germany**, on *Lycopersicon esculentum*, (*Solanaceae*), 1976, G.H. Boerema, CBS 102875 = PD 76/1097.

Paraphoma chrysanthemicola (Hollós) Gruyter, Aveskamp & Verkley, **comb. nov.**
MycoBank MB514650.
Basionym: *Phoma chrysanthemicola* Hollós, Ann. Hist.-Nat. Mus. Natl. Hung. 5: 456. 1907.
= *Phoma radicis-oxycocci* Ternetz, Jahrb. Wiss. Bot. 44: 365. 1907.

Specimens examined: **United Kingdom**, Kent, on stem of *Chrysanthemum morifolium* (*Asteraceae*), 1963, H.J. Wilcox, **neotype** of *Phoma chrysanthemicola* Hollós CBS H-5161, culture ex-neotype CBS 522.66. **Germany**, Berlin. On root of *Chrysanthemum morifolium*, Oct 1967, R. Schneider, CBS 172.70.

Note: Dorenbosch (1970) selected a neotype because the original herbarium material had been lost.

Paraphoma fimeti **(**Brunaud) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514651.
Basionym: *Phoma fimeti* Brunaud, Bull. Soc. Mycol. France 36 [= Vol II, ser. 11]: 338. 1889.

Specimens examined: **The Netherlands**, Zwijndrecht, from soil, Dec 1966, M.A. de Waard,

neotype of *Phoma fimeti* Brunaud CBS H-2011, culture ex-neotype CBS 170.70. **Switzerland**, on *Juniperus communis* (*Cupressaceae*), Jun 1991, G.H. Boerema, CBS 368.91 = PD 78/1096.

Note: The original herbarium material was lost so a neotype was designated (Dorenbosch 1970).

Pyrenochaeta acicola (Moug. & Lév.) Sacc., Syll. Fung. 3: 220. 1884 [as '(Lév.) Sacc.'].
Basionym: *Vermicularia acicola* Moug. & Lév. apud Léveillé, Ann. Sci. Nat., Bot. Ser. 3, 9: 259. 1848 [as 'Moug. Lév.']; not *Phoma acicola* sensu Saccardo, Syll. Fung. 3: 100. 1884 [as '(Lév.) Sacc.'; = *Sclerophoma pythiophila* (Corda) Höhn.].
≡ *Phoma leveillei* Boerema & G.J. Bollen, Persoonia 8: 115. 1975, var. *leveillei*.

For full synonymy see Boerema *et al*. (2004).

Specimens examined: **The Netherlands**, Someren, on waterpipe, Dec. 1995, Y. Driessen, **neotype designated here** CBS H-20314, culture ex-neopitype CBS 812.95). **The Netherlands**, Arnhem, on *Fragaria* (×) *ananassa* (*Rosaceae*), 1976, M.M.J. Dorenbosch, CBS 101634 = PD 76/416. **The Netherlands**, On *Fragaria* (×) *ananassa*, 1970, M.M.J. Dorenbosch, CBS 124142 = PD 70/407.

Note: The original herbarium material of *Vermicularia acicola* Moug. & Lév. could not be located and is presumed missing so a neotype was herein designated.

Pyrenochaeta cava (Schulzer) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514652.
Basionym: *Phoma cava* Schulzer, Verh. Zool.-Bot. Ges. Wien 21: 1248. 1871.
≡ *Pleurophoma cava* (Schulzer) Boerema, Loerakker & Hamers, Persoonia 16: 172. 1996.

For full synonymy see Boerema & Dorenbosch (1973) and van der Aa & Vanev (2002).

Specimens examined: **Croatia**, Vinkovci, from *Cydonia vulgaris* (*Rosaceae*), 1871, Schulzer von Müggenburg, illustrated plate 13, fig. 28, **holotype** of *Phoma cava* Schulzer. **Germany**, Kiel-Kitzeberg, from wheat-field soil, **epitype designated here** CBS H-20320, culture ex-epitype CBS 257.68 = IMI 331911. **Italy**, on branch of *Quercus cerris* (*Fagaceae*), CBS 115953.

Pyrenochaetopsis Gruyter, Aveskamp & Verkley, **gen. nov.** MycoBank MB514653.
Etymology: *–opsis* Greek = resembling, refers to the resemblance to *Pyrenochaeta*.

Pycnidia solitaria vel confluentia, superficialia vel in agaro immersa, globosa vel subglobosa, setosa, ostiolis papillatis vel non-papillatis, mellea/citrina vel olivacea/olivaceo-nigra, parietes cellulis pseudoparenchymatis. Cellulae conidiogenae hyalinae, phialidicae, discretae, vel integratae in conidiophoris acropleurogenis septatis. Conidia continua, cylindrica/ellipsoidea vel allantoidea, guttulata.

Pycnidia solitary to confluent, on upper surface or submerged in agar, globose to subglobose, setose, with non-papillate or papillate ostiole, honey/citrine to olivaceous/olivaceous-black, the wall with pseudoparenchymatal cells. Conidiogenous cells hyaline, phialidic, discrete, or

integrated in septate, acropleurogenous conidiophores. Conidia aseptate, cylindrical/ellipsoidal to allantoid, guttulate.

Type species: *Pyrenochaetopsis leptospora* (Sacc. & Briard) Gruyter, Aveskamp & Verkley.

Pyrenochaetopsis leptospora (Sacc. & Briard) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514654.
Basionym: *Pyrenochaeta leptospora* Sacc. & Briard, Revue Mycol. (Toulouse) 11: 16. 1889; not *Pyrenochaeta leptospora* Speg., Anales Mus. Nac. Buenos Aires 13: 354. 1910; not *Phoma leptospora* Speg., Fungi Chilens. 145. 1910, nor *Phoma leptospora* Sacc., Ann. Mycol. 11: 553. 1913.

≡ *Pyrenochaeta spegazziniana* Trotter, Sylloge Fung. 25: 190. 1931 (illegitimate: superfluous name, Boerema *et al.* 2004).
≡ *Phoma briardii* Gruyter & Boerema, Persoonia 17: 555. 2002 [‘2001’].

Specimens examined: **France**, near Troyes, on stem pieces of *Milium effusum* (*Poaceae*), May 1888, **holotype** *Pyrenochaeta leptospora* Sacc. & Briard, coll. P.A. Briard ‘no. 6’ PAD. **The Netherlands**, on *Secale cereale* (*Poaceae*), **epitype designated here** CBS H-20313, culture ex-epitype CBS 101635 = PD 71/1027. **Germany**, Kiel, Kitzeberg, on wheat field soil, CBS 536.66. **The Netherlands,** on sheep dung, 27 Oct 1968, H.A. van der Aa, culture CBS 131.69. **The Netherlands**, on *Hordeum vulgare* (*Poaceae*), J.W. Veenbaas, CBS 122789 = PD 07/03486800.

Pyrenochaetopsis decipiens (Marchal) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514655.
Basionym: *Pyrenochaeta decipiens* Marchal, Bull. Soc. Roy. Bot. Belg. 30: 139. 1891, not *Phoma decipiens* Mont., Fl. chil. cell. 7: 488. 1852.

= *Phoma terricola* Boerema, Versl. Meded. Plantenziektenk. Dienst Wageningen 163 (Jaarb. 1984): 38. 1985. Not *Phoma terricola* ‘Agnihothrudu’, Soil Sci. 91: 135. 1961 [a nomen nudum erroneously adopted in Mathur, Coelom. India: 185. 1979].

Specimens examined: **The Netherlands**, Hoofddorp, On cyst of *Globodera pallida*, May 1985, D. Hugo, no 727, **neotype** CBS H-20315, culture ex-neotype CBS 343.85 = IMI 386097. **The Netherlands**, Marknesse, on roots of *Hordeum vulgare* (*Poaceae*), M. Barth, nr. 830, 1988, CBS 165.89.

Note: The original herbarium material is lost, so a neotype was designated by Schneider (1979).

Pyrenochaetopsis indica **(**T.S. Viswan.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514656.
Basionym: *Pyrenochaeta indica* T.S. Viswan., Curr. Sci. 26(4): 118. 1957.

≡ *Phoma indica* (T.S. Viswan.) Gruyter & Boerema, Persoonia 17: 556. 2002.

Specimen examined: INDIA, Poona. On leaf spot of *Saccharum officinarum* (*Poaceae*), **holotype** of *Pyrenochaeta indica* AMH-11, culture ex-holotype IMI 062569 = CBS 124454.

Pyrenochaetopsis microspora (Gruyter & Boerema) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514657.
Basionym: *Phoma leveillei* var. *microspora* Gruyter & Boerema, Persoonia 17: 553. 2002 [‘2001’].

Specimens examined: **Montenegro,** Lake of Skadar, water, 1975, **holotype** of *Phoma leveillei* var. *microspora* Gruyter & Boerema HLB 999-242399, culture ex-type CBS 102876 = PD 75/911, **China**, Hong Kong. Lichen *Buellia*, Jun 1998, A. Aptroot, CBS 101333. **Brazil**, Minas Gerias, Lavras, on leaf of *Coffea arabica*, (*Rubiaceae*), L.H. Pfenning, CBS 119739.

Setophoma Gruyter, Aveskamp & Verkley, **gen. nov.** MycoBank MB514658.
Etymology: *seto* Lat. = bristle. Refers to the setose pycnidia.

Pycnidia solitaria vel confluentia, superficialia vel in agaro immersa, globosa vel subglobosa, setosa, ostiolis papillatis, mellea vel olivaceo-nigra, parietes cellulis pseudoparenchymatis. Cellulae conidiogenae hyalinae, phialidicae, discretae. Conidia continua, ellipsoidea vel subcylindrica/subfusoidea, guttulata.

Pycnidia solitary to confluent, on upper surface or submerged in agar, globose to subglobose, setose, with papillate ostioles, honey to olivaceous/olivaceous-black, the wall with pseudoparenchymatal cells. Conidiogenous cells hyaline, phialidic, discrete. Conidia aseptate, ellipsoidal to subcylindrical/subfusoid, guttulate.

Type species: *Setophoma terrestris* (H.N. Hansen) Gruyter, Aveskamp & Verkley.

Setophoma terrestris (H.N. Hansen) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514659.
Basionym: *Phoma terrestris* H.N. Hansen, Phytopathology 19: 699. 1929.
≡ *Pyrenochaeta terrestris* (H.N. Hansen) Gorenz, J.C. Walker & Larson, Phytopathology 38: 838. 1948; not *Phoma terrestris* R.K. Saksena, Nand & A.K. Sarbhoy, Mycopath. Mycol. Appl. 29: 86. 1966 (= *Phoma multirostrata* Boerema).

Specimens examined: **North America**, on root of *Allium sativum* (*Alliaceae*), Dec 1929, H.N. Hansen, **Lectotype designed here** CBS H-20311, culture ex-lectotype CBS 335.29). On root of *Allium cepa*, Aug 1952, R.H. Larson, CBS 377.52, **Senegal**, Dakar, on *Allium cepa*, May 1987, CBS 335.87.

Note: Type material was not indicated in the original description (Hansen 1929) and therefore, a dried specimen of isolate CBS 335.29, deposited by the author, is designated here as lectotype.

Setophoma sacchari **(**Bitancourt) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB514660.
Basionym: *Pyrenochaeta sacchari* Bitancourt, Arq. Inst. Biol. (Sao Paulo) 9: 301. 1938.
= *Pyrenochaeta setariae* H.C. Greene, Trans. Wisconsin Acad. Sci. 53: 211. 1964.
= *Pyrenochaeta penniseti* J. Kranz, Sydowia 22: 360. 1968.
= *Phoma setariae* (H.C. Greene) Gruyter & Boerema, Persoonia 17: 559. 2002 [‘2001’].

Specimens examined: BRAZIL, Cantareira, Sao Paulo. On leaves of *Saccharum officinarum* (*Poaceae*), 11-13 Oct 1937, nr. 2769, IBI, coll. A.A. Bitancourt, **holotype** of *Pyrenochaeta sacchari* Bitancourt. On leaves of *Saccharum officinarum* var. EK28, Jul 1939, nr 3064, A.A. Bitancourt, **epitype designed here**, CBS H-20312, culture ex-epitype CBS 333.39.

DISCUSSION

Phoma section *Paraphoma* was shown to be polyphyletic in the *Pleosporales* based on the SSU and LSU nrDNA phylogeny obtained in this study. *Phoma radicina*, the type species of the section *Paraphoma* (Morgan-Jones & White 1983), grouped with two related *Phoma* species in a subclade being assigned to *Phaeosphaeriaceae.* The anamorph genus *Paraphoma* is re-introduced here and comprises *Pa. radicina*, *Pa. chrysanthemicola* and *Pa. fimeti*. The conidiogenous cells are typical for *Phoma*: phialidic, ampulliform to doliiform. *Paraphoma radicina* and *Pa. chrysanthemicola* produce pycnidia with long setae, also interpreted as mycelial hairs, exceeding 200 µm in length. However, this character is variable in the genus *Paraphoma* because *Pa. fimeti* produces glabrous pycnidia. The phylogenetic relationship of *Pa. radicina* and *Pa. chrysanthemicola* is congruent with a similar ecological niche as soil-borne organisms that often are associated with root surfaces in temperate zones in Australia, Eurasia and North America. *Paraphoma fimeti* is also a soil-borne fungus often isolated from dead tissue of various herbaceous and woody plants. *Paraphoma chrysanthemicola* originally was described in *Phoma* section *Peyronellaea* based on its formation of aggregates chlamydospores and pseudosclerioid masses (Boerema 1993). However, the type species of this section, *Ph. glomerata*, belongs in *Didymellaceae* (de Gruyter *et al.* 2009), as do the majority of the other species classified in *Phoma* sect. *Peyronellaea* (Aveskamp *et al.* 2009a). The closest teleomorph we could find for these three species was the monotypic genus *Setomelanomma* (Rossman *et al.* 2002). *Setomelanomma holmii* recently was redescribed and placed in *Phaeosphaeriaceae*, supported by SSU rDNA phylogeny (Rossman *et al.* 2002). This species with unknown anamorph is associated with needle chlorosis and needle drop of *Picea pungens* and *P. glauca* in Europe and North America.

The genera *Setophoma* and *Neosetophoma* are introduced here, although the subclade comprises both genera is weakly supported (52 %). The species in both genera are only related distantly to the well supported *Paraphoma* clade. A relation with other coelomycetes or with a teleomorph could not be found, and the species certainly could not be maintained in the genus *Phoma*, classified in *Didymellaceae*. Therefore, we introduced both genera to accommodate these species in *Phaeosphaeriaceae*, although more phylogenetic relatives need to be found for a better understanding of this clade. *Setophoma terrestris* and *S. sacchari* have been recorded associated with monocotyledonous plants. *Neosetophoma samarorum*, type species of the genus, is closely related genetically to these species, but morphologically distinct based on its typically yellowish conidia that are attenuate at one end and the stagonosporoid synanamorph, *Stagonosporopsis fraxini*. The phylogeny of the *Neosetophoma samarorum* isolates suggest a species complex to be studied in more detail. The genera *Paraphoma, Setophoma and Neosetophoma* grouped in sister clades closely related to the teleomorph genera *Ophiosphaerella*, *Phaeosphaeria*, and *Phaeosphaeriopsis*, mainly recorded on *Poaceae*. The *Phaeosphaeria* species included in this study represent 'clade B' in an 18S rDNA sequence analysis by Câmara *et al.* (2002). The associated anamorphs in this clade are variable and described as '*Stagonospora*-like', '*Coniothyrium*-like', or as *Microsphaeropsis* sp. (Câmara *et al.* 2002, 2003). In some species an *Aposphaeria*-like or *Phoma*-like synanamorph has been observed *in vitro* (Leuchtmann 1984).

Although *Chaetosphaeronema hispidulum* and *C. coonsii* proved to be closely related to the teleomorphs *Phaeosphaeria* and *Phaeosphaeriopsis*, both species are recorded on dicotyledons. The genus *Chaetosphaeronema* is characterised by setose, dark brown pycnidia with thick-walled outer cell layers, producing hyaline, septate condia (Sutton 1980). In addition, the thick-walled cells of the pycnidia of *Chaetosphaeronema coonsii* may become scleroplectenchymatous and the conidia produced are always aseptate (Boerema *et al.* 2004). Re-examination of the ex-type culture of *C. coonsii* revealed that the pycnidia are covered by brown mycelial hairs, resembling setae.

The *Pyrenochaeta* species with distinct elongated, septate, acropleurogenous conidiophores produced in pycnidia usually covered by long setae, 200 µm in length or more, grouped in several related subclades in clade A, such as *Py. nobilis*, the type species of the genus (Figs 12–13), *Py. lycopersici* and *Py. parasitica* (teleom. *Herpotrichia parasitica*). Furthermore, *Py. berberidis* (teleom. *Cucurbitaria berberidis*) also grouped among these species. The teleomorph genera *Cucurbitaria* and *Herpotrichia* have been classified respectively in *Cucurbitariaceae* and *Lophiostomataceae* (Barr 1987b). In molecular studies it was demonstrated that *C. berberidis*, being the type species of *Cucurbitaria*, was only distantly related to *Herpotrichia juniperi* and *Herpotrichia diffusa*, both species with an unknown anamorph (Berbee 1996, Silva-Hanlin & Hanlin 1999). In a subsequent molecular study *H. juniperi* and *H. diffusa* were classified in *Melanommataceae* (Câmara *et al.* 2003). The basal clustering of *H. parasitica* with *Cucurbitaria berberidis* in *Cucurbitariaceae* demonstrate that both species with a *Pyrenochaeta* anamorph are related and that *H. parasitica* does not fit in the genus *Herpotrichia*. *Phoma leveillei* var. *leveillei* proved to be a species complex, and three isolates, all producing pycnidia covered by long setae, group in *Pyrenochaeta*; and the correct name for these isolates is *Pyrenochaeta acicola*. The conidiogenesis is *Phoma*-like (Fig. 14), and it is likely that the further development into branched, filiform, septate conidiophores is a lost character in *Py. acicola*. Therefore, this species morphologically has more affinity with the species described here in the genus *Paraphoma* because the latter only produce *Phoma*-like conidiogenous cells. In clade A two subclades comprise *Pyrenochaeta* species with pycnidia covered by relatively short setae, up to 100 µm, producing branched, filiform, septate, acropleurogenous conidiophores as well as *Phoma*-like conidiogenous cells. Species included are *Pyrenochaeta unguis-hominis*, *Py. quercina* and *Py. corni*.

Two cultures preserved as *Phialophorophoma litoralis* (CBS 234.92, 102828) were similar to *Py. corni* according to the SSU/LSU sequences and morphological characters. This finding was confirmed by sequence analyses of the actin, β-tubulin genes and the ITS 1 & 2 regions (data not shown). The characters observed in both isolates, including the formation of setose pycnidia and conidiogenesis (Figs 15–16), confirmed that these isolates belong to *Py. corni*. A third *Phialophorophoma litoralis* culture (CBS 297.74) clustered with *Py. quercina*. It is likely that *Phialophorophoma litoralis*, producing glabrous pycnidia (Linder 1944, Sutton 1980), refers to a different species.

The presence of two types of conidiogenesis is also a key character for the genus *Pleurophoma* (Boerema *et al.* 2004). Therefore, it was suggested that *Py. corni* could be considered as a 'setose' species of *Pleurophoma*, having more affinity with *Phoma* than with true species of *Pyrenochaeta* (de Gruyter & Boerema 2002). However, the present study demonstrate that both *Py. corni* and *Pleurophoma cava* can be attributed to the genus *Pyrenochaeta*.

The novel genus *Pyrenochaetopsis* is characterised by setose pycnidia similar to those of the genus *Pyrenochaeta*, but the conidiogenesis is usually *Phoma*-like because only incidentically elongated, septate conidophores may be observed (Figs 17–18). The genus *Pyrenochaetopsis* morphologically resembles the genus *Paraphoma* based on the *Phoma*-

like conidiogenesis. However, both genera are only distantly related based on the molecular phylogeny, and grouped in two distinct families (i.e. *Cucurbitariaceae* and *Phaeosphaeriaceae* respectively). Morphological criteria for delimitation of both genera are very limited, and the genus *Pyrenochaetopsis* so far includes only species that were formerly described in *Phoma* section *Paraphoma* such as *Phoma leveillei* var. *microspora*, *Ph. briardii*, *Ph. terricola*, *Ph. indica* and *Ph. leveillei* var. *leveillei*. In general the conidia of species in the genus *Pyrenochaetopsis* are relatively narrow, usually not exceeding 2 µm, similar to those of the *Pyrenochaeta* species included in this study but narrower than those observed in *Paraphoma*. These species are reclassified here in the new genus *Pyrenochaetopsis* as *Pyr. microspora*, *Pyr. leptospora*, *Pyr. decipiens* and *Pyr. indica*. These species are all soil-borne and mainly associated with gramineous plants. The species accommodated in the different subclades in clade A share ecological and morphological characters. The *Pyrenochaeta* species *Py. corni*, *Py. quercina* and *Py. cava* are found mostly as endophytes or saprobes on dicotyledons, trees and shrubs in particular. *Pyrenochaeta nobilis*, *Py. acicola*, *Py. berberis*, *Py. lycopersici* and *Py. parasitica* are recognised as plant pathogens on dicotyledons. These data demonstrate a parallel evolution on gramineous plants of the *Pyrenochaetopsis* species in clade A, and *Setophoma* spp. in clade B.

The phylogeny generated here demonstrates that the presence and appearance of some morphological characteristics, such as setose pycnidia and conidiogenous cells can be variable in *Pyrenochaeta* and *Pyrenochaetopsis*, probably due to degeneration or even loss of characters. The combination of elongated conidiogenous cells and setose pycnidia is not found in all species involved. Two other *Pyrenochaeta* species included in this study, the human pathogens *P. romeroi* and *P. mackinnonii*, were found in distinct clades in *Pleosporales*, unrelated to the type species of *Pyrenochaeta*. Both species can be differentiated from genus *Pyrenochaeta* by non-septate phialidic conidiogenous cells and conidia that are catenulate or in chains, very small, not exceeding 3 × 2 µm (Borelli 1959, 1976). A GenBank Blast-search of the LSU and ITS sequences (data not shown) of *Py. romeroi* and *Py. mackinnonii* revealed only a relatively high similarity for the LSU sequence with *Versicolorisporium triseptatum* (AB330081), a morphologically quite different coelomycete recently described from bamboo in Japan (Hatakeyama 2008). New genera to accommodate both species have to be introduced, but their phylogenetic relatives first need to be clarified.

Phoma gardeniae, producing pycnidia with short setae, clustered with *Phoma herbarum* in *Didymellaceae*. In *Phoma herbarum* occasionally setae-like outgrowths from outer wall cells have been recorded on older pycnidia *in vitro*, such as in the case of the *Didymellaceae*-associated species *P. glomerata* and *P. sorghina* (Boerema *et al.* 2004). Also *Chaetopyrena penicillata*, producing setose pycnidia and *Phoma*-like conidiogenous cells grouped with *Phoma herbarum* in *Didymellaceae*. *Phoma carteri*, *Phoma septicidalis*, *Phoma glycinicola* and *Pyrenochaeta dolichii* grouped in a distinct clade (Clade C), but their taxonomic position remains to be elucidated. Due to their close association with *Leptosphaeriaceae*, a further subsequent study dealing with *Phoma* species currently classified in *Phoma* section *Plenodomus* is required before the taxonomy of these four setose species can be redefined properly.

ACKNOWLEDGEMENTS

The Dutch Ministry of Agriculture, Nature and Food Quality has supported this research through an endowment of the FES program “Versterking infrastructuur Plantgezondheid”. We thank

Karin Rosendahl for technical assistance, Marjan Vermaas for helping with the photoplate and Machiel E. Noordeloos for critical reading of the manuscript. Paul Tooley kindly supplied two isolates of *Phoma glycinicola*.

CHAPTER 4

Redisposition of *Phoma*-like anamorphs in *Pleosporales*

J. de Gruyter[1–3], J.H.C. Woudenberg[1], M.M. Aveskamp[1], G.J.M. Verkley[1], J.Z. Groenewald[1], P.W. Crous[1, 3, 4]

[1]CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands;
[2]National Reference Centre, National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands;
[3]Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands;
[4]Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Studies in Mycology 75: 1–36. 2013.

Key words: coelomycetes, *Coniothyriaceae*, *Cucurbitariaceae*, *Leptosphaeriaceae*, *Melanommataceae*, molecular phylogeny, *Montagnulaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Sporormiaceae*, taxonomy, *Trematosphaeriaceae*.

Abstract: The anamorphic genus *Phoma* was subdivided into nine sections based on morphological characters, and included teleomorphs in *Didymella*, *Leptosphaeria*, *Pleospora* and *Mycosphaerella*, suggesting the polyphyly of the genus. Recent molecular, phylogenetic studies led to the conclusion that *Phoma* should be restricted to *Didymellaceae*. The present study focuses on the taxonomy of excluded *Phoma* species, currently classified in *Phoma* sections *Plenodomus*, *Heterospora* and *Pilosa*. Species of *Leptosphaeria* and *Phoma* section *Plenodomus* are reclassified in *Plenodomus*, *Subplenodomus* gen. nov., *Leptosphaeria* and *Paraleptosphaeria* gen. nov., based on the phylogeny determined by analysis of sequence data of the large subunit 28S nrDNA (LSU) and Internal Transcribed Spacer regions 1 & 2 and 5.8S nrDNA (ITS). *Phoma heteromorphospora*, type species of *Phoma* section *Heterospora*, and its allied species *Phoma dimorphospora*, are transferred to the genus *Heterospora* stat. nov. The *Phoma acuta* complex (teleomorph *Leptosphaeria doliolum*), is revised based on a multilocus sequence analysis of the LSU, ITS, small subunit 18S nrDNA (SSU), ß-tubulin (TUB), and chitin synthase 1 (CHS-1) regions. Species of *Phoma* section *Pilosa* and allied *Ascochyta* species were determined to belong to *Pleosporaceae* based on analysis of actin (ACT) sequence data. Anamorphs that are similar morphologically to *Phoma* and described in *Ascochyta*, *Asteromella*, *Coniothyrium*, *Plectophomella*, *Pleurophoma* and *Pyrenochaeta* are included in this study. *Phoma*-like species, which grouped outside the *Pleosporineae* based on a LSU sequence analysis, are transferred to the genera *Aposphaeria*, *Paraconiothyrium* and *Westerdykella*. The genera *Medicopsis* gen. nov. and *Nigrograna* gen. nov. are introduced to accommodate the medically important species formerly known as *Pyrenochaeta romeroi* and *Pyrenochaeta mackinnonii*, respectively.

Taxonomic novelties: New genera: ***Medicopsis*** Gruyter, Verkley & Crous, ***Nigrograna*** Gruyter, Verkley & Crous, ***Paraleptosphaeria*** Gruyter, Verkley & Crous, ***Subplenodomus*** Gruyter, Verkley & Crous. **New species:** ***Aposphaeria*** *corallinolutea* Gruyter, Aveskamp & Verkley, ***Paraconiothyrium*** *maculicutis* Verkley & Gruyter. **New combinations**: ***Coniothyrium*** *carteri* (Gruyter & Boerema) Verkley & Gruyter, *C. dolichi* (Mohanty) Verkley & Gruyter, *C. glycines* (R.B. Stewart) Verkley & Gruyter, *C. multiporum* (V.H. Pawar, P.N. Mathur & Thirum.) Verkley & Gruyter, *C. telephii* (Allesch.) Verkley & Gruyter, ***Heterospora*** (Boerema, Gruyter & Noordel.) Gruyter, Verkley & Crous, *H. chenopodii* (Westend.) Gruyter, Aveskamp & Verkley, *H. dimorphospora* (Speg.) Gruyter, Aveskamp & Verkley, ***Leptosphaeria*** *errabunda* (Desm.) Gruyter, Aveskamp & Verkley, *L. etheridgei* (L.J. Hutchison & Y. Hirats.) Gruyter, Aveskamp & Verkley, *L. macrocapsa* (Trail) Gruyter, Aveskamp & Verkley, *L. pedicularis* (Fuckel) Gruyter, Aveskamp & Verkley, *L. rubefaciens* (Togliani) Gruyter, Aveskamp & Verkley, *L. sclerotioides* (Preuss ex Sacc.) Gruyter, Aveskamp & Verkley, *L. sydowii* (Boerema, Kesteren & Loer.) Gruyter, Aveskamp & Verkley, *L. veronicae* (Hollós) Gruyter, Aveskamp & Verkley, ***Medicopsis*** *romeroi* (Borelli) Gruyter, Verkley & Crous, ***Nigrograna*** *mackinnonii* (Borelli) Gruyter, Verkley & Crous, ***Paraconiothyrium*** *flavescens* (Gruyter, Noordel. & Boerema) Verkley & Gruyter, *Paracon. fuckelii* (Sacc.) Verkley & Gruyter, *Paracon. fusco-maculans* (Sacc.) Verkley & Gruyter, *Paracon. lini* (Pass.) Verkley & Gruyter, *Paracon. tiliae* (F. Rudolphi) Verkley & Gruyter, ***Paraleptosphaeria*** *dryadis* **(**Johanson) Gruyter, Aveskamp & Verkley, *Paralept. macrospora* **(**Thüm.) Gruyter, Aveskamp & Verkley, *Paralept. nitschkei* (Rehm ex G. Winter) Gruyter, Aveskamp & Verkley, *Paralept. orobanches* (Schweinitz : Fr.) Gruyter, Aveskamp & Verkley, *Paralept. praetermissa* (P. Karst.) Gruyter, Aveskamp & Verkley, ***Plenodomus*** *agnitus* (Desm.) Gruyter, Aveskamp & Verkley, *Plen. biglobosus* (Shoemaker & H. Brun) Gruyter, Aveskamp & Verkley, *Plen. chrysanthemi* **(**Zachos, Constantinou & Panag.) Gruyter, Aveskamp & Verkley, *Plen. collinsoniae* **(**Dearn. & House) Gruyter, Aveskamp & Verkley, *Plen. confertus* **(**Niessl ex Sacc.) Gruyter, Aveskamp & Verkley, *Plen. congestus* **(**M.T. Lucas) Gruyter, Aveskamp & Verkley, *Plen. enteroleucus* (Sacc.) Gruyter, Aveskamp & Verkley, *Plen. fallaciosus* **(**Berl.) Gruyter, Aveskamp & Verkley, *Plen. hendersoniae* (Fuckel) Gruyter, Aveskamp & Verkley, *Plen. influorescens* (Boerema & Loer.) Gruyter, Aveskamp & Verkley, *Plen. libanotidis* (Fuckel) Gruyter, Aveskamp & Verkley, *Plen. lindquistii* (Frezzi) Gruyter, Aveskamp & Verkley, *Plen. lupini* (Ellis & Everh.) Gruyter, Aveskamp & Verkley, *Plen. pimpinellae* (Lowen & Sivan.) Gruyter, Aveskamp & Verkley, *Plen. tracheiphilus* (Petri) Gruyter, Aveskamp & Verkley, *Plen. visci* (Moesz) Gruyter, Aveskamp & Verkley, ***Pleospora*** *fallens* (Sacc.) Gruyter & Verkley, *Pleo. flavigena* (Constantinou & Aa) Gruyter & Verkley, *Pleo. incompta* (Sacc. & Martelli) Gruyter & Verkley, ***Pyrenochaetopsis pratorum*** **(**P.R. Johnst. & Boerema) Gruyter, Aveskamp & Verkley, ***Subplenodomus*** *apiicola* (Kleb.) Gruyter, Aveskamp & Verkley, *Subplen. drobnjacensis* (Bubák) Gruyter, Aveskamp & Verkley, *Subplen. valerianae* (Henn.) Gruyter, Aveskamp & Verkley, *Subplen. violicola* (P. Syd.) Gruyter, Aveskamp & Verkley, ***Westerdykella*** *capitulum* (V.H. Pawar, P.N. Mathur & Thirum.) de Gruyter, Aveskamp & Verkley, *W. minutispora* (P.N. Mathur ex Gruyter & Noordel.) Gruyter, Aveskamp & Verkley. **New names**: ***Pleospora*** *angustis* Gruyter & Verkley, *Pleospora halimiones* Gruyter & Verkley.

INTRODUCTION

The anamorphic genus *Phoma* includes many important plant pathogens. The taxonomy of *Phoma* has been studied intensively in the Netherlands for more than 40 years resulting in the development of a generic concept as an outline for identification of *Phoma* species (Boerema 1997). In this concept species of the genus *Phoma* are classified based on their morphological characters into nine sections: *Phoma, Heterospora, Macrospora, Paraphoma, Peyronellaea, Phyllostictoides, Pilosa, Plenodomus* and *Sclerophomella* (Boerema 1997). The species placed in each of the sections were systematically described culminating in the publication of the "*Phoma* Identification Manual" (Boerema *et al.* 2004), which contained the descriptions of 223 specific and infra-specific taxa of *Phoma*, and more than 1000 synonyms in other coelomycetous genera. The classification of the *Phoma* species in sections based on morphology is artificial (Boerema *et al.* 2004), and several species can be classified in more than one section as they reveal multiple "section-specific" characters.

A large, well-studied *Phoma* culture collection that includes more than 1100 strains of *Phoma* is the valuable result of the extensive morphological studies conducted in The Netherlands. That culture collection is the basis of an intensive molecular phylogenetic study of the genus *Phoma*, which commenced in 2006. Molecular studies of species of *Phoma* prior to the onset of this project concentrated on the development of molecular detection methods for specific, important plant pathogenic *Phoma* species, such as *Ph. macdonaldii, Ph. tracheiphila, Stagonosporopsis cucurbitacearum* (as *Ph. cucurbitacearum*) and *Boeremia foveata* (as *Ph. foveata*) (Aveskamp *et al.* 2008). The phylogeny of the type species of the nine *Phoma* sections and morphologically similar coelomycetes was determined utilising the sequence data of the large subunit 28S nrDNA (LSU) and the small subunit 18S nrDNA (SSU) regions (de Gruyter *et al*. 2009). Results of that study demonstrated that the type species of the nine *Phoma* sections all grouped in *Pleosporales*. The type species of five *Phoma* sections, *Phoma, Phyllostictoides, Sclerophomella, Macrospora* and *Peyronellaea* and similar genera, grouped in a distinct clade in *Didymellaceae*. The type species of the remaining four *Phoma* sections, *Heterospora, Paraphoma, Pilosa* and *Plenodomus*, clustered in several clades outside *Didymellaceae* based on the LSU and SSU sequence analysis leading to the conclusion that these species should be excluded from *Phoma* (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010).

The molecular phylogeny of the *Phoma* species in *Didymellaceae* was determined in a subsequent study (Aveskamp *et al.* 2010) and, as the phylogenetic placement of the sectional type species already suggested, included species mainly from sections *Phoma, Phyllostictoides, Sclerophomella, Macrospora* and *Peyronellaea*. The molecular phylogeny of 11 *Phoma* species classified in *Phoma* section *Paraphoma* based on their setose pycnidia was investigated using LSU and SSU sequences (de Gruyter *et al.* 2010) and this section was highly polyphyletic, with species clustering mainly in *Phaeosphaeriaceae* and *Cucurbitariaceae*.

The purpose of the present study was to clarify the molecular phylogeny of the *Phoma* species currently classified in sections *Plenodomus* and *Pilosa*, along with *Phoma* species which were determined to be distantly related to the generic type species *Ph. herbarum* in previous molecular studies. Additionally, *Phoma*-like isolates of coelomycetes currently classified in *Ascochyta* and *Coniothyrium* and clustering outside the *Didymellaceae* (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010) are included in this study along with a number of *Phoma*-like species that do not belong to *Pleosporineae*.

In the present study, the initial focus was to determine the molecular phylogeny of *Phoma betae* (teleom. *Pleospora betae*) and *Ph. lingam* (teleom. *Leptosphaeria maculans*), type

species of the *Phoma* sections *Pilosa* and *Plenodomus*, respectively, at the generic rank based on the sequence data of the LSU and the SSU regions. In a subsequent study, the sequence data of both the LSU and the ITS regions were used for a revised classification of the *Phoma* species currently classified in *Phoma* section *Plenodomus*. Only a limited number of the species currently classified in this section have a confirmed *Leptosphaeria* teleomorph.

The *Phoma acuta* species complex was subject of a more detailed study. The teleomorph of *Ph. acuta* is *Leptosphaeria doliolum*, type species of the genus *Leptosphaeria*. A multilocus analysis of sequence data of the SSU, LSU, ITS, ß-tubulin (TUB), and chitin synthase 1 (CHS-1) regions was performed. The phylogeny of *Phoma* species of section *Pilosa*, with a *Pleospora* teleomorph (*Pleosporaceae*) was studied utilising actin (ACT) sequence data.

Phoma-like species currently attributed to the genera *Aposphaeria*, *Asteromella*, *Coniothyrium*, *Phoma*, *Plenodomus*, *Pleurophoma* and *Pyrenochaeta*, which could not be classified in the *Pleosporineae* based on their molecular phylogeny, were included in a LSU sequence analysis. All *Phoma* taxa that are unrelated to *Didymellaceae* and treated in this paper are redisposed to other genera.

A further aim of this study was to establish a single nomenclature for well-resolved anamorph–teleomorph genera as discussed by Hawksworth *et al*. (2011). In cases where one anamorph–teleomorph generic relation is involved in a monophyletic lineage, one generic name was chosen based on priority and the other named teleomorph or anamorph state is treated as a synonym. Similar approaches towards single nomenclature have been employed in *Botryosphaeriales* (Crous *et al*. 2006, 2009a, b, Phillips *et al*. 2008), *Pleosporales* (Aveskamp *et al*. 2010), and *Hypocreales* (Lombard *et al*. 2010a–c, Chaverri *et al*. 2011, Gräfenhan *et al*. 2011, Schroers *et al*. 2011).

MATERIALS AND METHODS

Isolate selection, culture studies and DNA extraction

The generic abbreviations used in this study are: *Ascochyta* (*A.*), *Coniothyrium* (*C.*), *Heterospora* (*H.*), *Leptosphaeria* (*L.*), *Paraconiothyrium* (*Paracon.*), *Paraleptosphaeria* (*Paralep.*), *Phoma* (*Ph.*), *Plenodomus* (*Plen.*), *Pleospora* (*Pleo.*), *Pyrenochaeta* (*Py.*), *Subplenodomus* (*Subplen.*) and *Westerdykella* (*W.*). The isolates included in this study were obtained from the culture collections of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS-KNAW) and the Dutch National Plant Protection Organization, Wageningen, The Netherlands (PD) (Table 1). The freeze-dried isolates were revived overnight in 2 mL malt/peptone (50 % / 50 %) liquid medium and subsequently transferred and maintained on oatmeal agar (OA) (Crous *et al*. 2009c). The isolates, which were stored at –196 °C, were directly transferred to OA. Cultures growing on OA and malt extract agar (MEA) (Crous *et al*. 2009c) were studied morphologically as described in detail by Boerema *et al*. (2004). The genomic DNA isolation was performed 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 × in milliQ water and stored at 4 °C before use.

PCR and sequencing

For nucleotide sequence comparisons, partial regions of SSU, LSU and ITS, as well as part of the ACT, TUB and CHS-1 genes were amplified. The SSU region was amplified with the primers NS1 and NS4 (White *et al*. 1990) and the LSU region was amplified with the primers

Table 1. Isolates used in this study and their GenBank accession numbers. Name changes and newly generated sequences are indicated in bold.

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
***Aposphaeria corallinolutea* sp. nov.**	*Pleurophoma sp.*	131286	PD 83/367			**JF740329**				*Kerria japonica (Rosaceae)*	Netherlands
	Pleurophoma sp.	131287	PD 83/831			**JF740330**				*Fraxinus excelsior (Oleaceae)*	Netherlands
Aposphaeria populina		543.70				EU754130				*Populus canadensis (Salicaceae)*	Netherlands
	Pyrenochaeta sp.	350.82				**JF740265**				*Picea abies (Pinaceae)*	Germany
	Pleurophoma sp.	130330	PD 84/221			**JF740328**				*Cornus mas (Cornaceae)*	Netherlands
Beverwykella pulmonaria		283.53	ATCC 32983, IFO 6800			GU301804				*Fagus sylvatica (Fagaceae)*	Netherlands
Byssothecium circinans		675.92	ATCC 52767, ATCC 52678, IMI 266220			AY016357				*Medicago sativa* (*Fabaceae)*	USA
Chaetodiplodia sp.	*Chaetodiplodia* sp.	453.68					**JF740115**			*Halimione portulacoides (Chenopodiaceae)*	Netherlands
Chaetosphaeronema hispidulum		216.75			EU754045	EU754144				*Anthyllis vulneraria (Fabaceae)*	Germany
Cochliobolus sativus			DAOM 226212		DQ677995	DQ678045				*(Poaceae)*	unknown
***Coniothyrium carteri* comb. nov.**	*Phoma carteri*	101633	PD 84/74	**JF740180**		GQ387593				*Quercus* sp. *Fagaceae)*	Netherlands
	Phoma carteri	105.91		**JF740181**	GQ387533	GQ387594				*Quercus robur (Fagaceae)*	Germany

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
***Coniothyrium dolichi* comb. nov.**	*Pyrenochaeta dolichi*	124143	IMI 217261	**JF740182**		GQ387610				*Dolichos biforus (Fabaceae)*	India
	Pyrenochaeta dolichi	124140	IMI 217262	**JF740183**	GQ387550	GQ387611				*Dolichos biforus (Fabaceae)*	India
***Coniothyrium glycines* comb. nov.**	*Phoma glycinicola*	124455	IMI 294986	**JF740184**	GQ387536	GQ387597				*Glycine max (Fabaceae)*	Zambia
	Phoma glycinicola	124141	PG-1	**JF740185**		GQ387598				*Glycine max (Fabaceae)*	Zimbabwe
***Coniothyrium multiporum* comb. nov.**	*Phoma multipora*	501.91	PD 83/888	**JF740186**		GU238109				unknown	Egypt
	Phoma multipora	353.65	IMI 113689, ATCC 16207, HACC 164	**JF740187**		**JF740268**				saline soil	India
Coniothyrium palmarum		400.71		AY720708	EU754054	EU754153				*Chamaerops humilis (Arecaceae)*	Italy
***Coniothyrium telephii* comb. nov.**	*Phoma septicidalis*	188.71		**JF740188**	GQ387538	GQ387599				air	Finland
	Phoma septicidalis	856.97		**JF740189**	GQ387539	GQ387600				mineral wool	Finland
	Phoma septicidalis	101636	PD 86/1186	**JF740190**	GQ387540	GQ387601				*Glycine max (Fabaceae)*	Zimbabwe
Cucurbitaria berberidis, anam. *Pyrenochaeta berberidis*		363.93		**JF740191**	GQ387545	GQ387606				*Berberis vulgaris (Berberidaceae)*	Netherlands
Didymella exigua		183.55			EU754056	EU754155				*Rumex arifolius (Polygonaceae)*	France
Didymella lycopersici, anam. *Boeremia lycopersici*		378.67			**JF740097**	GU237950				*Lycopersicon esculentum (Solanaceae)*	Netherlands

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
Falcisormispora lignatilis			BCC 21118			GU371827				*Elaeis guineensis (Arecaceae)*	Thailand
Herpotrichia juniperi		200.31				DQ678080				*Juniperus nana (Cupressaceae)*	Switzerland
***Heterospora chenopodii* comb. nov.**	*Phoma heteromorphospora*	448.68		FJ427023	EU754088	EU754187				*Chenopodium album (Chenopodiaceae)*	Netherlands
	Phoma heteromorphospora	115.96	PD 94/1576	**JF740227**		EU754188				*Chenopodium album (Chenopodiaceae)*	Netherlands
***Heterospora dimorphospora* comb. nov.**	*Phoma dimorphospora*	345.78	PD 76/1015	**JF740203**		GU238069				*Chenopodium quinoa (Chenopodiaceae)*	Peru
	Phoma dimorphospora	165.78	PD 77/884	**JF740204**	**JF740098**	**JF740281**				*Chenopodium quinoa (Chenopodiaceae)*	Peru
Leptosphaeria conoidea	*Leptosphaeria conoidea*, anam. *Phoma doliolum*	616.75	ATCC 32813, IMI 199777, PD 74/56	**JF740201**	**JF740099**	**JF740279**				*Lunaria annua (Brassicaceae)*	Netherlands
	Leptosphaeria conoidea, anam. *Phoma doliolum*	125977	PD 82/888	**JF740202**		**JF740280**				*Senecio* sp. *(Asteraceae)*	Netherlands
Leptosphaeria doliolum	*Leptosphaeria doliolum* subsp. *doliolum* var. *doliolum*, anam. *Phoma acuta* subsp. *acuta*	505.75	PD 75/141	**JF740205**	GQ387515	GQ387576	**JF740126**	**JF740144**	**JF740162**	*Urtica dioica (Urticaceae)*	Netherlands

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
	Leptosphaeria doliolum subsp. *errabunda*, anam. *Phoma acuta* subsp. *errabunda*	541.66	PD 66/221	**JF740206**		**JF740284**	**JF740127**	**JF740145**	**JF740163**	*Rudbeckia* sp. *(Asteraceae)*	Netherlands
	Phoma acuta subsp. *acuta* f.sp. *phloxis*	155.94	PD 77/80	**JF740207**		**JF740282**	**JF740128**	**JF740146**	**JF740164**	*Phlox paniculata (Polemoniaceae)*	Netherlands
	Phoma acuta subsp. *acuta* f.sp. *phloxis*	125979	PD 78/37	**JF740208**		**JF740283**	**JF740129**	**JF740147**	**JF740165**	*Phlox paniculata (Polemoniaceae)*	Netherlands
	Leptosphaeria doliolum subsp. *doliolum* var. *doliolum*, anam. *Phoma acuta* subsp. *acuta*	504.75	PD 74/55	**JF740209**			**JF740130**	**JF740148**	**JF740166**	*Urtica dioica (Urticaceae)*	Netherlands
	Leptosphaeria doliolum subsp. *doliolum* var. *doliolum*, anam. *Phoma acuta* subsp. *acuta*	130000	PD 82/701	**JF740210**			**JF740131**	**JF740149**	**JF740167**	*Urtica dioica (Urticaceae)*	Netherlands
***Leptosphaeria errabunda* comb. nov.**	*Leptosphaeria doliolum* subsp. *errabunda*, anam. *Phoma acuta* subsp. *errabunda*	617.75	ATCC 32814, IMI 199775, PD 74/201	**JF740216**		**JF740289**	**JF740132**	**JF740150**	**JF740168**	*Solidago* sp. (hybrid) *(Asteraceae)*	Netherlands
	Leptosphaeria doliolum subsp. *errabunda*, anam. *Phoma acuta* subsp. *errabunda*	125978	PD 74/61	**JF740217**		**JF740290**	**JF740133**	**JF740151**	**JF740169**	*Delphinium* sp. *(Ranunculaceae)*	Netherlands

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
	Leptosphaeria doliolum subsp. *errabunda*, anam. *Phoma acuta* subsp. *errabunda*	129999	PD 78/569	**JF740218**			**JF740134**	**JF740152**	**JF740170**	*Aconitum* sp. *(Ranunculaceae)*	Netherlands
	Leptosphaeria doliolum subsp. *errabunda*, anam. *Phoma acuta* subsp. *errabunda*	129998	PD 84/462	**JF740219**			**JF740135**	**JF740153**	**JF740171**	*Gailardia (Asteraceae)*	Netherlands
	Leptosphaeria doliolum subsp. *errabunda*, anam. *Phoma acuta* subsp. *errabunda*	129997	PD 78/631	**JF740220**			**JF740136**	**JF740154**	**JF740172**	*Achillea millefolium (Apiaceae)*	Netherlands
***Leptosphaeria etheridgei* comb. nov.**	*Phoma etheridgei*	125980	DAOM 216539, PD 95/1483	**JF740221**		**JF740291**				*Populus tremuloides (Salicaceae)*	Canada
***Leptosphaeria macrocapsa* comb. nov.**	*Phoma macrocapsa*	640.93	PD 78/139	**JF740237**		**JF740304**	**JF740138**	**JF740156**	**JF740174**	*Mercurialis perennis (Euphorbiaceae)*	Netherlands
***Leptosphaeria pedicularis* comb. nov.**	*Phoma pedicularis*	126582	PD 77/710	**JF740223**		**JF740293**				*Gentiana punctata (Gentianaceae)*	Switzerland
	Phoma pedicularis	390.80	PD 77/711	**JF740224**		**JF740294**	**JF740137**	**JF740155**	**JF740173**	*Pedicularis* sp. *(Scrophulariaceae)*	Switzerland
***Leptosphaeria rubefaciens* comb. nov.**	*Phoma rubefaciens*	387.80	IMI 248432, ATCC 42533, PD 78/809	**JF740242**		**JF740311**				*Tilia* (x) *europea (Malvaceae)*	Netherlands
	Phoma rubefaciens	223.77		**JF740243**		**JF740312**				*Quercus* sp. *(Fagaceae)*	Switzerland

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
***Leptosphaeria sclerotioides* comb. nov.**	*Phoma sclerotioides*	144.84	CECT 20025, PD 82/1061	**JF740192**		**JF740269**				*Medicago sativa (Fabaceae)*	Canada
	Phoma sclerotioides	148.84	PD 80/1242	**JF740193**		**JF740270**				*Medicago sativa (Fabaceae)*	Canada
Leptosphaeria slovacica	*Leptosphaeria slovacica*, anam. *Phoma leonuri*	389.80	PD 79/171	**JF740247**	**JF740101**	**JF740315**				*Balota nigra (Lamiaceae)*	Netherlands
	Leptosphaeria slovacica, anam. *Phoma leonuri*	125975	PD 77/1161	**JF740248**		**JF740316**				*Balota nigra (Lamiaceae)*	Netherlands
***Leptosphaeria sydowii* comb. nov.**	*Phoma sydowii*	385.80	PD 74/477	**JF740244**		**JF740313**	**JF740139**	**JF740157**	**JF740175**	*Senecio jacobaea (Asteraceae)*	UK
	Phoma sydowii	125976	PD 84/472	**JF740245**		**JF740314**	**JF740140**	**JF740158**	**JF740176**	*Senecio jacobaea (Asteraceae)*	Netherlands
	Phoma sydowii	297.51		**JF740246**			**JF740141**	**JF740159**	**JF740177**	*Papaver rhoeas (Papaveraceae)*	Switzerland
***Leptosphaeria veronicae* comb. nov.**	*Phoma veronicicola*	145.84	CECT 20059, PD 78/273	**JF740254**		**JF740320**	**JF740142**	**JF740160**	**JF740178**	*Veronica chamaedryoides (Scrophulariaceae)*	Netherlands
	Phoma veronicicola	126583	PD 74/227	**JF740255**		**JF740321**	**JF740143**	**JF740161**	**JF740179**	*Veronica* 'Shirley Blue' *(Scrophulariaceae)*	Netherlands
Massaria platani		221.37			DQ678013	DQ678065				*Platanus occidentalis (Platanaceae)*	USA
Massarina eburnea			H 3953, HHUF 26621, JCM 14422		AB521718	AB521735				*Fagus sylvatica (Fagaceae)*	UK
		473.64	ETH 2945		GU296170	GU301840				*Fagus sylvatica* (*Fagaceae*)	Switzerland

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
***Medicopsis romeroi* comb. nov.**	*Pyrenochaeta romeroi*	252.60	ATCC 13735, FMC 151, UAMH 10841		EU754108	EU754207				Human, maduromycosis	Venezuela
	Pyrenochaeta romeroi	122784	PD 84/1022			EU754208				*Hordeum vulgare (Gramineae)*	unknown
Melanomma pulvis-pyrius		371.75				GU301845				wood	France
		400.97			DQ678020	DQ678072				*Fagus* sp. (Fagaceae)	Belgium
Neophaeosphaeria filamentosa		102202	BPI 802755	**JF740259**	GQ387516	GQ387577				*Yucca rostrata (Agavaceae)*	Mexico
Neosetophoma samarorum		138.96	PD 82/653		GQ387517	GQ387578				*Phlox paniculata (Polemoniaceae)*	Netherlands
Neottiosporina paspali		331.37			EU754073	EU754172				*Paspalum notatum* (*Poaceae*)	USA
***Nigrogana mackinnonii* comb. nov.**	*Pyrenochaeta mackinnonii*	674.75	FMC 270		GQ387552	GQ387613				black grain mycetoma, human	Venezuela
	Pyrenochaeta mackinnonii	110022				GQ387614				Human, mycetoma	Mexico
***Paraconiothyrium flavescens* comb. nov.**	*Phoma flavescens*	178.93	PD 82/1062			GU238075				soil	Netherlands
***Paraconiothyrium fuckelii* comb. nov.**	*Coniothyrium fuckelii*	797.95			GU238204	GU237960				*Rubus* sp. (*Rosaceae*)	Denmark
***Paraconiothyrium fusco-maculans* comb. nov.**	*Plenodomus fusco-maculans*	116.16				EU754197				*Malus* sp.	USA

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
***Paraconiothyrium lini* comb. nov.**	*Phoma lini*	253.92	PD 70/998			GU238093				Wisconsin tank	Netherlands
***Paraconiothyrium maculicutis* sp. nov.**	*Pleurophoma pleurospora*	101461	IMI 320754, UTHSC 87-144			EU754200				Human, cutaneous lesions	USA
Paraconiothyrium minitans		122788	PD 07/03486739		EU754074	EU754173				unknown	UK
		122786	PD 99/1064-1			EU754174				*Clematis* sp. *(Ranunculaceae)*	Netherlands
***Paraconiothyrium tiliae* comb. nov.**	*Asteromella tiliae*	265.94				EU754139				*Tilia platyphyllos (Tiliaceae)*	Austria
***Paraleptosphaeria dryadis* comb. nov.**	*Leptosphaeria dryadis*	643.86		**JF740213**		GU301828				*Dryas octopetala (Rosaceae)*	Switzerland
***Paraleptosphaeria macrospora* comb. nov.**	*Phoma macrospora*	114198	UPSC 2686	**JF740238**		**JF740305**				*Rumex domesticus (Chenopodiaceae)*	Norway
***Paraleptosphaeria nitschkei* comb. nov.**	*Leptosphaeria nitschkei*	306.51		**JF740239**		**JF740308**				*Cirsium spinosissimum (Asteraceae)*	Switzerland
***Paraleptosphaeria orobanches* comb. nov.**	*Phoma korfii*	101638	PD 97/12070	**JF400230**		**JF740299**				*Epifagus virginiana (Orobanchaceae)*	USA
***Paraleptosphaeria praetermissa* comb. nov.**	*Leptosphaeria praetermissa*	114591		**JF740241**		**JF740310**				*Rubus idaeus (Rosaceae)*	Sweden
Paraphaeosphaeria michoti		652.86	ETH 9483		GQ387520	GQ387581				*Typha latifolia (Typhaceae)*	Switzerland
Paraphoma radicina		111.79	IMI 386094, PD 76/437		EU754092	EU754191				*Malus sylvestris (Rosaceae)*	Netherlands
Phaeosphaeria nodorum		110109			EU754076	EU754175				*Lolium perenne (Gramineae)*	Denmark

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
Phoma herbarum		615.75		FJ427022	EU754087	EU754186				*Rosa multiflora (Rosaceae)*	Netherlands
Phoma paspali		560.81	PD 92/1569		GU238227	G238124				*Paspalum dilatum (Poaceae)*	New Zealand
***Plenodomus agnitus* comb. nov.**	*Leptosphaeria agnita*, anam. *Phoma agnita*	121.89	PD 82/903	**JF740194**		**JF740271**				*Eupatorium cannabinum (Asteraceae)*	Netherlands
	Leptosphaeria agnita, anam. *Phoma agnita*	126584	PD 82/561	**JF740195**		**JF740272**				*Eupatorium cannabinum (Asteraceae)*	Nehterlands
***Plenodomus biglobosus* comb. nov.**	*Leptosphaeria biglobosa*	119951		**JF740198**	**JF740102**	**JF740274**				*Brassica rapa (Brassicaceae)*	Netherlands
		127249	DAOM 229269	**JF740199**		**JF740275**				*Brassica juncea* (*Brassicaceae*)	France
***Plenodomus chrysanthemi* comb. nov.**	*Phoma vasinfecta*, synanam. *Phialophora chrysanthemi*	539.63		**JF740253**	GU238230	GU238151				*Chrysanthemum* sp. *(Asteraceae)*	Greece
***Plenodomus collinsoniae* comb. nov.**	*Leptosphaeria collinsoniae*	120227	JCM 13073, MAFF 239583	**JF740200**		**JF740276**				*Vitis coignetiae (Vitaceae)*	Japan
***Plenodomus confertus* comb. nov.**	*Leptosphaeria conferta*, anam. *Phoma conferta*	375.64		AF439459		**JF740277**				*Anacyclus radiatus (Asteraceae)*	Spain
***Plenodomus congestus* comb. nov.**	*Leptosphaeria congesta*, anam. *Phoma congesta*	244.64		AF439460		**JF740278**				*Erigeron canadensis (Asteraceae)*	Spain
***Plenodomus enteroleucus* comb. nov.**	*Phoma enteroleuca var. enteroleuca*	142.84	PD 81/654, CECT20063	**JF740214**		**JF740287**				*Catalpa bignonioides (Bignoniaceae)*	Netherlands

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
	Phoma enteroleuca var. enteroleuca	831.84		**JF740215**		**JF740288**				*Triticum aestivum (Poaceae)*	Germany
***Plenodomus fallaciosus* comb. nov.**	*Leptosphaeria fallasiosa*	414.62	ETH 2961	**JF740222**		**JF740292**				*Satureia montana (Lamiaceae)*	France
***Plenodomus hendersoniae* comb. nov.**	*Phoma intricans*	113702	UPSC 1843	**JF740225**		**JF740295**				*Salix cinerea (Salicaceae)*	Sweden
	Phoma intricans	139.78		**JF740226**		**JF740296**				*Pyrus malus (Rosaceae)*	Netherlands
***Plenodomus influorescens* comb. nov.**	*Phoma enteroleuca var. influorescens*	143.84	PD 78/883; CECT 20064	**JF740228**		**JF740297**				*Fraxinus excelsior (Oleaceae)*	Netherlands
	Phoma enteroleuca var. influorescens		PD 73/1382	**JF740229**		**JF740298**				*Lilium* sp. *(Liliaceae)*	Netherlands
***Plenodomus libanotidis* comb. nov.**	*Leptosphaeria libanotis*	113795	UPSC 2219	**JF740231**		**JF740300**				*Seseli libanotis (Apiaceae)*	Sweden
***Plenodomus lindquistii* comb. nov.**	*Leptosphaeria lindquistii*, anam. *Phoma macdonaldii*	386.80	PD 77/336	**JF740232**		**JF740301**				*Helianthus annuus (Asteraceae)*	former Yugoslavia
	Leptosphaeria lindquistii, anam. *Phoma macdonaldii*	381.67		**JF740233**		**JF740302**				*Helianthus annuus (Asteraceae)*	Canada
Plenodomus lingam	*Leptosphaeria maculans*, anam. *Phoma lingam*	275.63	MUCL 9901; UPSC 1025	**JF740234**	**JF740103**	**JF740306**				*Brassica* sp. *(Brassicaceae)*	UK
	Leptosphaeria maculans, anam. *Phoma lingam*	260.94	PD 78/989	**JF740235**		**JF740307**	**JF740116**			*Brassica oleracea (Brassicaceae)*	Netherlands

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
	Leptosphaeria maculans, anam. *Phoma lingam*	147.24					**JF740117**			Unknown	Unknown
***Plenodomus lupini* comb. nov.**	*Phoma lupini*	248.92	PD 79/141	**JF740236**		**JF740303**				*Lupinus mutabilis (Fabaceae)*	Peru
***Plenodomus pimpinellae* comb. nov.**	*Leptosphaeria pimpinellae*, anam. *Phoma pimpinellae*	101637	PD 92/41	**JF740240**		**JF740309**				*Pimpinella anisum (Apiaceae)*	Israel
***Plenodomus tracheiphilus* comb. nov.**	*Phoma tracheiphila*	551.93	PD 81/782	**JF740249**	**JF740104**	**JF740317**				*Citrus limonium (Rutaceae)*	Israel
	Phoma tracheiphila	127250	PD 09/04597141	**JF740250**		**JF740318**				*Citrus* sp. *(Rutaceae)*	Italy
Plenodomus visci comb. nov.	*Plectophomella visci*	122783	PD 74/1021	**JF740256**	EU754096	EU754195				*Viscum album (Viscaceae)*	France
Plenodomus wasabiae	*Phoma wasabiae*	120119	FAU 559	**JF740257**		**JF740323**				*Wasabia japonica (Brassicaceae)*	Taiwan
	Phoma wasabiae	120120	FAU 561	**JF740258**		**JF740324**				*Wasabia japonica (Brassicaceae)*	Taiwan
Pleomassaria siparia		279.74				AY004341				*Betula verrucosa* (*Betulaceae*)	Netherlands
***Pleospora angustis* nom. nov.**	*Leptosphaeria clavata*	296.51					**JF740122**			Uncertain	Switzerland
Pleospora betae	*Pleospora betae*, anam. *Phoma betae*	523.66	PD 66/270, IHEM 3915		EU754080	EU754179	**JF740118**			*Beta vulgaris (Chenopodiaceae)*	Netherlands
	Pleospora betae, anam. *Phoma betae*	109410	PD 77/113			EU754178	**JF740119**			*Beta vulgaris (Chenopodiaceae)*	Netherlands

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
Pleospora calvescens	*Pleospora calvescens, anam. Ascochyta caulina*	246.79	PD 77/655		EU754032	EU754131	**JF740120**			*Atriplex hastata (Chenopodiaceae)*	Germany
	Pleospora calvescens, anam. Ascochyta caulina	343.78					**JF740121**			*Atriplex hastata* (*Chenopodiaceae*)	Netherlands
Pleospora chenopodii	*Ascochyta hyalospora*	206.80	PD 74/1022		**JF740095**	**JF740266**	**JF740109**			*Chenopodium quinoa (Chenopodiaceae)*	Bolivia
	Pleospora calvescens, anam. Ascochyta caulina	344.78	PD 68/682				**JF740110**			*Atriplex hastata (Chenopodiaceae)*	Netherlands
***Pleospora fallens* comb. nov.**	*Phoma fallens*	161.78	LEV 1131				**JF740106**			*Olea europaea (Oleaeceae)*	New Zealand
	Phoma glaucispora	284.70	PD 97/2400				**JF740107**			*Nerium oleander (Apocynaceae)*	Italy
***Pleospora flavigena* comb. nov.**	*Phoma flavigena*	314.80	PD 91/1613				**JF740108**			water	Romania
***Pleospora halimiones* nom. nov.**	*Ascochyta obiones*	432.77	IMI 282137		**JF740096**	**JF740267**	**JF740113**			*Halimione portulacoides (Chenopodiaceae)*	Netherlands
	Ascochyta obiones	786.68					**JF740114**			*Halimione portulacoides (Chenopodiaceae)*	Netherlands
Pleospora herbarum		191.86	IMI 276975		GU238232	GU238160	**JF740123**			*Medicago sativa (Fabaceae)*	India
***Pleospora incompta* comb. nov.**	*Phoma incompta*	467.76					**JF740111**			*Olea europaea (Oleaeceae)*	Greece
	Phoma incompta	526.82					**JF740112**			*Olea europaea (Oleaeceae)*	Italy

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
Pleospora typhicola	*Pleospora typhicola*, anam. *Phoma typharum*	132.69			**JF740105**	**JF740325**	**JF740124**			*Typha angustifolia (Typhaceae)*	Netherlands
	Pleospora typhicola, anam. Phoma typharum	602.72					**JF740125**			*Typha* sp. *(Typhaceae)*	Netherlands
Pleurophoma pleurospora	*Pleurophoma* sp.	116668				**JF740326**				*Citysus scoparius (Fabaceae)*	Netherlands
	Pleurophoma sp.	130329	PD 82/371			**JF740327**				*Lonicera* sp. *(Caprifoliaceae)*	Netherlands
Preussia funiculata		659.74			GU296187	GU301864				soil	Senegal
Pseudorobillarda phragmitis		398.61	IMI 070678			EU754203				*Phragmitis australis (Poaceae)*	UK
Pyrenochaeta cava		257.68	IMI 331911	**JF740260**	EU754100	EU754199				wheat field soil	Germany
Pyrenochaeta lycopersici		267.59		**JF740261**	GQ387551	GQ387612				*Lycopersicon esculentum (Solanaceae)*	Netherlands
Pyrenochaeta nobilis		407.76		EU930011	EU754107/ DQ898287	EU754206				*Laurus nobilis (Lauraceae)*	Italy
Pyrenochaetopsis leptospora		101635	PD 71/1027	**JF740262**	GQ387566	GQ387627				*Secale cereale (Poaceae)*	Europe
***Pyrenochaetopsis pratorum* comb. nov.**	*Phoma pratorum*	445.81	PDDCC 7049, PD 80/1254	**JF740263**		GU238136				*Lolium perenne, leaf (Poaceae)*	New Zealand
	Phoma pratorum	286.93	PD 80/1252	**JF740264**		**JF740331**				*Dactylis glomerata (Poaceae)*	New Zealand
Pyrenophora tritici-repentis			OSC 100066		AY544716	AY544672				*(Poaceae)*	Italy

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
Roussoella hysterioides		125434	HH 26988			AB524622				*Sasa kurilensis (Poaceae)*	Japan
Setomelanomma holmii		110217			GQ387572	GQ387633				*Picea pungens (Pinaceae)*	USA
Setophoma terrestris		335.29			GQ387526	GQ387587				*Allium sativum (Alliaceae)*	USA
Sporormiella minima		524.50			DQ678003	DQ678056				dung of goat	Panama
Stagonosporopsis cucurbitacearum		133.96			GU238234	GU238181				*Cucurbita* sp. *(Cucurbitaceae)*	New Zealand
***Subplenodomus apiicola* comb. nov.**	*Phoma apiicola*	285.72		**JF740196**		GU238040				*Apium graveolens* var. *rapaceum (Umbelliferae)*	Germany
	Phoma apiicola	504.91	PD 78/1073	**JF740197**		**JF740273**				*Apium graveolens (Umbelliferae)*	Netherlands
***Subplenodomus drobnjacensis* comb. nov.**	*Phoma drobnjacensis*	269.92	PD 88/896	**JF740211**	**JF740100**	**JF740285**				*Eustoma exaltatum (Gentianaceae)*	Netherlands
	Phoma drobnjacensis	270.92	PD 83/650	**JF740212**		**JF740286**				*Gentiana makinoi* 'Royal Blue' *(Gentianaceae)*	Netherlands
***Subplenodomus valerianae* comb. nov.**	*Phoma valerianae*	630.68	PD 68/141	**JF740251**		GU238150				*Valeriana phu (Valerianaceae)*	Netherlands
	Phoma valerianae	499.91	PD 73/672	**JF740252**		**JF740319**				*Valeriana officinalis (Valerianaceae)*	Netherlands
***Subplenodomus violicola* comb. nov.**	*Phoma violicola*	306.68		FJ427083	GU238231	GU238156				*Viola tricolor (Violaceae)*	Netherlands
	Phoma violicola	100272		FJ427082		**JF740322**				*Viola tricolor (Violaceae)*	New Zealand

Table 1. (Continued).

Species name, final identification	Formerly identified	CBS nr.	Others	ITS	SSU	LSU	ACT	B-TUB	CHS	Host, substrate	Country
Thyridaria rubronotata		419.85				GU301875				*Acer pseudoplatanus* (*Aceraceae*)	Netherlands
Trematosphaeria pertusa		122368				FJ201990				*Fraxinus excelsior* (*Oleaceae*)	France
***Westerdykella capitulum* comb. nov.**	*Phoma capitulum*	337.65	PD 91/1614, ATCC 16195, HACC 167, IMI 113693			GU238054				saline soil	India
***Westerdykella minutispora* comb. nov.**	*Phoma minutispora*	509.91	PD 77/920			GU238108				saline soil	India
Westerdykella ornata		379.55				GU301880				mangrove mud	Mozambique

LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990). The ITS and TUB regions were amplified as described by Aveskamp *et al*. (2009a) using the primer pair V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al*. 1990) for the ITS and the BT2Fw and BT4Rd primer pair (Woudenberg *et al.* 2009) for the TUB locus. The ACT and CHS regions were amplified using the primer pairs ACT-512F / ACT-783R and CHS-354R / CHS-79F (Carbone & Kohn 1999). The amplification reactions were performed and analysed as described by de Gruyter *et al*. (2009).

Sequencing of the PCR amplicons was conducted using the same primer combinations, although the primer LR5 (Vilgalys & Hester 1990) was used as an additional internal sequencing primer for LSU. The sequence products were purified using Sephadex columns (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 both forward and reverse sequences using the Bionumerics v. 4.61 software package (Applied Maths, Sint-Martens-Latem, Belgium) and were lodged with GenBank. All sequences of reference isolates included in this study were obtained from GenBank (Table 1).

Phylogenetic analyses

To determine the phylogeny of *Phoma betae* and *Ph. lingam* at rank, the SSU and LSU sequence data of two isolates were aligned with the sequences of 46 reference isolates in the *Pleosporales* that were obtained from GenBank (Table 1), 14 of which were classified in *Pleosporaceae* or *Leptosphaeriaceae*. The phylogeny of *Phoma* section *Plenodomus* was determined with the combined data set of LSU and ITS sequences of 87 isolates, including 53 isolates currently classified in *Leptosphaeria* and *Phoma* section *Plenodomus*. *Phoma apiicola, Ph. dimorphospora, Ph. heteromorphospora, Ph. lupini, Ph. valerianae, Ph. vasinfecta* and *Ph. violicola* classified in *Phoma* sections *Phoma* or *Heterospora* (Boerema *et al.* 2004) grouped in previous molecular phylogenetic studies outside *Didymellaceae* (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010), and are therefore treated here.

In the study of the *Leptosphaeria doliolum* complex, that includes the subspecies of *Ph. acuta*, viz. subsp. *acuta, errabunda* and also *Ph. acuta* subsp. *acuta* f. sp. *phlogis,* a phylogenetic analysis was performed utilising the ITS, ACT, TUB, CHS-1 sequences of 18 isolates. *Phoma macrocapsa, Ph. sydowii* and *Ph. veronicicola* being closely related to this species complex were included.

The species concept of *Phoma*-like anamorphs in *Pleosporaceae* was determined by alignments of the ACT sequences of 15 isolates and five reference isolates. *Phoma fallens, Ph. glaucispora* and *Ph. flavigena* were also included. These species were originally classified in *Phoma* sect. *Phoma* (de Gruyter & Noordeloos 1992, de Gruyter *et al.* 1998). However, a molecular phylogenetic study demonstrated that these species grouped in a clade representing *Leptosphaeriaceae* and *Pleosporaceae* (Aveskamp *et al.* 2010). Sequence data were compared with those of isolates currently classified in the genera *Phoma, Ascochyta* and *Coniothyrium*, as well as isolates of *Leptosphaeria clavata* and the generic type species *Pleospora herbarum*. *Phoma incompta* is the only species classified in *Phoma* section *Sclerophomella,* which proved to be unrelated to *Didymellaceae* (Aveskamp *et al.* 2010).

The *Phoma*-like species that could not be attributed to *Pleosporineae* (Zhang *et al*. 2009, 2012) were studied with the LSU sequences of 40 isolates, including 20 reference isolates representing the anamorph genera *Beverwykella, Neottiosporina, Paraconiothyrium,* as well as the teleomorph genera *Byssothecium, Falciformispora, Herpotrichia, Massaria, Melanomma,*

Paraphaeosphaeria, Pleomassaria, Preussia, Roussoella, Sporormiella, Thyridaria, Trematosphaeria and *Westerdykella*. Four *Phoma* species were included which are currently described in *Phoma* section *Phoma*, viz. *Ph. capitulum*, *Ph. flavescens*, *Ph. lini*, and *Ph. minutispora* (de Gruyter & Noordeloos 1992, de Gruyter *et al.* 1993). In addition, the human pathogens *Pyrenochaeta romeroi and Py. mackinnonii*, which could not be classified in a recent study dealing with *Phoma*-like species with setose pycnidia (de Gruyter *et al.* 2010), were included.

The multiple alignments were automatically calculated by the BioNumerics software package, but manual adjustments for improvement were made by eye where necessary. For multilocus alignments, the phylogenetic analyses were done for each dataset individually, and where similar tree topologies were obtained, an analysis was performed on the combined alignment of all the gene regions in the multilocus alignment. Neighbour-Joining (NJ) distance analyses were conducted using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (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 MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) in two parallel runs, using the default settings but with the following adjustments: the GTR model (trees 1–3, 5) with gamma-distributed rate and the HKY+ γ-model (tree 4) were selected for the partitions using the Findmodel freeware (http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html), and a MCMC heated chain was set with a "temperature" value of 0.05. The number of generations and sample frequencies were set at 5 million and 10 (trees 3–5) or 100 (trees 1, 2) respectively and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01. The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and alignments and trees were deposited into TreeBASE (www.treebase.org).

RESULTS

The data for the aligned sequence matrices for the trees obtained in the different studies are provided below. In the case that alignments of multiple loci are involved, the topologies of the obtained trees for each locus were compared by eye to confirm that the overall tree topology of the individual datasets were similar to each other and to that of the tree obtained from the combined alignment. The NJ analyses with the three substitution models showed similar tree topologies and were congruent to those obtained in the Bayesian analyses. The results of the molecular phylogenetic analyses are supplied below; the summarised additional ecology and distribution data of the taxa involved were adopted from Boerema *et al.* (2004), where the references to original literature are provided.

Phylogeny of *Phoma lingam* and *Ph. betae*, the type species of *Phoma* sections *Plenodomus* and *Pilosa* (Pleosporineae)

The aligned sequence matrix obtained for the SSU and LSU regions had a total length of 2 671 nucleotide characters, 1 367 and 1 304 respectively. In the alignment, an insertion in the SSU at the positions 478–832 was observed for the cultures CBS 216.75, CBS 165.78, CBS 138.96, CBS 331.37 and CBS 674.75. This insertion was excluded from further phylogenetic analyses. The combined dataset used in the analyses included 48 taxa and contained 2 316 characters with 101 and 213 unique site patterns for SSU and LSU, respectively. The tree (Fig. 1) was rooted to

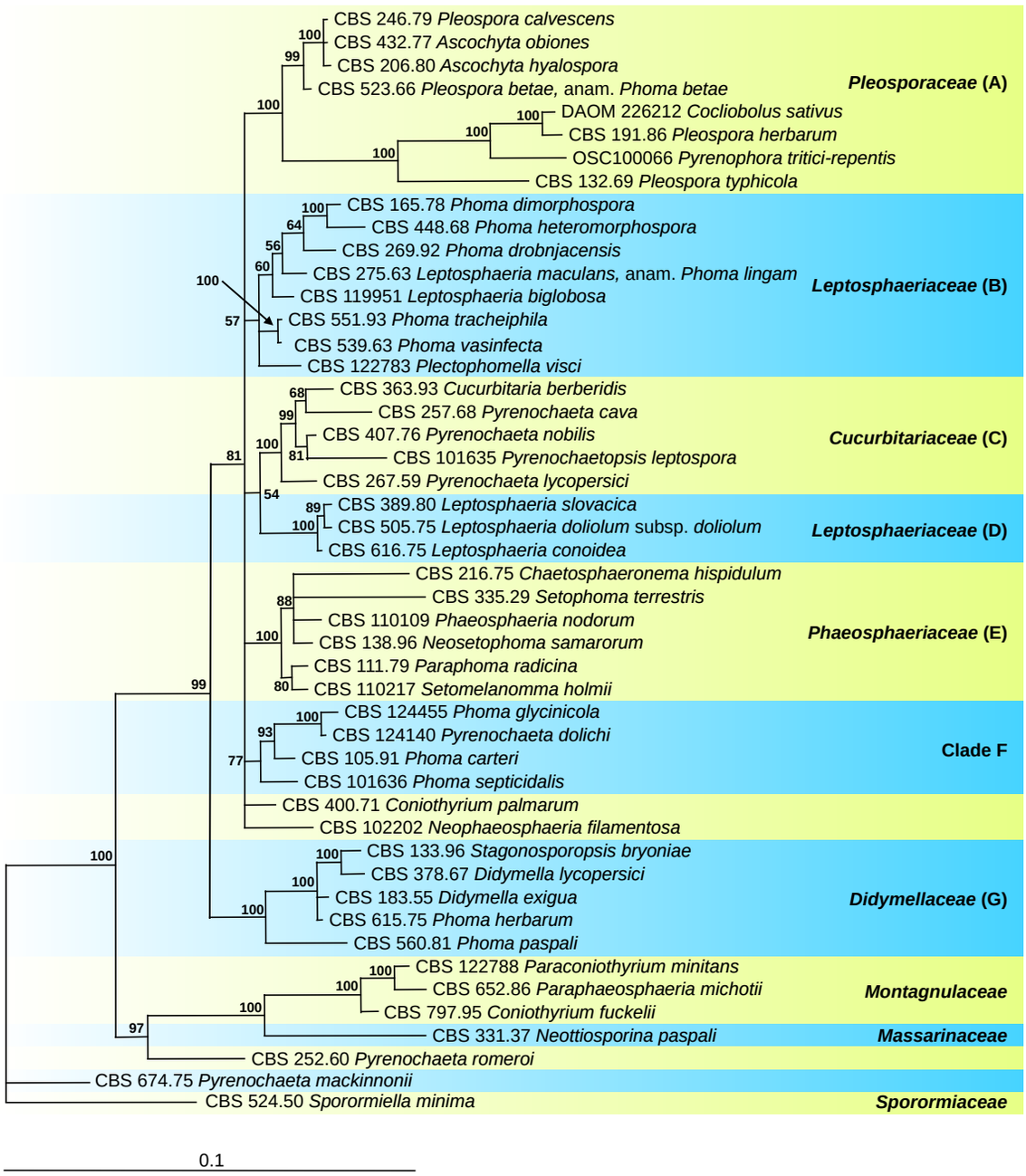


Fig. 1. The phylogeny of *Phoma lingam* and *Phoma betae*, the type species of *Phoma* sections *Plenodomus* and *Pilosa*, based on the strict consensus tree from a Bayesian analysis of 48 LSU/SSU sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted to *Sporormiella minima* (CBS 524.50).

Sporormiella minima (CBS 524.50). The Bayesian analysis resulted in 6 5442 trees after 3 272 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 56 028 trees (Fig. 1).

The families that belong to *Pleosporineae*, represented by the species grouping in clades A–G, clustered in a strongly supported clade (99 % posterior probability). Clade A, representing those species classified in *Pleosporaceae*, was strongly supported (100 %) and included two subclades. *Pleospora betae* (anam. *Ph. betae*), clustered with *Pleospora calvescens* (anam. *Ascochyta*

caulina), *A. obiones* and *A. hyalospora*; all recorded as pathogens on *Chenopodiaceae*. The generic type species *Pleospora herbarum*, a plurivorous species, grouped with *Cochliobolus sativus*, *Pyrenophora tritici-repentis* and *Pleospora typhicola* (anam. *Ph. typhina*), all recorded from *Poaceae*. Clade B includes *Leptosphaeria maculans* (anam. *Ph. lingam*) and clustered with *Leptosphaeria biglobosa*. In clade B also other important plant pathogens of *Phoma* section *Plenodomus* can be found, such as *Ph. tracheiphila*, *Ph. vasinfecta*, *Ph. drobnjacensis*, and *Plectophomella visci*. *Phoma heteromorphospora*, type species of *Phoma* section *Heterospora* (Boerema *et al.* 1997) and *Ph. dimorphospora* also grouped in this *Leptosphaeria* clade, in congruence with previous findings (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010).

Leptosphaeria doliolum (anam. *Ph. acuta*), type species of the genus *Leptosphaeria*, is found in Clade D, clustering with *L. conoidea* and *L. slovacica*. *Leptosphaeria doliolum* and its relatives comprise a sister clade C with species classified in *Cucurbitariaceae*, including *Cucurbitaria berberidis*, the three *Pyrenochaeta* species, *Py. cava*, *Py. lycopersici* and *Py. nobilis*, and *Pyrenochaetopsis leptospora*.

Phaeosphaeria nodorum and its relatives *Neosetophoma samarorum*, *Setophoma terrestris*, *Chaetosphaeronema hispidulum*, *Paraphoma radicina* and *Setomelanomma holmii*, represent *Phaeosphaeriaceae* in clade E as has previously been found (de Gruyter *et al.* 2009, 2010).

A distinct clade F includes *Ph. glycinicola*, *Ph. carteri*, *Ph. septicidalis*, and the taxonomic confusing species *Pyrenochaeta dolichi* (Grondona *et al.* 1997). The position of *Coniothyrium palmarum* and *Neophaeosphaeria filamentosa* could not be clarified, but both species are also treated below in a phylogeny including close relatives based on ITS and LSU regions (Fig. 2). *Didymella exigua*, type species of the genus *Didymella*, and *Ph. herbarum* represent *Didymellaceae*, and clustered in a well-supported clade (G) in congruence with previous studies (de Gruyter *et al.* 2009, 2010, Aveskamp *et al.* 2010). The molecular phylogeny of species which group in this analysis outside of Pleosporineae in *Montagnulaceae*, *Massarinaceae* and *Sporormiaceae* were further analyzed utilizing LSU sequence data of a broader range of taxa (Fig. 5).

Phoma section _Plenodomus_ and close allies

The aligned sequence matrix obtained for the LSU and ITS regions had a total length of 1 921 nucleotide characters, 1 332 and 589 respectively. The combined dataset used in the analyses included 87 taxa and contained 1921 characters with 298 and 118 unique site patterns for LSU and ITS respectively. The tree (Fig. 2) was rooted to *Ph. herbarum* (CBS 615.75), the representative isolate of the type species of *Phoma* (Boerema *et al.* 2004). The Bayesian analysis resulted in 100 002 trees after 5 000 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 90 930 trees (Fig. 2).

The species currently classified in *Leptosphaeria* and *Phoma* section *Plenodomus* grouped in clades A and B representing *Leptosphaeriaceae*, including the type species *Ph. lingam* and *Leptosphaeria doliolum*, respectively. Isolates of the taxa that represent *Cucurbitariaceae*, *Cucurbitaria berberidis* and its related species *Pyrenochaeta cava*, *Py. nobilis*, *Py. lycopersici* and *Pyrenochaetopsis leptospora*, clustered in a distinct clade D only distantly related to *Leptosphaeriaceae*. This finding agrees with a recent study (de Gruyter *et al.* 2010). *Phoma pratorum* clustered with *Pyrenochaetopsis leptospora*.

Leptosphaeria biglobosa grouped in a subclade A1 with *Ph. wasabiae*, the cause of black rot disease on *Wasabia japonica* (*Brassicaceae*) and *Ph. pimpinellae*, a necrotroph on *Pimpinella anisum* (*Apiaceae*). *Leptosphaeria maculans*, considered as closely related to the *L. biglobosa* complex, proved to be more distantly related in clade A1. In this subclade, other important pathogens can be found, such as *Ph. tracheiphila*, a quarantine organism on *Citrus* spp.

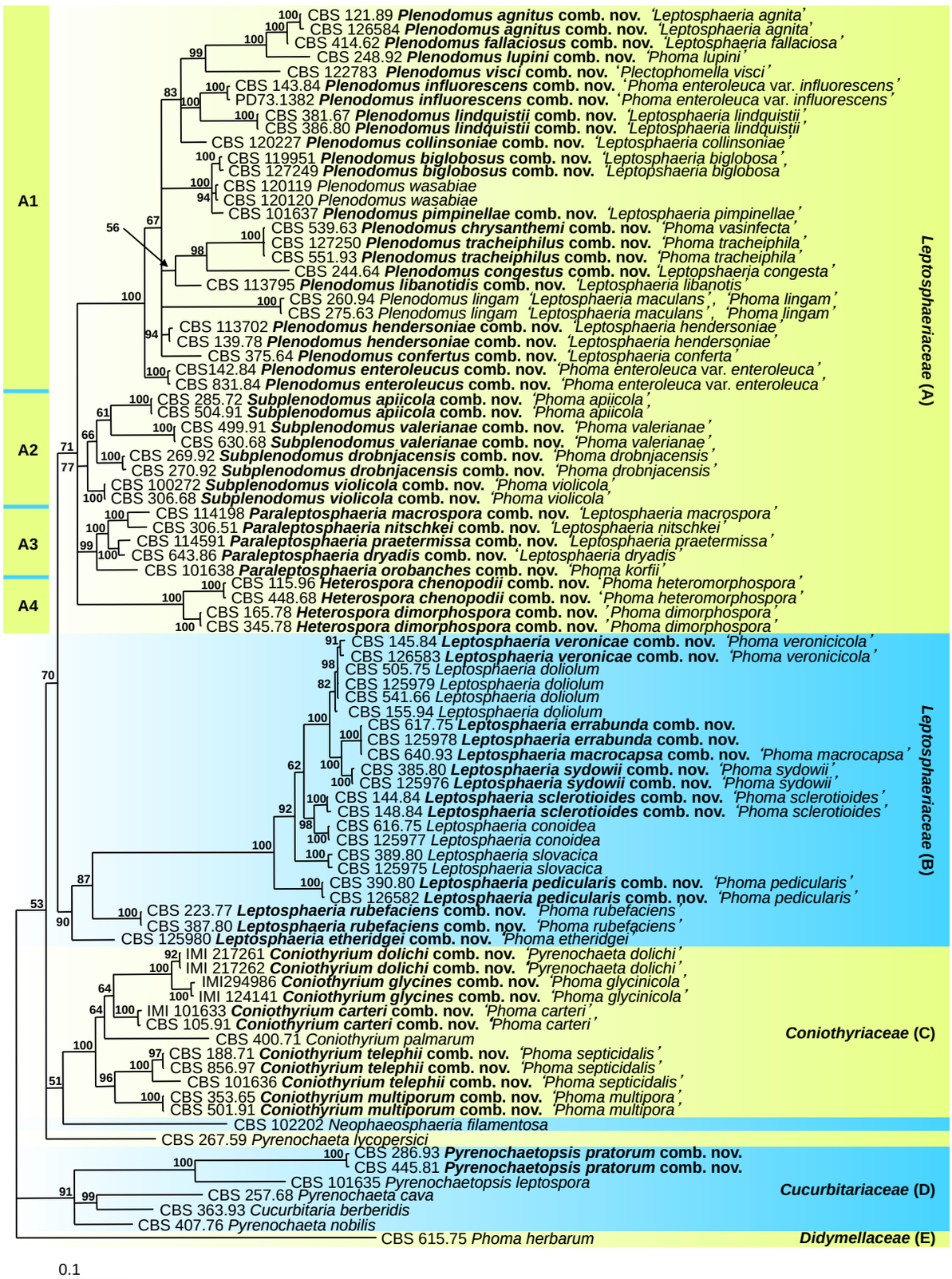


Fig. 2. The phylogeny of *Phoma* section *Plenodomus* and *Leptosphaeria*, based on the strict consensus tree from a Bayesian analysis of 87 LSU/ITS sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted to *Phoma herbarum* (CBS 615.75).

(*Rutaceae)*, *Ph. vasinfecta*, a pathogen on *Chrysanthemum* spp. (*Asteraceae*), *L. lindquistii* (anam. *Ph. macdonaldii*), a worldwide pathogen on *Helianthus annuus* (*Asteraceae*) and *Ph. lupini*, a seed borne pathogen known from *Lupinus* spp. (*Fabaceae*). Subclade A1 also comprises both varieties of *Ph. enteroleuca*, opportunistic pathogens on deciduous trees and shrubs, and the necrotrophic species *L. agnita* (anam. *Ph. agnita*), *Ph. congesta* (both recorded on *Asteraceae*), *Ph. conferta* (mainly on *Brassicaceae*), *L. hendersoniae* (on *Salicaceae*), *L. fallaciosa*, *L. collinsoniae* (mainly on *Lamiaceae)* and *L. libanotis* (on *Apiaceae*). *Plectophomella visci*, recorded from leaves of *Viscum album* (*Viscaceae*), also clustered in the *Leptosphaeriaceae*. The genus *Plenodomus* is re-introduced here to accommodate the species in subclade A1, which are allied to *Ph. lingam*.

Subclade A2 comprises pathogenic species often causing leaf spots such as *Ph. apiicola* on *Apium graveolens* (*Apiaceae*), *Ph. drobnjacensis* (on *Gentianaceae)*, *Ph. violicola* (on *Violaceae*) as well as the necrotrophic species *Ph. valerianae*, on *Valeriana* spp. (*Valerianaceae*). *Phoma apiicola* and *Ph. valerianae* were classified in *Phoma* section *Phoma*, and *Ph. violicola* was classified in *Phoma* sect. *Peyronellaea*; however, the relationship of these species in *Leptosphaeriaceae* is clearly demonstrated (Fig. 2), and therefore the species are transferred to the new genus *Subplenodomus*. These results are in congruence with a recent study where *Ph. violicola*, *Ph. apiicola* and *Ph. valerianae* clustered in a clade representing both *Leptosphaeriaceae* and *Pleosporaceae* (Aveskamp *et al.* 2010).

Four *Leptosphaeria* species, *L. macrospora* (soil) and the necrotrophic species *L. nitschkei* (on *Asteraceae*), *L. praetermissa*, on *Rubus idaeus* (*Rosaceae*) and *L. dryadis*, on *Dryas* spp. (*Rosaceae*) grouped in a subclade A3 and are transferred here to a new genus *Paraleptosphaeria*. *Phoma korfii* also clustered in this subclade. The European species *Ph. heteromorphospora*, type species of *Phoma* section *Heterospora*, and the American counterpart *Ph. dimorphospora*, both pathogens on *Chenopodiaceae*, grouped in a distinct subclade A4. *Phoma* sect. *Heterospora* is raised to generic rank to accommodate both species in *Leptosphaeriaceae*.

Clade B comprises necrotrophic species related to the type species *L. doliolum* (anam. *Ph. acuta*). The phylogeny of this species complex, and the closely related species *Ph. veronicicola*, *Ph. macrocapsa* and *Ph. sydowii*, is treated below. The necrotrophic species *Ph. sclerotioides*, *L. conoidea* (anam. *Ph. doliolum*), *L. slovacica* (anam. *Ph. leonuri*) and *Ph. pedicularis* also proved to be related. The species *Ph. rubefaciens* and *Ph. etheridgei* also belong to clade B, but these species, both recorded on trees, are more distantly related.

The *Phoma* species in clades A and B are in majority currently described as anamorphs of the genus *Leptosphaeria*, or belong to *Phoma* section *Plenodomus*. These *Phoma* anamorphs are only distantly related to the type species *Ph. herbarum* and its relatives in *Didymellaceae*, and therefore these species described in section *Plenodomus* are excluded from the genus *Phoma*. Clade C is more distantly related to *Leptosphaeriaceae* and comprises species that are related to *Coniothyrium palmarum* in *Coniothyriaceae.* Two subclades are recognised in clade C: *Ph. glycinicola*, *Py. dolichi* and *Ph. carteri* group with the generic type species *C. palmarum*, whereas two isolates of *Ph. septicidalis* group with *Ph. multipora*. The teleomorph *Neophaeosphaeria filamentosa* clustered basal to this clade. Clade D includes the genera *Cucurbitaria*, *Pyrenochaetopsis* and *Pyrenochaeta*, which represent *Cucurbitariaceae*. This finding is in congruence with previous studies (de Gruyter *et al.* 2010).

Phylogeny of the *Leptosphaeria doliolum* complex

The aligned sequence matrix obtained for the ITS, ACT, TUB and CHS-1 regions had a total length of 1 345 nucleotide characters; ITS 522, ACT 240, TUB 332 and CHS-1 251, respectively.

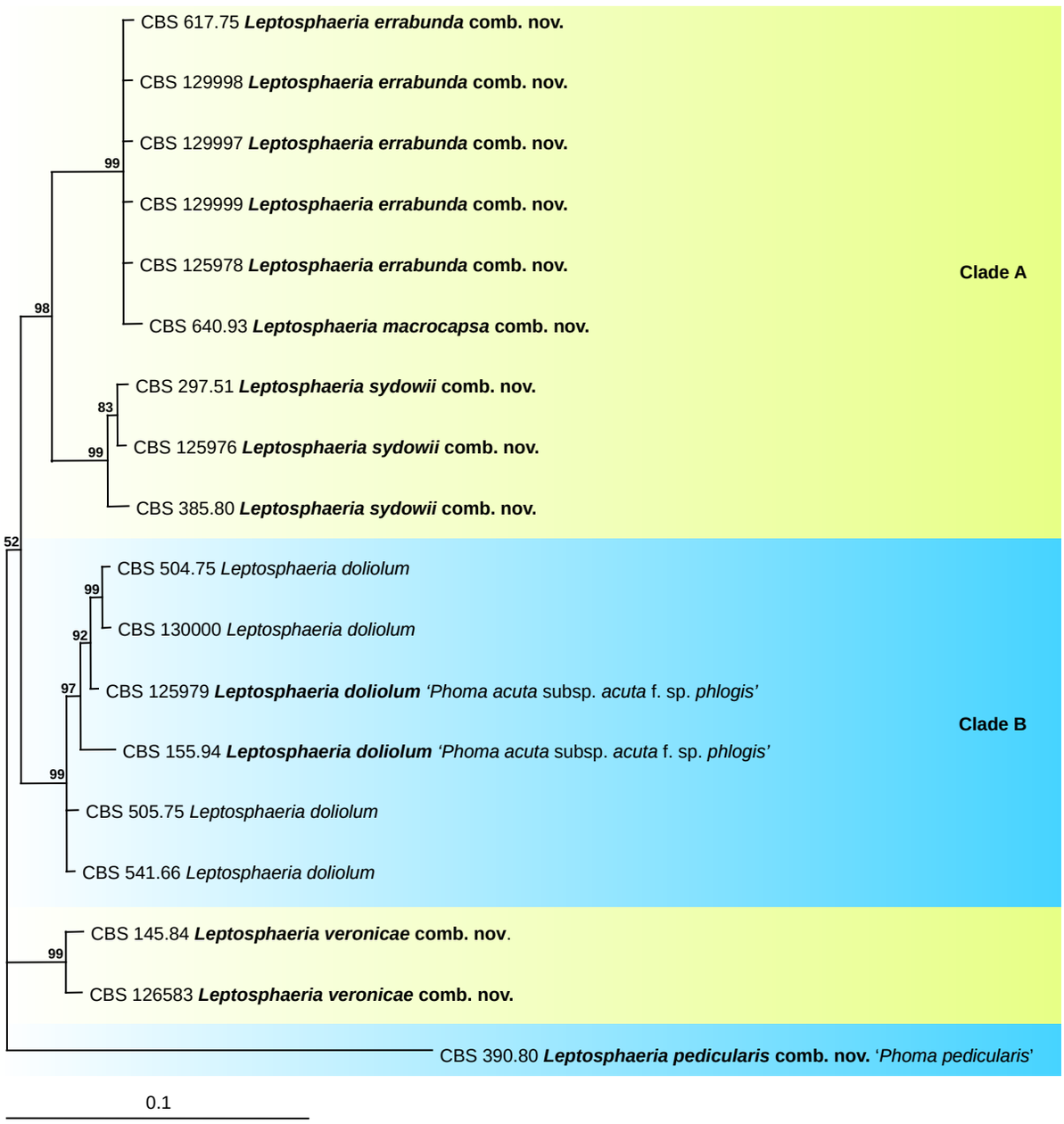


Fig. 3. The phylogeny of the *Leptosphaeria doliolum* complex, based on the strict consensus tree from a Bayesian analysis of 18 ITS/ACT/TUB/CHS-1 sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted to *Leptosphaeria pedicularis* comb. nov. (CBS 390.80).

The combined dataset used in the analyses included 18 taxa and contained 1 345 characters with 98 unique site patterns. The tree (Fig. 3) was rooted to "*Ph. pedicularis*" (CBS 390.80). The Bayesian analysis resulted in 6 002 trees after 30 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 3 341 trees.

The phylogenetic tree revealed two clades with high posterior probabilities, 98 and 99 % respectively, clade A with *Ph. acuta* subsp. *errabunda* and *Ph. macrocapsa*, and clade B with *Ph. acuta* subsp. *acuta* (anamorph of *Leptosphaeria doliolum*) and *Ph. acuta* subsp. *acuta* f. sp. *phlogis*. *Phoma sydowii*, a necrotroph on *Asteraceae*, *Senecio* spp. in particular, proved to

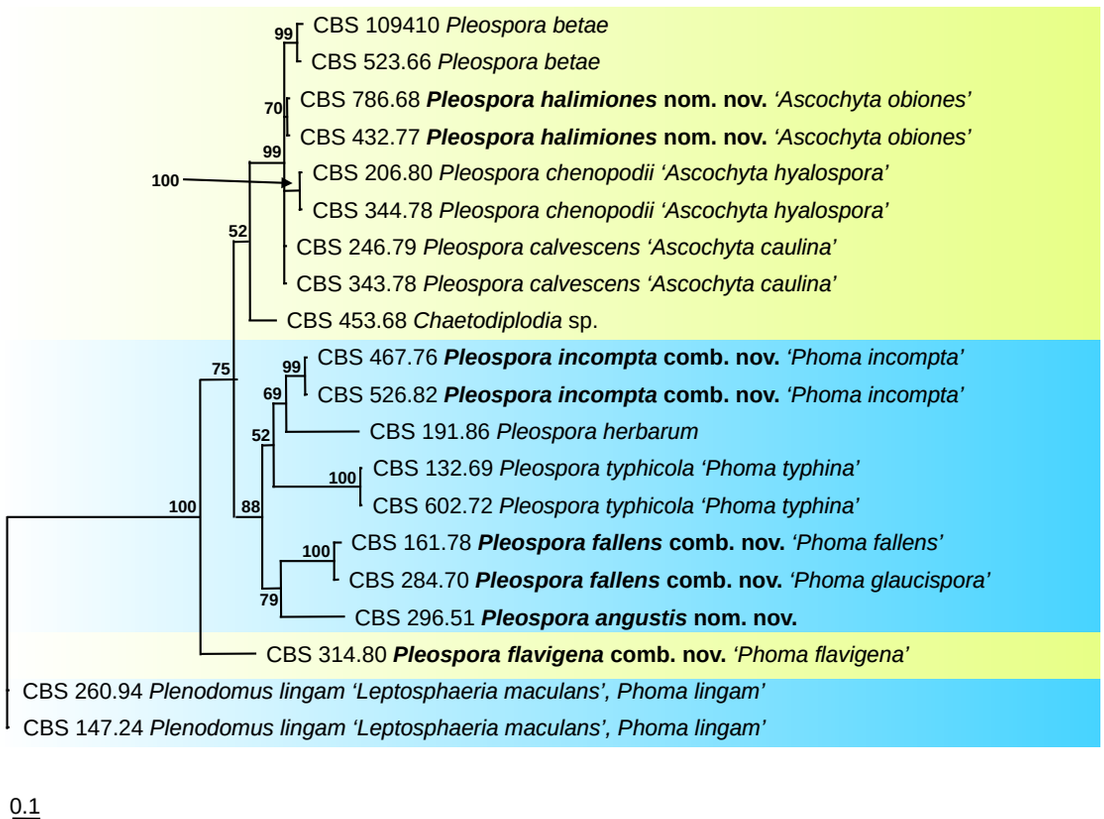


Fig. 4. The phylogeny of *Phoma*-like anamorphs in the *Pleosporaceae* based on the strict consensus tree from a Bayesian analysis of 20 ACT sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted to *Plenodomus lingam* (CBS 147.24, 260.94).

be closely related to *Ph. acuta* subsp. *errabunda*. The isolate CBS 297.51 preserved as *Ph. acuta* is similar to *Ph. sydowii*, a synonym of *L. sydowii*, see below. *Phoma veronicicola*, as a necrotroph specifically occurring on *Veronica* spp. (*Scrophulariaceae*), also proved to be related to *Leptosphaeria doliolum*.

Phylogeny of *Phoma* section *Pilosa*

The aligned sequence matrix obtained for the ACT region had a total length of 252 nucleotide characters (20 taxa), and contained 165 unique sites. The tree was rooted to *Ph. lingam* (CBS 147.24 and CBS 260.94). The Bayesian analysis resulted in 34 802 trees after 174 000 generations, from which the burn-in was discarded, and the consensus tree and posterior probabilities were calculated based on 11 728 trees (Fig. 4).

The phylogenetic tree representing *Pleosporaceae* includes *Ph. betae*, type species of *Phoma* section *Pilosa*. This section is characterised by producing pycnidia that are covered by mycelial hairs. *Phoma betae* clearly groups with other pycnidial fungi pathogenic on *Chenopodiaceae*, including *Ascochyta obiones, A. hyalospora* and *A. caulina* and *Chaetodiplodia* sp. All species produce similar hairy pycnidia, but are classified in *Ascochyta* or *Coniothyrium* due to conidial septation, or brown pigmentation of conidia, respectively.

A subclade comprises the cosmopolitan *Pleospora herbarum* and related species. The species involved are associated with various hosts or substrates. The most closely related *Ph. incompta* is a specific pathogen on *Olea europea* (*Oleaceae*). *Phoma incompta* was classified in *Phoma*

section *Sclerophomella* because of its thick-walled pycnidia (de Gruyter & Noordeloos 1992, Boerema & de Gruyter 1998). The pycnidial characters of *Ph. incompta*, pycnidia covered with mycelial hairs and with an indistinct ostiole visible as a pallid spot (de Gruyter & Noordeloos 1992) however, agrees with those of *Ph. betae* and *Ph. typhina*.

Phoma fallens proved to be closely related to *Ph. glaucispora* in keeping with the similar *in vitro* characters, especially the low growth-rate and the size and shape of its conidia (Boerema *et al*. 2004). Both species originate from southern Europe, and have been associated with spots on fruits and leaves of *Olea europea*, or leaf spots on *Nerium oleander,* respectively. An isolate preserved as *Leptosphaeria clavata*, CBS 259.51, proved to be closely related. The origin of the isolate, deposited by E. Müller, is unknown; however, it is likely that the isolate was obtained from *Poaceae*, *Triticum vulgare* or *Dactylis glomerata* (Müller 1950). *Phoma flavigena*, once isolated from water and also recorded from southern Europe, proved to be more distantly related in *Pleosporaceae*.

Phylogeny of *Phoma*-like anamorphs excluded from the suborder Pleosporineae

The aligned sequence matrix obtained for the LSU regions had a total length of 808 nucleotide characters, with 208 unique site patterns. The phylogenetic tree (Fig. 5) was rooted to *Pseudorobillarda phragmitis* (CBS 398.61). The Bayesian analysis resulted in 48 402 trees after 242 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 24 876 trees.

Clade A includes the reference isolates of the teleomorph *Paraphaeosphaeria* and the anamorph *Paraconiothyrium* classified in *Montagnulaceae*. This teleomorph/anamorph relation agrees with previous molecular phylogenetic studies (Verkley *et al.* 2004, Damm *et al.* 2008, de Gruyter *et al.* 2009). Other *Phoma*-like species in this clade are *Ph. lini*, *Plenodomus fusco-maculans*, *Pleurophoma pleurospora* (CBS 101461) and *Asteromella tilliae*. *Phoma lini*, a saprobe frequently recorded on dead stems of *Linum* spp., was described in *Phoma* section *Phoma* (de Gruyter *et al*. 1993). Re-examination of the conidia revealed that they are hyaline and thin-walled; however, also darker, greenish to yellowish *Coniothyrium*-like conidia were observed. The conidiogenous cells are *Phoma*-like, doliiform to ampulliform.

The isolate *Asteromella tiliae* (CBS 265.94) clearly represents a species of *Paraconiothyrium*, and therefore, the teleomorph name *Didymosphaeria petrakiana*, *Didymosphaeriaceae*, is probably incorrect. It was already mentioned by Butin & Kehr (1995) that "considering the taxonomical placement of the teleomorph, the authors were informed about forthcoming taxonomic changes".

The morphological characters of the isolate CBS 101461, considered as representing the generic type species *Pleurophoma pleurospora*, resembles *Paraconiothyrium* as was previously discussed (de Gruyter *et al.* 2009). The sterile ex-type strain of *Plenodomus fusco-maculans*, CBS 116.16, recorded from *Malus* sp., also grouped with the *Paraconiothyrium* isolates.

Coniothyrium fuckelii clustered in the *Paraphaeosphaeria*/*Paraconiothyrium* clade, in agreement with previous studies (Damm *et al*. 2008, Aveskamp *et al.* 2010), and therefore, the species is transferred to the genus *Paraconiothyrium*. Two *Phoma*-like species obtained from *Citysus scoparius* and *Lonicera* sp. respectively (CBS 116668 and CBS 130329), cluster near *Montagnulaceae* and *Massarinaceae*. The morphological characters of the species are typical for *Pleurophoma pleurospora*. The taxonomic position of both isolates at familial rank could not be determined. The morphology of *Phoma flavescens* proved to be most similar to that of *Paraconiothyrium*, it definitely does not belong to *Phoma*, and therefore the species is transferred to *Paraconiothyrium*. Sequence data of additional species clustering nearby

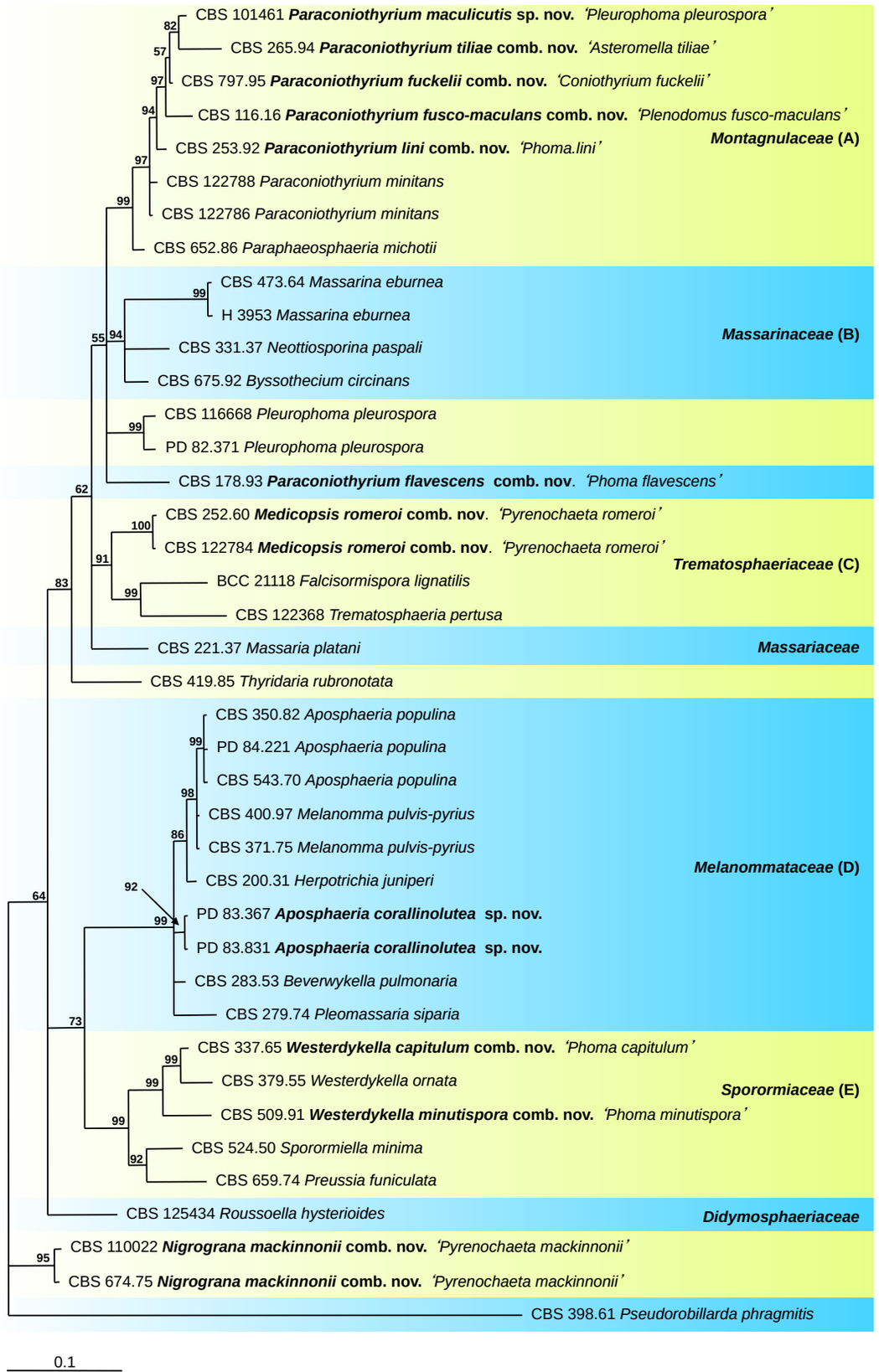


Fig. 5. The phylogeny of *Phoma*-like isolates excluded from the *Pleosporineae*, based on the strict consensus tree from a Bayesian analysis of 40 LSU sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted to *Pseudorobillarda phragmitis* (CBS 398.61).

are required to resolve the current classification of *Ph. flavescens*. None of the *Phoma*-like anamorphs included in this study grouped in clade B, which represents *Massarinaceae*.

Clade C includes the recently assigned ex-epitype strain of *Trematosphaeria pertusa*, isolate CBS 122368 (Zhang *et al.* 2008) and *Falcisformispora lignatilis*. Both *T. perusa* and *F. lignatilis* represent *Trematosphaeriaceae* (Suetrong *et al.* 2009). A second isolate preserved as *Trematosphaeria pertusa*, CBS 400.97, proved to be only distantly related, and clustered in clade D with *Aposphaeria populina* and *Melanomma pulvis-pyrius* in *Melanommataceae*. This isolate is considered as an incorrect identification (Mugambi & Huhndorf 2009), and we consider this sterile isolate as representative of *Melanomma pulvis-pyrius*. Clade C also comprises the human pathogen *Pyrenochaeta romeroi*. This species certainly does not belong to *Pyrenochaeta* (de Gruyter *et al.* 2010) and therefore, we describe the new genus *Medicopsis* in *Trematosphaeriaceae* to accommodate this species.

A well-supported clade D represents *Melanommataceae* and includes *Melanomma pulvis-pyrius, Herpotrichia juniperi* and *Beverwijkella pulmonaria*, in congruence with Zhang *et al.* (2009). There were four *Phoma*-like isolates present in the collections of CBS and PD, i.e. CBS 350.82, PD 83/367, PD 83/831 and PD 84/221, which could not be identified according to their morphological characters. The isolates were preserved as *Pleurophoma* spp. This study demonstrates that two strains represent *Aposphaeria populina*, whereas the other two strains represent the new species described here as *Aposphaeria corallinolutea*. Further studies in *Melanommataceae* are needed to clarify the phylogeny of *Aposphaeria* in *Melanommataceae*.

Sporormiaceae (clade E) is represented by *Sporormiella minima* and *Preussia funiculata*. *Phoma capitulum* and *Ph. minutispora*, well-defined soil-borne fungi from Asia, group in this clade. Both species are related with the anamorph *Westerdykella ornata*, and therefore the species are transferred to *Westerdykella* in *Sporormiaceae*.

Pyrenochaeta mackinnonii could not be assigned to familial rank. A blast search in GenBank with its LSU sequence suggested a relation with *Versicolorisporum triseptum*. However, the typical 3-septate conidia of this anamorph are different. Neither could *V. triseptum* be assigned at familial rank in *Pleosporales* (Tanaka *et al.* 2009). We therefore introduce the new genus *Nigrograna* to accommodate *Py. mackinnonii*.

TAXONOMY

Leptosphaeriaceae M.E. Barr, Mycotaxon 29: 503. 1987.

Heterospora (Boerema, Gruyter & Noordel.) Gruyter, Verkley & Crous, **stat. nov.** MycoBank MB564701.
Basionym: *Phoma* sect. *Heterospora* Boerema, Gruyter & Noordel., Persoonia 16: 336. 1997.

Type species: *Heterospora chenopodii* (Westend.) Gruyter, Aveskamp & Verkley, see below (= *Phoma heteromorphospora* Aa & Kesteren).

Heterospora chenopodii **(**Westend.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564702.
Basionym: *Phyllosticta chenopodii* Westend., Bull. Acad. Roy. Sci. Belgique Ser. 2, 2: 567. 1857; not *Phyllosticta chenopodii* Sacc., Syll. Fung. 3: 55. 1884 = *Phoma exigua* Desm. var. *exigua*; not *Plenodomus chenopodii* (P. Karst. & Har.) Arx, Verh. Kon. Ned. Akad. Wetensch.,

Afd. Natuurk., Sect. 2. 51: 72. 1957 ≡ *Phoma chenopodiicola* Gruyter, Noordel. & Boerema, Persoonia 15: 395. 1993; not *Phoma chenopodii* Pavgi & U.P. Singh, Mycopathol. Mycol. Appl. 30: 265. 1966. nom. illeg. = *Phoma chenopodii* S. Ahmad, Sydowia 2: 79. 1948.

≡ *Septoria westendorpii* G. Winter, Hedwigia 26: 26. 1887. nom. nov.; not *Phoma westendorpii* Tosquinet, Westend., Bull. Acad. Roy. Sci. Belgique Ser. 2, 2: 564. 1857.

≡ *Phoma variospora* Aa & Kesteren, Persoonia 10: 268. 1979. nom. nov., nom. illeg.; not *Phoma variospora* Shreem., Indian J. Mycol. Pl. Pathol. 8: 221. 1979 ("1978").

≡ *Phoma heteromorphospora* Aa & Kesteren, Persoonia 10: 542. 1980. nom. nov.

Specimens examined: **Belgium,** Beverloo, from leaves of *Chenopodium suecicum* (*album*) and *Chenopodium urbicum* (*Chenopodiaceae*), no date, G.D. Westendorp, Herb. Crypt. (Ed. Beyaert-Feys), No. 959. BR, **holotype** of *Phyllosticta chenopodii* Westend. ex herb. G.D. Westendorp. **The Netherlands**, Baarn, from leaf spots in *Chenopodium album*, 3 July 1968, H.A. van der Aa, **epitype designated here** CBS H-16386, culture ex-epitype CBS 448.68; Heelsum, from leaf spots in *Chenopodium album*, Sep. 1994, J. de Gruyter, CBS 115.96 = PD 94/1576.

Notes: Van der Aa & van Kesteren (1979) provided a nom. nov. since the epithet '*chenopodii*' was occupied in *Phoma*. For more details of the taxonomy of the species see van der Aa & van Kesteren (1979). Although *Leptosphaeria chenopodii-albi* was described from leaves of *Chenopodium album* (Crane & Shearer 1991) no cultures are available for comparison.

Heterospora dimorphospora (Speg.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564703.

Basionym: *Phyllosticta dimorphospora* Speg., Anales Mus. Nac. Buenos Aires 13: 334. 1910.

≡ *Phoma dimorphospora* (Speg.) Aa & Kesteren, Persoonia 10: 269. 1979.

= *Stagonospora chenopodii* Peck, Rep. (Annual) New York State Mus. Nat. Hist. 40: 60. 1887. (sometimes erroneously listed as *Stag. chenopodii* "House").

Specimens examined: **Argentina**, La Plata, from leaves of *Chenopodium hircinum* (*Chenopodiaceae*), 13 Oct. 1906, C. Spegazzini, Colect. micol. Museo Inst. Spegazzini, No. 11.353, LPS, **holotype** of *Phyllosticta dimorphospora* Speg. **Lima**, from stem of *Chenopodium quinoa*, 1977, L.J. Turkensteen, CBS 165.78 = PD 77/884. **Peru**, from lesions in stems of *Chenopodium quinoa*, 1976, V. Otazu, **epitype designated here** CBS H-16203, culture ex-epitype CBS 345.78 = PD 76/1015.

Note: For more details of the taxonomy of the species see van der Aa & van Kesteren (1979).

Leptosphaeria Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 234. 1863.

Type species: *Leptosphaeria doliolum* (Pers.: Fr.) Ces. & De Not., see below.

= *Leptophoma* Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 124: 73. 1915.

For full synonymy, including the species listed below, see Crane & Shearer (1991) and Boerema *et al.* (2004).

Leptosphaeria conoidea (De Not.) Sacc., Fungi Venet. Nov. Vel. Crit. Ser. 2: 314. 1875.
Basionym: *Leptosphaeria doliolum* var. *conoidea* De Not., Mycoth. Veneti, No. 76. 1873.
= *Leptosphaeria doliolum* subsp. *pinguicula* Sacc., Michelia 2: 598. 1882.
= *Phoma acuta* subsp. *amplior* Sacc. & Roum., Rev. Mycol. 6: 30. 1884.
≡ *Phoma hoehnelii* subsp. *amplior* (Sacc. & Roum.) Boerema & Kesteren, Trans. Brit. Mycol. Soc. 67: 299. 1976.
= *Phoma doliolum* P. Karst., Meddel. Soc. Fauna Fl. Fenn. 16: 9. 1888.
= *Plenodomus microsporus* Berl., Bull. Soc. Mycol. France 5: 55. 1889.

Specimens examined: **The Netherlands**, Zaltbommel, from dead stem of *Lunaria annua* (*Brassicaceae*), Jan. 1974, G.H. Boerema, CBS 616.75 = ATCC 32813 = IMI 199777 = PD 74/56; Montfoort, *Senecio* sp. (*Asteraceae*), 1982, CBS 125977 = PD 82/888.

Leptosphaeria doliolum (Pers. : Fr.) Ces. & de Not., Comment. Soc. Crittog. Ital. 1: 234. 1863.
Basionym: *Sphaeria doliolum* Pers. : Fr., Icon. Desc. Fung. Min. Cognit. (Leipzig) 2: 39. 1800.
= *Sphaeria acuta* Hoffm. : Fr, Veg. cryptog. 1: 22. 1787, Syst. Mycol. 2: 507. 1823.
≡ *Phoma acuta* (Hoffm. : Fr.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 125. 1870 (as "*acutum*").
≡ *Leptophoma acuta* (Hoffm. : Fr.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 124: 73. 1915.
≡ *Plenodomus acutus* (Hoffm. : Fr.) Bubák, Ann. Mycol. 13: 29. 1915. [as "(Fuckel)"].
= *Phoma phlogis* Roum., Rev. Mycol. 6: 160. 1884.
= *Phoma hoehnelii* var. *urticae* Boerema & Kesteren, Trans. Brit. Mycol. Soc. 67: 299. 1976.

Specimens examined: **The Netherlands**, from stem of *Rudbeckia* sp. (*Asteraceae*), Sep. 1966, M.M.J. Dorenbosch, CBS 541.66 = PD 66/221; from stem of *Urtica dioica* (*Urticaceae*), 1974, G.H. Boerema, CBS 504.75 = PD 74/55; Rhenen, from *Urtica dioica*, Feb. 1975, G.H. Boerema, CBS 505.75 = PD 75/141; Wageningen, from stem of *Phlox paniculata* (*Polemoniaceae*), 1977, G.H. Boerema, CBS 155.94 = PD 77/80; from stem of *Phlox paniculata*, 1978, G.H. Boerema, CBS 125979 = PD 78/37; from stem of *Urtica dioica*, 1982, G.H. Boerema, CBS 130000 = PD 82/701.

Notes: Isolate CBS 541.66 was preserved as *Phoma acuta* subsp. *errabunda* (teleom. *Leptosphaeria errabunda*, see below); however, the isolate clustered with *L. doliolum*. Both isolates CBS 155.94 and CBS 125979 were considered as *forma specialis "phlogis"* (Boerema *et al.* 1994) of the anamorph *Ph. acuta* subsp. *acuta*. The subspecies *acuta* was created by the differentiation of *Phoma acuta* subsp *amplior* Sacc. & Roum., but the latter is a synonym of *Ph. doliolum*, reclassified here as *L. conoidea*, see above. *Sphaeria acuta* Hoffm. was applied as basionym for different anamorphs an a teleomorph of various species of *Leptosphaeria* leading to a confusing nomenclature. The epitet has been unambiguously tied to *Ph. acuta* by Boerema & Gams (1995).

Leptosphaeria errabunda (Desm.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564704.
Basionym: *Phoma errabunda* Desm., Ann. Sci. Nat., Bot. Ser. 3, 11: 282. 1849.
≡ *Phoma acuta* subsp. *errabunda* (Desm.) Boerema, Gruyter & Kesteren, Persoonia 15: 465. 1994.

= *Leptophoma doliolum* Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 124: 75. 1915; not *Phoma doliolum* P. Karst. = *Leptosphaeria conoidea* (De Not.) Sacc., see above.

≡ *Plenodomus doliolum* (Höhn.) Höhn., Ber. Deutsch. Bot. Ges. 36: 139. 1918.

≡ *Phoma hoehnelii* Kesteren, Netherlands J. Pl. Pathol. 78: 116. 1972. nom. nov.

= *Leptosphaeria doliolum* subsp. *errabunda* Boerema, Gruyter & Kesteren, Persoonia 15: 466. 1994.

Specimens examined: **The Netherlands**, Leeuwarden, from stem of *Delphinium* sp. (*Ranunculaceae*), 1974, CBS 125978 = PD 74/61; Ferwerderadeel, from *Solidago* sp., hybrid (*Asteraceae*), Mar. 1974, G.H. Boerema, CBS 617.75 = ATCC 32814 = IMI 199775 = PD 74/201; from stem of *Aconitum* sp. (*Ranunculaceae*), CBS 129999 = PD 78/569; from stem of *Achillea millefolium* (*Asteraceae*), CBS 129997 = PD 78/631; from *Gailardia* sp. (*Asteraceae*), 1984, G.H. Boerema, CBS 129998 = PD 84/462.

Notes: The isolate CBS 617.75 = ATTC 32814 was deposited as the anamorph *Ph. hoehnelii* var. *hoehnelii*, but interpreted as *L. doliolum* subsp. *conoidea* (Dong *et al*. 1998). The isolate clustered with *L. errabunda* in this study.

Leptosphaeria etheridgei (L.J. Hutchison & Y. Hirats.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564712.
Basionym: *Phoma etheridgei* L.J. Hutchison & Y. Hirats., Canad. J. Bot. 72: 1425. 1994.

Specimen examined: **Canada**, Alberta, from bark of gall, on trunck of *Populus tremuloides* (*Salicaceae*), July 1989, P. Crane, **holotype** DAOM 216539, culture ex-holotype DAOM 216539 = CBS 125980 = PD 95/1483.

Leptosphaeria macrocapsa (Trail) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564713.
Basionym: *Phoma macrocapsa* Trail, Scott. Naturalist (Perth) 8: 327. 1886.

≡ *Plenodomus macrocapsa* (Trail) H. Ruppr., Sydowia 13: 20. 1959.

Specimen examined: **The Netherlands**, from stem of *Mercurialis perennis* (*Euphorbiaceae*), 1978, G.H. Boerema, CBS 640.93 = PD 78/139.

Leptosphaeria pedicularis (Fuckel) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564714.
Basionym: *Phoma pedicularis* Fuckel, Reisen Nordpolarmeer 3: 318. 1874 (as "*pedicularidis*"); not *Phoma pedicularis* Wehm., Mycologia 38: 319. 1946 (= *Phoma herbicola* Wehm).

= *Sphaeronaema gentianae* Moesz, Bot Közlem. 14: 152. 1915 (as "*Sphaeronema*").

≡ *Plenodomus gentianae* (Moesz) Petr., Ann. Mycol. 23: 54. 1925.

Specimens examined: **Switzerland**, Kanton Graubünden, Albulapass, from dead stem of *Pedicularis* sp. (*Scrophulariaceae*), 1977, CBS 390.80 = PD 77/711 = ATCC 42535 = IMI 248430; Zürich, from *Gentiana punctata* (*Gentianaceae*), 1977, CBS 126582 = PD 77/710.

Leptosphaeria rubefaciens (Togliani) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564715.
Basionym: *Phoma rubefaciens* Togliani, Ann. Sper. Agr. II, 7: 1626. 1953.

Specimens examined: **Switzerland**, Zürich, Albis, from twig of *Quercus* sp. (*Fagaceae*), Aug. 1976, W. Gams, CBS 223.77. **The Netherlands**, Oploo, from wood of *Tilia* (´) *europaea* (*Tiliaceae*), 1978, G.H. Boerema, CBS 387.80 = ATCC 42533 = IMI 248432 = PD 78/809.

Leptosphaeria sclerotioides (Sacc.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564716.
Basionym: *Phoma sclerotioides* Sacc., Fungi Herb. Bruxelles 21. 1892; Syll. Fung. 11: 492. 1895.
= *Plenodomus sclerotioides* Preuss, Klotzsch. Herb. Vivum Mycol. Sistems Fungorum German., No. 1281. 1849. nom. nud. (no description).
= *Plenodomus meliloti* Mark.-Let., Bolezni Rast. 16: 195. 1927.

Specimens examined: **Canada**, British Columbia, from *Medicago sativa*, 1980, J. Drew Smith, CBS 148.84 = PD 80/1242; Alberta, from root of *Medicago sativa* (*Fabaceae*), Mar. 1984, G.H. Boerema, CBS 144.84 = CECT 20025 = PD 82/1061.

Note: Seven varieties of this species have been recognised (Wunsch *et al.* 2011) in a phylogenetic analysis using 10 loci.

Leptosphaeria slovacica Picb., Sborn. Vysoké Skoly. Zemed. v Brno 7: 7. 1927.
= *Phoma leonuri* Letendre, Revue Mycol. 6: 229. 1884.
≡ *Plenodomus leonuri* (Letendre) Moesz & Smarods in Moesz, Magyar Bot. Lapok 31: 38. 1932.

Specimens examined: **The Netherlands**, from dead stem of *Ballota nigra* (*Lamiaceae*), 1977, CBS 125975 = PD 77/1161; Arnhem, from dead stem of *Ballota nigra*, 1979, G.H. Boerema, CBS 389.80 = PD 79/171.

Leptosphaeria sydowii (Boerema, Kesteren & Loer.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564717.
Basionym: *Phoma sydowii* Boerema, Kesteren & Loer., Trans. Brit. Mycol. Soc. 77: 71. 1981. nom. nov.
= *Sphaeronaema senecionis* Syd. & P. Syd., Ann. Mycol. 3: 185. 1905; not *Phoma senecionis* P. Syd., Beibl. Hedwigia 38: 136. 1899.
≡ *Plenodomus senecionis* (Syd. & P. Syd.) Bubák, Ann. Mycol. 13: 29. 1915.
≡ *Plenodomus senecionis* (Syd. & P. Syd.) Petr., Ann. Mycol. 19: 192. 1921. Isonym.
= *Plenodomus rostratus* Petr., Ann. Mycol. 21: 199. 1923; not *Phoma rostrata* O'Gara, Mycologia 7: 41. 1915; not *Leptosphaeria rostrata* M.L. Far & H.T. Horner, Nova Hedwidgia 15: 250. 1968.

Specimens examined: **Switzerland**, Kt. Zürich, Zollikon, from *Papaver rhoeas* (*Papaveraceae*), Oct. 1949, E. Müller, CBS 297.51. **The Netherlands**, from *Senecio jacobaea* (*Asteraceae*), G.H. Boerema, 1984, CBS 125976 = PD 84/472. **UK**, Scotland, Isle of Lewis, Hebrides, from dead stem of *Senecio jacobaea*, 1974, R.W.G. Dennis, CBS 385.80 = PD 74/477.

Notes: *Leptosphaeria senecionis* (Fuckel) G. Winter was suggested as the possible teleomorph (Boerema *et al.* 2004). Because the teleomorph connection has not been proven, however, we did not include it as a synonym that would have priority as the correct name. The isolate CBS 297.51 was originally identified as *L. doliolum* var. *doliolum*.

Leptosphaeria veronicae (Hollós) Gruyter, Aveskamp & Verkley **comb. nov.** MycoBank MB564718.
Basionym: *Sphaeronaema veronicae* Hollós, Ann. Hist.-Nat. Mus. Natl. Hung. 4: 341. 1906.
≡ *Phoma veronicicola* Boerema & Loer., Trans. Brit. Mycol. Soc. 84: 297. 1985. nom. nov.; not *Phoma veronicae* Roum., Revue Mycol. 6: 160. 1884.

Specimens examined: **The Netherlands**, from stem of *Veronica* "Shirley Blue" (*Scrophulariaceae*), 1974, CBS 126583 = PD 74/227; Huis ter Heide, from dead stem of *Veronica chamaedryoides*, Mar. 1978, H.A. van Kesteren, **neotype** CBS H-7632, culture ex-neotype CBS 145.84 = CECT 20059 = PD 78/273.

Paraleptosphaeria Gruyter, Verkley & Crous, **gen. nov.** MycoBank MB564720.

Pseudothecia immersed, subglobose, solitary or aggregated, thick-walled, pseudoparenchymatous to scleroplectenchymatous, ostiolate, unilocular. *Asci* bitunicate, broadly ellipsoidal, 8-spored, interascal filaments pseudoparaphyses, *Ascospores* biseriate, broadly fusiform, transversally 3–5 septate, hyaline to yellow-brownish. *Conidiomata* pycnidial, globose to subglobose, scleroplectenchymatous, with papillate pore, unilocular. *Conidiogenous cells* phialidic, ampulliform to doliiform. *Conidia* hyaline, aseptate, oblong to ellipsoidal. Sclerotia sometimes produced.

Type species: *Paraleptosphaeria nitschkei* (Rehm ex G. Winter) Gruyter, Aveskamp & Verkley (see below).

Notes: Munk (1957) recognised *Leptosphaeria* section *Para-Leptosphaeria*, an invalid taxon, as a heterogenous group. The section was differentiated from *Eu-Leptosphaeria*, which included the generic type species *L. doliolum*. *Leptosphaeria nitschkei* was considered a typical representative of section *Eu-Leptosphaeria* (Müller & von Arx 1950). However, this molecular phylogeny demonstrates that *L. nitschkei* is only distantly related to *L. doliolum*. We introduce *Paraleptosphaeria* to accomodate *L. nitschkei* and its relatives. These necrotrophic species are morphologically closely allied to *Leptosphaeria*. The former classification of *Leptosphaeria* in sections *Eu-Leptosphaeria* and *Para-Leptosphaeria* cannot be upheld from a evolutionary point of view, as two other species attributed to section *Eu-Leptosphaeria*, namely *L. agnita* and *L. maculans* (Munk 1957), were found to group in *Plenodomus*.

Paraleptosphaeria dryadis **(**Johanson) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564721.
Basionym: *Melanomma dryadis* Johanson, Hedwigia 29: 160. 1890.
≡ *Leptosphaeria dryadophila* Huhndorf, Bull. Illinois Nat. Hist. Surv. 34: 484 (1992). nom. illeg. via nom. superfl.
= *Leptosphaeria dryadis* Rostr., Bot. Tidsskr. 25: 305. 1903.

Specimen examined: **Switzerland**, Kt. Ticino, Leventina, Alpe Campolungo, from *Dryas octopetala* (*Rosaceae*), 24 July 1980, A. Leuchtmann, CBS 643.86.

Note: An explanation of the nomenclature of *Leptosphaeria dryadis* has been provided by Chen *et al*. (2002).

Paraleptosphaeria macrospora **(**Thüm.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564722.
Basionym: *Leptosphaeria macrospora* Thüm. Mycotheca Univ. 1359. 1879. nom. nov.
≡ *Metasphaeria macrospora* (Fuckel) Sacc., Syll. Fung. 2: 158. 1883.
Replaced synonym: *Pleospora macrospora* Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 138. 1870. nom. illeg., Art. 53.1.; not *Pleospora macrospora* (De Not.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 218. 1863.

Specimen examined: **Norway**, Troms, Tromsöya, from *Rumex domesticus* (*Polygonaceae*), 20 Aug. 1988, K. & L. Holm, CBS 114198 = UPSC 2686.

Paraleptosphaeria nitschkei (Rehm ex G. Winter) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564723.
Basionym: *Leptosphaeria nitschkei* Rehm ex G. Winter, Ascomyceten, Fascicle 1, No. 15. 1870. nom. nud.; Flora, Jena und Regensburg 55: 510. 1872.

Specimen examined: **Austria**, Ötscher in Niederösterreich, c. 4500', from *Cacalia* sp. (= *Adenostyles* sp, *Asteraceae*), June 1869, Lojka, **holotype** of *Leptosphaeria nitschkei* Rehm Ascomyceten 15b, S. **Switzerland**, Kt. Graubünden, Lü, from *Cirsium spinosissimum* (*Asteraceae*), 16 July 1948, E. Müller, **epitype designated here** CBS H-20822, culture ex-epitype CBS 306.51.

Note: The name *Leptosphaeria nitschkei* was considered a nom. nud. by Crane and Shearer (1991) who cited Art. 32.1 but gave no further explanation. In Flora, Jena und Regensburg 55: 510. 1872 Rehm refers to additional notes by G. Winter that include a Latin description. Therefore, we consider this name as valid, following Müller (1950) who provided a detailed description *in vivo*.

Paraleptosphaeria orobanches (Schweinitz : Fr.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564724.
Basionym: *Sclerotium orobanches* Schweinitz, Schriften Naturf. Ges. Leipzig 1: 57. 1822: Fr., Syst. Mycol. 2: 257. 1822.
= *Phoma korfii* Boerema & Gruyter, Persoonia 17: 275. 1999.

Specimen examined: **USA**, Ringwood Swamp, Lloyd-Cornell, from stem of *Epifagus virginiana* (*Orobanchaceae*), 13 Sep. 1995, T. Uturriaga, R.P. Korf, P. Mullin**, holotype** of *Sclerotium orobanches* Schweinitz, CUP 63537, culture ex-holotype CBS 101638 = PD 97/12070.

Note: A *Phoma* synanamorph of *Sclerotium orobanches* was reported by Yáñez-Morales *et al.* (1998) and described as *Phoma korfi* (Boerema & Gruyter 1999).

Paraleptosphaeria praetermissa (P. Karst.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564725.
Basionym: *Sphaeria praetermissa* P. Karst., Bidrag Kannedom Finlands Natur Folk 23: 89. 1873.
≡ *Leptosphaeria praetermissa* (P. Karst.) Sacc., Syll. Fung. 2: 26. 1883.

Specimen examined: **Sweden**, Dalarna, Folkärna, from *Rubus idaeus* (*Rosaceae*), 21 Mar. 1993, K. & L. Holm, CBS 114591.

Plenodomus Preuss, Linnaea 24: 145. 1851.
≡ *Phoma* sect. *Plenodomus* (Preuss) Boerema, Kesteren & Loer., Trans. Brit. Mycol. Soc. **77**: 61. 1981.
= *Diploplenodomus* Diedicke, Ann. Mycol. 10: 140. 1912.
= *Plectophomella* Moesz, Magyar Bot. Lapok 21: 13. 1922.
= *Apocytospora* Höhn., Mitt. Bot. Lab. TH Wien 1: 43. 1924.
= *Deuterophoma* Petri, Boll. R. Staz. Patalog. Veget. Roma 9: 396. 1929.

Type species: *Plenodomus rabenhorstii* Preuss, Linnaea 24: 145. 1851 (dubious synonym, see below) = *Plenodomus lingam* (Tode : Fr.) Höhn., see below.

Note: For full synonymy of the anamorph names of the species listed below, see Boerema *et al.* (1994). For additional synonyms of the teleomorph names of the species below that have been recorded from Asteraceous hosts, see Khashnobish *et al.* (1995).

Plenodomus agnitus (Desm.) Gruyter, Aveskamp & Verkley, **comb. nov**. MycoBank MB564726.
Basionym: *Sphaeria agnita* Desm., Ann. Sci. Nat., Bot. Ser. 3, 16: 313. 1851.
≡ *Leptosphaeria agnita* (Desm.) Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 236. 1863.
= *Plenodomus chondrillae* Died, Ann. Mycol.. 9: 140. 1911; Krypt.-fl. Brandenburg 9: 236. 1912.
= *Phoma agnita* Gonz. Frag., Mem. Real Acad. Ci. Barcelona 15: 6. 1920.

Specimens examined: **The Netherlands**, from stem of *Eupatorium cannabinum* (*Asteraceae*), 1982, W.M. Loerakker, CBS 126584 = PD 82/561; from stem of *Eupatorium cannabinum*, 1982, W.M. Loerakker, CBS 121.89 = PD 82/903.

Plenodomus biglobosus (Shoemaker & H. Brun) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564727.
Basionym: *Leptosphaeria biglobosa* Shoemaker & H. Brun, Canad. J. Bot. 79: 413. 2001.

Specimens examined: **France**, Le Rheu, from stem of *Brassica juncea* (*Brassicaceae*), CBS 127249 = DAOM 229269. **The Netherlands**, from *Brassica rapa* (*Brassicaceae*), 2006, R. Veenstra, CBS 119951.

Notes: *Leptosphaeria biglobosa* was originally described as a less virulent segregate of *L. maculans* (Shoemaker & Brun 2001). The species, also indicated as Tox^0 isolates, has been described from cultivated *Brassica* species as the cause of upper stem lesions and considered as less damaging than *L. maculans* (West *et al.* 2002). However, in Poland *L. biglobosa* is the

predominant cause of these symptoms (Jedryczka *et al*. 1999, Huang *et al.* 2005). The current species concept of *L. biglobosa* is broadly defined with six distinct subclades recognised by multilocus phylogenetic analyses of ITS, β-tubulin and actin sequences (Mendes-Pereira *et al.* 2003, Vincenot *et al.* 2008). These subclades are named after the host or geographic origin of the isolates involved. It has been suggested that the clades represent distinct subspecies formed over time by reproductive isolation (Mendes-Pereira *et al.* 2003). Alignments of the ITS sequences of *Ph. wasbiae*, *Ph. pimpinellae* and *L. biglobosa* isolates were compared with those of the representative strains of the *L. biglobosa* subclades obtained from GenBank, and both *Ph. wasbiae* and *Ph. pimpinellae* grouped in this species complex (unpubl. data). Both species are maintained here, awaiting a redescription of the taxa representing all clades in the *L. biglobosa* complex.

Plenodomus chrysanthemi **(**Zachos, Constantinou & Panag.) Gruyter, Aveskamp & Verkley, **comb. nov**. MycoBank MB564728.
Basionym: *Cephalosporium chrysanthemi* Zachos, Constantinou & Panag., Ann. Inst. Phytopath. Benaki, N.S. 55. 1960.
≡ *Phialophora chrysanthemi* (Zachos, Constantinou & Panag.) W. Gams, Cephalosporium-artige Schimmelpilze (Stuttgart): 207. 1971.
= *Phoma vasinfecta* Boerema, Gruyter & Kesteren, Persoonia 15: 484. 1994.

Specimen examined: **Greece**, from *Chrysanthemum* sp. (*Asteraceae*), Apr. 1963, D.G. Zachos, **holotype** CBS H-7576, culture ex-holotype CBS 539.63.

Note: The species was also described as *Phoma tracheiphila* f. sp. *chrysanthemi* (Baker *et al*. 1985).

Plenodomus collinsoniae (Dearn. & House) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564729.
Basionym: *Leptosphaeria collinsoniae* Dearn. & House, Bull. New York State Mus. Nat. Hist. 233–234: 36. 1921.

Specimen examined: **Japan**, Osawa river, Komukai, Miyagi, from *Vitis coignetiae* (*Vitaceae*), 27 Sep. 2003, Y. Takahashi, CBS 120227 = JCM 13073 = MAFF 239583.

Plenodomus confertus **(**Niessl ex Sacc.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564730.
Basionym: *Leptosphaeria conferta* Niessl ex Sacc., Syll. Fung. 2: 20. 1883.
= *Phoma conferta* P. Syd. ex Died., Krypt.-fl. Brandenburg 9: 142. 1912.

Specimen examined: **Spain**, Cais do Tejo, from dead stem of *Anacyclus radiatus* (*Asteraceae*), Mar. 1961, M.T. Lucas, CBS 375.64.

Plenodomus congestus **(**M.T. Lucas) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564731.
Basionym: *Leptosphaeria congesta* M.T. Lucas, Trans. Brit. Mycol. Soc. 46: 362. 1963.
= *Phoma congesta* Boerema, Gruyter & Kesteren, Persoonia 15: 461. 1994.

Specimen examined: **Spain**, Póvoa de Santa Iria, Estremadura, from stem *of Erigeron canadensis* (*Asteraceae*), Mar. 1961, M.T. Lucas, **holotype** of *Leptosphaeria congesta* M.T. Lucas, dried culture LISE 1638, culture ex-holotype CBS 244.64.

Plenodomus enteroleucus (Sacc.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564753.
Basionym: *Phoma enteroleuca* Sacc. var. *enteroleuca*, Michelia 1: 358. 1878.

Specimens examined: **France**, Alencon, from *Pyrus communis* (*Rosaceae*), 1878, C. C. Gillet, **holotype** of *Phoma enteroleuca* var. *enteroleuca*, Herb. Sacc. '19', PAD. **Germany**, Monheim, from leaf spots of *Triticum aestivum* (*Poaceae*), 15 Aug. 1984, M. Hossfeld, CBS H-3684, culture CBS 831.84. **The Netherlands**, Bennekom, from discoloured wood of *Catalpa bignonioides* (*Bignoniaceae*), 1981, G.H. Boerema, **epitype designated here** CBS H-16209, culture ex-epitype CBS 142.84 = PD 81/654 = CECT 20063.

Plenodomus fallaciosus **(**Berl.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564732.
Basionym: *Leptosphaeria fallaciosa* Berl., Bull. Soc. Mycol. France 5: 43. 1889.

Specimen examined: **France**, Var, Ste. Baume, from *Satureia montana* (*Lamiaceae*), July 1951, E. Müller, CBS 414.62 = ETH 2961.

Plenodomus hendersoniae (Fuckel) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564754.
Basionym: *Cucurbitaria hendersoniae* Fuckel, Symb. Myc. p. 172. 1870.
≡ *Melanomma hendersoniae* (Fuckel) Sacc., Syll. Fung. 2: 109. 1883.
≡ *Chiajaea hendersoniae* (Fuckel) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 129: 152. 1920.
≡ *Leptosphaeria hendersoniae* (Fuckel) L. Holm, Symb. Bot. Upsal. 14: 26. 1957.
= *Phoma intricans* M.B. Schwarz, Meded. Phytopath. Lab. Willie Commelin Scholten 8: 44. 1922.

Specimens examined: **Sweden**, Uppland, Jerusalem, from *Salix cinerea* (*Salicaceae*), 10 Apr. 1986, K. & L. Holm, CBS 113702 = UPSC 1843. **The Netherlands**, Wilhelminadorp, from bark of *Pyrus malus* (*Rosaceae*), June 1977, H.A.Th. van der Scheer, CBS 139.78.

Plenodomus influorescens (Boerema & Loer.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564755.
Basionym: *Phoma enteroleuca* var. *influorescens* Boerema & Loer., Trans. Brit. Mycol. Soc. 84: 290. 1985.

Specimens examined: **The Netherlands**, from *Lilium* sp. (*Liliaceae*), 1973, G.H. Boerema, PD 73/1382; Emmeloord, from *Fraxinus excelsior* (*Oleaceae*), 1978, J.D. Janse, **holotype** of *Phoma enteroleuca* var. *influorescens*, CBS H-16208, culture ex holotype CBS 143.84 = PD 78/883 = CECT 20064.

Note: The isolate PD 73/1382 is no longer available for study.

Plenodomus libanotidis (Fuckel) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564756.
Basionym: *Pleospora libanotidis* Fuckel, Jahrb. Nassauischen Vereins Naturk. 27–28: 24. 1873 as "*libanotis*".
≡ *Leptosphaeria libanotidis* **(**Fuckel) Sacc., Syll. Fung. 2: 16. 1883 as "*libanotis*".
= *Phoma sanguinolenta* Rostr., Tidsskr. Landokon. 5(7): 384. 1888; not *Phoma sanguinolenta* Grove, J. Bot. 23: 164. 1885.
≡ *Phoma rostrupii* Sacc., Syll. Fung. 11: 490. 1895. nom. nov.

Specimen examined: **Sweden**, Uppland, Gröna strand, from *Seseli libanotis* (*Apiaceae*), 19 May 1987, K. & L. Holm, CBS 113795 = UPSC 2219.

Plenodomus lindquistii (Frezzi) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564757.
Basionym: *Leptosphaeria lindquistii* Frezzi, Revista Invest. Agropec., Sér. 5, 5: 79. 1968.
= *Phoma macdonaldii* Boerema, Persoonia 6: 20. 1970.

Specimens examined: **Canada**, from *Helianthus annuus* (*Asteraceae*), 1967, W.C. McDonald, CBS 381.67. Former **Yugoslavia**, from stem of *Helianthus annuus*, 1977, A. Maric, CBS 386.80 = PD 77/336.

Note: Strain CBS 381.67 is ex-holotype of *Phoma macdonaldii* Boerema, pycnidial state of *Leptosphaeria lindquistii* Frezzi (Boerema 1970).

Plenodomus lingam (Tode : Fr.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 120: 463. 1911.
Basionym: *Sphaeria lingam* Tode: Fr., Fungi mecklenb. 2: 51. 1791.: Fr., Syst. Mycol. 2: 507. 1823.
≡ *Phoma lingam* (Tode : Fr.) Desm., Ann. Sci. Nat., Bot. Ser. 3, 11: 281. 1849.
= *Sphaeria maculans* Desm., Ann. Sci. Nat., Bot. Ser. 3, 6: 77. 1846. nom. illeg.
≡ *Leptosphaeria maculans* (Desm.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 235. 1863.
= *Plenodomus rabenhorstii* Preuss, Linnaea 24: 145. 1851. nom. dub.

Specimens examined: **The Netherlands**, near Goes, from *Brassica oleracea* (*Brassicaceae*), 1978, M.M.J. Dorenbosch, CBS 260.94 = PD 78/989. Origin unknown, Mar. 1924, A. Weber, CBS 147.24. **UK**, from *Brassica* sp. (*Brassicaceae*), 1963, B.C. Sutton, CBS 275.63 = MUCL 9901= UPSC 1025.

Notes: The combination *Plen. lingam* as published by van Höhnel (1911) was preferred over *Plen. rabenhorstii* Preuss (1851) by Boerema & van Kesteren (1964) because the type material of *Plen. rabenhorstii* had been lost during the Second World War. Therefore, *Plen. rabenhorstii* is indicated here as a *nomen dubium*. *Leptosphaeria maculans* causes a serious stem base canker (blackleg) on cultivated *Brassica* spp. (*Brassicaceae*) in Europe, Australia and North America (West *et al.* 2001, Fitt *et al.* 2006).

Plenodomus lupini (Ellis & Everh.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564758.
Basionym: *Phoma lupini* Ellis & Everh., Bull. Washburn Lab. Nat. Hist. 1: 6. 1884.
≡ *Asteromella lupini* (Ellis & Everh.) Petr., Sydowia 9: 495. 1955; not *Phoma lupini* N.F. Buchw., Møller, Fungi Faeröes 2: 153. 1958. nom. illeg.

Specimen examined: **Peru**, Andes region, from stem lesion of *Lupinus mutabilis* (*Fabaceae*), May 1992, J. de Gruyter, CBS 248.92 = PD 79/141.

Plenodomus pimpinellae (Lowen & Sivan.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564759.
Basionym: *Leptosphaeria pimpinellae* Lowen & Sivan., Mycotaxon 35: 205. 1989.
= *Phoma pimpinellae* Boerema & Gruyter, Persoonia 17: 278. 1999.

Specimen examined: **Israel**, Mt Carmel near Kibbutz Oren, from dead stems of *Pimpinella anisum* (*Apiaceae*), 9 Dec. 1987, R. Rowen, 523-88 NY, **holotype** of *Leptosphaeria pimpinellae* Lowen & Sivan, culture ex-holotype CBS 101637 = PD 92/41.

Plenodomus tracheiphilus (Petri) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564760.
Basionym: *Deuterophoma tracheiphila* Petri, Boll. Staz. Patol. Veg. Roma 9: 396. 1929.
≡ *Bakerophoma tracheiphila* (Petri) Cif., Ist. Bot. Reale Univ. Reale Lab. Crittog. Pavia Atti Ser. 5, 5: 307. 1946.
≡ *Phoma tracheiphila* (Petri) L.A. Kantsch. & Gikaschvili, Trudy Inst. Zasch. Rast. Tibilisi 5: 20. 1948.

Specimens examined: **Israel**, from *Citrus limonium* (*Rutaceae*), Oct. 1993, J. de Gruyter, CBS 551.93 = PD 81/782. **Italy**, from *Citrus* sp. (*Rutaceae*), CBS 127250 = PD 09/04597141.

Note: The species produces a *Phialophora*-like synanamorph.

Plenodomus visci (Moesz) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564761.
Basionym: *Plectophomella visci* Moesz, Magyar Bot. Lapok 21: 13. 1922.
= *Apocytospora visci* Höhn., Mitt. Bot. Lab. TH Wien 1: 43. 1924.

Specimen examined: **Hungary**, Tata-Tóváros, from leaves of *Viscum album* (*Viscaceae*), 22 Oct. 1911, G. von Moesz, BP, **holotype** of *Plectophomella visci* Moesz. **France**, from *Viscum album*, 1974, **epitype designated here** CBS H-20823, culture ex-epitype CBS 122783 = PD 74/1021.

Notes: *Plectophomella visci* is the type species of the genus *Plectophomella*. This genus was accepted by Sutton (1980) based on the eustromatic conidiomata; branched, septate conidiophores, phialidic conidiogenesis and small, hyaline conidia. However, the phylogenetic analyses clearly demonstrated the placement of *Plectophomella* grouping in the *Plenodomus* clade and therefore it is treated as a synonym.

Plenodomus wasabiae (Yokogi) J.F. White & P.V. Reddy, Canad. J. Bot. 76: 1920. 1999 (1998).
Basionym: *Phoma wasabiae* Yokogi, Ann. Phytopathol. Soc. Japan 2: 549. 1933.

Specimens examined: **Taiwan**, from *Wasabia japonica* (syn. *Eutrema wasabi*) (*Brassicaceae*), A. Rossman, CBS 120119 = FAU 559; from *Wasabia japonica*, A. Rossman, CBS 120120 = FAU 561.

Subplenodomus Gruyter, Verkley & Crous, **gen. nov.** MycoBank MB564769.

Conidiomata pycnidial, globose to papillate, or with an elongated neck, solitary or aggregated, thin-walled pseudoparenchymatous, or thick-walled scleroplectenchymatous, ostiolate, unilocular. *Conidiogenous cells* phialidic, ampulliform to doliiform. *Conidia* hyaline, aseptate, ellipsoid to cylindrical. *Chlamydospores* sometimes produced, olivaceous, unicellular in chains, or multicellular, dictyosporous-botryoid or forming pseudosclerotioid structures.

Type species: *Subplenodomus violicola* (P. Syd.) Gruyter, Aveskamp & Verkley (see below)

Etymology: Although the genus resembles *Plenodomus* in the production of thick-walled pycnidia, the pycnidial cell wall of *Subplenodomus* often remains pseudoparenchymatous, similar to the pycnidial wall of species of *Phoma*.

Subplenodomus apiicola (Kleb.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564770.
Basionym: *Phoma apiicola* Kleb., Z. Pflanzenkrankh. 20: 22. 1910.

Specimens examined: **Germany**, from tuber of *Apium graveolens* var. *rapaceum* (*Apiaceae*), Feb. 1972, Diercks, culture CBS 285.72. **The Netherlands**, from stem base of *Apium graveolens*, 1978, J. de Gruyter, CBS 504.91 = PD 78/1073.

Subplenodomus drobnjacensis (Bubák) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564771.
Basionym: *Phoma drobnjacensis* Bubák, Bot. Közlem. 14: 63. 1915
= *Pyrenochaeta gentianae* Chevassut, Bull. Soc. Mycol. France 81: 36. 1965.

Specimens examined: **The Netherlands**, from stem base of *Gentiana makinoi* "Royal Blue" (*Gentianaceae*), 1983, M.M.J. Dorenbosch, CBS 270.92 = PD 83/650; Naaldwijk, from red-brown root of *Eustoma exaltatum* (*Gentianaceae*), 1988, M.M.J. Dorenbosch, CBS 269.92 = PD 88/896.

Subplenodomus valerianae (Henn.) Gruyter, Aveskamp & Verkley, **comb. nov**. MycoBank MB564772.
Basionym: *Phoma valerianae* Henn., Nyt Mag. Naturvidensk. 42: 29. 1904.
= *Phyllosticta valerianae-tripteris* f. *minor* Unamuno, Mem. Real Soc. Esp. Hist. Nat. 15: 348. 1929.

Specimens examined: **The Netherlands**, Arnhem, from dead stem of *Valeriana phu* (*Valerianaceae*), Sep. 1968, G.H. Boerema, CBS 630.68 = PD 68/141; Elburg, from stem base of *Valeriana officinalis*, 1973, M.M.J. Dorenbosch, culture CBS 499.91 = PD 73/672.

Subplenodomus violicola (P. Syd.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564774.
Basionym: *Phoma violicola* P. Syd., Beibl. Hedwigia 38: 137. 1899.
= *Phyllosticta violae* f. *violae-hirtae* Allesch. Rabenh.-Fl., Ed. 2, Pilze 6: 156. 1898.
= *Phoma violae-tricoloris* Died., Ann. Mycol. 2: 179. 1904.
= *Phyllosticta violae* f. *violae-sylvaticae* Gonz. Frag., Trab. Mus. Nac. Ci. Nat., Ser. Bot. 7: 35. 1914.

Specimens examined: **New Zealand**, Auckland, Henderson, from leaf spot in *Viola tricolor* (*Violaceae*), 1997, J. Jury, CBS 100272. **The Netherlands**, Baarn, from leaf spot in *Viola tricolor*, 10 Mar. 1968, H.A. van der Aa, CBS 306.68.

Coniothyriaceae W.B. Cooke. Revista Biol. (Lisbon) 12: 289. 1983.

Coniothyrium carteri (Gruyter & Boerema) Verkley & Gruyter, **comb. nov.** MycoBank MB564775.
Basionym: *Phoma carteri* Gruyter & Boerema, Persoonia 17(4): 547. 2002 (“2001”). nom. nov.
Replaced synonym: *Pyrenochaeta minuta* J.C. Carter, Bull. Illinois Nat. Hist. Surv. 21: 214. 1941; not *Phoma minuta* Wehm., Mycologia 38: 318. 1946, nor *Phoma minuta* Alcalde, Anales Inst. Bot. Cavanilles 10: 235. 1952; not *Coniothyrium minutum* (Berl.) O. Kuntze, Revis. Gen. Pl. 3: 459. 1898 = *Phoma cava*, syn. of *Pyrenochaeta cava*; not *Coniothyrium minutum* (Died) Petr. & Syd., Feddes Repert. Spec. Nov. Regni Veg. Beih. 42: 349. 1927.

Specimens examined: **Germany,** isolated from *Quercus robur* (*Fagaceae*), 1991, CBS 105.91. **The Netherlands**, from shoot of *Quercus* sp. (*Fagaceae*), 1984, M.M.J. Dorenbosch, CBS 101633 = PD 84/74.

Coniothyrium dolichi (Mohanty) Verkley & Gruyter, **comb. nov.** MycoBank MB564776.
Basionym: *Pyrenochaeta dolichi* Mohanty, Indian Phytopathol. 11: 85. 1958.

Specimens examined: **India**, Nani Tal, Sarichuan, from leafspot of *Dolichos biflorus* (*Fabaceae*), 20 Oct. 1955, N.N. Mohandy, CBS 124140 = IMI 217262, CBS 124143 = IMI 217261.

Note: A synanamorph was noted and described as a *Coniosporium* state based on the dark brown to black, dictyosporous conidia (Mohanty 1958). This synanamorph was considered later as *Monodictys*-like (Grodona *et al.* 1997).

Coniothyrium glycines (R.B. Stewart) Verkley & Gruyter, **comb. nov.** MycoBank MB564777.
Basionym: *Pyrenochaeta glycines* R.B. Stewart, Mycologia 49: 115. 1957.
≡ *Phoma glycinicola* Gruyter & Boerema, Persoonia 17: 554. 2002 (“2001”). nom. nov. nom. inval.; not *Phoma glycines* Sawada, Special. Publ. Coll. Agric., Natl. Taiwan Univ. 8: 129. 1959. nom. inval. ≡ *Phoma glycines* Sawada ex J.K. Bai & G.Z. Lu, Fl. Fungorum Sin. 15: 33. 2003.

Specimens examined: **Zambia**, on Mt. Makulu, from leaf of *Glycine max* (*Fabaceae*), Mar. 1985, J.M. Waller, CBS 124455 = IMI 294986. **Zimbabwe,** from a leaf of *Glycine max* (*Fabaceae*), 2001, C. Lavy, CBS 124141 = PG1.

Coniothyrium multiporum (V.H. Pawar, P.N. Mathur & Thirum.) Verkley & Gruyter, **comb. nov.** MycoBank MB564778.
Basionym: *Phoma multipora* V.H. Pawar, P.N. Mathur & Thirum., Trans. Brit. Mycol. Soc. 50: 260. 1967.
≡ *Phoma multipora* V.H. Pawar & Thirum., Nova Hedwigia 12: 501. 1966. nom. nud.

Specimens examined: **Egypt**, CBS 501.91 = PD 83/888. **India,** Bombay, Bandra, from saline soil, 15 Jan. 1958, M.J. Thirumalachar, **Isotype** CBS H-16492, culture ex-isotype CBS 353.65 = ATCC 16207 = HACC 164 = IMI 113689.

Coniothyrium palmarum Corda, Icon. Fungorum. (Corda) 4: 38. 1840.
≡ *Clisosporium palmarum* (Corda) Kuntze, Revis. Gen. Pl. 3: 458. 1898.
≡ *Microdiplodia palmarum* (Corda) Died., Ann. Mycol. 11: 47. 1913.

Specimens examined: **Italy**, Sardegna, near Dorgali, from a dead petiole of *Chamaerops humilis* (*Arecaceae*), Aug. 1970, W. Gams, CBS H-10891–10893, culture CBS 400.71.

Coniothyrium telephii (Allesch.) Verkley & Gruyter, **comb. nov.** MycoBank MB564779.
Basionym: *Pyrenochaeta telephii* Allesch., Ber. bayer. bot. Ges. 4: 33. 1896.
≡ *Phoma septicidalis* Boerema, Versl. Meded. Plantenziektenk. Dienst Wageningen 153 (Jaarb. 1978): 20. 1979. nom. nov.; not *Phoma telephii* (Vestergr.) Kesteren, Netherlands J. Pl. Pathol. 78: 117. 1972.

Specimens examined: **Finland**, Helsinki, Asko Kahanpää, obtained from air, Jan. 1971, CBS H-16567, culture CBS 188.71; Oulu, from mineral wool between walls, Dec. 1996, K. Poldmaa, CBS 856.97. **Zimbabwe**, from leaf of *Glycine max* (*Fabaceae)*, CBS 101636 = PD 86/1186.

Cucurbitariaceae G. Winter. Rabenh, Krypt.-Fl., Ed 2, 308. 1885.

Neophaeosphaeria filamentosa **(**Ellis & Everh.) Câmara, M.E. Palm & A.W. Ramaley, Mycol. Res. 107: 519. 2003.
Basionym: *Leptosphaeria filamentosa* Ellis & Everh., J. Mycol. 4: 76. 1888.
≡ *Paraphaeosphaeria filamentosa* **(**Ellis & Everh.) M.E. Barr, Mycotaxon 43: 392. 1992.

Specimen examined: **Mexico**, from *Yucca rostrata* (*Asparagaceae*), Stevens, CBS 102202 = BPI 802755.

Pyrenochaetopsis pratorum **(**P.R. Johnst. & Boerema) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564780.
Basionym: *Phoma pratorum* P.R. Johnst. & Boerema, New Zealand J. Bot. 19: 395. 1981.

Specimen examined: **New Zealand,** Rakura, near Hamilton, from a leaf of *Lolium perenne* (*Poaceae*), 1980, P.R. Johnston, isotype CBS H-7625, CBS H-7626, culture CBS 445.81 = PDDCC 7049 = PD 80/1254; *Dactylis glomerata* (*Poaceae*), 1980, CBS 286.93 = PD 80/1252.

Pleosporaceae Nitschke, Verh. Naturhist. Vereines Preuss. Rheinl. 26: 74. 1869.

Pleospora angustis Gruyter & Verkley, **nom. nov.** MycoBank MB564781.
≡ *Leptosphaeria clavata* A.L. Guyot, Revue Mycol. (Paris) 11: 62. 1946.
≡ *Massariosphaeria clavata* (A.L. Guyot) Shoemaker & C.E. Babc., Canad. J. Bot. 67: 1582.1989; not *Pleospora clavata* Gucevič (”as *clavatis*”), Novosti Sist. Nizsh. Rast. 7: 168. 1970.

Specimen examined: **Switzerland**, 1951, E. Müller, CBS 296.51.

Notes: The origin of the isolate deposited by E. Müller is unknown; however, it is likely that the isolate was obtained from *Poaceae, Triticum vulgare* or *Dactylis glomerata* (Müller 1950). *Pleospora clavata* Gucevič was obtained from *Lonicera alseuosmoides* and refers to a different species.

Pleospora betae (Berl.) Nevod., Grib. ross. Exs., No. 247. 1915.
Basionym: *Pyrenophora echinella* var. *betae* Berl. Nuovo Giorn. Bot. Ital. 20: 208. 1888.
= *Pleospora betae* Björl., Bot. Not. 1944: 218. 1944. (later homonym). nom. illeg.
≡ *Pleospora bjoerlingii* Byford, Trans. Brit. Mycol. Soc. 46: 614. 1963. nom. nov.
= *Phoma betae* A.B. Frank, Z. Rúbenzucker-Ind. 42: 904, tab. 20. 1892.
= *Phyllosticta betae* Oudem., Ned. Kruidk. Arch. Ser. 2, 2: 181. 1877.
= *Gloeosporium betae* Dearn. & E.T. Barthol., Mycologia 9: 356. 1917.

Specimens examined: **The Netherlands**, Wageningen, from *Beta vulgaris* (*Chenopodiaceae*), Sep. 1966, M.M.J. Dorenbosch, CBS H-16156, culture CBS 523.66 = IHEM 3915 = PD 66/270; from *Beta vulgaris*, 1977, G.H. Boerema, CBS 109410 = PD 77/113.

Note: The name *Phoma betae* A.B. Frank has been conserved against *Phyllosticta tabifica* and any combination based on that name (Shoemaker & Redhead 1999).

Pleospora calvescens (Fr.) Tul. & C. Tul., Selecta Fung. Carpol. (Paris) 2: 266. 1863.
Basionym: *Sphaeria calvescens* Fr., Ann. Sci. Nat., Bot. Ser. 2, 19: 353. 1843.
≡ *Leptosphaeria calvescens* (Fr.) Sacc., Syll. Fung. 2: 24. 1883.
≡ *Pyrenophora calvescens* (Fr.) Sacc., Syll. Fung. 2: 279. 1883.
= *Chaetodiplodia caulina* P. Karst., Hedwigia 23: 62. 1884.
≡ *Ascochyta caulina* (P. Karst.) v.d. Aa & Kesteren, Persoonia 10: 271. 1979.
= *Microdiplodia henningsii* Staritz, Hedwigia 53: 163. 1913.

Specimens examined: **Germany**, Munkmarsch, from leaf spots in *Atriplex hastata* (*Chenopodiaceae*), 20 July 1977, G.H. Boerema, CBS H-8980, culture CBS 246.79 = PD 77/655. **The Netherlands**, Texel, from dead stem of *Atriplex hastata*, June 1978, H.A. van der Aa, CBS H-8976, culture CBS 343.78.
Note: For additional synonyms see Boerema *et al.* (1993).

Pleospora chenopodii Ellis & Kellerman, J. Mycol. 4: 26. 1888.
= *Diplodia hyalospora* Cooke & Ellis, Grevillea 7: 5. 1878; not *Pleospora hyalospora* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia. 42: 238. 1890.
≡ *Ascochyta hyalospora* (Cooke & Ellis) Boerema, S.B. Mathur & Neerg., Netherlands J. Pl. Pathol. 83: 156. 1977.
= *Diplodina ellisii* Sacc., Syll. Fung. 3: 417. 1884

Specimens examined: **Bolivia**, isolated from *Chenopodium quinoa* (*Chenopodiaceae*), 1974, S.B. Mathur, CBS H-9051, CBS H-9052, culture CBS 206.80 = PD 74/1022. **The Netherlands**, Zoutelande, from *Atriplex hastata* (*Chenopodiaceae*), Aug. 1968, H.A. van Kesteren, CBS 344.78 = PD 68/682.

Note: Isolate CBS 344.78 was originally identified as *Ascochyta caulina* but was identical to *Pleospora chenopodii* in the present study.

Pleospora fallens (Sacc.) Gruyter & Verkley, **comb. nov.** MycoBank MB564782.
Basionym: *Phoma fallens* Sacc., Syll. Fung. 10: 146. 1892.
= *Phyllosticta glaucispora* Delacr., Bull. Soc. Mycol. France 9: 266. 1893.
≡ *Phoma glaucispora* (Delacr.) Noordel. & Boerema, Versl. Meded. Plantenziektenk. Dienst Wageningen 166 (Jaarb. 1987): 108. 1989 (“1988”).
= *Phyllosticta oleandri* Gutner, Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 2, Sporov. Rast. 1: 306. 1933.

Specimens examined: **Italy**, Capri, Villa Jovis, from a leaf spot of *Nerium oleander* (*Apogynaceae*), CBS H-16639, culture CBS 284.70 = PD 97/2400. **New Zealand**, Levin, from leaf spot of *Olea europaea* (*Oleaceae*), 1978, G.F. Laundon, CBS 161.78 = LEV 1131.

Pleospora flavigena **(**Constantinou & Aa) Gruyter & Verkley, **comb. nov.** MycoBank MB564783.
Basionym: *Phoma flavigena* Constantinou & Aa, Trans. Brit. Mycol. Soc. 79: 343. 1982.

Specimen examined: **Romania**, Bucuresti, isolated from water, 1980, K. Fodor, CBS H-1418, **holotype** of *Phoma flavigena* Constantinou & Aa, culture ex-holotype CBS 314.80 = PD 91/1613.

Pleospora halimiones Gruyter & Verkley, **nom. nov.** MycoBank MB564784.
≡ *Diplodina obiones* Jaap (as “*obionis*”), Verh. Bot. Vereins Prov. Brandenburg 47: 96. 1905; not *Pleopora obiones* P. Crouan & H. Crouan, Fl. Finistère: 22. 1867.
≡ *Ascochytula obiones* (Jaap) Died., Ann. Mycol. 10: 141. 1912.
≡ *Ascochyta obiones* (Jaap) P.K. Buchanan, Mycol. Pap. 156: 28 1987.
= *Coniothyrium obiones* Jaap (as “*obionis*”), Schriften Naturwiss. Vereins Schleswig-Holstein 14: 29. 1907.

Specimens examined: **The Netherlands**, Texel, from leaf spots in *Halimione portulacoides* (*Chenopodiaceae*), 27 Oct. 1968, H.A. van der Aa, CBS H-9127, CBS H-9129, culture CBS 786.68; Texel, De Cocksdorp, from dead stems of *Halimione portulacoides*, 6 July 1977, H.A. van der Aa, CBS H-9126, CBS H-9125, culture CBS 432.77 = IMI 282137.

Notes: Isolate CBS 453.68 preserved as *Chaetodiplodia* sp. and also isolated from dying stems and leaf sheaths of *Halimione portulacoides* on Texel, is not the same as *Pleo. halimiones* and is probably a different species.

Pleospora herbarum (Pers.) Rabenh., Bot. Zeitung (Berlin) 15: 428. 1857; Klotzschii Herb. Viv. Mycol. **2**: no. 547 (1854.) *Basionym*: *Sphaeria herbarum* Pers., Syn. Meth. Fung. 1: 78. 1801.
= *Stemphylium herbarum* E.G. Simmons, Sydowia 38: 291. 1986 (1985).

Specimen examined: **India**, Uttar Pradesh, from a leaf of *Medicago sativa* (*Fabaceae*), 1986 (isolated in 1983), E.G. Simmons, CBS 191.86 = IMI 276975.

Note: This isolate is the ex-type culture of *Stemphylium herbarum*.

Pleospora incompta (Sacc. & Martelli) Gruyter & Verkley, **comb. nov.** MycoBank MB564785. *Basionym*: *Phoma incompta* Sacc. & Martelli, Syll. Fung. 10: 146. 1892.

Specimens examined: **Greece**, Crete, from branch of *Olea europaea* (*Oleaceae*), 1976, N. Malathrakis, CBS H-16394, culture CBS 467.76. **Italy**, from branch of *Olea europaea*, Mar. 1982, CBS H-16392, culture CBS 526.82.

Pleospora typhicola (Cooke) Sacc., Syll. Fung. 2: 264. 1883.
Basionym: *Sphaeria typhicola* Cooke, Grevillea 5: 121. 1877.
≡ *Clathrospora typhicola* (Cooke) Höhn., Ann. Mycol. 16: 88. 1918.
≡ *Pyrenophora typhicola* (Cooke) E. Müll., Sydowia 5: 256. 1951.
≡ *Macrospora typhicola* (Cooke) Shoemaker & C.E. Babc., Canad. J. Bot. 70: 1644. 1992.
= *Phyllosticta typhina* Sacc. & Malbr., Sacc., Michelia 2: 88. 1880.
≡ *Phoma typhina* (Sacc. & Malbr.) Aa, van der Aa & Vanev, A revision of the species described in *Phyllosticta*: 468. 2002.
= *Phoma typharum* Sacc., Syll. Fung. 3: 163. 1884.

Specimens examined: **The Netherlands,** Texel, from dead leaves of *Typha angustifolia* (*Typhaceae*), 1969, W. Gams, CBS H-16597, culture CBS 132.69; Staverden, from leaf spots of *Typha* sp., 24 June 1972, G.S. de Hoog, CBS H-16598, culture CBS 602.72.

Phoma-like anamorphs excluded from the suborder *Pleosporineae*

Montagnulaceae M.E. Barr, Mycotaxon **77**: 194. 2001.

Paraconiothyrium Verkley, Stud. Mycol. 50: 327. 2004.

Type species: *Paraconiothyrium estuarinum* Verkley & M. da Silva, Stud. Mycol. 50: 327. 2004.

Paraconiothyrium flavescens **(**Gruyter, Noordel. & Boerema) Verkley & Gruyter, **comb. nov.** MycoBank MB564786.

Basionym: *Phoma flavescens* Gruyter, Noordel. & Boerema, Persoonia 15(3): 375. 1993.

Specimen examined: **The Netherlands**, Nagele, from soil, rhizosphere of *Solanum tuberosum* (*Solanaceae*), CBS 178.93 = PD 82/1062.

Paraconiothyrium fuckelii (Sacc.) Verkley & Gruyter, **comb. nov.** MycoBank MB564787.
Basionym: *Coniothyrium fuckelii* Sacc., Nuovo Giorn. Bot. Ital. 8: 200. 1876; Michelia 1: 207. 1878
≡ *Clisosporium fuckelii* (Sacc.) Kuntze, Revis. Gen. Pl. 3: 458. 1898.
≡ *Microsphaeropsis fuckelii* (Sacc.) Boerema, 2003, Persoonia 18: 160. 2003.

Specimen examined: **Denmark**, Geelskov, from a dead stem of *Rubus* sp. (*Rosaceae*), 1995, A.M. Dahl-Jensen, CBS 797.95.

Note: *Coniothyrium fuckelii* var. *sporulosum* has been redisposed as *Paraconiothyrium sporulosum* (Verkley *et al*. 2004) and it is clearly different from *Paraconiothyrium fuckelii* (Damm *et al*. 2008).

Paraconiothyrium fusco-maculans (Sacc.) Verkley & Gruyter, **comb. nov.** MycoBank MB564788.

Basionym: *Phoma fusco-maculans* Sacc., Michelia 2: 275. 1881
≡ *Plenodomus fusco-maculans* (Sacc.) Coons, J. Agric. Res. 5: 714. 1916.

Specimen examined: **Italy**, Selva, from decorticated wood of *Malus pumila* (*Rosaceae*), Oct. 1880, PAD, **holotype** of *Phoma fusco-maculans* Sacc. **USA**, from wood of *Malus* sp. (*Rosaceae*), July 1916, G.H. Coons, **epitype designated here** CBS H-20825, culture ex-epitype CBS 116.16.

Notes: *Plenodomus fusco-maculans* was discussed by Boerema & Loerakker (1985) and de Gruyter *et al*. (2010). The holotype of the basionym *Aposphaeria fusco-maculans* was studied and considered to be *Aposphaeria pulviscula* (Boerema *et al*. 1996). However, the description of *A. fusco-maculans* given by Boerema *et al*. (1996) fits the generic concept of *Paraconiothyrium*, in congruence with the molecular phylogeny of the culture CBS 116.16.

Paraconiothyrium lini (Pass.) Verkley & Gruyter, **comb. nov.** MycoBank MB564789.
Basionym: *Phoma lini* Pass., Diagn. Funghi Nuovi 4, No. 81. 1890.

Specimen examined: **The Netherlands**, from Wisconsin tank, 1970, CBS 253.92 = PD 70/998.

Paraconiothyrium maculicutis Verkley & Gruyter, **sp. nov.** MycoBank MB564796. (Fig. 6).
Etymology: Latin, cutis = skin; maculae = spots.

Pycnidia in vitro 50–125 µm diam, globose to subglobose, glabrous or with mycelial outgrowth, scattered, non-ostiolate or ostiolate, pycnidial wall made up of 5–7 layers of cells. *Conidiogenous cells* 1.5–3 × 0.5–2.5 µm, indeterminate or ampulliform to filiform in a later state, up to 10 µm

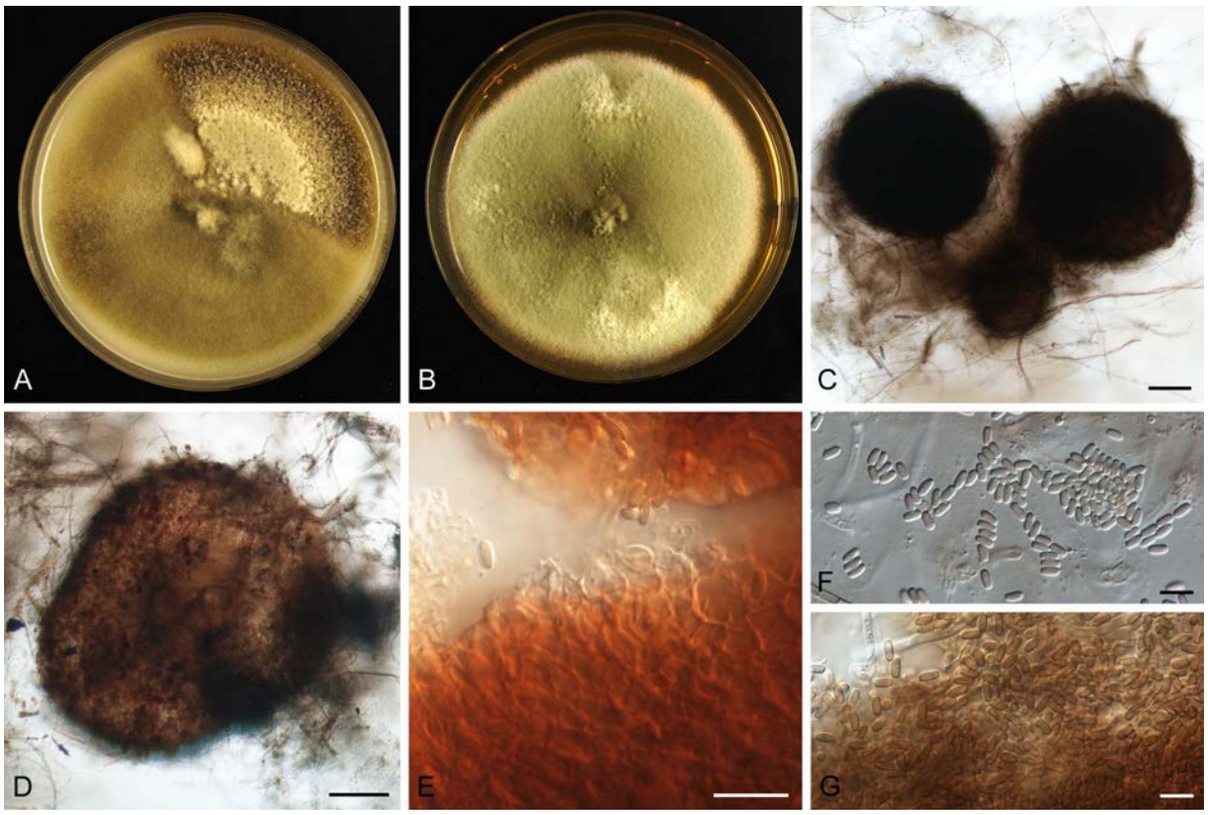


Fig. 6. *Paraconiothyrium maculicutis* sp. nov. CBS 101461. A–B. Fourteen day old cultures on OA (A) and MA (B). C–D. Pycnidia. E. *Phoma*-like conidiogenous cells. F–G. Conidia, initially hyaline to pale olivaceous (F), then becoming olivaceous (G). Scale bars: C–D = 20 µm; E = 10 µm; F–G = 5 µm.

in length. *Conidia* 1.5–2.5 × 0.5–1.5 µm, ellipsoidal, initially hyaline, then discolouring to olivaceous.

Description *in vitro*

Colonies on OA 50–52 mm diam after 7 d, margin entire; colony olivaceous buff to greenish olivaceous/grey olivaceous, with greenish olivaceous to pale olivaceous grey, finely floccose to woolly aerial mycelium; reverse smoke-grey to greenish olivaceous, with olivaceous patches. Colonies on MEA 43–44 mm diam after 7 d, margin entire; colony pale olivaceous grey to greenish olivaceous, with isabelline to cinnamon at centre, with compact pale olivaceous grey, finely floccose to woolly aerial mycelium; reverse buff to honey, isabelline to olivaceous near margin. *Pycnidia* globose to subglobose, olivaceous to brick, finally olivaceous black, scattered, mainly on the agar, 50–125 µm diam, glabrous or with mycelial outgrowth, non-ostiolate or ostiolate, pycnidial wall made up of 5–7 layers of cells. *Conidiogenous cells* 1.5–3 × 0.5–2.5 µm, ampulliform to filiform in a later state, up to 10 µm in length. *Conidia* 1.5–2.5 × 0.5–1.5 µm, av. 1 × 2 µm, length/width ratio = 1.5–3.2, av. 2.2, ellipsoidal, initially hyaline, then discolouring to olivaceous. *Chlamydospores* absent. NaOH spot test: negative. *Crystals* absent.

Specimen examined: **USA**, Texas; San Antonio**,** Fort Sam Houston, from human, cutaneous lesions, 1989, D.P. Dooley, **holotype** CBS H-20824, culture ex-holotype CBS 101461 = IMI 320754 = UTHSC 87-144.

Note: Isolate CBS 101461 was identified as *Pleurophoma pleurospora* (Dooly *et al*. 1989). However, *in vitro* data and the molecular phylogeny demonstrate that this isolate does not belong to *Pleurophoma pleurospora*, see below, and therefore is described as a new species in the genus *Paraconiothyrium*.

Paraconiothyrium minitans (W.A. Campb.) Verkley, Stud. Mycol. 50: 332. 2004.
Basionym: *Coniothyrium minitans* W.A. Campb., Mycologia 39: 191. 1947.

Specimens examined: **The Netherlands**, Boskoop, from stem of *Clematis* sp. (*Ranunculaceae*), 1999, J. de Gruyter, CBS 122786 = PD 99/1064-1. **UK**, CBS 122788 = PD 07/03486739.

Paraconiothyrium tiliae (F. Rudolphi) Verkley & Gruyter, **comb. nov.** MycoBank MB564790.
Basionym: *Asteroma tiliae* F. Rudolphi, Linnaea 4: 514. 1829.
≡ *Asteromella tiliae* (F. Rudolphi) Butin & Kehr, Mycol. Res. 99: 1193. 1995. nom. inval., Art. 33.4.

Specimen examined: **Austria**, Amlach, from a leaf of *Tilia platyphyllos* (*Tiliaceae*), 10 Sep. 1993, H. Butin, **neotype** IMI 362854, **lectotype designated here** CBS H-20826, culture ex-lectotype CBS 265.94.

Pleurophoma pleurospora (Sacc.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 123: 117. 1914. (Fig. 7).
Basionym: *Dendrophoma pleurospora* Sacc., Michelia 2: 97. 1880.

Description *in vitro*
Colonies on OA 14–18 mm diam after 7 d (18–28 mm after 14 d), margin entire to undulate; colony greenish olivaceous/olivaceous to rosy-buff and sepia, with white, felty aerial mycelium; reverse olivaceous grey to greenish olivaceous/ olivaceous. Colonies on MEA 11–16 mm diam after 7 d (19–29 mm after 14 d), colony margin undulate; colony pale olivaceous grey/ olivaceous grey to dark mouse-grey with rosy-buff tinges, with white, floccose, compact aerial mycelium, reverse umber/brown olivaceous to olivaceous/olivaceous black. *Pycnidia* globose to subglobose,olivaceous to olivaceous black, abundant, scattered, mainly on the agar, 30–120 µm diam, solitary or aggregated, covered by mycelial outgrowths or setae-like hyphae, up to 50 µm, non-papillated, without or with ostiole, walls made up of 2–5 layers of cells, outer layer(s) pigmented; conidial exudate not observed. *Conidiogenous cells* of two types; ampulliform to doliiform, 4–6.5 × 2–5.5 µm, or filiform, septate, branched, acropleurogenous, up to 60 µm long. *Conidia* 3.5–5.5 × 1.5–2.5 µm, av. 4.5 × 2 µm, length/ width ratio = 1.5–3, av. 2.1, cylindrical to oblong, without or with some minute, polar orientated guttules. *Chlamydospores* absent. NaOH spot test: a weak reddish discolouring may occur on MA, not specific. *Crystals* absent.

Specimens examined: **France**, Perpignan, from leaf of *Laurus nobilis* (*Lauraceae*), PAD, **holotype** of *Dendrophoma pleurospora* Sacc. **The Netherlands**, from wood of *Lonicera* sp. (*Caprifoliaceae*), **lectotype designated here** CBS H-20626, culture ex-lectotype CBS 130329 = PD 82/371; Molenhoek, Heumense Schans, from twig lesions of *Cytisus scoparius* (*Fabaceae*), 23 Aug. 2004, G. Verkley & M. Starink, CBS 116668.

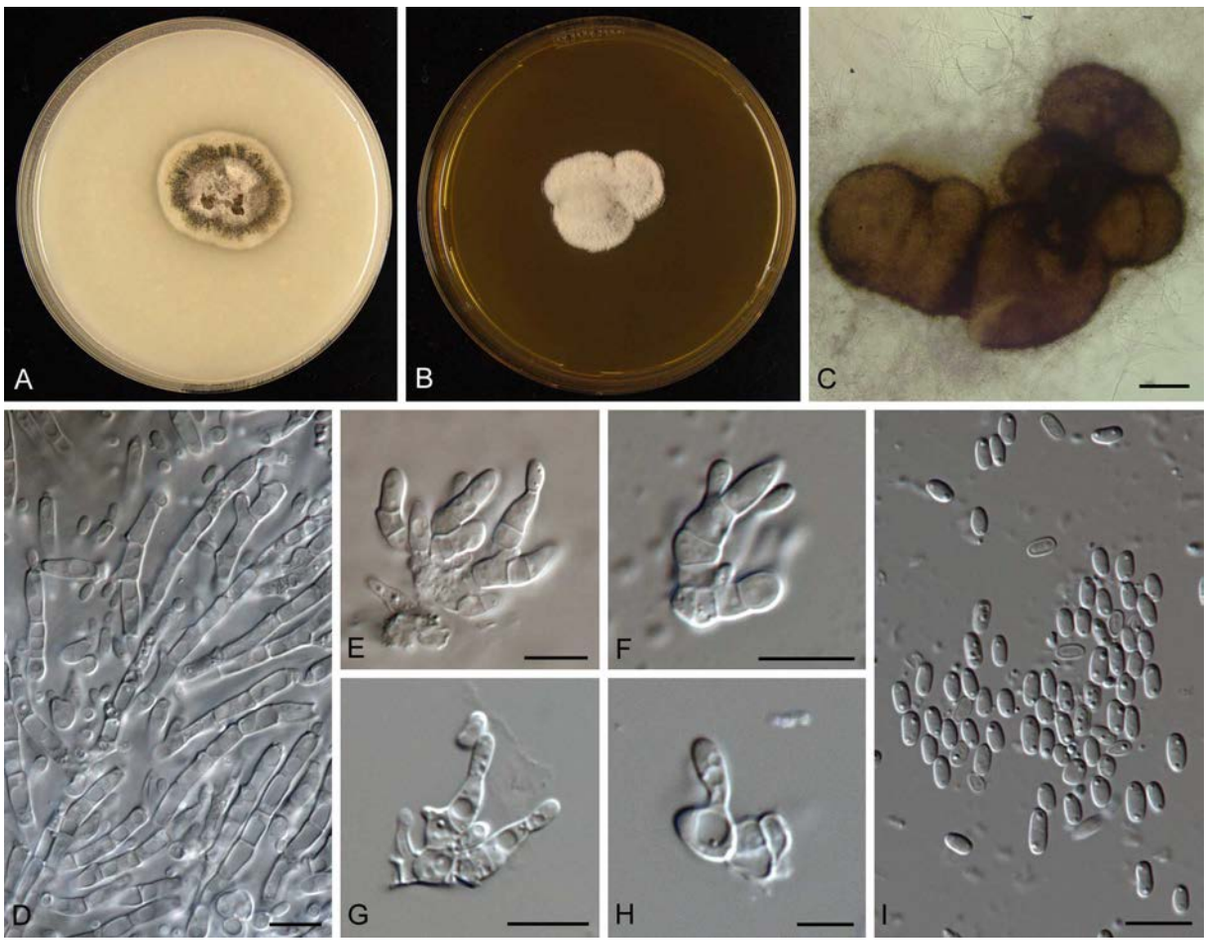


Fig. 7. *Pleurophoma pleurospora*. CBS 130329. A–B. Fourteen day old cultures on OA (A) and MA (B). C. Pycnidia. D–H. Conidiogenous cells, septate conidiophores with acropleurogenous conidiogenesis (D– G) or *Phoma*-like (H). I. Conidia. Scale bars: C = 50 µm; D–G, I = 10 µm; H = 5 µm.

Notes: A specimen derived from isolate CBS 130329 is assigned here as lectotype of *Pleurophoma pleurospora*, the type species of the genus (von Höhnel 1914). The species is known from branches and bare wood of trees and shrubs (Sutton 1980, Boerema *et al.* 1996) and the isolate from *Cytisus scoparius* demonstrates that the species also may occur on green twigs. The isolates showed two types of conidiogenesis characteristic for the genus *Pleurophoma*; *Phoma*-like, ampulliform to doliiform conidiogenous cells, as well as *Pyrenochaeta*-like branched, filiform, septate, acropleurogenous. As a result, species of the genus *Pleurophoma* can easily be confused with taxa classified in the genera *Phoma, Paraphoma, Pyrenochaeta* and *Pyrenochaetopsis*.

Paraphaeosphaeria michotii **(**Westend.) O.E. Erikss., Arkiv før Botanik 6: 406. 1967.
Basionym: *Sphaeria michotii* Westend., Bull. Acad. Roy. Sci. Belgique Ser. 2, 7: 87. 1859.

Specimen studied: **Switzerland**, Kt. Obwalden, from *Typha latifolia* (*Typhaceae*), 18 May 1980, A. Leuchtmann, CBS 652.86 = ETH 9483.

Massarinaceae Munk, Friesia 5: 305. 1956.

Byssothecium circinans Fuckel, Bot. Zeitung (Berlin) 19: 251. 1861.
≡ *Leptosphaeria circinans* (Fuckel) Sacc., Syll. Fung. 2: 88. 1883.
≡ *Passeriniella circinans* (Fuckel) Sacc., Syll. Fung. 11: 326. 1895.
≡ *Trematosphaeria circinans* (Fuckel) G. Winter, Rabenh. Krypt.-Fl., ed 1(2): 277. 1887.
≡ *Heptameria circinans* (Fuckel) Cooke, Grevillea 18: 30. 1889.
= *Melanomma vindelicorum* Rehm, Ber. Nat. Ver. Augsburg: 116. 1881.
≡ *Trematosphaeria vindelicorum* (Rehm) Sacc., Syll. Fung. 2: 122. 1883.

Specimen examined: **USA**, South Dakota, from rotten crown of *Medicago sativa* (*Fabaceae*), G. Semeniuk, CBS 675.92 = ATCC 52767 = ATCC 52678 = IMI 266220.

Massarina eburnea (Tul. & C. Tul.) Sacc., Syll. Fung. 2: 153. 1883.
Basionym: *Massaria eburnea* Tul. & C. Tul., Select. Fung. Carpol. (Paris) 2: 239. 1863.

Specimens examined: **Switzerland**, Zürich, from *Fagus sylvatica* (*Fagaceae*), S.K. Bose, CBS 473.64 = ETH 2945. **UK**, Wales, isolated from dead branch of *Fagus sylvatica*, HHUF 26621, JCM 14422 = H3953.

Neottiosporina paspali (G.F. Atk.) B. Sutton & Alcorn, Austral. J. Bot. 22: 519. 1974.
Basionym: *Stagonospora paspali* G.F. Atk., Bull. Cornell Univ. (Science) 3: 33. 1897.

Specimen examined: **USA**, Florida, from *Paspalum notatum* (*Poaceae*), Oct. 1937, R.K. Voorhees, CBS 331.37.

Trematosphaeriaceae Suetrong *et al*. Cryptogamie 32: 347. 2011.

Falciformispora lignatilis K.D. Hyde, Mycol. Res. 96: 27. 1992.

Specimen examined: **Thailand**, Pinruan Ban Bang, from *Elaeis guineensis* (*Arecaceae*), BCC 21118.

Medicopsis Gruyter, Verkley & Crous, **gen. nov.** MycoBank MB564791.
Etymology: refers to Medi- medica, Latin, -opsis, refers to, Greek. The description of the type species as the cause of a mycetoma suggest this is a human pathogen. However, the mycetoma described was secondary to a wound produced by a thorn of Palito blanco tree, and the species was found later on *Hordeum vulgare*.

Pycnidia solitary or confluent, on upper surface of the agar, globose to pyriform with elongated neck, setose, ostiolate, olivaceous to olivaceous-black, the wall with pseudoparenchymatal cells. *Conidiogenous cells* hyaline, phialidic, ampulliform to doliiform, to elongated. *Conidia* sub-hyaline to yellowish, ellipsoid, aseptate, catenulate.

Type species: *Medicopsis romeroi* (Borelli) Gruyter, Verkley & Crous (see below).

Medicopsis romeroi (Borelli) Gruyter, Verkley & Crous, **comb. nov.** MycoBank MB564792.
Basionym: *Pyrenochaeta romeroi* Borelli, Dermatol. Venez. 1: 326. 1959.

Specimens examined: **Venezuela**, from human, maduromycosis, no date, D. Borelli, UAMH 2892, **holotype** of *Pyrenochaeta romeroi* Borelli, culture ex-holotype CBS 252.60 = ATCC 13735 = FMC 151 = UAMH 10841. Country unknown, from *Hordeum vulgare* (*Poaceae*), 1984, M.M.J. Dorenbosch, CBS 122784 = PD 84/1022.

Notes: The species was described as a human pathogen of tropical origin, and it may cause suppurative subcutaneous or deep nonmycetomatous infections, or a subcutaneous phaeohyphomycotic cyst (Badali *et al.* 2010). However, the species also occurs in plant material.

Trematosphaeria pertusa (Pers.) Fuckel, Jahrb. Nassauischen Vereins Naturk 23–24: 161. 1870.
Basionym: *Sphaeria pertusa* Pers., Syn. Meth. Fung. 1: 83. 1801.

Specimen examined: **France**, Deux Sèvres, from bark of a dead stump of *Fraxinus excelsior* (*Oleaceae*), 25 Apr. 2004, Jacques Fournier, **epitype** IFRD 2002, culture ex-epitype CBS 122368.

Note: The epitype IFRD 2002 was designated by Zhang *et al.* (2008).

Massariaceae Nitschke. Verh. Naturhist. Vereines Preuss. Rheinl. 26: 73. 1869.

Massaria platani Ces., Fungi Eur. Exsicc. Klotzsch. Herb. Vivi Mycol. no. 323. 1861.

Specimen examined: **USA**, from *Platanus occidentalis* (*Platanaceae*), Jan. 1937, C.L. Shear, CBS 221.37.

Melanommataceae G. Winter, Rabenh. Krypt.-Fl., ed 1(2): 220 (1885) [as '*Melanommeae*']

Aposphaeria corallinolutea Gruyter, Aveskamp & Verkley, **sp. nov.** MycoBank MB564798. (Fig. 8).

Etymology: The name refers to the coral coloured colony on OA, and the luteous exudate diffusing into the agar medium.

Pycnidia in vitro 65–215 µm diam, solitary or aggregated to confluent, globose to subglobose, ostiolate or non-ostiolate. *Conidiogenous cells* 7–9 × 2–4 µm, ampuliform to filiform. *Conidia* 3–5 × 1–2 µm, ellipsoidal to allantoid, eguttulate or with some small, polar guttules.

Description *in vitro*
Colonies on OA 13–15 mm diam after 14 d, margin entire to somewhat lobated; colony vinaceous to brick, with white at centre, ochraceous near margin due to a diffusible pigment,

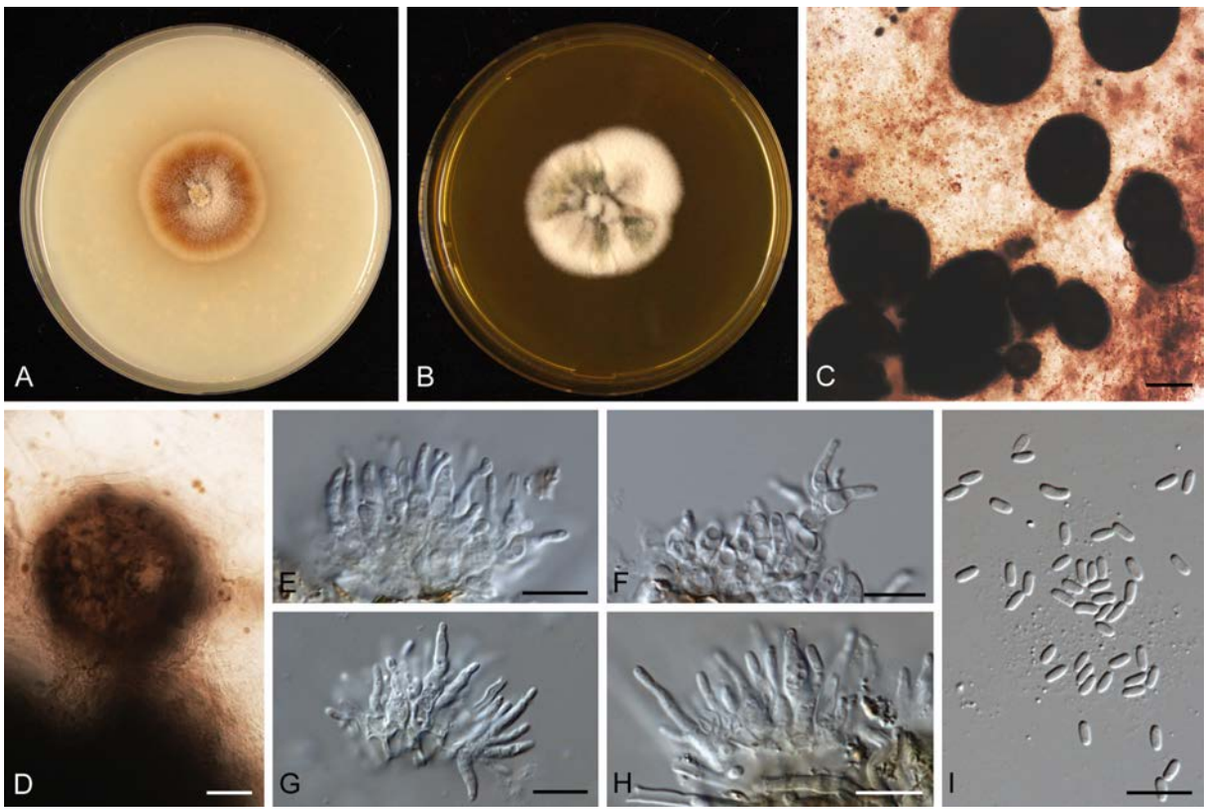


Fig. 8. *Aposphaeria corallinolutea* sp. nov. CBS 131287. A–B. Fourteen day old cultures on OA (A) and MA (B). C–D. Pycnidia. E–H. Conidiogenous cells. I. Conidia. Scale bars: C = 50 µm; D = 20 µm; E–I = 10 µm.

with white, felty or poorly developed aerial mycelium; reverse cinnamon to brick. Colonies on MEA 15–20 mm diam after 14 d, margin entire to somewhat lobated; colony white with dull green and grey olivaceous sectors and primrose tinges, with white, felty aerial mycelium; reverse sepia to brown olivaceous, greenish grey at centre, white near margin. *Pycnidia* globose to subglobose, olivaceous to brick, then olivaceous black, solitary or aggregated, 65–215 µm diam, non-setose or with short setae-like outgrowths up to 25 µm long, with or without distinct ostiole, pycnidial wall consisting of 3–5 layers of cells. *Conidiogenous cells* 7–9 × 2–4 µm, ampulliform to filiform. *Conidia* 3–5 × 1–2 µm, av. 4 × 1.5 µm, length/width ratio is 1.7–3.3, av. = 2.5, ellipsoidal to allantoid, eguttulate or with some small, polar guttules. *Chlamydospores* absent, NaOH test negative. *Crystals* produced in the agar, small, orange coloured.

Specimens examined: The **Netherlands**, from wood of *Fraxinus excelsior* (*Oleaceae*), 1983, M.M.J. Dorenbosch, **holotype** CBS H-20625, culture ex-holotype CBS 131287 = PD 83/831; from wood of *Kerria japonica* (*Rosaceae*), 1983, M.M.J. Dorenbosch, CBS 131286 = PD 83/367.

Aposphaeria populina Died., Krypt.-Fl. Brandenburg 9: 206. 1912 (vol. dated "1915"). (Fig. 9).

Description *in vitro*
Colonies on OA 21–24 mm diam after 7 d (32–37 mm diam after 14 d), margin entire to undulate; colony grey olivaceous/olivaceous to pale luteous/luteous, with white to pale olivaceous

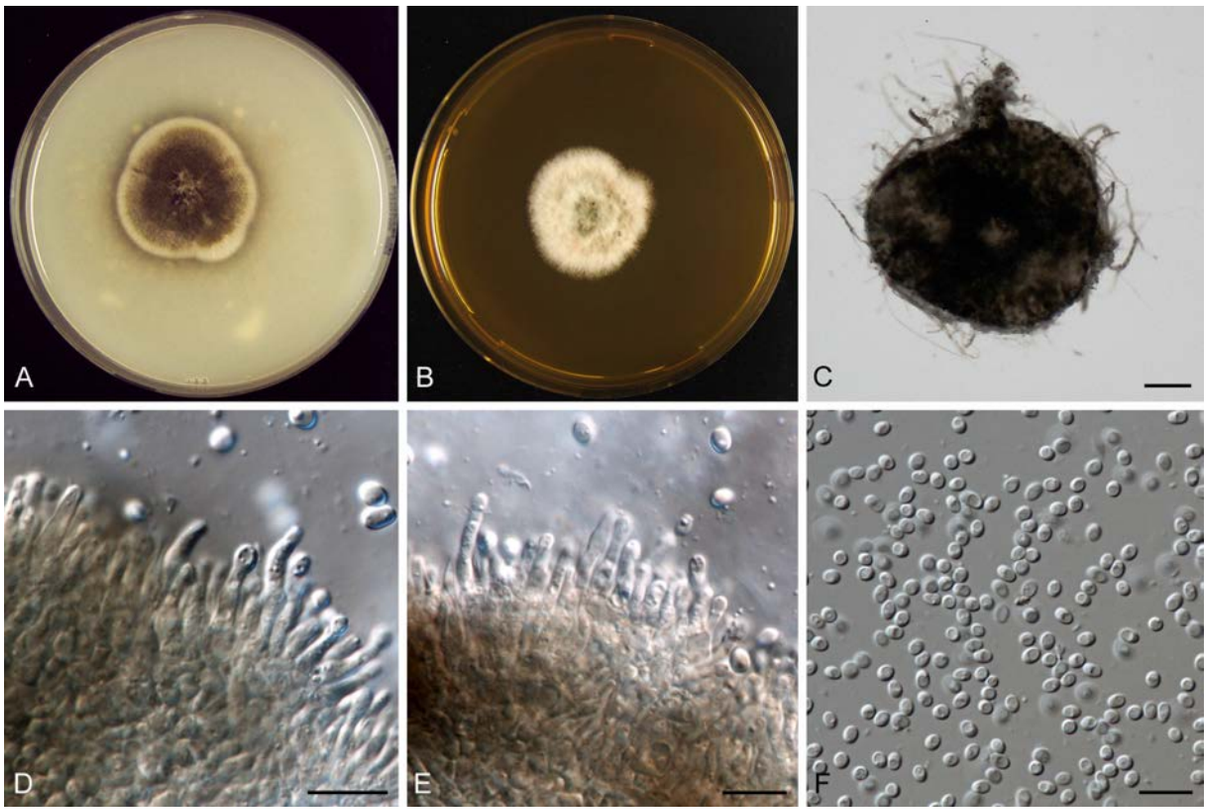


Fig. 9. *Aposphaeria populina*. CBS 543.70. A–B. Fourteen day old cultures on OA (A) and MA (B). C. Pycnidium with mycelial outgrowths. CBS 130330. D–E. Conidiogenous cells. F. Conidia. Scale bars: C = 20 µm; D–E = 10 µm; F = 5 µm.

grey, finely felty to woolly aerial mycelium; reverse luteous to orange, greenish olivaceous to olivaceous or grey olivaceous/olivaceous grey to iron-grey, a rosy-buff discolouring near margin may occur. Colonies on MEA 16–20 mm diam after 7 d (30–37 mm diam after 14 d), margin entire to undulate; colony pale olivaceous grey with rosy-vinaceous tinges to peach or olivaceous grey, with white, woolly aerial mycelium; reverse saffron to pale olivaceous/olivaceous grey, sometimes with dark vinaceous tinges, rosy-buff near margin. *Pycnidia* globose to subglobose, olivaceous to olivaceous black, scattered, 55–305 µm diam, glabrous or with mycelial outgrowths, non-ostiolate or ostiolate, pycnidial wall composed of up to 10 layers of cells. *Conidiogenous cells* 5–11.5 × 1.5–3 µm, ampulliform to filiform. *Conidia* hyaline, subglobose to ellipsoidal, with 1–3 minute guttules, 1–2 × 1–1.5 µm, av. 1.5 × 1 µm, length/width ratio is 1.0–2.0, av. = 1.4. *Chlamydospores* and crystals absent, NaOH test negative.

Specimens examined: **Germany**, Triglitz, from twigs of *Populus canadensis* (*Salicaceae*), Mar. 1904. O. Jaap, B, **holotype**; from branch scars of *Picea abies*, (*Pinaceae*), Feb. 1982, H. von Aufess, CBS 350.82. **The Netherlands**, Valkenswaard, from fallen twig of *Populus canadensis* (*Salicaceae*), 23 Mar. 1970, H.A. van der Aa**, epitype designated here** CBS H-9336, culture ex lectotype CBS 543.70; from wood of *Cornus mas* (*Cornaceae*), 1984, M.M.J. Dorenbosch, CBS 130330 = PD 84/221.

Beverwykella pulmonaria (Beverw.) Tubaki, Trans. Mycol. Soc. Japan 16: 139. 1975.
Basionym: *Papulaspora pulmonaria* Beverw., Antonie van Leeuwenhoek 20: 11. 1954.

Specimen examined: **The Netherlands**, Baarn, from submerged leaf in rain water barrel of *Fagus sylvatica* (*Fagaceae*), Apr. 1953, A.L. van Beverwijk, culture CBS 283.53 = ATCC 32983 = IFO 6800.

Herpotrichia juniperi (Duby) Petr., Ann. Mycol. 23: 43. 1925.
Basionym: *Sphaeria juniperi* Duby, Klotzsch. Herb. Vivum Mycol. Sistems Fungorum German., no. 1833. 1854.

Specimen examined: **Switzerland**, Andermatt, from *Juniperus nana* (*Cupressaceae*), Nov. 1931, E. Gäumann, CBS 200.31.

Melanomma pulvis-pyrius (Pers.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 160. 1870. (Fig. 1).
Basionym: *Sphaeria pulvis-pyrius* Pers., Syn. Meth. Fung. 1: 86. 1801.

Specimens examined: **Belgium**, from wood of *Fagus* sp. (*Fagaceae*), CBS 400.97. **France,** Vosges, Bot. Garden Le Chitelet, from unidentified decaying wood, CBS 371.75.

Notes: *Phoma*-like anamorphs have been reported by Chesters (1938) and Sivanesan (1984), but no anamorphic stage was observed in IFRDCC 2044, CBS 109.77 or CBS 371.75 after culturing 3 mo on PDA (Zhang *et al.* 2008). CBS 400.97 was preserved as *Trematosphaeria pertusa*.

Pleomassaria siparia (Berk. & Broome) Sacc., Syll. Fung. 2: 239. 1883.
Basionym: *Sphaeria siparia* Berk. & Broome, Ann. Mag. Nat. Hist. Ser. 2(9): 321. 1852.

Specimens examined: **The Netherlands**, Uden, from dead branch of *Betula verrucosa* (*Betulaceae*), 8 Dec. 1973, W.M. Loerakker, CBS H-258, CBS H-260, culture CBS 279.74.

Sporormiaceae Munk, Dansk Bot. Ark. 17(1): 450. 1957. (nom. inval., art. 36.1.)

Preussia funiculata (Preuss) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 91. 1870 (1869–70).
Basionym: *Perisporium funiculatum* Preuss, Linnaea 24(1): 143. 1851.

Specimen examined: **Senegal**, from soil, CBS 659.74.

Sporormiella minima (Auersw.) S.I. Ahmed & Cain, Canad. J. Bot. 50: 449. 1972.
Basionym: *Sporormia minima* Auersw., Hedwigia 7: 66. 1868.

Specimen examined: **Panama**, from dung of goat, CBS 524.50.

Westerdykella Stolk, Trans. Brit. Mycol. Soc. 38: 422. 1955.

Type species: *Westerdykella ornata* Stolk, see below.

Westerdykella capitulum (V.H. Pawar, P.N. Mathur & Thirum) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564801.
Basionym: *Phoma capitulum* V.H. Pawar, P.N. Mathur & Thirum., Trans. Brit. Mycol. Soc. 50: 261. 1967.

≡ *Phoma capitulum* V.H. Pawar & Thirum., Nova Hedwigia 12: 502. 1966 (as "*capitula*"). nom. nud., nom. inval.

= *Phoma ostiolata* V.H. Pawar, P.N. Mathur & Thirum., Trans. Brit. Mycol. Soc. 50: 262. 1967, var. *ostiolata*.

≡ *Phoma ostiolata* V.H. Pawar & Thirum., Nova Hedwigia 12: 502. 1966. nom. nud., nom. inval.

= *Phoma ostiolata* var. *brunnea* V.H. Pawar, P.N. Mathur & Thirum., Trans. Brit. Mycol. Soc. 50: 263. 1967.

≡ *Phoma ostiolata* var. *brunnea* V.H. Pawar & Thirum., Nova Hedwigia 12: 502. 1966. nom. nud. nom. inval.

Specimen examined: **India**, Bandra, Bombay, from saline soil, 15 Jan. 1958, M.J. Thirumalachar, **Isotype** CBS H-7602, culture ex-isotype CBS 337.65 = ATCC 16195 = HACC 167 = IMI 113693 = PD 91/1614.

Westerdykella minutispora (P.N. Mathur ex Gruyter & Noordel.) Gruyter, Aveskamp & Verkley, **comb. nov.** MycoBank MB564793.
Basionym: *Phoma minutispora* P.N. Mathur ex Gruyter & Noordel., Persoonia 15: 75. 1992 (as "collection name" originally also referred to Thirumalachar; = depositor).
Replaced synonym: *Phoma oryzae* Cooke & Massee, Grevillea 16: 15. 1887; not *Phoma oryzae* Catt., Arch. Triennale Bot. Crittog. Pavia 2–3: 118. 1879. nom. illeg.

≡ *Phyllosticta oryzae* (Cooke & Massee) I. Miyake. J. Coll. Agric. Imp. Univ. Tokyo 2: 252. 1910. nom. illeg.

Specimen examined: **India**, from saline soil, 1977, M.J. Thirumalachar, CBS H-5941, culture CBS 509.91 = PD 77/920.

Westerdykella ornata Stolk, Trans. Brit. Mycol. Soc. 38: 422. 1955.

Specimen examined: **Mozambique**, from mangrove mud, CBS 379.55.

Didymosphaeriaceae Munk, Dansk Bot. Ark. 15(2): 128. 1953.

Roussoella hysterioides (Ces.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Abt. 1. 128: 563. 1919.
Basionym: *Dothidea hysterioides* Ces., Atti Accad. Sci. Fis. 8: 24. 1879.

Specimen examined: **Japan**, Aomori, Shimokita Yagen, from culms of *Sasa kurilensis* (*Poaceae*)**,** Y. Ooki, culture CBS 125434 = HH 26988.

Family *incertae sedis*

Nigrograna Gruyter, Verkley & Crous, **gen. nov.** MycoBank MB564794.
Etymology: refers to Nigro- black, Latin, -grana, grains, Latin. The description refers to the black grains produced by the type species.

Pycnidia solitary or rarely confluent, on upper surface or submerged in agar, globose to subglobose or pyriform, with dark brown, septate mycelial outgrowths, with papillate ostioles, olivaceous to olivaceous-black, the wall with pseudoparenchymatous cells. *Conidiogenous cells* hyaline, phialidic, discrete. *Conidia* sub-hyaline, brown in mass, aseptate, ellipsoidal.

Type species: *Nigrograna mackinnonii* **(**Borelli) Gruyter, Verkley & Crous (see below).

Nigrograna mackinnonii **(**Borelli) Gruyter, Verkley & Crous, **comb. nov.** MycoBank MB564795.
Basionym: *Pyrenochaeta mackinnonii* Borelli, Castellania 4: 230. 1976.

Specimens examined: **Mexico**, from a mycetoma of a human, Feb. 2002, R. Arenas, CBS 110022; **Venezuela**, from a black grain mycetoma of human, Aug. 1975, D. Borelli, **holotype** FMC 270, culture ex-holotype CBS 674.75.

Thyridaria rubronotata (Berk. & Broome) Sacc., Syll. Fung. 2: 141. 1883.
Basionym: *Melogramma rubronotatum* Berk. & Broome, Ann. Mag. Nat. Hist. Ser. 3(3): 20. 1859.

Specimen examined: **The Netherlands**, Zuidelijk Flevoland, from a dead branch of *Acer pseudoplatanus* (*Aceraceae*), 13 Apr. 1985, N. Ernste, CBS H-18824, culture CBS 419.85.

DISCUSSION

The genus *Phoma* has been shown to be highly polyphyletic and *Phoma* is now restricted to taxa in the *Didymellaceae* (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010). *Phoma* anamorphs and *Phoma*-like species in *Coniothyriaceae*, *Leptosphaeriaceae*, *Melanommataceae*, *Montagnulaceae*, *Pleosporaceae*, *Sporormiaceae* and *Trematosphaeriaceae* are redisposed here as a result of this and previous studies.

The delimitation of *Leptosphaeriaceae* in *Pleosporineae* from *Cucurbitariaceae*, *Didymellaceae*, *Phaeosphaeriaceae* and *Pleosporaceae* agrees with recent studies of *Phoma*-like species in *Pleosporales* (de Gruyter *et al.* 2009, Aveskamp *et al.* 2010, de Gruyter *et al.* 2010). *Cucurbitariaceae* is recognised as the fifth family in *Pleosporineae* in addition to the four families accepted by Zhang *et al.* (2009) which are *Didymellaceae*, *Leptosphaeriaceae Phaeosphaeriaceae and Pleosporaceae*.

The genera *Leptosphaeria, Paraleptosphaeria, Plenodomus, Subplenodomus* and *Heterospora*

Plenodomus lingam and *L. doliolum*, the type species of *Plenodomus* and *Leptosphaeria* respectively, were found to be distant genetically, which agrees with findings of previous

Table 2. Characteristics of ascospores, mitosporic state and pathogenicity of *Leptosphaeria, Paraleptosphaeria, Plenodomus* and *Subplenodomus in vivo*.

Genus	Ascospores	Mitosporic state	Pathogenicity
Leptosphaeria	ascospores 3-septate, (dark) brown	mitosporic state common, pycnidial cell wall usually directly scleroplectenchymatous, conidia mostly aseptate	necrotrophic
Paraleptosphaeria	ascospores 3 (–5) septate, hyaline to yellow/brown	mitosporic state rare, pycnidial cell wall directly scleroplectenchymatous, conidia aseptate	necrotrophic
Plenodomus	ascospores 3–7 septate, light yellow to brown	mitosporic state common, pycnidial cell wall initially pseudoparenchymatous, later scleroplectenchymatous, conidia aseptate	necrotrophic or plant pathogenic
Subplenodomus	No known sexual state	mitosporic state common, pycnidial cell wall mainly pseudoparenchymatous, conidia aseptate	necrotrophic or plant pathogenic
Heterospora	No known sexual state	mitosporic state common, pycnidial cell wall pseudoparenchymatous, conidia of two types: small aseptate and large septate	plant pathogenic

molecular phylogenetic studies (Jasalavic *et al.* 1995, Morales *et al.* 1995, Dong *et al.* 1998, Câmara *et al.* 2002, Eriksson & Hawksworth 2003, Wunsch & Bergstrom 2011). In our study the generic type species grouped in sister clades, which represent *Leptosphaeria* and *Plenodomus*. Species of *Leptosphaeria* produce dark brown, 3-septate ascospores, which have been considered the primitive state with more recently evolved species producing ascospores that are paler in colour, longer and narrower, and more than 3-septate (Wehmeyer 1946). This hypothesis is supported by the results obtained in our study.

Paraleptosphaeria is distinct but seems to be most closely related to *Leptosphaeria* producing 3(–5)-septate, yellow/brown or hyaline ascospores. Both genera include only necrotrophic species. *Plenodomus* and *Subplenodomus* include necrotrophs and plant pathogens. Ascospores in *Plenodomus* are 3–7-septate, whereas in *Subplenodomus* no sexual state has thus far been recorded. The scleroplectenchymatous pycnidial cell wall is typical for *Plenodomus*, whereas in *Subplenodomus* the pycnidial cell wall is pseudoparenchymatous. *Heterospora* is closely allied to *Subplenodomus* and no sexual state has been recorded for this genus either. The distinctive characterisitics of the genera *Heterospora, Leptosphaeria, Paraleptosphaeria, Plenodomus* and *Subplenodomus* are summerised in Table 2. A blast search in GenBank using ITS sequences of five selected species of *Leptosphaeriaceae*, namely *L. doliolum, L. etheridgei, Plen. lingam, H. dimorphospora* and *Subplen. drobnjacensis*, did not reveal close matches to other teleomorphic or anamorphic genera.

Plectophomella visci grouped in *Plenodomus* in this study and in *Leptosphaeriaceae* in a previous molecular phylogeny of *Phoma* and allied anamorph genera (de Gruyter *et al.* 2009). *Plectophomella visci* is the type species of *Plectophomella* (Moesz 1922) and three additional species have been described in the genus. Two species were described from the bark of *Ulmus* spp., *viz. Plectophomella ulmi* (basionym *Dothiorella ulmi*) and *Plectophomella concentrica* (Redfern & Sutton 1981). *Dothiorella ulmi* is considered the appropriate name for *Plectophomella ulmi* (Crous *et al.* 2004). A third species, *Plectophomella nypae*, was described from *Nypa fruticans* (*Arecaceae*) (Hyde & Sutton 1992). As a result of the transfer of the type species *Plectophomella visci* to *Plenodomus*, the taxonomy of both *Plectophomella concentrica* and *P. nypae* needs to be reconsidered based on the outcome of a molecular study.

Plenodomus chrysanthemi could not be differentiated from *Plen. tracheiphilus* based on comparison of their LSU and ITS sequences. *Plenodomus vasinfecta* was proposed by Boerema *et al.* (1994) for the species originally described as *Phoma tracheiphila* f. sp. *chrysanthemi* (Baker *et al.* 1985). Because these are part of the *Plenodomus* clade the name *Plenodomus chrysanthemi* is proposed with *P. tracheiphila* f. sp. *chrysanthemi* and *P. vasinfecta* as synonyms. *Plenodomus chrysanthemi* and *Plen. tracheiphilus* are host specific (*Chrysanthemum* and *Citrus*, respectively) and the scleroplectenchymatous conidiomatal wall of *Plen. tracheiphilus* differentiates this species from *Plen. chrysanthemi*, where only a parenchymatous wall has been observed (Boerema *et al*. 1994). The results of this molecular study and the production of a *Phialophora* synanamorph by both species demonstrate the close relationship of both taxa.

Plenodomus enteroleucus and *Plen. influorescens* have a similar ecological niche as opportunistic pathogens on woody plants in Europe. Both taxa were formerly described as varieties of *Ph. enteroleuca*, vars. *enteroleuca* and *influorescens,* and could be differentiated only by the fluorescence of var. *enteroleuca* under black light. However, the molecular phylogeny demonstrates the two varieties are only distantly related and they are raised from varietal status to species rank. The close relation of *Plen. wasabiae* with *Plen. biglobosus* agrees with the results of a previous study on the production of Phomalignin A and other yellow pigments, as well as ITS sequence analyses (Pedras *et al.* 1995).

Subplenodomus apiicola, Subplen. drobnjacensis, Subplen. valerianae and *Subplen. violicola* all produce pycnidia with an elongated neck, resembling *Plenodomus*. The pycnidial wall remains usually pseudoparenchymatous. Pycnidia with a scleroplectenchymatous wall are only observed in *Subplen. drobnjacensis*. *Subplenodomus apiicolus*, *Subplen. drobnjacensis* and *Subplen. valerianae* produce relatively small conidia, up to 4.5 × 2 µm (de Gruyter & Noordeloos 1992) in congruence with many of the *Plenodomus* species described; however, in contrast *Subplen. violicola* produces relatively large conidia, up to 11 × 3 µm (Boerema 1993).

The grouping of species of *Phoma* section *Plenodomus* based on the host being either herbaceous plants or wood of trees and shrubs (Boerema 1982, Boerema *et al.* 1994) is not supported by the molecular phylogeny. The grouping of the species into two categories based on the production of pseudoparenchymatous pycnidia that become scleroplectenchymatous pycnidia (type I), versus always scleroplectenchymatous pycnidia (type 2) (Boerema *et al.* 1981), is partly supported by the molecular phylogeny. In the *Leptosphaeria* clade most species directly develop scleroplectenchymatous pycnidia, whereas in the *Plenodomus* clade the pycnidia generally are pseudoparenchymatous and become scleroplectenchymatous.

Heterospora is established for two species of *Phoma* sect. *Heterospora* that cluster in *Leptosphaeriaceae, viz H. chenopodii* and *H. dimorphospora.* All other species of *Phoma* sect. *Heterospora* are in *Didymellaceae* (Aveskamp *et al*. 2010).

The *Leptosphaeria doliolum* species complex

The taxonomy of the generic type species *Leptosphaeria doliolum* and *Phoma* anamorphs is complex with a number of subspecies and varieties described in literature. *Leptosphaeria doliolum* subsp. *doliolum* and *L. doliolum* subsp. *errabunda* are morphologically very similar, as well as the anamorphs *Ph. acuta* subsp. *errabunda* and *Ph. acuta* subsp. *acuta*. It has been suggested that both taxa represent originally American and European counterparts (Boerema *et al.* 1994). Both subspecies of *L. doliolum* proved to be closely related in a phylogenetic analysis utilising LSU and ITS. A detailed multilocus phylogenetic study including the ITS, ACT, TUB and CHS genes, however, demonstrated that both subspecies could be clearly differentiated, and represent two subclades in the *L. doliolum* complex.

All species allied with *L. doliolum* and *L. errabunda* are necrotrophic species. Surprisingly, *L. macrocapsa* grouped with the *L. errabunda* isolates. *Leptosphaeria macrocapsa* is described as a host-specialised necrotroph on *Mercurialis perennis* (*Euphorbiaceae*) in Europe (Boerema *et al.* 1994). The species is characterised by large pycnidia (Grove, 1935), with a conspicuously broad, long cylindrical neck (Boerema *et al*. 1994). This is different to the sharply delimited papilla or neck of variable length of the pycnidia of *L. errabunda*. *Leptosphaeria sydowii*, a necrotroph on *Senecio* spp. in particular (*Asteraceae*), proved to be closely related to *L. errabunda*. It can be concluded that the *Leptosphaeria doliolum* complex includes several necrotrophic species, with adapted host specificity.

The genus *Coniothyrium*

Coniothyrium palmarum is the type species of the genus *Coniothyrium*. *Coniothyrium* is characterised by ostiolate pycnidial conidiomata, annellidic conidiogenous cells, the absence of conidiophores, and brown, thick-walled, 0- or 1-septate, verrucose conidia. *Coniothyrium* is similar morphologically to some species in the genus *Microsphaeropsis*. However, *Microsphaeropsis* is characterised by the production of phialidic conidiogenous cells with periclinal thickening, and thin-walled, pale greenish brown conidia.

Coniothyrium, *Microsphaeropsis* and *Paraconiothyrium* clearly grouped in different clades in a study of the partial SSU nrDNA (Verkley *et al.* 2004). In a subsequent study utilising SSU and LSU sequences, the generic type species *Microsphaeropsis olivacea* grouped in *Didymellaceae*, whereas *Coniothyrium palmarum* clustered with the genus *Leptosphaeria* in *Leptosphaeriaceae* (de Gruyter *et al.* 2009). In the present study *C. palmarum* and its relatives grouped in a distinct clade, which represents *Coniothyriaceae*. *Phoma carteri*, *Ph. glycinicola*, *Ph. septicidalis* and *Pyrenochaeta dolichi* grouped in this clade and are transferred to the genus *Coniothyrium*. The inclusion of these species with setose pycnidia and conidiogenesis with elongated conidiophores expands the morphological circumscription of *Coniothyrium*. Species with those characters are also found in other genera treated in this paper in *Cucurbitariaceae*, *Didymellaceae*, *Phaeosphaeriaceae*, *Leptosphaeriaceae*, *Montagnulaceae* and *Sporormiaceae*, indicating convergent evolution.

The *Coniothyrium* species included here are plurivorous or soil-borne, such as *C. palmarum*, *C. septicidalis* and *C. multiporum*, or are associated with a specific host such as *C. carteri* on *Quercus* spp. (*Fagaceae*), *C. glycinicola* on *Glycine max* (*Fabaceae*) and *C. dolichii* on *Dolichos biflorus* (*Fabaceae*). The species also are diverse geographically.

Coniothyrium palmarum was frequently found associated with leaf spots on *Phoenix dactylifera* (*Arecaceae*) in India and Cyprus (Sutton 1980). The *C. palmarum* isolates regularly used in phylogenetic studies are CBS 758.73, from leaf spots on *Phoenix dactylifera* in Israel, and CBS 400.71, from a dead petiole of *Chaemeropsis humulis* (*Arecaceae*) in Italy. The

subtropical distribution of these species is similar to that of the most closely allied *C. dolichi* and *C. glycinicola*. *Coniothyrium multiporum*, recorded from marine soil, also is found in warm regions. *Coniothyium carteri*, in contrast, is reported from North America and Europe.

Coniothyrium dolichi produces setose pycnidia with hyaline conidia (Mohanty 1958). The conidiogenesis was studied in detail later. *Phoma*-like ampulliform conidiogenous cells as well as conidiogenous cells on filiform, septate conidiophores were found in the same pycnidia leading to confusion regarding the classification of this species in *Phoma* or *Pyrenochaeta* (Grodona *et al.* 1997). This study clearly supports the classification in *Coniothyrium*. *Coniothyrium glycinicola* was originally placed in the genus *Pyrenochaeta* as *Py. glycines* due to its setose pycnidia (Stewart 1957). The conidiogenesis and hyaline conidia are *Phoma*-like and therefore, it was reclassified as *Ph. glycinicola* in *Phoma* sect. *Paraphoma* (de Gruyter & Boerema 2002). However, in the original description it was noted that the conidia were greenish-yellow in mass (Stewart 1957), resembling *Microsphaeropsis* or *Coniothyrium*-like conidia. This study clearly supports the classification in *Coniothyrium*. *Coniothyrium carteri* produces setose pycnidia with hyaline conidia and therefore, the species was classified in *Phoma* section *Paraphoma* (de Gruyter & Boerema 2002). In spite of this similarity, *C. carteri* was determined to be only distantly related to the generic type species *Paraphoma radicina* (de Gruyter *et al.* 2010). *Coniothyrium multiporum* was described in *Phoma* section *Phoma*; however, it proved to be unrelated to *Phoma* in *Didymellaceae* (Aveskamp *et al.* 2010). The conidiogenesis may comprise elongated conidiophores (Pawar *et al.* 1967). Two isolates originally described as *Ph. septicidalis* are placed here in *Coniothyrium telephii.* Other strains deposited as *Ph. septicidalis* proved to be *Pyrenochaeta unguis-hominis* (de Gruyter *et al.* 2010).

The anamorph of the genus *Neophaeosphaeria* was described as *Coniothyrium*-like, producing pigmented, aseptate conidia from holoblastic, percurrently proliferating conidiogenous cells with conspicuous annellations (Câmara *et al.* 2003). Although *Neophaeosphaeria* is related to *Coniothyrium* based on the molecular data, *Neophaeosphaeria* probably belongs to a separate phylogenetic clade. The grouping of *N. filamentosa* with the *Coniothyrium* species included in this study was poorly supported and *N. filamentosa* proved to be more distantly related in previous molecular phylogenetic studies (Verkley *et al.* 2004, Damm *et al.* 2008, de Gruyter *et al.* 2010).

Both anamorph genera *Cyclothyrium* and *Cytoplea* were considered to be related to *Coniothyrium* and *Microsphaeropsis* (Sutton 1980) based on morphological similarities. *Cyclothyrium* also resembles *Paraconiothyrium* but produces conidiogenous cells that are more elongated than in most species of *Paraconiothyrium* and the conidia are almost truncate at the base, or at least they are much less rounded at the base than the conidia of *Paraconiothyrium* (Verkley *et al.* 2004). The generic type species *Cyclothyrium juglandis*, the anamorph of *Thyridaria rubronotata*, proved to be related to *Roussoella hysterioides*, teleomorph of *Cytoplea* (Verkley *et al.* 2004). Based on present results *R. hysterioides* could not be assigned to familial rank. The clustering of this species in *Massariaceae* (Zhang *et al.* 2009) could not be confirmed. Moreover, *Roussoella* probably is not a monophyletic genus (Tanaka *et al.* 2009). *Thyridaria rubronotata*, the teleomorph of *Cyclothyrium juglandis*, proved to be related to *Massariosphaeria phaeospora* but was not assigned to familial rank (Schoch *et al.* 2009).

Coniothyrium-like anamorphs also have been linked to *Mycosphaerella* in the past. However, these species were subsequently accommodated in *Colletogloeopsis* (Cortinas *et al.* 2006), *Readeriella/Kirramyces* (Crous *et al.* 2007) and are now known to be species of *Teratosphaeria* (Crous *et al.* 2009b).

The genus *Pleospora*

Pleospora is a large genus in *Pleosporaceae*, *Pleosporales*, and includes important pathogens that occur on both monocotyledons and dicotyledons. Anamorphs of *Pleospora s. lat.* have been described in various genera of coelomycetes and hyphomycetes as summarised by Zhang *et al.* (2009). A delimitation of *Pleospora* into two sections, *Pyrenophora* and *Eu-Pleospora* was made based on the size of fruiting bodies and ascospore septation and colour (Munk 1957). The genus *Pyrenophora* (*Drechslera* anamorphs) is recognised at the generic rank. However, *Pleospora* remains heterogenous (Wehmeyer 1961, Berbee 1996) and molecular phylogenetic studies demonstrated that *Pleospora* is polyphyletic in *Pleosporaceae* (Kodsueb *et al.* 2006, Wang *et al.* 2007, Inderbitzin *et al.* 2009). Taxa with a *Stemphylium* anamorph such as *Pleospora sedicola* and *Pleo. tomatonis*, as well as *Pleo. halophola* with no known anamorph, are closely related to *Cochliobolus*, whereas *Pleo. herbarum* and *Pleo. ambigua* were more distantly related in the *Pleosporaceae* (Kodsueb *et al.* 2006, Wang *et al.* 2007). A phylogenetic study of the genus *Massariosphaeria* demonstrated the polyphyly in the genera *Pleospora, Kirschsteiniothelia, Massarina, Melanomma, Trematosphaeria and Massariosphaeria* in the *Loculoascomycetes* (Wang *et al.* 2007) and the paraphyletic character of the genus *Cochliobolus* was demonstrated (Kodsueb *et al.* 2006, Mugambi & Huhndorf 2009). These findings support the previous speculation by several authors that ascomatal and ascospore morphologies have undergone convergent evolution among *Pleosporales* (Wang *et al*. 2007).

Pleospora betae groups ambiguously in *Pleosporaceae (*Dong *et al*. 1998). SSU nrDNA sequence data supported the affinity of *P. betae* to *Leptosphaeriaceae*. Partial LSU nrDNA data supported the affinity of *Pleo. betae* to *Pleosporaceae* (Dong *et al*. 1998), but bootstrap support values in that study were low. In a multigene phylogenetic study *Pleo. betae* was found as being basal to *Pleosporaceae* (Zhang *et al*. 2009). Our results demonstrate the sister group relationship of *Pleo. betae* and its relatives to the generic type species *Pleo. herbarum.*

Pleospora betae has been often confused with *Pleo. calvescens* as was discussed by Boerema *et al.* (1987). Both species are pathogens of *Chenopodiaceae* and are morphologically rather similar and therefore, a phylogenetic relation of both species was inferred (Boerema 1984). In addition *Ascochyta hyalospora*, originally found on the American continent on *Chenopodiaceae*, also was supposed to be closely related. Our results demonstrate that *Pleo. betae* and *Pleo. calvescens* could be recognised at species rank and confirmed that *A. hyalospora* is related supporting our transfer to *Pleospora* as *Pleo. chenopodii*. The delimitation of both halophytic species *Pleo. chenopodii* and *Pleo.calvescens* needs further study; both species could not be clearly differentiated based on the ACT sequences alone. Additional studies are underway to elucidate these species boundaries, in which also the recently described halophyte, *Ascochyta manawaorae* (Verkley *et al.* 2010), will be included. *Pleospora fallens* and *Pleo. incompta*, formerly described in *Phoma* sect. *Phoma* and producing mainly glabrous pycnidia, grouped in the *Pleo. herbarum* clade. *Pleospora typhicola,* producing pilose pycnidia, also grouped in this clade.

***Phoma*-like species excluded from the Pleosporineae**

The genus *Paraconiothyrium* was introduced by Verkley *et al*. (2004) as the anamorph of *Paraphaeosphaeria*. The morphological characters of *Paraconiothyrium* are variable. The conidiomata can be eustromatic to pycnidial, the phialidic conidiogenous cells are discrete or integrated, and the thin-walled conidia are aseptate or septate, smooth-walled or minutely warted, and hyaline to brown in a later stage (Verkley *et al.* 2004). The morphological characters of *Ph. lini* and *Asteromella tilliae*, redisposed here in *Paraconiothyrium*, fit this description.

Paraconiothyrium fuckelii is a serious plant pathogen of *Rosaceae* (Horst & Cloyd 2007), but it also is recorded as an opportunistic human pathogen as summarised by de Hoog *et al*. (2000). The teleomorph is currently known as *Leptosphaeria coniothyrium*, but this is not likely considering the phylogeny of *Leptosphaeriaceae* in *Pleosporales* (Fig. 1). The species was also described as *Melanomma coniothyrium* (Holm 1957); however, *Melanomma* is more distantly related in *Melanommataceae*.

Neottiosporina paspali proved to be related to *Paraconiothyrium*. However, this species is characterised by conidia with an apical appendage (Sutton 1980) and resembles members of *Massarinaceae*. *Pyrenochaeta romeroi* is redescribed in the new genus *Medicopsis*, and its taxonomic position is most close to *Trematosphaeriaceae*.

Aposphaeria corallinolutea could be recognised as a new species in *Melanommataceae*. *Phoma capitulum* and *Ph. minutispora* (*Phoma* section *Phoma*) clustered in *Sporormiaceae*, most closely related to the holotype isolate of *Westerdykella ornata*. Other *Phoma*-like anamorphs have been recorded in *Sporormiaceae*, such as anamorphs of *Sporormia aemulans* (≡ *Preussia aemulans*) and *Westerdykella dispersa* (≡ *Pycnidiophora dispersa*) (von Arx & Storm 1967). The *in vitro* characters of *W. capitulum* and *W. oryzae* agree with the *in vitro* characters of *Phoma*-like anamorphs in *Sporormiaceae* summarised by Boerema *et al.* (2004). The conidia produced are small, mostly 2–3 × 1–2 µm, arising from undifferentiated cells, but sometimes also elongated conidiogenous cells are observed. The colonies, often with a pink-yellow-red discolouration on OA, usually produce little aerial mycelium, whereas pycnidia are often produced in abundance. No matching sequences were found in a blast search in GenBank using the partial LSU sequences of *W. capitulum* and *W. minutispora*. *Westerdykella minutispora* from India was most similar to a sequence of *Westerdykella nigra*, isolate CBS 416.72, obtained from soil in Pakistan, and *W. capitulum* was most similar to a sequence of *W. dispersa*, isolate CBS 297.56, obtained from a seedling of *Phlox drummondii*, USA. These blast results support the redisposition of both species in the genus *Westerdykella*.

ACKNOWLEDGEMENTS

This project,"Strengthening the Plant Health Infrastructure", was supported by The Dutch Ministry of Economic Affairs, Agriculture and Innovation. We thank Mrs Trix Merkx and Mrs Karin Rosendahl-Peters for providing the strains from the culture collection of CBS and PD respectively and for their assistance in the deposit of strains. Mrs Arien van Iperen kindly helped us with the deposit of herbarium material. Thanks are due to Marjan Vermaas for her assistance in preparing the photoplates. We are indebted to Machiel E. Noordeloos and both reviewers for critical reading the manuscript.

CHAPTER 5

The development of a validated real-time (TaqMan) PCR for detection of *Stagonosporopsis andigena* and *S. crystalliniformis* in infected leaves of potato and tomato

J. de Gruyter[1–3], M.P.E. van Gent-Pelzer[4], J.H.C. Woudenberg[1], P.C.J. van Rijswick[2], E.T.M. Meekes[5], P.W. Crous[1, 3, 6], P.J.M. Bonants[4]

[1]CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands;
[2]National Reference Centre, National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands;
[3]Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands;
[4]Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands;
[5]Naktuinbouw, P.O. Box 40, 2370 AA Roelofarendsveen, The Netherlands;
[6]Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

European Journal of Plant Pathology (2012). DOI 10.1007/s10658-012-9990-8.

Key words: Actin, *Boeremia*, molecular phylogeny, *Peyronellaea*, *Phoma*.

Abstract: *Stagonosporopsis andigena* and *S. crystalliniformis* are serious foliage pathogens on potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*). As both species have been recorded only in the Andes area, *S. andigena* is listed as an A1 quarantine organism in Europe. The actin region of isolates of *Stagonosporopsis* and allied species of *Boeremia*, *Didymella*, *Peyronellaea* and *Phoma* was amplified using generic primers. DNA sequence differences of the actin gene were utilised to develop species-specific real-time (TaqMan) PCR assays for the detection of *S. andigena* and *S. crystalliniformis* in leaves of potato or tomato. The specificity of the TaqMan PCR assays was determined on genomic DNA extracted from two *S. andigena* and two *S. crystalliniformis* isolates and 16 selected isolates of *Stagonosporopsis*, *Phoma* and *Boeremia*, which are the closest relatives. The validation of the methods developed included the DNA extraction and the TaqMan PCR assays. The performance criteria specificity, analytical sensitivity, reproducibility, repeatability and robustness of the TaqMan PCR assays demonstrated the reliability of both methods for the detection of *S. andigena* and *S. crystalliniformis* in leaf material. The TaqMan PCR assays were tested on symptomatic leaves of potato and tomato that were obtained after artificial inoculation of detached leaves with both pathogens under quarantine conditions. In the artificial inoculation experiments both *S. andigena* and *S. crystalliniformis* caused leaf infections on potato and tomato.

INTRODUCTION

The molecular phylogeny of the genus *Phoma* has been studied intensively at the National Plant Protection Organization (Plantenziektenkundige Dienst, PD) and CBS-KNAW Fungal Biodiversity Centre in the Netherlands during the past few years to clarify the taxonomy of the genus and to provide tools for the development of molecular detection and identification methods. Results of these studies demonstrated that the taxonomy based on morphological characters does not reflect the molecular phylogeny, and therefore, many species described in *Phoma* have been reclassified in other genera (Aveskamp *et al*. 2009a, 2009b, 2010, de Gruyter *et al*. 2009, 2010). Several of these recently introduced genera are closely related to *Phoma*, including *Boeremia*, *Peyronellaea* and *Stagonosporopsis* (Aveskamp *et al*. 2009a, 2009b, 2010). These represent many destructive pathogens that occur in economically important crops. Several species that pose a threat in important food crops have been recorded only in restricted regions and are therefore listed on quarantine lists of countries in other parts of the world. However, other *Phoma*-like pathogens have already been spread more widely, such as *Boeremia foveata*, syn. *Phoma foveata*, the cause of gangrene on potatoes. This species probably originates from the Andes region (Otazú *et al*. 1979), and it is likely that this pathogen was spread by infected seed potatoes in the past. The international trade of plant material continuously extends, and to reduce the risk of introducing harmful pathogens, fast, reliable and validated molecular detection and identification methods are needed.

This paper deals with two *Stagonosporopsis* species that have been recently excluded from *Phoma*: *S. andigena* and *S. crystalliniformis* (Aveskamp *et al*. 2010). Both species have been respectively recorded as serious pathogens on potato and tomato in the Andes region. *Stagonosporopsis andigena* was originally described as Phoma andina (Turkensteen 1978), as the cause of 'black potato blight' and leaf spots on potato plants in Peru and Bolivia. *Stagonosporopsis crystalliniformis* was initially found as a new disease, locally known as 'carate' on tomato in Colombia (Navarro & Puerta 1981). The pathogen causes necrotic spots on all aerial parts of the plant and was reported as very destructive. A total plant necrosis and mummification of the fruits may occur. Later, the fungus was found on dying potato stems in Venezuela in 1980, and isolates were frequently obtained from potato leaf spots in Colombia (Loerakker *et al.* 1986). The pathogen was originally described as a variety of *Phoma andina*, namely *P. andina* var. *crystalliniformis* (Loerakker *et al*. 1986).

In a morphological study dealing with *Phoma* species that produce characteristic dendrite crystals in pure culture, it was concluded that both *P. andina* varieties could be recognised at species level as *P. andina* and *P. crystalliniformis* (Noordeloos *et al*. 1993). However, the name *P. andina* appeared to be a homonym and therefore, *P. andina* was renamed as *P. andigena* (Boerema *et al*. 1995). Both species have not been reported outside the Andes region so far. The European Plant Protection Organization (OEPP/EPPO) lists *S. andigena* as an A1 quarantine organism (OEPP/EPPO, 1984). The cultivation of potatoes and tomatoes is of significant importance in the Netherlands and, therefore, we developed a specific real-time PCR assay based on TaqMan® technology for the detection of both organisms to be applied in case suspected samples need to be investigated.

Phylogenetic studies in *Phoma* revealed that actin sequences provide a higher resolution for the delimitation of allied species compared to ITS sequences (Aveskamp, 2009b). Actin has also been used for the development of PCR methods for detection of pathogens that belong to other important genera, such as *Mycosphaerella* (Arzanlou *et al*. 2009) and *Cercospora* (Lartey *et al*. 2003).

The aim of this study was to develop TaqMan PCR assays for the direct detection of *Stagonosporopsis andigena* and *S. crystalliniformis* in leaves of potato or tomato. Both assays developed were based on DNA sequence differences of the actin gene. The validation of both assays developed included DNA extraction and TaqMan PCR of spiked samples. The results of the performance criteria specificity, analytical sensitivity, reproducibility, repeatability and robustness are provided. Both PCR assays were tested on symptomatic leaves of potato and tomato, which were obtained after artificial inoculation of detached leaves with both pathogens under quarantine conditions.

MATERIAL AND METHODS

Fungal isolates and plant material

The *Stagonosporopsis* isolates and additional isolates of *Boeremia*, *Didymella*, *Peyronellaea* and *Phoma* species that were included in this study were obtained from the culture collections of CBS, DAOM and PD (Table 1). The isolates were selected as being the most closely related species to *S. andigena* and *S. crystalliniformis* based on a multigene analysis of parts of 28S nrDNA (Large Subunit – LSU), ITS and ß-tubulin (Aveskamp *et al*. 2010). Additional isolates, which were formerly classified in *Phoma* and obtained from potato or tomato were added as well as some related species, which had been isolated from other crops in the Andes region in the past. The freeze-dried isolates were revived overnight in 2 ml malt/peptone (50 % / 50 %) liquid medium and subsequently transferred and maintained on oatmeal agar (OA) (Crous *et al*. 2009c). The isolates which were stored at –196 °C were directly transferred on OA. The plants used in this study were the potato cultivars ‘Bintje’, ‘Bionica’ and ‘Berthaultii’ and the tomato cultivars ‘Moneymaker’, ‘Microtom’ and ‘Heinz’.

DNA extractions

Genomic DNA isolation from fungal cultures was performed using the Ultraclean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, California, USA) according to the instructions of the manufacturer. All DNA extracts were diluted 10× in milliQ water and stored at 4 °C before use. Leaf plugs, 0.9 cm diam, which were taken from approximate six weeks old plants, were transferred to 1.5 ml microcentrifuge tubes with caps (QIAGEN Benelux bv, Venlo, The Netherlands), one per tube, and stored at –20 ºC. For DNA extraction, two stainless steel beads (3.2 mm diam) and 300 µl lysis buffer with 0.2 µl RNase (8 mg/ml) (Sbeadex® maxi plant kit, LGC Genomics, Berlin, Germany) were added. The tubes were shaken in a 96 rack in a beat mill (Mixer Mill MM300; Retsch GmbH, Haan, Germany) for 30 s on 30 r/s speed. The orientation of the rack was changed and the mixer mill was switched on a second time. After 30 min incubation at 65 ºC in a water bath the tubes were centrifuged for 5 min at 3,000 × *g*. The DNA isolation was performed on 50 µl supernatant using the KingFisher 96 magnetic particle processor (Thermo Electron Corporation, Breda, The Netherlands) with the reagents of the Sbeadex® maxi plant kit according to the manufacturer’s instructions. A negative control, which consisted of healthy leaf material, was included. A 0.9 cm diam leaf disk was spiked with mycelium scraped from one 0.9 cm diam agar plug to make spiked samples.

Actin amplifications and sequencing

The actin region was amplified using the primer pair ACT-512F / ACT-783R designed by Carbone & Kohn (1999) (Table 2). The amplification reactions were performed as described

by Aveskamp *et al*. (2009b). Consensus sequences were computed from forward and reverse sequences using the BioNumerics v4.60 software package (Applied Maths, Sint-Martens-Latem, Belgium) and were deposited in GenBank (Table 1).

Phylogenetic analyses

The obtained sequence data were aligned using the MAFFT multiple sequence alignment programme (Katoh *et al*. 2009). The phylogeny was rooted to *Phoma dimorphospora*, strain CBS 345.78. 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/hcvdb/findmodel/findmodel.html), 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 0.05, respectively and the run was automatically stopped as soon as the average standard deviation of split frequencies equalled 0.01. The resulting tree was printed with TreeView v1.6.6 (Page, 1996) and alignments and the tree was deposited in TreeBASE (www.treebase.org).

Design of the PCR primers and probes to detect *Stagonosporopsis andigena* and *S. crystalliniformis*

The actin sequences included in the phylogenetic study were aligned with MegAlign Software (DNA Star Inc., Madison, WI, USA). The primer pairs and probes were designed using Primer Express® software v3.0 (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands). The primer-probe combinations were blasted against the NCBI GenBank to minimise the likelihood of non-specific detection. The probes were 5'-end labelled with FAM (6-carboxy-flourescein) as the fluorescent reporter dye, and the 3'-end was modified with the non-fluorescence quencher dye Black Hole Quencher® 1 (BHQ-1). The primers and probes were manufactured by Biolegio bv, Nijmegen, The Netherlands.

TaqMan PCR amplification

PCR amplifications of genomic DNA were performed in PCR tubes in 96-well-plates (Bioplastics, Landgraaf, The Netherlands) in a total volume of 30 µl containing the following reaction mixture: 1× TaqMan® Universal PCR Master Mix (Applied Biosystems), 250 nM of each primer, 83.3 nM probe, and 1 µl of genomic DNA. The cycle parameters were 10 min at 95 ºC to activate the hot start Taq DNA polymerase, followed by 40 cycles of a 2-step amplification (15 s 95 ºC; 1 min 60 ºC). The TaqMan PCR was carried out in an ABI PRISM 7500 Sequence detector (Applied Biosystems). Each series of amplification reactions included sterile MQ water as an external negative control to test for contamination with DNA as well as the DNA from the reference strains *S. andigena* (CBS 101.80) and *S. crystalliniformis* (CBS 713.85) as a positive control.

A generic COX TaqMan PCR, to amplify a conserved region in the plant cytochrome oxidase (COX) gene was included to follow PCR inhibitors and potential inefficiencies of the DNA extraction if plant material was involved. The PCR performed included a 30 µl reaction mixture which contained 15 µl Premix Ex Taq™ (Takara BIO Europe SAS, Saint-Germain-en-Laye, France), 0.5 µl ROXII (Takara), 200 nM of each primer COX-F (Weller *et al*. 2000) and COX-RW (Mumford *et al*. 2004), 100 nM COXSOL1511T probe (Mumford *et al*. 2004) and 1 µl of DNA obtained from a spiked sample or artificially infected leaf material. The probe was 5'-end labelled with the reporter dye Yakima Yellow® (YY) and the 3'-end was modified with the non-fluorescence quencher dye BHQ-1 (Table 2).

Table 1. List of isolates included in this study. Newly generated sequences are indicated in bold.

Species name	Isolate Nr.	GenBank accession	Host, substrate	Country
Boeremia exigua var. *exigua*	CBS 431.74; PD 74/2447	EU880854	*Solanum tuberosum*	Netherlands
Boeremia exigua var. *gilvescens*	CBS 101156, PD 90/731	EU880848	*Solanum tuberosum*	Philippines
Boeremia foveata	CBS 341.67; CECT 20055; IMI 331912	EU880894	*Solanum tuberosum*	UK
Boeremia lycopersici	CBS 378.67; PD 76/276	EU880898	*Solanum lycopersicum*	Netherlands
Boeremia noackiana	CBS 101203; PD 79/1114	EU880882	*Phaseolus vulgaris*	Colombia
Paraphoma chrysanthemicola	CBS 522.66	**JN251989**	*Chrysanthemum morifolium*	UK
Peyronellaea anserina	CBS 360.84	**JN251981**	Potato flour	Netherlands
Peyronellaea glomerata	CBS 528.66; PD 63/590	FJ426905	*Chrysanthemum* sp.	Netherlands
Peyronellaea pomorum var. *pomorum*	CBS 539.66; ATCC 16791; IMI 122266; PD 64/914	FJ426946	*Polygonum tataricum*	Netherlands
Peyronellaea subglomerata	CBS 110.92; PD 76/1010	FJ426966	*Triticum* sp.	USA
Phoma chenopodiicola	CBS 128.93; PD 79/140	**JN251985**	*Chenopodium quinoa*	Peru
Phoma destructiva var. *destructiva*	CBS 133.93; PD 88/961; IMI 173142	**JN251987**	*Solanum lycopersicum*	Guadeloupe
Phoma destructiva var. *diversispora*	CBS 162.78; PD 77/725	**JN251988**	*Solanum lycopersicum*	Netherlands
Phoma eupyrena	CBS 374.91; PD 78/391	FJ426892	*Solanum tuberosum*	Netherlands
Phoma herbarum	CBS 615.75; PD 73/665; IMI 199779	EU880896	*Rosa multiflora*	Netherlands
Phoma huancayensis	CBS 105.80; PD 75/908	**JN251986**	*Solanum* sp.	Peru
Phoma labilis	CBS 124.93; PD 87/269	**JN251979**	*Solanum lycopersicum*	Netherlands
Phoma macrostoma var. *incolorata*	CBS 109173; PD 83/908	**JN251984**	*Malus sylvestris*	Netherlands
Phoma macrostoma var. *macrostoma*	CBS 482.95	**JN251983**	*Larix decidua*	Germany
Phoma nemophilae	CBS 249.38	**JN251964**	*Nemophila insignis*	Denmark

Table 1. (Continued).

Species name	Isolate Nr.	GenBank accession	Host, substrate	Country
Phoma subherbarum	CBS 249.92; PD 78/1088	**JN251982**	*Solanum* sp.	Peru
Stagonosporopsis actaeae	CBS 106.96; PD 94/1318	**JN251974**	*Actaea spicata*	Netherlands
Stagonosporopsis ajacis	CBS 177.93; PD 90/115	**JN251962**	*Delphinium* sp.	Kenya
Stagonosporopsis andigena	CBS 101.80; PD 75/909; IMI 386090	**JN251958**	*Solanum* sp.	Peru
Stagonosporopsis andigena	CBS 269.80; PD 75/914	**JN251959**	*Solanum* sp.	Peru
Stagonosporopsis artemisiicola	CBS 102636; PD 73/1409	**JN251971**	*Artemisia dracunculus*	France
Stagonosporopsis astragali	CBS 178.25; MUCL 9915	**JN251963**	*Astragalus* sp.	Unknown
Stagonosporopsis caricae	CBS 248.90	**JN251969**	*Carica papaya*	Chile
Stagonosporopsis crystalliniformis	CBS 713.85; ATCC 76027; PD 83/826	**JN251960**	*Solanum lycopersicum*	Colombia
Stagonosporopsis crystalliniformis	CBS 771.85; IMI 386091; PD 85/772	**JN251961**	*Solanum tuberosum*	Colombia
Stagonosporopsis cucurbitacearum	CBS 133.96; PD 79/127	**JN251968**	*Cucurbita* sp.	New Zealand
Stagonosporopsis dennisii	CBS 135.96; IMI 19337; PD 95/4756	**JN251975**	*Solidago canadensis*	Canada
Stagonosporopsis dorenboschii	CBS 426.90; IMI 386093; PD 86/551	**JN251980**	*Physostegia virginiana*	Netherlands
Stagonosporopsis heliopsidis	DAOM 221138; PD 95/6189;	**JN251970**	*Ambrosia artemisiifolia*	Canada
Stagonosporopsis hortensis	CBS 572.85; PD 79/269	**JN251966**	*Phaseolus vulgaris*	Netherlands
Stagonosporopsis ligulicola var. *inoxydabilis*	CBS 425.90; PD 81/520	**JN251972**	*Chrysanthemum parthenii*	Netherlands
Stagonosporopsis ligulicola var. *ligulicola*	CBS 500.63; MUCL 8090	**JN251973**	*Chrysanthemum indicum*	Germany
Stagonosporopsis loticola	CBS 562.81; PDDCC 6884	**JN251978**	*Lotus pedunculatus*	New Zealand
Stagonosporopsis lupini	CBS 375.84; PD 80/1250	**JN251967**	*Lupinus mutabilis*	Peru

Table 1. (Continued).

Species name	Isolate Nr.	GenBank accession	Host, substrate	Country
Stagonosporopsis oculo-hominis	CBS 634.92; IMI 193307	**JN251976**	Human	USA
Stagonosporopsis trachelii	CBS 379.91; PD 77/675	**JN251977**	*Campanula isophylla*	Netherlands
Stagonosporopsis valerianellae	CBS 329.67; PD 66/302	**JN251965**	*Valerianella locusta* var. *oleracea*	Netherlands
Outgroup				
Phoma dimorphospora	CBS 345.78; PD 76/1015	**JN251990**	*Chenopodium quinoa*	Peru

The cycle parameters were similar to those described above. The Ct-value was automatically calculated for each PCR by the algorithm ABI PRISM system software. Genomic DNA of the isolates as well as healthy plant material was included as positive and negative controls.

ITS amplifications

In case the results of the specific Taqman PCR were negative, an ITS-PCR was performed to demonstrate the PCR-ability of the isolated DNA. PCR amplifications were performed in duplicate in PCR tubes, total volume of 25 µl, which contained the following reaction mixture: 2.5 µl 10× buffer (Roche, Almere, The Nederlands), 60 µM dNTP's , 0.625 U Taq polymerase, 200 nM of the primers ITS1 and ITS4 (White *et al*., 1990) (Table 2), and 1 µl of genomic DNA. The cycle parameters were 2 min at 95 ºC, 35 cycles of a 3-step amplification (30 s 95 ºC; 30 s 57 ºC; 1 min 72 ºC), finally 10 min 72 ºC. The ITS PCR was performed in a DNA Engine (PTC-200) instrument (BioRad, Veenendaal, Nederland) in 96-wells-plates. A negative control, Milli-Q water, was included in each run. The PCR products were separated on a 1 % agarose gel and stained with GelRed (Biotium Inc, Hayward, CA, USA).

Assessment of TaqMan assay performance criteria

The TaqMan PCR methods for the detection of *Stagonosporopsis andigena* and *S. crystalliniformis* were validated for several performance characteristics. The specificity of the assays was tested with the TaqMan PCR on 1 µl genomic DNA extracted from the two *S. andigena* and two *S. crystalliniformis* isolates and 16 additional isolates of the allied *Stagonosporopsis*, *Phoma* and *Boeremia* species (Table 3). The analytical sensitivity (detection limit) of both TaqMan PCR assays was determined in duplicate in a 10-fold serial dilution of the genomic DNA in sterile MQ water in a range of approximately 1 ng–1 fg obtained from pure cultures of *S. andigena* (CBS 101.80) and *S. crystalliniformis* (CBS 713.85). In addition, the analytical sensitivity was determined in duplicate on DNA extracted from leaf material of the potato cv 'Bintje' or tomato cv 'Moneymaker' spiked with mycelium of isolate CBS 101.80 or CBS 713.85 in a serial dilution with DNA extracted from healthy leaves. A 0.9 cm diam leaf disk was spiked with mycelium scraped from one 0.9 cm diam agar plate. After the DNA extraction, a serial dilution 1:0, 1:1, 1:5, 1:20 and 0:1 in a total volume of 20 µl was made of the DNA of the spiked samples with DNA extracted from healthy leaves. One µl of the mixtures was tested in duplicate with the TaqMan PCR assays. The effect of host plant material on the amplification of DNA of the target

Table 2. Characteristics of primers and TaqMan probes designed for the detection of *S. andigena* and *S. crystalliniformis*, potato cytochrome oxidase DNA and conventional primers used for amplification of ITS1-ITS4 and actin regions.

	Sequence (5' – 3')	**Dye**	**Amplification**
TaqMan primers/probes			
S.andF2	TCT TCC GTA AGT CCT CCA AT C		Actin, *S. andigena*
S.andR1	GTG TTG TCA GTG GGA GGT TCA C		
S.andP1 probe	ACC TGG CAG CAG CAG CGT TCC T	5' end FAM; 3' end BHQ-1	
S.crysF2	GCA GTC TT CCGT AAG TCC C		Actin, *S. crystalliniformis*
S.crysR1	TCG CGG GCG TT TGCT		
S.crysP1 probe	CTG GCA GCA ACA GCA GCA GCG TTA CT	5' end FAM; 3' end BHQ-1	
COX-F	CGT CGC ATT CCA GAT TAT CCA		Cox, universal
COX-RW	CAA CTA CGG ATA TAT AAG RRC CRR AAC TG		
COXSOL1511T probe	AGG GCA TTC CAT CCA GCG TAA GCA	5' end YY; 3' end BHQ-1	
Conventional primers			
ACT-512F	ATG TGC AAG GCC GGT TTC GC		Actin, universal
ACT-783R	TAC GAG TCC TTC TGG CCC AT		
ITS1	TCC GTA GGT GAA CCT GCG G		ITS, universal
ITS4	TCC TCC GCT TAT TGA TAT GC		

Table 3. Results of the specificity test with the primer pairs designed for *Stagonosporopsis andigena* and *S. crystalliniformis*.

Isolate		Strain nr.	TaqMan PCR, Ct values	
			S. andigena	***S. crystalliniformis***
1	*Stagonosporopsis andigena*	CBS 269.80	22.82	nd[1]
2	*Stagonosporopsis crystalliniformis*	CBS 713.85	nd	22.21
3	*Boeremia exigua* var. *exigua*	CBS 431.74	nd	nd
4	*Boeremia foveata*	CBS 341.67	nd	nd
5	*Boeremia lycopersici*	CBS 378.67	nd	nd
6	*Boeremia noackiana*	CBS 101203	nd	nd
7	*Stagonosporopsis valerianellae*	CBS 329.67	nd	nd
8	*Peyronellaea anserina*	CBS 360.84	nd	nd
9	*Stagonosporopsis andigena*	CBS 101.80	23.84	nd
10	*Stagonosporopsis crystalliniformis*	CBS 771.85	nd	22.23
11	*Stagonosporopsis cucurbitacearum*	CBS 133.96	nd	nd
12	*Stagonosporopsis lupini*	CBS 375.84	nd	nd
13	*Peyronellaea glomerata*	CBS 528.66	nd	nd
14	*Peyronellaea pomorum* var. *pomorum*	CBS 539.66	nd	nd
15	*Peyronellaea subglomerata*	CBS 110.92	nd	nd
16	*Phoma chrysanthemicola*	CBS 522.66	nd	nd
17	*Phoma eupyrena*	CBS 374.91	nd	nd
18	*Phoma herbarum*	CBS 615.75	nd	nd
19	*Phoma huancayensis*	CBS 105.80	nd	nd
20	*Phoma labilis*	CBS 124.93	nd	nd

[1]nd: not detected.

organisms was tested in duplicated with spiked samples consisting of DNA of *S. andigena* (CBS 101.80) or *S. crystalliniformis* (CBS 713.85) with leaf material of potato 'Bintje', 'Bionica', 'Bethaultii' and tomato 'Moneymaker', 'Microtom' and 'Heinz' respectively. The robustness of the assays was assessed by changing two important parameters, the PCR machine and the Taq polymerase kit. The TaqMan PCR assays were repeated on another AB7500 PCR machine using the same samples of the serial dilution of the *S. andigena* and *S. crystalliniformis* isolates as described above, and the Taq polymerase kit was altered by use of the Premix Ex Taq™ (Takara).

The repeatability and reproducibility were determined following the Dutch guideline for the validation of detection and identification methods for plant pathogens and pests (2010) and the OEPP/EPPO guideline PM 7/98 (1) (2010), to obtain information about the sensitivity of the analysis for small variation in the execution under routine-like circumstances. At eight different times, 0.9 cm diam leaf disks of potato cv. 'Bintje' and tomato cv 'Moneymaker' were spiked with mycelium scraped from 0.45–1.8 cm diam plugs taken from colonies of the isolates *S. andigena* (CBS 101.80) and *S. crystalliniformis* (CBS 713.85) in eight replicates. DNA was extracted as described above in duplicate by two different technicians (A, B). One µl of the DNA extract was tested with the TaqMan PCR assays which were performed once (A) or in

duplicate (B) under the same laboratory conditions. A positive control, genomic DNA of both strains, and sterile MQ water as a negative control were included.

Testing the TaqMan PCR assays on artificially inoculated potato and tomato leaves
Detached leaves from 5 weeks old plants of potato 'Bintje' and tomato 'Moneymaker' were transferred into 18 × 14 × 5.5 cm transparent plastic boxes, one leaf per box. Each leaf was inoculated on the upper side with three to four 6 mm diam mycelium bearing agar plugs of one of the isolates *S. andigena* (CBS 101.80, 269.80) or *S. crystalliniformis* (CBS 713.85, 771.85) taken from a 10 days old culture grown on OA. The boxes were incubated during 2 days at 10 ºC in darkness, followed by 5 days at 20 ºC in daylight. At 7 days after inoculation, 0.9 cm diam. plugs were taken from the infected leaf tissue at the margin of the lesion and collected into a 1.5 ml Qiagen microtube, and stored at -20 ºC until DNA extraction. After DNA extraction, both TaqMan PCR assays developed for *S. andigena* and *S. crystalliniformis* were performed in duplicate as described above.

RESULTS

DNA phylogeny of the actin gene
The aligned sequence length obtained for the actin region was 314 nucleotides long. Of these 314 characters, 191 unique site patterns were present. The analysis run in MrBayes resulted in 46 002 trees after 230 000 generations, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated based on 25 819 trees. A main clade which represents the genus *Stagonosporopsis* was found (support 96 %, Fig. 1) in agreement with a multigene analysis based on LSU, ITS and ß-tubulin (Aveskamp *et al.* 2010). In this clade, *Stagonosporopsis andigena* and *S. crystalliniformis* could be recognised in a well-supported subclade demonstrating that both species are closely allied. The clade representing *Stagonosporopsis* also includes *Phoma nemophilae* CBS 249.38. *Boeremia* represents a well-supported monophyletic clade, while the subclade that represents *Peyronellaea* is poorly supported.

Selection of the Primer/probe combinations
The primer/probe combinations that were selected using actin sequence differences between *Stagonosporopsis andigena*, *S. crystalliniformis* and their related species are given in Table 2. The primer pairs produce fragments of the actin gene of 97 and 89 base pairs respectively. The partial sequence alignments of the forward and reverse primers of *Stagonosporopsis andigena* and *S. crystalliniformis* contained one SNP and two SNP's difference respectively, however, the probes developed contained seven different SNP's. The blast results with the primers and probes in GenBank demonstrated no homology with sequences of organisms present in the database.

Assessment of TaqMan assay performance criteria
The Ct values of the TaqMan PCR assays performed on DNA extracts obtained from pure cultures of *S. andigena* and *S. crystalliniformis*, as well as those of the related species of *Stagonosporopsis* included in this study, are given in Table 3. The Ct values for the *S. andigena* isolates CBS 101.80 and CBS 269.80 were 23.84 and 22.82, and the Ct values for the *S. crystalliniformis* isolates CBS 713.85 and CBS 771.85 were 22.21 and 22.23 respectively. All other isolates tested showed negative results, while correct PCR products were obtained with the ITS PCR demonstrating the amplification of DNA (data not shown).

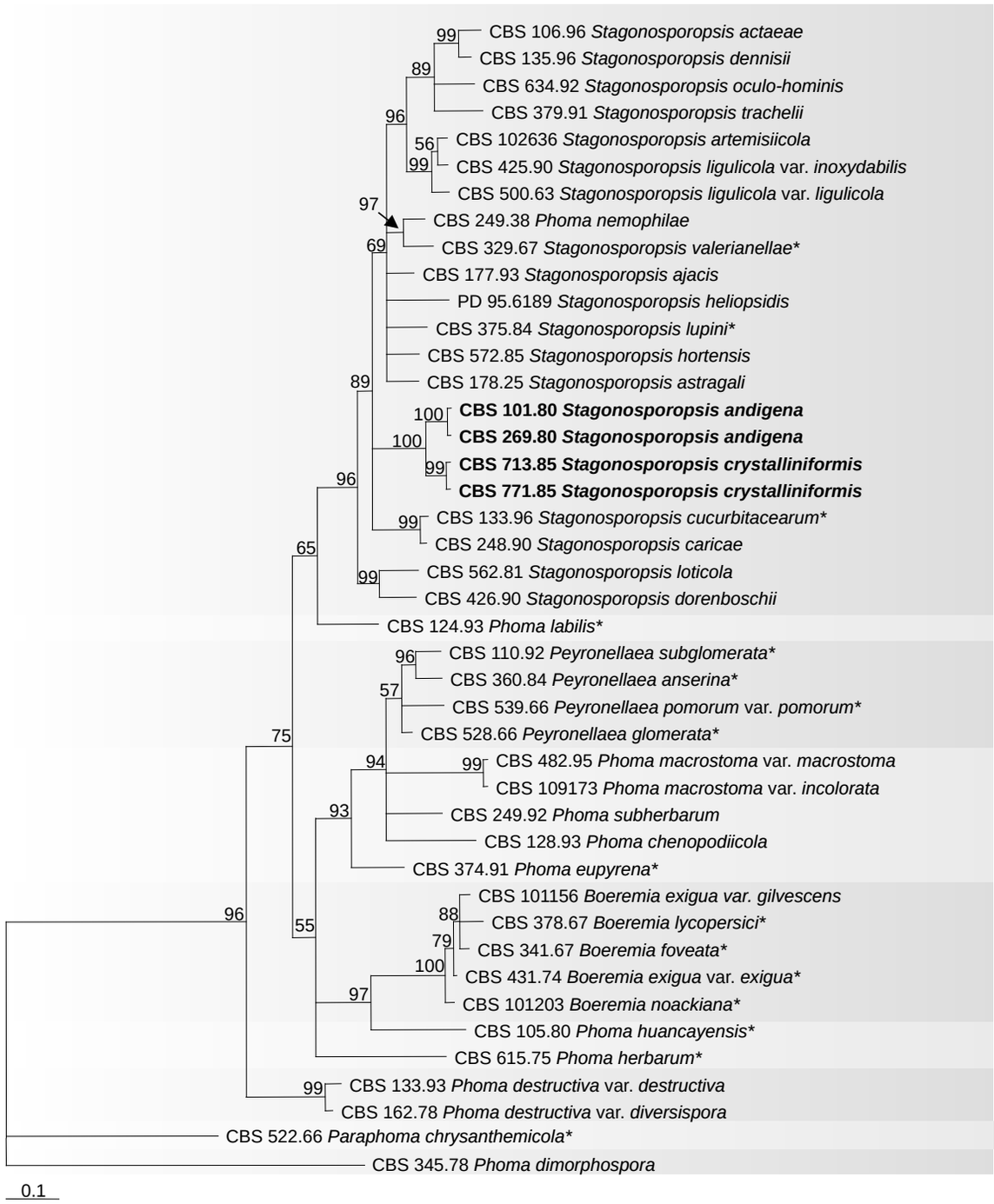


Fig. 1. The phylogenetic relationships of *Stagonosporopsis andigena* and *S. crystalliniformis* and species classified in the genera *Stagonosporopsis*, *Boeremia*, *Phoma*, and *Peyronellaea*, based on the strict consensus tree from a Bayesian analysis of 43 actin sequences. The Bayesian posterior probabilities are given at the nodes. The tree was rooted with *Phoma dimorphospora* (CBS 345.78). *Strains that were included in the specificity test (Table 3).

The results of the TaqMan PCR assays on a 10-fold serial dilution of *S. andigena* and *S. crystalliniformis* DNA in sterile MQ water are provided in Table 4. The PCR efficiency was estimated through the linear regression of the calculated calibration curve. The Ct values

Table 4. Ct values resulting from TaqMan PCR on genomic DNA in duplicate in a 10-fold serial dilution of pure cultures of *Stagonosporopsis andigena* (CBS 101.80) and *S. crystalliniformis* (CBS 713.85) to detemine the analytical sensitivity and robustness.

a	***S. andigena***		***S. crystalliniformis***		b	***S. andigena***		***S. crystalliniformis***	
	CBS 101.80		CBS 713.85			CBS 101.80		CBS 713.85	
1 ng	24.15	24.09	21.89	22.20	1 ng	23.84	25.02	22.21	23.83
100 pg	27.91	27.90	25.81	25.95	100 pg	28.57	nt[1]	25.56	nt
10 pg	31.37	31.79	29.84	28.96	10 pg	31.84	nt	28.71	nt
1 pg	37.45	34.92	32.93	32.78	1 pg	37.58	nt	32.94	nt
100 fg	37.02	36.06	35.15	35.54	100 fg	37.97	nt	35.15	nt
10 fg	nd[2]	nd	nd	36.27	MQ	nd	nd	nd	nd
1 fg	nd	nd	nd	nd					
MQ	nd	nd	nd	nd					

a. AB7500 machine 1, TaqMan® Universal PCR Master Mix Taq polymerase kit; b. AB7500 machine 2, Premix Ex Taq™ Taq polymerase kit, [1]nt: not tested, [2]nd: not detected.

obtained with the target DNA of 100 fg for *S. andigena* were discarded because the average value was out of the linear phase. The coefficients were R^2=0.998 and R^2=0.993, the amplification efficiency 92.4 % and 99.1 % for the TaqMan PCR assays for *S. andigena* and *S. crystalliniformis* respectively. The Ct threshold values 37.5 and 35.5 were determined qualitatively as the maximum values for a positive reaction for *S. andigena* and *S. crystalliniformis* respectively. These values correspond with the maximum Ct values found in the linear phases. The effect of host plant material on the amplification of DNA of the target organisms was tested with spiked samples consisting of DNA of *S. andigena* (CBS 101.80) or *S. crystalliniformis* (CBS 713.85) with leaf material of different cultivars of potato and tomato respectively (Table 5). The Ct values for *S. andigena* (29.48–30.96) and *S. crystalliniformis* (26.61–28.69) showed that it was possible to extract and amplify target DNA in the presence of plant material of the different cultivars. The TaqMan PCR assays on spiked samples of *S. andigena* and *S. crystalliniformis* with leaf of potato cv. 'Moneymaker' and tomato cv. 'Bintje' in a serial dilution demonstrated that a 20x dilution of the DNA extract still revealed a positive result (Table 6).

Similar results of the specific TaqMan PCR assays of *S. andigena* and *S. crystalliniformis* were obtained after changing the TaqMan polymerase or performing the PCR in another AB7500 PCR machine (Table 4). The results of the repeated experiments conducted over time and performed by two persons (A, B) were comparable (Table 7). Both species were detected in all spiked samples tested by both persons at the different times. The Ct values obtained in time with both PCR assays were similar. The 64 Ct values (eight time points with eight replicates) of the TaqMan PCR developed for *S. andigena* and applied on *S. andigena* mycelium spiked with potato leaf and for *S. crystalliniformis* on tomato were all below the Ct threshold values 37.5 and 35.5, respectively.

Detection of *S. andigena* and *S. crystalliniformis* in artificial infected potato and tomato leaves

The first symptoms on the detached leaves were obtained 3 days after the artificial inoculation of *S. andigena* and *S. crystalliniformis.* In the inoculation experiments on the detached leaves

Table 5. Ct values of TaqMan PCR determined in duplicate on spiked samples of *Stagonosporopsis andigena* and *S. crystalliniformis* with leaf plugs of *Solanum tuberosum*, cvs 'Bintje', 'Bionica' and 'Bethaultii', and *S. lycopersicum*, cv 'Moneymaker', 'Microtom' and 'Heinz'.

	Cultivar	***S. andigena* (CBS 101.80)**		***S. crystalliniformis* (CBS 713.85)**	
S. tuberosum	'Bintje'	29.48	29.68	28.54	27.91
	'Bionica'	30.00	30.03	27.75	27.80
	'Berthaultii'	30.30	29.77	28.69	28.50
S. lycopersicum	'Moneymaker'	30.56	30.79	26.61	26.74
	'Microtom'	30.66	30.50	27.98	27.89
	'Heinz'	30.96	30.29	28.53	28.41
	MQ	nd[1]	nd	nd	nd

Table 6. Ct values of TaqMan PCR on genomic DNA determined in duplicate in a serial dilution of spiked samples of pure cultures of *Stagonosporopsis* (*S*); *S. andigena* CBS 101.80 (*S. a.*) and *S. crystalliniformis* 713.85 (*S. c.*) with leaf material of *Solanum tuberosum* 'Bintje' (B) and *S. lycopersicum* 'Moneymaker' (M) to determine the analytical sensitivity.

***S.* + B : B**	***S. a.***	***S. a.***	***S. c.***	***S. c.***	***S.* + M : M**	***S. a.***	***S. a.***	***S. c.***	***S. c.***
1:00	31.71	31.56	30.02	29.80	1:00	32.05	31.98	27.87	28.17
1:01	32.58	32.42	30.90	30.97	1:01	32.42	32.71	28.58	29.01
1:05	33.63	34.34	32.36	32.35	1:05	33.85	34.33	30.20	30.42
1:20	34.24	35.18	33.14	33.09	1:20	35.95	35.17	32.29	31.92
0:01	nd[1]	nd	nd	nd	0:01	nd	nd	nd	nd
MQ	nd	nd	nd	nd	MQ	nd	nd	nd	nd
	COX detection					COX detection			
1:00	25.60	25.65	26.46	26.56	1:00	24.91	24.84	25.95	25.89
1:01	25.95	26.01	26.55	26.52	1:01	25.25	25.20	25.94	25.92
1:05	26.09	26.22	26.45	26.52	1:05	25.56	25.17	25.96	26.03
1:20	26.46	26.53	26.54	26.57	1:20	25.87	25.83	25.92	25.96
0:01	26.67	26.45	26.64	26.71	0:01	26.02	26.02	26.02	25.93
MQ	nd	nd	nd	nd	MQ	nd	nd	nd	nd

it became obvious that a 10 ºC initial incubation period is essential for infection. Both species caused infections on potato and tomato leaves. The symptoms caused by *Stagonosporopsis andigena* on tomato was a brown necrosis around the inoculum and developing along the veins with a chlorotic margin, comparable with those caused by *S. crystalliniformis.* In addition, *S. crystalliniformis* caused infections on potato leaves as stated by Loerakker *et al*. (1986).

The TaqMan PCR assays applied on infected leaf plugs demonstrated that both *Stagonosporopsis* species could be detected in infected potato and tomato leaf material. In one PCR test with the PCR developed for *S. andigena*, there was a positive PCR reaction on potato leaf infected with *S. crystalliniformis*, possibly due to a contamination (Table 8). The Ct values obtained for *S. andigena*, 26.43–28.37, were below the values obtained with spiked samples in

Table 7. Average Ct values of 8 replicates of real-time TaqMan PCR *Stagonosporopsis andigena* (CBS 101.80) and *S. crystalliniformis* (713.85), spiked with leaf disks of *L. esculentum* 'Moneymaker' and *S. tuberosum* 'Bintje', performed by 2 persons (A, single and B in duplicate) at 8 different times[1].

	Time	Potato			Tomato			Pc[2]	
S. andigena									
		A	B	B	A	B	B	A	B
	1	34.16	34.26	34.41	33.36	33.39	33.00	26.85	26.40
	2	32.86	33.44	33.61	32.82	33.34	33.11	26.49	26.45
	3	33.96	33.50	33.52	33.36	32.51	30.48	26.34	25.91
	4	32.86	33.86	33.53	32.82	31.25	32.00	25.64	25.84
	5	31.89	32.43	32.88	32.11	31.65	30.78	25.51	24.75
	6	32.53	34.09	33.64	32.71	32.58	31.67	25.94	25.96
	7	32.69	32.46	32.80	32.41	31.74	31.22	25.99	25.49
	8	34.41	34.00	34.15	33.94	33.78	31.82	26.99	26.79
S. crystalliniformis									
		A	B	B	A	B	B	A	B
	1	31.76	32.71	32.41	31.90	32.32	31.88	24.76	24.06
	2	31.00	32.71	32.77	31.99	32.23	31.76	25.59	26.45
	3	31.76	32.93	33.07	31.90	30.97	30.07	23.23	23.75
	4	31.00	32.06	32.41	31.99	32.35	30.28	23.66	23.98
	5	31.54	31.48	32.22	31.66	30.10	28.95	23.44	22.86
	6	28.73	30.98	30.77	29.67	30.63	29.48	23.31	24.16
	7	30.40	30.50	30.51	30.15	28.71	29.17	23.73	23.70
	8	33.18	33.05	33.28	32.71	32.30	31.44	24.73	24.96

[1]The MQ water controls were negative. [2]Pc: Positive control, genomic DNA.

a previous experiment (Table 5). Both reference cultures included showed negative or positive results for both PCR assays as expected, ITS amplicons were obtained with the ITS primers ITS1 and ITS4 that demonstrates the PCR-ability. Negative results with both PCR assays were obtained with non-infected leaf material and sterile MQ water.

DISCUSSION

Stagonosporopsis andigena and *S. crystalliniformis* are foliage pathogens that have presently not been reported from outside the Andes region. The isolates included in the present study were obtained in 1975 and 1985 respectively, and recent isolates could not be obtained. Both species are distinct from *Boeremia foveata*, a pathogen of potato tubers that also originates from the Andes region, but with a wider known distribution. *Stagonosporopsis andigena* has been recorded on wild and cultivated species of potato. In terms of quarantine the host range is important and the statement that *S. andigena* also occurs on tomato and other solanaceous weeds (OEPP/EPPO, 1984) could not be confirmed by any of the citations listed as was also noted

Table 8. Ct values of Taqman PCR amplification performed on DNA extracts after duplicate inoculation and extraction from leaves of *Solanum tuberosum* 'Bintje' (B) and *S. lycopersicum* 'Moneymaker' (M) infected by *Stagonosporopsis andigena* and *S. crystalliniformis*.

Target	**CBS nr.**	***S. andigena***		***S. crystalliniformis***	
infected plant material					
M + *S. andigena*	101.80	26.78	26.60	nd[1]	nd
B + *S. andigena*	101.80	28.35	28.14	nd	nd
M + *S. andigena*	269.80	27.48	26.43	nd	nd
B + *S. andigena*	269.80	27.12	28.37	nd	nd
M + *S. crystalliniformis*	713.85	nd	nd	27.82	25.29
B + *S. crystalliniformis*	713.85	nd	nd	26.43	26.22
M + *S. crystalliniformis*	771.85	nd	nd	25.03	25.45
B + *S. crystalliniformis*	771.85	36.04	nd	30.22	28.72
reference cultures					
S. andigena	101.80	24.18		nd	
S. crystalliniformis	713.85	nd		23.31	

[1]nd: not detected

recently (Cline, 2011). However, the results of our artificial inoculations with mycelial plugs on detached leaves to obtain infected leaves for PCR assays demonstrated that *S. andigena* might also infect tomato leaves. Although we tried to infect potato tubers, no disease symptoms could be obtained in congruence with previous data for *S. andigena* (Turkensteen 1978).

The molecular phylogeny utilising actin sequence data demonstrates that *S. andigena* and *S. crystallini-formis* are closely related species of *Stagonosporopsis*. This result is in agreement with the molecular phylogeny based on LSU, ITS and ß-tubulin sequences (Aveskamp *et al*. 2010). Both isolates of each species showed a high genetic homogeneity. No sexual state has thus far been recorded for either species. *Stagonosporopsis andigena* and *S. crystalliniformis* were previously classified in *Phoma* section *Heterospora* according to their morphological characters (Boerema *et al*. 1997). This *Phoma* section is characterised by the formation of dimorphic conidia, namely small 'phomoid' and distinctly larger 'ascochytoid-stagonosporoid' conidia, the latter also described as synanamorph in *Stagonosporopsis* (Boerema *et al*. 1997, 2004). Molecular studies demonstrated that *Phoma* section *Heterospora* is polyphyletic, and that only part of the species of this section grouped in a monophyletic clade, and have been described in the amended holomorph *Stagonosporopsis* (Aveskamp *et al*. 2010). *Stagonosporopsis cucurbitacearum* proved to be one of the most closely related species based on the alignments of actin sequences compared in this study. *Stagonosporopsis cucurbitacearum* is regarded world-wide as an important seed-borne pathogen on *Cucurbitaceae*, esp. *Cucumis*, *Cucurbita* and *Citrullus*, but has also been recorded associated with infected leaves of potato in Peru (Turkensteen & Pinedo, 1982). This finding supports the close relation of these species, and suggests a geographic origin in the Andes region.

The identification of *Phoma*-like isolates requires a high level of experience, it is time consuming, and the *in vitro* characters are often variable (Aveskamp *et al*. 2008), which may lead to misidentifications. Several molecular methods for the detection and identification of

important pathogenic *Phoma* species have been developed during the last decade. A PCR test was developed to distinguish *Boeremia foveata* (syn. *Phoma foveata*) from *B. exigua* (syn. *P. exigua*) and its varieties, using primers that were derived from a RAPD product (Macdonald *et al*. 2000). A PCR-ELISA method was developed for the detection of *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*, *Phoma cucurbitacearum*) in fruit crops that belong to *Cucurbitaceae* (Somai *et al*. 2002). Primers derived from the Internal Transcribed Spacer regions 1 & 2 (ITS) and 5.8S rDNA were developed for the detection of *D. bryoniae* and *P. foveata* (Koch & Utkhede 2004, Cullen *et al*. 2007). In addition, PCR assays based on the ITS region were developed for the detection and identification of *P. tracheiphila* in citrus fruits (Balmas *et al*. 2005, Licciardella *et al*. 2006, Demontis *et al*. 2008). Actin sequence data provided tools for the development of TaqMan PCR assays for *S. andigena* and *S. crystalliniformis*. The performances characteristics were determined, and demonstrated that the DNA extraction and PCR methods are suitable for the detection and identification of *S. andigena* an *S. crystalliniformis* in leaves of potato and tomato.

The allied *Stagonosporopsis* species and more distantly related species of *Boeremia*, *Peyronellaea* and *Phoma* were not detected. Both TaqMan PCR methods were sensitive, and reliability and repeatability could be demonstrated. Extraction and amplification of target DNA occurred in the presence of plant material of the cultivars of potato ‘Bintje’, ‘Bionica’, ‘Bethaultii’ and tomato ‘Moneymaker’, ‘Microtom’ and ‘Heinz’. The Ct values obtained with artificially inoculated leaves of potato ‘Bintje’ and tomato ‘Moneymaker’ were all below or comparable with those obtained with spiked samples.

ACKNOWLEDGEMENT

The Dutch Ministry of Economics, Innovation and Agriculture has supported this research through an endowment of the FES program ‘Reinforcement Infrastructure Plant Health’.

CHAPTER 6

General discussion

Phoma* and allied genera in *Didymellaceae

The identification of species in *Phoma* and morphologically similar coelomycetes is complicated and needs a high level of morphological expertise. Key characteristics are not always easily observed, and sometimes may even be absent. Moreover, there are many *Phoma* species that show ambiguous characters in relation to section-classification. As an example, *Ph. dictamnicola*, a pathogen of *Dictamnus* spp. was treated in *Phoma* section *Phoma* because of its formation of very small, aseptate conidia in pseudoparenchymatous pycnidia (de Gruyter & Noordeloos 1992). The pseudoparenchymatous pycnidia produced by *Ph. dictamnicola* are relatively thick-walled and initially closed. Therefore, this species was classified in section *Sclerophomella* (Boerema & de Gruyter 1998) because this section is characterised by thick-walled pycnidia, producing an opening in a later stage (the development of a pore instead of a predetermined ostiole). An additional complicating taxonomic character is that in leaf spots *Ph. dictamnicola* may produce distinctly large, 1-septate conidia, in contrast with the small conidia that are produced *in vitro* and on dead stems. *Phoma* species that may produce both types of conidia are classified in *Phoma* section *Heterospora* (Boerema *et al.* 1997) and therefore, the species could have been placed in this section as well. *Phoma* species with ambiguous characters have been included in the keys of the different sections in the *Phoma* Identification Manual (Boerema *et al.* 2004), but it illustrates the difficulties that may arise during identification based solely on morphological characters.

DNA sequence data of several genes were employed in this study including the 18S nrDNA (SSU), the 28S nrDNA (LSU), the Internal Transcribed Spacer (ITS) regions 1 & 2 with intervening 5.8S nrDNA and partial gene sequences of actin (ACT), ß-tubulin (TUB) and chitin synthase 1 (CHS-1). The SSU, and to a minor extent the LSU are relatively conserved and valuable for phylogenetic studies at generic level in *Pleosporales* (Silva-Hanlin *et al.* 1999, Kruys *et al.* 2006, de Gruyter *et al.* 2009, de Gruyter *et al.* 2010). A combination of the LSU and the more variable ITS regions appeared suitable for molecular phylogenetic studies at species level (de Gruyter *et al.* 2012).

The TUB sequences appeared to be valuable to identify the genetic diversity of the related species *Phaeosphaeria nodorum* and *Phaeo. avenaria* (Malkus *et al.* 2005), or as part of a multilocus molecular phylogenetic analysis to clarify the molecular phylogeny of the *Phoma* species in *Didymellaceae* (Aveskamp *et al.* 2010). The ACT sequences appeared suitable for phylogenetic studies below genus level in the former *Ph. exigua* complex (Aveskamp *et al.* 2009b) and CHS-1 was useful as part of a multilocus molecular phylogenetic analysis of *Colletotrichum* species with curved conidia (Damm *et al.* 2009).

Therefore, the phylogeny of closely related taxa was demonstrated in the present study in a concatenated sequence alignment of ITS and the house-keeping genes encoding actin, ß-tubulin and chitin synthase 1, respectively (de Gruyter *et al.* 2012).

Results of the molecular phylogenetic studies on *Phoma* and allied species described in chapter 2 formed the basis for a rigorous redisposition of the genera and species in *Pleosporales*. The phylogeny obtained demonstrated that the classification of the species into the nine *Phoma* sections is highly artificial and does not reflect the evolutionary relationships. *Phoma herbarum*, the type species of *Phoma*, grouped with allied *Phoma* and *Didymella* species in the newly introduced family *Didymellaceae* (de Gruyter *et al.* 2009). It clearly demonstrates that *Didymella* does not belong to *Mycosphaerellaceae*, *Pleosporaceae*, *Phaeosphaeriaceae* or *Venturiaceae* as previously proposed. A neotype of the generic type species *D. exigua* could be defined by combining *in vivo* and *in vitro* data provided by Corbaz (1957), with the LSU/SSU sequence data of a *D. exigua* strain that was isolated from the same host and location in France, deposited in the CBS collection in 1955.

The type species of the *Phoma* sections *Phoma, Phyllostictoides, Sclerophomella, Macrospora* and *Peyronellaea* are allied to *Didymellaceae*. This finding could be expected for the sections *Phoma, Peyronellaea, Phyllostictoides* and *Macrospora* with a high similarity in morphological characters. The size and septation of conidia or formation of dictyochlamydospores instead of unicellular chlamydospores appeared to be of minor importance from an evolutionary point of view.

Phoma sections *Phoma* and *Phyllostictoides* include species with a teleomorph described in *Didymella*. The teleomorph of section *Macrospora* has been controversially placed in *Didymella* and *Mycosphaerella,* but the results of this study demonstrate its close relation with *Didymella*.

The relatively thick-walled, initially closed pseudoparenchymatous pycnidia of *Phoma complanata*, type species of *Phoma* sect. *Sclerophomella*, suggested a close relation with species described in *Phoma* section *Plenodomus*, reclassified in *Plenodomus* and allied genera in *Leptosphaeriaceae* (de Gruyter *et al.* 2012). The finding of *Phoma complanata* in *Didymellaceae* illustrates that thick-walled pycnidia with the development of a pore instead of predetermined ostiole is a convergent character in *Pleosporales.*

The type species of *Phoma* sections *Heterospora, Paraphoma, Pilosa* and *Plenodomus* grouped outside *Didymellaceae.* Therefore, these *Phoma* sections cannot be maintained in *Phoma* and moreover, they are polyphyletic.

The close relation of *D. exigua* with the generic type species *Leptosphaerulina australis* in *Didymellaceae* (de Gruyter *et al.* 2009) was not expected because of the distinct morphological differences in ascospore septation. A remarkable example of parallel evolution was observed with the genera *Mycosphaerella* and *Sphaerulina* in *Dothideales* (Crous *et al.* 2003). It demonstrates that such a notable difference in ascospore septation may exist in closely allied genera.

The generic type species *Ascochyta pisi* and *Microsphaeropsis olivacea* also grouped in *Didymellaceae* (de Gruyter *et al.* 2009). *Ascochyta*, morphologically very similar to *Phoma* section *Phyllostictoides* because of its hyaline, septate conidia, includes several species with a *Didymella* teleomorph (Peever *et al.* 2007) such as the recently described teleomorph of the type species *Didymella pisi* (Chilvers *et al.* 2009). These results illustrate that conidial septation is of minor importance in the evolutionary history of these species. *Microsphaeropsis* is characterised by producing olivaceous conidia in contrast with the hyaline conidia of *Phoma*, but in this study both genera were found to be closely allied. It was already observed that dark conidia could be found in old pycnidia of *Phoma* species in sections *Peyronellaea* and *Sclerophomella* (Boerema *et al*. 2004), suggesting that this character should also be interpreted with care.

Phyllosticta was misapplied in the Saccardoan system by including various *Phoma*-like species that were found on leaves. *Phyllosticta* grouped with *Guignardia* and *Macrophomina phaseolina* in *Botryosphaeriaceae* in accordance with Schoch *et al.* (2006). *Sphaeropsis visci* and *Diplodia pinea* could also be attributed to this family. The *Phoma*-like genera *Selenophoma* and *Coleophoma* are only distantly related and clustered in *Dothideaceae, Dothideales* (de Gruyter *et al.* 2009).

The molecular phylogeny of *Phoma* and its allied genera in *Didymellaceae* was not within the scope of this thesis, and was studied by Aveskamp *et al*. (2009a,b, 2010; PhD thesis to be submitted).

Taxonomy of *Phoma*-like species with setose or pilose pycnidia

Phoma radicina was originally described as the type species of *Paraphoma* (Morgan-Jones & White 1983) and later transferred to *Phoma* as section *Paraphoma* (van der Aa *et al.* 1990).

This section of *Phoma* comprises species that produce setose pycnidia. Molecular phylogenetic studies on SSU and LSU demonstrated that *Phoma radicina* resides in *Phaeosphaeriaceae* rather than in *Phoma*, *Didymellaceae* (de Gruyter *et al.* 2009, 2010). Therefore, *Paraphoma* was reinstalled to accommodate *Ph. radicina* and two allied species. *Phoma* section *Paraphoma* was found to be highly polyphyletic and even the *Phoma* species transferred to *Paraphoma*, viz. *Ph. chrysanthemicola* and *Ph. fimeti*, were originally classified in the other *Phoma* sections *Peyronellaea* and *Phoma* (de Gruyter *et al.* 2010). *Phoma chrysanthemicola* produces pycnidia covered by setae-like mycelial hairs rather than stiff setae, and *Phoma fimeti* produces glabrous pycnidia. Pycnidial characters in *Paraphoma* are variable, and the ecological niche and soilborn nature are often shared among species belonging to this section. All other *Phoma* species classified in *Phoma* section *Paraphoma* grouped in several other families within the *Pleosporales*.

Two important diagnostic characters, viz. conidiogenesis (*Phoma*-like with (sub)globose conidiogenous cells or *Pyrenochaeta*-like with elongated conidiophores) and pycnidia (glabrous, pilose or setose) are variable in *Pleosporales*. This indicates a parallel evolutionary development, where degenerations or even frequent losses of these features have occurred. *Phoma terricola*, *Ph. indica*, *Ph. briardii*, *Ph. leveillei* var. *microspora* and strains of *Ph. leveillei* var. *leveillei* reside in the new genus *Pyrenochaetopsis*, allied to *Pyrenochaeta* in *Cucurbitariaceae*. The predominant *Phoma*-like conidiogenesis observed in *Pyrenochaetopsis* suggested a close relation with *Paraphoma* in *Phaeosphaeriaceae* rather than with *Pyrenochaeta*, characterised by the presence of elongate, septate conidiophores.

Phoma setariae and Ph. terrestris are distantly related to *Paraphoma* in *Phaeosphaeriaceae* and are reclassified in the new genus *Setophoma*. This genus was found to be closely related to *Pyrenochaetopsis* and both genera are associated with plants belonging to the *Graminaceae*. *Phoma samarorum* was reclassified as type species of the new genus *Neosetophoma* (de Gruyter *et al.* 2010), closely allied to *Setophoma*. *Neosetophoma samarorum* comprises several characters that do also occur in species belonging to other genera. The conidia produced are yellowish and resemble those produced by *Coniothyrium* (*Didymellaceae*) or *Paraconiothyrium* (*Montagnulaceae*). *Stagonosporopsis fraxini* has been described as a stagonosporoid synanamorph, but *Stagonosporopsis* has been revised and resides in *Didymellaceae* (Aveskamp *et al.* 2010). *Phoma carteri*, *Ph. septicidalis* and *Ph. glycinicola*, that also produce setose pycnidia, grouped in a distinct clade near *Leptosphaeriaceae*, represented by *Leptosphaeria doliolum* and *L. maculans* (de Gruyter *et al.* 2010). *Phoma gardeniae*, producing pycnidia with relatively short setae, even clustered with *Phoma herbarum* in *Didymellaceae*.

Pycnidia of *Ph. betae* and *Ph. typhina*, the only species classified in *Phoma* section *Pilosa* (Boerema 2003) are densely covered by hyphal outgrows, not setae, described as "pilose or hairy" pycnidia. These pilose pycnidia have also been observed in some species of the genera *Paraphoma* and *Neosetophoma*, such as *Par. chrysanthemicola* and *N. samarorum*.

Phoma betae and *Ph. typhina* are associated with the teleomorph *Pleospora*, viz. *Pl. betae* and *Pl. typhicola*. *Phoma betae*, type species of *Phoma* section *Pilosa*, is worldwide recorded as the cause of necrosis on seedlings, leaves, stems and roots of food crops of the *Chenopodiaceae*, beet (*Beta vulgaris*) and spinach (*Spinacia oleracea*) in particular (Boerema *et al*. 1987). *Phoma betae* has morphological characters that are very similar to other coelomycetes that have been described to occur on *Chenopodiaceae* (van der Aa & van Kesteren 1979) such as *A. caulina*, *A. hyalospora* and *Ascochyta obiones*. *Ascochyta hyalospora* was recorded from *Chenopodium album* and the food crop *Chenopodium quinoa* in North and South America, respectively (Boerema *et al.* 1977) and resembles the European *Ascochyta caulina* in many respects (van der

Aa & van Kesteren 1979). The conidia of both species are hyaline when associated with disease symptoms, but are remarkably pale-brown when found on dead plant material. Pathogenicity experiments demonstrated that symptoms caused by both species on *Chenopodium album* are similar (Boerema 1984).

The close relation of these *Phoma* and *Ascochyta* species resembles that observed between the generic type species *Phoma herbarum* and *Ascochyta pisi* in *Didymellaceae*. *Pleospora* is acknowledged as holomorph to accommodate the *Phoma*- and *Ascochyta*-like species in *Pleosporaceae* (de Gruyter *et al.* 2012).

Pleospora typhicola is commonly found on leaves and stems of *Typha* spp. in Europe, associated with spots or dead material (Boerma 2003). The different ecology of the species compared with that of *Pl. betae* is in agreement with the molecular phylogeny (de Gruyter *et al*. 2012). *Pleospora betae* represents a sister group of the generic type species *Pleospora herbarum* and its allied species including *Pl. typhicola* (de Gruyter *et al*. 2012). Further studies are needed to determine the phylogeny within *Pleosporaceae*. The occurrence of diverse hyphomycetous anamorphs such as *Stemphylium* (Simmons 1969), *Alternaria* and *Dendryphion* (von Arx 1981) and coelomycetes described in the genera *Ascochyta*, *Phoma* and *Coniothyrium* (van der Aa & van Kesteren 1979) illustrates the diversity in *Pleosporaceae.*

***Phoma*-like species with thick-walled pycnidia**

Pycnidia consisting of many layers of individual cells that become scleroplectenchymatous are characteristic for *Phoma* section *Plenodomus* in the morphological species concept of *Phoma* (Boerema 1997). Boerema & van Kesteren (1964) described the type species *Ph. lingam* at that time known as *Plenodomus lingam*, in detail. Initially it was concluded that *Plenodomus lingam* could not belong to the form-genus *Phoma* (Boerema 1964). The initially closed pycnidia of *Plenodomus lingam*, with an opening formed only during maturation (Cunningham 1927), were later indicated as pycnidia with developmental pores (Boerema 1976), to differentiate them from pycnidia with predetermined ostioles observed in pycnidia of the genus *Phoma* (Boerema & van Kesteren 1964). The cell wall structure is another important discriminative character. *Plenodomus lingam* may produce pycnidia with pseudoparenchymatous walls similar to those of *Phoma*, indicated as type I (Cunningham 1927). A further development of the pycnidial wall occurs with additional layers of thick-walled, scleroplectenchymatous cells between the outer cell layer and conidiogenous cells (Boerema & van Kesteren 1964). Pycnidia which developed such a cell wall were indicated as type II (Henderson 1918, Cunningham 1927). Pycnidia of type II may also develop directly from the pycnidial primordia. A third phenotype based on pycnidial development of *Pl. lingam*, indicated as type III, is referred to as the sterile scleroplectenchymatous pycnosclerotium. In addition, additional differences in the development of conidia were observed. *Plenodomus lingam* may produce conidiogenous cells with narrow protuberances, referred to as murogenous (Luttrell 1963), whereas in *Ph. herbarum* the conidiogenesis was described as a budding process, indicated as porogenous (Boerema 1964).

New definitions of the form-genus *Phoma* using conidiogenesis (Boerema & Bollen 1975) favoured the classification of *Plenodomus* in *Phoma*. This idea was adopted by von Arx (1970), who accommodated the genus in *Phoma* as section '*Plenodomus*' (Boerema 1976). This classification was formally proposed by Boerema *et al*. (1981). Genera included as synonyms are *Diploplenodomus, Deuterophoma* and *Leptophoma* (Boerema *et al.* 1981, 1994).

The teleomorph *Leptosphaeria* was described in a broad generic concept with a subdivision of the genus into four sections (Müller 1950, Munk 1957). Some of the sections were later raised

to genus level, and several genera were segregated (Holm 1957, Schoemaker 1984). At present, *Leptosphaeria* is classified in *Leptosphaeriaceae* (Barr 1987a) which only includes species that produce scleroplectenchymatous ascomata (Eriksson 1967, Hedjaroude 1968, Holm 1957, von Arx & Müller 1975, Schoemaker 1984, Barr 1987a). The scleroplectenchymatous cell wall structure of the ascomata is similar to that of pycnidia of *Phoma* species classified in section *Plenodomus*. *Leptosphaeria* has exclusively been recorded to occur on dicotyledonous plants, as all species that were described in *Plenodomus* (Boerema *et al.* 1994, Boerema & de Gruyter 1999).

The anamorph-teleomorph relationship of *Phoma* section *Plenodomus* and *Leptosphaeria* in the morphological generic concept of *Phoma* (Boerema 1997) was clearly demonstrated by its type species, *Ph. lingam*, teleomorph *Leptosphaeria maculans* (Smith & Sutton 1964) and *Leptosphaeria doliolum*, anamorph *Ph. acuta* subsp. *acuta* "as *Plenodomus*" (Riedl 1959, Lucas & Webster 1967).

The association of *Leptosphaeria* and *Phoma* section *Plenodomus* was confirmed in several molecular phylogenetic studies, whereas heterogeneity of *Phoma lingam* and the generic type species *Ph. herbarum* was reported (Reddy *et al*. 1998, Pethybridge *et al*. 2004, Torres *et al*. 2005, Schoch *et al*. 2006). The grouping of *Ph. herbarum* in the new family *Didymellaceae* and *Ph. lingam* in *Leptosphaeriaceae* (de Gruyter *et al.* 2009) demonstrated that the taxonomy of *Leptosphaeria* and *Phoma* section *Plenodomus* associated with *Phoma* could not be maintained.

The subsequent study described in this thesis (de Gruyter *et al.* 2012) demonstrates that the molecular phylogeny of species described in *Leptosphaeria/Plenodomus* is even more complex. Delimitation within *Phoma* section *Plenodomus* in the two categories A and B was made based on morphological criteria (Boerema *et al.* 1981). Category A comprises species with common pseudoparenchymatous pycnidia (phenotype I), as well as scleroplectenchymatous pycnidia (phenotype II). Species attributed to category A are usually pathogenic. The pycnidial phenotype I is associated with disease symptoms, whereas phenotype II is usually found on dead tissue, such as overwintering stems. The type species *Ph. lingam* fits in category A. Also *in vitro*, after initial development of pycnidial phenotype I, a subsequent development into phenotype II may occur. Category B always produces both *in vivo* and *in vitro* phenotype II pycnidia. This category includes both saprobic and parasitic species. *Leptosphaeria* indeed comprises species that directly develop scleroplectenchymatous pycnidia. The species included are necrotrophs. *Plenodomus* includes mainly pathogens, where the development from phenotype I into type II can usually be observed. The molecular phylogeny of *Leptosphaeria* and *Phoma* section *Plenodomus* appeared to be more complex. The new genera *Subplenodomus* and *Paraleptosphaeria* were recognised. *Subplenodomus* includes pathogens, and pycnidia produced are mainly of type I, whereas most of the species found in *Paraleptosphaeria* are necrotrophs that produce type II pycnidia. The development of type II pycnidia is usually associated with necrotrophs and this is characteristic for *Leptosphaeria* and *Paraleptosphaeria*. The pathogenic species grouped in *Plenodomus* and *Subplenodomus*, produce type I pycnidia in the parasitic phase.

A second grouping of species in section *Plenodomus* using host plant preference data, viz. group A, occurring on herbaceous plants, and group B associated with bark and bare wood of trees and shrubs (Boerema 1982, Boerema *et al.* 1994) did not corroborate with the results obtained in our molecular phylogenic studies. Additional morphological characters support the molecular phylogeny of the genera *Leptosphaeria*, *Paraleptosphaeria*, *Plenodomus* and *Subplenodomus*, but these characters are not always observed. *Leptosphaeria* species produce (dark) brown 3-septate ascospores, which have been considered as the ancient state for the species that more recently evolved in producing ascospores that are paler in colour, longer

and narrower, and containing more than 3-septa (Wehmeyer 1946). This observation is in agreement with the results obtained in our study. *Paraleptosphaeria* is most closely related to *Leptosphaeria* producing 3(–5) septate, yellow/brown or even hyaline ascospores. *Plenodomus* and *Subplenodomus* are more distantly related. Ascospores in *Plenodomus* are 3–7-septate and in *Subplenodomus* no sexual state has been recorded thus far.

***Phoma-like* species with conidial dimorphism**

Phoma section *Heterospora* comprises mainly pathogenic species with a specific host preference within various plant families. Most of the species produce two types of conidia associated with different stages in the disease cycle of the fungus. In fresh disease symptoms, in the initially developed pycnidia typical large conidia are produced. In older infection sites, the size of conidia in newly developed pycnidia becomes smaller. Finally, only pycnidia with small conidia are found on dead plant material, similar to those usually produced *in vitro* (Boerema *et al.* 1997).The conidial dimorphism might be related to the availability and quality of nutrients in the course of the infection which in turn might relate to the physiological state of the host plant (living versus necrotic cells).

This conidial dimorphism has also been observed in some species classified in other *Phoma* sections. These species usually produce both aseptate and septate conidia of normal phomoid size but occasionally the septate conidia may become extraordinary large and resemble those produced by species of *Phoma* section *Heterospora*. This phenomenon has been observed in *Phoma* section *Sclerophomella,* in species such as *Ph. dictamnicola, Ph. complanata* and *Ph. alecterolophi* (Boerema & de Gruyter 1998), the latter recently reclassified as *Peyronellaea alectorolophi* (Aveskamp *et al.* 2010). In addition, the production of distinctly larger, septate conidia has also been recorded in section *Phyllostictoides*, for example *Ph. ligulicola* var. *ligulicola* and *Ph. cucurbitacearum* (de Gruyter *et al.* 2002). Both species have been recently transferred to *Stagonosporopsis* (Aveskamp *et al.* 2010). Other examples are *Ph. protuberans,* originally described in *Phoma* section *Macrospora* (de Gruyter *et al.* 2002) and now classified in *Peyronellaea* (Aveskamp *et al.* 2010), and two species from *Phoma* section *Peyronellaea*, *Ph. clematidina* and *Ph. narcissi* (Boerema *et al.* 1997), the latter reclassified as *Peyronellaea curtissii* (Aveskamp *et al.* 2010). The species involved are also in majority host-specific plant pathogens.

These examples demonstrate that the ability to produce significantly large ascochytoid/stagonosporoid conidia is not a distinctive character for classification at generic level. The molecular phylogenetic studies demonstrate that *Phoma* section *Heterospora* is highly polyphyletic. The type species *Ph. heteromorphospora* is closely related to the ecologically and morphologically related *Ph. dimorphospora* (Aveskamp *et al.* 2010, de Gruyter *et al.* 2012). Both species grouped outside *Didymellaceae,* and are therefore accommodated in the new genus *Heterospora* (de Gruyter *et al.* 2012).

The remaining species of *Phoma* section *Heterospora* mainly grouped in *Didymellaceae*, at least in three different clades (Aveskamp *et al.* 2010). The majority of these species, some of them with a *Didymella*-like teleomorph, have been recombined in the emended holomorph *Stagonosporopsis*. *Stagonosporopsis* includes also species formerly classified in other sections of *Phoma* such as *S. cucurbitacearum*, syn. *Phoma cucurbitacearum*, teleom. *Didymella bryoniae* (Aveskamp *et al.* 2010). It demonstrates the heterogeneity of *Phoma* section *Heterospora*. The molecular phylogeny of *Stagonosporopsis* and its related genera in *Didymellaceae* provides tools for the development of identification and detection methods for plant pathogens. Sequence data of actin, combined with DNA amplification fingerprinting (DAF) with short, arbitrary, mini-

hairpin primers were used to identify taxon-specific sequence characterised amplified regions (SCARs). Specific primers could be developed for the identification of the different taxa in the *Phoma exigua* complex (Aveskamp *et al.* 2009b), accommodated now in *Boeremia* (Aveskamp *et al.* 2010). Taqman PCR methods for the detection of the important pathogens *S. andigena* and *S. crystalliniformis* in leaves of potato and tomato, respectively, could be developed using actin sequences (de Gruyter *et al.* 2012).

Concluding remarks

Our molecular studies demonstated that *Phoma*-like species are found in various genera within *Pleosporales*. *Phoma* showed a high degree of heterogeneity, and the generic concept with nine sections could not be maintained. The majority of the *Phoma* species have been redescribed in *Cucurbitariaceae, Didymellaceae, Leptosphaeriaceae, Phaeosphaeriaceae* and *Pleosporaceae*, suborder *Pleosporineae.* New genera have been introduced using the single nomenclature for well-resolved anamorph-teleomorph genera proposed by Hawksworth *et al.* (2011). The present molecular study of *Phoma* revealed a better understanding of the taxonomy of several species previously classified in the morphologically related anamorph genera *Ascochyta, Asteromella, Coniothyrium, Microsphaeropsis, Paraconiothyrium, Pleurophoma* and *Pyrenochaeta. Phoma*-like species were recognised in genera classified outside the suborder *Pleosporineae* such as *Paraconiothyrium* in *Paraphaeosphariaceae*, *Aposphaeria* in *Meanommataceae*, and *Westerdykella* in *Sporormiaceae*. Two medical *Phoma*-like species that were originally described in *Pyrenochaeta* (Borelli 1959, 1976) were recognised representing the new genera *Medicopsis* and *Nigrograna*.

The morphological characters such as cell wall structure of pycnidia and colour, size and septation of conidia are a result of multiple evolutionary events. Heterogeneity was observed not only in *Phoma*, but also in the morphologically similar coelomycetous fungi and further studies are needed to establish a single nomenclature. Several species such as *Neotiosporina*, closely related to *Paraconiothyrium*, still have to be assigned to family level.

The identification of *Phoma*-like species based on morphological characters *in vitro* and in *vivo* poses a risk without inclusion of supporting molecular sequence data. A combination of *in vitro* and *in vivo* characters with typical disease symptoms may lead to identification of plant pathogens. However, the existence of species complexes, absence of characters in isolates, and close similarities *in vitro* between species may easily lead to misidentifications. The development of morphologically similar saprobes and necrotrophs in single infection sites often occurs.

The online database Q-bank (www.q-bank.eu) has been developed as an identification and detection reference database for quarantine organisms. The standard procedure for the identification of unknown isolates can best be done by a blast search of ITS or actin sequences in the *Phoma* database of Q-bank. The combination of the molecular with *in vitro* data per species is a valuable tool for identification of quarantine fungi. The *in vitro* characters of an unknown isolate can be compared with data provided in Q-bank.

In cases where no matches are found, GenBank can be queried for strains that have been found with similar sequences, and whether these have possibly been described in other genera.

APPENDIX

REFERENCES

GLOSSARY

SUMMARY

SAMENVATTING

DANKWOORD

CURRICULUM VITAE

EDUCATION STATEMENT

REFERENCES

Aa HA van der, Boerema GH, Gruyter J de (2000). Contributions towards a monograph of *Phoma* (Coelomycetes) – VI-1. Section Phyllostictoides: Characteristics and nomenclature of its type species *Phoma exigua*. *Persoonia* **17**: 435–456.

Aa HA van der, Kesteren HA van (1979). Some pycnidial fungi occurring on *Atriplex* and *Chenopodium*. *Persoonia* **10**: 267–276.

Aa HA van der, Noordeloos ME, Gruyter J de (1990). Species concepts in some larger genera of the Coelomycetes. *Studies in Mycology* **32**: 3–19.

Aa HA van der, Vanev S (2002). *A revision of the species described in Phyllosticta*. Centraalbureau voor Schimmelcultures, Utrecht. Netherlands. 510 pp.

Abeln ECA, Stax AM, Gruyter J de, Aa HA van der (2002). Genetic differentiation of *Phoma exigua* varieties by means of AFLP fingerprints. *Mycological Research* **106**: 419–427.

Arx JA von (1970). A revision of the fungi classified as *Gloeosporium*. *Bibliotheca Mycologia* **24**: 1–203. J. Cramer, Lehre.

Arx, JA von (1981). *The Genera of Fungi Sporulating in Pure Culture*. 3rd edn. J. Cramer, Vaduz J. Cramer, Liechtenstein. 424 pp.

Arx JA von (1987). Plant pathogenic fungi. *Nova Hedwigia Beihefte* **87**: 1–288.

Arx JA von, Müller E (1975). A re-evaluation of the bitunicate ascomycetes with keys to families and genera. *Studies in Mycology* **9**: 1–159.

Arx JA von, Storm PK (1967). Über einige aus dem erdboden isolierte, zu *Sporormia*, *Preussia* und *Westerdykella* gehörende Ascomyceten. *Persoonia* **4**: 407–415.

Arzanlou M, Kema GHJ, Waalwijk C, Carlier J, Vries I de, Guzman M, Vargas MA, Helder J, Crous PW (2009). Molecular diagnostics in the *Mycosphaerella* leaf spot disease complex of banana and for *Radopholus similis*. *Acta Horticulturae* **828**: 237–244.

Aveskamp MM, Gruyter J de, Crous PW (2008). Biology and recent developments in the systematics of *Phoma*, a complex genus of major quarantine significance. *Fungal Diversity* **31**: 1–18.

Aveskamp MM, Gruyter J de, Woudenberg JHC, Verkley GJM, Crous PW (2010). Highlights of the *Didymellaceae*: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycology* **65**: 1–60.

Aveskamp MM, Verkley GJM, Gruyter J de, Murace MA, Perelló A, Woudenberg JHC, Groenewald JZ, Crous PW (2009a). DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. *Mycologia* **101**: 363–382.

Aveskamp MM, Woudenberg JHC, Gruyter J de, Turco E, Groenewald JZ, Crous PW (2009b). Development of taxon-specific sequence characterized amplified region (SCAR) markers based on actin sequences and DNA amplification fingerprinting (DAF): a case study in the *Phoma exigua* species complex. *Molecular Plant Pathology* **10**: 403–414.

Balmas V, Scherm B, Ghigone S, Salem AOM, Cacciola SO, Migheli Q (2005). Characterisation of *Phoma tracheiphila* by RAPD-PCR, microsatellite-primed PCR and ITS rDNA sequencing and development of specific primers for *in planta* PCR detection. *European Journal of Plant Pathology* **111**: 235–247.

Badali H, Chander J, Gulati N, Attri A, Chopra R, Najafzadeh MJ, Chhabra S, Meis JFGM, Hoog GS de (2010). Subcutaneous phaeohyphomycotic cyst caused by *Pyrenochaeta romeroi*. *Medical Biology* **48**: 763–768.

Baker KF, Davis-Clark LH, Wilhelm S, Snyder WC (1985). An aggressive vascular-inhabiting *Phoma* (*Phoma tracheiphila* f.sp. *chrysanthemi* nov. f. sp.) weakly pathogenic to

Chrysanthemum. *Canadian Journal of Botany* **63**: 1730–1735.
Barr ME (1979). A classification of the *Loculoascomycetes*. *Mycologia* **71**: 935–957.
Barr ME (1987a). New taxa and combinations in the loculoascomycetes. *Mycotaxon* **29:** 501–505.
Barr ME (1987b). Prodomus to Class *Loculoascomycetes*. Hamilton I. Newell, Inc.: Amherst, Massachussetts, USA.
Barr ME (2002). *Teichosporaceae*, another family in the *Pleosporales*. *Mycotaxon* **82**: 373–389.
Berbee ML (1996). Loculoascomycete origins and evolution of filamentous Ascomycete morphology based on 18s rRNA gene sequence data. *Molecular Biology and Evolution* **13**: 462–470.
Boerema GH (1964). *Phoma herbarum* Westend., the type-species of the form-genus *Phoma*. *Persoonia* **3**: 9–16.
Boerema GH (1965). Spore development in the form-genus *Phoma*. *Persoonia* **3**: 413–417.
Boerema GH (1967). The *Phoma* organisms causing gangrene of potatoes. *Netherlands Journal of Plant Pathology* **73**: 190–192.
Boerema GH (1970). Additional notes on *Phoma herbarum*. *Persoonia* **6**: 15–48.
Boerema GH (1976). The *Phoma* species studied in culture by Dr. R.W.G. Dennis. *Transactions of the British Mycological Society* **67**: 289–319.
Boerema GH (1982). Phoma-soorten van de sectie *Plenodomus*. *Verslagen en Mededelingen Plantenziektenkundige Dienst Wageningen* **158** (Jaarboek 1981): 28–30.
Boerema GH (1984). Mycologisch-taxonomisch onderzoek. *Ascochyta*'s met lichtbruine conidiën die pathogeen zijn voor *Chenopodiaceae*. *Verslagen en Mededelingen Plantenziektenkundige Dienst Wageningen* **162** (Jaarboek 1983): 31–34.
Boerema GH (1993). Contributions towards a monograph of *Phoma* (Coelomycetes) – II. Section *Peyronellaea*. *Persoonia* **15**: 197–221.
Boerema GH (1997). Contributions towards a monograph of *Phoma* (Coelomycetes) – V. Subdivision of the genus in sections. *Mycotaxon* **64**: 321–333.
Boerema GH (2003). Contributions towards a monograph of *Phoma* (Coelomycetes) – X. Section *Pilosa* (taxa with a *Pleospora* teleomorph) and nomenclatural notes on some other taxa. *Persoonia* **18**: 153–161.
Boerema GH, Bollen GJ (1975). Conidiogenesis and conidial septation as differentiating criteria between *Phoma* and *Ascochyta*. *Persoonia* **8**: 111–144.
Boerema GH and Coworkers (1993). *Check-list for scientific names of common parasitic fungi*. Libri botanici; Vol. 10. IHW-Verlag, Eching. 370 pp.
Boerema GH, Dorenbosch MMJ (1973). The *Phoma* and *Ascochyta* species described by Wollenweber and Hochapfel in their study on fruit–rotting. *Studies in Mycology* **3**: 1–55.
Boerema GH, Gams W (1995). What is *Sphaeria acuta* Hoffm. : Fr.? Mycotaxon **53**: 355–360.
Boerema GH, Gruyter J de (1998). Contributions towards a monograph of *Phoma* (Coelomycetes) – VII. Section *Sclerophomella*: Taxa with thick-walled pseudoparenchymatous pycnidia. *Persoonia* **17**: 81–95.
Boerema GH, Gruyter J de (1999). Contributions towards a monograph of *Phoma* (Coelomycetes) – III-Supplement: Additional species of section *Plenodomus*. *Persoonia* **17**: 273–280.
Boerema GH, Gruyter J de, Kesteren HA van (1994). Contributions towards a monograph of *Phoma* (Coelomycetes) – III-1. Section *Plenodomus*: Taxa often with a *Leptosphaeria* teleomorph. *Persoonia* **15**: 431–487.
Boerema GH, Gruyter J de, Noordeloos ME (1995). New names in *Phoma*. *Persoonia* **16**: 131.
Boerema GH, Gruyter J de, Noordeloos ME (1997). Contributions towards a monograph of

Phoma (Coelomycetes) – IV. Section *Heterospora*: Taxa with large sized conidial dimorphs, *in vivo* sometimes as *Stagonosporopsis* synanamorphs. *Persoonia* **16**: 335–371.

Boerema GH, Gruyter J de, Noordeloos ME, Hamers MEC (2004). *Phoma* identification manual. Differentiation of specific and infra-specific taxa in culture. Cabi publishing. 470 pp.

Boerema GH, Höweler LH (1967). *Phoma exigua* Desm. and its varieties. Persoonia **5**: 15–28.

Boerema GH, Kesteren HA van (1962). *Phoma*-achtige schimmels bij aardappel. Verslagen en Mededelingen Plantenziektenkundige Dienst Wageningen **136** (Jaarboek 1961): 201–209.

Boerema GH, Kesteren HA van (1964). The nomenclature of two fungi parasitizing *Brassica*. *Persoonia* **3**: 17–28.

Boerema GH, Kesteren HA van, Loerakker WM (1981). Notes on *Phoma*. *Transactions of the British Mycological Society* **77**: 61–74.

Boerema GH, Loerakker WM (1985). Notes on *Phoma* 2. *Transactions of the British Mycological Society* **84**: 289–302.

Boerema GH, Loerakker WM, Hamers MEC (1987). Check-list for scientific names of common parasitic fungi. Supplement Series 2a (additions and corrections): Fungi on field crops: beet and potato; caraway, flax and oilseed poppy. *Netherlands Journal of Plant Pathology* **93,** Suppl. **1**: 1–20.

Boerema GH, Loerakker WM, Hamers MEC (1996). Contributions towards a monograph of *Phoma* (Coelomycetes) – III-2. Misapplications of the type species-name and the generic synonyms of section *Plenodomus* (Excluded species). *Persoonia* **16**: 141–190.

Boerema GH, Mathur SB, Neergaard P (1977). *Ascochyta hyalospora* (Cooke & Ell.) comb. nov. in seeds of *Chenopodium quinoa*. *Netherlands Journal of Plant Pathology* **83**: 153–159

Boise J (1985). An amended description of *Trematosphaeria*. *Mycologia* **77**: 230–237.

Borelli D (1959). *Pyrenochaeta romeroi* n. sp. *Dermatologia-Venezolana* **1**: 325–327.

Borelli D (1976). *Pyrenochaeta mackinnonii* nova species agente de micetoma. *Castellania* **4**: 227–234.

Brewer JG, Boerema GH (1965). Electron microscope observations on the development of pycnidiospores in *Phoma* and *Ascochyta* spp. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen* **68**: 86–97.

Buchanan PK (1987). A reappraisal of *Ascochyta* and *Ascochytella* (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.

Câmara MPS, Palm ME, Berkum P van, O'Neill NR (2002). Molecular phylogeny of *Leptosphaeria* and *Phaeosphaeria*. *Mycologia* **94**: 630–640.

Câmara MPS, Ramaley AW, Castlebury LA, Palm ME (2003). *Neophaeosphaeria* and *Phaeosphaeriopsis*, segregates of *Paraphaeosphaeria*. *Mycological Research* **107**: 516–522.

Carbone I, Kohn LM (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002). A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.

Chaverri P, Salgado C, Hirooka Y, Rossman AY, Samuels GJ (2011). Delimitation of *Neonectria* and *Cylindrocarpon* (*Nectriaceae*, *Hypocreales*, *Ascomycota*) and related genera with *Cylindrocarpon*-like anamorphs. *Studies in Mycology* **68**: 57–78.

Chen C-Y, David JC, Hsieh WH (2002). *Leptosphaeria dryadis*. *IMI Descriptions of Fungi and Bacteria 154, Sheet 1533*. CABI Bioscience, Egham, Surrey, UK.

Chesters CGC (1938). Studies on British pyrenomycetes II. A comparative study of *Melanomma pulvis-pyrius* (Pers.) Fuckel, *Melanomma fuscidulum* Sacc. and *Thyridaria rubro-notata* (B. & Br.) Sacc. *Transactions of the British Mycological Society* **22**: 116–150.

Chilvers MI, Rogers JD, Dugan FM, Stewart JE, Chen W, Peever TL (2009). *Didymella pisi* sp. nov., the teleomorph of *Ascochyta pisi*. *Mycological Research* **113**: 391–400.

Cline E (2011). *Phoma andigena*. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved July 1, 2011, from http://nt.ars-grin.gov/sbmlweb/fungi/ index.cfm.

Corbaz R (1957). Récherches sur le genre *Didymella* Sacc. *Phytopathologische Zeitschift* **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.

Cortinas MN, Burgess T, Dell B, Xu DP, Crous PW, Wingfield BD, Wingfield MJ (2006). First record of *Colletogloeopsis zuluense* comb. nov., causing a stem canker of Eucalyptus in China. *Mycological Research* **110**: 229–236.

Crane, JL, Shearer CA (1991). A nomenclator of *Leptosphaeria* V. Cesati & G. de Notaris (Mycota-Ascomycotina-Loculoascomycetes). *Illinois Natural history Survey Bulletin* **34**: 195–355.

Crous PW, Braun U, Groenewald JZ (2007). *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**: 1–32.

Crous PW, Gams W (2000). *Phaeomoniella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea* **39**: 112–118.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004a). MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.

Crous PW, Groenewald JZ, Mansilla JP, Hunter GC, Wingfield MJ (2004b). 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, Schoch CL, Hyde KD, Wood AR, Gueidan C (2009a). Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology* **64**: 17–47.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WOF, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006). Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**: 235–253.

Crous PW, Summerell BA, Carnegie AJ, Wingfield MJ, Hunter GC, Burgess TI, Andijc V, Barber PA, Groenewald JZ (2009b). Unravelling *Mycosphaerella*: do you believe in genera? *Persoonia* **23**: 99–118.

Crous PW, Verkley GJM, Groenewald JZ, Samson RA (2009c). *Fungal Biodiversity.* CBS Laboratory Manual Series. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. 270 pp.

Cullen DW, Toth IK, Boonham N, Walsh K, Barker I, Lees AK (2007). Development and validation of conventional and quantitative polymerase chain reaction assays for the detection of storage rot potato pathogens, *Phytophthora erythroseptica*, *Pythium ultimum*, *Phoma foveata*. *Journal of Phytopathology* **155**: 309–315.

Cunningham GH (1927). Dry-rot of Swedes and turnips: its cause and control. Wellington, New Zealand: New Zealand Department of Agriculture: Bulletin 133.

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.

Damm U, Woudenberg JHC, Cannon PF and Crous PW (2009). *Colletotrichum* species with curved conidia from herbaceous hosts. *Fungal Diversity* **39**: 45–87.

Demontis MA, Cacciola SO, Orrù M, Balmas V, Chessa V, Maserti BE, Mascia L, Raudino F, Magnano di San Lio G, Migheli Q (2008). Development of real-time PCR systems based on SYBR® Green I and TaqMan® technologies for specific quantitative detection of *Phoma tracheiphila* in infected Citrus. *European Journal of Plant Pathology* **120**: 339–351.

Dennis RWG (1946). Notes on some British fungi ascribed to *Phoma* and related genera. *Transactions of the British Mycological Society* **29**: 11–42.

Diedicke H (1912). Die Abteilung Hyalodidymae der Sphaerioideen. *Annales Mycologica* **10**: 135–152.

Dong J, Chen W, Crane JL (1998). Phylogenetic studies of the *Leptosphaeriaceae*, *Pleosporaceae* and some other Loculoascomycetes based on nuclear ribosomal DNA sequences. *Mycological Research* **102**: 151–156.

Dooly 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. *The Journal of Infectious Diseases* **159**: 503–507.

Dorenbosch MMJ (1970). Key to nine ubiquitous soil-borne *Phoma*-like fungi. *Persoonia* **6**: 1–14.

Duan JX, Wu WP, Liu XZ (2007). Reinstatement of *Coleonaema* for *Coleophoma oleae* and notes on *Coleophoma*. *Fungal Diversity* **26**: 187–204.

Dutch guideline for the validation of detection and identification methods for plant pathogens and pests. (n.d.) Retrieved August 31, 2010, from http://www.naktuinbouw.nl/sites/naktuinbouw.eu/files/Dutch%20Validation%20Guideline%20v1.0.pdf.

Eriksson OE (1967). On graminicolous pyrenomycetes from Fennoscandia I, II, Ill. Phragmosporous and scolecosporous species. *Arkiv för Botanik* Ser 2, **6**: 381–440.

Eriksson OE, Hawksworth DL (2003). *Saccharicola*, a new genus for two *Leptosphaeria* species on sugar cane. *Mycologia* **95**: 426–433.

Fatehi J, Bridge PD, Punithalingam E (2003). Molecular relatedness within the "*Ascochyta pinodes*-complex". *Mycopathologia* **156**: 317–327.

Fitt BDL, Brun H, Barbetti MJ, Rimmer SR (2006). World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* **114**: 3–15.

Foister CE (1940). Descriptions of new fungi causing economic diseases in Scotland. *Transactions and Proceedings of the Botanical Society of Edinburgh* **33**: 65–68.

Freyer K von, Aa HA van der (1975). Über *Pyrenochaeta parasitica* spec. nov., die Nebenfruchtform von *Herpotrichia parasitica* (Hartig) E. Rostrup (= *Trichosphaeria parasitica*). *European Journal of Forest Pathology* **5**: 177–182.

Gams W, Verkley GJM, Crous PW (2007). *CBS course of mycology, 5th ed.* Centraalbureau voor Schimmelcultures, Utrecht. 242 pp.

Gräfenhan T, Schroers H-J, Nirenberg HI, Seifert KA (2011). An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Studies in Mycology* **68**: 79–113.

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.

Grodona I, Monte E, Garcia-Acha I, and Sutton BC, 1997. *Pyrenochaeta dolichi*: an example of a confusing species. *Mycological Research* **101**: 1404–1408.

Grove WB (1935). *British stem- and leaf-fungi (Coelomycetes) Vol. 1 Sphaeropsidales*. Cambridge, UK. Cambridge University Press. 488 pp.

Gruyter J de (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) – IX. Section *Macrospora*. *Persoonia* **18**: 85–102.

Gruyter J de, Aveskamp MM, Woudenberg JHC, Verkley GJM, Groenewald JZ, Crous PW (2009). Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycological Research* **113**: 508–519.

Gruyter J de, Boerema GH (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) – VIII. Section *Paraphoma*: Taxa with setose pycnidia. *Persoonia* **17** ("2001"): 541–561.

Gruyter J de, Boerema GH, Aa HA van der (2002). Contributions towards a monograph of *Phoma* (Coelomycetes) – VI-2. Section *Phyllostictoides*: Outline of its taxa. *Persoonia* **18**: 1–53.

Gruyter J de, Gent-Pelzer MPE van, Woudenberg JHC, Rijswick PCJ van, Meekes ETM, Crous PW, Bonants PJM (2012). The development of a validated real-time (TaqMan) PCR for detection of *Stagonosporopsis andigena* and *S. crystalliniformis* in infected leaves of potato and tomato. *European Journal of Plant Pathology*: DOI 10.1007/s10658-012-9990-8.

Gruyter J de, Noordeloos ME (1992). Contributions towards a monograph of *Phoma* (Coelomycetes) – I-1. Section *Phoma*: Taxa with very small conidia *in vitro*. *Persoonia* **15**: 71–92.

Gruyter J de, Noordeloos ME, Boerema GH (1993). Contributions towards a monograph of *Phoma* (Coelomycetes) – I-2. Section *Phoma*: Additional taxa with very small conidia and taxa with conidia up to 7 mm long. *Persoonia* **15**: 369–400.

Gruyter J de, Noordeloos ME, Boerema GH (1998). Contributions towards a monograph of *Phoma* (Coelomycetes) – I-3. Section *Phoma*: Taxa with conidia longer than 7 mm. *Persoonia* **16**: 471–490.

Gruyter J de, Woudenberg JHC, Aveskamp MM, Verkley GJM, Groenewald JZ, Crous PW (2010). Systematic reappraisal of species in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*. *Mycologia* **102**: 1066–1081.

Gruyter J de, Woudenberg JHC, Aveskamp MM, Verkley GJM, Groenewald JZ, Crous PW (2012). redisposition of *Phoma*-like anamorphs in *Pleosporales*. *Studies in Mycology* **75**: 1–36.

Hansen HN (1929). Etiology of the pink-root disease of onions. *Phytopathology* **19**: 691–704.

Hatakeyama S, Tanaka K, Harada Y (2008). Bambusicolous fungi in Japan (7): a new coelomycetous genus, *Versicolorisporium*. *Mycoscience* **49**: 211–214.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, *et al.* (2011). The Amsterdam declaration on fungal nomenclature. *IMA Fungus* **2**: 105–112.

Hedjaroude A (1968). Études taxonomiques sur les *Phaeosphaeria* Miyake et leurs formes voisines (ascomycètes). *Sydowia* **22**: 57 –107.

Henderson MP (1918). The black-leg disease of cabbage caused by *Phoma lingam* (Tode) Desm. *Phytopathology* **8**: 379–431.

Höhnel F von (1911). Fragmente zur Mykologie XIII (713): Über *Leptosphaeria maculans* (Desm.) und *Sphaeria lingam* Tode. *Sitzungsberichte der Kaiserlichen Akademie der*

Wissenschaften in Wien. (Mathematisch-naturwissenschaftliche Klasse (Abteilung I) **120**: 458–463.

Höhnel F von (1914). *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien. (Mathematisch-naturwissenschaftliche Klasse (Abteilung I)* **123**: 49–155.

Holm L (1957). Études taxonomiques sur les Pléosporacées. *Symbolae Botanicae Upsalienses* **14**: 5–188.

Holm L (1975). Nomenclatoral notes on *Pyrenomycetes*. *Taxon* **24**: 475–488.

Hoog GS de, Gerrits van den Ende AHG (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41**: 183–189.

Hoog GS de, Guarro J, Gené J, Figueras MJ (2000). *Atlas of Clinical Fungi*. 2nd edition. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. 1126 pp.

Horst RK, Cloyd R (2007). *Compendium of rose diseases and pests*. 2nd edition. APS press. 96 pp.

Huang YJ, Fitt BDL, Jedryczka M, Dakowska S, West JS, Gladders P, Steed JM, Li ZQ (2005). Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*L. biglobosa*). *European Journal of Plant Pathology* **111**: 263–277.

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.

Hyde KD, Eriksson OE, Yue JZ (1996). *Roussoella*, an ascomycete genus of uncertain relationships with a *Cytoplea* anamorph. *Mycological Research* **100**: 1522–1528.

Hyde KD, Sutton BC (1992). *Nypaella frondicola* gen. et sp. nov., *Plectophomella nypae* sp. nov. and *Pleurophomopsis nypae* sp. nov. (Coelomycetes) from intertidal fronds of *Nypa fruticans*. *Mycological Research* **96**: 210–214.

Inderbitzin P, Mehta YR, Berbee ML (2009). *Pleospora* species with *Stemphylium* anamorphs: a four locus phylogeny resolves new lineages yet does not distinguish among species in the *Pleospora herbarum* clade. *Mycologia* **101**: 329–339.

Jasalavic CA, Morales VM, Pelscher LE, Seguin-Swartz G (1995). Comparison of nuclear ribosomal DNA sequences from *Alternaria* species pathogenic to crucifers. *Mycological Research* **99**: 604–614.

Jedryczka M, Fitt BDL, Kachlicki P, Lewartowska E, Balesdent MH, Rouxel T (1999). Comparison between Polish and United Kingdom populations of *Leptosphaeria maculans*, cause of stem canker of winter oilseed rape. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **106**: 608–617.

Katoh K, Asimenos G, Toh H. (2009). Multiple Alignment of DNA Sequences with MAFFT. (In D. Posada (Ed), Bioinformatics for DNA Sequence Analysis, *Methods in Molecular Biology* **537**: 39–64.

Khashnobish A, Shearer CA, Crane JL (1995). Reexamination of species of *Leptosphaeria* on asteraceous hosts. *Mycotaxon* **54**: 91–106.

Kinsey GC (2002). *Phoma septicidalis*. IMI Descriptions of Plant Pathogenic Fungi and Bacteria No. 1508.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). *Ainsworth & Bisby's Dictionary of the Fungi.* 10th Edn. CABI Biosicence. 771 pp.

Koch CA, Utkhede RS (2004). Development of a multiplex classical polymerase chain reaction technique for detection of *Didymella bryoniae* in infected cucumber tissues and greenhouse air samples. *Canadian Journal of Plant Pathology* **26**: 291–298.

Kodsueb R, Dhanasekaran V, Aptroot A, Lumyong S, McKenzie EHC, Hyde KD, Jeewon R (2006). The family *Pleosporaceae*: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28SrDNA. *Mycologia* **98**: 571–583.

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.

Lartey RT, Weiland JJ, Caesar-TonThat T, Bucklin-Comiskey S (2003). A PCR protocol for rapid detection of *Cercospora beticola* in sugarbeet tissues. *Journal of Sugar Beet Research* **40**: 1–10.

Leuchtmann A (1984). Über *Phaeosphaeria* Miyake und andere bitunicate Ascomyceten mit mehrfach querseptierten Ascosporen. *Sydowia* **37**: 75–194.

Licciardella G, Grasso FM, Bella P, Cirvilleri G, Grimaldi V, Catara V (2006). Identification and detection of *Phoma tracheiphila*, causal agent of Citrus Mal Secco disease, by real-time polymerase chain reaction. *Plant Disease* **90**: 1523–1530.

Linder DH (1944). I. Classification of the Marine fungi. *Farlowia* **1**: 401–467.

Loerakker WM, Navarro R, Lobo M, Turkensteen LJ (1986). *Phoma andina* var. *crystalliniformis* var. nov., un patógeno nuevo del tomate y de la papa en los Andes. *Fitopatología* **21**: 99–102.

Lombard L, Crous PW, Wingfield BD, Wingfield, MJ (2010a). Species concepts in *Calonectria* (*Cylindrocladium*). *Studies in Mycology* **66**: 1–13.

Lombard L, Crous PW, Wingfield BD, Wingfield, MJ (2010b). Multigene phylogeny and mating tests reveal three cryptic species related to *Calonectria pauciramosa*. *Studies in Mycology* **66**: 15–30.

Lombard L, Crous PW, Wingfield BD, Wingfield, MJ (2010c). Phylogeny and systematics of the genus *Calonectria*. *Studies in Mycology* **66**: 31–69.

Lucas MT, Webster J (1967). Conidial states of British species of *Leptosphaeria*. *Transactions of the British Mycological Society* **50**: 85–121.

Lumbsch HT, Huhndorf SM (ed.) (2007). Outline of the *Ascomycota* – 2007. *Myconet* **13**: 1–58.

Luttrell ES (1963). Taxonomic criteria in *Helminthosporium*. *Mycologia* **55**: 643–674.

MacDonald JE, White GP, Coté MJ (2000). Differentiation of *Phoma foveata* from *P. exigua* using a RAPD generated PCR-RFLP marker. *European Journal of Plant Pathology* **106**: 67–75.

Malkus A, Reszka E, Chang C-J, Arseniuk E, Chang P-FL, Ueng PP (2005). Sequence diversity of β-tubulin (tubA) gene in *Phaeosphaeria nodorum* and *P. avenaria*. *FEMS Microbiology Letters* **249**: 49–56.

Mendes-Pereira E, Balesdent MH, Brun H, Rouxel T (2003). Molecular phylogeny of the *Leptosphaeria maculans - L. biglobosa* species complex. *Mycological Research* **107**: 1287–1304.

Moesz G (1922). Mycologiai közlemények V. (Mykologische Mitteilungen V.). *Magyar Botanikai Lapok* **21**: 5–16.

Mohanty NN (1958). An undescribed species of *Pyrenochaeta on Dolichos biflorus* Linn. *Indian Phytopathology* **8**: 85–87.

Monte E, Bridge PD, Sutton BC (1991). An integrated approach to *Phoma* systematics. *Mycopathologia* **115**: 89–103.

Morales VM, Jasalavich CA, Pelcher LE, Petrie GA, Taylor JL (1995). Phylogenetic relationship among several *Leptosphaeria* species based on their ribosomal DNA sequences. *Mycological Research* **99**: 593–603.

Morgan-Jones G, White JF (1983). Studies in the genus *Phoma*. III. *Paraphoma*, a new genus to accommodate *Phoma radicina*. *Mycotaxon* **18**: 57–65.

Mosch WHM, Mooi JC (1975). A chemical method to identify tuber rot in potato caused by *Phoma exigua* var. *foveata*. *Netherlands Journal of Plant Pathology* **81**: 86–88.

Mugambi GK, Huhndorf SM (2009). Molecular phylogenetics of *Pleosporales*: *Melanommataceae* and *Lophiostomataceae* re-circumscribed (*Pleosporomycetidae, Dothideomycetes*, Ascomycota). *Studies in Mycology* **64**: 103–121.

Müller E (1950). *Die schweizerischen arten der gattung Leptosphaeria und ihrer verwandten*. Sydowia, Annales Mycologici editi in Notitiam Scientiae Mycologicae Universalis. Horn, Austria **4**: 185–319.

Müller E, Arx JA von (1950). Einige aspecte zur systematik pseudosphärialer Ascomyceten. *Berichte der Schweizerichen Botanischen Gesellschaft* **21**: 329–397.

Müller E, Arx JA von (1962). Die Gattungen der didymosporen Pyrenomyceten. *Beiträge zur Kryptogamen flora der Schweiz* **11**: 1–922.

Mumford RA, Skelton AL, Boonham N, Postuma KI, Kirby MJ, Adams AN (2004). The improved detection of Strawberry Crinkle Virus using Real-Time RT-PCR (Taqman®). (In R.R. Martin (Ed.). ISHS Xth international symposium on small fruit virus diseases. Valencia, Spain, *Acta Horticulturae* **656**: 81–86.

Munk A (1957). *Danish Pyrenomycetes: a preliminary flora*. Dansk botanisk Arkiv 17, Dansk Botanisk Forening. Ejnar Munksgaard, Copenhagen, Denmark. 491 pp.

Navarro RA, Puerta OD (1981). Presencia de *Phoma* sp. en tomate (*Lycopersicon esculentum*). *Ascolfi Informa* **7**: 32.

Niekerk JM van, 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.

Noordeloos ME, Gruyter J de, Eijk GW van, Roeijmans HJ (1993). Production of dendritic crystals in pure cultures of *Phoma* and *Ascochyta* and its value as a taxonomic character relative to morphology, pathology and cultural characteristics. *Mycological Research* **97**: 1343–1350.

OEPP/EPPO (1984). Data sheets on quarantine organisms No. 141, *Phoma andina*. *Bulletin OEPP/EPPO Bulletin* **14**: 45–48.

OEPP/EPPO (2010). PM 7/98 (1): Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *Bulletin OEPP/EPPO Bulletin* **40**: 5–22.

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.

Otazú V, Boerema GH, Mooi JC, Salas B (1979). Possible geographical origin of *Phoma exigua* var. *foveata*, the principal causal organism of potato gangrene. *Potato Research* **22**: 333–338.

Page RDM (1996). Treeview: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.

Pawar, VH, Mathur, PN, Thirumalachar MJ (1967). Species of *Phoma* isolated from marine soils in India. *Transactions of the British Mycological Society* **50**: 259–265.

Pedras MSC, Taylor JL, Morales VM (1995). Phomaligin A and other yellow pigments in *Phoma lingam* and *P. wasabiae*. *Phytochemistry* **38**: 1215–1222.

Peever TL, Barve MP, Stone LJ, Kaiser WJ (2007). Evolutionary relationships among *Ascochyta* species infecting wild and cultivated hosts in legume tribes *Cicereae* and *Vicieae*. *Mycologia* **99**: 59–77.

Peters JC, Lees AK, Cullen DW, Sullivan L, Stroud GP, Cunnington AC (2008). Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. *Plant Pathology* **57**: 262–71.

Pethybridge SJ, Scott JB, Hay FS (2004). Genetic relationships among isolates of *Phoma lugilicola* from pyrethrum and chrysanthemum based on ITS and its detection by PCR. *Australasian Plant Pathology* **33**: 173–181.

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, English MP (1975). *Pyrenochaeta unguis-hominis* sp. nov. on human toe-nails. *Transactions of the British Mycological Society* **64**: 539–541.

Punithalingam E (1979). Graminicolous *Ascochyta* species. *Mycological Papers* **142**: 1–214.

Punithalingam E (1990). *Mycosphaerella zeae-maydis*. CMI descriptions of pathogenic fungi and bacteria 1015. *Mycopathologia* **112**: 49–50.

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. 159 pp.

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.

Redfern DB, Sutton BC (1981). Canker and dieback of *Ulmus glabra* caused by *Plectophomella concentrica*, and its relationship to *P. ulmi*. *Transactions of the British Mycological Society* **77**: 381–390.

Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.

Riedl H (1959). Kulturversuche zum Pleomorphismus einiger Pyrenomyzeten. *Osterreichische Botanische Zeitschrift* **106**: 477–545.

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.

Rossman AY, Far DF, Castlebury LA, Shoemaker R, Mengistu A (2002). *Setomelanomma holmii* (*Pleosporales*, Phaeosphaeriaceae) on living spruce twigs in Europe and North America. *Canadian Journal of Botany* **80**: 1209–1215.

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. 768 pp.

Saccardo PA (1884). Sylloge Sphaeropsidearum et Melanconiearum omnium hucusque cognitorum. *Sylloge Fungorum* **3**. Patavii. 860 pp.

Schneider R (1979). Die Gattung *Pyrenochaeta* De Notaris. *Mitteilungen aus der Biologischen Bundesanstalt für Land- Forstwirtschaft Berlin-Dahlem* **189**: 1–73.

Secor GA, Salas B (2001). Fusarium dry rot and Fusarium wilt. In: Stevenson WR *et al.* eds. Compendium of Potato Diseases, 2nd edn. St Paul, MN, USA. APS Press, 23–25.

Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI (2009). A class-wide phylogenetic assessment of *Dothideomycetes*. *Studies in Mycology* **64**: 1–15.

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006). A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* **98**: 1041–1052.

Schroers H-J, Gräfenhan T, Nirenberg HI, Seifert KA (2011). A revision of *Cyanonectria* and *Geejayessia* gen. nov., and related species with *Fusarium*-like anamorphs. *Studies in Mycology* **68**: 115–138**.**

Shoemaker RA (1984). Canadian and some extralimital *Leptosphaeria* species. *Canadian Journal of Botany* **62**: 2688–2729.

Shoemaker RA, Brun H (2001). The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. *Canadian Journal of Botany* **79**: 412–419.

Shoemaker RA, Redhead SA (1999). Proposals to conserve the names of four species of fungi (*Phoma betae*, *Helminthosporium avenae*, *Pyrenophora avenae* and *Pleospora tritici-repentis*) against competing earlier synonyms. *Taxon* **48**: 381–384.

Silva-Hanlin DMW, Hanlin RT (1999). Small subunit ribosomal RNA gene phylogeny of several *Loculoascomycetes* and its taxonomic implications. *Mycological Research* **103**: 153–160.

Simmons EG (1969). Perfect states of *Stemphylium*. *Mycologia* **61**: 1–26.

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. 701 pp.

Smith HC, Sutton BC (1964). *Leptosphaeria maculans* is the ascogenous state of *Phoma lingam*. *Transactions of the British Mycological Society* **47**: 159–165.

Somai, BM, Keinath AP, Dean RA (2002). Development of PCR-ELISA for detection and differentiation of *Didymella bryoniae* from related *Phoma* species. *Plant Disease* **86**: 710–716.

Stewart RB (1957). An undescribed species of *Pyrenochaeta* on soybean. *Mycologia* **49**: 115–117.

Suetrong S, Schoch CL, Spatafora JW, Kohlmeyer J, Volkmann-Kohlmeyer B (2009). Molecular systematics of the marine *Dothideomycetes*. *Studies in Mycology* **64**: 155–173.

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. 696 pp.

Swofford DL (2003). PAUP*: *Phylogenetic analysis using parsimony (*and other methods), version 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

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.

Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y, Sano T, Shirouzu T, Hosoya T (2009). Molecular taxonomy of bambusicolous fungi: *Tetraplosphaeriaceae*, a new pleosporalean family with *Tetraploa*-like anamorphs. *Studies in Mycology* **64**: 175–209.

Torres M, 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.

Turkensteen LJ (1978). Tizón foliar de la papa en el Perú. I, Especies de *Phoma* asociadas. *Fitopatología* **13**: 67–69.

Turkensteen LJ, Pinedo HM (1982). *Didymella bryoniae* como patógeno de la papa en las regiones Cálidas del Perú. *Fitopatología* **17**: 54–55.

Vanev SG, van der Aa HA (1998). An annotated list of the published names in *Asteromella*. *Persoonia* **17**: 47–67.

Verkley GJM, Crous PW, Groenewald JZ, Braun U, Aptroot A (2004a). *Mycosphaerella punctiformis* revisited: morphology, phylogeny, and epitypification of the type species of the genus *Mycosphaerella* (*Dothideales, Ascomycota*). *Mycological Research* **108**: 1271–1282.

Verkley GJM, Silva M da, Wicklow DT, Crous PW (2004b). *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, Woudenberg JHC, Gruyter J de (2010). *Ascochyta manawaorae* Verkley, Woudenberg & Gruyter, sp. nov. *Persoonia* **24**: 128–129.

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.

Vincenot L, Balesdent MH, Li H, Barbetti MJ, Sivasithamparam K, Gout L, Rouxel T (2008). Occurrence of a new subclade of *Leptosphaeria biglobosa* in Western Australia. *Phytopathology* **98**: 321–329.

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.

Wang HK, Aptroot A, Crous PW, Hyde KD, Jeewon R (2007). The polyphyletic nature of *Pleosporales*: an example from *Massariosphaeria* based on rDNA and RBP2 gene phylogenies. *Mycological Research* **111**: 1268–1276.

Wehmeyer LE (1946). Studies on some fungi of northwestern Wyoming. III. *Pleospora* and *Leptosphaeria*. *Lloydia. A quarterly Journal of Biological Science, Manasha* **9**: 203–240.

Wehmeyer LE (1961). *A world monograph of the genus Pleospora and its segregates*. Univ. Michigan Press. U.S.A. 451 pp.

Weller SA, Elphinstone JG, Smith NC, Boonham N, Stead DE (2000). Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Applied Environmental Microbiology* **66**: 2853–2558.

West JS, Balesdent MH, Rouxel T, Narcy JP, Huang YJ (2002). Colonization of winter oilseed rape tissues by A/Tox+ and B/Tox0 *Leptosphaeria maculans* (phoma stem canker) in France and England. *Plant Pathology* **51**: 311–321.

West JS, Kharbanda PD, Barbetti MJ, Fitt BDL (2001). Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. *Plant Pathology* **50**: 10–27.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*. Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. Academic press, San Diego, CA, U.S.A.: 315–322.

Winton LM, Stone JK, Hansen EM, Shoemaker RA, 2007. The systematic position of *Phaeocryptopus gaeumannii*. *Mycologia* **99**: 240–252.

Wollenweber HW, Hochapfel H (1936). Beiträge zur kenntniss parasitärer und saprophytischer Pilze. I. *Phomopsis*, *Dendrophoma*, *Phoma* und *Ascochyta* und ihre Beziehung zur fruchtfäule. *Zeitschrift für Parasitenkunde* **8**: 561–605.

Woudenberg JHC, Aveskamp MM, Gruyter J de, Spiers AG, Crous PW (2009). Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **22**: 56–62.

Wunsch MJ, Bergstrom GC (2011). Genetic and morphological evidence that *Phoma sclerotioides*, causal agent of brown root rot of alfalfa, is composed of a species complex. *Phytopathology* **101**: 594–610.

Yáñez-Morales M de J, Korf RP, Babcock JF (1998). Fungi on *Epifagus* (*Orobanchaceae*) – I. On *Sclerotium orobanches* and its *Phoma* synanamorph. Mycotaxon **67**: 275–286.

Zhang, Y, Crous PW, Schoch CL, Hyde KD (2012). *Pleosporales. Fungal Diversity* **53**: 1–221.

Zhang Y, Fournier J, Pointing SB, Hyde KD (2008). Are *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa* congeneric? *Fungal Diversity* **33**: 47–60.

Zhang Y, Schoch CL, Fournier J, Crous PW, Gruyter J de (2009). Multi-locus phylogeny of the *Pleosporales*: a taxonomic, ecological and evolutionary reevaluation. *Studies in Mycology* **64**: 85–102.

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.

GLOSSARY

acropleurogenous denoting spores formed at the tip and on the sides of fungal hyphae
aggregated near together, thereby forming a cluster or mass of individual units
allantoid curved, cylindrical (sausage-shaped)
ampulliform flask-like in form
anamorph asexual or mitosporic form of propagation (= imperfect state)
annellation elongation of a conidiogenous cell with progressive conidiogenesis
annellide a conidiogenous cell with annellations
ascoma (pl. ascomata) ascocarp, the general term for all fruiting-bodies containing asci
ascospore a sexual spore of ascomycetes formed inside an ascus
ascus (pl. asci) the sexual organ of ascomycetes in which meiospores are formed endogenously
aseptate having no cross walls
attenuate narrowed
basionym the name-bringing or epithet-bringing synonym on which a transfer or new combination is based
bitunicate a functionally two-layered wall structure of asci
blastic a mode of conidiogenesis in which conidia are blown out from a conidiogenous cell
chlamydospore a thick-walled resting-spore that is not easily liberated from the subtending hypha
clavate club-shaped; gradually thickening at one end
coelomycetes anamorphic fungi producing conidia in pycnidial, acervular or stromatic conidiomata
comb. nov. combinatio nova, a new combination formed from a previously published legitimate name and employing the same final epithet
conidia asexually produced spores; particularly used for anamorphs of ascomycetes and basidiomycetes (and permanently asexual fungi)
conidiogenesis the description of processes involved in conidium formation
conidiogenous cell the cell from which a conidium is formed
conidiomata the general term for hyphal aggregations containing conidia, such as pycnidium
conidiophore the assembly of all cells differentiated for conidium production (including the conidiogenous cell)
coprophilous an ecological preference for colonization of dung (after surviving passage through the intestines)
dictyo(chlamydo)spores with both transversely and longitudinally septate spores
didymosporous with two-celled spores
dimorphism the co-occurrence of two different distinct forms of parts within the same organism.
doliiform barrel-like in form
endophyte growing inside (green plants), usually without causing visible symptoms
epithet the final word in a binary combination
epitype a material designated to be representative of a species, while other type material in poorer shape is still present
eustromatic a mass or matrix of vegetative hyphae of fungal tissue only, in or on which spores or fruiting bodies bearing spores are produced
ex when a name has been proposed but not validly published by one author and thereafter validly published and ascribed to him by another author the word ‘ex’ is used to connect the names of both authors

filiform thread-like
gen. nov. genus novum, new genus
glabrous smooth, not hairy
guttulate having one or more oil-like drops (guttules) inside
holoblastic a budding mechanism in which the walls of the bud remain in continuous connection with the mother cell (in conidiogenesis and proliferation)
holotype the unique material deposited by an author as type of a new species
homonym an identical name used for a different organism; the younger homonym is illegitimate
hymenium the spore bearing layer of a fruit-body
integrated a conidiogenous cell formed as a direct continuation of the subtending conidiophore cells
isotype type material deposited in other herbaria at the same time as and identical with the holotype material
lectotype a single specimen that is selected as most representative of a species, after the publication from material deposited before that date
monotypic having only one representative, as a genus having only one species
murogenous old term, conidia developing as expansions of the entire conidiophore tip (aleuriospores)
mycelium the thallus of a fungus consisting of radiating hyphae
necrotroph a parasite that derives its energy from dead cells of the host
neotype a specimen designated to represent a species when all original material is lost
nom. illeg. illegitimate name, a name that conflicts with the rules of priority or homonymy
nom. nov. nomen novum, a name proposed as a substitute for a previously published name
nom. nud. nomen nudum, a name of a new taxon published without a description or diagnosis or reference to a description or diagnosis
ostiole the preformed opening of an ascoma or pycnidial conidioma
papillate having a papilla
percurrently growing through in the direction of the long axis
phialide a form of conidiogenous cell which produces a basipetal sequence (formed at the base) of conidia from a fixed conidiogenous locus
phragmospores with elongate spores (or conidia) with transverse septa
pilose covered with hairs
porogenous old term. conidia originating as protrusions through pores in the conidiophore wall (porospores, currently defined as tetric, conidiogenesis in which each conidium is delimited by an extension of the inner wall of the conidiogenous
proliferation a change in shape of a sporangiophore or conidiophore after the formation of a spore (conidium), either percurrent or sympodial (q.v.)
pseudoparaphyses hyphae originating above the level of the asci and growing downwards between the developing asci
pseudoparenchymatous composed of very thick-walled conglutinate cells
pycnidium sporulation on plant material enclosed by a wall of fungal material, with or without ostiole
pycnosclerotium, a more or less hard-walled structure resembling a pycnidial conidiomata but having no spores
pyriform pear-like in form
saccate like a sac or bag
sanctioned name The name of a fungus treated as if conserved against earlier homonyms

and competing synonyms, through acceptance in one of two sanctioning works of Fries or Persoon, indicated with colon such as *Sphaeria acuta* Hoffm. : Fr.

saprobe mode of heterotrophic nutrition, in which a fungus absorbs organic substrates from dead organic matter

scleroplectenchymatous thick tissue formed by hyphae composed of very thick-walled conglutinate cells

sclerotium a multicellular fungal resting structure of very different size, often differentiated into cortex and medulla

septate having a septum, a cross-wall in fungal hyphae or spores

setae a stiff, usually dark, erect vegetative hypha

setose covered with setae

spec. nov. species novum, new species

spore a general term for a reproductive structure in fungi, bacteria and cryptogamic plants

stat. nov. status novus, assignment of a taxon to a different rank within the taxonomic hierarchy, e.g. when an infraspecific taxon is raised to the rank of species or the inverse change occurs

substrate the chemically defined substance on which a fungus feeds

synanamorph a different kind of asexual sporulation occurring besides another one

synonym a name that applies to the same organism as another; an obligate synonym is based on the same type (º), a facultative synonym has a different type (=)

taxon (pl. taxa) referring to any rank of classification at, below and above species rank

teleomorph the sexual form of sporulation or perfect state of a fungus, characterised by the presence of meiospores

truncate end cut of horizontally

unicellular consisting of a single cell

valid publication the publication in printed form with Latin diagnosis and correct indication of type material

SUMMARY

The anamorphic genus *Phoma* includes many important plant pathogens. The identification of *Phoma* species based on studies in pure culture is difficult and time consuming and the *in vitro* characters are often variable. Moreover, the present classification of *Phoma* species into sections is ambiguous and morphological characters are shared with related genera. In the present study the molecular phylogeny of species of *Phoma* and allied genera was examined and the results obtained were used to revise the taxonomy. The DNA sequence data obtained provide tools for the development of detection and identification methods.

Chapter 1 provides a general introduction of the anamorph genus *Phoma* and the research that has been performed in The Netherlands during the last decades is described. *Phoma* is characterised by producing hyaline conidia in fruiting bodies called pycnidia. The genus includes many important plant pathogens. The taxonomy of *Phoma* has been studied intensively at the Plant Protection Service in the Netherlands for more than 50 years, resulting in the development of a generic concept in 1997 as an outline for identification of *Phoma* species. In this concept species of the genus *Phoma* are classified based on their morphological characters into the nine sections *Phoma, Heterospora, Macrospora, Paraphoma, Peyronellaea, Phyllostictoides, Pilosa, Plenodomus* and *Sclerophomella*. The species placed in each of the sections were systematically described culminating in the publication of the "*Phoma* Identification Manual" in 2004, with the descriptions of 223 specific and infra-specific taxa of *Phoma,* and more than 1000 synonyms in other coelomycetous genera. In the Netherlands the late Gerhard Boerema, former head of the Mycology Department at the Plant Protection Service, has been the driving force behind this *Phoma* research for decades.

The *Phoma* Identification Manual is a valuable tool for the morphological identification of isolates, but *in vitro* studies are very time consuming and need a high level of expertise. Moreover, the classification of *Phoma* species in sections based on morphological characters appeared artificial and several species can be classified in more than one section because of their multiple "section-specific" characters. In addition, distinctive characters of *Phoma* sections are shared among morphologically related coelomycetous genera including *Ascochyta*, *Asteromella*, *Microsphaeropsis*, *Phomopsis*, *Phyllosticta*, *Pleurophoma*, *Pyrenochaeta* and *Stagonospora*. *Phoma* sections are related to diverse teleomorph genera including *Didymella*, *Leptosphaeria*, *Mycosphaerella* and *Pleospora.* Synanamorphs of *Phoma* species have been recognised amongst the genera *Phaeomoniella, Stagonosporopsis, Epicoccum*, *Phialophora* and *Sclerotium* illustrating their heterogeneity.

A large, well-studied *Phoma* culture collection established at the Plant Protection Service and the "Centraalbureau voor Schimmelcultures" includes more than 1100 strains of *Phoma* species. This collection formed the basis of an intensive molecular phylogenetic study of the genus *Phoma* and morphologically similar genera, which commenced in 2006. Furthermore, a literature study identified sequences of genes that are suitable for phylogenic studies and elucidation of the evolutionary history of the genus *Phoma*.

Several potentially informative regions of the genome were sequenced in the first phase of the project as has been described in chapters 2–4. The phylogeny and DNA sequence data obtained have provided tools for the development of fast and reliable molecular detection and identification methods. The development of Real-time TaqMan PCR methods for the detection and identification of two important plant pathogenic (quarantine) species formerly described in *Phoma*, *Stagonosporopsis andigena* and *S. crystalliniformis,* is described in Chapter 5.

In **chapter 2** several genes were studied to elucidate the molecular phylogeny of *Phoma* and allied genera. Sequence data of the 18S nrDNA (SSU) and 28S nrDNA (LSU) regions of the type species of the *Phoma* sections and morphologically similar coelomycetes and related teleomorphs were compared. The results justified the introduction of the new family *Didymellaceae* to accommodate the generic type species *Didymella exigua* and *Phoma herbarum*. The type species of the *Phoma* sections *Phyllostictoides, Sclerophomella, Macrospora* and *Peyronellaea* also grouped in *Didymellaceae*.

The generic type species *Ascochyta pisi* and *Microsphaeropsis olivacea* also grouped in *Didymellaceae* and it shows that these genera are closely allied to *Phoma*. The type species of *Phoma* sections *Heterospora, Paraphoma, Pilosa* and *Plenodomus* grouped in various families outside *Didymellaceae* and were subject of following studies.

Chapter 3 provides a molecular phylogenetic re-evaluation on *Phoma*-like species that appeared only distantly related to the generic type species *Phoma herbarum* and its related *Didymella* teleomorph (*Didymellaceae*). *Phoma* section *Paraphoma*, characterised by setose pycnidia, resembles species of *Pyrenochaeta* and *Pleurophoma*. Sequence data from the SSU and LSU regions of the species classified in *Phoma* section *Paraphoma* were compared with those of representative isolates of *Pyrenochaeta* and *Pleurophoma*, and with those of the type species of the *Phoma* sections *Phoma, Pilosa* and *Plenodomus*. Unnamed, often sterile *Phoma*-like strains in the collections were included. The molecular phylogeny of species that were classified in *Phoma* section *Paraphoma* appeared to be highly polyphyletic and a thorough reclassification of the species is provided. *Paraphoma* was reinstalled and grouped with the new genera *Neosetophoma* and *Setophoma* in *Phaeosphaeriaceae*. *Pyrenochaeta* and the new genus *Pyrenochaetopsis*, including mainly taxa formerly described in *Phoma* section *Paraphoma*, were closely allied in *Cucurbitariaceae.*

In **chapter 4** the molecular phylogeny of species of *Phoma* sections *Plenodomus, Pleospora* and *Heterospora* was determined using LSU, SSU and ITS. In a "one species = one name" approach, the species described in *Phoma* section *Plenodomus* and its teleomorph *Leptosphaeria* were reclassified in *Leptosphaeria, Plenodomus* and the new genera *Paraleptosphaeria* and *Subplenodomus* in *Leptosphaeriaceae*. Two species of *Phoma* section *Heterospora*, the type species *Phoma heteromorphospora* and its allied species *Ph. dimorphospora*, were transferred to the new genus *Heterospora* that also grouped in *Leptosphaeriaceae*. *Leptosphaeria doliolum* comprises a species complex that was revised based on multilocus sequence data of LSU, ITS, SSU, ß-tubulin, and chitin synthase 1. The molecular phylogeny of species classified in *Ascochyta* and *Phoma*, section *Pilosa* in *Pleosporaceae* that produce morphologically similar pilose pycnidia, was determined based on analysis of actin sequence data. Several *Phoma*-like species grouped outside the suborder *Pleosporineae* in a LSU sequence analysis and were transferred to the genera *Aposphaeria* (*Melanommataceae*), *Paraconiothyrium* (*Montagnulaceae*) and *Westerdykella* (*Sporormiaceae*). *Coniothyrium palmarum* and related species were described in *Coniothyriaceae*. The new genera *Medicopsis* (*Trematosphaeriaceae*) and *Nigrograna*, of which the family is still unknown, are introduced to accommodate two medically important species formerly classified in *Pyrenochaeta*.

In **chapter 5** specific real-time (TaqMan) PCR assays were developed for the detection of the pathogens *Stagonosporopsis andigena* and *S. crystalliniformis* in leaves of potato and tomato. The molecular phylogeny with related species of *Stagonosporopsis*, *Boeremia* and *Phoma*

based on sequence polymorphisms in the actine gene, was determined. The reliability of the DNA extraction and TaqMan PCRs for the detection of *S. andigena* and *S. crystalliniformis* in leaf material was tested in performance studies and demonstrated the specificity, analytical sensitivity, reproducibility, repeatability and robustness of both assays.

SAMENVATTING

Het genus *Phoma* bevat een groot aantal belangrijke plant pathogene schimmels. De identificatie van *Phoma* soorten op basis van onderzoek aan isolaten in cultuur is moeilijk en tijdrovend en de determinatiekenmerken zijn vaak variabel. Bovendien is de huidige indeling van *Phoma* in secties niet éénduidig en komen morfologische kenmerken overeen met die van gerelateerde genera. De moleculaire fylogenie van soorten behorende tot *Phoma* en verwante genera werd onderzocht en de resultaten werden gebruikt voor een revisie van de taxonomie. De DNA-sequentie gegevens verkregen in dit onderzoek vormen de basis voor de ontwikkeling van detectie- en identificatiemethoden.

Hoofdstuk 1 geeft een algemene inleiding over het schimmelgeslacht *Phoma* en beschrijft het onderzoek dat afgelopen decennia in Nederland is uitgevoerd. *Phoma* wordt gekaraketiseerd door de vorming van hyaline sporen in pycniden, ongeslachtelijk gevormde vruchtlichamen. Het genus bevat een groot aantal belangrijke ziektenverwerkers van planten. De taxonomie van *Phoma* is intensief bestudeerd door de afdeling Mycologie van de voormalige Plantenziektenkundige Dienst in Wageningen gedurende een periode van meer dan 40 jaar. In dit onderzoek werden *in vitro* studies uitgevoerd, waarbij de morfologische kenmerken van de soorten, zichtbaar bij het kweken van de schimmels op voedingsbodems, werden vastgelegd. Dit heeft geleid tot de ontwikkeling van een genus concept voor *Phoma*. In dit concept worden de *Phoma* soorten op basis van specifieke morfologische kenmerken geklassificeerd in de negen secties te weten *Phoma*, *Heterospora*, *Macrospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Pilosa*, *Plenodomus* en *Sclerophomella*.

Dit soortconcept vormde de basis voor de systematische beschrijvingen van de ons bekende *Phoma* soorten die werden uitgevoerd in de periode 1991-2002. De resultaten van dit taxonomisch onderzoek werden samengevat in de "*Phoma* Identification Manual", uitgegeven in 2004. In dit handboek zijn de beschrijvingen van 223 *Phoma* soorten opgenomen, met meer dan 1000 synoniemen in andere morfologisch gerelateerde schimmelgenera. Gerhard Boerema, voormalig hoofd van de Mycologie afdeling van de Plantenziektenkundige Dienst, helaas te vroeg overleden in 2008, is tientallen jaren de drijvende kracht geweest achter dit *Phoma* onderzoek in Nederland.

De *Phoma* Identification Manual is het standaardwerk voor de morfologische identificatie van *Phoma* isolaten, maar de *in vitro* studies zijn zeer tijdrovend en vereisen een hoge mate van deskundigheid en ervaring. Bovendien is de indeling van *Phoma* soorten in secties op basis van de morfologie kunstmatig en verschillende soorten kunnen worden ingedeeld in meerdere secties omdat ze kenmerken bezitten die in meerdere secties voorkomen. Bovendien kunnen van *Phoma* soorten deze kenmerken gelijk zijn aan de karakteristieken van morfologisch gerelateerde asexuele genera die eveneens sporen in pycnidia vormen, zoals *Ascochyta*, *Asteromella*, *Microsphaeropsis*, *Phomopsis*, *Phyllosticta*, *Pleurophoma*, *Pyrenochaeta* en *Stagonospora*.

Een aantal *Phoma* secties zijn als ongeslachtelijke taxa verbonden met diverse geslachtelijke genera uit de Ascomyceten, waaronder *Didymella*, *Leptosphaeria*, *Mycosphaerella* en *Pleospora*. Ook zijn synanamorfen van *Phoma* soorten bekend die kenmerkend zijn voor genera als *Epicoccum*, *Phaeomoniella*, *Phialophora*, *Sclerotium* en *Stagonosporopsis*. Dit illustreert de heterogeniteit van *Phoma*.

De Nederlandse Voedsel en Waren Autoriteit (NVWA), waarin de voormalige Plantenziektenkundige Dienst is opgenomen, en het Centraalbureau voor Schimmelcultures in Utrecht beheren een uitgebreide, goed bestudeerde *Phoma* cultuurcollectie met meer dan 1100

Phoma isolaten. Deze collectie vormde de basis voor een intensieve moleculaire fylogenetische studie van het genus *Phoma* en morfologisch gerelateerde genera. Dit moleculaire onderzoek werd gestart in 2006 en uitgevoerd op het Centraalbureau voor Schimmelcultures. Een literatuurstudie leverde informatie op over moleculaire phylogeny bij *Phoma*-achtige schimmels die kon worden toegepast om evolutionaire verwantschappen binnen *Phoma* en gerelateerde genera aan te tonen.

In de eerste fase van het project werden de DNA sequenties bepaald van verschillende gebieden van het genoom, beschreven in de hoofdstukken 2 t/m 4. Met de fylogenie en de DNA-sequentie gegevens die werden verkregen in dit onderzoek is het mogelijk om voor *Phoma*-achtige soorten snelle en betrouwbare moleculaire detectie-en identificatiemethoden te ontwikkelen. Voor de detectie en identificatie van *Stagonosporopsis andigena* en *S. crystalliniformis*, twee belangrijke plantpathogene quarantaine soorten die voorheen beschreven waren in het genus *Phoma*, werden Real-timeTaqman PCR methoden ontwikkeld, beschreven in hoofdstuk 5.

In **hoofdstuk 2** werd de fylogenie van de type soorten van de *Phoma* secties, morfologisch verwante coelomyceten en hieraan gerelateerde teleomorfen, bepaald met behulp van de sequentiegegevens van de 18S nrDNA (SSU) en 28S nrDNA (LSU) gebieden. Aan de hand van de resultaten kon een nieuwe familie, *Didymellaceae*, worden beschreven waarin de type soorten van *Didymella* en *Phoma*, respectievelijk *Didymella exigua* en *Phoma herbarum*, werden ondergebracht. Daarnaast bleken niet alleen de type soorten van de *Phoma* secties *Phyllostictoide*s, *Sclerophomella*, *Macrospora* en *Peyronellaea* verwant in *Didymellaceae*, maar ook de generieke type soorten *Ascochyta pisi* en *Microsphaeropsis olivacea*. Deze genera blijken dus nauw verwant aan *Phoma*. Daarintegen bleken de type soorten van de *Phoma* secties *Heterospora, Paraphoma, Pilosa* en *Plenodomus* juist niet verwant; deze soorten clusterden in verschillende families buiten *Didymellaceae* en werden in het vervolgonderzoek nader bestudeerd.

Hoofdstuk 3 van dit proefschrift gaat verder met moleculaire fylogenetische studies aan *Phoma*-achtige soorten die nauwelijks verwant zijn aan de generieke type soort *Phoma herbarum* en de daarmee verbonden teleomorf *Didymella* in *Didymellaceae*. *Phoma* sectie *Paraphoma*, die wordt gekenmerkt door de vorming van pycniden bedekt met setae, lijkt daarmee op soorten uit de genera *Pyrenochaeta* en *Pleurophoma*. SSU en LSU sequentiegegevens van de soorten ingedeeld in *Phoma* sectie *Paraphoma* werden vergeleken met die van representatieve isolaten van *Pyrenochaeta* en *Pleurophoma* en die van de type soorten van de *Phoma* secties *Phoma*, *Pilosa* en *Plenodomus*. In dit onderzoek werden ook niet nader geïdentificeerde, vaak steriele *Phoma*-achtige isolaten meegenomen. Uit het onderzoek bleek dat de soorten behorende tot de *Phoma* sectie *Paraphoma* moleculair gezien vaak nauwelijks verwant bleken te zijn, een herziene indeling van de soorten van deze sectie wordt in dit hoofdstuk beschreven. *Paraphoma* werd opnieuw als genus geïntroduceerd en bleek verwant aan de in dit proefschift als nieuwe genera beschreven *Neosetophoma* en *Setophoma* in *Phaeosphaeriaceae*. *Pyrenochaeta* en het in dit proefschrift nieuw geïntroduceerde genus *Pyrenochaetopsis*, met voornamelijk soorten uit de voormalige *Phoma* sectie *Paraphoma*, bleken te behoren tot *Cucurbitariaceae*.

In **hoofdstuk 4** werd de moleculaire fylogenie van soorten beschreven in de *Phoma* secties *Plenodomus, Pleospora* en *Heterospora* bepaald met behulp van LSU, SSU en ITS. In een “één soort = één naam” benadering, waarin geslachtelijke (teleomorf) en ongeslachtelijke (anamorf) stadia tot één naam worden samengebracht, werden de soorten beschreven in *Phoma* sectie

Plenodomus met de teleomorf *Leptosphaeria* ingedeeld in *Leptosphaeria, Plenodomus* en de nieuwe genera *Paraleptosphaeria* en *Subplenodomus* in *Leptosphaeriaceae*. Twee soorten van *Phoma* sectie *Heterospora*, de type soort *Phoma heteromorphospora* en de verwante soort *Ph. dimorphospora*, werden ondergebracht in het nieuwe genus *Heterospora*, in *Leptosphaeriaceae*. De generieke type soort *Leptosphaeria doliolum* bestaat uit een soort-complex waarvan de taxonomie werd herzien op basis van de sequentiegegevens van LSU, ITS, SSU, ß-tubuline, en chitine synthase 1. De moleculaire verwantschap van *Phoma* sectie *Pilosa* en *Ascochyta* soorten, gekarakteriseerd door de vorming van pycniden bedekt met myceliumdraden geclassificeerd in *Pleosporaceae*, werd bepaald op basis van de analyse van actine sequentiegegevens. Tenslotte werd in een LSU sequentie-analyse de classificatie bepaald van verschillende *Phoma*-achtige soorten die niet behoren tot de *Pleosporineae*. Deze soorten werden aan de hand van de verkregen fylogenie overgebracht naar de genera *Aposphaeria* (*Melanommataceae*), *Paraconiothyrium* (*Montagnulaceae*) en *Westerdykella* (*Sporormiaceae*). *Coniothyrium palmarum* en verwante soorten werden beschreven in *Coniothyriaceae*. *Medicopsis* (*Trematosphaeriaceae*) en *Nigrograna*, waarvan de familie nog onbekend is, werden als nieuwe genera in dit proefschrift geïntroduceerd om twee medisch belangrijke soorten, voorheen geclassificeerd in *Pyrenochaeta*, onder te brengen.

In **hoofdstuk 5** werden real-time (TaqMan) PCR methodieken ontwikkeld voor de detectie van *Stagonosporopsis andigena* en *S. crystalliniformis* in bladeren van de aardappel en tomaat. De moleculaire fylogenie van beide *Stagonosporopsis* soorten met verwante soorten in de genera *Boeremia*, *Phoma* en *Stagonosporopsis* werd vastgesteld op basis van polymorfismen in de sequentie van het actine gen. De betrouwbaarheid van de ontwikkelde toetsen voor de detectie van *S. andigena* en *S. crystalliniformis* in bladmateriaal werd bepaald door het aantonen van de specificiteit, analytische gevoeligheid, reproduceerbaarheid, herhaalbaarheid en de robuustheid van de TaqMan PCR's voor beide quarantaine schimmels.

DANKWOORD

Ruim 6 jaar geleden kreeg ik de mogelijkheid om opnieuw aan *Phoma* te gaan werken in een onderzoeksproject binnen het Fonds Economische Structuurversterking (FES) programma "Plantgezondheid". Dit had ik nooit verwacht omdat met het uitkomen van de *Phoma* Identification Manual in 2004 voor mij een tijdperk was afgesloten. Nicolette, jij gaf mij eerst de kans om bij een interne reorganisatie verder te gaan als disciplineleider bij Mycologie, om vervolgens enkele maanden later voor te stellen om dit promotieonderzoek er voor één dag in de week bij te gaan doen met de opmerking 'het hoeft natuurlijk niet'. Ik ben de uitdaging aangegaan en ben je dankbaar voor de geboden kansen. Het onderzoek gaf mij de mogelijkheid betrokken te zijn bij de stormachtige ontwikkelingen rond de hernieuwde taxonomie van schimmels op basis van de moleculaire fylogenie. Het gaf ook een nieuwe inhoud aan mijn contacten met het Centraalbureau voor Schimmelcultures (CBS).

Pedro, jij werd mijn promotor en gaf me direct een plek binnen het CBS. Jouw enorme gedrevenheid en enthousiasme hebben mij gestimuleerd om door te zetten en dit werk te voltooien, ook in perioden waarin ik moeite had om alles in de lucht te houden. Jouw kritische blik heeft ook een verdieping gegeven van het onderzoek, erg bedankt voor je begeleiding.

Pierre, als co-promotor toonde jij je betrokkenheid en belangstelling voor dit onderzoek. Chiel, je bleef inhoudelijk belangstelling houden voor mijn werk aan *Phoma*, ik wil jullie beiden bedanken voor het doornemen van de manuscripten.

Op het CBS voelde het gelijk vertrouwd aan, ook al was ik er maar één dag in de week. Maikel, jij was mij al voor gegaan met eveneens een promotieonderzoek op *Phoma* en al een aantal maanden bezig bij het CBS. Je hebt mij wegwijs gemaakt in de moleculaire wereld, bedankt daarvoor. Arien, Mieke, Marizeth, mijn kamergenoten bij aanvang en Marijke en Henk die kort na mij startten met hun onderzoek, bedankt voor jullie gezelligheid. Joyce, jij kwam enkele maanden later binnen bij het CBS voor de uitvoering van het moleculaire onderzoek. Je pakte de zaken gelijk op en jouw werk heeft in hoge mate bijgedragen aan de resultaten beschreven in dit proefschrift. Je was ook altijd bereid om extra werk te doen als ik op de valreep weer aankwam met nieuwe isolaten om mee te nemen in het onderzoek. Bijzondere dank hiervoor. Het was ook leuk om te zien hoe je een eigen onderzoek oppakte en inmiddels ben je zelf met een promotieonderzoek bezig, fantastisch! Karin, Trix en Arien, bedankt voor het werk rond de collectie en herbarium.

Gerard en Ewald, bedankt voor jullie betrokkenheid bij de taxonomische artikelen.

Het praktische werk aan de methodenontwikkeling van de TaqMan PCR's, beschreven in dit proefschrift, werd voornamelijk uitgevoerd door Marga bij Plant Research International. De discussies met Marga, Peter en Ellis over de resultaten waren voor mij heel waardevol. De infectieproeven beschreven in dit onderzoek werden uitgevoerd door Patricia. Ellis en Rien, jullie voegden op mijn verzoek een stuk onderzoek toe aan de infectieproeven in het kader van FES. Allen erg bedankt voor jullie inzet en samenwerking!

Marjan zorgde voor het redigeren en het optimaliseren van de figuren en Manon voor de lay-out van dit proefschrift, bedankt!

Velen op het CBS toonden hun belangstelling, hetzelfde geldt voor collega's van de Nederlandse Voedsel en Waren Autoriteit (NVWA) en daarbuiten, voor familie en kennissen, het gaf mij de inspiratie om door te zetten.

De basis voor dit onderzoek ligt uiteindelijk in de resultaten die bij voorgaande morfologische studies aan *Phoma* zijn bereikt door Gerhard Boerema en zijn medewerkers Mieke Dorenbosch, Hennie van Kesteren, Mariëlle Hamers en Wim Loerakker. Mieke, jij hebt aan de basis gestaan

van de *Phoma* isolatencollectie die wij hebben gebruikt voor het moleculaire onderzoek. Jij hebt mij indertijd eind jaren tachtig een indrukwekkende, goed gedocumenteerde *Phoma* collectie overgedragen, die daarna verder is uitgebreid en geborgd op het CBS. Deze collectie werd eerst gebruikt voor het morfologische onderzoek door Gerhard, Chiel en mijzelf dat uiteindelijk leidde tot de *Phoma* Identification Manual, en vervolgens tot dit proefschrift.

Het is erg jammer dat Gerhard de uitkomsten van het onderzoek beschreven in dit proefschrift niet meer heeft kunnen meemaken. Hij begreep aanvankelijk niet goed dat ik *Phoma* opnieuw oppakte na het uitkomen van de Manual, echter toen ik hem de eerste fylogenetische bomen liet zien, kwam bij hem ook weer een hernieuwd enthousiasme voor *Phoma* boven.

Het onderzoek werd uitgevoerd binnen het FES programma. Harm, projectleider van het FES project en Lute-Harm, coördinator schimmels binnen FES, bedankt voor jullie inzet, dat geldt natuurlijk ook voor de leden van de begeleidingskommissie.

De afgelopen jaren was een periode met veel veranderingen bij de Plantenziektenkundige Dienst (PD), met uiteindelijk de fusie met de Voedsel en Waren Autoriteit en Algemene Inspectie Dienst tot de Nederlandse Voedsel en Waren Autoriteit. Mariëtte volgde Nicolette op als afdelingshoofd van het NRC, bedankt Mariëtte voor de geboden ruimte om dit onderzoek af te ronden.

Er was minder tijd voor het onderhouden van sociale contacten in de afgelopen jaren. Wat wel gelukkig bleef was het wekelijks biljarten op de vrijdagavond en het jaarlijkse biljartuitje bij Rob, de Schierclubactiviteiten rond Oud en Nieuw en natuurlijk het hardlopen bij ‘Onder Ons’ in Oosterbeek, allemaal bedankt voor de gezelligheid!

Er waren ook minder gelukkige tijden met een periode van zorg en het gemis na het overlijden van zowel mijn moeder als schoonmoeder. Het is jammer dat zij dit niet meer meemaken.

Lieve Mela, Corine en Mattijs, er veranderde veel in de afgelopen jaren. Corine en Mattijs, jullie gingen studeren en het is altijd gezellig als jullie weer thuis komen met verhalen over belevenissen. Corine, jij bent nu net klaar met je studie en misschien heb je inmiddels met Robbert een plekje gevonden.

Het proefschrift is afgerond en laten we hopen dat we meer gelegenheid krijgen om erop uit te trekken!

CURRICULUM VITAE

Hans de Gruyter was born on January 4, 1956 in The Hague. After he obtained his Mavo diploma in 1972, he continued at the Antonie van Leeuwenhoek Institute in Delft, where he received his HBO diploma botanical analytical specialist in 1975. He continued his study for biochemical analytical specialist at the same institute, but with more affinity with botany he took the opportunity to start as botanical analytical specialist at the Plant Protection Service (Plantenziektenkundige Dienst, PD), Department Control of Diseases, Wageningen in 1977.

His work comprised the testing of fungicides in laboratory and greenhouse conditions, as well as in field experiments. He followed the theoretical training on plant pathology for employees working in the Field Service of PD in the period 1979-1981. He participated for a further two years in research projects, namely the ecology of pathogens involved in the bottom-rot complex of lettuce, including *Rhizoctonia solani*, *Botrytis cinerea*, *Pythium* spp. and *Sclerotinia* spp., and the chemical control of *Erwinia amylovora* on *Cotoneaster* species and pear in field experiments. He was also involved in the biological control of the Dutch elm disease in field experiments.

In 1989, he started as mycological taxonomic specialist at the Mycology Department. He followed the courses General Mycology and Ascomycetes at CBS in 1990 and 1993. Besides the daily work on the diagnostics and identification of plant pathogens, he focused on the taxonomy of important genera of plant pathogenic fungi, *Phoma*, *Phytophthora* and *Fusarium* in particular. The morphological genus concept of *Phoma*, the specialty of the Mycology Department, was revised in co-operation with former workers of the Mycology department, and in collaboration with the Centraalbureau of Schimmelcultures (CBS) in the period 1990–2004. In the frame of EU twinning programs, he provided training in Mycology for diagnosticians at the laboratory of the Plant Protection Services in Estonia, Hungary, Poland, Romania, Slovenia and Turkey.

He participated in two EU research projects, the EU cost project alder-*Phytophthora* in 1996–2002 and the EU RAPRA project *Phytophthora ramorum* in 2004–2007. He became member of the working party managing the research projects at PD. He co-operated in the Dutch working party Aesculaap, were he was managing the research program phytopathology to find the cause of bleeding canker of horse chestnut trees.

He became head of the Mycology Department of the National Reference Centre (NRC) at the PPS in 2005. He followed the training program “management in government organisations” at Institute Leeuwendaal in 2005–2006. Since 2006, he is member of the FAO-IPPC panel for Diagnostic Protocols. The Plantenziektenkundige Dienst merged with the Voedsel en Waren Autoriteit (VWA) and the Algemene Inspectie Dienst (AID) in 2010 to form the “Netherlands Food and Consumer Product Safety Authority” (Nederlandse Voedsel en Waren Autoriteit, NVWA), that became reality in 2012. Hans de Gruyter was subsequently appointed as manager of the team Diseases, including Bacteriology, Mycology and Virology, as part of the National Reference Centre, National Plant Protection Organization.

EDUCATION STATEMENT

BioDIVERSITEIT

Appendix to PhD Proficiency certificate of Hans de Gruyter

	date	CE
1. Start-up phase (ca. 2 EC)		
First presentation of your project (mandatory)		
Phylogeny within the genus *Phoma*, and its relations to coelomycetous genera. Laboratory of Phytopathology, Wageningen University, Wageningen	May 11, 2007	2,0
Writing or rewriting a project proposal		
A proposal was written in 2006 and has been adapted and submitted in January 2007. Title: Phylogeny within the genus Phoma, and its relations to teleomorphs and related coelomycetous genera.	Jan 2007	4,5
Subtotal Start-up phase		**6,5**
2. Scientific Exposure (minimum 6 EC)		
PhD day (mandatory)		ca. 3
Biodiversity PhD Student day, NCB Naturalis, Leiden	Dec 4, 2008	0,3
Phylogeny of the anamorph genus *Phoma*. PhD day research school Biodiversity, Wageningen (Poster).	Dec 10, 2009	0,3
Conidiogenesis and pycnidial characters in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*; tools for identification?. PhD day research school Biodiversity, Wageningen (oral)	Dec 10, 2009	0,3
CBS/Naturalis/NHN/IBED/EPS colloquia, study days, etc. (mandatory)		ca. 1
Phylogeny within the genus *Phoma*, and its relations to coelomycetous genera. Laboratory of Phytopathology,	May 11, 2007	0,3
Wageningen University, Wageningen (oral) Phylogeny of the anamorph-genus *Phoma* and its teleomorph relations, Laboratory of Phytopathology,	Feb 22, 2008	0,3
Wageningen University, Wageningen (oral) Conidiogenesis and pycnidial characters in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*;	Nov 17, 2008	0,3
tools for identification? CBS, Utrecht (oral) Unraveling the genus *Phoma*: Section *Plenodomus*. CBS Utrecht (oral)	Sep 28, 2009	0,3
Studies in the revised genus *Phoma*. Laboratory of Phytopathology, Wageningen University, Wageningen (oral)	Feb 25, 2011	0,3
International symposia and congresses (mandatory)		
Phytophthora ramorum, developments in the Netherlands, European Mycological Network (EMN)/ EPPO, Wageningen (oral)	Apr 26-28, 2006	0,9
Barcoding the genus *Phoma*, IMCC 8th, Cairns, Australia (poster)	Aug 21-25, 2006	1,5
IPPC Technical Panel of Diagnostic Protocols, Valencia, Spain	Oct 16-20, 2006	1,5
Study of living plants with disease symptoms, CBS Course of Mycology, Utrecht (oral)	Feb 15, 2007	0,3
Training sessions on the Central Quarantine Laboratory in Bucharest, Romania (Twinning program, oral)	Mar 26-30, 2007	1,5
Opening symposium Quarantine Laboratory, Plant Protection Service, Wageningen	May 31-Jun 1, 2007	0,3
IPPC Technical Panel of Diagnostic Protocols. Buenos Aires, Argentina	Sept. 24-29, 2007	1,5
First workshop applied phytopathology, Naktuinbouw, Roelofarendsveen	Nov 7, 2007	0,3
Magical Mushooms Course, Laboratory of Phytopathology, Wageningen University, Wageningen (oral)	Oct 4, 2007	0,3

*Bio*DIVERSITEIT

BioDIVERSITEIT

IPPC Technical Panel of Diagnostic protocols, Braunchweig, Germany (chair)	Jun 2-8, 2008	1,5
Phylogeny of the anamorph-genus *Phoma* and allied genera. (poster), ICPP, Torino, Italy (poster)	Aug 24-28, 2008	1,5
Second workshop applied phytopathology, Naktuinbouw, Roelofarendsveen	Jan 15, 2009	0,3
Course Fungal Biodiversity, Diagnostics of plant pathogens, CBS, Utrecht (oral)	Mar 5, 2009	0,3
The genus *Phoma*. Annual meeting FES program, LNV, Ede (oral)	Jun, 10, 2009	0,3
Presentation FES project, EPPO, Paris, France (discussion)	Sept 18, 2009	0,3
Het schimmelgeslacht *Phoma*: klassieke taxonomie of moleculaire fylogenie? nVWA (oral)	Sep 28, 2009	0,3
Magical Mushooms Course, Laboratory of Phytopathology, Wageningen University, Wageningen (oral)	Oct 5, 2009	0,3
Introduction course Mycology for inspectors, nVWA, Wageningen (oral)	Feb 9, Mar 2, 2010	0,3
Phoma research in The Netherlands. Scientific Commitee Board, CBS, Utrecht (oral)	Apr 6, 2010	0,3
Redisposition of *Phoma* anamorphs in the *Leptosphaeriaceae* and *Pleosporaceae*. IMC 9, *Edinburgh*, United Kingdom (poster)	Aug 1-6, 2010	1,5
Wettelijke onderzoekstaken (WOT), LNV, Den Haag	Jan 19, 2010	0,3
FES openingssymposium Q-bank, Leiden (poster)	Jun 22, 2010	1,5
IPPC Technical Panel of Diagnostic Protocols, Washington, USA	Jul 26-30, 2010	1,5
Meeting Quarantine Barcoding of Life (QBOL), Bologna, Italy	Oct 20, 2010	0,3
Subtotal Scientific Exposure		**21,0**

3. In-Depth Training (minimum 12 EC)	*date*	*CE*
PhD courses (mandatory)		min 12
Molecular phylogeny, Wageningen University, Wageningen	Apr 14-17, 2008	1,2
Introduction to Phylogenetic Analysis, Nationaal Herbarium Nederland - Leiden Universiteit Branch, Leiden	Apr 3-7, 2006	1,2
Subtotal In-Depth Studies		**2,4**

4) Personal development (minimum 3 ECTS credit points)	*date*	*CE*
Skill training courses (mandatory)		ca. 2
Management in Overheidorganisaties. Adviesburo Leeuwendaal, Veldhoven, The Netherlands	Jan 18-19, 2006	0,6
	Feb 15-17, 2006	0,9
Cursus 'systeemlab', dynamiek en wetmatigheden in organisatiestructuren. Ardis, Den Haag, The Netherlands	Jan 14-15, 2009	0,6
Organisation of PhD students day, course or conference		ca. 2
Co-organiser meeting of the European Mycological Network/ EPPO), Wageningen, the Netherlands	Apr 26-28, 2006	1,0
Co-organiser workshop Implementation of EPPO standard PM 3/64, Wageningen, the Netherlands	May 31, 2007	1,0
Membership of Board, Committee or PhD council		pm
EPPO working party Diagnostic Protocols for Regulated Pests (Fungi)		1,0
Member IPPC Technical Panel of Diagnostic Protocols		1,0
Member European Mycological Network		1,0
Member werkgroep kennis, nVWA, Plant Protection Service		1,0
Secretary of the KNPV Commission 'Nederlandse namen van plantenziekten'		
Subtotal Personal Development		8,1
TOTAL NUMBER OF CREDIT POINTS*		**38,0**

*Bio*DIVERSITEIT

The research described in this thesis was conducted at the Centraalbureau voor Schimmelcultures / CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

Financial support for the research was provided by the Dutch Ministry of Agriculture, Nature and Food Quality through an endowment of the FES programme “Versterking infrastructuur Plantgezondheid” and the Royal Netherlands Academy of Arts and Sciences (KNAW).

Financial support from the Centraalbureau voor Schimmelcultures / CBS-KNAW Fungal Biodiversity Centre, Wageningen University and the Netherlands Food and Consumer Product Safety Authority for printing this thesis is gratefully acknowledged.

The Training and Supervision Plan was completed at the Research School Biodiversity.

Layout and design thesis:
Manon van den Hoeven-Verweij

Front and back cover:
Chaetosphaeronema coonsii CBS 141.84. Pycnidial cell wall with conidiogenous cells and conidia. Design: Arjen Werkman

Printed at:
GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede, The Netherlands