Phylogeny and DNA-based identification in *Phoma* and related genera

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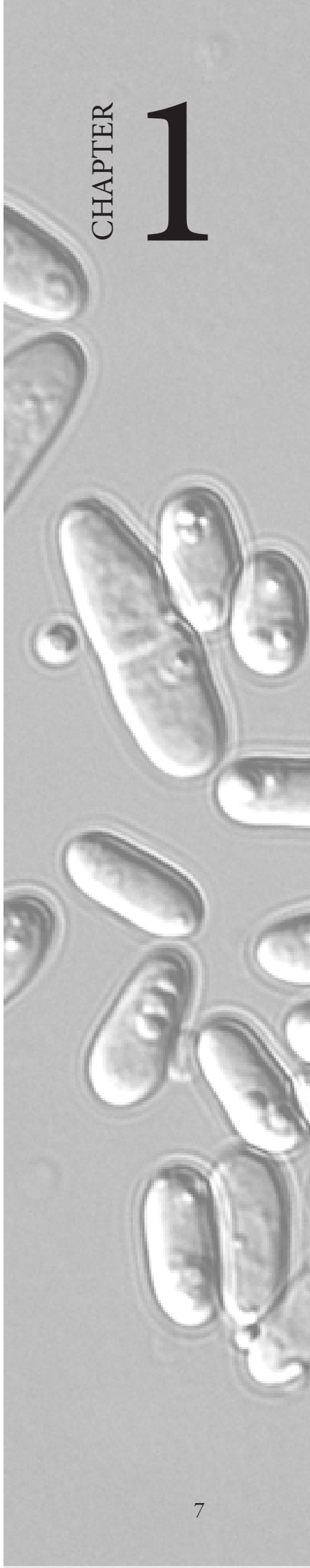
CONTENT

CHAPTER 1

General introduction



General introduction

This thesis addresses the taxonomy of a generic complex presently known as *Phoma* Sacc. emend Boerema & Bollen. The vast majority of the taxonomic work on this genus was conducted during the last 40 years of the previous century by researchers at the former Dutch Plant Protection Service (PD), and supported at the CBS-KNAW Fungal Biodiversity Centre (CBS). This elaborate work culminated in the publication of a monographic treatment of *Phoma* in 2004, in which the morphology *in vivo* and *in vitro*, as well as the ecology of 223 specific and infra-specific *Phoma* taxa was discussed in detail (Boerema *et al.* 2004).

This handbook marked the end of the era in which the taxonomy of this genus mainly relied on morphological observations and descriptions in culture. However, it can also be regarded as the starting point of the present study. In this project, it was aimed to integrate DNA-based identification methods into the taxonomic system established by these previous researchers. The major part of this study therefore deals with the validation of current generic and species concepts.

As a consequence of the extensive studies undertaken by Boerema and his co-workers, many well-vouchered strains, including a significant number of types, are present at the fungal collections in the Netherlands. These strains, which are currently housed at the CBS and PD culture collections and herbaria, form the basis of the research conducted in the studies described in the present thesis.

Fungal biodiversity

Of the seven kingdoms of life currently acknowledged, the regnum *Fungi* is considered to be one of the largest on earth. The enormous species richness in the fungi is only exceeded by that of the prokaryotes (Curtis *et al.* 2006) and the animal kingdom, which mainly can be contributed to the arthropods (Hamilton *et al.* 2010). The estimates for the worldwide fungal species richness vary significantly, between 500 000 (May 2000) and 9 900 000 species (Cannon 1997). In two subsequent studies, Hawksworth (1991, 2001) estimated the total diversity within the fungal kingdom at approximately 1 500 000 species. Schmit & Mueller (2007) estimated that there is a minimum of 712 000 extant fungal species worldwide, but reckon that the actual number is probably much higher, which supports the 1 500 000 figure. This number is accepted as feasible by most mycologists (Hawksworth 2004, Mueller & Schmit 2007), although two recent papers again postulate 611 000 (Mora *et al.* 2011), and 5 100 000 (Blackwell 2011), respectively, suggesting that this matter is far from settled.

In contrast to these huge numbers of fungal species richness, the amount of species that have been described in literature thus far is remarkably low: MycoBank (Crous *et al.* 2004, Robert *et al.* 2005) lists 452 442 names (27 Feb. 2013), but includes also many older names that have been synonymised or recombined in the past. Kirk *et al.* (2008) estimated the number of described species at 97 330 which is 6.5 % of the estimated total species number. This implies that approximately 1.4 million fungal species are still waiting to be described, and at the rate of 1 250 novelties per year (average over the past 10 years, based on MycoBank data), it will take more than 1.100 years to describe even the low estimate of 1.5 million species.

Various authors dealt with the question where to look for these novelties (Hawksworth & Rossman 1997, Hyde 2001, Crous *et al.* 2006a). One of the recommendations of Hawksworth and Rossman (1997) was that a closer look should be taken at cryptic species, i.e. a morphospecies that is so broadly defined that the circumscription actually applies to multiple evolutionary and genetically distinct taxonomic entities. Hawksworth & Rossman (1997) estimated that at least 20 000 species that still await formal description can be found in holdings of present culture collections. With the rise of molecular techniques in mycology (Bridge 2002), many novel species complexes and cryptic species have been identified, leading to accelerated species discovery. Sequence typing and careful morphological re-examination of current holdings in Biological Resource Centres (BRCs) will help to improve recognition of cryptic lineages and taxonomic entities within broadly defined morphospecies. Hawksworth *et al.* (2004) estimated that culture collections have around 16% of the presently known taxa in their holdings. In a generic overview paper, Hawksworth (2001) mentioned the presence of hundreds of undescribed taxonomic novelties that were present in such BRCs and herbaria, indicating that these institutions are the mycological treasure troves themselves.

Species and genus concepts

In order not to lose track among all the poorly defined and undescribed taxa in fungal collections, the use of a proper species concept is essential. Multiple species concepts have been defined throughout history. In this Darwin era, the most applied concept is the Evolutionary Species Concept (ESC; Simpson 1951, 1961, Wiley 1978, Wiley & Mayden 2000), which is also followed in this thesis. According the ESC, the term "species" is explained as "an entity composed of organisms which maintains its identity from other such entities through time and over space, and which has its own independent evolutionary fate and historical tendencies" (Wiley & Mayden 2000). This concept is widely applied as the "master concept" but does not include any criterion on which basis species can be distinguished. Therefore, it is not applied in species recognition and diagnosis.

More usable are the operational species concepts, or "species recognitions" that are compatible with the ESC. The three most applied types of species recognition are Morphological Species Recognition (MSR), Biological Species Recognition (BSR) and Phylogenetic Species Recognition (PSR) (Mayden 1997), which are also commonly used in fungal taxonomy.

The BSR is based on presence or absence of sexual reproduction between two organisms (Taylor *et al.* 2000), and cannot always be applied in mycology. As sexual structures in most fungal strains, including the vast majority of the strains ascribed to the genus *Phoma*, have never been observed (Aveskamp *et al.* 2008), the BSR cannot be followed in the present study.

MSR uses similarity in morphological characters as main criterion to ascribe an organism to a certain taxon. Harrington & Rizzo (1999) stated that: "A species is … the smallest aggregation of populations with a common lineage that shares unique, diagnosable phenotypic characters". As soon as it became clear that the host-based nomenclature was not a highly reliable method to differentiate species, the application of MSR became common practice in the 20th century mycology. Studies of strains in culture (Dennis 1946), and classification of morphological features under "standardised" laboratory conditions represented a giant step forward in the understanding of fungal systematics. Nevertheless also this operational species concept proved to be far from perfect, as many fungi cannot be cultured, or show loss of morphological features when grown on artificial media. Furthermore, appearance of morphological characters may be highly sensitive to the composition of media, and the culturing conditions employed, as is shown in a study on *Phoma* species from India (Rai 2000). The inability to effectively link different states of pleomorphic fungi with one another, also led to a confusing situation in which a single species may either carry a teleomorph name, as well as one or more anamorph names.

The genetic similarity between multiple organisms is the basis of PSR. Significant morphological or ecological differences in a new evolutionary species are often preceded by a series of changes in gene sequences. Therefore, PSR can be regarded as more consistent with the ESC than MSR or BSR (Taylor *et al.* 2000). However, the main pitfall is that currently the focus in scientific studies is too much on genetic similarity alone, frequently in total absence of pathogenicity data. Nowadays it appears to be common practice to "recognise" a species by means of a quick single sequence blast-search comparison with data present in public databases alone (Hyde & Soytong 2007). This approach has led to many misidentifications in the past, and subsequent deposit of new sequences of incorrectly identified organisms in sequence databases in the public domain (Bridge *et al.* 2003; Nilsson *et al.* 2006). A correctly applied PSR is based on genealogical concordance of multiple DNA sequences (Genealogical Concordance Phylogenetic Species Recognition – GCPSR) (Baum & Shaw 1995, Taylor *et al.* 2000). Furthermore, a comparison between strains on other features should always be made, which can be either morphological, biochemical, or ecological. In the present study, the GCPSR will be applied as an operational species concept, whereas microscopic features, such as the sizes and shapes of various types of spores and fruiting bodies will be studied to ratify the genetic clustering of strains in a phylogeny.

Defining *Phoma*

The anamorph genus *Phoma* is a member of the coelomycetes, a currently much debated taxonomic group. This group is characterised by the production of conidia from conidiogenous cells lining a cavity (pycnidia,

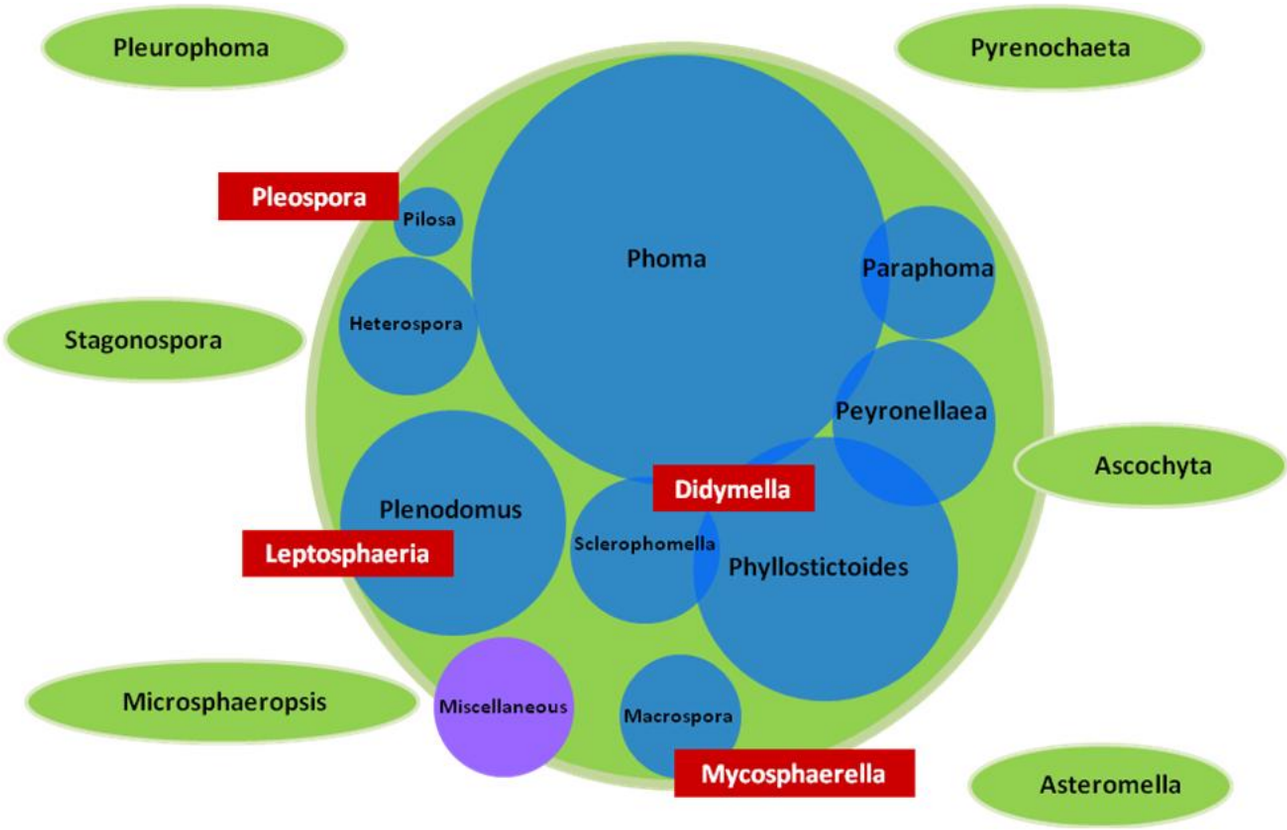


Figure 1.1. Diagrammatic representation of the distribution of species within the various sections of *Phoma* (blue/purple), the relationships between the sections and their similarity with teleomorphs (red) some other coelomycetous genera (green) (Figure reproduced from Boerema *et al*. 2004, courtesy of J. de Gruyter).

acervuli). As in the fungi in general, the total number of described species in this group is only a fraction of the estimated world species number: in 1994, Rossman (1994) stated that only 4.5 % of the expected 200 000 coelomycete species were known. The total "black hole" of species richness in the coelomycetes is probably much larger, as the author's estimate of the total number of fungi was rather conservative – one million –, and many of the taxa recognised in the early nineties have been merged since. Major efforts in this regard have been made in *Phoma*.

The genus *Phoma* is embedded in the order *Pleosporales* (Schoch *et al.* 2006, 2009a, Zhang *et al.* 2009). In the past, the genus contained more than 3 200 epithets (Sutton 1964, Monte *et al.* 1991), of which most are nowadays regarded as invalid. Currently, many of these names have been recombined into other genera (Mel'nik 2000, van der Aa & Vanev 2002) or synonymised (Boerema *et al.* 2004). The genus was defined by Brewer & Boerema (1965) and Boerema & Bollen (1975), and in subsequent studies the systematics was further refined (Boerema 1997, Boerema *et al.* 2004). *Phoma* was restricted to nine sections that were often based on morphological characters, or on teleomorph associations (Figure 1.1). A further introduction to the biology, systematics and taxonomic history of the genus *Phoma* will be provided in Chapter 2.

Outline of the thesis

The present study was initiated in 2005, shortly after the publication of the '*Phoma* Identification Manual' (Boerema *et al.* 2004), which summarises the work of more than 40 years of *Phoma* taxonomic research in the Netherlands. These taxonomic studies were mainly based on micromorphology, in addition to phytopathological data and culture characteristics.

To judge the current infrageneric taxonomy of *Phoma*, and to facilitate the development of robust molecular identification and detection techniques, a multi-locus DNA sequence dataset was established, comprising more than 1 000 well-vouchered strains of *Phoma* and related and convergent genera. The major part of this study has focused on the taxonomy of the *Phoma* species in *Didymellaceae*.

Chapter 1 provides an introduction to fungal biodiversity and taxonomy, and discusses the species concepts applied here. The genus *Phoma* is also briefly introduced.

In a literature overview provided in **Chapter 2**, the systematics of this genus before the initiation of this project is summarised. The progress made in recent years on the systematics of this genus is discussed, and the use of molecular typing tools for further delineation of *Phoma* is advocated. In addition, a general overview of the biology and ecology of the species is provided.

In **Chapter 3** the phylogeny of *Phoma* section *Peyronellaea* is reconstructed by means of multilocus sequence typing (MLST). Species in this section are characterised by the production of dictyochlamydospores, although this feature can also occur in other sections. In total 122 strains were analysed, revealing five species that were new to science, as well as the necessity to introduce new combinations for another five taxa. Further analysis of the dataset revealed that section *Peyronellaea* was polyphyletic.

Chapter 4 addresses the characterisation of taxon-specific primer combinations that can be used for rapid molecular identification of taxa in the *Phoma exigua* species complex. DNA sequences were obtained by means of sequence analysis of the actin locus, or, by analysing fingerprinting patterns obtained from Random amplified Polymorphic DNA Patterns (RAPDs) on agarose gels and subsequent sequence analysis of taxon-specific bands. The fingerprinting patterns in this part of the study were obtained by a novel RAPD-based technique, called DNA amplified fingerprinting (DAF) and are described in this chapter.

A re-evaluation of the taxonomy and systematics of the genus *Phoma* is presented in **Chapter 5**. The phylogenetic position of the genus in comparison to other morphologically similar pleosporalean fungi is determined using an analysis of 28S Large Subunit (LSU) and 18S Small Subunit (SSU) DNA sequence data. Anamorph-teleomorph relations are assessed and the subdivision of the genus as it is currently applied is validated. The chapter specially focuses on the systematics within the recently established family, *Didymellaceae*. An alignment consisting of LSU, ITS (Internal Transcribed Spacer regions 1 & 2 and the 5.8S gene), and portions of the β-tubulin gene revealed a heterogeneous group of taxa within this family.

In **Chapter 6**, the results obtained in this study are discussed and summarised. The results are placed in a broader context and compared to what was known prior to this study, leading to several suggestions for future research. Furthermore, the development of an on-line identification and reference database for *Phoma* and related genera is introduced in this chapter.

CHAPTER 2

Biology and recent developments in the systematics of *Phoma*, a complex genus of major quarantine significance

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Summary

Species of the coelomycetous genus *Phoma* are ubiquitously present in the environment, and occupy numerous ecological niches. More than 220 species are currently recognised, but the actual number of taxa within this genus is probably much higher, as only a fraction of the thousands of species described in literature have been verified *in vitro*. For as long as the genus exists, identification has posed problems to taxonomists due to the asexual nature of most species, the high morphological variability *in vivo*, and the vague generic circumscription according to the Saccardoan system. In recent years the genus was revised in a series of papers by Gerhard Boerema and co-workers, using culturing techniques and morphological data. This resulted in an extensive handbook, the "*Phoma* Identification Manual" which was published in 2004. The present review discusses the taxonomic revision of *Phoma* and its teleomorphs, with a special focus on its molecular biology and papers published in the post-Boerema era.

Keywords: coelomycetes, *Phoma*, systematics, taxonomy

Introduction

The genus *Phoma* is geographically widespread and consists of a large group of fungi that are found in numerous ecological niches. Besides several harmless saprobic species, *Phoma* has also been shown to be an important fungal plant pathogenic genus occurring on economically important crops. Several *Phoma* species are also of quarantine significance, posing serious problems to organisations that are involved in plant health quarantine regulation. Identification of isolates found on possible infected material is frequently carried out under extreme time constraints. Because morphological studies are time-consuming, expensive, and require highly skilled personnel, the chance of successfully identifying strains to species level in such laboratories is often low. Therefore, there is a need for the development of fast, reliable molecular methods for the detection of quarantine actionable *Phoma* species. Several molecular methods to detect quarantine species have been developed in the past decade, e.g. for *Ph. macdonaldii* (Miric *et al.* 1999), *Ph. cucurbitacearum* (Somai *et al.* 2002b, Koch & Utkhede, 2004), *Ph. foveata* (Macdonald *et al.* 2000, Cullen *et al.* 2007) and *Ph. tracheiphila* (Balmas *et al.* 2005, Licciardella *et al.* 2006), but the validation of these methods is heavily questioned as the species concepts of these taxa are not yet fully understood. In this literature review we will provide an outline of the biology of the genus, and circumscribe its taxonomic boundaries. We will also focus on the methodologies that can be used to obtain a better understanding of speciation within *Phoma*.

Phoma biology

The generic name *Phoma* was in the first instance solely reserved for plant stem pathogens (Saccardo 1880), but nowadays the genus comprises pathogens, opportunists as well as saprobes from a much wider range of substrates. The more ubiquitous species such as *Ph. herbarum*, *Ph. glomerata*, *Ph. pomorum* var. *pomorum* and *Ph. eupyrena* have been found on inorganic materials; isolates are known from asbestos, cement, oil-paint and plaster (*Ph. herbarum*), chemicals, paint (*Ph. glomerata*) and crockery (*Ph. pomorum* var. *pomorum*), along with many other inorganic substrates. These ubiquitous fungi probably also play an important role in the degradation of organic materials, together with other, more specialized species. In contrast to these harmless saprobes, more than 50% of the species described thus far are known to be able to occur in living tissue, either as opportunists or as primary pathogens.

Phoma infections commonly occur in humans and animals. Zaitz *et al.* (1997) and De Hoog *et al.* (2000) referred to nine species that were isolated from humans. Recently, an additional species, *Ph. exigua*, was added to the list of human pathogens (Balis *et al.* 2006). Several severe vertebrate diseases are also associated with *Phoma*, such as bovine mycotic mastitis (Costa *et al.* 1993) and fish-mycosis in salmon and trout (Ross *et al.* 1975, Hatai *et al.* 1986, Voronin 1989, Faisal *et al.* 2007). Soil associated organisms such as arthropods and nematodes can also be subject to *Phoma* infections. Chen *et al.* (1996) listed 11 *Phoma* species found in association with cyst nematodes. Furthermore, *Phoma* species have been found parasitizing other fungi and oomycetes (Hutchinson *et al.* 1994, Sullivan & White 2000). Although lichens have been poorly studied, a key to 14 lichenicolous *Phoma* species was provided by Hawksworth and Cole (2004). Still, the vast majority of the species appear to only colonise plant material. Some species are only known from decaying leaves or wood, whereas others play a role as secondary invaders of weakened plant tissue. However, more than 110 species are known to be primary plant pathogens, mainly specialising on a single plant genus or family.

The economically most important pathogens include the widespread species *Ph. medicaginis* (Broscious & Kirby 1988) and the two species that are involved in Black Leg in *Brassicaceae*: *Ph. lingam* and to a lesser extent the unnamed *Phoma* anamorph of *Leptosphaeria biglobosa* (Gugel & Petrie 1992, Fitt *et al.* 2006). Although recent estimations of financial losses are rare, the impact of this genus on agriculture is highly significant. *Phoma lingam* is regarded to be the most important pathogen of oilseed rape in the Northern Hemisphere, with yield losses of up to 25 % being recorded (Fitt *et al.* 2006). Besides the direct costs due to yield loss, indirect losses occur due to import and export restrictions to prevent introductions of possible pathogenic or quarantine relevant *Phoma* species. Rigorous measures, such as refusal or even destruction of a shipment that is suspected of infection with such quarantine organisms, are the cause of high additional costs to both the exporting and importing traders. These quarantine organisms include amongst others *Ph. andigena* and *Ph. tracheiphila* in the European

and Mediterranean regions (Smith *et al.* 1992), *Ph. foveata* (also known as *Ph. exigua* var. *foveata*) in southern America (Mendes *et al.* 2007), and *Ph. macdonaldii* in Australia (Miric *et al.* 1999).

Only in some cases the pathogenic nature of *Phoma* is regarded as helpful, by means of biocontrol agent of weeds and plant pathogens. The ubiquitous species *Ph. herbarum*, *Ph. exigua* and *Ph. macrostoma* may play a role as bioherbicide, effective against broadleaf weeds, such as dandelion (*Taraxacum* spp.), and chickweed (*Caryophyllaceae*) (Stewart-Wade and Boland, 2004, 2005, Zhou *et al.* 2004), clematis (*Clematis vitalba*) (Paynter *et al.* 2006) and salal (*Gaultheria* spp.) (Zhao *et al.* 2005, 2007). The antagonistic effect of *Ph. glomerata* and *Ph. etheridgei*, respectively against *Microsphaera penicillata* and *Phellinus tremulae*, has been studied in detail. However, their application as biocontrol agent is debatable, due to application problems and a low impact on the disease spread (Hutchinson *et al.* 1994, Sullivan & White, 2000).

The life cycle

Although the life cycle is influenced by the occupied niche, it is relatively similar for all plant pathogenic *Phoma* species. Primary infection of hosts may occur through wounds that are caused by cultivation practices, weather conditions, or interaction with other organisms. In several species, entering a host plant through stomata or directly through the epidermis also may occur (Williams 1992, Agrios 1997, Roustaee *et al.* 2000, West *et al.* 2001, van de Graaf *et al.* 2002). Initially, the fungal hyphae grow intercellulary through plant tissues (Hammond & Lewis, 1987). Following this symptomless stage, the fungus becomes necrotrophic. Host cells are subject to phytotoxification or to a hypersensitive response, after which the fungus has acces to the resources of the dead plant tissue. This can be observed macroscopically as the formation of lesions. After a short period, often dark-coloured, mostly globose or flask-shaped pycnidial conidiomata can be observed within the lesions, which are embedded in the plant's epidermis. These pycnidia contain numerous conidia, which ooze in a pale white to pinkish coloured matrix. A detailed description of the spore production is provided by Boerema (1965), Brewer & Boerema (1965) and Boerema & Bollen (1975). In some cases extradermal mycelium may be formed. Conidia, and in some species mycelial fragments, disperse easily by water-splash, misting or wind, and can thus infect new host plants. Also birds and insects may act as vectors (Perrotta & Graniti, 1988). In absence of a suitable host, due to crop harvesting for example, most species persist as saprobes on decaying organic material in the soil. In most cases, this material is the residue of plants that were previously infected. In this mode, the mould may survive periods of stress, such as drought or extreme cold. The structures which are most suitable for long-term survival are the conidiomata, and the uni- or multicellular chlamydospores, which are formed only by a small number of *Phoma* species. From this material infection may occur in newly planted crops, and from the exuding conidiomata new conidia can emerge to facilitate onward dispersal.

Sometimes also a meiotic cycle can be observed, besides the asexual one described above, in which ascospores are formed in pseudothecial ascomata. If such sexual structures are found in nature, they are mostly observed in the saprobic part of the life cycle (Williams 1992, West *et al.* 2001). However, such a sexual stage is quite uncommon within this genus, and is presently not known for more than 40 taxa, of which many produce the sexual reproductive structures only *in vivo*. Unfortunately, little is known about the induction of sex *in vitro*.

The abundant production of conidia, together with a relatively fast growth rate and the capability to invade a large number of hosts and substrates, are the main reasons why the genus is so cosmopolitan in distribution. The most frequent occurring species, *Ph. eupyrena, Ph. exigua*, *Ph. glomerata*, *Ph. herbarum*, and *Ph. macrostoma* are found worldwide, irrespective of the climatic conditions. *Phoma herbarum* is probably also indigenous to Antarctica (McRae & Seppelt 1999, Tosi *et al.* 2002). Several plant pathogenic species have been transmitted worldwide with the cultivated host on seeds and other plant material.

The *Phoma* generic concept

The first description of a fungus that belongs to the genus *Phoma* dates back to 1821 (Sutton 1980). Nevertheless, it took until 1880 before the generic name was officially introduced by Saccardo, and was later emended by Boerema and Bollen in 1975 (Saccardo 1880, Boerema & Bollen 1975). In the Saccardoan system, the genus name *Phoma* applied to filamentous fungi, which were capable of forming pycnidial conidiomata with aseptate, hyaline conidia that could inhabit plant stems. Fungi with the same

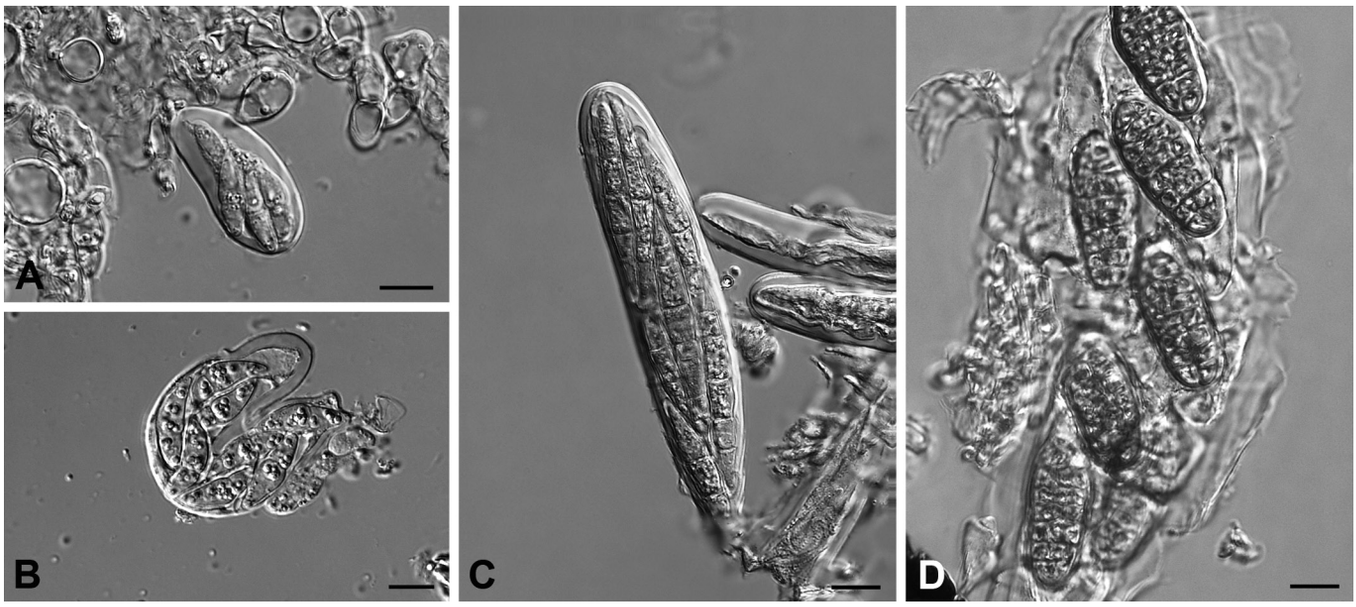

Figure 2.1. A–D. Teleomorphs of *Phoma*. Asci and ascospores of A–B. *Didymella zeae-maydis* (anam. *Ph. zeae-maydis*) C. *Leptosphaeria maculans* (anam. *Ph. lingam*) D. *Pleospora herbarum*, lectotype of the genus *Pleospora*. Scale bars = 10 µm.

morphological appearance, but found in association with leaf spots, were placed in the genus *Phyllosticta* (van der Aa & Vanev 2002, Boerema *et al.* 2004).

Identification of fungi occurred mainly *in vivo* until the fourth decade of the 20th century. Wollenweber & Hochapfel (1936) were the first to recognise the advantages of using growth characteristics on artificial substrates in *Phoma* taxonomy, whereas Dennis (1946) launched the use of basic morphology, physiology and biochemical tests *in vitro*. In a study on hyphomycetes, Hughes (1953) introduced conidiogenesis as an important taxonomic criterion, a feature that was later applied to all conidial fungi including species of *Phoma* (Sutton, 1964). In addition, new criteria for morphological differentiation were introduced to distinguish *Phoma* species from other coelomycetes such as *Ascochyta* (Boerema and Bollen, 1975), *Phyllosticta* (van der Aa *et al.* 1990), *Coniothyrium* and *Paraconiothyrium* (Verkley *et al.* 2005).

In 1964, eight years after the designation of *Phoma* as a conserved genus name in the International Code of Botanical Nomenclature (Lanjouw *et al.* 1956), the designated type species, *Phoma herbarum*, was restudied and lectotype material was selected (Boerema 1964). Morphology, synonymy and ecology of this type species were later extensively described by Boerema (1970) and Morgan-Jones (1988a).

Currently *Phoma* species can be defined as filamentous fungi that produce pycnidial conidiomata with monophialidic, doliiform to flask-shaped conidiogenous cells. A collarette is present at the apex of those cells after the production of the first conidium. *In vitro* the hyaline conidia are mainly single-celled, although in several species a small percentage of transversely septate conidia may also be observed. This definition applies to more than 220 inter- and intraspecific taxa (Boerema *et al.* 2004), but it should be borne in mind that this definition does not necessarily apply to samples in nature, as bicelled or even pigmented conidia occur more often *in vivo*.

The genus *Phoma* is generally considered by most modern mycologists to be taxonomically problematic due to ambiguous morphological criteria, but also due to uncertain phylogenetic affinities. The genus is currently linked to three different teleomorph genera. If teleomorphs are known, they reside in *Didymella*, *Leptosphaeria,* or occasionally *Pleospora* (Table 2.1, Figure 2.1). It should be noted that not all species in these genera form *Phoma* anamorphs; this is a feature that may have been lost over time, or developed multiple times in multiple ancestors. In literature, *Phoma* species have also been associated with other teleomorph genera within *Dothideomycetes*, including *Mycosphaerella* (Punithalingam 1990, Corlett 1991, de Gruyter *et al.* 2002), *Belizeana* (Kohlmeyer & Volkmann-Kohlmeyer 1987), and *Fenestella*, *Cucurbitaria*, *Preussia*, and *Westerdykella* (von Arx 1981). As many of these connections are based on association, they remain to be confirmed in culture. Furthermore, a range of synanamorphs of *Phoma* spp. have been recognised in *Stagonosporopsis*, *Epicoccum*, *Phialophora* and *Sclerotium* (Boerema & Bollen 1975, Sutton 1977, Boerema 1993, Boerema *et al.* 1994, 1997, Arenal 2000, 2004). Crous & Gams (2000) also described a phoma-like synanamorph of *Phaeomoniella chlamydospora* (*Chaetothyriales*), a fungus

Table 2.1. The nine sections of the genus *Phoma*, with their type species and associated teleomorph genera.

Section	Type species	Associated teleomorph genus
Heterospora	*Ph. heteromorphospora*	-
Macrospora	*Ph. zeae-maydis*	*Didymella*
Paraphoma	*Ph. radicina*	-
Peyronellaea	*Ph. glomerata*	-
Phoma	*Ph. herbarum*	*Didymella*
Phyllostictoides	*Ph. exigua* var. *exigua*	*Didymella*
Pilosa	*Ph. betae*	*Pleospora*
Plenodomus	*Ph. lingam*	*Leptosphaeria*
Sclerophomella	*Ph. complanata*	*Didymella*

associated with Petri disease of grapevines (Mostert *et al.* 2006). This complexity in taxonomy is a further complicating factor for the identification and differentiation of members of the genus *Phoma*.

Amongst the many scientists that attempted to order the numerous species belonging to *Phoma* are Saccardo (1884), Grimes *et al.* (1932), Wollenweber & Hochapfel (1936), Dennis (1946), Sutton (1964) Monte *et al.* (1990, 1991) and Rai & Rajak (1993). Morgan-Jones and co-workers described some important species in detail in several papers published in Mycotaxon (Morgan-Jones & White 1983a, b, White & Morgan-Jones 1983, 1986, 1987a, b, Morgan-Jones & Burch 1987a, b, 1988a, b, Morgan-Jones 1988a, b). The greatest effort made on *Phoma* systematics was by Boerema and co-workers, who published contributions towards a genus concept including numerous species descriptions and synonyms in a series of papers in Persoonia (de Gruyter & Noordeloos 1992, Boerema 1993, 1997, 2003, Boerema *et al.* 1994, 1996, 1997, 1999, Boerema & de Gruyter 1998, 1999, de Gruyter *et al.* 1998, 2002, van der Aa *et al.* 2000, de Gruyter, 2002, de Gruyter & Boerema, 2002). As a final product of their 40-year study on *Phoma* taxonomy, a taxonomic handbook was written based on the papers published in the previous decades (Boerema *et al.* 2004). The *Phoma* generic concept, and therefore also the identification key is based on the establishment of nine sections within the genus (Boerema 1997), which are listed in Table 2.1.

Although the key is helpful for identification of species, it is still uncertain if this division into sections can be considered natural from an evolutionary perspective. In this classification system, most sections are to be based on morphological characters that imply a certain evolutionary relationship, or comprise species that share a teleomorph in the same genus. Unfortunately, several characters that are linked to a certain section, sometimes also seem to occur in species that are placed in other taxonomic groups. As an example, Punithalingam (2004) mentioned the slightly pilose species, *Ph. anserina* and *Ph. leonuri* that are not accommodated in either section *Pilosa* or *Paraphoma*. Based on the appearance of their conidia, these species have been placed in the sections *Phoma* and *Plenodomus*, respectively. This ambiguous approach has resulted in multiple overlaps between the separate sections. Furthermore, the sections *Phoma* and *Phyllostictoides* are also considered to be artificial. Instead of comprising species with a shared feature, both sections seem to be a repository for species that lack the presence of good sectional characters. According to Boerema (2004), section *Phoma* comprises species "that have much in common with *Ph. herbarum*". But as the majority of the species are accommodated within this section, it shows the necessity to conduct further research on classification within the genus.

Towards a generic concept for *Phoma*

Macromolecular approaches to systematics were introduced in mycology in the late 80's (for a review see Bruns *et al.* 1991). These techniques, although accurate, were providing only a limited number of relevant characters. The introduction of nucleotide sequences as phylogenetic characters greatly advanced fungal systematics (Bridge 2002, Malgoire *et al.* 2004). Within the genus *Phoma* this gave rise to several novelties, and to new insights on the delimitation of *Phoma* and its teleomorphs, as described below.

The present classification system has been criticized for the unclear discrimination between *Phoma* and several related or even convergent genera. For example, Grondona *et al.* (1997) discussed the poor

delineation between the *Phoma* section *Paraphoma* and the genera *Pyrenochaeta* and *Pleurophoma*. Furthermore, the relation between *Ascochyta* and *Phoma* species that produce septate conidia remains unresolved. The confusion between the two genera is illustrated by the large number of shared synonyms. Although both genera have teleomorphs in *Didymella*, Boerema & Bollen (1975) differentiated *Phoma* from *Ascochyta* on differences in conidiogenesis and conidial septation. The conidia of *Ascochyta* are basically always 2-celled, due to early euseptation during conidiogenesis, whereas in *Phoma* septate conidia are rare in culture - although it is not uncommon in nature - and are formed by a late euseptation. Spectrometrical analysis of crystals formed by specimens of both genera seemed to support this differentiation (Noordeloos *et al.* 1993). However, later Faris-Mokaiesh *et al.* (1995) showed that *Ph. pinodella* and *A. pinoides* probably are closely related, basing their conclusions on a similarity in the banding pattern of PCR and RFLP products. This relatedness between the two species was supported by sequence analyses of various gene regions (Barve *et al.* 2003, Fatehi *et al.* 2003, Peever *et al.* 2007).

A section that has been the subject of continuous discussion is *Plenodomus*. In the mid-70s, the genus *Plenodomus* was incorporated into *Phoma* as *Phoma* section *Plenodomus* (Boerema *et al.* 1981). Presently 32 taxa are accommodated in this section (Boerema *et al.* 1994, 1996, 2004, Torres *et al.* 2005b). Already in 1964, even before the official recombination of this group of fungi into the genus *Phoma*, Boerema and van Kesteren (1964) questioned the link between *Plenodomus lingam* and the genus *Phoma* due to differences in pycnidial development. Later, the authors justified the recombination based on the similarity in conidiogenesis: both taxa produced conidia from unicellular, flask-shaped phialides, a feature that is considered to be specific for the genus *Phoma* (Boerema *et al.* 1981). This decision arose again as point of discussion in 1997, when molecular data of the ITS-region revealed that *Ph. lingam* and its relative *Ph. wasabiae* were only distantly related to other phomoid fungi (Reddy *et al.* 1998). This led to the hypothesis that the former genus *Plenodomus* had been incorrectly reduced to synonymy, and should be reinstated (Reddy *et al.* 1998). This idea agrees with other, more recent studies using other genes (Pethybridge *et al.* 2004, Torres *et al.* 2005b, Schoch *et al.* 2006), although the interspecific variation in *Plenodomus*-linked *Leptosphaeria* species is relatively high (Morales *et al.* 1995, Câmara *et al.* 2002).

The synanamorphs and teleomorphs of *Phoma* have been poorly investigated thus far, except for the *Ph. lingam* complex. Few synanamorph relationships have thus far been confirmed by means of molecular techniques. Arenal *et al.* (2000, 2004) demonstrated *Ph. epicoccina* and *Epicoccum nigrum* to be synanamorphs by employing ITS sequence data, and synonymised these species after further microscopical studies.

Towards a species concept for *Phoma*

Numerous *Phoma* strains have been identified and characterised using mainly morphological characters *in vitro*. As in most other fungal genera, these characters include the size and shape of conidiomata, conidia and chlamydospores, but also metabolite production and growth rates and patterns on various agar media.

Characters relating to pycnidial conidiomata are mainly used for sectional differentiation (Figure 2.2). Pycnidia are highly variable in shape and size, but in most species they are either globose or subglobose, or sometimes pyriform due to an elongated neck (section *Plenodomus*). In older cultures the pycnidia may aggregate. The colour varies per species from yellowish to brown olivaceous or olivaceous black and depends on the culturing conditions and age. Occasionally the pycnidia are setose as in section *Paraphoma* (Figure 2.2B), or pilose as in section *Pilosa* (Figure 2.2C). Single or multiple ostioles may be observed, although in some species (sections *Sclerophomella* and *Plenodomus*) pycnosclerotia are found (Figure 2.2D).

In contrast to most sections that produce thin-walled pseudoparenchymatous pycnidia, the species in *Sclerophomella* are characterised by the ability to produce thick-walled conidiomata, whereas species in section *Plenodomus* produce scleroplectenchyma in the pynidial wall. The presence of multicellular chlamydospores (Figure 2.3) is often a good indication that a strain belongs to the section *Peyronellaea*, although this might not always be the case. The shape of these chlamydospores is in most cases comparable to the multicellular conidia of *Alternaria* spp., so-called alternarioid or alternarioid-botryoid (Figure 2.3A–D), but in some species pseudoscleroid or epicoccoid chlamydospores can be found (Figure 2.3E and 3I respectively). The formation of unicellular chlamydospores (Figure 2.3F–H) is not regarded as characteristic for any particular section, but can aid identification at the species level. In the same way the characters of

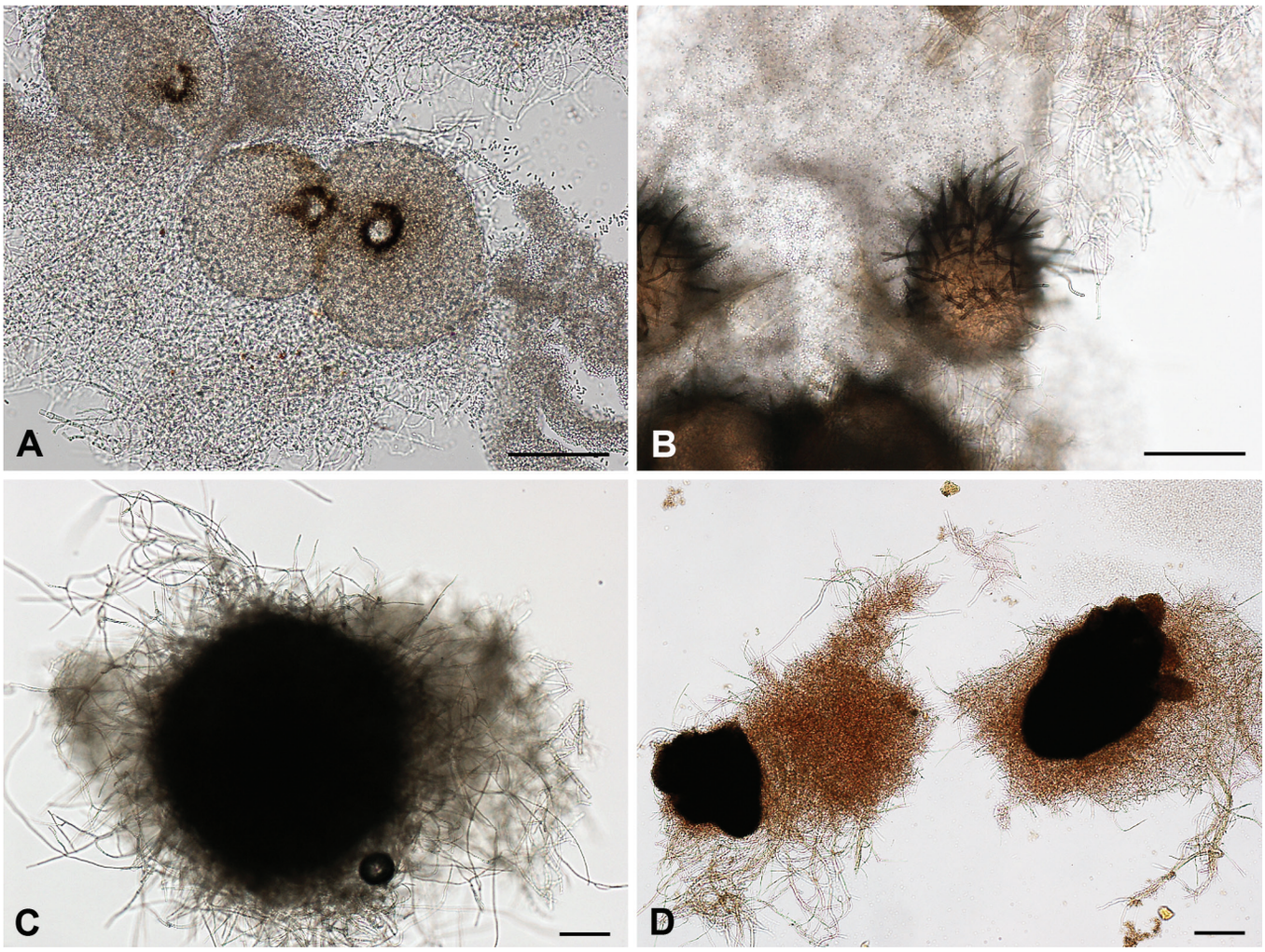

Figure 2.2. A–D. Pycnidial types. A. Regular glabrous as in *Ph. herbarum* B. Setose as in *Ph. carteri*. C. Pilose as in *Ph. betae*. D. Pycnosclerotia-like pycnidia as in *Ph. incompta*. Scale bars = 100 µm.

swollen hyphae, commonly regarded as ancestral to chlamydospores, are regarded as informative (Boerema 1993). Conidia are formed in pycnidia, and conidial shape and size are regarded as the most useful indicators for determining a strain up to species level, but are also used for differentiating some sections (Figure 2.4). The average conidium measures *ca.* 2.5–10 × 1–3.5 µm. However, in the section *Macrospora* enlarged conidia occur, which are up to 25 × 9 µm (Figure 2.4K–L). The section *Heterospora* comprises species that posses both normal sized conidia as well as so-called macroconidia (Figure 2.4I–J). Especially in these sections there might be a high variability in conidial size, even within a single species. Studying cultures *in vitro* should be done under standardised conditions: cultures grown on different media and under different conditions may prove to be highly variable (Rai 2000). Besides conidial measurements, conidial shape is of primary importance. Guttule number and size may provide additional valuable characters for species identification. The section *Phyllostictoides* (Figure 2.4E–H) can be distinguished from the section *Phoma* (Figure 2.4 A–D) by the presence of a low percentage of septate conidia in pure culture. The septation ratio is highly variable, even within species and can be influenced by the growth media. Therefore, this character is often regarded as uninformative (Onfroy *et al.* 1999). *In vivo*, the ratio of septate conidia may be up to 95 %, which has led to many misidentifications in *Ascochyta* in the past (Boerema & Bollen, 1975), and a large number of synonyms for *Phoma* species in *Ascochyta* (van der Aa *et al.* 2000).

Growth characteristics on media, such as growth rate, pigment formation and colony outline and pattern can also aid identification. The media types that are most intensively applied in *Phoma* identification include oatmeal agar, malt extract agar and, to a lesser extent, cherry decoction agar (for protocols see Boerema *et al.* 2004). Boerema *et al.* (2004) provide measurements of the diameter of *Phoma* colonies after 7 d of incubation at 20–22 ºC in complete darkness, and complete colony descriptions after a further

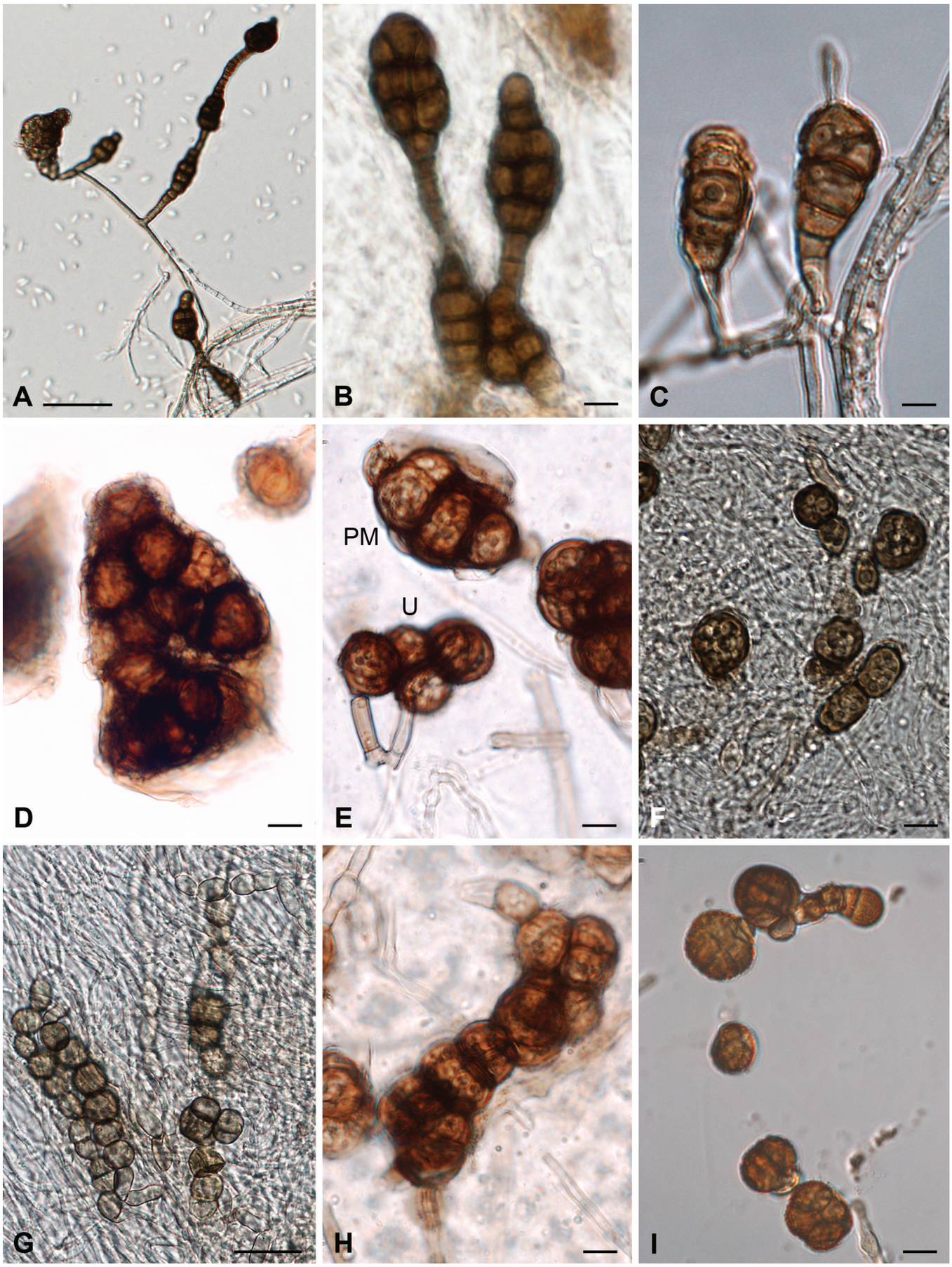

Figure 2.3. A–I. Chlamydospore morphology in *Phoma*. A. Chain of chlamydospores of *Ph. glomerata*. B. Alternaroid chlamydospores of *Ph. glomerata*. C. Alternaroid chlamydospores of *Ph. jolyana*. D. Botryoid chlamydospore as in *Ph. sorghina*. E. Unicellular (u) and pseudosclerotoid multicellular chlamydospores (pm) in *Ph. chrysanthemicola*. F. Unicellular chlamydospores of *Ph. pinodella*. G. Chain of unicellular chlamydospores in *Ph. clematidina*. H. Chain of unicellular chlamydospores in *Ph. chrysanthemicola*. I. Epicoccoid chlamydospores in *Ph. epicoccina*. Scale bars: A = 100 µm, G = 50 µm, B–F and H–I = 10 µm.

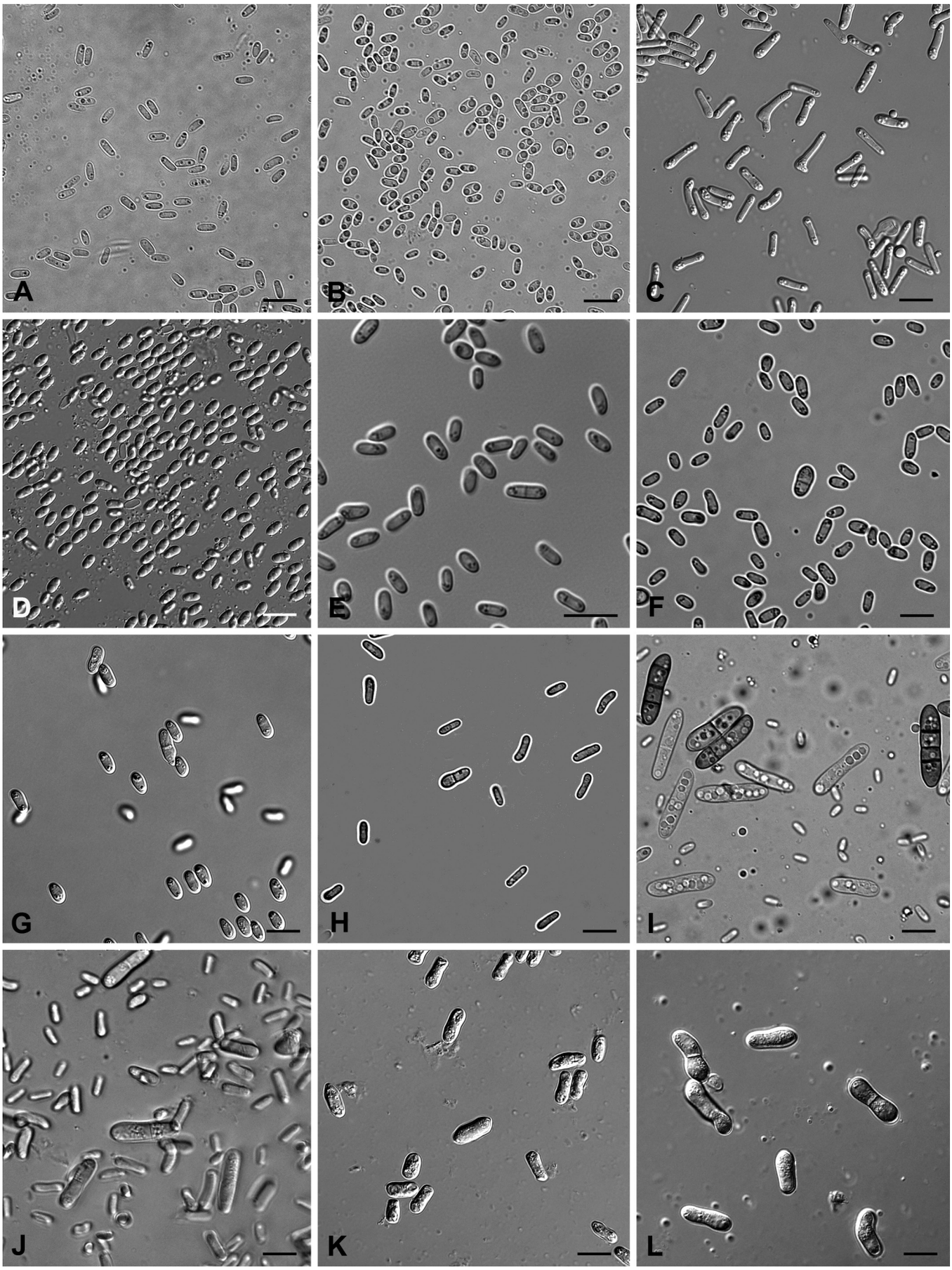

Figure 2.4. A–L. Conidial morphology in *Phoma*. A–D. Conidia hyaline, aseptate small-sized as in A. *Ph. herbarum* B. *Ph. multirostrata* C. *Ph. astragali* and D. *Ph. eupyrena*. E–H. Conidia hyaline, occasionally septate, small-sized as in E. *Ph. exigua* F. *Ph. cucurbitacearum* G. *Ph. macrostoma* and H. *Ph. polemonii*. I–J. Conidia generally hyaline small-sized, but always also large septate and often pigmented condia occur as in I. *Ph. actaeae* and J. *Ph. schneiderae*. K–L. Conidia hyaline, occasionally septate, but all relatively large as in K. *Ph. rabiei* and L. *Ph. xanthina*. Scale bars = 10 μm.

incubation period of 7 d at the same temperature, but at a UV : dark interval of 11:13 (Boerema *et al.* 2004). Unfortunately, within a single species, wide variability may be observed, especially the presence of sector formations as mycelial or pycnidial zones. Again, standardisation is essential, as slight alterations in the media may be the cause of observed differences in growth characteristics (Rai 2000).

The use of biochemical reactions and physiological tests to indicate the presence of certain metabolites was common practice in *Phoma* systematics (Wollenweber & Hochapfel 1936, Dennis 1946; Boerema & Höweler 1967, Dorenbosch 1970, Monte *et al.* 1990, 1991, Noordeloos *et al.* 1993). The application of alkaline reagents (KOH, NaOH) on fresh cultures is still used as it may change the colour of pH dependent metabolites and pigments (Boerema & Höweler 1967, Dorenbosch 1970). Furthermore, the biochemical analysis of dendritic crystals formed in older cultures can be used for the identification of a strain up to species level (Noordeloos *et al.* 1993). Although the development of such techniques in mycology is not yet fully optimised, the interest in such methods for species identification is decreasing as such specific methods can only be applied on a limited scale. Instead, molecular labs are more commonly equipped to perform more routine and popular DNA-based methods, which are thought to be more consistent.

DNA-based approaches in *Phoma* taxonomy

Modern DNA-based techniques can greatly contribute to identification and taxonomy of fungal species. Nevertheless, the actual number of studies using macromolecular approaches to define new species in *Phoma* and resolve species complexes within this genus is relatively low (e.g. Shoemaker & Brun 2001, Bridge *et al.* 2003, 2004, Torres *et al.* 2005a, b).

To identify strains up to species level, a high level of expertise is required. Nevertheless, misidentifications in morphological taxonomy of *Phoma* species cannot always be avoided, as not all isolates may fully express all the species-specific characters. As a result, up to 2003 almost one fifth of the total number of *Phoma* strains of which sequences were submitted to public sequence databases such as GenBank were found to be incorrectly identified (Bridge *et al.* 2003). This trend is not unique to *Phoma*, however, as this percentage is slowly increasing over the years, and by 2006, close to 27 % of the total public fungal sequences were derived from incorrectly identified strains (Nilsson *et al.* 2006). This may also be due to the existence of so-called species complexes, in which multiple morphologically indistinguishable taxa are occupying the same ecological niche, but are only distantly related from an evolutionary point of view. Further, it seems to have become common practice to sequence fungal strains without even identifying them morphologically (Hyde & Soytong, 2007). Bridge *et al.* (2004) suggested that the nucleotide sequences deposited as *Ph. herbarum* in GenBank probably represent at least two different taxa. Because *Ph. herbarum* is the type species of the genus *Phoma* (Boerema 1964, Morgan-Jones 1988a), it should be unambiguously clarified to which taxon the name *Ph. herbarum* should be applied.

Several other species complexes have been revealed using molecular techniques. In this context the *Ph. lingam* species complex can be mentioned. A natural variance in virulence and pathogenicity within *Ph. lingam* was observed in the United States (Pound 1947) and Canada (McGee & Petrie 1978). These findings were found to occur worldwide, as high genetic diversity amongst isolates of this species was observed by e.g. Johnson & Lewis (1990), Schäfer & Wöstemeyer (1992), Morales *et al.* (1993), Pongam *et al.* (1999), Williams & Fitt (1999), Purwantara *et al.* (2000), and Voigt *et al.* (2001). Molecular typing tools aided in distinguishing two species within *Ph. lingam* (teleomorph: *L. maculans*), which could hardly be separated using solely morphological characters. The teleomorph of the weakly aggressive variant was described as *L. biglobosa* with an unnamed anamorph in the genus *Phoma* (Shoemaker & Brun, 2001). *Leptosphaeria biglobosa* comprises 3 to 5 separate taxa itself, whereas the remaining isolates in *Ph. lingam* also seem to be a heterogeneous assemblage of cryptic taxa (Howlett *et al.* 2001, Mendes-Pereira *et al.* 2003, Barrins *et al.* 2004, Voigt *et al.* 2005).

Other species complexes that have been revealed to require further study include *Ph. cucurbitacearum* and *Ph. exigua*. Several studies on the population structure of *Didymella bryoniae*, the teleomorph of *Ph. cucurbitacearum*, revealed the presence of at least two separate subgroups within the American population (Somai *et al.* 2002a, Kothera *et al.* 2003). Supporting the theory of van der Aa *et al.* (2000) on the existence of 11 varieties within *Ph. exigua*, Abeln *et al.* (2002), using AFLP-data, showed a widely distributed variance within *Ph. exigua* var. *exigua*. New, unnamed variants of this taxon appear to represent some serious pathogens, such as a recently discovered pathogen on lettuce (Koike *et al.* 2006). The discovered species complexes are probably just the tip of an iceberg, as most species have been studied less intensively. Based

on their variable appearance, it can also be assumed that species such as *Ph. complanata*, *Ph. macrostoma* and *Ph. leveillei* are heterogeneous assemblages of multiple taxa.

Recently, a phylogenetic approach based on molecular data for the classification of fungi has aided in the discovery of two new species. This approach consisted of a DNA database, which was compared to several unidentified *Phoma* strains. The described species include *Ph. billsii*, that was isolated from Hawaiian soil samples, and a species from the American West Coast that provisionally - and as shown earlier in this paper, questionably - is described in the genus *Plenodomus* as *Ph. morganjonesii* (Torres *et al.* 2005a,b).

How many species are included in *Phoma*?

The unclear Saccardoan criteria for defining a new *Phoma* species and the tradition of host associated characterisation, together with a large host-range of isolates and the widespread presence of the genus, led to the description of 638 taxa in 1884 (Saccardo 1884). The number of *Phoma* "species" that have been characterised, grew to more than 2 800 in the first half of the past century (Sutton 1980, Monte *et al.* 1991). To date, almost 3 000 *Phoma* epithets have been recorded in MycoBank (Crous *et al.* 2004, Robert *et al.* 2005).

Based on the new morphological methods of identification (Boerema & Bollen 1975), the genus *Phoma* was revised. The recently published identification manual describes 223 specific and infra-specific taxa (Boerema *et al.* 2004). Although this manual describes most of the known plant pathogenic species and common saprobic species, we can assume that a large number of *Phoma* species still have to be (re-) described. Only 2 000 of the approximately 5 000 existing herbarium specimens have been studied by the Boerema and co-workers during the past 45 years, and although many of those could be recombined into other species, the total number of *Phoma* taxa listed in this work is probably incomplete. Ample research has also been conducted on lichenicolous species. At least 14 lichenicolous species could not be cultured, and their morphology is only known directly from the substrate (Hawksworth & Cole 2004). Furthermore, physiological and molecular data of those species are lacking, making a classification in the current *Phoma* taxonomic system uncertain.

A second reason why the present taxonomical system is probably lacking taxa is that a number of strains are still not recognised, as these are solely known under a synanamorph or teleomorph name. In the Identification Manual eight such species are listed, most of them known under their respective *Leptosphaeria* teleomorphs (Boerema *et al.* 2004). Further-more, many species show a high natural variation in shape and size of microscopical structures and cultural characters. Due to phenotypical characters, pathogenicity, host range or virulence, separate species in such complexes are often hard to recognise. The aid of molecular typing tools is thus needed for the differentiation of separate taxa.

On the other hand, the Identification Manual also contains some taxa of which the validity remains debatable. For example, the three intraspecific taxa of *Ph. multirostrata*, the varieties *multirostrata*, *microspora* and *macrospora*, can solely be distinguished based on their conidial dimensions (Boerema 1986). Those different varieties were not accepted by various other authors (Morgan-Jones 1988b, Rai 2000), because the conidial dimensions of the three varieties strongly overlap, and intermediate forms are regularly encountered (Boerema 1986, de Gruyter *et al.* 1993, Boerema *et al.* 2004). Nucleotide sequences from several housekeeping genes of the type strains of the three varieties do not show any differences (Aveskamp, unpubl. data), and the subdivision of *Ph. multirostrata* is therefore questionable.

In the same way as the *in vivo* phenotype may depend on the composition of the substrate, the high intraspecific variation in culture is the type and pH of the media used, as well as the growth temperature. This may have influenced the shape and size of pycnidia, conidia and chlamydospores, growth-rate, pigmentation and colony colour in many descriptive studies (Rai 2000, Onfroy 1999). Furthermore, the difference in condition of structures in herbarium material and freshly collected samples can be confusing in descriptive studies (Shin & Mel'nik 2004), and could have aided in the misunderstanding of generic boundaries.

The state of our knowledge of the systematics of the genus is not much further advanced than it was in the Saccardoan system. An idea of the descriptive work that still has to be carried out is seen in the 40-year study of Boerema and co-workers. Of the 223 taxa they accepted, 110 (almost 50 %) were recombined from other taxa or described as new. As only 40 % of the herbarium species has been re-examined thus far, this indicates how many years of taxonomical work may lie ahead.

The future of *Phoma* research

Currently the concept and limits of *Phoma* are still under debate. Understanding true phylogenetic relations of species currently classified in *Phoma* requires new studies on the type materials and reference strains of its taxa and their related genera, and the generation of both phenotypic and genetic data. The strains present in the fungal collections in the world should be combined for an intensive study based on the morphology and genotypic characters of the taxa within the genus complex to obtain a better understanding of the genus, and to define a sound generic concept. At the species level much research needs to be conducted on the species complexes, which in some cases can best be explained as a recent speciation event. It must always be kept in mind that strains that look alike, and that are assigned to as the same species, might quite well be phylogenetically distinct. This is certainly the case within the subclassification of *Phoma*, for example in the sections *Phyllostictoides*, *Heterospora* and *Phoma*. But as is shown at the generic level, it might also be the other way around: morphologically distinct taxa that only recently have evolved from a shared common ancestor. Understanding how speciation in *Phoma* occurs will be one of the main challenges for the future. In the apparently closely related genus *Ascochyta*, the mating type locus has been characterised in two species: *A. lentis* and *A. rabiei* (Barve *et al.* 2003, Chérif *et al.* 2006). The mating type related genes have proven to be highly informative in reconstructing phylogeny (Barve *et al.* 2003), probably because these are much more involved in the speciation process than the house-keeping genes and the nrDNA loci, which are more commonly used in phyogenetical studies. Also the mating type sequence of *Leptosphaeria maculans*, teleomorph of *Phoma lingam*, has been determined (Cozijnsen & Howlett 2003), and was valuable in phylogenetic reconstruction (Voigt *et al.* 2005).

To solve the problems in identification of isolates found at various ports of import and border controls, a comprehensive taxonomic system is urgently required to provide rapid identifications to aid in distinguishing plant pathogenic quarantine organisms from saprobic species and opportunists. The development of a polyphasic identification tool, based on a good collection of strains is needed to aid proper identification of isolates. One of the most promising initiatives that can aid in this is DNA-barcoding (Hebert *et al.* 2002), and the subse-quent development of microcodes (Summerbell *et al.* 2005), an initiative in which a short species-specific nucleotide sequence is sought for each taxon. This approach may be of high value to invasive species identification (Armstrong & Ball 2005). Recently, a joint programme was initiated between the CBS Fungal Biodiversity Centre and the Dutch Plant Protection Service to develop a new *Phoma* identification method and database, mainly based on this DNA-barcoding concept. The crux of this project is, however, to fully understand the species concepts before taxon-specific nucleotide sequences can be identified.

CHAPTER 3

DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties

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Summary

Species of the anamorph genus *Phoma* are commonly isolated from a wide range of ecological niches. They are notoriously difficult to identify due to the paucity of morphological features, and the plasticity of these when cultivated on agar media. Species linked to *Phoma* section *Peyronellaea* are typified by the production of dictyochlamydospores, and thus have additional characters to use in taxon delineation. However, the taxonomy of this section is still not fully understood. Furthermore, the production of such chlamydospores is also known in some other sections of *Phoma*. To clarify the phylogeny of *Phoma* taxa that produce dictyochlamydospores, DNA sequences were generated from three loci, namely ITS, actin, and β-tubulin. Results obtained were unable to support section *Peyronellaea* as a taxonomic entity. Dictyochlamydospore formation appears to be a feature that developed, or was lost, multiple times during the evolution of *Phoma*. Furthermore, based on the multi-gene analyses obtained, five new *Phoma* species could be delineated, while a further five required taxonomic revision to be consistent with the genetic variation observed.

Keywords: actin, β-tubulin, coelomycetes, dictyochlamydospores, ITS, multi-gene phylogeny, taxonomy.

Introduction

Although the genus *Phoma* Sacc. emend Boerema & Bollen is widely distributed and omnipresent, it is still poorly understood, and generally considered to be a taxonomically difficult group of mitosporic ascomycetes. *Phoma* is characterised by the production of single-celled, hyaline conidia in monophialidic, doliiform to flask-shaped conidiogenous cells in thin-walled pycnidia (Boerema & Bollen 1975). The present concept, however, also includes species that produce thick-walled pycnidia, or form septate conidia in addition to continuous conidia in pure culture (Boerema 1997, Boerema *et al.* 2004). Specimens have mainly been isolated from soil and from a wide range of plant hosts where they reside as primary pathogens, opportunists, saprobes or endophytes (Chapter 2). The existing subgeneric classification was defined by Boerema (1997) after a 40-year study of the morphological characters. The genus was divided into nine sections that are based on their morphological appearance: *Phoma*, *Heterospora*, *Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Sclerophomella*, *Plenodomus*, *Macrospora* and *Pilosa*. Although this subdivision is extremely helpful in identifying strains up to species level, it remains artificial, as several taxa exhibit features that are representative of different sections.

One of the most confusing sections in this regard is *Peyronellaea*, even though it has been intensively studied in the past (Boerema 1993, Boerema *et al.* 1965a, 1968, 1971, 1973, 1977, Morgan-Jones & Burch 1987, Morgan-Jones & White 1983, White & Morgan-Jones 1983, 1986, 1987). *Peyronellaea* was incorporated into the genus *Phoma* in 1990 (van der Aa *et al.* 1990). It accommodates fungi producing pycnidial conidiomata with phialidic conidiogenous cells as well as dictyochlamydospores, having both transverse and longitudinal septa. Three types of dictyochlamydospores are distinguished: (i) alternarioid to irregular botryoid, i.e. those that resemble the conidia of the genus *Alternaria*, and often also developing in chains (Luedemann 1961), (ii) epicoccoid, resembling the conidia of the genus *Epicoccum*, and (iii) pseudosclerotioid, resembling pseudosclerotia, often developing in aggregates of many unicellular chlamydospores (Boerema *et al.* 2004).

The section currently comprises 12 species and five infraspecific taxa (Boerema *et al.* 2004). However, several species accommodated in other sections of *Phoma* are also capable of producing comparable chlamydospores, including *Ph. gardeniae* (sect. *Paraphoma*), *Ph. clematidina* and *Ph. narcissi* (sect. *Heterospora*), *Ph. multirostrata* and *Ph. eupyrena* (sect. *Phoma*) and *Ph. zeae-maydis* and *Ph. boeremae* (sect. *Macrospora*). Furthermore, *Ph. exigua* has been previously erroneously included in sect. *Peyronellaea*, but in contrast to the species mentioned above, it does not produce multicellular chlamydospores (Boerema *et al.* 1977).

Several taxa that are currently incorporated in *Phoma* sect. *Peyronellaea* have features in common with other sections, or even with other genera. For example, two species from North America, *Ph. americana* and *Ph. subglomerata*, are characterised by the incidental production of uniseptate conidia, a key character for species placed in *Phoma* sect. *Phyllostictoides* (van der Aa *et al.* 1990). Furthermore, one species, *Ph. epicoccina*, produces thick-walled poroid pycnidia resembling those of *Phoma* sect. *Sclerophomella* (Boerema & de Gruyter 1998). Some strains of the type species of this section, *Ph. glomerata*, produce conidia that become pigmented after maturation. This feature is uncommon in *Phoma* and is actually regarded as a character of *Microsphaeropsis, Coniothyrium* or *Paraconiothyrium* (Verkley *et al.* 2004, Damm *et al.* 2008).

In the present paper we studied the genetic and morphological diversity among the taxa currently accommodated in *Peyronellaea*. A further aim was to clarify the phylogenetic relation with several chlamydospore-producing species currently accommodated in other sections, or that still remain to be described.

Materials and Methods

Culture and morphological studies

A total of 122 strains (Table 3.1) were obtained from the culture collections of CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), PD (Plant Protection Service, Wageningen, The Netherlands), IMI (International Mycological Institute, Kew, UK) and LEV (Plant Health and Diagnostic Station, Auckland, New Zealand). Freeze-dried strains were revived overnight in 2 mL malt/peptone (50/50

%) liquid medium. Subsequently, cultures were transferred and maintained on oatmeal agar (OA, Gams *et al.* 2007) at 10 °C and in complete darkness. Morphological studies of the strains were performed on OA, malt extract agar (MEA) and cherry decoction agar (CHA, Gams *et al.* 2007). Cultures were incubated as described in Boerema *et al.* (2004). Eight days after inoculation, the colony growth was measured. Colony colours were rated 15 d after incubation using Rayners' colour chart (Rayner 1970). Morphological features were studied following sporulation. Fungal structures were mounted in tap water using a scalpel blade and examined under a Nikon 80i light microscope. Sizes of the various structures were determined by averaging the measurements of 30 examples of each structure, except for conidiogenous cells, of which the size range was estimated based on *ca.* five structures. Fifth and 95th percentiles were determined for all measurements and are provided in parentheses. By application of a droplet of 1N NaOH, the production of metabolite E+ was determined (Dorenbosch 1970, Noordeloos *et al.* 1993). The structure of the pycnidial wall and shape of conidiogenous cells were studied using microtome sections of 6 µm thickness, prepared with a Leica CM3050 freezing microtome and mounted in lactic acid. Taxonomic novelties and descriptions were deposited in MycoBank (www.mycobank.org, Crous *et al.* 2004).

Molecular studies – DNA extraction, PCR and sequencing

Actively growing mycelium was scraped from culture plates and transferred to 2 mL collection tubes from the UltraClean™ Microbial DNA Kit (MoBio Laboratories Inc., Carlsbad, California). DNA isolation was carried out according to the manufacturer's recommendations. Obtained DNA samples were checked for purity and integrity by gel electrophoresis, after which the samples were diluted 10× and stored at 4 °C before further handling. The ITS1-5.8S-ITS2 region (ITS) of the nuclear ribosomal DNA operon was amplified using the V9G (De Hoog and Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990) primer pair. The actin gene (ACT) was partly amplified using primer pair ACT-512F and ACT-783R (Carbone and Kohn 1999). Two newly designed primers, TUB2Fd (5′- GTB CAC CTY CAR ACC GGY CAR TG – 3′) and TUB4Rd (5′ - CCR GAY TGR CCR AAR ACR AAG TTG TC – 3′) were used to amplify a part of the β-tubulin (TUB) gene. For the two housekeeping genes ACT and TUB, each PCR reaction had a total volume of 12.5 µL and contained 0.5 µL of 10× diluted gDNA, 1× PCR Buffer, 2 mM $MgCl_2$, 100 µM of each of the dNTP's, 0.2 µM of each of the primers, and 0.5 units of *Taq* DNA polymerase (Bioline, Luckenwalde, Germany). The reaction mixture prepared for ITS amplification was similar, except for a double concentration of dNTP's. The polymerase chain reactions (PCRs) were conducted in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) using an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation (95 °C for 30 s), annealing (48 to 55 °C for 30 s depending on the locus) and extension (72 °C for 80 s). The final extension phase was conducted at 72 °C for 7 min. Annealing temperatures varied per reaction, and were set at 48 °C, 52 °C, and 55 °C for ITS, TUB and ACT respectively.

Both strands of the amplified DNA fragments were sequenced using the same PCR primers and the BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's recommendations. The sequence products were purified using a 96-well multiscreen HV plate (Millipore, Billerica, Massachusetts) and Sephadex G-50 superfine columns (Amersham Biosciences, Roosendaal, The Netherlands). The products were analyzed on an ABI Prism 3700 DNA Sequencer (Applied Biosystems). A consensus sequence was assembled from the forward and reverse sequences with the BioNumerics v. 4.5 software package (Applied Maths, St-Martens-Lathem, Belgium). Obtained sequences were deposited in GenBank under accession numbers as listed in Table 3.1.

Phylogenetic analysis

The consensus sequences were aligned using BioNumerics and adjusted by hand where necessary. The best nucleotide substitution models were determined using MrModeltest v. 2.2 (Nylander 2004). A Bayesian tree inference (BI) analysis was performed with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001). One tree was saved per 100 generations and the run was automatically ended when the standard deviation of split frequencies was below 0.01. To avoid suboptimal trees being taking into account for the consensus tree, a 'burn-in' of 25 % of the saved trees was used. The resulting '50 % majority rule consensus' trees were printed with TreeView v. 1.6.6 (Page 1996) and are lodged with TreeBASE (www.treebase.org).

To obtain further evidence for branch supports, a series of Neighbour-Joining (NJ) analyses was conducted in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) with the uncorrected ("p"),

the Kimura-2-parameter and the HKY85 substitution models. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Ties encountered were broken randomly.
A third measure of branch support was obtained by conducting a Maximum Likelihood (ML) analysis using RAxML (Randomized Axelerated Maximum Likelihood) software (Stamakis *et al.* 2008) through the CIPRES website (www.phylo.org). The same three partitions were used as in the BI and NJ tests, but as RAxML only implements the GTR substitution model, the symmetrical model for the ITS partition was waived here. The robustness of the obtained trees in the NJ and ML analyses was evaluated by 1000 bootstrap replications.

To test whether the three different loci could be used in a combined analysis, phylogenies were estimated using maximum likelihood analyses for each data partition (ML bootstrap values > 70 %), and compared by eye for congruency. Congruence of these trees was further determined using the Shimodaira-Hasegawa test (SH test, Shimodaira & Hasegawa 1999), which is implemented in PAUP. The topology of the concatenated ML tree was compared to the topology of the ML trees obtained for each partition in a one-tailed bootstrap test using 1000 replications with full likelihood maximization to determine whether the trees were significantly different.

The SH test was also used to determine whether the species that are currently linked with *Phoma* section *Peyronellaea* represent a monophyletic group. Therefore, a constraint tree in which such a phylogeny was simulated was compared to the consensus tree obtained from the RAxML analysis of the ITS dataset. Subsequently, these trees were compared as described above.

Results

ITS phylogeny

Due to alignment difficulties of the house keeping genes, two alignments of DNA sequences were subjected to phylogenetic analyses. The first alignment consisted of 122 ITS sequences generated in this study and six obtained from GenBank. This ITS alignment consisted of 566 characters including alignment gaps, of which 237 were variable and 329 were constant. A GenBank sequence of *Pyrenochaeta romeroi* (DQ836802) was used as outgroup. The BI analysis was run using the 'best' model and parameters as determined, which were the symmetrical (SYM) substitution model with inverse gamma rates and equal dirichlet base frequencies. The temperature value set at 0.4. The analysis run of the ITS sequence matrix in MrBayes resulted in 11 039 trees, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated. The topology and support values of the BI tree were in congruence with those of the trees obtained by NJ and the optimal tree obtained in the ML analysis. The reconstructed phylogeny using the ITS dataset revealed 16 heterogenous strains to be a paraphyletic basal assemblage to a major clade consisting of 106 strains (Figure 3.1). The majority of the taxa belonging to *Phoma* sect. *Peyronellaea* were found in this major clade (support values 1, 100 % and 95 % for BI posterior probability, NJ and ML bootstrap supports respectively), although also several type species of other *Phoma* sections were accommodated here, such as *Ph. herbarum* (section *Phoma*), *Ph. exigua* var. *exigua* (section *Phyllostictoides*) and *Ph. zeae-maydis* (sect. *Macrospora*). Further, the *Peyronellaea* species *Ph. chrysanthemicola* and *Ph. violicola* were located amongst the basal lineages, indicating that the section *Peyronellaea* does not represent a monophyletic clade. This is supported by the SH test conducted on the ITS dataset, in which the hypothesis that the obtained tree in Figure 3.1 is in congruence with monophyly of *Peyronellaea* is rejected ($P < 0.01$).

Table 3.1. List of strains used, with their country of origin and the host. Sequences derived from other studies are marked in bold.

Species	Collection number[1]	Source	Origin	GenBank Accession Numbers		
				ACT	ITS	TUB
Phoma americana	CBS 185.85	*Zea mays*	USA	FJ426870	FJ426972	FJ427088
	CBS 256.65	*Phaseolus vulgaris*	Denmark	FJ426871	FJ426973	FJ427089
	CBS 568.97	*Glycine max*	USA	FJ426872	FJ426974	FJ427090
	CBS 112525	*Triticum aestivum*	Argentina	FJ426873	FJ426975	FJ427091
	IMI 361195	—	—	FJ426874	FJ426976	FJ427092
	PD 78/1089	*Zea mays*	South-Africa	FJ426875	FJ426977	FJ427093
	PD 79/58	*Sorghum vulgare*	Nigeria	FJ426876	FJ426978	FJ427094
	PD 80/1143	*Zea mays*	USA	FJ426877	FJ426979	FJ427095
	PD 82/1059	Nematode cyst	—	FJ426878	FJ426980	FJ427096
Ph. betae	CBS 523.66	*Beta vulgaris*	Netherlands	—	FJ426981	—
	—	*Beta vulgaris* var. *cicla*	Italy	—	**EU003450**	—
Ph. boeremae	CBS 109942	*Medicago littoralis*	Australia	FJ426879	FJ426982	FJ427097
Ph. calidophila	CBS 448.83	Desert soil	Egypt	FJ426948	FJ427059	FJ427168
	PD 84/109	*Cucumis sativus*	Europe	FJ426949	FJ427060	FJ427169
Ph. calorpreferens	CBS 109.92	Food	Netherlands	FJ426880	FJ426983	FJ427098
Ph. chrysanthemicola	CBS 172.70	*Chrysanthemum morifolium*	Germany	—	FJ426984	—
	CBS 522.66	*Chrysanthemum morifolium*	UK	—	FJ426985	—
	PD 87/153	*Cichorium intybus*	Netherlands	—	FJ426986	—
	PD 92/468	—	—	—	FJ426987	—
Ph. clematidina	CBS 102.66	*Clematis* sp.	UK	FJ426881	FJ426988	FJ427099
	CBS 108.79	*Clematis* sp.	Netherlands	FJ426882	FJ426989	FJ427100
	CBS 195.64	*Clematis jackmannii*	Netherlands	FJ426883	FJ426990	FJ427101
	CBS 201.49	*Clematis* sp.	Netherlands	FJ426884	FJ426991	FJ427102
	CBS 520.66	*Selaginella* sp.	Netherlands	FJ426885	FJ426992	FJ427103
Ph. coffeae-arabicae	CBS 123380	*Coffea arabica*	Ethiopia	FJ426886	FJ426993	FJ427104
	CBS 123398	*Coffea arabica*	Ethiopia	FJ426887	FJ426994	FJ427105
Ph. epicoccina	CBS 125.82	Human	Netherlands	FJ426888	FJ426995	FJ427106
	CBS 173.73	*Dactylis glomerata*	USA	FJ426889	FJ426996	FJ427107
	CBS 505.85	Soil	Germany	FJ426890	FJ426997	FJ427108
	CBS 115825	*Malus* sp.	Netherlands	FJ426891	FJ426998	FJ427109
Ph. eupyrena	CBS 374.91	*Solanum tuberosum*	Netherlands	FJ426892	FJ426999	FJ427110
	CBS 527.66	Wheat field soil	Germany	FJ426893	FJ427000	FJ427111
Ph. exigua var. *exigua*	CBS 431.74	*Solanum tuberosum*	Netherlands	**EU880854**	FJ427001	FJ427112
Ph. exigua var. *exigua*	CBS 118.94	*Phaseolus vulgaris*	Netherlands	—	**EU167567**	—
Ph. gardeniae	CBS 302.79	Air sample	Netherlands	FJ426894	FJ427002	FJ427113
	CBS 626.68	*Gardenia jasminoides*	India	FJ426895	FJ427003	FJ427114
Ph. glomerata	CBS 133.72	Church wall-fresco	Romania	FJ426896	FJ427004	FJ427115
	CBS 284.76	*Populus nigra*	Russia	FJ426897	FJ427005	FJ427116
	CBS 287.76	*Rubus idaeus*	Russia	FJ426898	FJ427006	FJ427117
	CBS 288.76	*Populus alba*	Russia	FJ426899	FJ427007	FJ427118
	CBS 289.76	*Allium nutans*	Russia	FJ426900	FJ427008	FJ427119

Table 3.1. (Ctd).

Species	Collection number[1]	Source	Origin	GenBank Accession Numbers		
				ACT	ITS	TUB
	CBS 290.76	*Ribes nigrum*	Russia	FJ426901	FJ427009	FJ427120
	CBS 293.36	*Solanum tuberosum*	Germany	FJ426902	FJ427010	FJ427121
	CBS 304.49	*Lycopersicon esculentum*	Netherlands	FJ426903	FJ427011	FJ427122
	CBS 464.97	Indoor (Bathroom)	Netherlands	FJ426904	FJ427012	FJ427123
	CBS 528.66	*Chrysanthemum* sp.	Netherlands	FJ426905	FJ427013	FJ427124
	CBS 834.84	*Hordeum sativum*	Germany	FJ426906	FJ427014	FJ427125
	CBS 120109	*Juniperus* sp.	USA	FJ426907	FJ427015	FJ427126
	CBS 112448	Indoor environment	Germany	FJ426908	FJ427016	FJ427127
	—	*Rosa* sp.	Mexico	—	**AY904060**	—
	PD 73/1415	*Heracleum* sp.	Russia	FJ426909	FJ427017	FJ427128
	PD 74/1023	Air sample	UK	FJ426910	FJ427018	FJ427129
	PD 77/47	*Medicago sativa*	Netherlands	FJ426911	FJ427019	FJ427130
	PD 81/767	*Cucumis sativus*	—	FJ426912	FJ427020	FJ427131
	PD 83/782	*Capsicum* sp.	—	FJ426913	FJ427021	FJ427132
Ph. herbarum	CBS 615.75	*Rosa multiflora*	Netherlands	**EU880896**	FJ427022	FJ427133
	—	Rhizosphere of *Picea mariana*	Canada	—	**DQ132841**	—
Ph. heteromorphospora	CBS 448.68	*Chenopodium album*	Netherlands	—	FJ427023	—
Ph. infossa	CBS 123394	*Fraxinus pennsylvanica*	Argentina	FJ426914	FJ427024	FJ427134
	CBS 123395	*Fraxinus pennsylvanica*	Argentina	FJ426915	FJ427025	FJ427135
Ph. jolyana	CBS 463.69	*Mangifera indica*	India	FJ426916	FJ427026	FJ427136
	PD 83/326	*Malus sylvestris*	India	FJ426917	FJ427027	FJ427137
Ph. lingam	—	*Brassica napus*	Australia	—	**M96384**	—
Ph. microchlamydospora	CBS 105.95	*Eucalyptus* sp.	UK	FJ426918	FJ427028	FJ427138
	CBS 491.90	Unidentified vegetable crop	UK	FJ426919	FJ427029	FJ427139
Ph. multirostrata	CBS 110.79	*Cucumis sativus*	Netherlands	FJ426920	FJ427030	FJ427140
	CBS 274.60	Soil from poultry farm	India	FJ426921	FJ427031	FJ427141
	CBS 380.67	*Lilium* sp.	—	FJ426922	FJ427032	FJ427142
	CBS 368.65	Soil	India	FJ426923	FJ427033	FJ427143
Ph. multirostrata	CBS 120115	Soil	Puerto Rico	FJ426924	FJ427034	FJ427144
	CBS 120116	Soil	Puerto Rico	FJ426925	FJ427035	FJ427145
	PD 77/508	*Philodendron* sp.	Netherlands	FJ426926	FJ427036	FJ427146
	PD 83/48	*Cucumis sativus*	Netherlands	FJ426927	FJ427037	FJ427147
Ph. narcissi	CBS 251.92	*Nerine* sp.	Netherlands	FJ426928	FJ427038	FJ427148
	PD 71/6	*Ismene* sp.	—	FJ426929	FJ427039	FJ427149
	PD 76/61	*Hippeastrum* sp.	—	FJ426930	FJ427040	FJ427150
	PD 92/1460	*Sprekelia* sp.	Netherlands	FJ426931	FJ427041	FJ427151
Ph. omnivirens	CBS 341.86	*Phaseolus vulgaris*	Belgium	FJ426932	FJ427042	FJ427152
	CBS 654.77	—	India	FJ426933	FJ427043	FJ427153

Table 3.1. (Ctd).

Species	Collection number[1]	Source	Origin	GenBank Accession Numbers		
				ACT	ITS	TUB
	CBS 991.95	Soil	Papua New Guinea	FJ426934	FJ427044	FJ427154
	CBS 992.95	Soil	Papua New Guinea	FJ426935	FJ427045	FJ427155
	CBS 123396	*Chrysanthemum indicum*	Netherlands	FJ426936	FJ427046	FJ427156
	CBS 123397	*Statice* sp.	Tanzania	FJ426937	FJ427047	FJ427157
Ph. paspali	CBS 560.81	*Paspalum dilatatum*	New Zealand	FJ426938	FJ427048	FJ427158
Ph. pimprina	CBS 246.60	Soil	India	FJ426939	FJ427049	FJ427159
	PD 77/1028	Soil	India	FJ426940	FJ427050	FJ427160
Ph. pinodella	CBS 318.90	*Pisum sativum*	Netherlands	FJ426941	FJ427051	FJ427161
	CBS 531.66	*Trifolium pratense*	USA	FJ426942	FJ427052	FJ427162
Ph. pomorum var. *circinata*	CBS 285.76	*Heracleum dissectum*	Russia	FJ426943	FJ427053	FJ427163
	CBS 286.76	*Allium nutans*	Russia	FJ426944	FJ427054	FJ427164
Ph. pomorum var. *cyanea*	CBS 388.80	*Triticum* sp.	South Africa	FJ426945	FJ427055	FJ427165
Ph. pomorum var. *pomorum*	CBS 539.66	*Polygonum tataricum*	Netherlands	FJ426946	FJ427056	FJ427166
	PD 81/592	*Ribes uva-crispa*	Netherlands	FJ426947	FJ427057	FJ427167
Ph. radicina	CBS 111.79	*Malus sylvestris*	Netherlands	—	FJ427058	—
Ph. samarorum	CBS 138.96	*Phlox paniculata*	Netherlands	—	FJ427061	—
	CBS 139.96	*Poa* sp.	Netherlands	—	FJ427062	—
Ph. sancta	CBS 281.83	*Ailanthus altissima*	South Africa	FJ426950	FJ427063	FJ427170
	CBS 644.97	*Opuntia ficus-indica*	Argentina	FJ426951	FJ427064	FJ427171
	LEV 15292	*Gleditsia triacantha*	—	FJ426952	FJ427065	FJ427172
Ph. schachtii	CBS 502.84	*Heterodera schachtii*	Netherlands	—	FJ427066	—
Ph. sorghina	CBS 179.80	*Sorghum vulgare*	Puerto Rico	FJ426953	FJ427067	FJ427173
	CBS 180.80	*Zea mays*	South Africa	FJ426954	FJ427068	FJ427174
	CBS 181.80	*Oryza sativa*	Guinea-Bissau	FJ426955	FJ427069	FJ427175
Ph. sorghina	CBS 293.72	*Panicum miliare*	India	FJ426956	FJ427070	FJ427176
	CBS 301.89	*Lycopersicon esculentum*	Martinique	FJ426957	FJ427071	FJ427177
	CBS 627.68	*Citrus* sp.	France	FJ426958	FJ427072	FJ427178
	CBS 846.68	*Coffea* sp.	India	FJ426959	FJ427073	FJ427179
	CBS 886.95	*Stellaria* sp.	Papua New Guinea	FJ426960	FJ427074	FJ427180
	CBS 986.95	Soil	Papua New Guinea	FJ426961	FJ427075	FJ427181
	PD 76/1025	*Aspidiotus destructor*	India	FJ426962	FJ427076	FJ427182
	PD 81/721	*Pinus* sp.	USA	FJ426963	FJ427077	FJ427183
	PD 88/549	*Lycopersicon esculentum*	Martinique	FJ426964	FJ427078	FJ427184
	PD 03486771	*Triticum* sp.	Netherlands	FJ426965	FJ427079	FJ427185
Ph. subglomerata	CBS 110.92	*Triticum* sp.	USA	FJ426966	FJ427080	FJ427186
	PD 78/1090	*Zea mays*	South-Africa	FJ426967	FJ427081	FJ427187
Ph. violicola	CBS 100272	*Viola tricolor*	New Zealand	—	FJ427082	—

Table 3.1. (Ctd).

Species	Collection number[1]	Source	Origin	GenBank Accession Numbers		
				ACT	ITS	TUB
	CBS 306.68	*Viola tricolor*	Netherlands	—	FJ427083	—
Ph. zantedeschiae	CBS 131.93	*Calla* sp.	Netherlands	FJ426968	FJ427084	FJ427188
	PD 69/140	*Calla* sp.	Netherlands	FJ426969	FJ427085	FJ427189
Ph. zeae-maydis	CBS 588.69	*Zea mays*	USA	FJ426970	FJ427086	FJ427190
	MA 0027	*Zea mays*	USA	FJ426971	FJ427087	FJ427191
Pyrenochaeta romeroi	IP 571.61	Human mycetoma	Senegal	—	DQ836802	—

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; IMI: International Mycological Institute, Kew, UK; IP: Pasteur Institute Collection of Fungi, Pasteur Institute, Paris, France; LEV: Plant Health and Diagnostic Station, Auckland, New Zealand; MA: Culture collection of Maikel Aveskamp, housed at CBS; PD: Plant Protection Service, Wageningen, The Netherlands.

Table 3.2. Log likelihood scores for data partition combinability using the Shimodaira-Hasegawa (SH) test. ($P < 0.05$).

Partition	Constraint	Score (-lnL)	Difference (-lnL)	Probability (P)
Actin	ACT	3353.198	—	—
	Concatenated	323.641	70.444	0.104
β-tubulin	TUB	305637488	—	—
	Concatenated	3103.207	46.832	0.327
ITS	ITS	1668.992	—	—
	Concatenated	1690.181	21.189	0.073

Concatenated phylogeny

The second alignment included 104 taxa, including one outgroup taxon (CBS 560.81 *Ph. paspali*), which was found to be basal to the major clade in Figure 3.1. No strongly conflicting nodes were detected in the phylogenies of the separate loci (ML bootstrap values > 70%). Topologies were congruent for each partition, although ITS showed a lower degree of resolution of the terminal taxa. Also the results of the SH tests (Table 3.2) suggest that the ITS tree differs most from the concatenated tree, although this is not significant ($P = 0.073$). Based on the similarity in topologies and the non-significant SH tests, the partitions used in the second dataset (ITS, ACT, TUB) could be concatenated.

The concatenated alignment had a total length of 1 148 characters (ITS: 500, ACT: 300, TUB: 348) including alignment gaps. Of these characters 381 (ITS: 96, ACT: 156, TUB: 131) were variable and 767 (ITS: 406, ACT: 144, TUB: 217) were constant. The SYM+I+G model was found to be optimal for the ITS partition, whereas the 'best' substitution model for the ACT and TUB sequence matrix was determined to be GTR+I+G. For the BI analysis, the temperature value was set at set at 0.5. The MrBayes run of the second dataset resulted in 3 340 trees, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated (Figure 3.2). Trees supporting the same clades were obtained irrespective of the analysis method used. Further phylogenetic results obtained are discussed below where applicable.

Taxonomy

Most *Peyronellaea* taxa and other chlamydospore-forming species studied here appeared to be properly described in the past. However, five novel dictyochlamydospore-forming species of *Phoma* could be identified in the present study. These species are described below. One species, *Ph. infossa*, was already known to science, but its description is amended as it appeared to produce dictyochlamydospores. Furthermore, five new combinations are proposed.

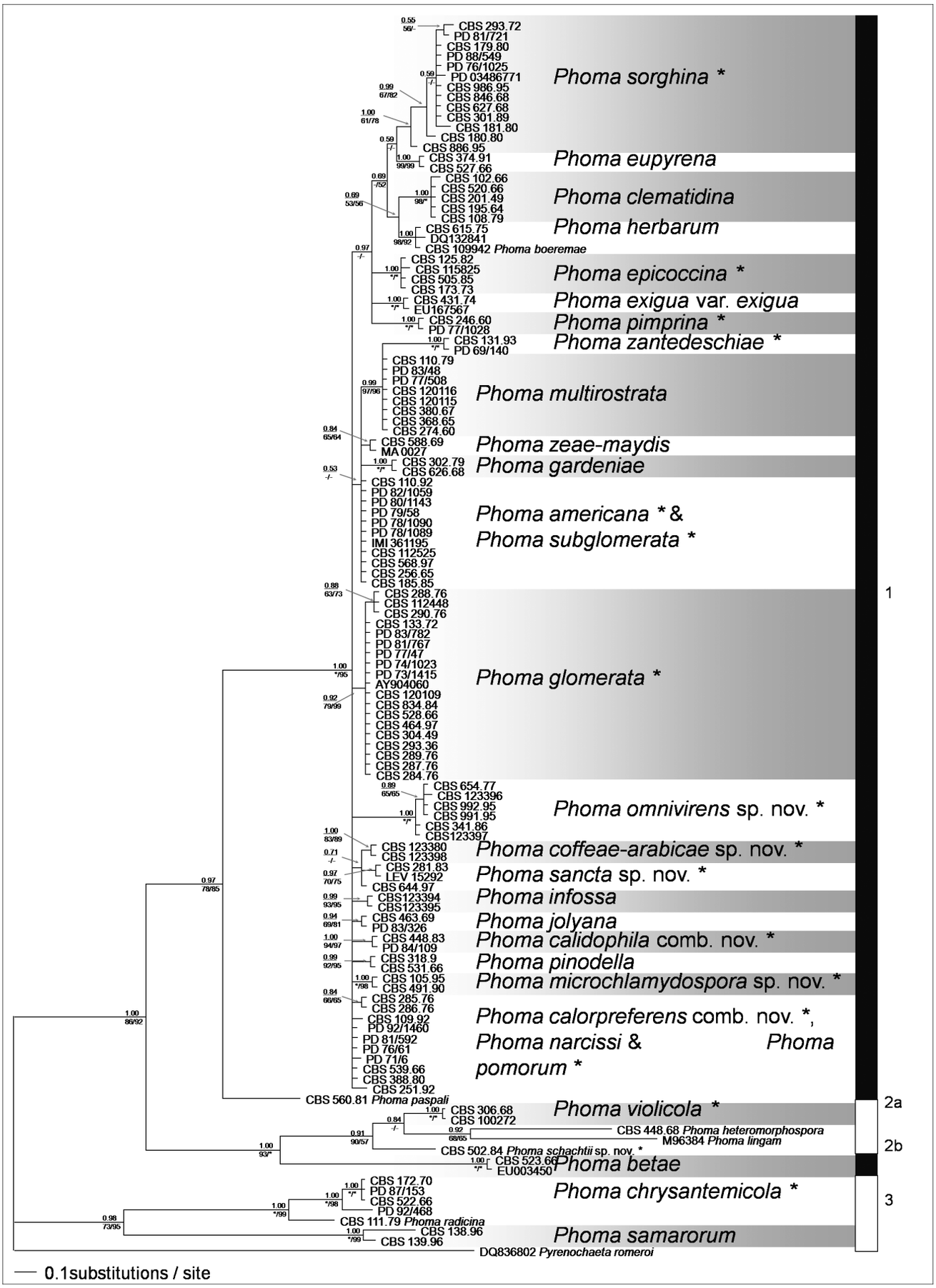

Figure 3.1. Fifty percent majority rule consensus tree from a BI analysis of ITS sequences of *Phoma* sect. *Peyronellaea* (n = 122). At the nodes, the BI Posterior Probabilities are presented above the branch and bootstrap percentages of the NJ analysis using the HKY85 substitution model and ML analysis are given below the branch. Branches that were less than 50 % supported in the NJ and ML analyses are indicated with a hyphen (-), whereas asterisks indicate full support. The bar indicates the number of substitutions per site. The tree is rooted with *Pyrenochaeta romeroi*.

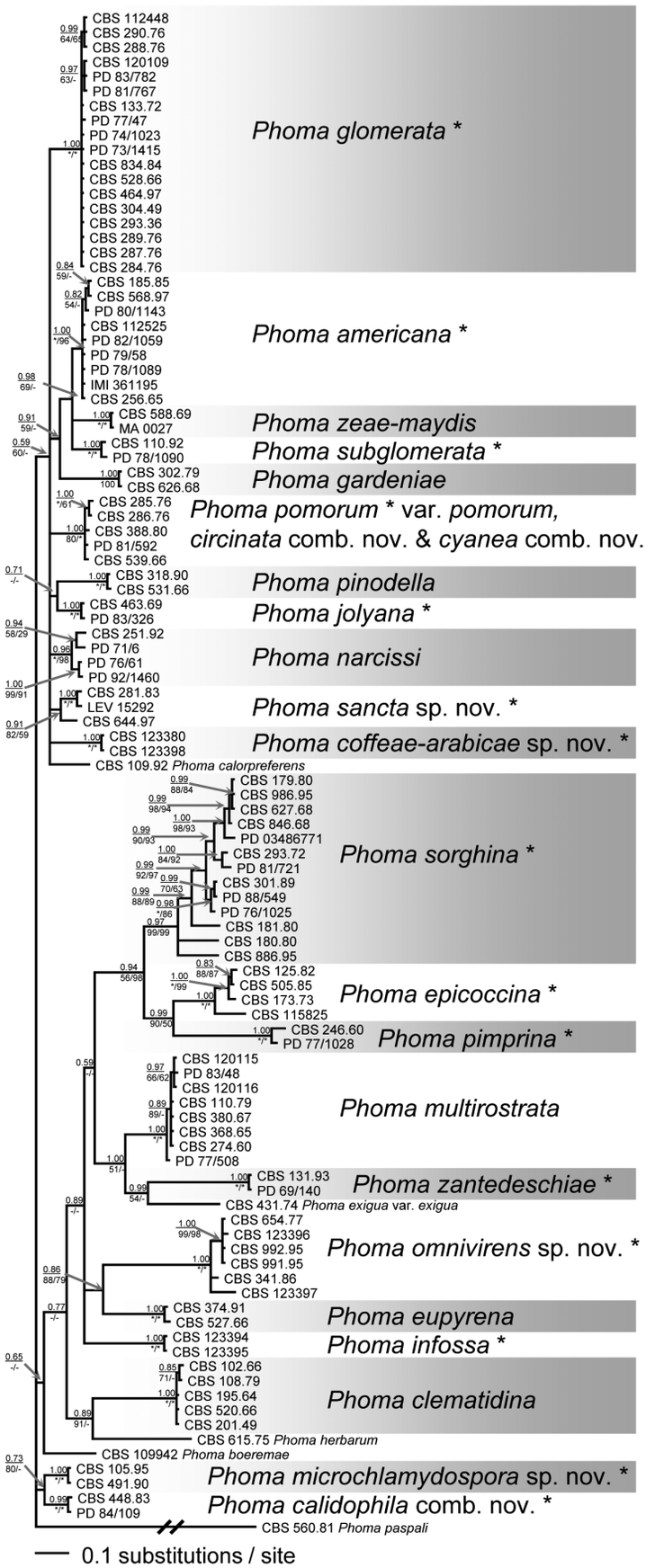

Figure 3.2. Reconstructed phylogeny based on a 50 % majority rule consensus tree of *Phoma* sect. *Peyronellaea* using a BI analysis of 104 concatenated ITS-ACT-TUB sequences. At the nodes, the BI Posterior Probabilities are presented above the branch and bootstrap percentages of the NJ analysis using the HKY85 substitution model and ML analysis are given below the branch. Branches that were less than 50 % supported in the NJ and ML analyses are indicated with a hyphen (-), whereas asterisks indicate full support. The bar indicates the number of substitutions per site. The tree is rooted with *Phoma paspali* CBS 560.81.

Phoma calidophila Aveskamp, Gruyter & Verkley, **nom. nov.** pro *Sphaeronema sahariense* Faurel & Schotter. MycoBank MB512566.
Basionym: *Sphaeronaema sahariense* Faurel & Schotter, *Revue Mycol*. **30**: 156. 1965; not *Phoma sahariensis* Faurel & Schotter, *Revue Mycol*. **30**: 154. 1965.
= *Phoma jolyana* var. *sahariensis* (Faurel & Schotter) Boerema, Dorenb. & Aa apud Boerema, *Versl. Med. Pl. ziektenk. Dienst* **159**: 27. 1983 [‘1982’].

Etymology: Name refers to this species’ preference for warmth.

For detailed descriptions see Boerema (1983, 1993).

Specimen examined: **Egypt**, from desert soil, Feb. 1980, *M.I.A. Abdel-Kader*, **neotype** designated here, CBS H-20168, ex neotype culture CBS 448.83.

Notes: The present species was previously known as a variety of *Ph. jolyana*. It is elevated to species level here due to the phylogenetic results obtained in the present study. Colony characters are similar to those of *Ph. jolyana*, although aerial mycelium in OA plates can be yellow olivaceous, and a yellow discoloration of the agar is present. Pycnidial formation is only induced at a temperature range of 28–30 ºC, indicating the high temperature preference of this species. The pycnidia resemble those of *Ph. jolyana* in shape and size, but in contrast, a pronounced neck may occur in *Ph. calidophila*. Furthermore, the conidia are shorter than those of *Ph. jolyana*, measuring 4–5.5(–6) × (2–)2.5–3 µm, giving them a somewhat ellipsoidal-obovoid appearance. Boerema (1983, 1993) reports the presence of a halo surrounding these chlamydospores when cultured.

Because a form of the preferred epithet ‘*sahariense*’ was already occupied, a new name is proposed here for this species. Type material of *Sphaeronaema sahariense* could not be traced, and therefore neotype material is designated here.

Phoma calorpreferens (Boerema, Gruyter & Noordel.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB512567.
Basionym: *Phoma pomorum* var. *calorpreferens* Boerema, Gruyter & Noordel., *Persoonia* **15**: 207. 1993.

For a detailed description see Boerema (1993).

Specimen examined: **The Netherlands**, from undefined food material, 1973, G.H. Boerema, Holotype L990.290 418, culture CBS 109.92 = CBS 264.74 = PD 73/1405).

Notes: This taxon was considered to be a warmth-preferring variety of *Ph. pomorum*, as it can grow at temperatures above 30 ºC. The phylogenetic studies reveal, however, that both taxa are only distantly related. Therefore, *Ph. pomorum* var. *calorpreferens* is elevated to species level here. It shares many characters with *Ph. pomorum*, but pycnidia are generally smoother and the conidial matrix is pinkish instead of cream-white (Boerema 1993). Furthermore, conidia (4–)5–8.5(–12) × 2–3(–3.5) µm, and chlamydopores (up to 25 µm), are generally larger than those of *Ph. pomorum* (Boerema 1993).

Phoma coffeae-arabicae Aveskamp, Verkley & Gruyter, **sp. nov.** MycoBank MB512568 Figures 3.3a–c, 3.4.

Conidia ellipsoidea usque ovoidea, hyalina, continua, (4–)4.5–6(–7) × (2.5–)3–4(–4.5) µm, eguttulata, vel guttulis polaribus minutis 1–4. Chlamydosporae multicellulares immersae, pseudosclerotioideae, dictyosporae, intercalares, solitariae, (23–)40–100(–190) × (11–)15–30 µm.

Etymology: Named after the host from which it was isolated, *Coffea arabica*.

Pycnidia mostly solitary or in chains, on the agar surface or submerged, variable in shape and size, mostly ovoid but also (sub-)globose or elongated, glabrous, (100–)150–310 × (100–)110–200(–240) µm, papillate

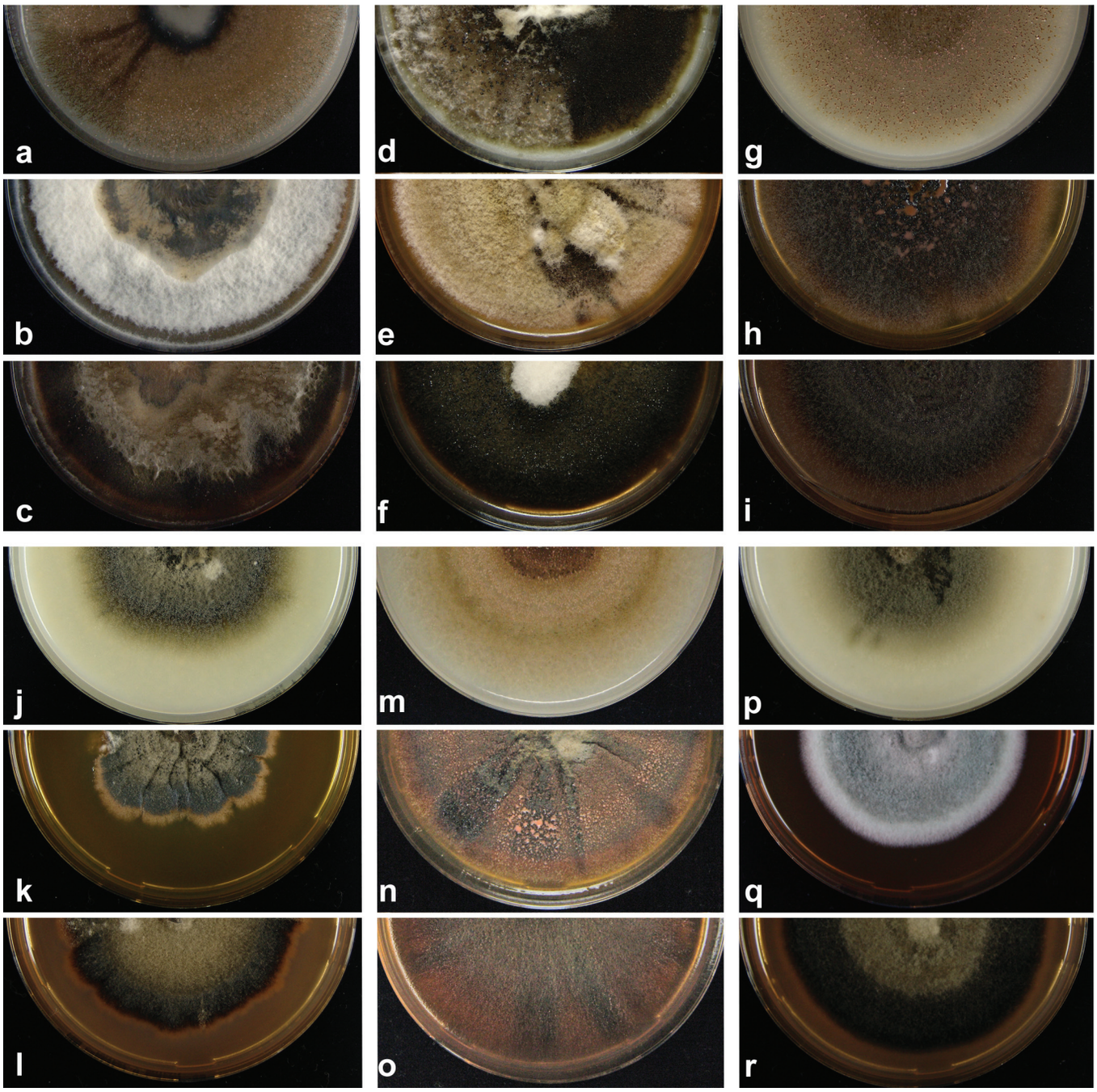

Figure 3.3. A–R. Two-week old colonies on OA (top), MEA (middle) and CHA (bottom). A–C. *Phoma coffeae-arabicae* CBS 123380. D–F. *Ph. infossa* CBS 123395. G–I. *Ph. microchlamydospora* CBS 105.95. J–L. *Ph. omnivirens* CBS 341.86. M–O. *Ph. sancta* CBS 281.83. P–Q. *Ph. schachtii* CBS 502.84.

or with an elongated neck, mostly uni- or bi-ostiolate. *Ostioles* variable in size, but sometimes relatively wide (up to 30 µm diam). *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–5 layers, 10–17 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped to globose, *ca.* 6–7.5 × 5.5–7 µm. *Conidia* ellipsoidal to ovoid, thin-walled, smooth, hyaline, always aseptate, variable in length, (4–)4.5–6(–7) × (2.5–)3–4(–4.5) µm, eguttulate or with 1–4 minute apolar guttules. *Conidial matrix* salmon to flesh. *Multicellular chlamydospores* immersed, brown, pseudosclerotioid, dictyosporous, intercalary, solitary but often with 2–3 elements on a single hypha, (23–)40–100(–190) × (11–)15–30 µm.

Colonies on OA 61–66 mm diam, with entire, smooth margins. Aerial mycelium sparse or absent, tufted, white. Immersed mycelium hyaline or greenish olivaceous, fuscous-black near center. Reverse concolorous. Colonies on MEA 57–70 mm diam, with entire, smooth, sharp margin. Aerial mycelium condensed, white with rosy-vinaceous tinges. Agar surface iron-grey. Reverse fulvous to amber, but leaden black in zones with abundant pycnidia. Colonies on CHA similar growth rate to MEA. Aerial mycelium compact or tufted, primrose to citrine-green, pale greenish glaucous near center, and leaden-grey near margin. Reverse leaden-black.

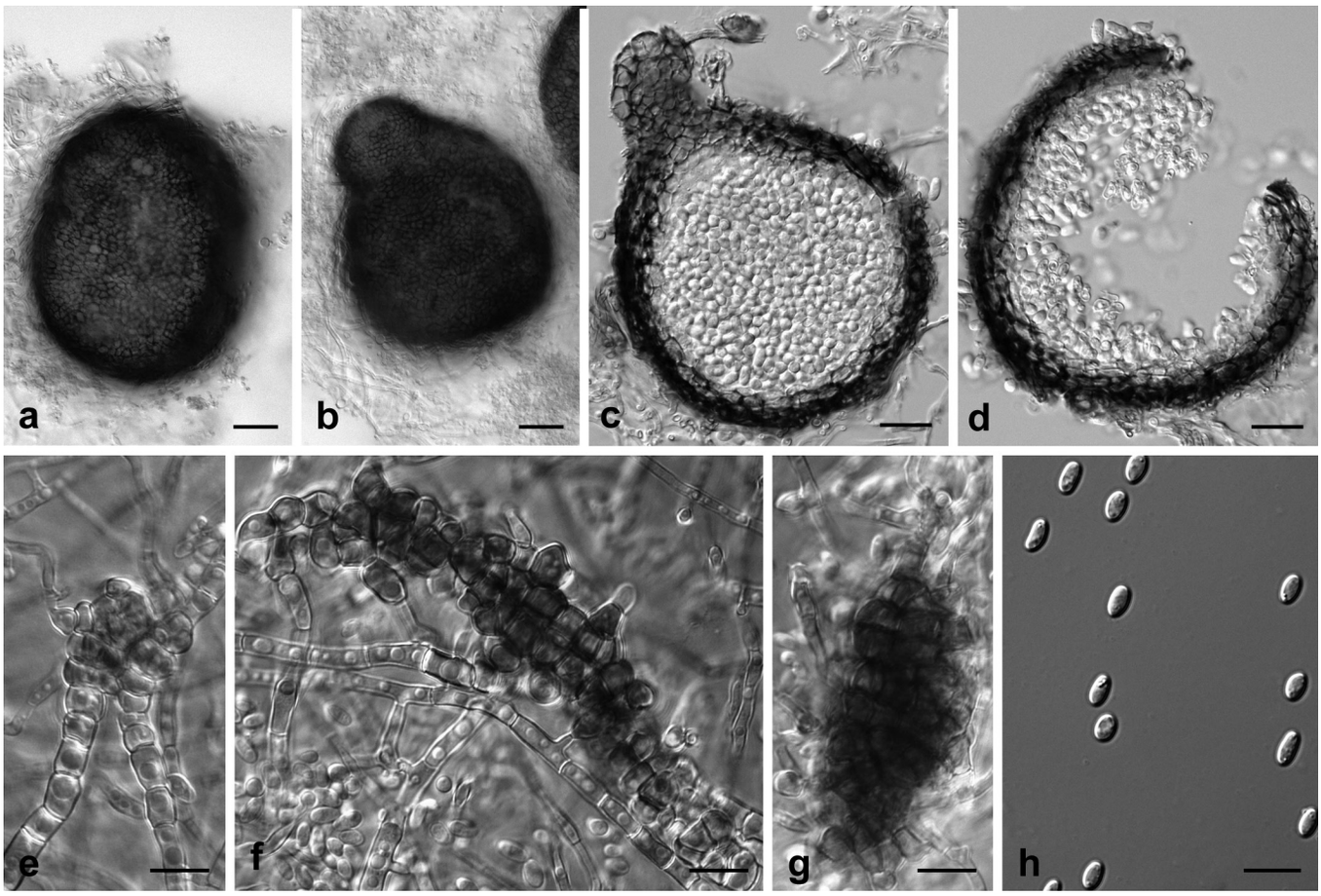

Figure 3.4. *Phoma coffeae-arabicae* (ex-holotype) A–B. Pycnidia. C–D. Pycnidial section. E–G. Chlamydospores. H. Conidia. Bars: A–B = 50 µm, C–G = 20 µm, H = 10 µm.

Specimens examined: **Ethiopia**, from *Coffea arabica*, 1984, M.M.J. Dorenbosch, **holotype designated here** CBS H-20143, ex-holotype culture CBS 123380 = PD 84/1013); From *Coffea arabica*, 1984, *M.M.J. Dorenbosch*, CBS H-20144, culture CBS 123398 = PD 84/1014).

Notes: Multiple *Phoma* species have been found in association with *Coffea arabica*, such as *Ph. coffeicola*, *Ph. coffeiphila*, *Ph. costarricensis*, *Ph. excelsa*, *Ph. pereupyrena* and *Ph. tarda*. However, none of those species produces multicellular chlamydospores, although unicellular, perennial structures have been described in *Ph. pereupyrena* (de Gruyter *et al.* 1993). Furthermore, the conidia of these species are more elongated than those of *Ph. coffeae-arabicae* (Saccas 1981, Boerema *et al.* 2004).

Although *Phoma coffeae-arabicae* forms pseudosclerotioid chlamydospores, it is phylogenetically related to a group that further mainly comprises *Peyronellaea* species forming alternarioid-botryoid chlamydospores (Figure 3.2). It is easily recognised by its conspicuously wide ostiole, comparable to that of *Ph. macrostoma* (White & Morgan-Jones 1984).

Phoma infossa Ellis & Everh., J. Myc. 4: 102. 1888. Figures 3.3d–f, 3.5.

Pycnidia mostly solitary on the agar surface, subglobose to elongated, but sometimes somewhat tapering towards the ostiolum, glabrous, (170–)190–250(–305) × (105–)140–180(–200) µm. *Ostioles* mostly single, (22–)40–75(–105) µm diam, papillate, or with an erumpent and obtusely-conic neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 5–9 layers, 28.5–55 µm thick. *Micropycnidia* sometimes emerge from pycnidia but also solitary, globose to subglobose, 45–80 µm diam. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, *ca*. 5.5–8(–11) × 5–5.5(–7) µm. *Conidia* from both pycnidial types indistinguishable, ovoid, thin-walled, smooth, hyaline but incidentally brown, aseptate, (4–)4.5–6 × 2.5–3.5 µm, eguttulate, or with (1–)3–6 minute polar guttules. Conidial matrix rosy-buff to salmon. *Multicellular chlamydospores* honey to cinnamon, commonly alternarioid–botryoid,

dictyosporous, but sometimes also phragmosporous, solitary or coalescing into long chains of up to 5 elements, terminal on hyphae, but occasionally intercalary, abundantly in the aerial mycelium, 18–32(–55) × 11.5–17(–22) µm.

Colonies on OA 45–55 mm diam, with entire, smooth margins; aerial mycelium occurring in sections, tufted, floccose, lavender-grey or white, *ca.* 2–3 mm high; immersed mycelium grey to grey olivaceous; near colony margin becoming hyaline or citrine, with zones of olivaceous black mycelium. Reverse slate-blue with dark mouse-grey tinges. Colonies on MEA 42–49 mm diam, with entire, smooth, sharp margins. Aerial mycelium compact, tufted, smoke-grey, but olivaceous or primrose near the center and rosy-vinaceous near the margin; sometimes with zones in which the aerial mycelium is absent and where the surface is covered by abundant black pycnidia. Occasionally sectors occur with more developed white to pale mouse-grey aerial mycelium. Reverse black, but primrose near the center and sienna at the margins. Colonies on CHA similar to MEA, but with moderate aerial mycelium occurring; reverse violaceous-black.

Specimens examined: **Argentina**, Provincia de Buenos Aires, La Plata, from *Fraxinus pennsylvanica*, 2008, *M.A. Murace*, **neotype designated here** CBS H-20145, ex-neotype culture CBS 123395 = CPC 15054; from *Fraxinus pennsylvanica*, 2008, *M.A. Murace*, CBS H-20146, culture CBS 123394 = CPC 15052.

Notes: The obtusely-conic, erumpent ostioles that are produced abundantly together with the simple, papillate ones are characteristic for *Ph. infossa.* This species is only rarely observed, and has been found before on dead limbs of *Fraxinus* in New York State (Ellis & Everhart 1888). To our knowledge however, this is the first time this species has been cultivated and preserved.

Phoma microchlamydospora Aveskamp & Verkley, **sp. nov.** MycoBank MB512569 Figures 3.3g–i, 3.6.

Conidia subglobosa usque ellipsoidea, hyalina, continua, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) µm, a Ph. pimprina guttulis majoribus differentia. Chlamydosporae unicellulares (sub)globosae, 4.5–6.5 µm diam, intercalares, plerumque catenulatae. Chlamydosporae multicellulares sparsae, botryoideae-dictyosporae, e cellulis usque septem compositae, globosae, semper solitariae, 4–13 µm diam.

Etymology: Named after its relatively small chlamydospores.

Pycnidia solitary or confluent, globose, glabrous, dark mouse-grey to black, immersed or superficial on the agar surface, as well as in the aerial mycelium, (110–)150–260(–380) × (110–)150–260(–340) µm. *Ostioles* 1–3(–5), papillate, but often on an elongated neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–5 layers, 10–18 µm thick. *Micropycnidia* abundant, pale brown, solitary, globose to elongated, (27.5–)35.5–71 × (27–)31–62(–70) µm. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped or broadly cymbiform, *ca*. 11 × 6 µm. *Conidia* from both pycnidial types indistinguishable, subglobose to ellipsoidal, hyaline, smooth, aseptate, (4–)4.5–6.5(–7) × 3.5–4.5(–5.5) µm, egutulate or with up to 4(–6) small guttules. Conidial matrix rosy-buff to rosy-vinaceous. *Unicellular chlamydospores* (sub-) globose, tan-brown, intercalary, often in chains, relatively small, measuring 4.5–6.5 µm diam, with many small to medium-sized guttules. *Multicellular chlamydospores* sparse, botryoid-dictyosporous, brown, consisting of up to 7 cells, globose, intercalary but sometimes laterally branched from hyphal strands, always solitary, measuring 4–13 µm diam, eguttulate or with many medium-sized guttules.

Colonies on OA 36–40 mm diam, with entire, smooth, sharp margins. Aerial mycelium normally absent, or dark aerial hyphae may appear near center. Immersed mycelium hyaline; reverse olivaceous. Sometimes with a saffron discoloration of the agar due to a diffusible pigment, which persists after application of NaOH. Colonies on MEA 28–34 mm diam, with entire, smooth, sharp margin. Immersed mycelium fuscous-black. Sometimes sectors with white compact aerial mycelium are present; reverse concolorous. Colony on CHA as on MEA, although sometimes a thin, pale olivaceous grey to iron-grey mycelial mat is covering the surface.

Specimens examined: **United Kingdom**. From leaves of *Eucalyptus* sp., 1994, A.M. Ainsworth, **holotype designated here** CBS H-20147, ex holotype culture CBS 105.95; From an unknown vegetable plant, 1990, D. Hyall, CBS H-20148, culture CBS 491.90.

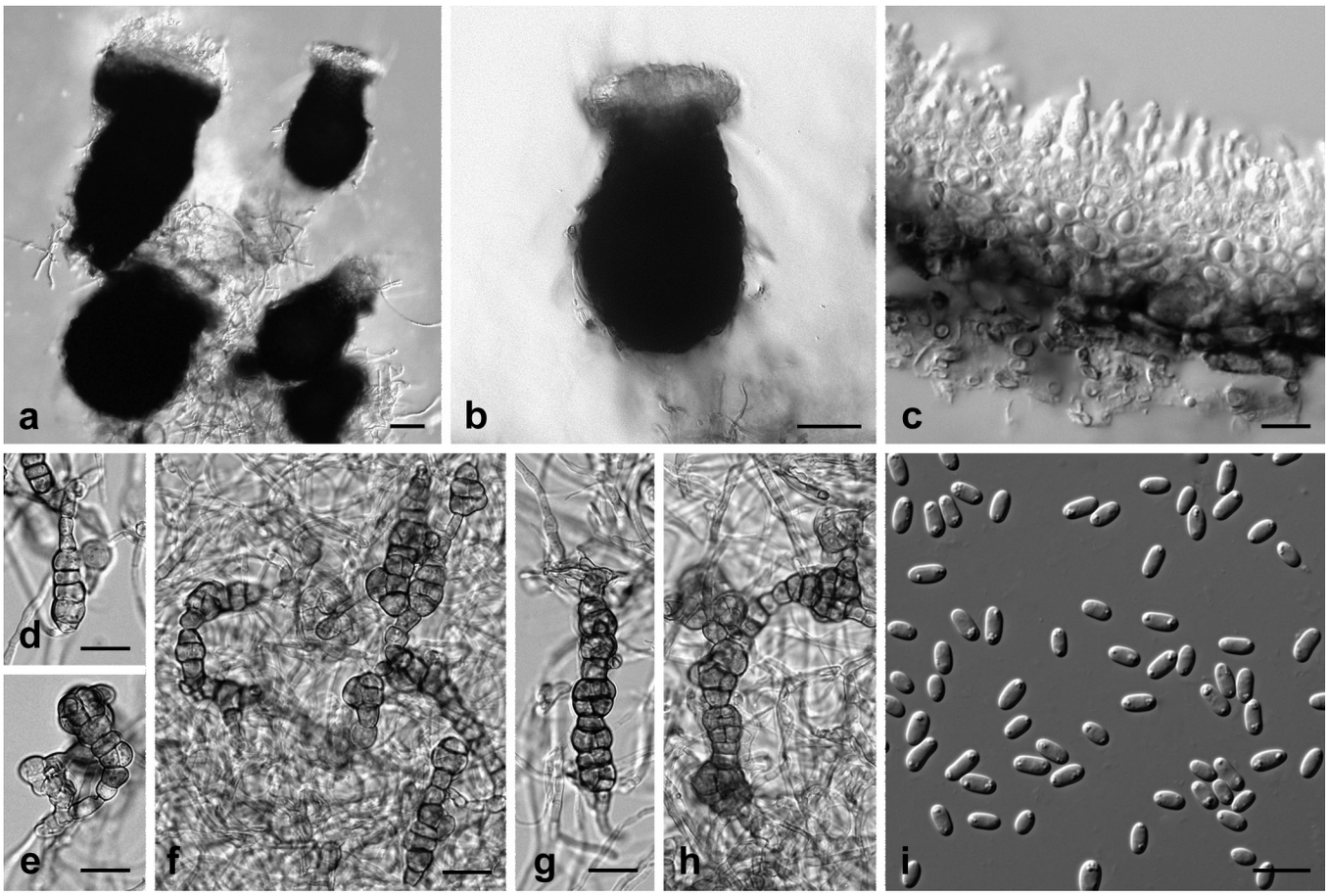

Figure 3.5. *Phoma infossa* (ex-neotype) A–B. Pycnidia. C. Pycnidial section. D–H. Chlamydospores. I. Conidia. Bars: A–B = 50 µm, C, I = 10 µm, D–H = 20 µm.

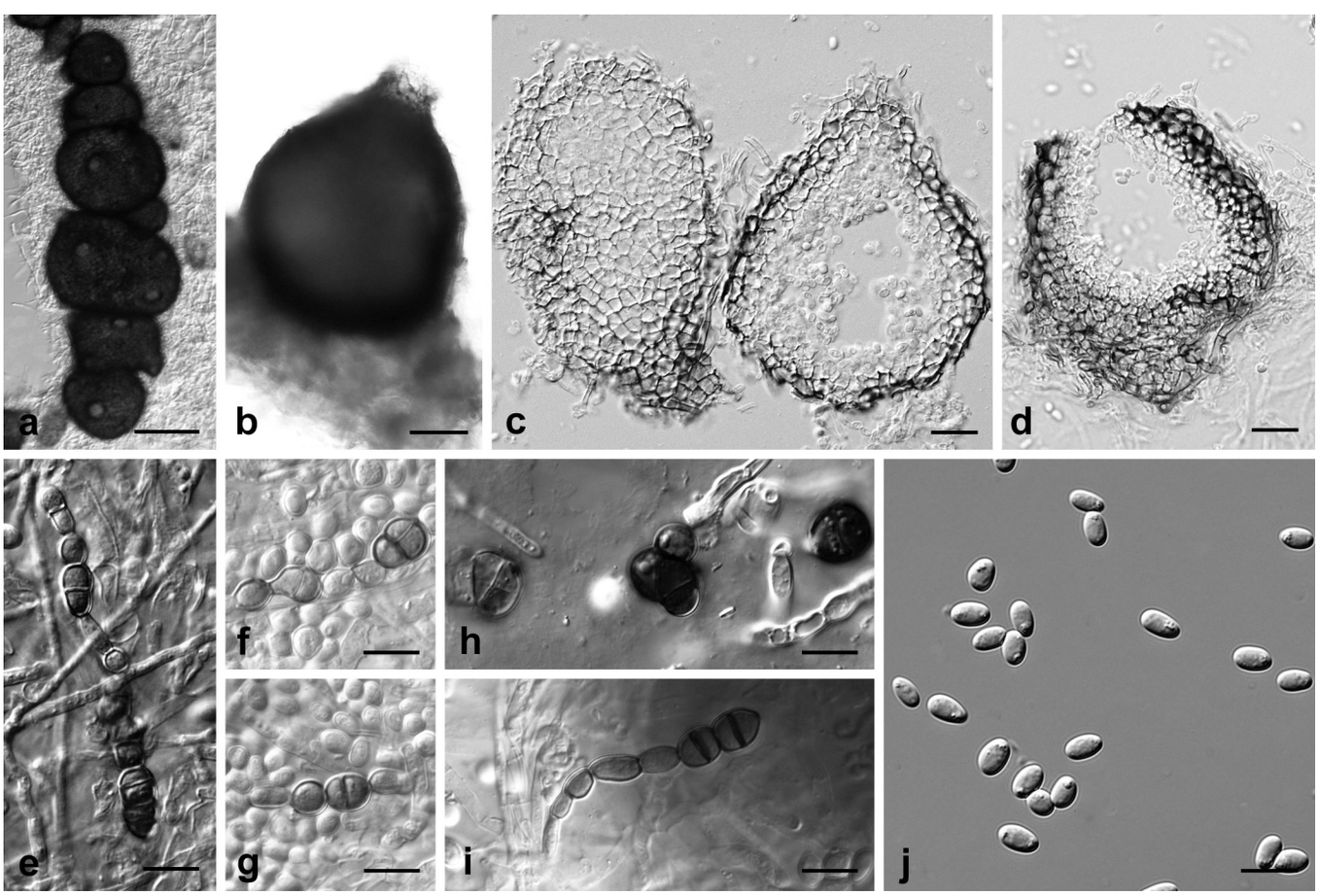

Figure 3.6. *Phoma microchlamydospora* (ex-holotype) A–B. Pycnidia. C–D. Pycnidial section. E–I. Chlamydospores. K. Conidia. Bars: A = 100 µm, B = 50 µm, C–I = 20 µm, J = 10 µm.

Notes: The chlamydospores of *Phoma microchlamydospora* are extremely small compared to most other botryoid dictyochlamydospore producing species, which produce on average structures of 8–20 µm diam (Boerema *et al.* 2004). Conidia are similar in shape and size to those of *Ph. pimprina*, but the guttules are larger in the present species. Phylogenetically, this species clusters with *Ph. calidophila*, although distinctive differences exist in pycnidial and chlamydopore morphology.

Phoma multirostrata (P.N. Mathur, S.K. Menon & Thirum.) Dorenb. & Boerema, *Mycopath. Mycol. Appl.* **50**: 255. 1973.
Basionym: *Sphaeronaema multirostratum* P.N. Mathur, S.K. Menon & Thirum., *Sydowia* **13**: 146. 1959 [as '*Sphaeronema multirostrata*'].
= *Phoma multirostrata* var. *macrospora* Boerema, *Versl. Med. Pl. ziektenk. Dienst* **164**: 29. 1986
= *Phoma multirostrata* var. *microspora* (Allesch.) Boerema, *Versl. Med. Pl. ziektenk. Dienst* **164**: 30. 1986.
For an extended synonymy see Boerema *et al.* (2004).

Pycnidia solitary or confluent, globose to subglobose or irregular, glabrous, brown to black, superficial or immersed, variable in size, 150–350(–720) µm diam. *Ostioles* multiple, conspicuous (10–25 µm diam), non-papillate or on elongated necks, up to 260 µm long. *Pycnidial wall* pseudoparenchymatous, composed of oblong to cylindrical or elongated cells, 4–5 layers, *ca*. 9–14.5 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped. *Conidia* oblong to ellipsoidal, thin-walled, smooth, hyaline, aseptate, highly variable in size, (3.5–)4.5–6.5(–8.5) × (1.5–)2–2.5(–3) µm, with 0–3(–4) polar guttules. Conidial matrix white to buff or rosy-buff. *Chlamydospores* mostly unicellular, 5–15 µm diam, ellipsoidal to oblong to somewhat pyriform, olivaceous or pale brown with greenish guttules, solitary or in chains, intercalary but incidentally also terminal. Regularly also a bunch of clustered unicellular chlamydospores can be observed, especially in older cultures. These structures are easily mistaken for pseudosclerotioid chlamydospores as in *Ph. violicola*.

Colonies on OA (60–)65–70(–80) mm diam, with entire, smooth, sharp margins. Aerial mycelium sparse, floccose or tufted, white to grey or completely absent. Agar surface olivaceous to chestnut with colourless sectors. Reverse concolorous. Colony on MEA 60–75 mm diam, with entire, smooth, sharp margin. Aerial mycelium felty, floccose or wooly, olivaceaous to olivaceous buff. Agar surface glaucous-grey. Reverse leaden-grey to olivaceous black. Colony on CHA 65–75 mm diam, with entire, smooth, sharp margin. Aerial mycelium floccose, white to grey, absent near the margin of the colony. Agar surface dark mouse-grey to greenish black. Reverse concolorous.

Specimens examined: **India**, Maharashtra, Poona, Talegaon, from poultry farm soil, March 1959, M.J. Thirumalachar, **isotype** CBS H-7616, ex isotypeculture CBS 274.60; from soil, March 1959, M.J. Thirumalachar, CBS H-16499, culture CBS 368.65; **The Netherlands**, Hoorn, greenhouse, from the stem of *Cucumis sativus*, Aug. 1967, G.H. Boerema, CBS H-16502, culture CBS 110.79.

Notes: The three varieties of *Ph. multirostrata* recognised by Boerema (1986), var. *multirostrata*, *macrospora* and *microspora* can no longer be retained as separate taxonomic entities. Taxonomic characters distinguish these varieties insufficiently, forcing Boerema *et al.* (2004) already to state that 'intermediate variants commonly occur'. Furthermore, no genetic differences consistent with those distinguishing the varieties were found in the DNA analysis conducted in the present study. Therefore all varieties are synonymized with the original species, *Ph. multirostrata*.

Phoma omnivirens Aveskamp, Verkley & Gruyter, **sp. nov.** MycoBank MB512570 Figures 3.3j–l, 3.7.

Conidia subcylindrica usque ellipsoidea, hyalina, continua, (3.5–)4–5.5(–7) × (1.5–)2–2.5(–3) µm, guttulis polaribus 1–2. Chlamydosporae unicellulares oblongae, plerumque in catenas longas positae, 7–14(–20) × (4–)4.5–8.5(–18) µm, pluriguttulatae. Chlamydosporae multicellulares irregulares, dictyosporae, botryoideae, intercalares, in agaro immersae, (12–)15–52.5(–70) µm diam.

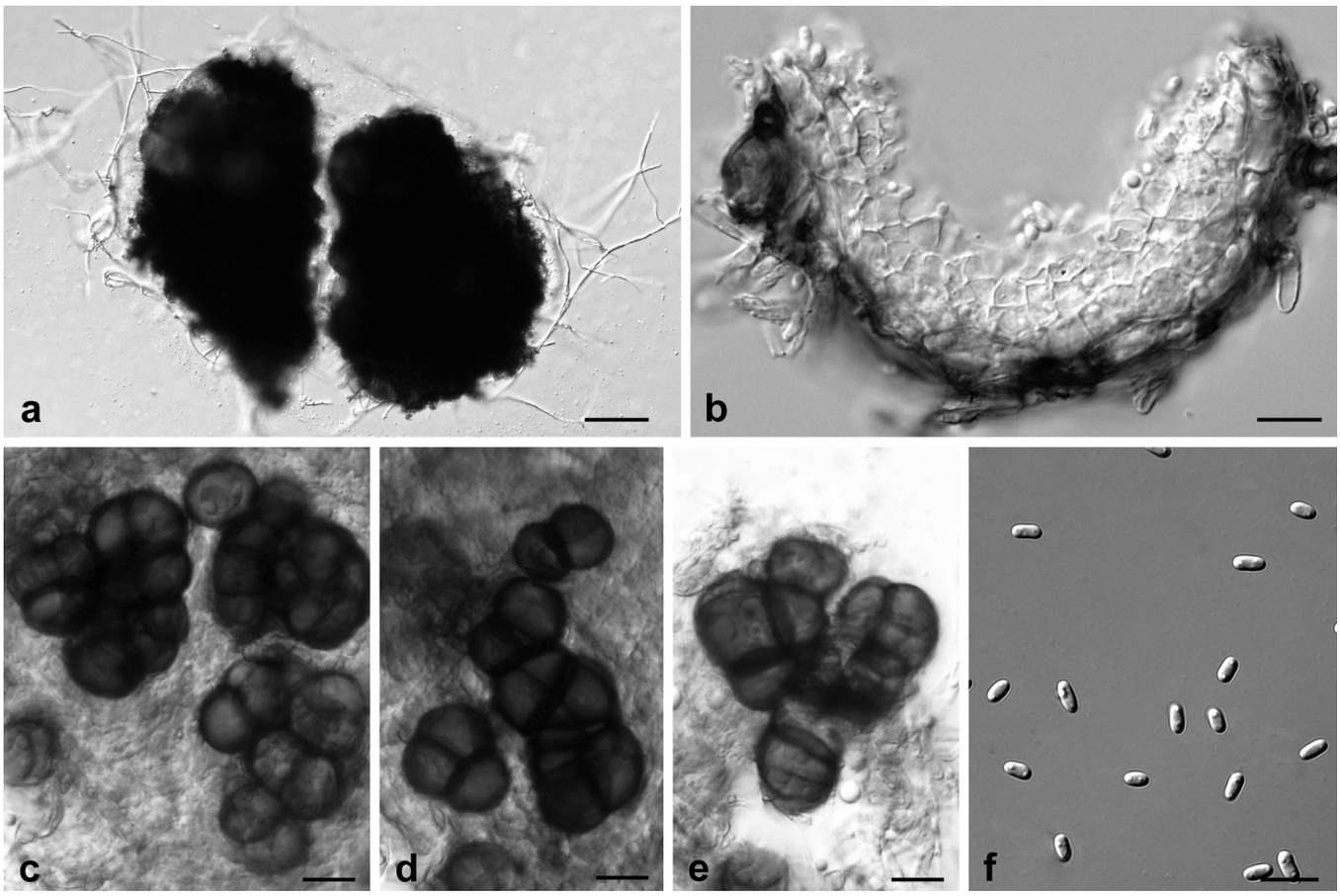

Figure 3.7. *Phoma omnivirens* (ex-holotype) A. Pycnidia. B. Pycnidial section. C–E. Chlamydospores. F. Conidia. Bars: A = 100 µm, B–F = 10 µm.

Etymology: Name refers to the omnipresence of this species, which has been isolated from a wide range of hosts and geographical locations.

Pycnidia solitary or confluent, immersed or on the agar surface, globose to slightly subglobose, with many hyphal outgrowths, dark brown to black, 100–260(–350) × (90–)100–240(–300) µm, uni-ostiolate, non-papillate, papillate or sometimes with a broad, elongated neck, giving the pycnidium a somewhat ovoid appearance. Pycnidial wall pseudoparenchymatous, composed of isodiametric to elongated cells, 2–6 layers, 10.5–16.5(–17.5) µm thick. *Micropycnidia* if present, generally darker than the regular pycnidia, solitary or confluent, globose, obpyriform or elongated, (40–)65–120 × (40–)60–100 µm.

Conidiogenous cells phialidic, hyaline, simple, smooth, globose to flask-shaped, *ca.* (4.5–)5–6 × 4.5–5.5 µm. *Conidia* from both pycnidial types indistinguishable, subcylindrical to ellipsoidal, thin-walled, smooth, hyaline, aseptate, (3.5–)4–5.5(–7) × (1.5–)2–2.5(–3) µm, with (1–)2 small to medium-sized polar guttules; conidial matrix buff. *Submerged hyphae* smooth, hyaline, thin-walled, but often becoming pigmented and swollen, attaining a width of up to 9.5 µm. *Unicellular chlamydospores* oblong, brownish, often in long chains, 7–14(–20) × (4–)4.5–8.5(–18) µm, with many guttules in each cell. *Multicellular chlamydospores* consisting of agglomerates of unicellular chlamydospores, irregularly shaped, dictyosporous, botryoid, brownish, intercalary, submerged in the agar, (12–)15–52.5(–70) µm diam.

Colony on OA 35–60 mm diam, with entire, smooth, sharp margin. Aerial mycelium tufted, floccose to compact, locally well-developed, white to (pale-) olivaceous grey, sometimes greenish olivaceous near margin. Immersed mycelium dark mouse-grey to leaden-black, towards the colony margin the colour fades away to dull-green and white. Reverse concolorous or greenish black. Often an amber, primrose or buff diffusible pigment can be observed on the agar. Colony on MEA 28–52 mm diam; margins entire, smooth, sharp, or lobate to crenate. Aerial mycelium, floccose, wooly or compact, white or with various shades of grey (pale mouse-grey, olivaceous grey, iron-grey). Reverse olivaceous grey to leaden-black. After application of NaOH the agar colour changes to bright green. Colony CHA as on MEA, but aerial mycelium less well developed.

Specimens examined: **Belgium**, Gembloux, from *Phaseolus vulgaris*, 1968, L. Obando, **holotype designated here** CBS H-20151, ex holotype culture CBS 341.86; **India**, Japalbur, from an unknown substrate, 1977, D.P. Tiwari, CBS H-20152, culture CBS 654.77; **Papua New Guinea**, Varirata National Park, from soil, Aug 1995, A. Aptroot, CBS H-20153, culture CBS 991.95; Varirata National Park, from soil, Aug 1995, A. Aptroot, CBS H-20154, culture CBS 992.95); **The Netherlands**, from *Chrysanthemum indicum*, 1981, J. de Gruyter, CBS H-20155,culture CBS 123396 = PD 81/122; **Tanzania**, from *Statice* sp., 1990, J. de Gruyter, CBS H-20156, culture CBS 123397 = PD 90/1555.

Notes: This species has been isolated from a wide variety of substrates and from geographically distinct locations. Isolates have previously erroneously been identified as *Ph. sorghina*, due to the similarity in shape of the chlamydospores, but this species is distinguishable by the absence of pink or reddish pigments in the colony.

Phoma pomorum* var. *circinata (Kusnezowa) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB512571.
Basionym: *Peyronellaea circinata* Kusnezowa, *Nov. sist. Niz. Rast*. **8**: 189. 1971.
= *Phoma jolyana* var. *circinata* (Kusnezowa) Boerema, Dorenb. & Kesteren, in *Kew Bull*. **31**: 535. 1977 ['1976'].
= *Peyronellaea nigricans* Kusnezowa, *Nov. sist. Niz. Rast.* **8**: 191. 1971.

For detailed descriptions see Boerema *et al.* (1977), Boerema (1993) and Morgan-Jones & Burch (1987).

Specimens examined: **Russia**, Siberia, Novosibirsk, Hortus Botanicus, from *Heracleum dissectum*, 1963, T.T. Kusnezowa, isotype CBS H-3747, ex isotype culture CBS 285.76 = ATCC 26241 = IMI 176742 = VKM F-1843; Siberia, Novosibirsk, Hortus Botanicus, from a leaf of *Allium nutans*, 1963, T.T. Kusnezowa, CBS H-16399, culture CBS 286.76 = ATCC 26242 = IMI 176743 = VKM F-1844.

Notes: This taxon, which was previously seen as a variety of *Ph. jolyana*, differs only 1 nucleotide in the ITS sequence from *Ph. pomorum* var. *pomorum* (CBS 539.66), whereas both ACT and TUB sequences do not show any consistent differences. Nevertheless, morphologically this taxon is distinct. *Phoma pomorum* var. *circinata* has somewhat larger conidia, (3.5–)5–9 × 2–3.5 µm than the type var., (4–)5–7(–8) × 1.5–2.5(–3) µm (Boerema 1993). Furthermore, unicellular chlamydospores are absent in *Ph. pomorum var. circinata.* Thus far, strains have only been reported from Novosibirsk, Russia.

Phoma pomorum var. ***cyanea*** (Jooste & Papendorf) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB512572.
Basionym: *Phoma cyanea* Jooste & Papendorf, *Mycotaxon* **12**: 444. 1981.

For detailed descriptions see Jooste and Papendorf (1981) and Boerema (1993).

Specimen examined: **South Africa**, Heilbron, from straw of *Triticum* sp., 1972, W.J. Jooste, holotype PREM 45736, culture CBS 388.80.

Notes: *Phoma pomorum* var. *cyanea* is a species that has thus far only been reported from South Africa. It is easily distinguishable from *Ph. pomorum* var. *pomorum* by the production of a bluish pigment in the hyphae, pycnidia and chlamydospores. The remaining morphological characters, however, fit within the scope of *Ph. pomorum*. Furthermore, the sequence analyses conducted in the present study show a 100 % similarity on ITS, ACT and TUB between the two taxa. It is therefore concluded that *Ph. cyanea* should be reduced to a variety of the older *Ph. pomorum*, as *Ph. pomorum* var. *cyanea*.

Phoma sancta Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB512573. Figures 3.3m–o, 3.8.

Conidia ovoidea, hyalina, continua, 5–7(–7.5) × 2.5–4(–4.5) µm, guttulis polaribus 3–9(–12). Chlamydosporae multicellulares alternariodeae, phragmosporae vel dictyosporae, (11–)16–26(–30) × (6.5–)7.5–11(–13.5) µm, solitariae, terminales, in hyphis aereis brevibus formatae.

Etymology: Named because of its association with the hosts *Gleditsia triacantha* (Christusdoorn in Dutch, meaning Christ's Thorn) and *Ailanthus altissima*, Tree of Heaven.

Pycnidia solitary or confluent, globose, glabrous or completely covered with short hyphal outgrows, superficially on the agar and in aerial mycelium, (80–)125–260 µm diam, conspicuously papillate; ostioles 1(–2), 20–40(–60) µm diam. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 3–7 layers, relatively thick, measuring 21–43(–51) µm thick. *Micropycnidia* formed in the aerial mycelium, generally paler than the regular pycnidia, or even hyaline, solitary, (sub-)globose, (40–)60–80(–110) µm diam. *Conidiogenous cells* phialidic, hyaline, simple, smooth, globose to flask-shaped, (5–)6–7 × (5–)5.5–6.5 µm. *Conidia* ovoid, thin-walled, smooth, hyaline, aseptate, 5–7(–7.5) × 2.5–4(–4.5) µm, with 3–9(–12) polar guttules. Conidial matrix salmon. *Chlamydospores* multicellular, alternarioid, phragmosporous or dictyosporous, (11–)16–26(–30) × (6.5–)7.5–11(–13.5) µm, dark brown, terminal on erect aerial hyphae, solitary.

Colonies on OA 45–60 mm diam, with entire, smooth, sharp margins. Aerial mycelium sparse or absent, tufted, grey to white. Immersed mycelium fawn, but fading away to grey olivaceous, becoming hyaline near margin; reverse concolorous. After application of NaOH, the agar near the hyphae becomes inconspicuously reddish brown. Colonies on MEA 52–57 mm diam, with entire, smooth, sharp margins. Aerial mycelium greenish olivaceous to white, floccose and abundant near center, towards the margin less well developed. Immersed mycelium iron-black with or without vinaceous sectors. Reverse concolorous. Colonies on CHA similar to MEA, but aerial mycelium less well developed.

Specimen examined: **South Africa,** from dead branches of *Ailanthus altissima*, Oct. 1982, C. Jansen, **holotype designated here**, CBS H-16332, ex holotype culture CBS 281.83.

Notes: *Phoma sancta* appears to be widespread, and clusters within a group in which amongst others *Ph. glomerata*, *Ph. pomorum* and *Ph. jolyana* are accommodated. This species is recognizable by the high percentage of phragmospores that are formed in culture. The latter feature may have been the reason for the previous identification as *Ph. jolyana*. The latter species produces its chlamydospores mainly in the agar and in the aerial mycelium on a wide range of media, whereas the multicellular chlamydospores of *Ph. sancta* are mainly formed on OA, and are terminally located on short, erect hyphae emerging from the agar surface.

Phoma schachtii Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB512574. Figures 3.3p–r, 3.9.

Conidia ellipsoidea, hyalina, continua, (4–)4.5–5.5(–6) × (1.5–)2–2.5 µm, eguttulata, vel guttulis polaribus 2(–3). Chlamydosporae multicellulares dictyosporae, alternariodeae vel botryoideae, (15.5–)31–81.5(–101.5) × (9.5–)19–50.5(–63) µm diam, viridulae, terminales, solitariae vel in catenas breves positae, in culturis vestioribus confertim aggregatae et pseudosclerotiodeae.

Etymology: Named after the host species on which the fungus was found, a cyst of the nematode *Heterodera schachtii.*

Pycnidia solitary or confluent, globose, completely covered with hyphal outgrows, submerged in the agar, (180–)220–600(–650) µm diam, papillate, or with an elongated neck, and a single inconspicuous ostiole. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric to oblong cells, 4–9 layers, (22.5–)26.5–37(–41.5) µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, *ca*. 5–7 × 4–6 µm. *Conidia* ellipsoidal, thin-walled, smooth, hyaline, aseptate, (4–)4.5–5.5(–6) × (1.5–)2–2.5 µm, eguttulate or with 2(–3) polar guttules. Conidial matrix cream white. *Multicellular chlamydospores* developing after

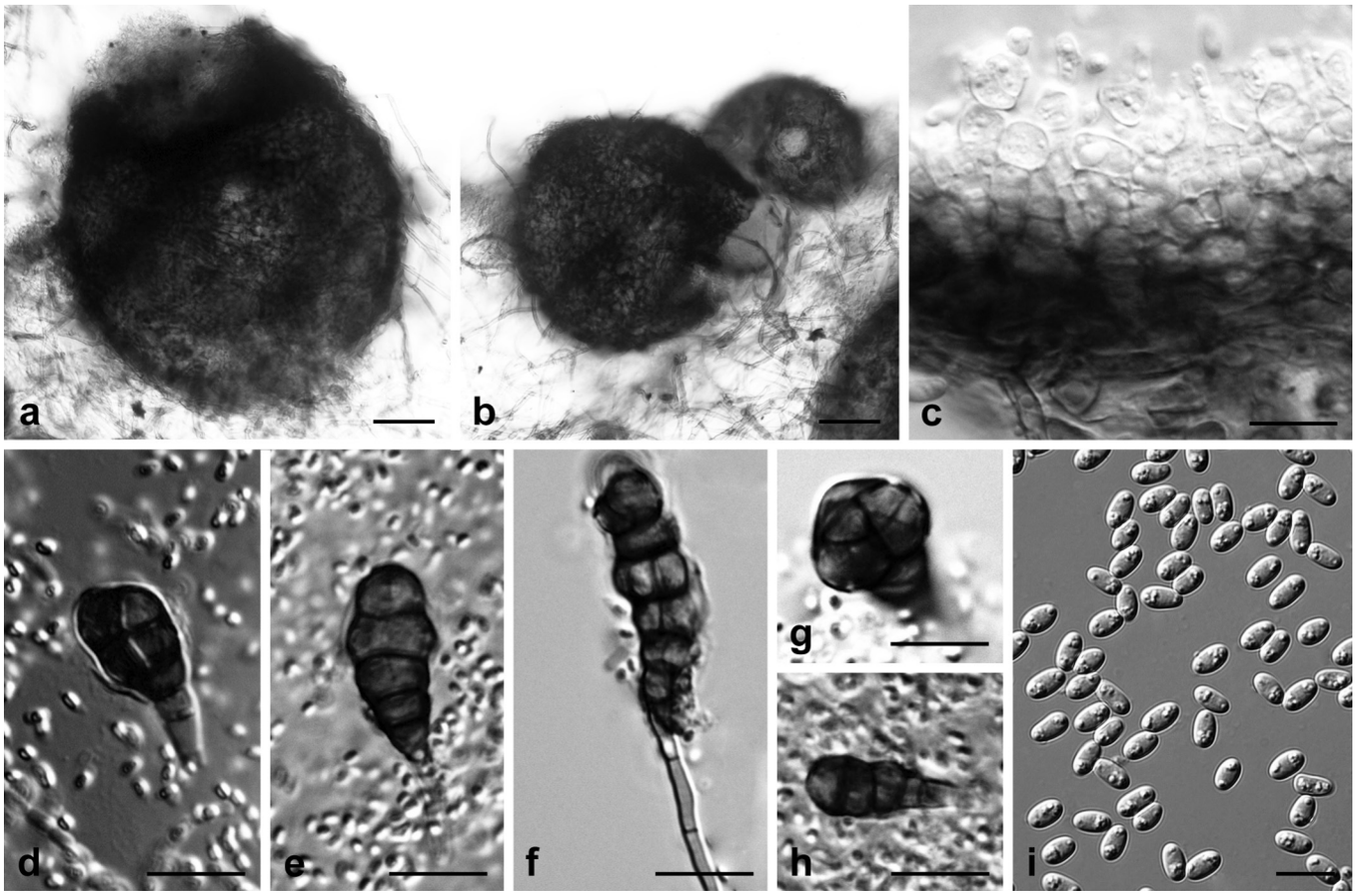

Figure 3.8. *Phoma sancta* (ex-holotype) A–B. Pycnidia. C. Pycnidial section. D–H. Chlamydospores. I. Conidia. Bars: A = 50 µm, B–I = 10 µm.

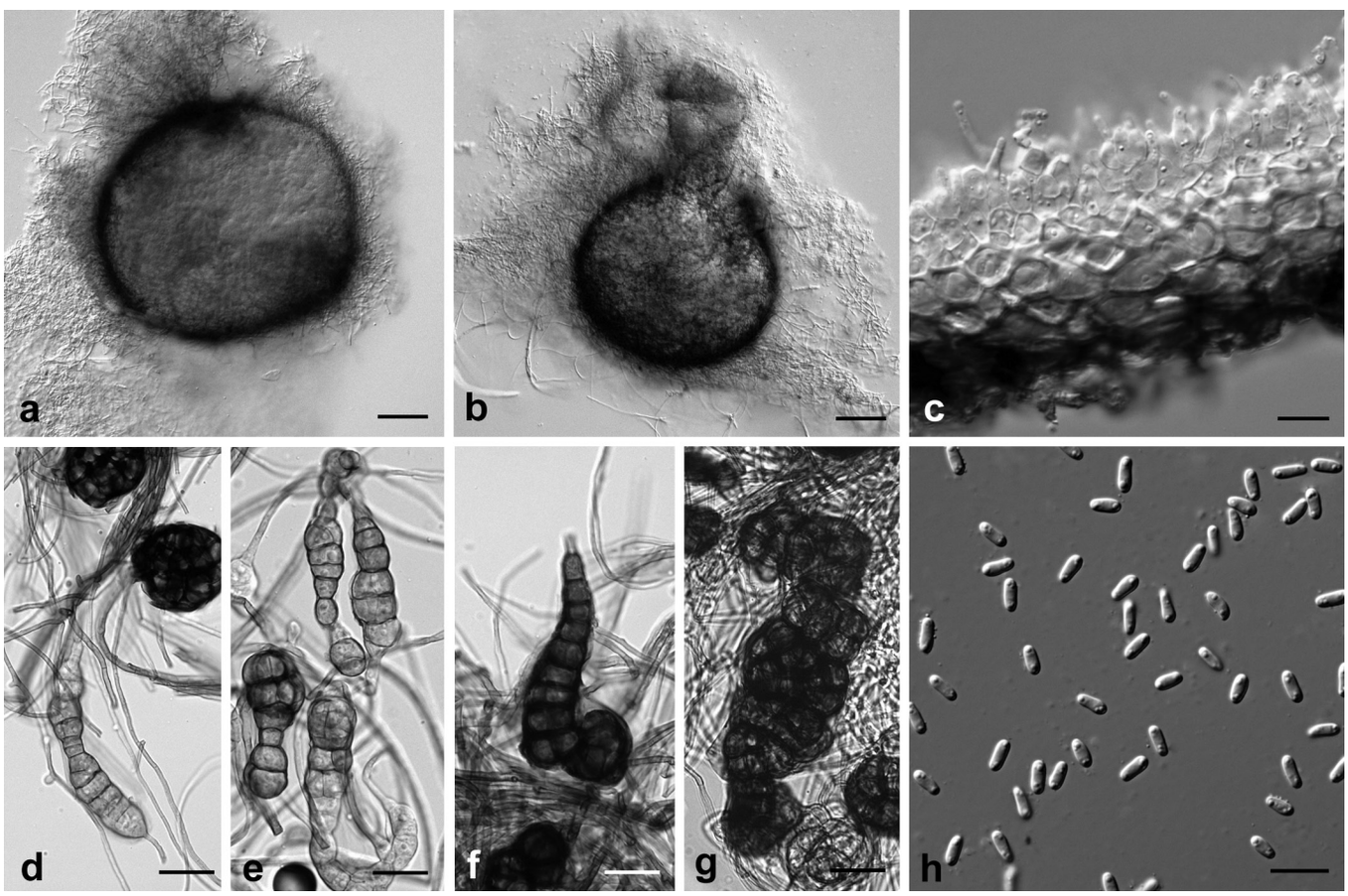

Figure 3.9. *Phoma schachtii* (ex-holotype) A–B. Pycnidia. C. Pycnidial section. D–G. Chlamydospores. H. Conidia. Bars: A–B = 50 µm, C, H = 10 µm, D–G = 20 µm.

several weeks, dictyosporous, alternarioid or botryoid, abundant in the aerial mycelium, (15.5–)31–81.5(–101.5) × (9.5–)19–50.5(–63) µm diam, greenish, terminal, single or in short chains with up to 3 elements, in older cultures aggregating into pseudosclerotioid masses.

Colonies on OA 26–32 mm diam, with entire, smooth, sharp margins; aerial mycelium felted, mostly olivaceous grey, near center iron-grey and smoke-grey near margins. Reverse olivaceous grey with some olivaceous zones. Colonies on MEA 20–24 mm diam, with entire, smooth, sharp margins. Aerial mycelium felted or floccose to tufted, greenish grey or pale olivaceous grey. Reverse olivaceous black to dark slate-blue, near margin somewhat brown-vinaceous. Colonies on CHA 28–32 mm diam, with entire, smooth, sharp margins, covered by a compact or felted mycelial mat, olivaceous grey to fuscous, near the center mouse-grey. Reverse concolorous.

Specimen examined: **The Netherlands**, Bergen op Zoom, from the cyst of *Heterodera schachtii*, 1984, W. Heybroek, **holotype designated here** CBS H-16188, ex holotype culture CBS 502.84.

Notes: At least nine *Phoma* species, of which most are capable of producing chlamydospores, have been isolated from the cysts of *Heterodera* spp. (Chen *et al.* 1996). *Phoma schachtii,* which has been found parasitizing a cyst nematode, has many characters in common with *Ph. chrysanthemicola*, which explains why it has not previously been recognised as a separate taxon. The two species can be distinguished by the clear alternarioid-botryoid chlamydodospores that are present in fresh cultures. In later stages, these will aggregate and form long pseudosclerioid masses. Those masses are generally more smooth than in *Ph. chrysanthemicola*, which has more warty chlamydospore walls.

Discussion

In their final publication after more than 40 years of morphological studies on the genus *Phoma*, Boerema and co-workers listed the 223 specific and infra-specific taxa that they recognised (Boerema *et al.* 2004). Since the publication of this identification manual, several studies on *Phoma* species have been conducted utilizing DNA sequence phylogenies, revealing *Phoma* to be a more complicated genus than previously considered (Reddy *et al.* 1998, Torres *et al.* 2005a, b). Besides the unclear generic definition (see Chapter 2), also the current morphology-based subdivision of *Phoma* appears not to be in congruence with its molecular phylogeny.

Two species that are regarded members of the section *Peyronellaea*, viz. *Ph. chrysanthemicola* and *Ph. violicola*, and also the newly described species *Ph. schachtii*, do not group with the majority of the Peyronellaea species in clade 1 but are found amongst the basal leneages, together with the type species of *Phoma* sections *Heterospora*, *Paraphoma*, and *Plenodomus* (Figure 3.1). Characters that are considered to be typical for these sections, namely pluriform conidia, setose pycnidia or scleroplectenchyma respectively, were however never observed in *Ph. chrysanthemicola*, *Ph. violicola* or *Ph. schachtii*. These species are all characterized by the formation of chlamydospores in so-called pseudosclerotioid masses.

Most dictyochlamydospore-producing taxa cluster together with *Ph. herbarum,* the type species of the genus *Phoma* and as a consequence also of the section *Phoma* in clade 1. Further, these taxa cluster with the type species of two other sections, viz., *Ph. exigua var. exigua* (sect. *Phyllostictoides*) and *Ph. zeae-maydis* (sect. *Macrospora*) (Figure 3.2). Chlamydospores produced by the taxa in this cluster represent the botryoid and alternarioid types, except for those of the novel species *Ph. coffeae-arabicae*, which are pseudosclerotioid. Based on these results the subdivision of the genus *Phoma* (Boerema (1997) can therefore be questioned. This observation is in congruence with the study of Torres *et al.* (2005a), who found major inconsistencies between the system of Boerema (1997) and their molecular data and advocated that the current taxonomy of the genus *Phoma* needs to be thoroughly revised.

The *Phoma* anamorph state is found in multiple Pleosporalean teleomorphs, including *Didymella*, *Leptosphaeria* and *Pleospora* (see Chapter 2). The backbone structure of Figure 3.1 can largely be explained by the clustering of *Peyronellaea* species with the different teleomorph groups. Most species studied cluster with *Ph. zeae-maydis* (teleomorph *D. zeae-maydis*) in clade 1, indicating that *Didymella* would be the most likely teleomorph for those species if a sexual state would be encountered. *Phoma violicola* and *Ph. schachtii* are found in clade 2a, in which also *Ph. lingam* is clustered, which has a teleomorph in *Leptosphaeria*. Clade 2b represents the *Pleospora*-associated clade. In clade 3, three species are grouped of which thus far, no teleomorph has been recorded. The species in this clade represent the sections *Peyronellaea* (*Ph.*

chrysanthemicola), *Heterospora* (*Ph. samarorum*) and *Paraphoma* (*Ph. radicina*, type species of its section). The clades found in this study resemble some of the groups found in the study of Schoch *et al.* (2006) in *Pleosporales*. The phylogenetic distances between clades 1 and 2 were observed before by Reddy *et al.* (1998) and Torres *et al.* (2005b), and forced these authors to advocate the reinstallation of the anamorph genus name *Plenodomus* for the *Leptosphaeria*-associated species. However, such a taxonomic recombination requires further evaluation of all *Phoma* species and associated genera.

Phoma identification is regarded to be problematic, and gives rise to many misidentifications (Bridge *et al.* 2003), but most strains studied here, have been classified properly. Strains that could not be identified upon collection due to overlapping species characters can now be delimited and defined using molecular characterization tools. In the present study we recognise five novel dictyochlamydospore-forming species that were preserved in culture collections under incorrect names, or as unidentified species. New combinations in a further five taxa were made to ensure consistency with the DNA data obtained in the present study. The species concepts defined in the past appear to be still valid for *Ph. americana* (1983a, Boerema 1993), *Ph. epicoccina* (Boerema 1993, Arenal *et al.* 2000), *Ph. glomerata*, *Ph. pomorum* var. *pomorum* (Boerema *et al.* 1965a, Boerema 1993), *Ph. chrysanthemicola, Ph. pimprina*, *Ph. subglomerata*, *Ph. violicola* and *Ph. zantedeschiae* (Boerema 1993). Also *Ph. sorghina* (Boerema *et al.* 1968, White & Morgan-Jones 1983) appears to be properly described and represents a monophyletic clade, although a high level of infraspecific genetic variation has been observed. Only two strains (CBS 991.95 and CBS 992.95) were morphologically and genetically clearly distinct and are reclassified in the novel species *Ph. omnivirens* here. The remaining 13 species clustered together in a *Ph. sorghina* superclade, in which no less than nine different, often well-supported subclades are recognised (Figure 3.2). The morphological variation was however, sparse, and all strains fitted within the scope of the species as described by Boerema *et al.* (1973) and White & Morgan-Jones (1983). Also the host association and the origin are too diverse to provide further information on a possible further classification. The high genetic variety in comparison to e.g. *Ph. glomerata* may indicate a high recombination rate. Sexual recombination, although a teleomorph has never been observed, may be one of the reasons for this phenomenon.

Much confusion still surrounds the identity of *Ph. jolyana*. A relatively wide species concept has previously been applied to this taxon (Boerema *et al.* 1965a, Morgan-Jones and Burch 1987b), which gave rise to many incorrectly identified isolates. At least two new taxa were encountered amongst the strains that were initially stored in the CBS and PD culture collections as *Ph. jolyana*, and are renamed *Ph. coffeae-arabicae* and *Ph. sancta* in this study. Previously, three varieties were recognised within this species, of which the type variety was widespread, whereas var. *circinata* and *sahariensis* had been collected on only a few occasions from isolated places (Boerema *et al.* 2004). In this study both varieties have been recombined: var. *sahariensis* is elevated to species level, as *Ph. calidophila*, whereas var. *circinata* has been recombined to a variety of *Ph. pomorum*. Given the isolated origins of these isolates we expect that many more dictyochlamydospore-producing taxa will be encountered in the future once these origins are sampled more.

This study also addresses the problem that single morphological characters cannot always be discriminative between taxa. A good example of this is the genetic similarity of *Ph. pomorum* and *Ph. cyanea*. Although *Ph. cyanea* was easily distinguishable due to the obvious production of a bluish pigment in its hyphae, pycnidia and chlamydospores, sequence analysis proved it to be highly similar to *Ph. pomorum*. Because several other morphological characters showed high similarity between the two taxa, it was concluded that *Ph. cyanea* should be reduced to a variety of the older *Ph. pomorum*.

The taxa that clustered in section *Peyronellaea* resemble a genetically heterogenous group. The ability to produce dictyochlamydospores has probably been lost and gained multiple times in the evolution of the *Pleosporales*. This character is also easily lost in culture, as has repeatedly been reported in literature (Boerema *et al.* 1965a, Dorenbosch 1970). Chlamydospore production in fungi is generally considered to be a survival strategy due to harsh conditions by perennation (Kirk *et al.* 2008). Although the strains that are used in this study were collected from a wide variety of environments, a relatively high number was retrieved from plant material belonging to *Gramineae*. Also, many of the chlamydospore-forming species have been found in association with cyst nematodes (Heteroderidae, Chen *et al.* 1996). It is tempting to link the similarity in hosts with the capability to produce chlamydospores. Therefore, it may very well be that production of such thick-walled spores may serve other ecological purposes besides long-term survival. Further research should be conducted on the functioning of these structures.

CHAPTER 4

Development of taxon-specific SCAR markers based on actin sequences and DAF : a case study in the *Phoma exigua* species complex

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Summary

Phoma exigua is considered to be an assemblage of at least nine varieties that are mainly distinguished on the basis of host specificity and pathogenicity. However, these varieties are also recorded as weakness pathogens and secondary invaders from non-host tissue. In practice it is difficult to distinguish *Ph. exigua* from its close relatives, and to correctly identify isolates up to variety level, due to their low genetic variation and high morphological similarity. Because of quarantine issues and phytosanitary measures, a robust DNA-based tool is required for robust identification of the separate taxa in this species complex. The present study is therefore aimed at developing such a tool that is based on unique nucleotide sequence identifiers. More than 60 strains of *Ph. exigua* and related species were compared on partial actin gene sequences, or analysed using DNA Amplification Fingerprinting (DAF) with short, arbitrary mini-hairpin primers. Fragments in the fingerprint that were unique to a single taxon were identified, purified and sequenced. Alignment of the sequence data and subsequent primer trials led to the identification of taxon-specific sequence characterised amplified regions (SCARs), and to a set of specific oligonucleotide combinations that can be used to identify these organisms in plant quarantine inspections.

Keywords: diagnostics, coelomycetes, systematics

Introduction

The coelomycete fungus *Phoma exigua* Sacc. is an omnipresent soil inhabitant that occurs with regular incidence as an above-ground plant pathogen, and is presently known from more than 200 plant genera. In many cases, the associated diseases are regarded as serious (e.g. Álvarez *et al.* 2005, Shamoun & Zhao 2005, Koike *et al.* 2006), although *Ph. exigua* is also regularly reported to colonise weakened plant material as an opportunistic secondary invader (Boerema *et al.* 2004). Even animal or human tissue may be colonised (Balis *et al.* 2006). The complexity of the species has been known for quite some time; Boerema & Höweler (1967) were the first to recognise different varieties within *Ph. exigua*. Based on the assumptions of a relatively strict host specificity and appearance on agar media, four infra-specific taxa were proposed, including the varieties *foveata* and *sambuci-nigrae*. These species are currently recognised as separate taxa due to differences in colony characters (Boerema *et al.* 1987) or the absence of certain morphological and physiological characters *in vitro* (Monte *et al.* 1990, 1991). Later, van der Aa *et al*. (2000) discussed the presence of 11 varieties within the *Ph. exigua* complex, of which nine were eventually recognised after AFLP analyses (Abeln *et al.* 2002). The infraspecific taxa currently accepted include the type *Ph. exigua* var. *exigua*, and the varieties *diversispora*, *forsythiae*, *heteromorpha, lilacis, linicola*, *noackiana, populi*, and *viburni*. Boerema *et al*. (2004) provided some cultural –but no microscopical– characters to distinguish these infraspecific taxa of *Ph. exigua*. However, the recognition of these varieties is still considered problematical due to the natural variation within the taxa and the fact that the expression of many cultural characters depend on small variations in the substrate and growing conditions (Rai 2000). Therefore, identification of these isolates is still mainly driven by host association. Closely related species, which are morphologically often confused with *Ph. exigua*, often play an important role as causal agents of plant diseases. For example, the quarantine organism *Ph. foveata* is known as causal agent of potato gangrene, as is the ubiquitous *Ph. exigua* var. *exigua* (Boerema 1967, Boyd 1972). *Phoma sambuci-nigrae* is known from elder (*Sambucus nigra*) (Boerema & Höweler 1967), whereas *Ph. lycopersici* is a widely distributed pathogen of tomato (*Lycopersicon esculentum*) (Morgan-Jones & Burch 1988b).

Organisations involved in assessing plant health are currently in need of a quick, easy to handle protocol to detect and identify isolates of *Phoma* up to species or variety level. A rapid and robust DNA-based tool that could discriminate between the various taxa in this species complex, is the perfect choice for such a protocol. The DNA barcode initiative is, to date, the best approach to identify species-specific nucleotide sequences (Hebert *et al.* 2002). Identification of such DNA barcodes in the genus *Phoma* can aid rapid detection of serious plant pathogens. Although this initiative is generally focussed on the identification of nucleotide sequences that are specific for a single species, we aimed to develop such molecular identifiers for taxa at subspecies level. In recent years, various genes have been proposed as standard loci to use for DNA barcoding in fungi, such as the internal transcribed spacers of the rDNA operon ITS region (Druzhinina *et al.* 2005), and *cytochrome c oxidase subunit 1* (*cox1*) (Seifert *et al.* 2007). However, various studies reported the low discriminatory power of ITS sequences within the *Ph. exigua* complex (Abeln *et al.* 2002; Cullen *et al.* 2007). Furthermore, also *cox1* analysis applied on a subset of 36 *Phoma exigua* related strains according to the methodology of Seifert *et al*. (2007) did not reveal conserved nucleotide differences between taxa (J.H.C. Woudenberg, unpubl. data). In order to develop unique sequence identifiers in the *Ph. exigua* complex, other loci need to be screened, and more suitable genes identified.

Genes that have often been used in Multilocus Sequence Typing (MLST) are the so-called housekeeping genes; these are genes that encode for proteins that are required for the basic functioning of every cell. One of those genes is actin, of which the nucleotide sequence shows high discriminative powers at species level (Voigt & Wöstemeyer 2000). Further fingerprinting based methods may be applied as Abeln *et al*. (2002) showed by means of Amplified Fragment Length Polymorphism (AFLP), could be of great value in discriminating the different varieties and species in the *Phoma* complex. However, developing taxon-specific primers (SCARs – sequence characterised amplified regions) based on AFLP-fingerprints is expensive and laborious, and therefore not preferred. Other fingerprinting tools can nevertheless be helpful in the design of species-specific oligonucleotides, such as RAPD-derived PCR-RFLP or microsatellite analysis.). The RAPD based RFLP method however, results in a considerably low number of bands (Macdonald *et al.* 2000). And although microsatellite analysis is highly reproducible, the development of specific primers is quite laborious (Groenewald *et al.* 2007). Another method that could be of value to achieve the development of taxon-specific primers, is DNA Amplification Fingerprinting (DAF) using mini-hairpin primers (Caetano-

Anollés & Gresshoff 1994). Mini-hairpin primers consist of a 7 or 8 nucleotide long DNA-loop, and an arbitrary core of three to five nucleotides in length. Such short, but very stable, oligonucleotide primers have proven to be helpful in the estimation of the genetic relationships between species in several agricultural crops and weeds (Caetano-Anollés *et al.* 1995), and for the identification of markers linked to a specific mutated locus in pea (Men *et al.* 1999). Besides its usefulness in assessing the genetic variation in various plant populations (Caetano-Anollés 1998, Caetano-Anollés *et al.* 1999, Sen *et al.* 1999), this method has further successfully been applied in a study on the genetic diversity within the plant pathogenic fungi *Fusarium oxysporum* f. sp. *cubense* (Bentley & Bassam 1996) and *Discula destructiva* (Caetano-Anollés *et al.* 1996). To our knowledge, the use of these primers has never been applied in the development of taxon-specific nucleotide markers for plant associated fungi. Therefore, in this paper our aims are (i) to apply the DAF technique on the *Ph. exigua* complex, (ii) identify fingerprint bands specific to the varieties and (iii) convert the DAF profile to a specific SCAR marker. Further we will determine whether (iv) sequence comparison of the partial actin gene can aid in identifying the multiple infraspecific taxa in this complex.

Materials and Methods

Strains and DNA extraction

A total of 58 strains, belonging to the 12 different taxa within the *Ph. exigua* complex, were selected for study (Table 4.1). The reference strains indicated by Boerema *et al*. (2004) were included. Furthermore, six strains from *Ph. herbarum*, *Ph. strasseri*, and *Ph. telephii* were selected as outgroups. The strains were cultured on oatmeal agar medium (OA; Gams *et al.* 2007) and incubated at 24 ºC in complete darkness. After 14 d of growth, mycelium was scraped off the plates using a sterile scalpel blade and collected in a Microbead tube (Ultraclean Microbial DNA Isolation Kit, Mo Bio Laboratories, Carlsbad, CA). Isolation of DNA was conducted using the Ultraclean Microbial DNA Isolation Kit (Mo Bio Laboratories) according to the manufacturer's instructions. The DNA yield was diluted 10 times and stored at 4 ºC until further handling.

Actin PCR and sequence typing

The actin gene region was partially amplified using 0.2 µM of each primer ACT-512F and ACT-783R (Carbone and Kohn, 1999). The reaction mixture further contained *ca.* 5 ng of DNA, 1× PCR Buffer, 1 mM $MgCl_2$, 48 µM dNTPs, 0.5 µM of each of the primers, and 0.25 units of *Taq* DNA polymerase (Bioline, Luckenwalde, Germany). Each reaction was made up to a final volume of 12.5 µL. The polymerase chain reaction (PCR) was performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA), using the following amplification conditions: 94 ºC for 10 min, followed by 40 cycles of denaturation (94 ºC for 30 s), annealing (55 ºC, for 30 s) and elongation (72 ºC for 80 s), and a final extension step at 72 ºC for 6 min. Sequencing of the amplicons was performed with the PCR primers in both directions using the BigDye terminator chemistry v. 3.1 (Applied Biosystems) according to the manufacturer's recommendations. Sequence products were purified with Sephadex G-50 Fine (GE Healthcare Bio-Sciences, Uppsala, Sweden) and subsequently separated and analysed on an ABI Prism 3730 DNA Sequencer (Applied Biosystems).

Consensus sequences were computed from the forward and reverse sequences using the BioNumerics v. 4.5 software package (Applied Maths, St-Martens-Lathem, Belgium). The consensus sequences were assembled and aligned using the same software and adjusted manually where necessary. The phylogenetic analyses of the sequence data were done in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) and consisted of Neighbour-Joining analysis with the uncorrected ("p"), the Jukes-Cantor and the HKY85 substitution models. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. The robustness of the resulting phylogenetic trees was evaluated by 1000 bootstrap replications (Hillis & Bull 1993) and the trees were printed with TreeView v. 1.6.6 (Page 1996). Obtained consensus sequences are deposited in GenBank (For Accession numbers see Table 4.1).

DAF-PCR and fingerprint analysis

Five arbitrarily mini hairpin primers were designed and consisted of seven nucleotides forming the stem and loop (5' GCGAAGC 3') followed by three nucleotides forming an arbitrary core. The designed oligonucleotide primers were 5' H-CAA 3', 5' H-CAG 3', 5' H-CCA 3', 5' H-CCT 3' and 5' H-CTC 3', in which H stands for the stem and loop nucleotides. Amplification of 42 strains was performed using the

following amplification conditions: 95 ºC for 5 min, followed by 40 cycles of denaturation, annealing and elongation (96, 30, and 72 ºC during 30 s for each step), and a final extension step at 72 ºC for 6 min. Each PCR reaction had a total volume of 15 µL and contained 1.0 µL of 10× diluted g enomic DNA, 1× PCR Buffer, 2 mM $MgCl_2$, 240 µM of each of the dNTPs, 3 µM of the primer, and 2 units of *Taq* DNA polymerase (Bioline).

The total volume of obtained PCR product (15 µL) was separated through gel electrophoresis at 110 V for *ca.* 120 min 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 and 0.01M EDTA, pH = 7.85). The molecular weight marker Hyperladder I (Bioline) was used as DNA standard. Subsequently, the DNA fragments were visualised and photographed under UV light.

The fingerprints were analysed for each primer separately with the BioNumerics v4.5 software package. The similarity between the patterns was calculated using the Jaccard-UPGMA algorithm. Analyses were first performed with all five banding patterns separately, with all visible bands having equal weights. This was followed by a combined unweighted analysis using the simple matching binary coefficient, as on average an equal number of bands were present for all five amplification reactions.

Primer development

Two well-vouchered strains were selected per taxon for gel band isolation. Fragments resulting from DAF-PCR unique to each taxon were cut out of the agarose gel using sterile scalpel blades. The DNA was re-suspended by application of the Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare, Little Chalfont, UK) and ligated into a pGEM®-T Easy vector, using the pGEM®-T Easy Vector System I (Promega, Madison, USA), according to the manufacturer's instructions. The vector with insert was cloned into competent *Escherichia coli* strain JM109 cells (Promega) by applying a heat-shock for 50 s at 42 ºC. The recombinants were visualised with blue/white screening on standard LB agar plates supplemented with 100 µg/mL ampicillin, 0.1 mM IPTG and 40 µg/mL X-Gal. The white colonies were picked up with a pipette tip and resuspended in 10 µL LB solution for colony PCR. The colony PCR was performed with the universal M13F and M13R primers and the reaction mixture contained 1.5 µL liquid colony, 0.2 µM of each primer, 0.4 unit *Taq* polymerase E (Genaxxon Bioscience, Biberach, Germany), 0.03 mM dNTP's, 1.5 mM $MgCl_2$ and 1× PCR buffer E 'incomplete' (Genaxxon) in a total reaction volume of 10 µL. The initial denaturation step of 5 min at 94 ºC was followed by 35 cycles of 94 ºC (20 s), 55 ºC (20 s), and 72 ºC (100 s) with a final elongation step at 72 ºC (7 min).

Sequencing of the amplicons was performed in the same way as for the actin amplicons, but with the exception that the universal primers M13F and M13R were applied. Using the Seqman programme from the Lasergene package (DNAstar, Madison, Wisconsin, USA), the vector's DNA was subtracted, consensus sequences were computed and sequences were aligned. Obtained consensus sequences were compared to sequences deposited in GenBank using the BLAST search algorithm.

To obtain a conventional PCR assay to identify the separate taxa included in this species complex, primer sets unique to each taxon were designed based on the actin region or to the taxon-specific DAF-bands using the Primer3 web application (http://primers.sourceforge.net). In the case of the DAF-based primers, primers were based on taxon-specific bands that did not show any genetic variation in the sequence analyses. At least one of the designed oligonucleotides contained the complete arbitrary core of the mini-hairpin primer. For the actin-based primer combinations, either the universal primer ACT-783R (Carbone and Kohn, 1999) or the newly designed forward primer ACT1Fd (5'— GCYGCBCTCGTYATYGACAATGG — 3'), was used as an anchor primer whilst the second primer was designed on the region of high interspecific variation. It was attempted to design all primers (Table 4.2) with an annealing temperature of circa 60 °C to guarantee standardisation of PCR conditions. The sequences are deposited in GenBank, trace files are lodged with MycoBank.

Results

Actin sequence analysis

PCR products of *ca.* 270 bp long were obtained and 59 informative sites were observed between all strains analysed (Table 4.1). Four uninformative SNPs were found in the alignment, which was limited to single strains, whilst one single nucleotide insertion was observed. Almost identical sequences were obtained for all samples,

Table 4.1. List of *Phoma* strains studied. GenBank accession numbers refer to the actin sequences obtained in this study. Representative strains designated by Boerema *et al*. (2004) are marked with an asterisk (*). Isotype strains are indicated with a hash mark (#).

Strain no.	Other collection no.	GenBank no.	Species	Variety	Host	Origin
CBS 101151	PD 82/1022	EU880846	*Ph. exigua*	var. *exigua*	*Dahlia* sp.	Netherlands
CBS 101152	PD 90/835-3	EU880847	*Ph. exigua*	var. *exigua*	*Digitalis* sp.	Netherlands
CBS 101156	PD 90/731	EU880848	*Ph. exigua*	var. *exigua*	*Solanum tuberosum*	Philippines
CBS 113.36		EU880849	*Ph. exigua*	var. *exigua*	*Dahlia variabilis*	Germany
CBS 114.56		EU880850	*Ph. exigua*	var. *exigua*	*Hydrangea macrophylla*	Italy
CBS 236.28			*Ph. exigua*	var. *exigua*	*Solanum tuberosum*	Netherlands
CBS 342.67		EU880851	*Ph. exigua*	var. *exigua*	*Solanum tuberosum*	UK, Northern Ireland
CBS 373.61		EU880852	*Ph. exigua*	var. *exigua*	*Ulmus* sp.	Netherlands
CBS 391.84		EU880853	*Ph. exigua*	var. *exigua*	*Foeniculum vulgare*	Germany
CBS 431.74*	PD 74/2447	EU880854	*Ph. exigua*	var. *exigua*	*Solanum tuberosum*	Netherlands
CBS 446.83		EU880855	*Ph. exigua*	var. *exigua*	*Thymus* sp.	Netherlands
CBS 534.75		EU880856	*Ph. exigua*	var. *exigua*	*Atropa belladonna*	Belgium
CBS 761.70		EU880857	*Ph. exigua*	var. *exigua*	*Digitalis purpurea*	Netherlands
CBS 833.84		EU880858	*Ph. exigua*	var. *exigua*	*Hordeum vulgare*	Germany
PD 87/720		EU880859	*Ph. exigua*	var. *exigua*	*Helianthus annuus*	Netherlands
PD 94/1396		EU880860	*Ph. exigua*	var. *exigua*	*Solanum tuberosum*	Netherlands
CBS 102.80*	PD 79/61	EU880861	*Ph. exigua*	var. *diversispora*	*Phaseolus vulgaris*	Kenya
CBS 101214	PD 86/695, IMI 373349	EU880862	*Ph. exigua*	var. *diversispora*	*Phaseolus lunatus*	Zambia
CBS 531.86		EU880863	*Ph. exigua*	var. *diversispora*	*Vigna* sp.	Burundi
CBS 101194	PD 79/687, IPO 89-19	EU880864	*Ph. exigua*	var. *diversispora*	*Phaseolus vulgaris*	Netherlands
PD 86/694	BBA 3329	EU880865	*Ph. exigua*	var. *diversispora*	*Vigna radiata*	Germany
CBS 101197	PD 95/721	EU880866	*Ph. exigua*	var. *forsythiae*	*Forsythia* sp.	Netherlands
CBS 101212	PD 95/8846	EU880867	*Ph. exigua*	var. *forsythiae*	*Forsythia* sp.	Netherlands
CBS 101213*	PD 92/959	EU880868	*Ph. exigua*	var. *forsythiae*	*Forsythia* sp.	Netherlands
CBS 443.94*		EU880869	*Ph. exigua*	var. *heteromorpha*	*Nerium oleander*	Italy
CBS 101196	PD 79/176	EU880870	*Ph. exigua*	var. *heteromorpha*	*Nerium oleander*	France
CBS 548.90		EU880871	*Ph. exigua*	var. *heteromorpha*	*Nerium oleander*	Italy
PD 88/304		EU880872	*Ph. exigua*	var. *heteromorpha*	*Nerium oleander*	Netherlands
CBS 101206	PD 88/118	EU880873	*Ph. exigua*	var. *lilacis*	*Syringa vulgaris*	Netherlands
CBS 101207	PD 94/614	EU880874	*Ph. exigua*	var. *lilacis*	*Syringa vulgaris*	Netherlands
CBS 569.79*	PD 72/741	EU880875	*Ph. exigua*	var. *lilacis*	*Syringa vulgaris*	Netherlands
CBS 489.94		EU880876	*Ph. exigua*	var. *lilacis*	*Forsythia*	Netherlands
CBS 109.49		EU880877	*Ph. exigua*	var. *linicola*	*Linum usitatissimum*	Unknown
CBS 112.28		EU880878	*Ph. exigua*	var. *linicola*	*Linum usitatissimum*	Former USSR
CBS 114.28		EU880879	*Ph. exigua*	var. *linicola*	*Linum usitatissimum*	Netherlands
CBS 116.76*	ATCC 32332, CECT 20022, CECT 20023, IMI 197074, IPO 5987	EU880880	*Ph. exigua*	var. *linicola*	*Linum usitatissimum*	Netherlands

Table 4.1. (Ctd).

Strain no.	Other collection no.	GenBank no.	Species	Variety	Host	Origin
CBS 100353*	PD 87/718	EU880881	*Ph. exigua*	var. *noackiana*	*Phaseolus vulgaris*	Guatemala
CBS 101203	PD 79/1114, IPO 50987	EU880882	*Ph. exigua*	var. *noackiana*	*Phaseolus vulgaris*	Colombia
CBS 101215	PD 87/717	EU880883	*Ph. exigua*	var. *noackiana*	*Phaseolus vulgaris*	Central America
CBS 101216	PD 79/1113	EU880884	*Ph. exigua*	var. *noackiana*	*Phaseolus vulgaris*	Colombia
CBS 100167	PD 93/217	EU880885	*Ph. exigua*	var. *populi*	*Populus (x) euramericana*	Netherlands
CBS 100168	PD 94/138	EU880886	*Ph. exigua*	var. *populi*	*Populus (x) euramericana*	Netherlands
CBS 101198	PD 97/12185	EU880887	*Ph. exigua*	var. *populi*	*Populus* sp.	Netherlands
CBS 101202	PD 82/942	EU880888	*Ph. exigua*	var. *populi*	*Salix* sp.	Netherlands
CBS 100354*	PD 84/448	EU880889	*Ph. exigua*	var. *viburni*	*Viburnum opulus*	Netherlands
CBS 101209	PD 70/717	EU880890	*Ph. exigua*	var. *viburni*	*Viburnum* sp.	Netherlands
CBS 101211	PD 93/838	EU880891	*Ph. exigua*	var. *viburni*	*Lonicera* sp.	Netherlands
CBS 109176*	PD 94/1394, CECT 2828	EU880892	*Ph. foveata*		*Solanum tuberosum*	Bulgaria
CBS 200.37#		EU880893	*Ph. foveata*		*Solanum tuberosum*	UK, England
CBS 341.67*	CECT 20051, MUCL 9928	EU880894	*Ph. foveata*		*Solanum tuberosum*	UK, Northern Ireland
CBS 502.91	PD 86/276	EU880895	*Ph. herbarum*		*Nerium* sp.	Netherlands
CBS 615.75*	PD 73/665, CECT 20014-16, IHEM 3803, IMI 199779, LCP 90.2647, ATCC 2499	EU880896	*Ph. herbarum*		*Rosa multiflora*	Netherlands
CBS 196.36	ATCC 11847, MUCL 9560	EU880897	*Ph. lycopersici*		*Lycopersicon esculentum*	Germany
CBS 378.67*	PD 67/276	EU880898	*Ph. lycopersici*		*Lycopersicon esculentum*	Netherlands
CBS 735.74		EU880899	*Ph. lycopersici*		*Lycopersicon esculentum*	Netherlands
CBS 101199	PD 79/392	EU880900	*Ph. lycopersici*		*Lycopersicon esculentum*	Netherlands
CBS 101200	PD 72/863	EU880901	*Ph. lycopersici*		*Lycopersicon esculentum*	Netherlands
CBS 104.68	CECT 20048, IMI 331913	EU880902	*Ph. sambucinigrae*		*Sambucus nigra*	Netherlands
CBS 629.68*#	PD 67/753		*Ph. sambucinigrae*		*Sambucus nigra*	Netherlands
CBS 109170	PD 75/796	EU880903	*Ph. sambucinigrae*		*Sambucus nigra*	Netherlands
CBS 126.93	PD 73/642	EU880904	*Ph. strasseri*		*Mentha* sp.	Netherlands
CBS 261.92*	PD 92/318, ATCC 24146	EU880905	*Ph. strasseri*		*Mentha piperita*	USA, Oregon
CBS 109175	PD 79/524	EU880906	*Ph. telephii*		*Sedum spectabile*	Netherlands
CBS 760.73*	PD 71/1616, IMI 300060	EU880907	*Ph. telephii*		*Sedum telephium*	Netherlands

Table 4.2. Taxon-specific primers designed for the identification of species and varieties in the *Ph. exigua* complex. The GenBank accession number of the sequence of DAF fragment from which the primers were designed is shown in the last column.

Taxon name	Marker	Primer ID	Primer sequence (5'-3')	Product size (bp)	Tm (ºC)	GenBank no.
Ph. exigua var. *diversispora*	Actin	ACTdiv76F	CAGCAGCACCCCTCCATTA	190	62	
		ACT-783R*	TACGAGTCCTTCTGGCCCAT			
Ph. exigua var. *exigua*	H-CCT	Ex2-1	GCCCTGCAGACAATTTACGA	368	60	EU880838
		Ex2-2	GCCGATGAAAAGGAAAACCT			
Ph. exigua var. *forsythiae*	H-CAA	For1-1	GCCAAGCGATATTGTACGCTAG	378	64	EU880845
		For1-2	ACCTCCGAGACATGAACGAC			
Ph. exigua var. *heteromorpha*	H-CAA	ExpoF	CAAACTGTACACGACTCTTC	363	58	EU880841
		Expo364R	GAATACACTGGTCGGTAGTT			
Ph. exigua var. *lilacis*	Actin	ACT1Fd	GCYGCBCTCGTYATYGACAATGG	139	60	
		ACTlil103R	GTGGGCGCAATGTAGCC			
Ph. exigua var. *linicola*	Actin	ACTlin74L	GGCAGCAGCACCCAAG	195	59	
		ACT-783R*	TACGAGTCCTTCTGGCCCAT			
Ph. exigua var. *noackiana*	H-CCT	Noa1-1	AGCCTCCAACGATCATAAG	482	60	EU880842
		Noa1-2	TCAAGCACAAGTAGCACAAAAA			
Ph. exigua var. *populi*	H-CAA	Pop3-1	AACGGGCAGTACTGATGCAAT	400	64	EU880843
		Pop3-2	CCGACACGTACCCACAATTT			
Ph. exigua var. *viburni*	H-CAA	Vib4-1	CAAGAGATGCCTGGACCGTT	753	64	EU880844
		Vib4-8	AGTTTCTCAACTTTGACGCCTAGC			
Ph. foveata	H-CTC	Fov1-1	GCCTCAACGGTGAAGATGG	572	58	EU880839
		Fov1-2	GCCTCGAGTTCTAAATGAGTTTG			
Ph. lycopersici	Actin	ACT1Fd ACTlyc145R	GCYGCBCTCGTYATYGACAATGG AGTCAGGACAATGTTCTCC	174	54	
Ph. sambuci-nigrae	H-CAA	Sam1-1	CCAAGGTGAGGGTCGAGAAG	253	60	EU880840
		Sam1-4	CCATGGCACTTTGGAGTCTA			

* Primer developed by Carbone & Kohn (1999)

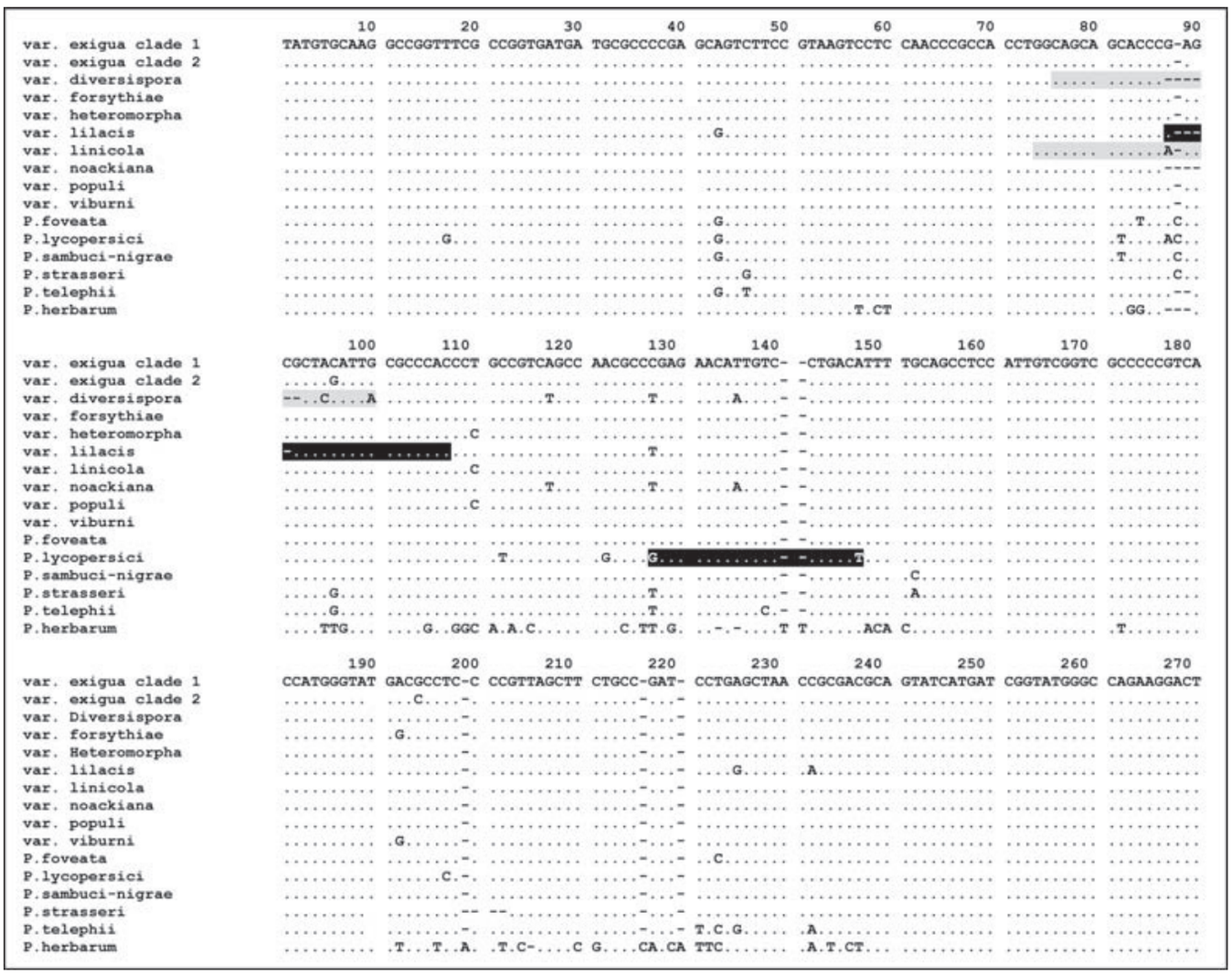

Figure 4.1. Alignment of actin sequences of 13 *Phoma* strains belonging to the *Ph. exigua* complex and three outgroup species. Strains included are *Ph. exigua* var. *exigua* (CBS 431.74 and CBS 101156), var. *diversispora* (CBS 531.86), var. *forsythiae* (CBS 101213), var. *heteromorpha* (CBS 443.94), var. *lilacis* (CBS 569.79), var. *linicola* (CBS 116.76), var. *noackiana* (CBS 100353), var. *populi* (CBS 100167), var. *viburni* (CBS 100354), *Ph. sambuci-nigrae* (CBS 109170), *Ph. foveata* (CBS 341.67), and *Ph. lycopersici* (CBS 378.67). As outgroup species, *Ph. strasseri* (CBS 261.92), *Ph. telephii* (CBS 760.73) and *Ph. herbarum* (CBS 615.75) were included. Primers designed for this study are indicated by marked blocks: grey sequence blocks represent a forward primer sequence, black blocks a reverse primer. The full stops indicate characters identical to those in the first line and dashes represent alignment gaps. Primer ACTdiv76F spans var. *diversispora* nucleotides 76–100, primer ACTlil103R spans var. *lilacis* nucleotides 87–107, primer ACTlin74L spans var. *linicola* nucleotides 74-90, and primer ACTlyc145R spans *Ph. lycopersici* nucleotides 127–147.

except for some SNP's and an intron region between position 51–152 (*Ph. exigua* var. *exigua*, CBS 431.74, GenBank Accession EU880854), where multiple dissimilarities were found that were highly conserved over the individuals of a single taxon (Figure 4.1). The *Ph. exigua* var. *diversispora*, *lilacis*, and *linicola* and the species *Ph. lycopersici* showed a series of deletions and point mutations between taxa that were suitable for primer development. The designed taxon-specific primers are listed in Table 4.2. Phylogenetic results based on the actin sequence alignment (Figure 4.2) show that the outgroup species *Ph. herbarum* and *Ph. strasseri* are basal to the species in the *Ph. exigua* complex. Furthermore, several of the species related to the *Ph. exigua* complex, *Ph. foveata*, *Ph. lycopersici* and *Ph. sambuci-nigrae* could be clearly distinguished, with bootstrap support values of 85, 99 and 86 %, respectively. Remarkably, also *Ph. telephii* clusters amidst the taxa in the *Ph. exigua* complex, with high bootstrap support for the strains of this species (98 %). This may however be an artefact of the tree itself, due to a high level of basal polytomy. Many of the *Ph. exigua* varieties appear to be

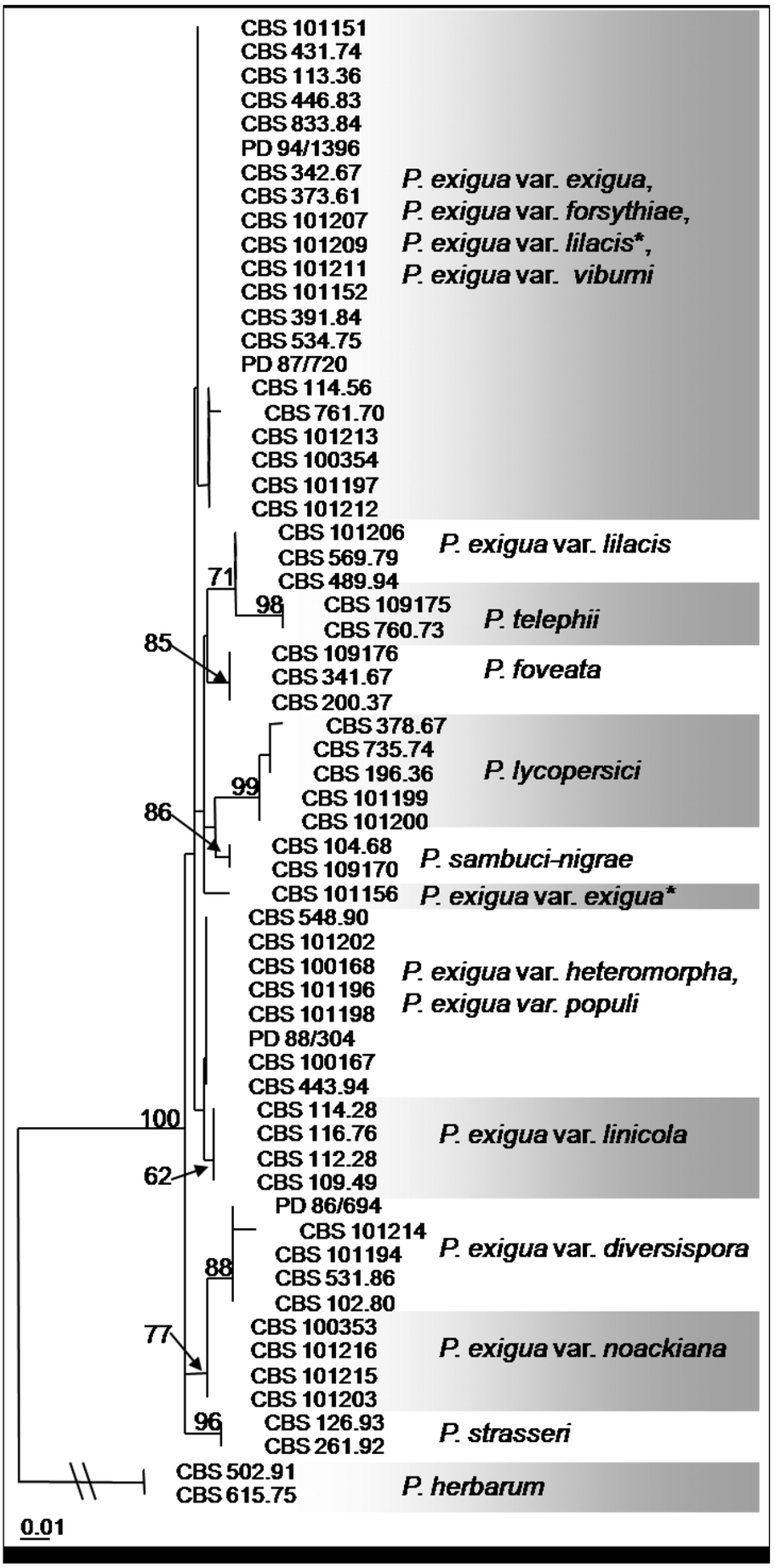

Figure 4.2. Neighbour-Joining phylogenetic tree obtained from partial actin sequences using the HKY85 substitution model (n = 62). Bootstrap support values are based on 1000 replicates and are shown at the nodes when higher than 60 %. The scale bar represents 0.01 substitutions per site. The tree is rooted with two strains of *Ph. herbarum* (CBS 615.75 and CBS 502.91). The taxa marked with asterisks are considered to be misidentifications in the culture database.

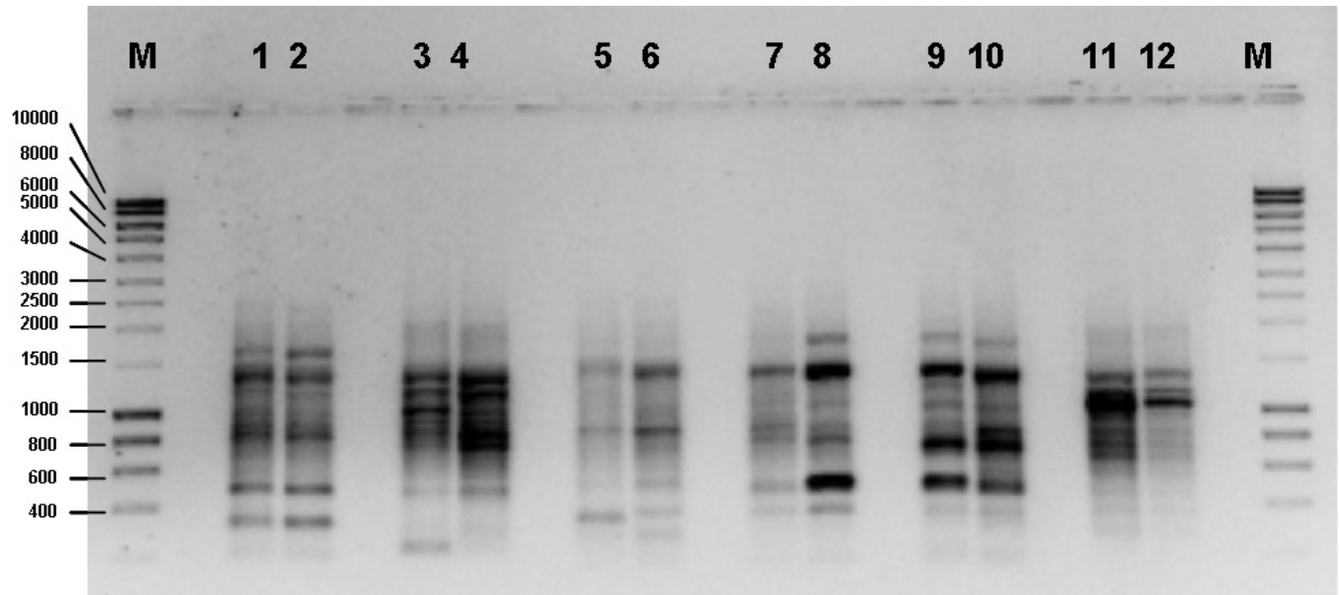

Figure 4.3. Example of a DAF assay on selected isolates from the *Ph. exigua* complex using the H-CCT primer. Lanes 1 & 2, *Ph. exigua* var. *heteromorpha* CBS 443.94 and CBS 101196. Lanes 3 & 4, *Ph. exigua* var. *noackiana* CBS 100353 and CBS 101203. Lanes 5 & 6, *Ph. exigua* var. *populi* CBS 100167 and CBS 101202. Lanes 7 & 8, *Ph. exigua* var. *viburni* CBS 100354 and CBS 101211. Lanes 9 & 10, *Ph. exigua* var. *forsythiae* CBS 101213 and CBS 101197. Lanes 11 & 12, *Ph. lycopersici* CBS 378.67 and CBS 101199. Lanes M, Hyperladder I (Bioline).

monophyletic, although this monophyly is based on individual SNP's and a very short sequence length, which may explain the lack of high support values in the trees' backbone structure. Two of the analysed strains, CBS 101156 and CBS 101207, identified as var. *lilacis* and *exigua* respectively, fall outside the main clusters for these taxa. These morphologically atypical strains are marked with an asterisk.

Analysis of the DAF products

DNA extracts of 42 strains belonging to the *Ph. exigua*-complex were analysed using five mini-hairpin primers. Each of the fingerprints contained 1–13 visible bands between 300 and 2000 bp (av. = 6.9 bands/primer) (Figure 4.3). The results were well-reproducible as multiple amplification reactions in different thermal cyclers and in different amplification runs provided similar banding patterns (data not shown). Considerable variation was observed between the included species and varieties, and none of the bands was consistent over all samples, but often shared bands were found between multiple strains of a single species or variety that were unique for that taxon. Taxon-specific bands of various sizes were obtained with different mini hairpin primers (Table 4.2). Taxon-specific primers were developed for *Ph. foveata* and *Ph. sambuci-nigrae* and for *Ph. exigua* var. *heterospora*, *forsythiae*, *noackiana*, *populi*, and *viburni*. Also for the *Ph. exigua* var. *exigua* strains that cluster with the reference strains CBS 431.74 and CBS 101152, a specific primer combination was developed.

A combined UPGMA analysis of the five generated fingerprints is presented in Figure 4.4. Results indicate that many of the taxa included in this study were represented by one well-supported cluster (bootstrap support value > 70 %). Those included species and varieties, with the exception of var. *lilacis* and var. *exigua* proved to be monophyletic. Similar to the data represented in Figure 4.2, strain CBS 101207, which was originally identified as *Ph. exigua* var. *lilacis*, is not clustering with the main body of the var. *lilacis* strains. The type variety *exigua* appears to consist of two separate clusters. One, indicated in Figure 4.4 as "*Phoma exigua* var. *exigua* 1" comprises both reference strains, but has long-branched taxa and a low support value.

A second cluster comprising eight other strains that also originally were identified as *Ph. exigua* var. *exigua*, is indicated in Figure 4.4 as "*Phoma exigua* var. *exigua* 2" and is well-supported (bootstrap support value = 92 %). Also nucleotide sequence data of the actin region of all var. *exigua* strains did not reveal consistent significant differences (Figures 4.1, 4.2). Nevertheless, due to the DAF analysis results, the taxonomical status of those diverging strains appears questionable. Therefore, the strains in this clade were excluded from the primer design phase of this study.

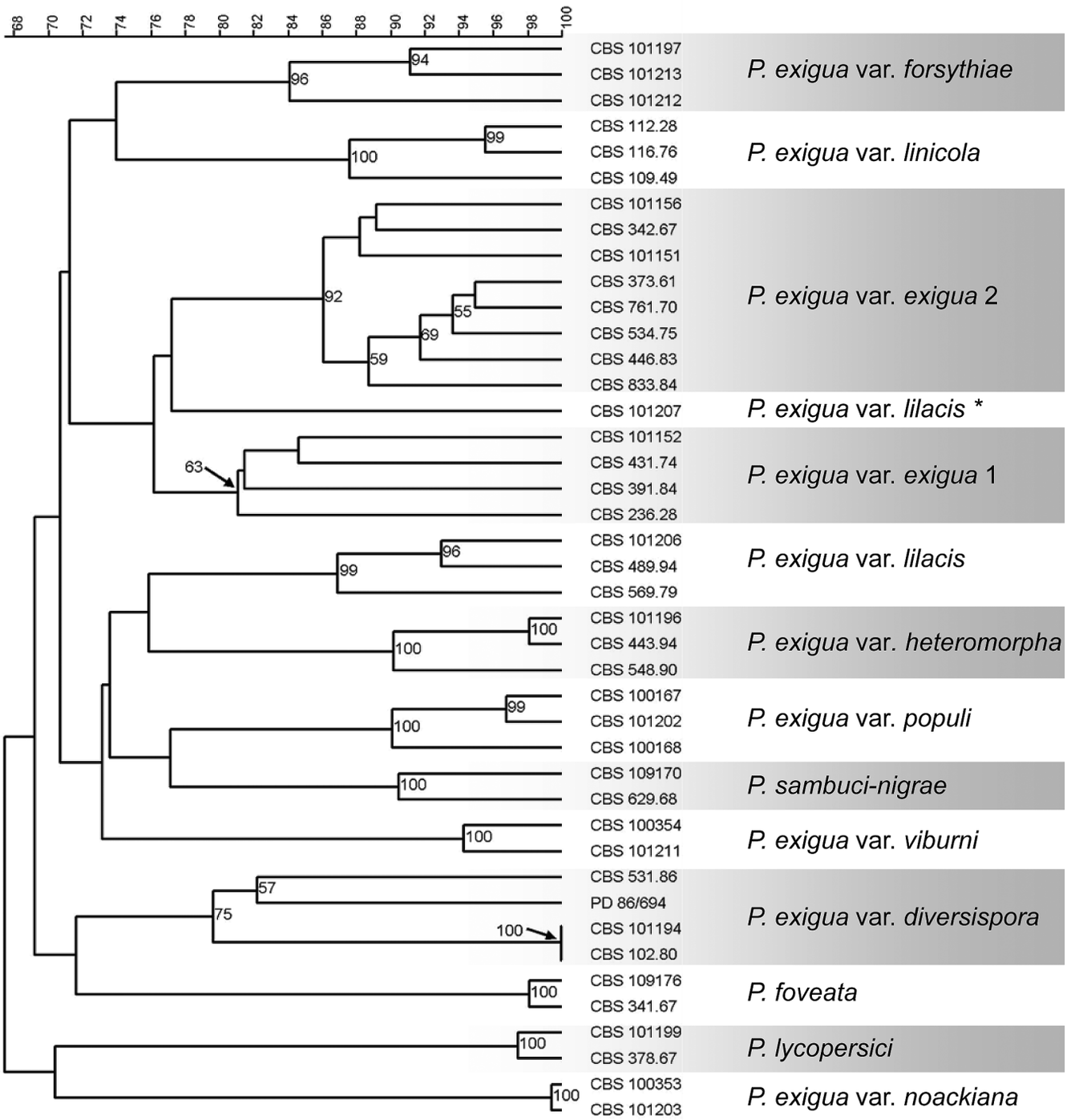

Figure 4.4. UPGMA analysis based on the combination of all five DAF mini-hairpin patterns using the simple matching binary coefficient to calculate the similarity between the strains. Bootstrap support is based on 1000 replicates and values higher than 50 % are shown. The scale at the top indicates percentage similarity. The taxon marked with an asterisk is considered to be a misidentification in the culture database.

Specificity of taxon-specific PCR assays

Twelve taxon-specific primer combinations were developed to distinguish the currently known *Ph. exigua* varieties and three species that are often confused with *Ph. exigua*. Four of these combinations anneal within the actin gene, eight were developed based on the taxon-specific bands generated with the DAF-minihairpin protocol (Table 4.2). Specific fragments resulting from the DAF were selected from two strains per taxon. The fragments were ligated, cloned and sequenced. Fragments, in which genetic variation was absent, were used for primer design. The sequences obtained from the DAF fragments did not match with any of the sequences currently available in GenBank.

The developed primer combinations were tested on specificity on a selection of 47 *Phoma* strains present in the CBS culture collection, which belonged to 36 different species. Furthermore, the primers were

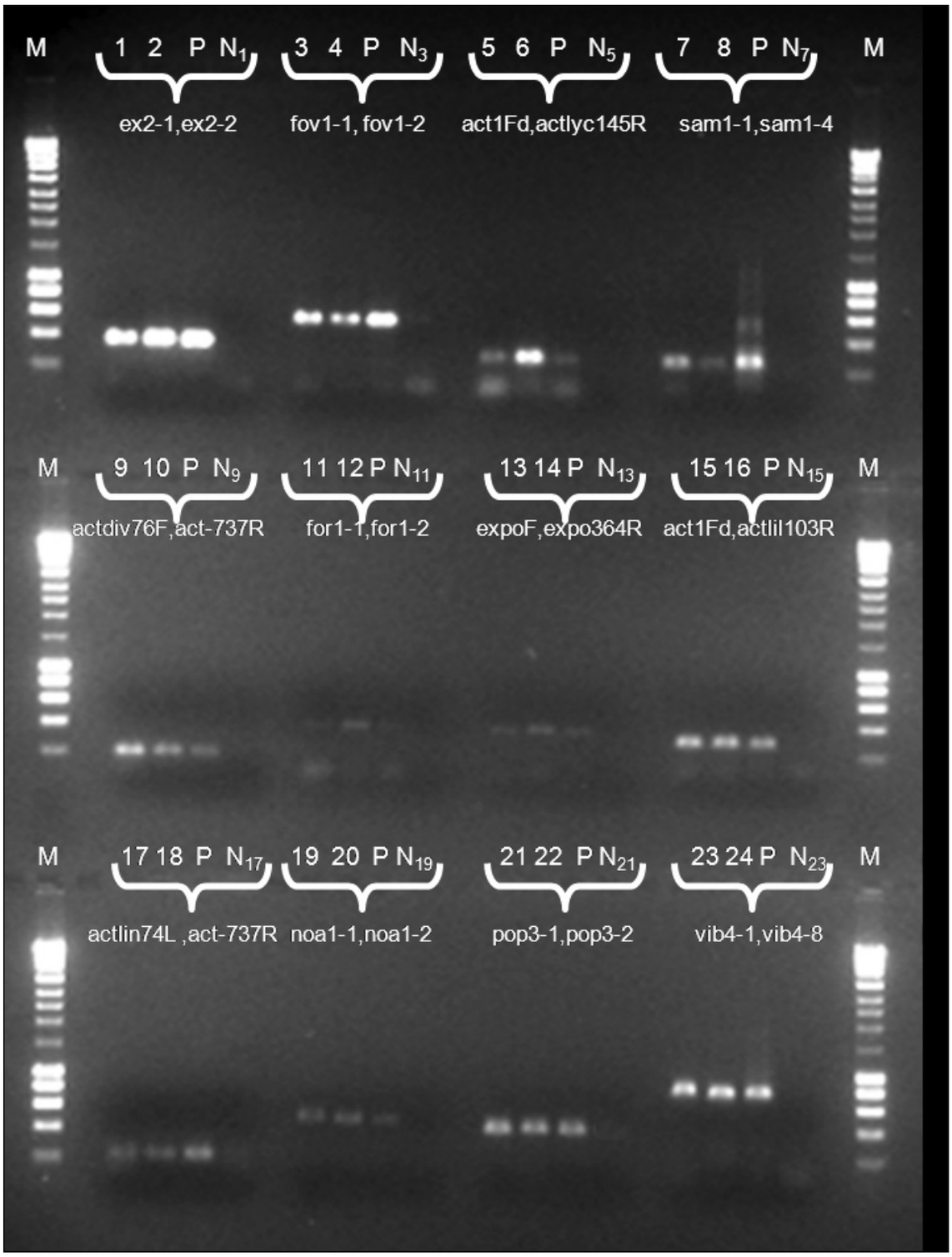

Figure 4.5. PCR Products amplified from genomic DNA using the species-specific primer combinations indicated below the wells. Lane 1, *Ph. exigua* var. *exigua* (CBS 431.74); Lane 2, idem (CBS 101152); Lane 3, *Ph. foveata* (CBS 341.67); Lane 4, idem (CBS 109176); Lane 5, *Ph. lycopersici* (CBS 378.67); Lane 6, idem (CBS 101199); Lane 7, *Ph. sambuci-nigrae* (CBS 629.68); Lane 8, idem (CBS 109170); Lane 9, *Ph. exigua* var. *diversispora* (CBS 102.80); Lane 10, idem (CBS 101194); Lane 11, *Ph. exigua* var. *forsythiae* (CBS 101213); Lane 12, idem (CBS 101197); Lane 13, *Ph. exigua* var. *heteromorpha* (CBS 443.94); Lane 14, idem (CBS 101196); Lane 15, *Ph. exigua* var. *lilacis* (CBS 569.79); Lane 16, idem (CBS 101207); Lane 17, *Ph. exigua* var. *linicola* (CBS 116.76) ; Lane 18, idem (CBS 112.28); Lane 19, *Ph. exigua* var. *noackiana* (CBS 100353); Lane 20, idem (CBS 101203); Lane 21, *Ph. exigua* var. *populi* (CBS 100167) ; Lane 22, idem (CBS 101202); Lane 23, *Ph. exigua* var. *viburni* (CBS 100354); Lane 24, idem (CBS 101211). Lane P comprises genomic DNA from pooled isolates from all varieties (CBS 431.74, CBS 341.67, CBS 378.67, CBS 629.68, CBS 102.80, CBS 101213, CBS 101196, CBS 443.94, CBS 569.79, CBS 116.76, CBS 100353, CBS 100167, CBS 100354 and CBS 101156 = *Ph. exigua* var. *exigua* type 2) amplified with the respective primer pairs. Lanes N_1-N_{23} contains the same pooled DNA as in lane P, but excluding the target DNA of the specific applicable primer pair. Lanes M, Hyperladder I (Bioline).

tested on all strains of the target taxa, listed in Table 4.1. This assay resulted in finally obtaining 12 primer combinations, which showed no false positives or false negatives (data not shown), giving confidence on the specificity of the developed primers.

As a final trial, the 12 primer combinations were tested to confirm that they were taxon-specific. Therefore, DNA of target strains was amplified using the developed primers. As a positive control, pooled DNA of the 12 taxa as well as from the second *Ph. exigua* var. *exigua* taxon (represented by CBS 101156) were included. As a negative control, the same DNA was pooled, with exclusion of that of the target taxon. Results show that the designed primer combinations successfully amplified DNA of the target taxa (Figure 4.5), whereas the negative controls did not show any amplification product.

Discussion

Prior to the development of DNA-based identification and detection methods, the different taxa in the *Ph. exigua* species complex could only be discriminated based on a certain level of host-specificity, cultural characters and minute morphological differences *in vitro* (Boerema & Höweler 1967, van der Aa *et al.* 2000, Boerema *et al.* 2004). Previous attempts to design sets of taxon-specific oligonucleotides on basis of ITS did not differentiate between *Ph. foveata* and the several *Ph. exigua* varieties (Cullen *et al.* 2007), and a technique developed by Macdonald *et al*. (2000) required an additional endonuclease digestion, only to distinguish *Ph. foveata* from the *Ph. exigua* varieties *exigua* and *diversispora* and from *Ph. sambuci-nigrae*. As DNA barcoding has proven to be a useful method in taxon identification, it was preferred to identify such taxon-specific oligonucleotide sequences. Unfortunately, both gene regions currently applied for DNA barcoding in fungi, *cox1* and ITS, have only limited discriminatory power at the sub-species level, which is required for the *Ph. exigua* complex. Furthermore, nucleotide sequence analyses of a number of other gene regions (calmodulin, β-tubulin, translation elongation factor 1-α, 18S and 28S nrDNA) on a selection of at least 36 well-vouchered strains of *Ph. exigua* and related species provided only a few nucleotide differences, that proved to not be conserved within a taxon (M.M. Aveskamp, unpubl. data). As the actin gene can discriminate between several closely related *Phoma* taxa, apparently with little phylogenetic overlap between sister taxa, its use as standard barcode region for the genus *Phoma* deserves further investigation. Although the same locus has been applied before in developing identification techniques and is highly reliable for deep-level phylogeny (Voigt &Wöstemeyer 2000), its potential as barcoding gene has not been fully studied. Nevertheless, our data show that it was not possible to design actin-based DNA barcodes for all species or varieties in the complex studied, let alone develop taxon-specific sets of oligonucleotide primers based on this locus. Future work on the use of actin as candidate barcoding gene for *Phoma* could involve the use of a longer amplification product of the gene, as we only focussed in this study on a product shorter than 300 bp in length. However, design and development of a robust new primer combination is required before the chances of actin being employed as a barcoding gene for *Phoma* can be estimated.

When comparing fungal strains at species level or higher, an MLST approach is often sufficient to differentiate the separate taxa. However, when multiple infraspecific taxa are involved, such as in the *Ph. exigua* species complex, the need for further phylogenetic analysis using fingerprinting methods is often required. Arbitrarily Primed Technology (APT) methods use single arbitrarily chosen primers to produce simple fingerprints of complex genomes. However, most of these methods, have been considered to be poorly reproducible between different laboratories or even between different thermal cyclers (MacPherson *et al.* 1993; Penner *et al.* 1993). To increase the robustness and reproducibility of this technique, the DAF methodology was developed, which is based on the use of very short oligonucleotide primers (Caetano-Anollés 1993). A modified protocol in which the mini-hairpin primers were implemented was later adopted (Caetano-Anollés & Gresshoff 1994). Mini-hairpin primers produce very consistent banding patterns due to the short arbitrary core: in a study on genetic variability of flowering dogwood (*Cornus florida*) less than 2 % variation in band mobility and less than 3 % variation in product yield were observed (Caetano-Anollés *et al.* 1999). The reproducibility of this technique was confirmed in the present study as duplicate reactions resulted in identical banding patterns (data not shown).

However, from an evolutionary point of view, this technique, in combination with the set of strains used, is of less significance as the evolutionary history and the relation between the taxa in this complex could not be derived from the results obtained. Most individual taxa in the phylogenetic tree obtained (Figure

4.4) were long branched, and bootstrap support for the trees' backbone structure was low. This may be due to the relatively low number of bands obtained and the method of scoring the bands – the stricter a band is scored, the longer the branches will become. The number of bands obtained per reaction is relatively low compared to what is observed when this method is applied to plant material. One of the reasons may be that, in general, plant genomes are much larger than those of fungi. For example, the genome of *Leptosphaeria maculans*, teleomorph of *Ph. lingam*, is estimated to consist of *ca.* 34 Mb (Howlett *et al.* 2001). This is 4–400 times smaller than an average plant genome (Paterson 2006). Consequence of this is that in a DAF assay on plant samples, more bands will be obtained, and thus more bands could be consistent over multiple taxa, which may lead to higher support values for the basal nodes. Nevertheless, for development of taxon-specific SCAR markers the low phylogenetic applicability is not relevant, as single nucleotide differences can be sufficient to identify taxa.

The lack of support at the deeper nodes in the DAF-based tree may be one of the explanations why the *Ph. exigua* complex as a whole seems to be paraphyletic; however, also in the actin-based phylogeny (Figure 4.2), there are some indications that the species borders of *Ph. exigua* are not in congruence with the present taxonomical information, although this phylogenety is based on a limited number of SNPs from a single locus. The figure mainly illustrates the existence of a so-called 'barcoding-gap', and does not necessarily represents the 'correct' evolutionary history of the taxa included. Further research is required to obtain a better understanding of the evolutionary history of this species complex. In contrast, most of the individual varieties included in this study formed separate clusters in the DAF data set, which supports the subdivision of *Ph. exigua* as proposed by van der Aa *et al*. (2000). Also the closely related *Ph. lycopersici*, *Ph. sambuci-nigrae* and *Ph. foveata* appeared to be monophyletic. However, a group of strains that all previously have been identified as *Ph. exigua* var. *exigua*, formed a clearly distinct banding pattern in all analyses conducted, and represent a separate clade in the phylogenetic analysis. The existence of this clade is in congruence with an observation of Abeln *et al*. (2002), in which one strain, CBS 101156, clustered outside the main *Ph. exigua* var. *exigua* clade in their AFLP study. The authors hypothesised that the alternate banding pattern reflected the distinct geographical origin, as this strain was collected from the Philippines. However, present results show that the strains in this cluster occur worldwide on a wide range of hosts. Further analysis of this group is required to determine the taxonomic status of these strains. Again, Abeln *et al*. (2002) mention 'strain CBS 101207, a *Ph. exigua* var. *lilacis* isolate, being genetically totally different compared to the three other *Ph. exigua* var. *lilacis* isolates'. The present study supports the observation that this deviating strain probably belongs to another, yet undescribed variety.

DAF using mini-hairpin primers appears to be a helpful technique to discriminate closely related fungal taxa. Therefore, this technique is considered to be a promising tool for assessing infraspecific genetic variation. Using the obtained DAF-profiles, we were able to design oligonucleotide primer combinations that can aid in identifying taxa at sub-species level. One of the pitfalls of SCAR-based identification of *Ph. exigua* varieties is that it is based on the absence or presence of two extreme short nucleotide sequences. Mutations or sexual genetic recombination may affect the target DNA of the developed primers, causing false negative identification results. However, it should be noted that thus far no evidences have been found for sexual reproduction of *Ph. exigua*. Although the complete set of well-vouchered strains that are housed in the public collections of the CBS and PD was used, the amount of control strains was limited only three for some taxa. Therefore, it is recommended to further validate the developed primers on a larger set of strains. The PCR assays have been conducted using only pure cultures *in vitro*, it is hoped that the data generated could eventually be implemented in a test to distinguish *Phoma* species and varieties *in planta*. The availability of such a test would greatly contribute to the reduction of invasive *Phoma* species and facilitate the utilisation of proper phytosanitairy measures.

The borders between the species rank and the variety level of a taxon in the *Ph. exigua* species complex have been a major point of discussion in the past. For example, *Ph. foveata* has been recombined as a variety into *Ph. exigua* (Boerema 1967) and reinstalled again (Boerema *et al.* 1987), mainly in order to avoid confusion with the plurivorous *Ph. exigua*, - but reaching quite the opposite. In this study it was shown that the species and varieties in this complex are genetically highly similar. To further elucidate the evolutionary history of *Phoma* and to redefine taxonomical concepts in this genus will be one of the main challenges for the future.

CHAPTER 5

Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera

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Summary

Fungal taxonomists routinely encounter problems when dealing with asexual fungal species due to poly- and paraphyletic generic phylogenies, and unclear species boundaries. These problems are aptly illustrated in the genus *Phoma*. This phytopathologically significant fungal genus is currently subdivided into nine sections which are mainly based on a single or just a few morphological characters. However, this subdivision is ambiguous as several of the section-specific characters can occur within a single species. In addition, many teleomorph genera have been linked to *Phoma*, three of which are recognised here. In this study it is attempted to delineate generic boundaries, and to come to a generic circumscription, which is more correct from an evolutionary point of view by means of multilocus sequence typing. Therefore, multiple analyses were conducted utilising sequences obtained from 28S nrDNA (Large Subunit - LSU), 18S nrDNA (Small Subunit - SSU), the Internal Transcribed Spacer regions 1 & 2 and 5.8S nrDNA (ITS), and part of the β-tubulin (TUB) gene region. A total of 324 strains were included in the analyses of which most belonged to *Phoma* taxa, whilst 54 to related pleosporalean fungi. In total, 206 taxa were investigated, of which 159 are known to have affinities to *Phoma*. The phylogenetic analysis revealed that the current Boeremaean subdivision is incorrect from an evolutionary point of view, revealing the genus to be highly polyphyletic. *Phoma* species are retrieved in six distinct clades within the *Pleosporales*, and appear to reside in different families. The majority of the species, however, including the generic type, clustered in a recently established family, *Didymellaceae*. In the second part of this study, the phylogenetic variation of the species and varieties in this clade was further assessed. Next to the genus *Didymella*, which is considered to be the sole teleomorph of *Phoma s. str*., we also retrieved taxa belonging to the teleomorph genera *Leptosphaerulina* and *Macroventuria* in this clade. Based on the sequence data obtained, the *Didymellaceae* segregate into at least 18 distinct clusters, of which many can be associated with several specific taxonomic characters. Four of these clusters were defined well enough by means of phylogeny and morphology, so that the associated taxa could be transferred to separate genera. Aditionally, this study addresses the taxonomic description of eight species and two varieties that are novel to science, and the recombination of 61 additional taxa.

Keywords: *Boeremia*, coelomycetes, *Didymella*, *Didymellaceae*, DNA phylogeny, *Epicoccum*, *Leptosphaerulina*, *Macroventuria*, *Peyronellaea*, *Phoma*, *Pleosporales*, taxonomy, *Stagonosporopsis*

Introduction

Coelomycetous fungi (Grove 1935) are geographically widespread and are found in numerous ecological niches. Sutton (1980) mentions exponents of this anamorph group inhabiting soil, organic debris, and water, as well as species that parasitize other fungi, lichens, insects and vertebrates. A substantial percentage of the coelomycetes is associated with plant material, either as opportunists or as primary pathogens (Sutton 1980).

Difficulties in morphological identification have resulted in a poor understanding of the generic and species boundaries in the coelomycetes (Sutton 1977, 1980, Nag Raj 1981, van der Aa *et al.* 1990, Torres *et al.* 2005a, b, de Gruyter *et al*. 2009). In an attempt to improve the classification of the coelomycetes, Sutton (1980) proposed to divide the order into six suborders, which unfortunately proved to be highly artificial from an evolutionary perspective (de Gruyter *et al*. 2009).

The current common procedure for isolate identification, which chiefly relies on similarity of DNA sequences to those found in public DNA libraries (Hyde & Soytong 2007), combined with the high level of incorrectly identified sequences in these databases (Bridge *et al.* 2003, 2004, Nilsson *et al.* 2006) placed the likelihood of achieving correct identifications of coelomycetous fungi under intense scrutiny. As pointed out by de Gruyter *et al.* (2009), for appropriate morphological identifications within the coelomycete genera *in vitro* studies are essential, for example in the cases in which quarantine pathogens are involved (Aveskamp *et al.* 2008). For the current generic delimitation of this class, the use of conidiogenesis characters as taxonomic criteria is of major importance (Hughes 1953; Boerema 1965, Boerema & Bollen 1975, Sutton 1964, 1977, 1980, Singh *et al.* 1997).

Phoma

The genus *Phoma* Sacc. emend. Boerema & G.J. Bollen (*Pleosporales*) is a good example of a coelomycetous genus made fascinating by its great ecological diversity, but taxing investigators with profound difficulties in making identifications. The majority of the taxa within this mitosporic genus have been found in association with land plants, causing mainly leaf and stem spots (Aveskamp *et al*. 2008). Approximately 50 % of the *Phoma* taxa that were redescribed by Boerema *et al.* (2004) are recognised as relevant phytopathogenic fungi, including a series of pathogens with quarantine status (Boerema *et al.* 2004, Aveskamp *et al*. 2008). Although most taxa are continuously present in the environment as saprobic soil organisms, many species switch to a pathogenic lifestyle when a suitable host is encountered (Aveskamp *et al*. 2008). The genus further comprises several species and varieties that are recognised as endophytic, fungicolous and lichenicolous fungi (e.g. Hawksworth 1981, Xianshu *et al*. 1994, Sullivan & White 2000, Hawksworth & Cole 2004, Diederich *et al.* 2007). In addition, approximately 10 species are known as pathogens of humans (e.g. De Hoog *et al*. 2000, Balis *et al.* 2006) and other vertebrates, such as cattle (Costa *et al.* 1993) and fish (Ross *et al*. 1975, Hatai *et al*. 1986, Voronin 1989, Faisal *et al*. 2007). Next to such an active role in vertebrate pathology, *Phoma* spp. may indirectly affect animal health by the production of toxic secondary metabolites (Bennett 1983, Pedras & Biesenthal 2000, Rai *et al.* 2009), as is known for *Ph. sorghina* in straw roofs in South Africa (Rabie *et al.* 1975) and may be the case in *Ph. pomorum* in cattle feed (Sørensen *et al.* 2010). An almost completely unexplored habitat of *Phoma* spp. is the marine environment (Kohlmeyer & Volkmann-Kohlmeyer 1991), in which *Phoma* species are regularly found that are completely new to science (e.g. Osterhage *et al*. 2000, Yarden *et al*. 2007).

The genus *Phoma* has always been considered to be one of the largest fungal genera, with more than 3 000 infrageneric taxa described (Monte *et al*. 1991). The number of species described in *Phoma* rose to this level due to the common practice of host associated nomenclature, in combination with the paucity in micromorphological characters and a high variability in cultural characteristics. These factors have resulted in the fact that the systematics of the genus never has been fully understood (Aveskamp *et al.* 2008). Based on various morphological features depicted by earlier workers, probably less than one-tenth of the 3 200 species listed in MycoBank (www.mycobank.org, Crous *et al.* 2004, Robert *et al.* 2005) can currently still be recognised as a separate *Phoma* taxon. Many of those names were thus already reduced to synonymy after an extensive study of the genus (Boerema *et al*. 2004), and after a thoroughly revised generic concept of the morphologically similar genera *Ascochyta* (Boerema & Bollen 1975) and *Phyllosticta* (van der Aa 1973, van der Aa & Vanev 2002). Many other species could be recombined into other coelomycete genera,

Table 5.1. Overview of the characters of the various *Phoma* sections in the Boeremaean classification system. Adapted from Boerema *et al*. (2004).

Section	Teleomorph	Synanamorph	Sectional character
Heterospora	-	*Stagonosporopsis*	Production of distinctly large conidia in addition to the regular conidia
Macrospora	*Mycosphaerella*	-	Conidia large, measuring 8–19 × 3–7 µm
Paraphoma	-	-	Setose pycnidia
Peyronellaea	-	*Epicoccum* *	Multicellular chlamydospores
Phoma	*Didymella*	*Phialophora* *	-
Phyllostictoides	*Didymella*	-	Small septate conidia in addition to the regular conidia
Pilosa	*Pleospora*	-	Pycnidia covered by pilose outgrows
Plenodomus	*Leptosphaeria*	*Sclerotium* * *Phialophora* *	Pycnidia scleroplectenchymatous
Sclerophomella	*Didymella*	-	Pycnidia thick-walled

*Synanamorph only recorded in a single species.

such as *Asteromella*, *Microsphaeropsis*, *Phomopsis*, *Pleurophoma*, *Pyrenochaeta* and *Stagonospora* (Sutton 1964, 1980, Boerema & Bollen 1975). In addition, *Coniothyrium* and *Paraconiothyrium* have regularly been mistaken for *Phoma* (Verkley *et al.* 2004, Damm *et al.* 2008, Woudenberg *et al*. 2009). In their studies, Boerema *et al.* (2004) recognised a total of 215 *Phoma* taxa and eight teleomorph species with an unnamed *Phoma* anamorph, although this is probably just the tip of the iceberg as, thus far, only 40 % of the herbarium species mentioned in literature could be recovered and studied properly. Additionally, novel species are described regularly in this genus (e.g. Hawksworth & Cole 2004, Torres *et al.* 2005a, Li *et al.* 2006, Diederich *et al.* 2007, Aveskamp *et al.* 2009a, Davidson *et al.* 2009).

A subdivision of the asexual genus *Phoma* that is currently widely applied divides the genus into nine sections, including the sections *Phoma*, *Heterospora*, *Macrospora, Paraphoma*, *Peyronellaea*, *Phyllostictoides*, *Pilosa, Plenodomus* and *Sclerophomella* (Boerema 1997). These sections are primarily based on just a few morphological or physiological characters and have not been confirmed as biologically realistic by molecular biological studies. The number of taxa per section may vary, ranging from almost 70 species in section *Phoma* to only two in section *Pilosa*. In Table 5.1, a list is provided with the main characters of every section (Boerema 1997). This subdivision into sections has led to an identification system that is considered to be extremely helpful in morphological identification (Boerema *et al.* 2004). However, as was hypothesised by Boerema *et al.* (2004), the classification has proved to be artificial. Molecular evidence has shown that the sections are linked to phylogenetically distinct teleomorph genera (Reddy *et al*. 1998, Torres *et al.* 2005b, de Gruyter *et al*. 2009). Even these teleomorph genera are not always monophyletic (Morales *et al.* 1995, Câmara *et al.* 2002, Kodsueb *et al*. 2006, Inderbitzin *et al.* 2009). In addition, characters that are thought to be specific for a certain section appeared to be polyphyletic, as is illustrated for dictyochlamydospores and setose pycnidia, the main characters for the sections *Peyronellaea* (Aveskamp *et al.* 2009a) and *Paraphoma* (Grondona *et al*. 1997, de Gruyter *et al.* 2010) respectively. Furthermore, *Phoma* section *Phoma*, a group of species which is characterised by the absence of chlamydospores, septate conidia, and pycnidial ornamentation or wall thickening, is considered to be a repository for degenerated and insufficiently understood species that could not be placed elsewhere.

The genus *Phoma* is typified by *Phoma herbarum* (Boerema 1964). This species has thus far not been linked to any teleomorph, but several other species that are currently accommodated in *Phoma* do have a sexual state. The species in the section *Pilosa* are linked to the teleomorph genus *Pleospora*, while many species in the section *Plenodomus* have a sexual state in *Leptosphaeria*. As mentioned above, *Leptosphaeria* is para- or possibly polyphyletic (Morales *et al.* 1995, Câmara *et al.* 2002). The poorly studied genus *Didymella* is associated with approximately 40 *Phoma* species placed in sections *Phoma*, *Phyllostictoides* and *Sclerophomella* (Boerema *et al*. 2004). Only recently, the teleomorph of the newly described species *Ph. muscivora* is taxonomically placed in the genus *Atradidymella* (Davey & Currah 2009) which, thus far, only comprises one species. In older literature, *Phoma* has been linked to several other teleomorph genera, such as *Mycosphaerella* (Corlett 1991, de Gruyter 2002), *Belizeana* (Kohlmeyer & Volkmann-Kohlmeyer 1987),

and *Fenestella*, *Cucurbitaria*, *Preussia*, and *Westerdykella* (von Arx 1981). None of these hypothesised teleomorph-anamorph linkages is supported by molecular evidence. All must be investigated by study of type material. However, these associations are unlikely as the mentioned teleomorph genera are not linked to the *Pleosporales*. The species and teleomorph relations are also not recognised by Boerema *et al*. (2004), except for two *Phoma* species of the section *Macrospora*, *Ph. rabiei* and *Ph. zeae-maydis* which were linked to "*Mycosphaerella*" teleomorphs as *M. rabiei* (Kaiser 1997, de Gruyter 2002) and *M. zeae-maydis* (Mukunya & Boothroid 1973) respectively. Both species also have names in *Didymella*. The use of those names is recommended, since *Mycosphaerella* has been shown to be phylogenetically widely separated from all known *Phoma* species (de Gruyter *et al.* 2009, Crous *et al*. 2009a).

Characteristic strains of the genus concerned have been used in a Multilocus Sequence Typing (MLST) study of the *Dothideomycetes*, which indicated that *Phoma* is phylogenetically embedded in the *Pleosporales* (Schoch *et al*. 2006, 2009b). A similar, but smaller scale study aiming to delineate the species in the unofficial suborder Phialopycnidiineae (Sutton 1980), revealed that *Phoma* is highly polyphyletic, as reference species of the various sections were recovered in distinct clades of the reconstructed phylogeny (de Gruyter *et al.* 2009). Type species of the sections *Heterospora, Plenodomus*, *Paraphoma* and *Pilosa* appeared to be ancestral to a cluster comprising types of the other sections, as well as to members of the anamorph genera *Ascochyta*, *Microsphaeropsis*, *Chaetasbolisia, Coniothyrium* and *Paraconiothyrium.* This group has been elevated to family level and is now recognised as the *Didymellaceae* (de Gruyter *et al.* 2009). A Blast-search in public sequence libraries revealed a high genetic similarity between species ascribed to the *Didymellaceae* and two other teleomorph genera, *Macroventuria* and *Leptosphaerulina*, although these genera are morphologically clearly distinct from *Didymella* (van der Aa 1971, von Arx 1981). The genetic similarity between those two genera has been observed before by Kodsueb *et al.* (2006), but the phylogenetic relationship with the genus *Didymella* was not noted in their study. Members of these two genera have therefore also been included in this study.

To solve the problems in quarantine species identification of isolates taken from samples obtained during phytosanitary border controls, a comprehensive taxonomic system is required (Aveskamp *et al.* 2008). As DNA-based techniques do become more and more important in identification and detection of plant pathogens (Bridge 2002), such a taxonomic system should be in line with sequence data. One of the major initiatives in this field is the development of DNA Barcodes (Hebert *et al.* 2003, Summerbell *et al*. 2005), which has been promising in the rapid detection of potentially serious plant pathogens (Armstrong & Ball 2005).

Three genes have in recent years been proposed as standard loci for use in DNA barcoding in fungi. These comprise the internal transcribed spacers (ITS) of the rDNA operon ITS region (Druzhinina *et al.* 2005), actin (ACT, Aveskamp *et al.* 2009b), and cytochrome *c* oxidase subunit I (COI, Seifert *et al.* 2007). The last locus was successfully applied in DNA Barcoding of *Penicillium* (Seifert *et al.* 2007, Chen *et al.* 2009). However, COI analysis applied to a subset of *Ph. exigua* related strains, did not reveal taxon-specific conserved SNPs (Aveskamp *et al.* 2009b), whilst in an attempt to barcode *Aspergillus*, COI was found to have limited value (Geiser *et al.* 2007). Although ACT has proven helpful in resolving the phylogeny of *Phoma exigua* below species level (Aveskamp *et al.* 2009b), it could not be applied in the present study, as interspecific variation proved to be too high to align the obtained sequences properly. The use of ITS as fungal barcode locus is most popular (Seifert 2009) and has been applied in several taxonomic groups, such as *Trichoderma* and *Hypocrea* (Druzhinina *et al.* 2005), and *Trichophyton* (Summerbell *et al.* 2007) and in ecological groups such as wood-inhabiting fungi (Naumann *et al.* 2007). The power of this locus for barcoding lies in the multiple copies that are present within each cell; this phenomenon results in lower detection thresholds than can be obtained with single-copy loci. Despite the general practicality of using ITS in barcoding, the locus is relatively conservative and may oversimplify species delimitations or blur generic boundaries in some groups (Nilsson *et al.* 2008). In the present study, a combination of four loci is therefore applied. These include two loci that are renowned for their capacity to resolve phylogenies above family level, namely parts of the LSU (Large Subunit – 28S) and SSU (Small Subunit – 18S) nrDNA. Furthermore, two loci were applied that mainly provide resolution at species level – or even below. In addition to the abovementioned ITS regions, also part of the β-tubulin gene was utilised, which was successfully applied in a preliminary study on *Phoma* species of the section *Peyronellaea* (Aveskamp *et al.* 2009a).

For the present study, four objectives were defined.

1. The main objective of this study was to reach consensus on the circumscription of the genus *Phoma*. A modified definition of the genus is not only helpful in taxonomy, but will also be of interest to plant quarantine officers (Aveskamp *et al.* 2008). Teleomorph associations of *Phoma* are still uncertain, and here we attempt to shed light on the sexual state of *Phoma s. str*. Species representing all *Phoma* sections were included and DNA sequences were compared with those of other species in the *Pleosporales*.
2. Secondly, we aimed to integrate morphological and cultural features with DNA sequence data to resolve the generic limits of taxa currently placed in *Didymellaceae*. The number of genera in this family is still unclear. Although de Gruyter *et al.* (2009) found a series of genera that, according to their reconstructed phylogeny, clustered in this family, many were not clearly defined or were morphologically distant from each other, although all anamorph taxa found are accommodated in the coelomycetes (Sutton 1980). Examples of these taxa were included in this study, although the number of *Ascochyta*, *Coniothyrium* and *Microsphaeropsis* species is too high to take all infrageneric taxa of these adjacent genera into account.
3. Further, we aimed to validate the *Phoma* sections, which are widely applied in *Phoma* species recognition. Are the sections representing evolutionary units, and what is the taxonomical value of the characters used to define the sections? To judge the value of the Boeremaean taxonomic system, representative species of all sections were studied, including the sectional type species. The main focus was, however, to resolve the sections associated with *Didymellaceae*.
4. Finally, we aimed to assess the molecular variation within species that have historically been placed in *Phoma*. Genes were tested for their potential reliability as standard barcoding genes for *Phoma* species.

For this study, a sequence data set was generated and morphological data assembled for the more than 300 well-vouchered strains available in the culture collections of CBS (CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands) and PD (Plantenziektenkundige Dienst, Dutch Plant Protection Service, Wageningen, the Netherlands). In addition, five species recognised in a recent study in the section *Peyronellaea* (Aveskamp *et al.* 2009a) have also been included, as well as several strains that could not be associated with any of the species that were accepted in *Phoma* by Boerema *et al.* (2004), and that were maintained as unnamed *Phoma* species in the culture collections mentioned above. These strains were recognised as taxonomic novelties and are described at species or variety level in the present paper. Furthermore, several species were relocated to more appropriate genera based on the results obtained.

Materials and Methods

Strain selection

A total of 324 strains, belonging to 206 species were selected for the present study. The majority of these species (159) belonged to the genus *Phoma* or its associated teleomorphs, the remainder to genera that are regularly confused with this genus and that belong to the *Pleosporales* according to the studies published by de Gruyter *et al.* (2009). Besides the anamorphous species that were included, representatives of the teleomorph genera *Didymella*, *Leptosphaeria*, *Leptosphaerulina*, *Macroventuria* and *Pleospora* were also included. The genus *Atradidymella* was not included in our study, as the genus is only recently described (Davey & Currah 2009).

Strains were obtained from CBS and PD culture collections in lyophilised form or from the liquid nitrogen collection. Freeze-dried strains were revived overnight in 2 mL malt/peptone (50 % / 50 %) liquid medium. Subsequently, the cultures were transferred and maintained on oatmeal agar (OA, Crous *et al.* 2009b). The strains that were stored at -196 °C were directly plated on the same agar medium.

DNA extraction, amplification and sequence analysis

Genomic DNA extraction was performed using the Ultraclean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), according to the instructions of the manufacturer. All DNA extracts were diluted 10 × in milliQ water and stored at 4 ºC before their use as PCR templates.

For nucleotide sequence comparisons fragments of four loci were analysed: LSU, SSU, ITS, and TUB. Amplification of LSU and SSU was conducted utilising the primer combination LR0R (Rehner &

Samuels 1994) and LR7 (Vilgalys & Hester 1990) for LSU sequencing and the primer pair NS1 and NS4 (White *et al.* 1990) for SSU. The PCRs were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) in a total volume of 12.5 µL. The PCR mixture contained 0.5 µL 10 × diluted genomic DNA, 0.2 µM of each primer, 0.5 Unit Taq polymerase E (Genaxxon Bioscience, Germany), 0.04 mM (SSU) or 0.06 mM (LSU) of each of the dNTP, 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 °C, followed by 35 cycles of denaturation, annealing and elongation and a final elongation step of 7 min at 72 °C. For the SSU amplification, the 35 cycles consisted of 30 s at 94 °C, 50 s at 48 °C and 90 s at 72 °C; for the LSU 45 s at 94 °C, 45 s at 48 °C and 2 min at 72 °C. The loci ITS and TUB were amplified as described by Aveskamp *et al*. (2009a), using the primer pairs V9G (De Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990) for ITS sequencing and the BT2Fw and BT4Rd primer pair (Aveskamp *et al.* 2009a) for sequencing of the TUB locus. PCR products were analysed by electrophoresis in a 1.0 % (w/v) agarose gel containing 0.1 ug/mL ethidium bromide in 1 × TAE buffer (0.4 M Tris, 0.05 M glacial acetetic acid 0.01 M ethylenediamine tetraacetic acid [EDTA], pH 7.85). The amplicons were visualised under UV light. Hyperladder I (Bioline, Luckenwalde, Germany) was applied as size standard.

The obtained amplicons were sequenced in both directions using the same primer combinations, except for LSU, where an additional primer, LR5 (White *et al.* 1990) was further required to assure complete coverage of the locus. Sequencing reactions were prepared with the BigDye terminator chemistry v. 3.1 (Applied Biosystems) according to the manufacturer's recommendations. Sequence products were purified with Sephadex G-50 Fine (Amersham Biosciences, Roosendaal, the Netherlands) and subsequently separated and analysed on an ABI Prism 3730 DNA Sequencer (Applied Biosystems). Consensus sequences were computed from the forward and reverse sequences using the BioNumerics v. 4.61 software package (Applied Maths, St-Martens-Latem, Belgium). The consensus sequences are deposited in GenBank (For GenBank accession numbers see Tables 5.2 & 5.3).

Obtained consensus sequences were assembled and aligned using the same BioNumerics software and adjusted manually where necessary. As SSU was highly conserved in deeper node phylogenies, revealing almost no phylogenetic informative nuclear polymorphisms, and as ITS and TUB proved to be unalignable due to a high level of polymorphism if all taxa studied would be taken into account, it was decided to conduct two separate analyses. The first analysis comprised SSU and LSU loci, and was applied to 76 taxa of which most species included belonged to genera that were often confused with *Phoma* (Sutton 1980, de Gruyter *et al.* 2009). A second set of analyses was conducted on 274 taxa, and focussed on the species that had proven to be related to the *Didymellaceae* from preliminary studies.

Each of the phylogenetic analyses consisted of two methods: Bayesian Interference (BI) and Maximum Likelihood (ML). For BI analysis, the nucleotide substitution models were determined for each locus separately with MrModeltest v. 2.2 (Nylander 2004). According to this software, the General Time Reversible substitution was determined to be the best model for SSU, TUB and LSU in both data sets, with inverse gamma rates and dirichlet base frequencies (GTR + I + G). For the ITS dataset, the software suggested the Symmetrical Model as the best model for substitution of nucleotides. Also in this locus, the inverse gamma rates and dirichlet base frequencies were used (SYM + I + G). The actual Bayesian calculations were performed in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001). One tree was saved per 100 generations, and the run was automatically ended when the standard deviation of split frequencies was below 0.01. The temperature value of the Bayesian run was set at 0.2. To avoid suboptimal trees being taking into account for the consensus tree, a burn-in of 25 % of the saved trees was used. The resulting "50 % majority rule consensus" trees were visualised with TreeView v. 1.6.6 (Page 1996).

A second measure of branch support was obtained by conducting a ML analysis using RAxML software (Stamatakis *et al.* 2005) through the CIPRES Website (www.phylo.org). The same partitions were used as in the BI analyses, but because RAxML implements only the GTR substitution model, the symmetrical model for the ITS partition was waived. The robustness of trees in the ML analyses was evaluated by bootstrapping the datasets. The number of bootstrap replicates was automatically determined by the RAxML software (Stamatakis *et al.* 2008). The obtained trees in both analyses are lodged with TreeBASE (www.treebase.org).

Morphology
Morphological studies of the strains were performed on OA, malt extract agar (MEA) and cherry decoction agar (CHA) (Crous *et al*. 2009b). The cultures were incubated according to the methodologies described by Boerema *et al*. (2004). Eight days after inoculation, the colony growth was measured. At the 15th day after incubation, the colony colours were rated using the colour charts of Rayner (1970). Micromorphological features were studied after maturation of the pycnidia. Therefore, fungal structures were mounted in tap water using a scalpel blade and examined under a stereo light microscope. Perennial structures that were formed in the agar medium, such as chlamydospores, were cut out from the medium, and mounted in lactic acid. Remaining agar was removed from these samples by gently heating the glass slides. The sizes of the various structures were determined by averaging the measurements of 30 samples of each structure, except for conidiogenous cells and pycnidial wall characters, of which the size ranges were estimated based on 5–10 samples. Fifth and 95th percentiles were determined for all measurements and are provided in parentheses. By application of a droplet of 1N NaOH, the production of metabolite E+ was determined (Dorenbosch 1970, Noordeloos *et al*. 1993). The structure of the pycnidial wall and shape of conidiogenous cells were studied using microtome sections of 6 µm thickness, prepared with a Leica CM3050 freezing microtome and mounted in lactic acid. Taxonomic recombinations and novel species and descriptions were deposited in MycoBank.

Results

Systematics of the genus *Phoma*

DNA phylogenetical analysis
Due to alignment difficulties multiple datasets, consisting of different sets of loci, were utilised. For a generic overview, LSU and SSU were included in the first alignment, which consisted of 76 taxa. A list of species names and numbers, original substrates, geographical origins and GenBank accession numbers of the strains used in this study is provided in Table 5.2. The aligned sequence matrix had a total length of 2 210 characters including alignment gaps (LSU: 1 258 and SSU: 952 bp). Of those characters, 1 809 (LSU: 994 and SSU: 815) were constant and 401 were variable (LSU: 264 and SSU: 137). The Bayesian analysis run was aborted after 10 000 000 generations as a point of stationarity was reached in the average standard deviation of split frequencies, at a value of 0.0288. The applied "burn-in" percentage of 25 % was well after stationarity in the probability of the trees was reached. The tree topologies and support values of the ML analysis, differed only slightly from the trees obtained from the Bayesian analyses, supporting the probability of the tree. The tree is rooted to *Pseudorobillarda phragmitis* (CBS 398.61).

Based on the LSU-SSU phylogenetic study performed here for the various anamorph and teleomorph species in the *Phoma* complex, eight clades were revealed (Figure 5.1), including one which only comprises the outgroup specimen. The various clades will be treated below, but for additional synonymy on the *Phoma* species we refer to Boerema *et al.* (2004). The findings in these clades are largely in congruence with the observations of de Gruyter *et al.* (2009).

Species that were ascribed to the *Phoma* section *Phoma* by Boerema *et al.* (2004) appear to be genetically highly heterogeneous, as these species are recovered in almost every clade. Species that were ascribed to *Phoma* section *Heterospora* appear to be linked to at least three distinct clades. Also polymorphism is observed for sections *Paraphoma*, *Peyronellaea* and *Sclerophomella*, as well as for *Coniothyrium* and *Ascochyta*. The type species of this latter genus, *A. pisi*, is not included in the present tree, but is genetically similar to the *Didymellaceae*.

Treatment of the clades
Clade 1, Outgroup: *Pseudorobillarda phragmitis* was selected as outgroup on the basis of the studies conducted by de Gruyter *et al*. (2009). This species, although being recognised as a coelomycete, is not only phylogenetically, but also morphologically distinct from *Phoma*, although Sutton (1980) classified it in the Phialopycnidiineae.

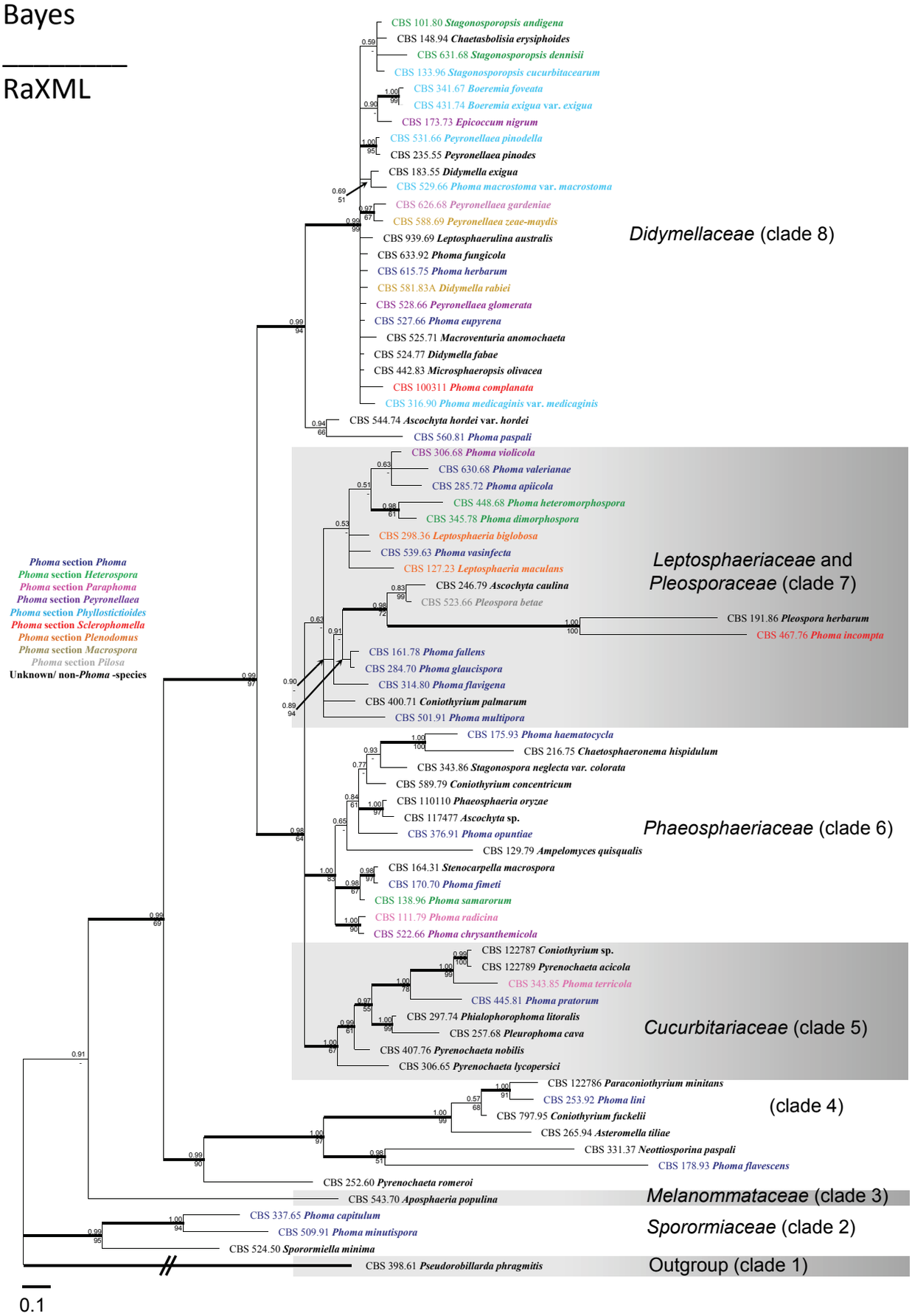


Figure 5.1. Fifty percent majority rule consensus tree from a BI analysis of Large and Small subunit sequences of *Phoma* and related genera (n = 76). At the nodes the BI Posterior Probabilities are presented above the branch, and bootstrap percentages of the ML analysis are given below the branch. Branches that were less than 50 % supported in the ML analyses are indicated with a hyphen. The bar indicates the number of substitutions per site. The tree is rooted with *Pseudorobillarda phragmitis* (CBS 398.61).

Table 5.2. Isolates of *Phoma* and related genera used for DNA analyses. The GenBank accession numbers in bold have been obtained from other studies.

Strain no.[1]	Holomorph[2]	GenBank no.		Original substrate	Locality
		SSU	LSU		
CBS 129.79	*Ampelomyces quisqualis*	**EU754029**	**EU754128**	Mildew on *Cucumis sativus*	Canada
CBS 543.70	*Aposphaeria populina*	**EU754031**	**EU754130**	*Populus canadensis*	Netherlands
CBS 246.79; PD 77/655	*Ascochyta caulina* T	**EU754032**	**EU754131**	*Atriplex hastata*	Germany
CBS 544.74	*Ascochyta hordei* var. *hordei*	**EU754035**	**EU754134**	*Triticum aevestum*	South Africa
CBS 117477	*Ascochyta* sp.	GU238202	GU237926	*Salicornia australis*	New Zealand
CBS 265.94	*Asteromella tiliae*	**EU754040**	**EU754139**	*Tilia platyphilos*	Austria
CBS 431.74; PD 74/2447	*Boeremia exigua* var. *exigua* B	**EU754084**	**EU754183**	*Solanum tuberosum*	Netherlands
CBS 341.67; CECT 20055; IMI 331912	*Boeremia foveata* B	GU238203	GU237947	*Solanum tuberosum*	UK
CBS 148.94	*Chaetasbolisia erysiphoides*	**EU754041**	**EU754140**	Unknown	Unknown
CBS 216.75; PD 71/1030	*Chaetosphaeronema hispidulum*	**EU754045**	**EU754144**	*Anthyllis vulneraria*	Germany
CBS 589.79	*Coniothyrium concentricum*	**EU754053**	**EU754152**	*Yucca* sp.	Netherlands
CBS 797.95	*Coniothyrium fuckelii*	GU238204	GU237960	*Rubus* sp.	Denmark
CBS 400.71	*Coniothyrium palmarum*	**EU754054**	**EU754153**	*Chamaerops humilis*	Italy
CBS 122787; PD 03486691	*Coniothyrium* sp.	**EU754052**	**EU754151**	Unknown	Germany
CBS 183.55	*Didymella exigua* T	**EU754056**	**EU754155**	*Rumex arifolius*	France
CBS 524.77	*Didymella fabae*	**EU754034**	**EU754133**	*Phaseolus vulgaris*	Belgium
CBS 581.83A	*Didymella rabiei*	GU238205	GU237970	*Cicer arietinum*	Syria
CBS 173.73; ATCC 24428; IMI 164070	*Epicoccum nigrum* T	GU238206	GU237975	*Dactylis glomerata*	USA
CBS 298.36	*Leptosphaeria biglobosa*	GU238207	GU237980	*Brassica napus* var. *napobrassica*	Unknown
CBS 127.23; MUCL 9930	*Leptosphaeria maculans*	**EU754090**	**EU754189**	*Brassica* sp.	Netherlands
CBS 939.69	*Leptosphaerulina australis*	**EU754068**	**EU754167**	Soil	Netherlands
CBS 525.71	*Macroventuria anomochaeta* T	GU238208	GU237984	Decayed canvas	South Africa
CBS 442.83	*Microsphaeropsis olivacea*	**EU754072**	**EU754171**	*Taxus baccata*	Netherlands
CBS 331.37	*Neottiosporina paspali*	**EU754073**	**EU754172**	*Paspalum notatum*	USA
CBS 122786; PD 99/1064-1	*Paraconiothyrium minitans*	**EU754075**	**EU754174**	Unknown	Unknown
CBS 626.68; IMI 108771	*Peyronellaea gardeniae* T	**GQ387534**	**GQ387595**	*Gardenia jasminoides*	India
CBS 528.66; PD 63/590	*Peyronellaea glomerata* B	**EU754085**	**EU754184**	*Chrysanthemum* sp.	Netherlands
CBS 531.66	*Peyronellaea pinodella* B	GU238209	GU238017	*Trifolium pratense*	USA

Table 5.2. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.		Original substrate	Locality
		SSU	LSU		
CBS 235.55	*Peyronellaea pinodes*	GU238210	GU238021	Unknown	Netherlands
CBS 588.69	*Peyronellaea zeae-maydis* T	**EU754093**	**EU754192**	*Zea mays*	USA
CBS 110110	*Phaeosphaeria oryzae*	**GQ387530**	**GQ387591**	*Oryza sativa*	South Korea
CBS 297.74	*Phialophorophoma litoralis*	**EU754078**	**EU754177**	Sea water	Montenegro
CBS 285.72	*Phoma apiicola* B	GU238211	GU238040	*Apium graveolens* var. *rapaceum*	Germany
CBS 337.65; ATCC 16195; IMI 113693	*Phoma capitulum* B	GU238212	GU238054	Soil	India
CBS 522.66	*Phoma chrysanthemicola* T	**GQ387521**	**GQ387582**	*Chrysanthemum morifolium*	UK
CBS 100311	*Phoma complanata*	**EU754082**	**EU754181**	*Heracleum sphondylium*	Netherlands
CBS 345.78; PD 76/1015	*Phoma dimorphospora*	GU238213	GU238069	*Chenopodium quinoa*	Peru
CBS 527.66	*Phoma eupyrena* B	GU238214	GU238072	Soil	Germany
CBS 161.78	*Phoma fallens* B	GU238215	GU238074	*Olea europaea*	New Zealand
CBS 170.70; ATCC 22707; CECT 20011; IMI 163514; PD 70/Alk	*Phoma fimeti* T	**GQ387523**	**GQ387584**	*Apium graveolens*	Netherlands
CBS 178.93; PD 82/1062	*Phoma flavescens* T	GU238216	GU238075	Soil	Netherlands
CBS 314.80	*Phoma flavigena* T	GU238217	GU238076	Water	Romania
CBS 633.92; ATCC 36786; VKM MF-325	*Phoma fungicola*	**EU754028**	**EU754127**	*Microsphaera alphitoides* on *Quercus* sp.	Ukraine
CBS 284.70	*Phoma glaucispora* B	GU238218	GU238078	*Nerium oleander*	Italy
CBS 175.93; PD 92/370	*Phoma haematocycla* T	GU238219	GU238080	*Phormium tenax*	New Zealand
CBS 615.75; PD 73/665, IMI 199779	*Phoma herbarum* B	**EU754087**	**EU754186**	*Rosa multiflora*	Netherlands
CBS 448.68	*Phoma heteromorphospora* B	**EU754088**	**EU754187**	*Chenopodium album*	Netherlands
CBS 467.76	*Phoma incompta* B	GU238220	GU238087	*Olea europaea*	Greece
CBS 253.92; PD 70/998	*Phoma lini* B	GU238221	GU238093	Water	USA
CBS 529.66; PD 66/521	*Phoma macrostoma* var. *macrostoma* B	GU238222	GU238098	*Malus sylvestris*	Netherlands
CBS 316.90	*Phoma medicaginis* var. *medicaginis*	GU238223	GU238103	*Medicago sativa*	Czech Republic
CBS 509.91; PD 77/920	*Phoma minutispora*	GU238224	GU238108	Saline soil	India
CBS 501.91; PD 83/888	*Phoma multipora* B	GU238225	GU238109	Unknown	Egypt
CBS 376.91; CBS 328.78, PD 77/1177	*Phoma opuntiae* B	GU238226	GU238123	*Opuntia ficus-indica*	Peru

Table 5.2. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.		Original substrate	Locality
		SSU	LSU		
CBS 560.81; PD 92/1569; PDDCC 6614	*Phoma paspali* T	GU238227	GU238124	*Paspalum dilatatum*	New Zealand
CBS 445.81; PDDCC 7049	*Phoma pratorum* T	GU238228	GU238136	*Lolium perenne*	New Zealand
CBS 111.79; PD 76/437, IMI 386094	*Phoma radicina* B	**EU754092**	**EU754191**	*Malus sylvestris*	Netherlands
CBS 138.96; PD 82/653	*Phoma samarorum* B	**GQ387517**	**GQ387578**	*Phlox paniculata*	Netherlands
CBS 343.85; IMI 386097	*Phoma terricola* T	**GQ387563**	**GQ387624**	*Globodera pallida*	Netherlands
CBS 630.68; PD 68/141	*Phoma valerianae* B	GU238229	GU238150	*Valeriana phu*	Netherlands
CBS 539.63	*Phoma vasinfecta* T	GU238230	GU238151	*Chrysanthemum* sp.	Greece
CBS 306.68	*Phoma violicola* B	GU238231	GU238156	*Viola tricolor*	Unknown
CBS 523.66; PD 66/270	*Pleospora betae* B	**EU754080**	**EU754179**	*Beta vulgaris*	Netherlands
CBS 191.86; IMI 276975	*Pleospora herbarum* T	GU238232	GU238160	*Medicago sativa*	India
CBS 257.68; IMI 331911	*Pleurophoma cava*	**EU754100**	**EU754199**	Soil	Germany
CBS 398.61; IMI 070678	*Pseudorobillarda phragmitis* T	**EU754104**	**EU754203**	*Phragmites australis*	UK
CBS 122789; PD 03486800	*Pyrenochaeta acicola*	**EU754105**	**EU754204**	*Hordeum vulgare*	Unknown
CBS 306.65	*Pyrenochaeta lycopersici* T	**EU754106**	**EU754205**	*Lycopersicon esculentum*	Germany
CBS 407.76	*Pyrenochaeta nobilis* T	**EU754107**	**EU754206**	*Laurus nobilis*	Italy
CBS 252.60; ATCC 13735	*Pyrenochaeta romeroi* T	**EU754108**	**EU754209**	Man	Venezuela
CBS 524.50	*Sporormiella minima*	**DQ678003**	**DQ678056**	Goat dung	Panama
CBS 343.86	*Stagonospora neglecta* var. *colorata*	**EU754119**	**EU754218**	*Phragmites australis*	France
CBS 101.80; PD 75/909; IMI 386090	*Stagonosporopsis andigena* B	GU238233	GU238169	*Solanum* sp.	Peru
CBS 133.96; PD 79/127	*Stagonosporopsis cucurbitacearum*	GU238234	GU238181	*Cucurbita* sp.	New Zealand
CBS 631.68; PD 68/147	*Stagonosporopsis dennisii* B	GU238235	GU238182	*Solidago floribunda*	Netherlands
CBS 164.31	*Stenocarpella macrospora*	**EU754121**	**EU754220**	*Zea mays*	Unknown

[1]ATCC: American Type Culture Collection, Virginia, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CECT: Coleccion Espanola de Cultivos Tipo, Valencia University, Spain; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; MUCL: Mycotheque de l'Universite catholique de Louvain, Louvain-la-Neuve, Belgium; PD: Plant Protection Service, Wageningen, the Netherlands; PDDCC: Plant Diseases Division Culture Collection, Auckland, New Zealand VKM: All-Russian Collection of Microorganisms, Pushchino, Russia.

[2]T: Type strain; B: Reference strain according to Boerema *et al*. (2004).

Table 5.3. Strains from the *Didymellaceae* used for DNA analyses. The GenBank accession numbers in bold have been obtained from other studies.

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 544.74	*Ascochyta hordei* var. *hordei*	**EU754134**	GU237887	GU237488	*Triticum aevestum*	South Africa
CBS 109.79; PD 77/747	*Boeremia crinicola* B	GU237927	GU237737	GU237489	*Crinum powellii*	Netherlands
CBS 118.93; PD 70/195	*Boeremia crinicola*	GU237928	GU237758	GU237490	*Crinum* sp.	Netherlands
CBS 101194; PD 79/687; IMI 373349	*Boeremia diversispora*	GU237929	GU237716	GU237491	*Phaseolus vulgaris*	Netherlands
CBS 102.80; PD 79/61; CECT 20049; IMI 331907	*Boeremia diversispora* B	GU237930	GU237725	GU237492	*Phaseolus vulgaris*	Kenya
CBS 119730	*Boeremia exigua* var. *coffeae*	GU237942	GU237759	GU237504	*Coffea arabica*	Brazil
CBS 109183; IMI 300060; PD 2000/10506	*Boeremia exigua* var. *coffeae* B	GU237943	GU237748	GU237505	*Coffea arabica*	Cameroon
CBS 431.74; PD 74/2447	*Boeremia exigua* var. *exigua* B	**EU754183**	**FJ427001**	**FJ427112**	*Solanum tuberosum*	Netherlands
CBS 101150; PD 79/118	*Boeremia exigua* var. *exigua*	GU237933	GU237715	GU237495	*Cichorium intybus*	Netherlands
CBS 101197; PD 95/721	*Boeremia exigua* var. *forsythiae*	GU237931	GU237718	GU237493	*Forsythia* sp.	Netherlands
CBS 101213; PD 92/959	*Boeremia exigua* var. *forsythiae* B	GU237932	GU237723	GU237494	*Forsythia* sp.	Netherlands
CBS 101196; PD 79/176	*Boeremia exigua* var. *heteromorpha*	GU237934	GU237717	GU237496	*Nerium oleander*	France
CBS 443.94	*Boeremia exigua* var. *heteromorpha* B	GU237935	GU237866	GU237497	*Nerium oleander*	Italy
CBS 569.79; PD 72/741	*Boeremia exigua* var. *lilacis* B	GU237936	GU237892	GU237498	*Syringa vulgaris*	Netherlands
CBS 114.28	*Boeremia exigua* var. *linicola*	GU237937	GU237752	GU237499	*Linum usitatissimum*	Netherlands
CBS 116.76; ATCC 32332; CECT 20022; CECT 20023; IMI 197074	*Boeremia exigua* var. *linicola* B	GU237938	GU237754	GU237500	*Linum usitatissimum*	Netherlands
CBS 100167; PD 93/217	*Boeremia exigua* var. *populi* T	GU237939	GU237707	GU237501	*Populus* (x) *euramericana*	Netherlands
CBS 101202; PD 82/942	*Boeremia exigua* var. *populi*	GU237940	GU237719	GU237502	*Salix* sp.	Netherlands
CBS 101207; PD 94/614	*Boeremia exigua* var. *pseudolilacis* T	GU237941	GU237721	GU237503	*Syringa vulgaris*	Netherlands
CBS 100354; PD 84/448	*Boeremia exigua* var. *viburni* B	GU237944	GU237711	GU237506	*Viburnum opulus*	Netherlands
CBS 101211; PD 93/838	*Boeremia exigua* var. *viburni*	GU237945	GU237722	GU237507	*Viburnum* sp.	Netherlands
CBS 109176; CECT 2828; PD 94/1394	*Boeremia foveata* B	GU237946	GU237742	GU237508	*Solanum tuberosum*	Bulgaria
CBS 341.67; CECT 20055; IMI 331912	*Boeremia foveata* B	GU237947	GU237834	GU237509	*Solanum tuberosum*	UK

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 366.91; PD 70/811	*Boeremia hedericola*	GU237948	GU237841	GU237510	*Hedera helix*	Netherlands
CBS 367.91; PD 87/229	*Boeremia hedericola* B	GU237949	GU237842	GU237511	*Hedera helix*	Netherlands
CBS 378.67; PD 76/276	*Boeremia lycopersici* B	GU237950	GU237848	GU237512	*Lycopersicon esculentum*	Netherlands
CBS 109172; PD 84/143	*Boeremia lycopersici*	GU237951	GU237739	GU237513	*Lycopersicon esculentum*	Netherlands
CBS 100353; PD 87/718	*Boeremia noackiana* B	GU237952	GU237710	GU237514	*Phaseolus vulgaris*	Guatemala
CBS 101203; PD 79/1114	*Boeremia noackiana*	GU237953	GU237720	GU237515	*Phaseolus vulgaris*	Colombia
CBS 109170; PD 75/796	*Boeremia sambuci-nigrae*	GU237954	GU237738	GU237516	*Sambucus nigra*	Netherlands
CBS 629.68; CECT 20048; IMI 331913; PD 67/753	*Boeremia sambuci-nigrae* T	GU237955	GU237897	GU237517	*Sambucus nigra*	Netherlands
CBS 126.93; PD 73/642	*Boeremia strasseri*	GU237956	GU237773	GU237518	*Mentha* sp.	Netherlands
CBS 261.92; ATCC 244146; PD 92/318	*Boeremia strasseri*	GU237957	GU237812	GU237519	*Mentha piperita*	USA
CBS 109175; PD 79/524	*Boeremia telephii* B	GU237958	GU237741	GU237520	*Sedum spectabile*	Netherlands
CBS 760.73; PD 71/1616	*Boeremia telephii* B	GU237959	GU237905	GU237521	*Sedum spectabile*	Netherlands
CBS 148.94	*Chaetasbolisia erysiphoides*	**EU754140**	GU237785	GU237522	Unknown	Unknown
CBS 187.83; PD 82/128	*Didymella adianticola* B	GU238035	GU237796	GU237576	*Polystichum adiantiforme*	USA
CBS 258.92; PD 89/1887	*Didymella adianticola*	GU238036	GU237811	GU237577	*Polystichum adiantiforme*	Costa Rica
CBS 102634; PD 75/248	*Didymella applanata*	GU237997	GU237726	GU237555	*Rubus idaeus*	Netherlands
CBS 205.63	*Didymella applanata* T	GU237998	GU237798	GU237556	*Rubus idaeus*	Netherlands
CBS 234.37	*Didymella cannabis*	GU237961	GU237804	GU237523	*Cannabis sativa*	Unknown
CBS 102635; PD 77/1131	*Didymella catariae*	GU237962	GU237727	GU237524	*Nepeta catenaria*	Netherlands
CBS 183.55	*Didymella exigua* T	**EU754155**	GU237794	GU237525	*Rumex arifolius*	France
CBS 524.77	*Didymella fabae*	GU237963	GU237880	GU237526	*Phaseolus vulgrais*	Belgium
CBS 649.71	*Didymella fabae*	GU237964	GU237902	GU237527	*Vicia faba*	Netherlands
PD 83/492	*Didymella fabae*	GU237965	GU237917	GU237528	*Phaseolus vulgaris*	Netherlands
PD 84/512	*Didymella macropodii*	GU237966	GU237919	GU237529	Crucifer	Unknown
CBS 100190; PD 82/736	*Didymella macropodii*	GU237967	GU237708	GU237530	*Brassica napus*	Germany
CBS 126.54	*Didymella pisi*	GU237968	GU237772	GU237531	*Pisum sativum*	Netherlands

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 122785; PD 78/517	*Didymella pisi*	GU237969	GU237763	GU237532	*Pisum sativum*	Netherlands
CBS 534.65	*Didymella rabiei*	GU237970	GU237886	GU237533	*Cicer arietinum*	India
CBS 581.83a	*Didymella rabiei*	GU237971	GU237894	GU237534	*Cicer arietinum*	Syria
CBS 121.75; ATCC 32164; IHEM 3403; IMI 194767; PD 73/584	*Didymella urticicola* T	GU237972	GU237761	GU237535	*Urtica dioica*	Netherlands
PD 73/570	*Didymella urticicola*	GU237973	GU237914	GU237536	*Urtica dioica*	Netherlands
CBS 454.64	*Didymella vitalbina*	**FJ515646**	**FJ515605**	**FJ515623**	*Clematis vitalba*	France
CBS 138.25	*Diplodina coloradensis*	**EU754158**	GU237784	GU237537	*Senecio* sp.	Unknown
CBS 172.34	*"Dothiorella ulmi"*	**EU754160**	GU237789	GU237538	*Ulmus* sp.	USA
CBS 125.82; IMI 1331914; CECT 20044	*Epicoccum nigrum*	GU237974	**FJ426995**	**FJ427106**	Human	Netherlands
CBS 173.73; ATCC 24428; IMI 164070	*Epicoccum nigrum* T	GU237975	**FJ426996**	**FJ427107**	*Dactylis glomerata*	USA
CBS 246.60; ATCC 22237; ATCC 16652; IMI 081601	*Epicoccum pimprinum* T	GU237976	**FJ427049**	**FJ427159**	Soil	India
PD 77/1028	*Epicoccum pimprinum*	GU237977	**FJ427050**	**FJ427160**	Unknown	Unknown
CBS 179.80; PD 76/1018	*Epicoccum sorghi*	GU237978	**FJ427067**	**FJ427173**	*Sorghum vulgare*	Puerto Rico
CBS 627.68; PD 66/926	*Epicoccum sorghi*	GU237979	**FJ427072**	**FJ427178**	*Citrus* sp.	France
CBS 213.55	*Leptosphaerulina americana*	GU237981	GU237799	GU237539	*Trifolium pretense*	USA
CBS 275.59; ATCC 13446	*Leptosphaerulina arachidicola*	GU237983	GU237820	GU237543	*Arachis hypochea*	Taiwan
CBS 317.83	*Leptosphaerulina australis*	**EU754166**	GU237829	GU237540	*Eugenia aromatica*	Indonesia
CBS 939.69	*Leptosphaerulina australis*	**EU754167**	GU237911	GU237541	Soil	Netherlands
CBS 235.58	*Leptosphaerulina trifolii*	GU237982	GU237806	GU237542	*Trifolium* sp.	Netherlands
CBS 525.71	*Macroventuria anomochaeta* T	GU237984	GU237881	GU237544	Decayed canvas	South Africa
CBS 502.72	*Macroventuria anomochaeta*	GU237985	GU237873	GU237545	*Medicago sativa*	South Africa
CBS 526.71	*Macroventuria wentii*	GU237986	GU237881	GU237546	Unidentified plant material	USA
CBS 432.71	*Microsphaeropsis olivacea*	GU237987	GU237863	GU237548	*Sorothamus* sp.	Netherlands
CBS 233.77	*Microsphaeropsis olivacea*	GU237988	GU237803	GU237549	*Pinus laricio*	France
CBS 442.83	*Microsphaeropsis olivacea*	**EU754171**	GU237865	GU237547	*Taxus baccata*	Netherlands
CBS 132.96; PD 93/853	*Peyronellaea alectorolophi* T	GU237989	GU237778	GU237550	*Rhinanthus major*	Netherlands

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 185.85; PD 80/1191	*Peyronellaea americana* B	GU237990	**FJ426972**	**FJ427088**	*Zea mays*	USA
CBS 568.97; PD 94/1544; ATCC 44494	*Peyronellaea americana*	GU237991	**FJ426974**	**FJ427090**	*Glycine max*	USA
PD 82/1059	*Peyronellaea americana*	GU237992	**FJ426980**	**FJ427096**	Nematode cyst	Unknown
CBS 360.84	*Peyronellaea anserina* B	GU237993	GU237839	GU237551	Potatoflour	Netherlands
CBS 363.91; PD 79/712	*Peyronellaea anserina*	GU237994	GU237840	GU237552	*Pisum sativum*	Netherlands
CBS 315.90; PD 80/1190	*Peyronellaea arachidicola*	GU237995	GU237827	GU237553	*Arachis hypogaea*	Zimbabwe
CBS 333.75; ATCC 28333; IMI 386092; PREM 44889	*Peyronellaea arachidicola* T	GU237996	GU237833	GU237554	*Arachis hypogaea*	South Africa
CBS 269.93; PD 78/1087	*Peyronellaea aurea* B	GU237999	GU237818	GU237557	*Medicago polymorpha*	New Zealand
CBS 444.81; PDDCC 6546	*Peyronellaea australis* T	GU238000	GU237867	GU237558	*Actinidia chinensis*	New Zealand
PD 77/919	*Peyronellaea australis*	GU238001	GU237915	GU237559	*Actinidea chinensis*	Unknown
CBS 109.92; PD 73/1405	*Peyronellaea calorpreferens* T	GU238002	**FJ426983**	**FJ427098**	Undefined food material	Netherlands
CBS 630.97; ATCC 96683; IMI 361196; PD 96/2022	*Peyronellaea calorpreferens*	GU238004	GU237925	GU237560	*Heterodera glycines*	USA
CBS 875.97; PD 93/1503	*Peyronellaea calorpreferens*	GU238003	GU237908	GU237561	Indoor environment	USA
CBS 123380; PD 84/1013	*Peyronellaea coffeae-arabicae* T	GU238005	**FJ426993**	**FJ427104**	*Coffea arabica*	Ethiopia
CBS 123398; PD 84/1014	*Peyronellaea coffeae-arabicae*	GU238006	**FJ426994**	**FJ427105**	*Coffea arabica*	Ethiopia
PD 92/1460	*Peyronellaea curtisii*	GU238012	**FJ427041**	**FJ427151**	*Sprekelia*	Netherlands
CBS 251.92; PD 86/1145	*Peyronellaea curtisii* B	GU238013	**FJ427038**	**FJ427148**	*Nerine* sp.	Netherlands
CBS 377.91; PD 79/210	*Peyronellaea eucalyptica* B	GU238007	GU237846	GU237562	*Eucalyptus* sp.	Australia
CBS 508.91; PD 73/1413	*Peyronellaea eucalyptica*	GU238008	GU237878	GU237563	Water	Croatia
CBS 302.79; PD 79/1156	*Peyronellaea gardeniae*	**GQ387596**	**FJ427002**	**FJ427113**	Air	Netherlands Antilles
CBS 626.68; IMI 108771	*Peyronellaea gardeniae* T	**GQ387595**	**FJ427003**	**FJ427114**	*Gardenia jasminoides*	India
CBS 464.97; MUCL 9882	*Peyronellaea glomerata*	GU238009	**FJ427012**	**FJ427123**	Indoor environment	Netherlands
CBS 528.66; PD 63/590	*Peyronellaea glomerata* B	**EU754184**	**FJ427013**	**FJ427124**	*Chrysanthemum* sp.	Netherlands
CBS 103.25	*Peyronellaea lethalis*	GU238010	GU237729	GU237564	Unknown	Unknown
CBS 463.69	*Peyronellaea musae* B	GU238011	**FJ427026**	**FJ427136**	*Mangifera indica*	India
CBS 377.93; PD 80/976	*Peyronellaea obtusa* B	GU238014	GU237847	GU237565	*Daucus carota*	Netherlands

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 391.93; PD 80/87	*Peyronellaea obtusa* B	GU238015	GU237858	GU237566	*Spinacia oleracea*	Netherlands
CBS 318.90; PD 81/729	*Peyronellaea pinodella*	GU238016	**FJ427051**	**FJ427161**	*Pisum sativum*	Netherlands
CBS 531.66	*Peyronellaea pinodella* B	GU238017	**FJ427052**	**FJ427162**	*Trifolium pratense*	USA
CBS 100580; PD 98/1135	*Peyronellaea pinodella*	GU238018	GU237713	GU237567	*Glycine max*	Hungary
CBS 567.97; PD 97/2160	*Peyronellaea pinodella*	GU238019	GU237891	GU237568	*Glycine max*	Hungary
CBS 159.78b	*Peyronellaea pinodes*	GU238020	GU237786	GU237569	*Pisum sativum*	Iraq
CBS 285.49	*Peyronellaea pinodes*	GU238022	GU237823	GU237571	*Primula auricula*	Switzerland
CBS 235.55	*Peyronellaea pinodes*	GU238021	GU237805	GU237570	Unknown	Netherlands
CBS 525.77	*Peyronellaea pinodes*	GU238023	GU237883	GU237572	*Pisum sativum*	Belgium
CBS 525.77a	*Peyronellaea pinodes*	GU238024	GU237882	GU237573	*Pisum sativum*	Belgium
CBS 539.66; ATCC 16791; IMI 122266; PD 64/914	*Peyronellaea pomorum* var. *pomorum* B	GU238028	**FJ427056**	**FJ427166**	*Polygonum tataricum*	Netherlands
CBS 285.76; ATCC 26241; IMI 176742; VKM F-1843	*Peyronellaea pomorum* var. *circinata* T	GU238025	**FJ427053**	**FJ427163**	*Heracleum dissectum*	Russia
CBS 286.76; ATCC 26242; IMI 176743; VKM F-1844	*Peyronellaea pomorum* var. *circinata*	GU238026	**FJ427054**	**FJ427164**	*Allium nutans*	Russia
CBS 388.80; PREM 45736	*Peyronellaea pomorum* var. *cyanea* T	GU238027	**FJ427055**	**FJ427165**	*Triticum* sp.	South Africa
CBS 381.96; PD 71/706	*Peyronellaea protuberans* B	GU238029	GU237853	GU237574	*Lycium halifolium*	Netherlands
CBS 281.83	*Peyronellaea sancta* T	GU238030	**FJ427063**	**FJ427170**	*Ailanthus altissima*	South Africa
LEV 15292	*Peyronellaea sancta*	GU238031	**FJ427065**	**FJ427172**	*Gleditsia triacantha*	Unknown
CBS 110.92; PD 76/1010	*Peyronellaea subglomerata* B	GU238032	**FJ427080**	**FJ427186**	*Triticum* sp.	USA
PD 78/1090	*Peyronellaea subglomerata*	GU238033	**FJ427081**	**FJ427187**	*Zea mays*	Unknown
CBS 588.69	*Peyronellaea zeae-maydis* T	**EU754186**	**FJ427086**	**FJ427190**	*Zea mays*	USA
CBS 179.97	*Phoma acetosellae*	GU238034	GU237793	GU237575	*Rumex hydrolapathum*	Netherlands
CBS 379.93; PD 82/945	*Phoma aliena*	GU238037	GU237851	GU237578	*Berberis* sp.	Netherlands
CBS 877.97; PD 94/1401	*Phoma aliena*	GU238038	GU237910	GU237579	*Buxus sempervirens*	Netherlands
CBS 381.91; PD 79/1110	*Phoma anigozanthi* B	GU238039	GU237852	GU237580	*Anigozanthus maugleisii*	Netherlands
CBS 107.96; PD 73/598	*Phoma aquilegiicola* B	GU238041	GU237735	GU237582	*Aconitum pyramidale*	Netherlands
CBS 108.96; PD 79/611	*Phoma aquilegiicola* B	GU238042	GU237736	GU237583	*Aquilegia* sp.	Netherlands
CBS 125.93; PD 77/1029	*Phoma arachidis-hypogaeae* B	GU238043	GU237771	GU237584	*Arachis hypogaea*	India

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 383.67; PD 65/223	*Phoma aubrietiae* B	GU238044	GU237854	GU237585	*Aubrietia hybrida* cv. Superbissima	Netherlands
CBS 627.97; PD 70/714	*Phoma aubrietiae* B	GU238045	GU237895	GU237586	*Aubrietia* sp.	Netherlands
CBS 714.85; PD 74/265	*Phoma bellidis* B	GU238046	GU237904	GU237587	*Bellis perennis*	Netherlands
PD 94/886	*Phoma bellidis*	GU238047	GU237923	GU237581	*Bellis* sp.	Netherlands
CBS 109942; PD 84/402	*Phoma boeremae* T	GU238048	**FJ426982**	**FJ427097**	*Medicago littoralis* cv. Harbinger	Australia
CBS 120105	*Phoma brasiliensis* T	GU238049	GU237760	GU237588	*Amaranthus* sp.	Brazil
CBS 357.84	*Phoma bulgarica* T	GU238050	GU237837	GU237589	*Trachystemon orientale*	Bulgaria
CBS 124515; PD 82/1058	*Phoma bulgarica*	GU238051	GU237768	GU237590	*Trachystemon orientale*	Bulgaria
CBS 448.83	*Phoma calidophila* T	GU238052	**FJ427059**	**FJ427168**	Soil	Egypt
PD 84/109	*Phoma calidophila*	GU238053	**FJ427060**	**FJ427169**	*Cucumis sativus*	Europe
CBS 128.93; PD 79/140	*Phoma chenopodiicola* B	GU238055	GU237775	GU237591	*Chenopodium quinoa* cv. Sajana	Peru
CBS 129.93; PD 89/803	*Phoma chenopodiicola*	GU238056	GU237776	GU237592	*Chenopodium quinoa* cv. Sajana	Peru
CBS 102.66	*Phoma clematidina*	**FJ515630**	**FJ426988**	**FJ427099**	*Clematis* sp.	UK
CBS 108.79; PD 78/522	*Phoma clematidina* T	**FJ515632**	**FJ426989**	**FJ427100**	*Clematis* sp.	Netherlands
CBS 507.63; MUCL 9574; PD 07/03486747	*Phoma clematidis-rectae* T	**FJ515647**	**FJ515606**	**FJ515624**	*Clematis* sp.	Netherlands
PD 95/1958	*Phoma clematidis-rectae*	**FJ515648**	**FJ515607**	**FJ515625**	*Clematis* sp.	Netherlands
CBS 100409	*Phoma commelinicicola* B	GU238057	GU237712	GU237593	*Tradescantia* sp.	New Zealand
CBS 100311	*Phoma complanata*	**EU754181**	GU237709	GU237594	*Heracleum sphondylium*	Netherlands
CBS 268.92; PD 75/3	*Phoma complanata*	**EU754180**	GU237815	GU237595	*Angelica sylvestris*	Netherlands
CBS 506.91; IMI 215229; PD 91/876	*Phoma costarricensis* B	GU238058	GU237876	GU237596	*Coffea* sp.	Nicaragua
CBS 497.91; PD 79/209	*Phoma costarricensis*	GU238059	GU237870	GU237597	*Coffea arabica*	Unknown
CBS 193.82	*Phoma crystallifera* T	GU238060	GU237797	GU237598	*Chamaespartium sagittale*	Austria
CBS 124513 ; PD 73/1414	*Phoma dactylidis* T	GU238061	GU237766	GU237599	*Dactylis glomerata*	USA

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 133.93; PD 88/961; IMI 173142	*Phoma destructiva* var. *destructiva*	GU238064	GU237779	GU237602	*Solanum lycopersicum*	Guadeloupe
CBS 378.73; CECT 2877	*Phoma destructiva* var. *destructiva* B	GU238063	GU237849	GU237601	*Lycopersicon esculentum*	Tonga
CBS 162.78; PD 77/725	*Phoma destructiva* var. *diversispora*	GU238062	GU237788	GU237600	*Lycopersicon esculentum*	Netherlands
CBS 507.91; PD 74/148	*Phoma dictamnicola* B	GU238065	GU237877	GU237603	*Dictamnus albus*	Netherlands
CBS 109179; PD 90/835-1	*Phoma digitalis*	GU238066	GU237744	GU237604	*Digitalis* sp.	Netherlands
CBS 229.79; LEV 7660	*Phoma digitalis* B	GU238067	GU237802	GU237605	*Digitalis purpurea*	New Zealand
CBS 346.82	*Phoma dimorpha* T	GU238068	GU237835	GU237606	*Opuntiae* sp.	Spain
CBS 186.83; PD 82/47	*Phoma draconis* B	GU238070	GU237795	GU237607	*Dracaena* sp.	Rwanda
CBS 123.93; PD 77/1148	*Phoma eupatorii* B	GU238071	GU237764	GU237608	*Eupatorium cannabinum*	Netherlands
CBS 374.91; PD 78/391	*Phoma eupyrena* B	GU238072	**FJ426999**	**FJ427110**	*Solanum tuberosum*	Netherlands
CBS 527.66; ATCC 22238	*Phoma eupyrena* B	GU238073	**FJ427000**	**FJ427111**	Soil	Germany
CBS 633.92; ATCC 36786; VKM MF-325	*Phoma fungicola*	**EU754127**	GU237900	GU237609	*Microsphaera alphitoides* on *Quercus* sp.	Ukraine
CBS 112.96	*Phoma glaucii*	GU238077	GU237750	GU237610	*Dicentra* sp.	Netherlands
CBS 114.96; PD 94/888	*Phoma glaucii* B	**FJ515649**	**FJ515609**	**FJ515627**	*Chelidonium majus*	Netherlands
CBS 377.67	*Phoma gossypiicola* B	GU238079	GU237845	GU237611	*Gossypium hirsutum*	USA
CBS 104.80; PD 74/1017	*Phoma henningsii* B	GU238081	GU237731	GU237612	*Acacia mearnesii*	Kenya
CBS 502.91; PD 86/276	*Phoma herbarum*	GU238082	GU237874	GU237613	*Nerium* sp.	Netherlands
CBS 615.75; PD 73/665; IMI 199779	*Phoma herbarum* B	**EU880896**	**FJ427022**	**FJ427133**	*Rosa multiflora*	Netherlands
CBS 629.97; PD 76/1017	*Phoma herbicola* B	GU238083	GU237898	GU237614	Water	USA
CBS 105.80; PD 75/908	*Phoma huancayensis* T	GU238084	GU237732	GU237615	*Solanum* sp.	Peru
CBS 390.93; PD 77/1173	*Phoma huancayensis*	GU238085	GU237857	GU237616	*Chenopodium quinoa*	Peru
CBS 220.85	*Phoma humicola* B	GU238086	GU237800	GU237617	*Franseria* sp.	USA
CBS 123394	*Phoma infossa*	GU238088	**FJ427024**	**FJ427134**	*Fraxinus pennsylvanica*	Argentina

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 123395	*Phoma infossa* T	GU238089	**FJ427025**	**FJ427135**	*Fraxinus pennsylvanica*	Argentina
CBS 252.92; PD 80/1144	*Phoma insulana* B	GU238090	GU237810	GU237618	*Olea europaea*	Greece
CBS 124.93; PD 87/269	*Phoma labilis* B	GU238091	GU237765	GU237619	*Solanum lycopersicum*	Netherlands
CBS 479.93; PD 70/93	*Phoma labilis*	GU238092	GU237868	GU237620	*Rosa* sp.	Israel
CBS 347.82	*Phoma longicolla*	GU238094	GU237836	GU237621	*Opuntiae* sp.	Spain
CBS 124514; PD 80/1189; VPRI 1239	*Phoma longicolla* T	GU238095	GU237767	GU237622	*Opuntiae* sp.	Spain
CBS 223.69	*Phoma macrostoma* var. *incolorata* B	GU238096	GU237801	GU237623	*Acer pseudoplatanus*	Switzerland
CBS 109173; PD 83/908	*Phoma macrostoma* var. *incolorata* B	GU238097	GU237740	GU237624	*Malus sylvestris*	Netherlands
CBS 529.66; PD 66/521	*Phoma macrostoma* var. *macrostoma* B	GU238098	GU237885	GU237625	*Malus sylvestris*	Netherlands
CBS 482.95	*Phoma macrostoma* var. *macrostoma*	GU238099	GU237869	GU237626	*Larix decidua*	Germany
CBS 259.92; IMI 286996; PD 91/272	*Phoma matteuciicola* B	GU238100	GU237812	GU237627	*Matteuccia struthiopteris*	Canada
CBS 112.53	*Phoma medicaginis* var. *macrospora* B	GU238101	GU237749	GU237628	*Medicago sativa*	USA
CBS 404.65; IMI 116999	*Phoma medicaginis* var. *macrospora* B	GU238102	GU237859	GU237629	*Medicago sativa*	Canada
CBS 316.90	*Phoma medicaginis* var. *medicaginis*	GU238103	GU237828	GU237630	*Medicago sativa*	Czech Republic
CBS 105.95	*Phoma microchlamydospora* T	GU238104	**FJ427028**	**FJ427138**	*Eucalyptus* sp.	UK
CBS 491.90	*Phoma microchlamydospora*	GU238105	**FJ427029**	**FJ427139**	Unidentified vegetable	UK
CBS 315.83	*Phoma minor*	GU238106	GU237826	GU237631	*Syzygium aromaticum*	Indonesia
CBS 325.82	*Phoma minor* T	GU238107	GU237831	GU237632	*Syzygium aromaticum*	Indonesia
CBS 110.79; PD 65/8875; MUCL 8247	*Phoma multirostrata*	GU238110	**FJ427030**	**FJ427140**	*Cucumis sativus*	Netherlands
CBS 274.60; IMI 081598	*Phoma multirostrata* T	GU238111	**FJ427031**	**FJ427141**	Soil	India
CBS 368.65; PD 92/1757; HACC 154	*Phoma multirostrata*	GU238112	**FJ427033**	**FJ427143**	Soil	India
PD 83/48	*Phoma multirostrata*	GU238113	**FJ427037**	**FJ427147**	*Cucumis sativus*	Unknown
CBS 117.93; PD 83/90	*Phoma nebulosa*	GU238114	GU237757	GU237633	*Mercurialis perennis*	Netherlands
CBS 503.75; ATCC 32163; DSM 63391; IMI 194766; PD 75/4	*Phoma nebulosa* B	GU238115	GU237875	GU237634	*Urtica dioica*	Austria

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 358.71	*Phoma negriana* B	GU238116	GU237838	GU237635	*Vitis vinifera*	Germany
PD 79/74	*Phoma negriana*	GU238117	GU237916	GU237636	*Vitis vinifera*	Netherlands
CBS 116.96; PD 95/7930	*Phoma nigripycnidia* B	GU238118	GU237756	GU237637	*Vicia cracca*	Russia
CBS 114.93; PD 74/228	*Phoma novae- verbascicola*	GU238119	GU237753	GU237638	*Verbascum* sp.	Netherlands
CBS 127.93; PD 92/347	*Phoma novae-verbascicola* B	GU238120	GU237774	GU237639	*Verbascum densiflorum*	Netherlands
CBS 654.77	*Phoma omnivirens*	GU238122	**FJ427043**	**FJ427153**	Unknown	India
CBS 991.95	*Phoma omnivirens*	GU238121	**FJ427044**	**FJ427154**	Soil	Papua New Guinea
CBS 560.81; PD 92/1569; PDDCC 6614	*Phoma paspali* T	GU238124	**FJ427048**	**FJ427158**	*Paspalum dilatatum*	New Zealand
CBS 561.81; PDDCC 6615	*Phoma paspali*	GU238125	GU237889	GU237640	*Lolium perenne*	New Zealand
CBS 124516; PD 84/453	*Phoma pedeiae*	GU238126	GU237769	GU237641	Orchidaceae	Netherlands
CBS 124517; PD 92/612A	*Phoma pedeiae* T	GU238127	GU237770	GU237642	*Schefflera elegantissima*	Netherlands
CBS 267.92; PD 76/1014	*Phoma pereupyrena* T	GU238128	GU237814	GU237643	*Coffea arabica*	India
CBS 268.93; CBS 108.93; PD 88/720	*Phoma piperis* B	GU238129	GU237816	GU237644	*Peperomia pereskifolia*	Netherlands
PD 90/2011	*Phoma piperis*	GU238130	GU237921	GU237645	*Peperomia* sp.	Netherlands
CBS 284.93; PD 75/907	*Phoma plurivora*	GU238131	GU237822	GU237646	*Medicago sativa*	Australia
CBS 558.81; PDDCC 6873	*Phoma plurivora* T	GU238132	GU237888	GU237647	*Setaria* sp.	New Zealand
CBS 109181; PD 83/757	*Phoma polemonii* B	GU238133	GU237746	GU237648	*Polemonium caeruleum*	Netherlands
CBS 116.93; PD 71/884	*Phoma poolensis* B	GU238134	GU237755	GU237649	*Antirrhinum majus*	Netherlands
CBS 113.20; PD 92/774	*Phoma poolensis*	GU238135	GU237751	GU237650	Unknown	Unknown
CBS 372.91; PD 75/690	*Phoma putaminum* B	GU238137	GU237843	GU237651	*Ulmus* sp.	Netherlands
CBS 130.69; CECT 20054; IMI 331916	*Phoma putaminum* B	GU238138	GU237777	GU237652	*Malus sylvestris*	Denmark
CBS 109177; LEV 15165; PD 2000/9941	*Phoma rhei* B	GU238139	GU237743	GU237653	*Rheum rhaponticum*	New Zealand
CBS 298.89	*Phoma saxea*	GU238140	GU237824	GU237654	Limestone	Germay

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 419.92	*Phoma saxea* T	GU238141	GU237860	GU237655	Corroded mediterranean marble	Germany
CBS 122.93; PD 77/1049	*Phoma selaginellicola* B	GU238142	GU237762	GU237656	*Selaginella* sp.	Netherlands
CBS 160.78; LEV 11451	*Phoma senecionis* B	GU238143	GU237787	GU237657	*Senecio jacobaea*	New Zealand
CBS 249.92; PD 78/1088	*Phoma subherbarum*	GU238144	GU237808	GU237658	*Solanum* sp.	Peru
CBS 250.92; DAOM 171914; PD 92/371	*Phoma subherbarum* B	GU238145	GU237809	GU237659	*Solanum* sp.	Peru
CBS 305.79A; DAOM 170848	*Phoma subherbarum*	GU238146	GU237825	GU237660	*Zea mays*	Peru
CBS 135.93; PD 83/87	*Phoma sylvatica* B	GU238147	GU237781	GU237661	*Melampyrum pratense*	Netherlands
CBS 874.97; PD 93/764	*Phoma sylvatica* B	GU238148	GU237907	GU237662	*Melampyrum pratense*	Netherlands
CBS 436.75	*Phoma tropica* T	GU238149	GU237864	GU237663	*Saintpaulia ionantha*	Germany
CBS 876.97; PD 82/1008	*Phoma versabilis* B	GU238152	GU237909	GU237664	*Silene* sp.	Netherlands
PD 2000/1379	*Phoma versabilis*	GU238153	GU237913	GU237665	*Stellaria media*	Netherlands
CBS 500.91; PD 83/322	*Phoma viburnicola* B	GU238154	GU237871	GU237666	*Ilex aquifolium*	Netherlands
CBS 523.73; PD 69/800	*Phoma viburnicola* B	GU238155	GU237879	GU237667	*Viburnum cassioides*	Netherlands
CBS 383.68	*Phoma xanthina* B	GU238157	GU237855	GU237668	*Delphinium* sp.	Netherlands
PD 84/407	*Phoma xanthina*	GU238158	GU237918	GU237669	*Delphinium* sp.	Netherlands
CBS 131.93; PD 69/140	*Phoma zantedeschiae*	GU238159	**FJ427084**	**FJ427188**	*Calla* sp.	Netherlands
CBS 105.96; PD 74/230	*Stagonosporopsis actaeae* B	GU238165	GU237733	GU237670	*Cimicifuga simplex*	Netherlands
CBS 106.96; PD 94/1318	*Stagonosporopsis actaeae* T	GU238166	GU237734	GU237671	*Actaea spicata*	Netherlands
CBS 176.93; PD 86/547	*Stagonosporopsis ajacis*	GU238167	GU237790	GU237672	*Delphinium* sp.	Netherlands
CBS 177.93; PD 90/115	*Stagonosporopsis ajacis* T	GU238168	GU237791	GU237673	*Delphinium* sp.	Kenya
CBS 101.80; PD 75/909; IMI 386090	*Stagonosporopsis andigena* B	GU238169	GU237714	GU237674	*Solanum* sp.	Peru
CBS 269.80; PD 75/914	*Stagonosporopsis andigena*	GU238170	GU237817	GU237675	*Solanum* sp.	Peru
CBS 102636; PD 73/1409	*Stagonosporopsis artemisiicola* B	GU238171	GU237728	GU237676	*Artemisia dracunculus*	France
CBS 178.25; MUCL 9915	*Stagonosporopsis astragali* B	GU238172	GU237792	GU237677	*Astragalus* sp.	Unknown
CBS 248.90	*Stagonosporopsis caricae*	GU238175	GU237807	GU237680	*Carica papaya*	Chile
PD 06/03082531	*Stagonosporopsis caricae*	GU238176	GU237912	GU237681	*Carica papaya*	Brazil
CBS 282.76	*Stagonosporopsis caricae*	GU238177	GU237821	GU237682	*Brassica* sp.	Indonesia

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 713.85; ATCC 76027; PD 83/826	*Stagonosporopsis crystalliniformis* T	GU238178	GU237903	GU237683	*Lycopersicon esculentum*	Colombia
CBS 771.85; IMI 386091; PD 85/772	*Stagonosporopsis crystalliniformis*	GU238179	GU237906	GU237684	*Solanum tuberosum*	Colombia
CBS 109171; PD 91/310; PDDCC 272	*Stagonosporopsis cucurbitacearum*	GU238180	GU237922	GU237685	*Cucurbita* sp.	Netherlands
CBS 133.96; PD 79/127	*Stagonosporopsis cucurbitacearum*	GU238181	GU237780	GU237686	*Cucurbita* sp.	New Zealand
CBS 631.68; PD 68/147	*Stagonosporopsis dennisii* B	GU238182	GU237899	GU237687	*Solidago floribunda*	Netherlands
CBS 135.96; IMI 19337; PD 95/4756	*Stagonosporopsis dennisii*	GU238183	GU237782	GU237688	*Solidago canadensis*	Canada
CBS 320.90; PD 86/932	*Stagonosporopsis dorenboschii* B	GU238184	GU237830	GU237689	*Physostegia virginiana*	Netherlands
CBS 426.90; IMI 386093; PD 86/551	*Stagonosporopsis dorenboschii* T	GU238185	GU237862	GU237690	*Physostegia virginiana*	Netherlands
CBS 109182; PD 74/231	*Stagonosporopsis heliopsidis* B	GU238186	GU237747	GU237691	*Heliopsis patula*	Netherlands
PD 95/6189; DAOM 221138	*Stagonosporopsis heliopsidis*	GU238187	GU237924	GU237692	*Ambrosia artemisiifolia*	Canada
CBS 104.42	*Stagonosporopsis hortensis* B	GU238198	GU237730	GU237703	Unknown	Netherlands
CBS 572.85; PD 79/269	*Stagonosporopsis hortensis* B	GU238199	GU237893	GU237704	*Phaseolus vulgaris*	Netherlands
CBS 425.90; PD 81/520	*Stagonosporopsis ligulicola* var. *inoxydabilis* T	GU238188	GU237861	GU237693	*Chrysanthemum parthenii*	Netherlands
PD 85/259	*Stagonosporopsis ligulicola* var. *inoxydabilis*	GU238189	GU237920	GU237694	*Matricaria* sp.	Netherlands
CBS 500.63; MUCL 8090	*Stagonosporopsis ligulicola* var. *ligulicola* B	GU238190	GU237872	GU237695	*Chrysanthemum indicum*	Germany
CBS 137.96; PD 84/75	*Stagonosporopsis ligulicola* var. *ligulicola* B	GU238191	GU237783	GU237696	*Chrysanthemum indicum*	Netherlands
CBS 562.81; PDDCC 6884	*Stagonosporopsis loticola* T	GU238192	GU237890	GU237697	*Lotus pedunculatus*	New Zealand
CBS 628.97; PD 79/72; PDDCC 3870	*Stagonosporopsis loticola*	GU238193	GU237896	GU237698	*Lotus tenuis*	New Zealand
CBS 101494; PD 98/5247	*Stagonosporopsis lupini* B	GU238194	GU237724	GU237699	*Lupinus albus*	UK
CBS 375.84; PD 80/1250	*Stagonosporopsis lupini*	GU238195	GU237844	GU237700	*Lupinus mutabilis*	Peru
CBS 634.92; IMI 193307	*Stagonosporopsis oculo-hominis* T	GU238196	GU237901	GU237701	Human	USA

Table 5.3. (Ctd).

Strain no.[1]	Holomorph[2]	GenBank no.			Original substrate	Locality
		LSU	ITS	TUB		
CBS 109180; PD 79/175	*Stagonosporopsis rudbeckiae* B	GU238197	GU237745	GU237702	*Rudbeckia bicolor*	Netherlands
CBS 379.91; PD 77/675	*Stagonosporopsis trachelii* B	GU238173	GU237850	GU237678	*Campanula isophylla*	Netherlands
CBS 384.68	*Stagonosporopsis trachelii* B	GU238174	GU237856	GU237679	*Campanula isophylla*	Sweden
CBS 273.92; PD 76/1019	*Stagonosporopsis valerianellae*	GU238200	GU237819	GU237705	*Valerianella locusta*	Netherlands
CBS 329.67; PD 66/302	*Stagonosporopsis valerianellae* B	GU238201	GU237832	GU237706	*Valerianella locusta* var. *oleracea*	Netherlands

[1]ATCC: American Type Culture Collection, Virginia, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CECT: Coleccion Espanola de Cultivos Tipo, Valencia University, Spain; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; HACC: Research Laboratory, Hindustan Antibiotics Ltd., Pimpri Poona, India; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; LEV: Plant Health and Diagnostic Station, Auckland, New Zealand; MUCL: Mycotheque de l'Universite catholique de Louvain, Louvain-la-Neuve, Belgium; PD: Plant Protection Service, Wageningen, the Netherlands; PDDCC: Plant Diseases Division Culture Collection, Auckland, New Zealand; PREM: National Collection of Fungi: Culture Collection, Pretoria, South Africa; VKM: All-Russian Collection of Microorganisms, Pushchino, Russia; VPRI: Victorian Plant Disease Herbarium, Victoria, Australia.

[2]T: Type strain; B: Reference strain according to Boerema *et al*. (2004).

Clade 2, *Sporormiaceae*: In the basal lineages, *Sporormiella minima* (CBS 524.50) was recovered, representing the *Sporormiaceae*, which was recently recircumscribed (Barr 2000). In the same clade, two species were recovered that are described in *Phoma* section *Phoma*: *Ph. capitulum* and *Ph. minutispora*.

Both species are distinguishable from other species in this Boeremaean section by the production of relatively small subglobose conidia (measuring *ca*. 2–5 × 1.5–3 µm) with a few, large guttules. Within the *Sporormiaceae*, teleomorphs species have been reported with phoma-like anamorphs, such as *Westerdykella dispersa* (von Arx 1981). Two *Sporormiaceae*-associated genera, *Sporormia* and *Preussia*, have been mentioned as possible teleomorph for *Ph. deserticola* (von Arx & Storm 1967), a species that was regarded as miscellaneous by Boerema *et al.* (2004). Also these anamorphs produce minute (sub-)globose conidia (von Arx 1981, Boerema *et al.* 2004). Although the *Sporormiaceae* belongs to the *Pleosporales* (Barr 2000, 2002), it forms a rather basal clade to most of the other *Phoma* species, and a taxonomic revision of *Ph. capitulum* and *Ph. minutispora* should therefore be considered.

Clade 3, *Melanommataceae*: One species that belongs to the *Melanommataceae* was included in the phylogenetical reconstruction of the phomoid *Pleosporales*. This species, *Aposphaeria populina* (CBS 543.70), is recovered in the basal lineages of the reconstructed tree. The close association of this family with the *Sporormiaceae* and their phylogenetic placement in the basal lineages of the *Pleosporales* is in congruence with results obtained in earlier studies (Kruys *et al.* 2006, de Gruyter *et al.* 2009). Although some earlier workers regularly mistook several *Phoma* species for members of the genus *Aposphaeria* (e.g. Saccardo 1884), none of the *Phoma* species included in this study were clustering with the *Melanommataceae*.
Clade 4, This clade comprises a range of species that almost all belong to different genera. *Phoma lini* and *Ph. flavescens* are the two *Phoma* representatives found in this clade, although they are not sister species. Based on morphological data, both species were accommodated in *Phoma* section *Phoma* (de Gruyter *et al*. 1993). Both species produce a yellow diffusible pigment *in vitro*, although a positive reaction to NaOH is only observed in *Ph. lini*. Both *Ph. flavescens* and *Ph. lini* are closely related to *Paraconiothyrium minitans* (≡ *Coniothyrium minitans*; Verkley *et al.* 2004). With this formal recombination into *Paraconiothyrium*, it was aimed to differentiate *Par. minitans*, which produces complex, thick-walled pycnidia from other *Coniothyrium* species that normally produce more phomoid pycnidia (Verkley *et al.* 2004). The close relationship between *Par. minitans* with *C. fuckelii* that is found here is in congruence with the observations of Damm *et al.* (2008), although the teleomorph name, *Leptosphaeria coniothyrium*, would suggest a association with the *Leptosphaeriaceae* (clade 8). The likeliness of the findings of *Pyrenochaeta romeroi* (CBS 252.60), *Asteromella tiliae* (CBS 265.94) and *Neottiosporina paspali* (CBS 331.37) in this clade was already discussed by de Gruyter *et al.* (2009).

Clade 5, Cucurbitariaceae: Clade 5 comprises mainly taxa with setose pycnidia, including several representative species of the genus *Pyrenochaeta.* In addition, a *Coniothyrium* sp., *Phialophorophoma litoralis* and *Pleurophoma cava* grouped in this clade, as well as two *Phoma* species, *Ph. pratorum* (section *Phoma*) *and Ph. terricola*, (section *Paraphoma*). Another representative of the section *Paraphoma* that is included in this study is *Ph. radicina*, which is however found in clade 6. The taxonomy of setose species that are currently classified in *Phoma* section *Paraphoma*, *Pyrenochaeta* and *Pleurophoma* is revised (de Gruyter *et al.* 2010). Also in several non-*Paraphoma* species in the genus *Phoma* setose or semi-pilose pycnidia do occur incidentally (Boerema *et al.* 2004). However, thus far, no setae-forming *Ph. pratorum* strains have been recorded. The finding of this species in the present clade is thus highly remarkable.

The *Coniothyrium* strain in this clade (CBS 122787) was previously identified as *C. cerealis*, and is found to be closely related to *Pyrenochaeta acicola* (BPP = 0.99, RBS = 100 %). As was illustrated in a previous study of Muthumeenakshi *et al.* (2001) *C. cerealis* is quite distantly related to other *Coniothyrium* species. However, based on comparison with sequence data available in GenBank, it is unlikely that its previous identification was correct. This finding further illustrates the polyphyly of the genus *Coniothyrium*, which further has been retrieved in clades 4, 6 (*Phaeosphaeriaceae*), 7 (*Leptosphaeriaceae* and *Pleosporaceae*) and 8 (*Didymellaceae*). As mentioned before, some species of this genus have been associated with the teleomorph genus *Leptosphaeria*, and are thus expected to cluster with the *Leptosphaeriaceae* (clade 7). None of the species recovered in clade 5 has been associated with a teleomorph.

Clade 6, Phaeosphaeriaceae: The species that are found in the well-supported clade 6 (BPP = 1.00; RBS = 83 %), belong to the morphologically heterogeneous group of the *Phaeosphaeriaceae*. Most findings in this clade have already been discussed in the previous paper of de Gruyter *et al.* (2009). In addition to that study, six *Phoma* species are retrieved in this clade. *Phoma radicina*, type of *Phoma* section *Paraphoma*, is found in close association with *Ph. chrysanthemicola* (BPP = 1.00; RBS = 90 %). The association between *Ph. radicina* and the *Phaeosphaeriaceae* is further discussed by de Gruyter *et al.* (2010). Its close association with *Ph. chrysanthemicola* has been observed before by Aveskamp *et al.* (2008a), but the link with the *Phaeosphaeriaceae* has not been established. Strains of *Ph. chrysanthemicola* exhibit some semi-setose pycnidia that are, however, often fully covered by mycelial hairs (Boerema 1993). This is a feature that is in common with *Ph. radicina*, which has, as type species of the section *Paraphoma*, clearly visible setae. In contrast, the main characteristic of *Ph. chrysanthemicola*, the presence of pseudosclerotioid masses, has never been observed in the latter species. However, also not all strains of *Ph. chrysanthemicola* exhibit this character (Dorenbosch 1970).

Phoma fimeti forms a subclade with *Ph. samarorum* and a strain that was previously identified as *Stenocarpella macrospora* (BPP = 0.98; RBS = 67 %), but that is probably misidentified (de Gruyter *et al.* 2009). Especially the finding of *Ph. samarorum* is noteworthy, as it is found rather distinct from two clusters of other species belonging to the section *Heterospora*, which are retrieved among the *Leptosphaeriaceae* and *Didymellaceae* (clades 7 and 8). In contrast to these other *Heterospora* species, the large conidia of *Ph. samarorum* that can be observed *in planta* are clearly distinct by the subulate top cells, and measures up to 17 × 3.5 µm (Boerema *et al.* 1997). The strain identified as *Stenocarpella macrospora* is now sterile and therefore not studied morphologically. This species is known to produce similar-shaped, septate conidia, which are however pigmented and considerably larger, 44–82 × 7.5–11.5 µm (Sutton 1980). The close association with *Ph. fimeti* is therefore remarkable as this species is known to produce only minute, aseptate conidia, measuring (2–)2.5–4(–5) × (1.5–)2–2.5(–3) µm (de Gruyter & Noordeloos 1992).

The remaining two *Phoma* species in this clade, *Ph. haematocycla* and *Ph. opuntiae*, also produce such minute conidia. *Phoma haematocyla*, a flax-associated species from New Zealand, is retrieved in a subclade that also accommodates *Chaetasphaeronema hispidulum* (BPP = 1.00; RBS = 100 %).

All *Phoma* species found here are morphologically rather distinct, hence their placement in four different *Phoma* sections (Boerema *et al.* 2004). None of the *Phoma* species accommodated in this clade is associated with a teleomorph. The main teleomorph associated with the *Phaeosphaeriaceae* is *Phaeosphaeria,* although also incidentally a *Leptosphaeria* species is associated with this family (Câmara *et al.* 2002). An anamorph genus that is often confused with *Phoma* is *Microsphaeropsis* (Boerema 1997), which is linked to *Phaeosphaeria* (Câmara *et al.* 2002). Both anamorph genera differ in conidial pigmentation, which is commonly only present in mature conidia of *Microsphaeropsis*. Younger conidia are, however, often colourless. It may be that the *Phoma* species in this clade actually belong to what is now known as *Microsphaeropsis*, but have lost the pigmentation character during evolution.

Clade 7, Leptosphaeriaceae and *Pleosporaceae*: Clade 7 is a large clade comprising many *Phoma* species from various Boeremaean sections. Three reference species encountered here have been associated with the *Leptosphaeriaceae* before, these include *Leptosphaeria maculans*, *L. biglobosa* and *Coniothyrium palmarum* (Reddy *et al.* 1998, Verkley *et al.* 2004, de Gruyter *et al.* 2009), or with the *Pleosporaceae*, such as *Pleospora herbarum*, *Ascochyta caulina* and *Ph. betae* (Dong *et al.* 1998, Kodsueb *et al.* 2006, Inderbitzin *et al.* 2009, de Gruyter *et al.* 2009).

The two *Leptosphaeria* species in this study that were associated with a *Phoma* anamorph cluster together in the present clade: *L. maculans* (anam *Ph. lingam*) and *L. biglobosa*, which produces an unnamed, phomoid anamorph that is highly similar to *Ph. lingam* (Shoemaker & Brun 2001). Both species are serious pathogens of *Brassicaceae* (Fitt *et al.* 2006). *Leptosphaeria biglobosa* was found to be closely related to *Ph. lingam* in previous studies (Mendes-Perreira *et al.* 2003) and was for a long time recognised as a weakly pathogenic variety of the latter species (Johnson & Lewis 1990, Schäfer & Wöstemeyer 1992, Morales *et al*. 1993, Pongam *et al*. 1999, Williams & Fitt 1999, Purwantara *et al*. 2000, Shoemaker & Brun 2001, Voigt *et al*. 2001).

The phylogenic relation of *Phoma* species currently classified in sections *Pleonodomus* and *Pilosa* is currently investigated (de Gruyter *et al*. 2013). However, the present results reveal that a number of species from other *Phoma* sections fits in the *Leptosphaeriaceae* and *Pleosporaceae*. These include *Ph. apiicola*, *Ph. fallens*, *Ph. flavigena*, *Ph. glaucispora*, *Ph. multipora*, *Ph. valerianeae* and *Ph. vasinfecta*. In contrast

to the species that are accommodated in sections *Pilosa* and *Plenodomus*, pilose or scleroplectenchymatous pycnidia have never been recorded in these seven species; hence the placements in section *Phoma*.

Phoma multipora was ascribed to section *Phoma*. However, the original morphological description mentions the presence of elongated conidiophores (Pawar *et al.* 1967), which indicates that this species does not belong to the genus *Phoma* according to the present-day concept.

In addition, some representatives of other sections are found in clade 7, such as *Ph. incompta* (section *Sclerophomella*) and *Ph. violicola*, which is associated with the section *Peyronellaea*. Based on previous studies in the section *Peyronellaea* however, also *Ph. chrysanthemicola* and *Ph. schachtii* may be expected to cluster with the species in this clade (Aveskamp *et al*. 2009a). Remarkably, also two representatives of the section *Heterospora* are found in this clade. *Phoma heteromorphospora* is the assigned type species of this section (Boerema *et al.* 1997), whereas *Ph. dimorphospora* is morphologically closely allied, in congruence with the molecular results obtained here. Both species have a slow growth-rate and occur on *Chenopodium* spp., but can be distinguished by the absence of the conidial dimorphism in *Ph. dimorphospora in vitro*. Moreover, the latter species is commonly found in North and South America, whilst *Ph. heteromorphospora* occurs mainly in Europe (Boerema *et al*. 2004).

With the exception of *Ph. samarorum* (clade 6 – *Phaeosphaeriaceae*), the other species of the section *Heterospora* are found in clade 8, which represents the *Didymellaceae*. The major difference between the *Heterospora* species in the present clade in contrast to those in the *Didymellaceae* is the size of the septate conidia, which are up to 9 × larger in vivo than the regular conidia in *Ph. heteromorphospora* and *Ph. dimorphospora*, whereas, in the *Didymellaceae* clade, the septate conidia are only 1.5–4.5 × larger.

Also, *Coniothyrium palmarum*, which represents the type of its genus, clusters in this clade. Just as in *Phoma*, the species in *Coniothyrium* have only a limited number of morphological features that can aid in taxonomy. This has led to an unwanted situation in which species morphologically placed in this genus have been shown in phylogenetic examination to be dispersed among multiple families (Verkley *et al*. 2004). Although, based on type species, an anamorph-teleomorph link has been established between *Coniothyrium* and *Leptosphaeria* (Crous 1998), many heterogeneous species are *Coniothyrium*-like, and belong phylogenetically to different families or even classes (Cortinas *et al*. 2006). In this study we found "*Coniothyrium*" species accommodated in at least three different clades (Figure 5.1). *Coniothyrium clematidis-rectae* is phylogenetically linked to the Didymellaceae (Figure 5.2 – see below). *Phoma* and *Coniothyrium* are considered to be highly similar and are only distinguished on basis of the pigmentation of the conidia and the structure of the pycnidial wall (Boerema *et al*. 2004).

This clade also accommodates *Pleospora betae*, a notorious leaf and seed pathogen of beet (*Beta vulgaris*, Bugbee & Cole 1981), and *Pl. herbarum*, which is the type species of the genus *Pleospora*. The genetic distance between the two species was already observed in a study utilising SSU nrDNA sequences (Dong *et al*. 1998). Also three *Phoma* species that are found in close association with these "true" *Pleosporaceae* and that are found basal to this clade, *Ph. fallens*, *Ph. flavigena* and *Ph. glaucispora* have glabrous pycnidia and, like *Ph. betae*, aseptate conidia, hence their link to *Phoma* section *Phoma*. Absence of an ostiole is only recorded in *Ph. glaucispora* (de Gruyter *et al*. 1998).

Pleospora is linked to the anamorph genus *Stemphylium* (Simmons 1969), *Alternaria* and *Dendryphion* (von Arx 1981). The pluriform nature of the *Pleospora* anamorphs strongly contrasts with the relatively uniform morphology of the teleomorphic structures (Holm 1962, Kodsueb *et al*. 2006, Inderbitzin *et al*. 2009). The polyphyletic nature of *Pleospora* has been hypothesized by Holm (1962) and Berbee (1996), but only recently have molecular studies confirmed its taxonomic complexity (Dong *et al*. 1998, Kodsueb *et al*. 2006, Inderbitzin *et al*. 2009).

Clade 8, Didymellaceae: The major cluster observed in the generic phylogeny is the top clade in Figure 5.1, which represents the *Didymellaceae* clade. This clade is well supported (BPP = 0.99, RBS = 94 %), but with the loci used, a high level of basal polytomy is recorded within the clade. The ancestral species in this clade are the *Gramineae*-pathogens, *Ascochyta hordei* and *Ph. paspali*. The latter species has been considered to be an indigenous pathogen of grasses in Australia and New Zealand (Johnston 1981, Boerema *et al*. 2004), but based on sequence comparisons this species is probably also present in Europe (Wirsel *et al*. 2001, C. Gueidan pers. comm.).

Clade 8 comprises most *Phoma* species, including CBS 615.75, the representative strain of *Ph. herbarum* (Boerema *et al*. 2004), which is type species of the genus (Boerema 1964). This clade also includes the type

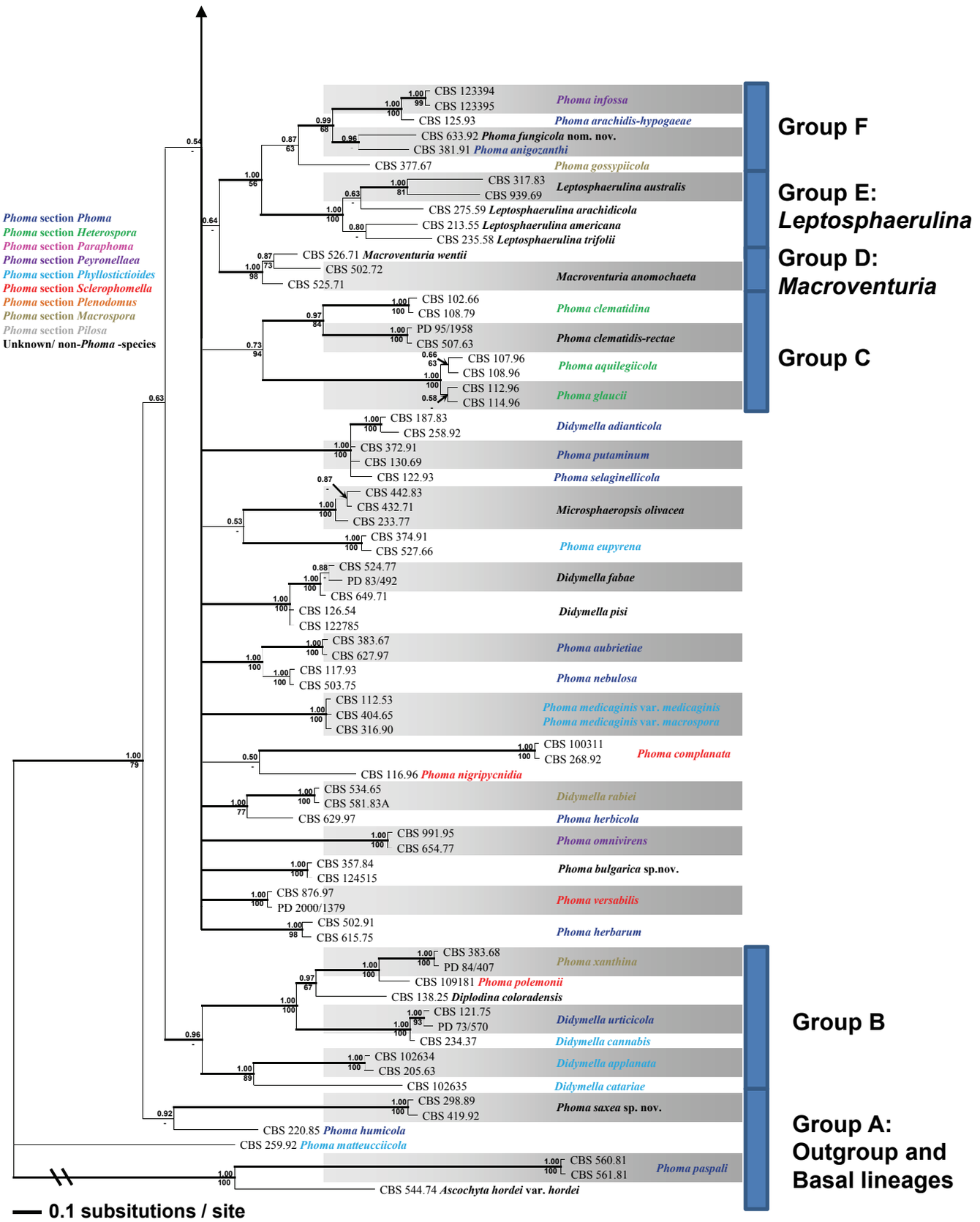


Figure 5.2. Fifty percent majority rule consensus tree from a BI analysis of LSU, ITS and TUB sequences of *Didymellaceae* (n = 274). At the nodes the BI Posterior Probabilities are presented above the branch, and bootstrap percentages of the analysis are given below the branch. Branches that were less than 50 % supported in the ML analyses are indicated with a hyphen. The bar indicates the number of substitutions per site. The tree is rooted with *Ascochyta hordei* var. *hordei* (CBS 544.74) and *Phoma paspali* (CBS 560.81 & CBS 561.81).

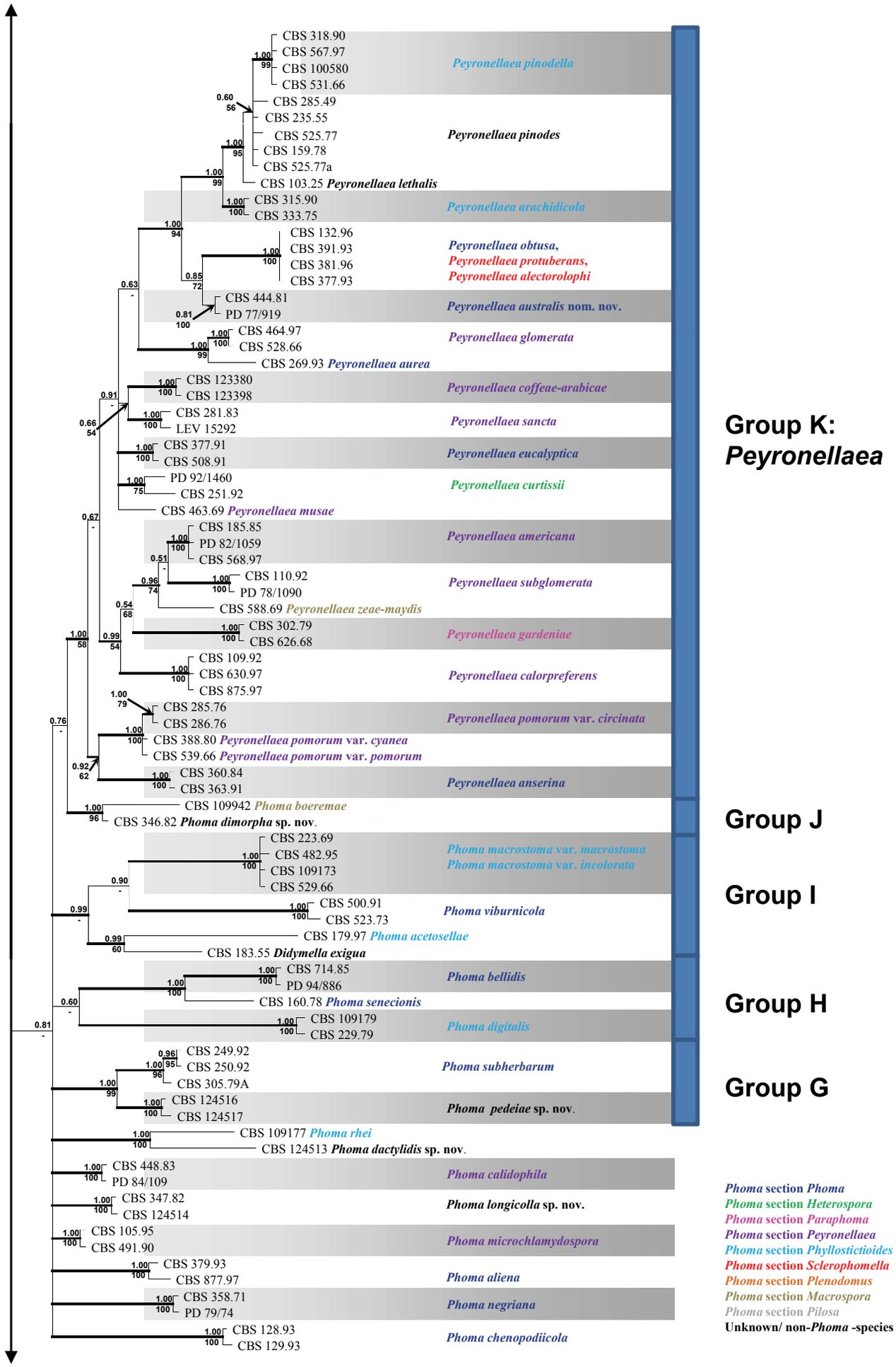


Figure 5.2. (Ctd).

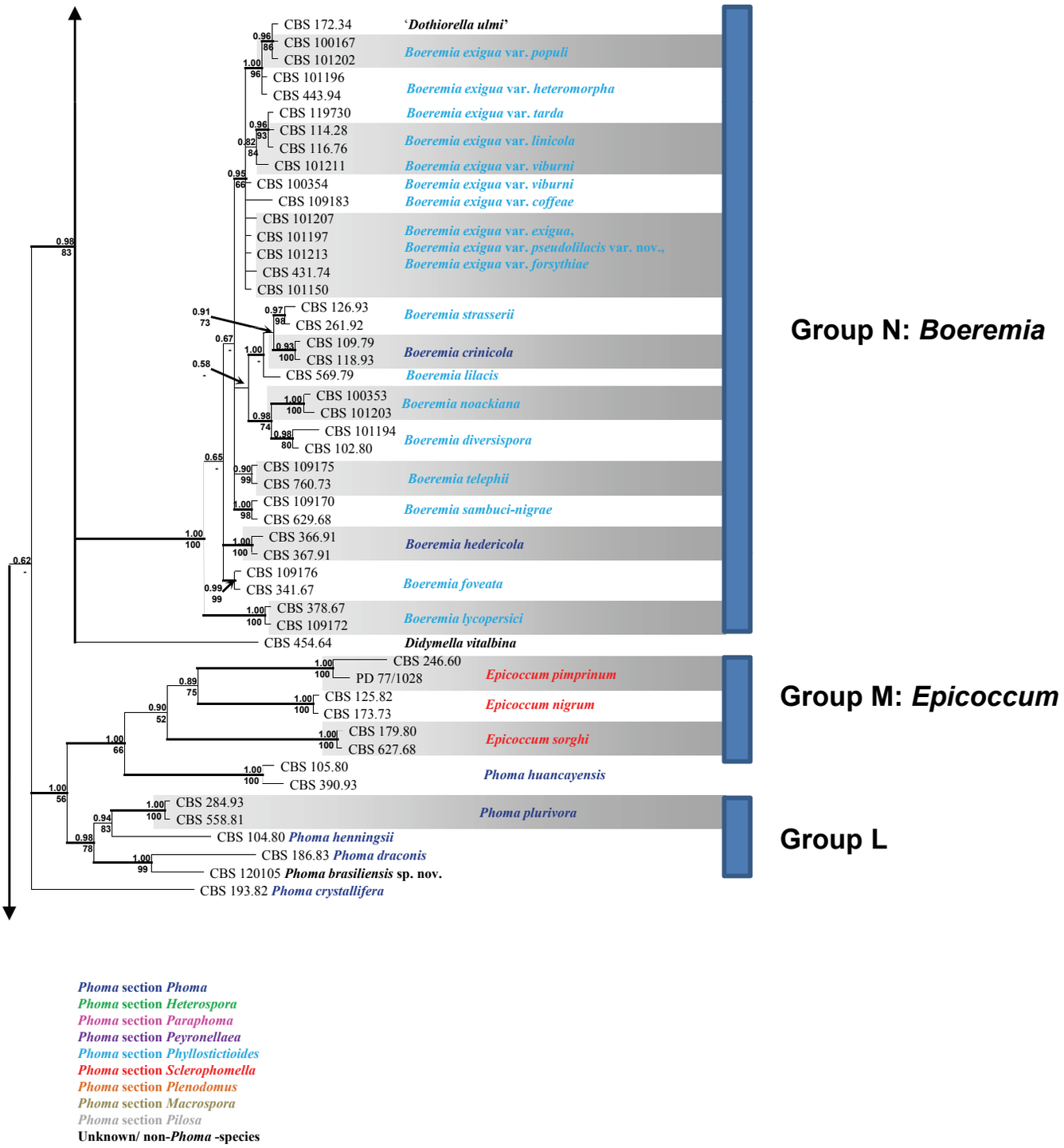


Figure 5.2. (Ctd).

species of the *Phoma* sections *Phoma*, *Peyronellaea*, *Phyllostictoides*, *Sclerophomella* and *Macrospora*. Some phytopathologically and medically relevant species of the section *Heterospora* are also associated with this clade, although some species of this section are found in other clades, such as *Ph. samarorum* (clade 6) and *Ph. dimorphospora,* and the sectional type *Ph. heteromorphospora* (clade 7). Finally, a single species of the setose section *Paraphoma*, *Ph. gardeniae*, is found in the *Didymellaceae*. Based on the sequence data obtained in this study, it is estimated that approximately 70 % of the species recognised by Boerema *et al*. (2004) can be associated with the *Didymellaceae*.

Besides the many *Phoma* species, several other anamorph fungi are found within this clade, including *Ampelomyces quercinus, Ascochyta fabae* (teleom. *Didymella fabae*), *Asc. hordei* var. *hordei*, *Asc. pinodes* (teleom. *Didymella pinodes*), *Chaetasbolisia erysiphoides*, *Didymella exigua*, *Epicoccum nigrum* (synanamorph *Ph. epicoccina*) and *Microsphaeropsis olivacea*. Of these species, *Asc. pisi*, *C. erysiphoides*

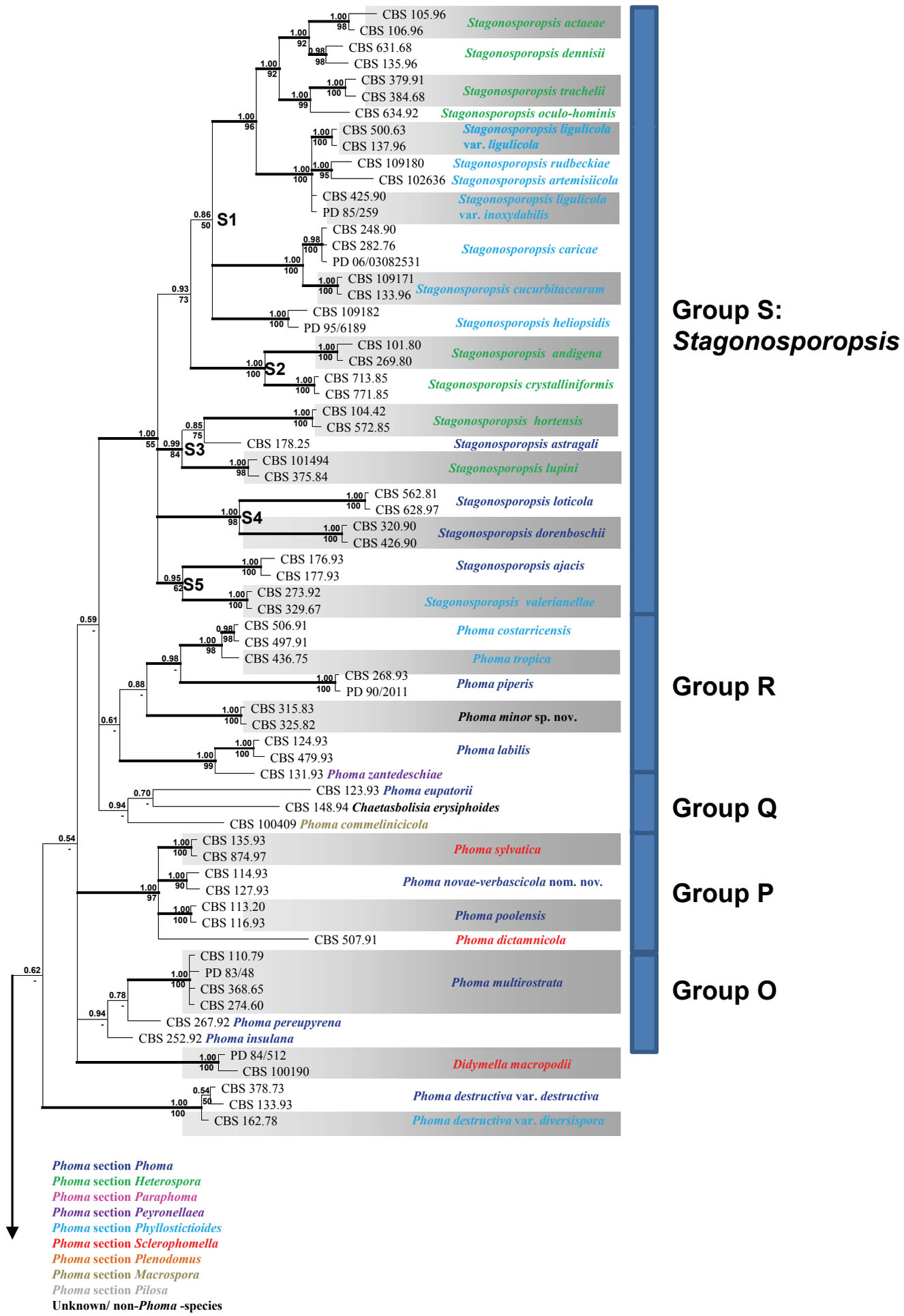


Figure 5.2. (Ctd).

and *M. olivacea* are recognised as type species for their respective genera. de Gruyter *et al*. (2009) already discussed the probability of finding most of these non-*Phoma* taxa in the *Didymellaceae* clade.

It should be noted that not all *Ascochyta* species are found within this clade, indicating that this genus is also polyphyletic. Whereas *A. hordei* var. *hordei* is found to be one of the basal taxa of clade 8, the legume associated pathogens *A. fabae*, *A. pinodes* and the type species *A. pisi* are found in close association with several species of *Phoma*. This result is in congruence with the observations in the study of Peever *et al.* (2007). Also the recently described *Didymella clematidis* has an anamorph state in *Ascochyta* and is closely related to *Phoma* taxa in this major clade (Woudenberg *et al*. 2009). A representative strain *Asc. caulina* and a new *Ascochyta* species that is still due to be published (G.J.M. Verkley, pers. comm.), however, have been found to be only distantly related and are found in clades 7 and 6, respectively.

Where a sexual state is known for the *Phoma* and *Ascochyta* species in clade 8, it is *Didymella*. The type species of this teleomorph genus, *D. exigua*, is also found within this clade, although it is not associated with a *Phoma* anamorph state. The family *Didymellaceae* was introduced for this group by de Gruyter *et al*. (2009). However, type species of two other teleomorph genera have also been found within this clade. DNA sequences of *Leptosphaerulina australensis* resemble a high level of similarity with those of the various *Phoma* and *Didymella* strains, although none are identical. Also sequences of LSU and ITS sequence data obtained from GenBank of *L. americana*, *L. argentinensis*, *L. chartarum*, *L. crassiasca* and *L. trifolii* (GenBank accession no. AY278318, AY849949, EU272493, U79485, AY8315585 respectively) were highly similar or even identical to the *Didymellaceae* sequences obtained in the present study (data not shown). These observations are in congruence with the results obtained by Silva-Hanlin & Hanlin (1999), who found that *D. bryoniae* (anam. *Ph. cucurbitacearum*) was closely related with *L. chartarum* and *L. crassiasca*. Also *Macroventuria anomochaeta*, which represents the genus *Macroventuria* (van der Aa 1971) groups in *Didymellaceae*. The close genetical resemblance of *Macroventuria* and *Leptosphaerulina* found in the present study is in congruence with the results of Kodsueb *et al.* (2006).

The loci employed here for phylogenetic analysis are sufficient to identify clades at the family level, but for proper resolution at generic level or lower, additional gene regions need to be sequenced. As the majority of *Phoma* species is embedded in the *Didymellaceae* clade, we will define further generic and species boundaries within this recently established family in the subsequent part of this paper.

Systematics of *Didymellaceae*

DNA phylogenetic analysis

The alignment that was used to delineate the *Didymellaceae* consisted of 274 sequences belonging to 196 species. A list of the species names and numbers, original substrates, geographical origins and GenBank Accession numbers of the strains used in this study is provided in Table 5.3. The sequence matrix had a total length of 2 188 characters including the alignment gaps (LSU: 1 327; ITS: 508 and TUB: 353). Of those characters, 1 788 (LSU: 1 233; ITS: 374 and TUB: 181) were constant, whereas 400 characters (LSU: 94; ITS: 144 and TUB: 192) were variable.

The analysis run of the LSU-ITS-TUB sequence matrix in MrBayes was aborted after obtaining 20 000 trees, which was well after stationarity in the probability of the trees was reached, whereas the standard deviation of split frequencies was below 0.02. From the obtained tree population, the 25 % burn-in was discarded and the consensus tree and posterior probabilities were calculated. The topology and support values of the BI tree were in congruence with the optimal tree obtained in the ML analysis.

Systematics: treatment of clades

As most other anamorph genera, *Phoma* has largely been used as a convenient form genus, rather than a phylogenetic entity. With the number of *Phoma* species that are being analysed on DNA sequence level rapidly increasing, the question is raised whether form genera should be maintained or that more natural groupings, merging both phylogeny and morphological data, should be erected. Of course, as greater numbers of taxa are collected and analysed, the taxonomic boundaries of more clades will be resolved. However, for the present, only those genera that could be resolved based on available cultures are treated. The groups mentioned below refer to those indicated A–R in Figure 5.2. The unresolved clades are left untreated, and are thus not discussed.

The taxa in this part of the study were selected based on genetic and/or morphological similarities with the species that were associated with the *Didymellaceae* in Figure 5.1. Although numerous taxa from various genera have been associated with "*Phoma*", the number of genera that could be included in the selection for *Didymellaceae* was limited. Next to *Phoma*, the only species found were those accommodated on basis of previous morphological studies in either *Ampelomyces*, *Ascochyta*, *Chaetasbolisia*, *Coniothyrium*, *Didymella*, *Diplodina*, *Dothiorella*, *Epicoccum*, *Leptosphaerulina*, *Macroventuria*, or *Microsphaeropsis*. Of three of these generic representatives, viz. *Chaetasbolisia, Diplodina* and *Dothiorella*, we suspect that some cultures have been preserved under an incorrect name. The species representing *Ampelomyces*, *A. quercinus,* was correctly identified, but as suggested earlier, the taxonomic placement in this genus appears to be incorrect (Szentiványi *et al.* 2005).

Strains belonging to a single species proved to be genetically identical or at least highly similar, indicating that the initial identification of these strains had been carried out correctly.

Several well-supported clusters are recognised within this family that are treated here as novel groups of *Didymellaceae*. In this section these separate groups are treated. However, although multiple genes were employed in this study to generate a phylogenetic reconstruction of the family, high levels of basal polytomy were observed as well (Figure 5.2). Application of general nrDNA loci alone did not reduce this high level of polytomy, whilst interspecies variation in several well-supported clades was reduced drastically.

Group A - outgroup and basal lineages:

The tree presented in Figure 5.2 is rooted to *Ascochyta hordei* and *Ph. paspali*, which proved to be ancestral to the *Didymellaceae* in Figure 5.1. The latter species was described by Johnston (1981) as a species from grasses in New Zealand and Australia, but in recent years, isolates with similar genotypes were isolated from iron-rich volcanic soil from France (C. Gueidan, pers. comm.), and from common reed (*Phragmites australis*) in Germany (Wirsel *et al.* 2001). These isolates were, however, never studied morphologically.

Another species used as outgroup is *Ascochyta hordei* var. *hordei* (CBS 544.74), which was obtained from a South African *Triticum aestivum*, indicating that also within *Didymellaceae*, species that are ascribed to *Ascochyta* do not form a monophyletic group. Also CBS 259.92, the isotype of *Ph. matteuciicola*, proved to be basal to most other *Phoma* species. *Phoma matteuciicola* is commonly known as a pathogen of many fern species (de Gruyter *et al.* 2002). Within the basal lineages, also a group comprising *Ph. humicola* and the novel species *Ph. saxea* is found, although this group is only supported by BI analysis (BPP = 0.92, RBS < 50%). Although *Phoma humicola* is known as a saprobic soil fungus, it is sometimes mistaken for the notorious potato pathogen *Ph. foveata* (Group N), due to a similar biochemical reaction to NaOH and the formation of citrine green crystals on MEA (de Gruyter *et al.* 1998). However, conidia of *Ph. humicola* are always eguttulate in contrast to those of *Ph. foveata*. *Phoma saxea* has been found twice in Germany on rock material, and will be further described below.

Phoma humicola J.C. Gilman & E.V. Abbott, *Iowa St. Coll. J. Sci*. **1** (3): 266. 1927.

Specimen examined: **USA**, Nevada, Death Valley, from a dead leaf of *Franseria* sp., 1971, G.H. Boerema, CBS H-16390, culture CBS 220.85.

Phoma matteuciicola Aderkas, Gruyter, Noordel. & Strongman, *Canad. J. Pl. Pathol.* **14** (3): 227. 1992.

Specimen examined: **Canada**, Nova Scotia, Five Mile River, from leaf base of *Matteuccia struthiopteris*, May 1981, Ph. von Aderkas, **holotype** DAOM 183092, culture ex-holotype CBS 259.92 = IMI 286996 = PD 91/272.

Notes: Gangrene in ostrich fern was originally attributed to *Ph. exigua* var. *foveata* (von Aderkas & Brewer 1983), which is here recombined as *Boeremia foveata*, but Von Aderkas *et al.* (1992) recognised a new species as causal agent of this disease. The phylogeny presented here supports these observations, as *Ph. matteuciicola* is found rather distinct from *B. foveata*.

Phoma paspali Ph.R. Johnst., *New Zealand J. Bot.* **19** (2): 181. 1981.

Specimens examined: **New Zealand**, Auckland, Kaikohe, from a dead leaf of *Paspalum dilatatum*, Jan. 1979, Ph.K. Buchanan, **isotype** CBS H-7623, culture ex-isotype CBS 560.81 = PD 92/1569; Waikato District, Ruakura, from *Lolium perenne*, Jan. 1979, G.H. Boerema, CBS 561.81 = PDDCC 6615.

Phoma saxea Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515591. Figure 5.3.

Conidia dimorpha, intra idem pycnidia formata. Conidia typus 1 (sub)globosa, glabra, hyalina, continua, (3–)3.5–5.5 µm diam., (0–)3–10(–15) guttulis praedita. Conidia typus 2 cylindrica vel ellipsoidea, glabra, hyalina, continua, (3.5–)4.5–7(–7.5) × 2.5–3.5(–4) µm, plerumque eguttulata, vel 1–3 guttulis praedita. Matrix conidiorum salmonea. Chlamydosporae continuae, globosae, viridulae, in catenas usque 35 positae, (8.5–)10–16.5(–17.5) × (6–)8–12.5(–14) µm.

Etymology: Refers to the substratum on which both isolates of this species were found, stone material.

Pycnidia solitary, (sub-)globose, glabrous or covered with hyphal outgrows, (90–)135–280(–310) × (90–)105–260(–275) µm. *Ostioles* single, papillate, with wide openings, *ca*. 40–80 µm diam. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 2–3 layers, 10–17 µm thick, outer cell layer brown pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, variable in appearance, flask-shaped, oblong or isodiametric *ca*. 5.5–7.5 × 3–4 µm. *Conidia* of two types, both originating from the same pycnidia. Conidia of type 1: (sub-)globose, thin-walled, smooth, hyaline, aseptate (3–)3.5–5.5 µm diam, with (0–)3–10(–15) guttules. Conidia of type 2: cylindrical to ellipsoidal, thin-walled, smooth, hyaline, aseptate, (3.5–)4.5–7(–7.5) × 2.5–3.5(–4) µm, mainly egutullate or with up to 3 minute guttules. *Conidial matrix* salmon. *Chlamydospores* ubiquitously present in the agar, unicellular, globose, in long chains of up to 35 elements, greenish pigmented, measuring (8.5–)10–16.5(–17.5) × (6–)8–12.5(–14) µm.

Culture characteristics: Colonies on OA, 45–50 mm diam after 7 d, margin regular. Immersed mycelium flat, olivaceous to greenish olivaceous, citrine-green or coral near the colony margin. Aerial mycelium absent, but sometimes some grey erect tufts are encountered near the colony centre; reverse concolourous. Colonies on MEA 20–25 mm diam after 7 d, margin regular. Immersed mycelium violet-slate, but saffron near the colony margin. Abundant pycnidia are present on the agar surface; reverse iron-grey, saffron near the colony margin. Colonies on CHA similar as on MEA, but somewhat slower growing, 10–15 mm diam. after 7 d, and some sparse white aerial mycelia hyphae are present in the colony. Application of NaOH results in a greenish yellow discolouration of the agar, best to be observed on OA medium.

Specimens examined: **Germany**, Oldenburg, from corroded Mediterranean marble, June 1992, J. Kuroczkin, **holotype designated here** CBS H-20240, culture ex-holotype CBS 419.92; Oldenburg, from limestone, 1987, J. Kuroczkin, CBS 298.89.

Notes: The pycnidial wall of *Phoma saxea* is extremely thin and almost hyaline when the conidia have exuded. Older pycnidia collapse and remain as a double-layered, disc-like structure on the agar.

Both strains of this species have been isolated from stone material, such as limestone (CBS 298.89) and corroded Mediterranean marble (CBS 419.92). Although the genus is known from all kinds of substrates, the number of rock-inhabiting *Phoma* isolates is relatively low. Selbmann *et al.* (2002) report on *Ph. herbarum* from Antarctic rock, and Boerema *et al.* (2004) list several species from rock-like materials, such as cement (*Ph. herbarum*), wall-plaster (*Ph. heteroderae* – here recombined into *Ph. calorpreferens*) and crockery (*Ph. pomorum*). In addition, multiple species are recorded from rock-inhabiting lichens. These species, listed by Hawksworth & Cole (2004) are, however, unculturable and could therefore not be compared with *Ph. saxea in vitro*. However, the morphological descriptions suggest that the mentioned species and *Ph. saxea* are different taxonomic entities.

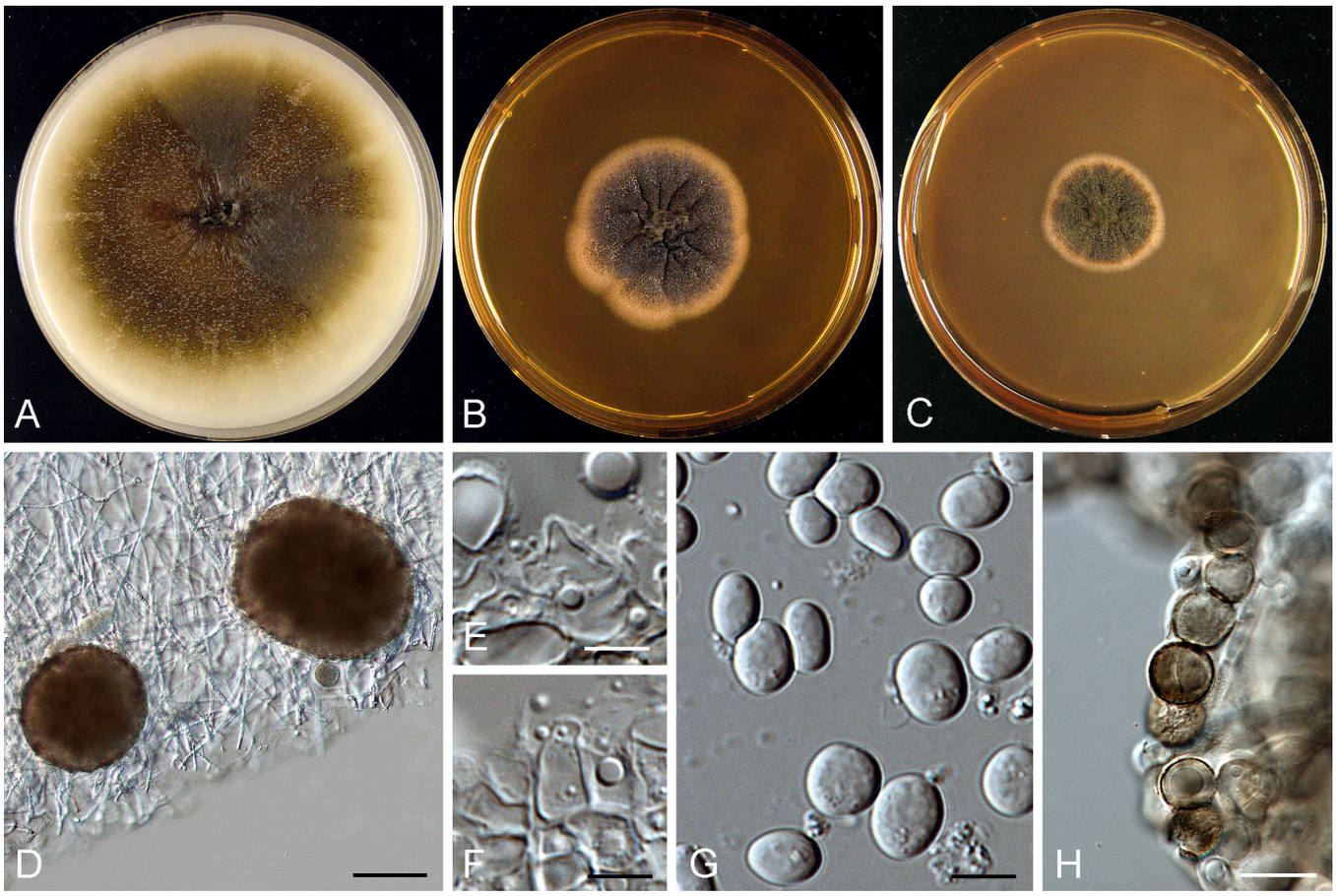


Figure 5.3. *Phoma saxea* (CBS 419.92). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D. Pycnidia. E–F. Conidiogenous cells. G. Conidia. H. Chain of unicellular chlamydospores. Scale bars: D = 100 µm, E–G = 5 µm, H = 20 µm.

Group B:
Four of the six species clustering in Group B produce a *Didymella* teleomorph. Only *Ph. polemonii* and *Ph. xanthina* presently have no known sexual state. The species in this clade are collected from a wide variety of dicots, although all individual taxa appear to be host-specific (Boerema *et al.* 2004). Also the micromorphological features of these species are highly variable.

A single strain that was kept in the CBS collection as *Diplodina coloradensis* was found in this clade as well. However, this genus name has been accommodated in the *Gnomoniaceae* (*Diaporthales*), indicating that this strain has been preserved under an incorrect name and should be renamed. However, as this strain proved to be sterile, no proper redescription of the material could be provided.

Didymella applanata (Niessl) Sacc., *Syll. Fung.* **1**: 546. 1882.
Basionym: *Didymosphaeria applanata* Niessl, *Oesterr. Bot. Z.* **25**(4): 129. 1875.
Anamorph: ***Phoma argillacea*** (Bres.) Aa & Boerema, in de Gruyter, Boerema & van der Aa, *Persoonia* **18** (1): 17. 2002.
Basionym: *Phyllosticta argillacea* (Bres.), *Hedwigia* **1894**: 206. 1894.

Specimens examined: **The Netherlands**, Baarn, from *Rubus idaeus*, Sep. 1963, A. van Dijkman, CBS H-11943, culture CBS 205.63; from *Rubus idaeus*, 1975, G.H. Boerema, CBS 102634 = PD 75/248.

Didymella cannabis (G. Winter) Arx, in Müller & Arx, *Beitr. Kryptogamenfl. Schweiz* **11** (2): 365. 1962.
Basionym: *Sphaerella cannabis* G. Winter, *Hedwigia* **11**(10): 145. 1872.
Anamorph: ***Phoma cannabis*** (L.A. Kirchn.) McPartl., *Mycologia* **86** (6): 871. 1995.

Basionym: *Depazea cannabis* L.A. Kirchn., *Lotos* **6**: 183. 1856.

Specimen examined: Unknown origin, from *Cannabis sativa*, Oct. 1937, K. Röder, CBS 234.37.

Notes: The studied culture (Röder 1937) is now sterile, and could therefore not be described here morphologically.

Didymella catariae (Cooke & Ellis) Sacc., *Syll. Fung.* **1**: 557. 1882.
Basionym: *Sphaeria catariae* Cooke & Ellis, *Grevillea* **5**: 96. 1876.
Anamorph: *Phoma nepeticola* (Melnik) Dorenb. & Gruyter, *Persoonia* **18** (1): 18. 2002.
Basionym: *Ascochyta nepeticola* Melnik, *Novoste Sist. Nizsh. Rast.* **1968**: 178. 1968.

Specimen examined: **The Netherlands**, from the stem of *Nepeta cataria*, 1977, M.M.J Dorenbosch, CBS 102635 = PD 77/1131.

Didymella urticicola Aa & Boerema, in Boerema, *Trans. Brit. Mycol. Soc.* **67** (2): 303. 1976.
Anamorph: ***Phoma urticicola*** Aa & Boerema, in Boerema, *Trans. Brit. Mycol. Soc.* **67** (2): 303. 1976.
Specimens examined: **The Netherlands**, Wageningen, from a dead stem tip of *Urtica dioica*, Mar. 1973, G.H. Boerema, holotype CBS H-11971, culture ex-holotype CBS 121.75 = ATCC 32164 = IHEM 3403 = IMI 194767 = PD 73/584; from *Urtica dioica*, 1973, G.H. Boerema, PD 73/570.

Phoma polemonii Cooke, *Grevillea* **13** (68): 94. 1885.

Specimen examined: **The Netherlands**, from *Polemonium caeruleum*, 1983, J. de Gruyter, CBS 109181 = PD 83/757.

Phoma xanthina Sacc., *Michelia* **1** (4): 359. 1884.

Specimens examined: **The Netherlands**, Baarn, from leaves of *Delphinium* sp., May 1968, H.A. van der Aa, CBS H-8938, culture CBS 383.68; from *Delphinium* sp., 1984, G.H. Boerema, PD 84/407.

Group C:

The species in Group C cluster in two subgroups: One comprising the *Clematis* pathogens *Ph. clematidina* and *Coniothyrium clematidis-rectae*, the other subgroup comprising *Ph. aquilegiicola* and *Ph. glaucii*, two pathogens of *Ranunculaceae* and *Papaveraceae,* respectively. All three *Phoma* species in this group were morphologically linked to the section *Heterospora* (Boerema *et al.* 1997), but are distinct from the species in clade S by the absence of conidia that represent the *Stagonosporopsis* synanamorph in culture, although smaller septate conidia do occur. In these species the *Stagonosporopsis* synanamorph is only known from *in vivo* material (Boerema 1993, Boerema *et al*. 1997).

The several species that were associated with the *Ph. clematidina* morphotype have recently been distinguished in a study of Woudenberg *et al.* (2009). In the same study, the authors showed that *C. clematidis-rectae* is closely related and, based on sequence analysis, a member of the family *Didymellaceae*. The major character on which this species is regarded as distinct from *Ph. clematidina* is by the production of pale brown pigmented conidia. In addition, the conidiogenesis of *Coniothyrium* is annellidic with percurrent proliferation, in contrast to the conidiogenesis in *Phoma*, which is considered to be solely phialidic with periclinal thickening (Boerema & Bollen 1975, Sutton 1980). Evidence for the presence of annellides has, however, not been observed in *C. clematidis-rectae*, while conidial pigmentation is relatively pale in comparison to other *Coniothyrium* species. Pigmented conidia have also been observed in various *Phoma* species before (Dorenbosch 1970, Boerema *et al.* 2004, Aveskamp *et al.* 2009a). These features may indicate that this species is actually a *Phoma* with early conidial pigmentation. Therefore *C. clematidis-rectae* is recombined into *Phoma* below.

Phoma aquilegiicola M. Petrov, *Acta Inst. Bot. Acad. Sci. USSR Pl. Crypt. [Trudy Bot. Inst. Akad. Nauk SSSR] Fasc.* **1**: 281. 1933.

Specimens examined: **The Netherlands**, from a stem of *Aconitum pyramidale*, 1973, G.H. Boerema, CBS 107.96 = PD 73/598; from a stem of *Aquilegia* sp., 1979, G.H. Boerema, CBS 108.96 = PD 79/611.

Phoma clematidina (Thüm.) Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* (Jaarboek 1978) **153**: 17. 1979. emend. Woudenberg *et al. Persoonia* **22**: 59. 2009.
Basionym: *Ascochyta clematidina* Thüm., *Bull. Soc. Imp. Naturalistes Moscou* **55**: 98. 1880.

Specimens examined: **Russia**, Minussinsk, from leaves of *Clematis glaucae*, N. Martianoff, **isotype** LE 40082; **The Netherlands**, Spaubeek, from the stem of *Clematis* sp., July 1978, *G.H. Boerema*, **epitype** CBS H-16193, culture ex-epitype CBS 108.79 = PD 78/522; from *Clematis* sp., I. de Boer, Nov. 1949, CBS 201.49; Boskoop, from *Clematis jackmanii*, C. Dorsman, Oct. 1962, CBS 195.64; Wageningen, from *Selaginella* sp. M.M.J. Dorenbosch, 1966, CBS 520.66; **UK**, England, from *Clematis* sp., Jan. 1966, F.T. Last, CBS 102.66.

Phoma clematidis-rectae (Petr.) Aveskamp, Woudenberg & Gruyter, ***comb. nov.*** MycoBank MB515592.
Basionym: *Coniothyrium clematidis-rectae* Petr., *Fungi Polon.* **576**. 1921.

Pycnidia solitary or confluent, immersed or produced on the agar surface, globose, glabrous, (80–)85–130(–155) µm diam, in older cultures pycnidia may become larger and grow after maturation to 220–250 µm diam. *Ostioles* 1(–4), wide, non-papillate to papillate or, in older cultures, on a elongated neck. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 4–5 layers, (10–)11–19(–19.5) µm thick, outer 1–2 layers pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, ampulliform to doliiform, measuring 3–4.5(–5) × 2.5–4.5 µm. *Conidia* ellipsoidal to cylindrical, thin-walled, smooth, aseptate, (3–)4–7(–8) × 2–3(–3.5) µm, with (2–)5–12 guttules, initially hyaline, but mature conidia become slightly brownish pigmented. *Conidial matrix* sepia.

Culture characteristics: Colonies on OA 42–52 mm diam after 7 d, margin regular. Immersed mycelium dark brick to sepia or iron-grey, but hyaline near the colony margin. Pycnidia in concentric rings give the colony an olivaceous tinge. Aerial mycelium absent; reverse concolourous. Colonies on MEA 27–52 mm diam after 7 d, margin regular. Aerial mycelium incidentally occurs in sectors in some strains, grey to olivaceous. Immersed mycelium rosy-buff to rosy-vinaceous with olivaceous and grey tinges; reverse olivaceous iron-grey to saffron. Application of NaOH did not have any effect.

Specimens examined: **The Netherlands**, Boskoop, from *Clematis* sp., 1963, G.H. Boerema, CBS H-20275, culture CBS 507.63 = PD 07/03486747 = MUCL 9574; from *Clematis* sp., 1995, J. de Gruyter, PD 95/1958.

Notes: In congruence with the studies of Woudenberg *et al.* (2009), this species was found to be closely related to *Ph. clematidina* and other *Didymellaceae* species. In contrast, it is only distantly related to the type species of *Coniothyrium*, *C. palmarum*. Therefore, a recombination into *Phoma* is proposed here. The present species is clearly distinct from *Ph. clematidina* by the production of pigmented conidia, although the level of pigmentation is low, which distinguishes *Ph. clematidis-rectae* from the species remaining in *Coniothyrium* that produce darker, olivaceous conidia.

Phoma glaucii Brunaud, "*Ph. glauci" Ann. Soc. Sci. Nat. La Rochelle* **1892**: **97**. 1892.

Specimens examined: **The Netherlands**, near Lisse, from *Dicentra* sp., 1979, G.H. Boerema, CBS 112.96; Wageningen, from a leaf of *Chelidonium majus*, 1994, G.H. Boerema, CBS 114.96 = PD 94/888.

Groups D & E – Leptosphaerulina and Macroventuria:
The most remarkable findings in the *Didymellaceae* are the *Leptosphaerulina* and *Macroventuria* (clade E) teleomorph genera. The species belonging to these teleomorphs are found amidst the *Didymellaceae*, causing the genus *Didymella* to be paraphyletic. The species in both genera are closely related to each other, as was already pointed out by Kodsueb *et al.* (2006), who, however, missed the link with *Didymella*. A phomoid anamorph state has, thus far, not been recorded for any of the species in these teleomorph genera.

Leptosphaerulina is morphologically distinct from *Macroventuria* and *Didymella*, although all three genera are known for their hyaline ascospores (van der Aa 1971, von Arx 1981). *Leptosphaerulina* produces large, longitudinally and transversally septated ascospores, resembling those of *Pleospora* and *Cucurbitaria*, although the ascospores of these genera are pigmented. The major difference between *Didymella* and *Macroventuria* is the presence of setae on the pseudothecia of the latter genus, whereas *Didymella* ascomata are commonly glabrous. According to the original description (van der Aa 1971), *Macroventuria* strains resemble *Venturia* by their setose pycnidia, but differ in their restricted number of the asci.

Leptosphaerulina americana (Ellis & Everh.) J.H. Graham & Luttr., *Phytopathology* **51**: 686. 1961.
Basionym: *Pleospora americana* Ellis & Everh., in *North American Pyrenomycetes*: 336. 1892, nom. nov. pro *Pleospora hyalospora* Ellis & Everh., *Proc. Acad. Nat. Sci. Philadelphia*: 238. 1890, non *Pleospora hyalospora* Speg.

Specimen examined: **USA**, Georgia, from *Trifolium pratense*, Apr. 1954, E.S. Luttrell, CBS 213.55.

Leptosphaerulina arachidicola W.Y. Yen, M.J. Chen & K.T. Huang, *J. Agric. Forest*. **10**: 167. 1956.

Specimen examined: **Taiwan**, from a leaf of *Arachis hypogaea*, 1956, K.T. Huang, CBS 275.59 = ATCC 13446.

Note: CBS 275.59 is degenerated and forms only very tiny sclerotia *in vitro.*

Leptosphaerulina australis McAlpine, *Fungus Diseases of stone-fruit trees in Australia*: 103. 1902.

Specimens examined: **Indonesia**, Lampung, from *Eugenia aromatica*, Dec. 1982, H. Vermeulen, CBS 317.83. **The Netherlands**, Baarn, from soil, Sep. 1969, J.A. Stalpers, CBS 939.69.

Leptosphaerulina trifolii (Rostr.) Petr., *Sydowia* **13**: 76. 1959.
Basionym: *Sphaerulina trifolii* Rostr., *Bot. Tidsskr.* **22**: 265. 1899.

Specimen examined: **The Netherlands**, from *Trifolium* sp., 1958, CBS 235.58.

Macroventuria anamochaeta Aa, *Persoonia* **6** (3): 362. 1971.

Specimens examined: **South Africa**, Karroo Desert, from decayed canvas, Aug. 1971, M.C. Papendorf, **holotype** CBS H-14192, ex-holotype culture CBS 525.71; Cape Province, from a trunk of *Medicago sativa*, June 1972, W.F.O. Marasas, CBS 502.72.

Macroventuria wentii Aa, *Persoonia* **6** (3): 361. 1971.

Specimen examined: **USA**, Nevada, Death Valley, from plant litter, Aug. 1971, F.W. Went, **holotype** CBS H-14195, ex-holotype culture CBS 526.71.

Group F:
As a sister group to *Leptosphaerulina*, several host-specific *Phoma* species are found that induce leaf spots on a variety of plant species, including *Ph. infossa*, *Ph. anigozanthi*, *Ph. arachidis-hypogaeae* and *Ph. gossypiicola*. The latter species causes leaf spots and stem canker on cotton plants (*Gossypium* spp.). However, other plant species may also become symptomatic when deliberately infected (Holliday & Punithalingam 1970). *Phoma infossa* has originally been reported from stems of ash trees (*Fraxinus* sp.) in New York State (Ellis & Everhart 1888), but has recently been associated with a severe foliar disease of green ash (*F. pennsylvanica*) in Argentina (Aveskamp *et al.* 2009a). All species produce aseptate conidia in culture, although *Ph. gossypiicola* is known to also produce 2- to multi-celled conidia *in vivo*, hence the *Ascochyta gossypii* synonym (de Gruyter 2002).

In contrast to these plant pathogens, a fungicolous species also occurs in the present clade. Species from the genera *Phoma* and *Ampelomyces* have been "frequently confused with each other" (Sullivan & White 2000), which explains why *Ph. fungicola* is found here. This species was previously known as *Amp. quercinus* and is recombined in the subsequent taxonomical section. The finding of this species in the *Didymellaceae* is in congruence with sequence results obtained by Sullivan & White (2000) and Szentiványi *et al.* (2005). Also *Amp. humuli*, another fast-growing species, proved to be phylogenetically similar to species that currently represent the *Didymellaceae* (Kiss & Nakasone 1998). Additionally, it has been suggested that the fast growing species *Amp. artemisiae* and *Amp. uncinulae* (Rudakov 1979, Kiss 1997) actually do, in fact, not represent *Ampelomyces*, but belong to the genus *Phoma*; these species were incorrectly identified based on their host-association (Kiss *et al.* 2004). The species in *Ampelomyces* are all recognised as parasites of fungi that cause powdery mildew (Kiss 1997). However, it is suggested that also the ubiquitous species *Ph. glomerata* has fungicolous capacities, and may be suited as mycoparasitic control agent of powdery mildew (Sullivan & White 2000).

Only one of the *Phoma* species embedded in this clade has been associated with a teleomorph. In the description of *Ph. anigozanthi*, the sexual state is recorded as *Sphaerella millepunctata* (apud Gruyter & Noordeloos 1992). *Sphaerella* is practically synonymised with *Mycosphaerella* (e.g. Aptroot 2006), but as described above, several of the *Mycosphaeralla* species have subsequently been recombined into *Didymella*. In the present study no evidence of teleomorph formation *in vitro* has been observed, which is in congruence with the results of Gruyter & Noordeloos (1992). As also type material of *Ph. anigozanthi* and *S. millepunctata* could not be obtained, this taxonomic link is still to be confirmed.

Phoma anigozanthi Tassi, *Boll. Reale Orto Bot. Siena* **3** (2 – '1899'): 148. 1900.

Specimen examined: **The Netherlands**, from a leaf of *Anigozanthus maugleisii*, 1979, H. Cevat CBS H-5199, culture CBS 381.91 = PD 79/1110.

Phoma arachidis-hypogaeae (V.G. Rao) Aa & Boerema, *Persoonia* **15**(3): 388. 1993.
Basionym: *Phyllosticta arachidis-hypogaeae* V.G. Rao, *Sydowia* **16** ('1962'): 275. 1963.

Specimen examined: **India**, Madras, from a leaf of *Arachis hypogaea*, 1977, CBS 125.93 = PD 77/1029.

Phoma fungicola Aveskamp, Gruyter & Verkley, **nom. nov.** pro *Cicinobolus quercinus* Syd. *Ann. Mycol*. **13**: 42. 1915. MycoBank MB515593.
Basionym: *Cicinobolus quercinus* Syd., *Ann. Mycol*. **13**: 42. 1915.
≡ *Ampelomyces quercinus* (Syd.) Rudakov, *Mikol. Fitopatol.* **13** (2): 109. 1979. not *Phoma quercina* Sacc & Roum. *Syll. fung*. **3**: 96. 1881, = *Phomopsis quercina* Sacc.) Höhn., not *Phoma quercina (*Peck) Sacc. *Syll. fung.* **3**: 96. 1884.

Etymology: Epithet refers to the fungicolous lifestyle of this species.

Pycnidia always solitary, produced on the agar surface, globose, peroblate to suboblate, glabrous, measuring (50–)65–130(–150) × (65–)95–200(–220) µm with a single, conspicuous, non-papillate ostiole. *Pycnidial wall* pale brown, pseudoparenchymatous, composed of isodiametric cells, 3–5 layers, (6–)8.5–14.5(–16) µm thick, outer 1–2 layers slightly pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, doliiform to ampulliform, variable in size, *ca*. (3–)3.5–5 × 3–4(–5) µm. *Conidia* variable in shape and size, subglobose to oval or obtuse, thin-walled, smooth, aseptate, measuring (5–)5.5–7.5(–8.5) × 3–4.5(–5) µm, with 0–2(–3) minute guttules, initially hyaline, but brown at maturity. Conidial exudates not recorded.

Culture characteristics: Colonies on OA 55–68 mm diam after 7 d, margin regular. Aerial mycelium white, floccose to woolly. Immersed mycelium greenish olivaceous to olivaceous near the colony centre. Abundant black pycnidia are scattered over the medium; reverse concolourous. Colonies on MEA 65–75 mm diam after 7 d, margin regular. Aerial mycelium covering the whole colony, compact, white to pale grey, with olivaceous tinges near the colony centre; reverse olivaceous black.

Specimen examined: **Ukraine**, Crimea, in the vicinity of Feodosiya, on *Microsphaera alphitoides* from *Quercus* sp., 1979, O.L. Rudakov, CBS H-20276, culture CBS 633.92 = ATCC 36786, VKM MF-325.

Notes: The epithet used for the description of this species in the genera *Cicinobolus* and *Ampelomyces* could not be transferred to the genus *Phoma* as *Ph. quercina* is already occupied. This name, however, refers to a *Phyllosticta* species (van der Aa & Vanev 2002). Therefore, a new name is proposed here for the present species.

Kiss & Nakasone (1998) already found that several fast-growing *Ampelomyces* species were phylogenetically distinct from the type species, which is characterised by a rather slow growth rate, and suggested that *A. quercinus* belonged to *Phoma*. This finding was supported by results obtained in later studies (Sullivan & White 2000, Szentiványi *et al.* 2005).

Phoma gossypiicola Gruyter, *Persoonia* **18** (1): 96. 2002.

Specimen examined: **USA**, Texas, from a leaf of *Gossypium* sp., 1963, L.S. Bird CBS H-9006, culture CBS 377.67.

Phoma infossa Ellis & Everh., *J. Mycol.* **4**(10): 102. 1888, emend. Aveskamp *et al. Mycologia* **101**. 373. 2009.

Specimens examined: **Argentina**, Buenos Aires Province, La Plata, from leafs of *Fraxinus pennsylvanica*, 2008, M. Murace, **neotype** CBS H-20145, culture ex-neotype CBS 123395; Buenos Aires Province, La Plata, from leafs of *Fraxinus pennsylvanica*, 2008, M. Murace, CBS 123394.

Group G:

This group (BPP = 1.00, RBS = 99 %) consists of *Ph. subherbarum* and *Ph. pedeiae* sp. nov. Although the first species name suggests a close resemblance with the type species *Ph. herbarum*, it is phylogenetically distinct. Both *Ph. herbarum* and *Ph. subherbarum* are accommodated in section *Phoma*, but are distinct in colony characters: in contrast to *Ph. herbarum, Ph. subherbarum* does not react to the application of a droplet of NAOH (de Gruyter *et al.* 1993). The growth rate of *Ph. subherbarum* is also considerably faster, as a colony can cover the plate surface within 1 wk.

Boerema *et al.* (2004) hypothesized that *Ph. subherbarum* is from American origin. In contrast, both strains of *Ph. pedeiae* were found in the Netherlands. Both species in this clade appear to have a plurivorous nature. The novel species *Ph. pedeiae* is described below.

Phoma pedeiae Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515594. Figure 5.4.

Conidia ellipsoidea vel cylindrica, glabra, hyalina, continua, 3–4.5 × 1.5–2.5 µm, 0–2(–3) guttulis praedita. Matrix conidiorum cremeo-alba.

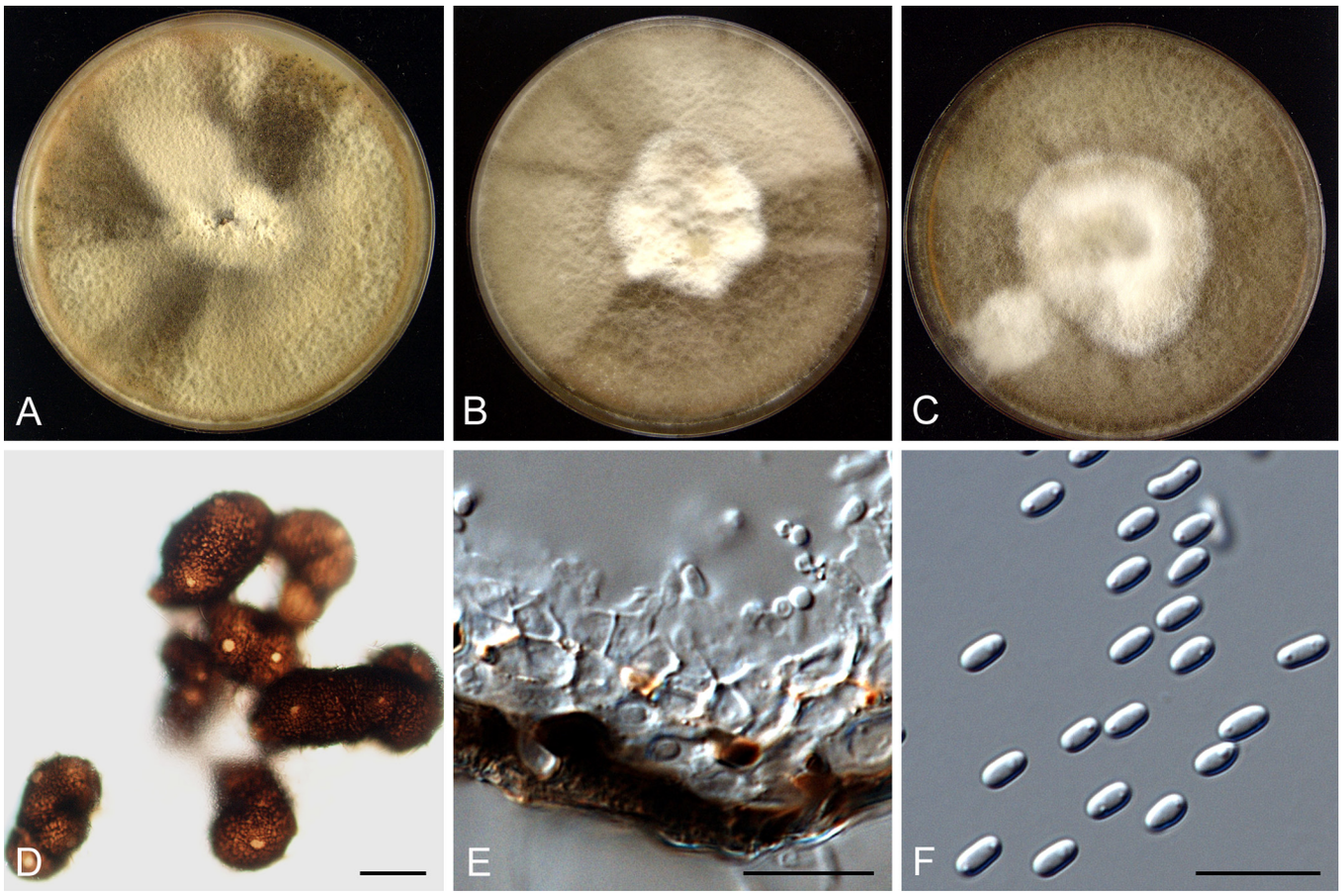


Figure 5.4. *Phoma pedeiae* (CBS 124517). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D. Pycnidia. E. Section of the pycnidial wall. F. Conidia. Scale bars: D = 100 µm, E–F = 10 µm.

Etymology: Named after the institute that has facilitated most of the research on the taxonomy of the genus *Phoma* and affiliated genera in the past decade, the PD (Plantenziektenkundige Dienst – Dutch Plant Protection Service). Both isolates of this species were collected and preserved by employees of this institute.

Pycnidia solitary or confluent, produced on the agar surface, globose to ellipsoidal, glabrous, (90–)100–230(–255) × (75–)90–155(–165) µm with 1–2 conspicuous, non-papillate ostioles. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, 3–5 layers, 11–17 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, relatively small, *ca.* 3.5–4(–4.5) × 3–4 µm. *Conidia* ellipsoidal to cylindrical, thin-walled, smooth, hyaline, aseptate 3–4.5 × 1.5–2.5 µm, with 0–2(–3) guttules. *Conidial matrix* crème-white.

Culture characteristics: Colonies on OA, 65–75 mm diam after 7 d, margin regular. Immersed mycelium olivaceous. Aerial mycelium floccose, white or smoke-grey to greenish olivaceous. Abundant black pycnidia are scattered over the medium; reverse concolourous with some reddish tinges. Colonies on MEA 55–65 mm diam after 7 d, margin regular. Aerial mycelium covering the whole colony, floccose, smoke-grey to greenish olivaceous, white near the centre of the colony; reverse olivaceous black or bay. Agar colour changes to bay due to diffusible pigments produced by the fungus. Colonies on CHA similar as on MEA, but somewhat faster growing, 70–80 mm diam after 7 d. Application of NaOH did not have any effect.

Specimens examined: **The Netherlands**, Aalsmeer region, on *Schefflera elegantissima*, 1992, J. de Gruyter, **holotype designated here** CBS H-20239, culture ex-holotype CBS 124517 = PD 92/612A; on *Orchidaceae* sp., 1984, J. de Gruyter, CBS 124516 = PD 84/453.

Notes: *Phoma pedeiae* has been found in association with several tropical ornamental pot plants in Dutch greenhouses. Only mild disease symptoms were recorded from this species, and therefore the fungus was

not further studied. Phylogenetically, this species is found in close relation to *Ph. subherbarum* (BPP = 1.00; RBS = 99 %), which is probably a weak pathogen and saprobe of different plant substrates occurring on the American continent (de Gruyter *et al.* 1993).

Phoma subherbarum Gruyter, Noordel. & Boerema, *Persoonia* **15**(3): 387. 1993.

Specimens examined: **Canada**, from *Zea mays*, **holotype** L 992.177.439, culture ex-holotype CBS 250.9292 = DAOM 171914 = PD 92/371; from *Zea mays*, May 1978, G.A. Neish, CBS 305.79A = DAOM 170848; **Peru**, from *Solanum* sp., CBS 249.92 = PD 78/1088.

Group H:

Phoma bellidis and *Ph. senecionis* are found in association with two plant genera from the *Compositae* family: *Bellis* spp. and *Senecio* spp. respectively (de Gruyter *et al.* 1993). The distantly related *Ph. digitalis* is a pathogen of *Digitalis* spp. (*Scrophulariaceae*), but shares the feature with *Ph. bellidis* that it is also recorded as a seed-pathogen (Boerema & Dorenbosch 1979). In contrast, *Ph. senecionis* is only known as a necrophyte.

Phoma bellidis Neerg., *Friesia* **4**: 74. 1950.

Specimens examined: **The Netherlands**, from seed of *Bellis perennis*, 1985, G.H. Boerema, CBS H-5200, culture CBS 714.85 = PD 74/265; from *Bellis* sp., 1994, J. de Gruyter, PD 94/886.

Phoma digitalis Boerema apud Boerema & Dorenbosch, *Verslagen Meded. Plziektenk. Dienst Wageningen* **153**: 19. 1979.

Specimen examined: **The Netherlands**, Ommen, from *Digitalis* sp., 1990, J. de Gruyter, CBS 109179 = PD 90/835-1.

Phoma senecionis Ph. Syd., *Hedwigia*, Beibl. **38**: 136. 1899.

Specimen examined: **New Zealand**, Raetihi, from a stem of *Senecio jacobaea*, Feb. 1977, S. Ward, CBS 160.78 = LEV 11451.

Group I:

Group I comprises three *Phoma* taxa (*Ph. acetosellae*, *Ph. macrostoma* var. *macrostoma* and var. *incolorata*) that were placed in the section *Phyllostictoides* on the basis of the presence of septate conidia (Gruyter *et al.* 2002), but also accommodates *Ph. viburnicola.* The placement of this species in section *Phoma* can be debated, as a single septate conidium has been observed in strain CBS 500.91, one of the strains that was designated as reference strain (de Gruyter & Noordeloos 1992). Also *D. exigua* (CBS 183.55 - Neotype) is found in this clade, the type species of the genus *Didymella* (de Gruyter *et al.* 2009), which does however not produce an anamorph state. The four species do not exhibit a shared pathological feature or geographic origin. The variety *incolorata* differs from var. *macrostoma* in lacking a red to violet pigment in the hyphae and any reaction to NaOH.

Phoma acetosellae (A.L. Sm. & Ramsb.) Aa & Boerema, in de Gruyter, Boerema & van der Aa, *Persoonia* **18**(1): 16. 2002.
Basionym: *Phyllosticta acetosellae* A.L. Sm. & Ramsb., *Trans. Brit. Mycol. Soc.* **4**: 173. 1912.

Specimens examined: **France**, Corrèze, Monteil sur Bois, from a leaf of *Rumex acetosella*, 1976, H.A. van der Aa, CBS H-16138, culture 631.76. **The Netherlands**, Baarn, from a stem of *Rumex hydrolapathum*, March 1996, H.A. van der Aa, CBS 179.97.

Phoma macrostoma var. ***macrostoma*** Mont., *Annls Sci. Nat.*, *Bot*. III **11**: 52. 1849.

Specimens examined: **Germany**, near München, from the bark of *Larix decidua*, 1995, G.J. Verkley, CBS 482.95. **The Netherlands**, Wageningen, from wood of *Malus sylvestris*, Sep. 1969, G.H. Boerema, CBS H-16431, culture CBS 529.66 = PD 66/521.

Phoma macrostoma var. ***incolorata*** (A.S. Horne) Boerema & Dorenb., *Persoonia* **6**(1): 55. 1970.
Basionym: *Polyopeus purpureus* var. *incolorata* A.S. Horne, *J. Bot.* **58**: 240. 1920.

Specimens examined: **Switzerland**, Vierwaldstättersee, near Brunnen, from a leaf of *Acer pseudoplatanus*, Oct. 1968, J. Gemmen, CBS H-20240, culture CBS 223.69. **The Netherlands**, from *Malus sylvestris*, 1983, J. de Gruyter, CBS 109173 = PD 83/908.

Phoma viburnicola Oudem., *Contr. Flora Mycol. d. Pays-Bas* **17**: 247. 1901.

Specimens examined: **The Netherlands**, Wageningen, Aboretum, from *Viburnum cassioides*, 1969, G.H. Boerema, CBS H-16605, culture CBS 523.73 = PD 69/800; from *Chamaecyparis lawsoniana*, 1981, G.H. Boerema, CBS 371.91 = PD 81/413; Baarn, from a leaf of *Ilex aquifolium*, 1993, J. de Gruyter, CBS 500.91 = PD 83/222.

Group J:
This small group (BPP = 1.00, RBS = 96 %) comprises only two species. Because of the production of dictyochlamydospores, *Phoma boeremae* was suggested to belong to the section *Peyronellaea* (Group K, Aveskamp *et al*. 2009a), to which the present group is closely related. No such structures were, however, observed in its sister species, *Ph. dimorpha* sp. nov. This species is known from a single strain, which sporulates poorly and may be degenerated.

Phoma boeremae Gruyter, *Persoonia* **18**(1): 91. 2002.
Specimen examined: **Australia**, Victoria, Burnley Gardens, from seed of *Medicago littoralis* cv. Harbinger, Febr. 1982, M. Mebalds, **neotype** L 996.294.536, ex-neotype culture CBS 109942 = PD 84/402.

Phoma dimorpha Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515595. Figure 5.5.

Conidia dimorpha, in vitro cylindrica, glabra, hyalina, continua, 8–9.5(–10.5) × (2–)2.5–3(–3.5) µm, (5–)6–8(–10) guttulis minutis apolaribus praedita, in vivo eguttulata, (8–)9–12(–12.5) × (4.5–)5–5.5(–6.5) µm.

Etymology: The epithet refers to the two different conidial types that are observed.

Pycnidia produced only scarcely *in vitro*, in clusters of *ca*. 4–10 elements, globose, glabrous, non-papillate, produced on the agar surface, relatively small, measuring (65–)85–170(–190) µm diam. *Ostioles* single, non-pappillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–7 layers, 14–20 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, *ca*. 5.5–7 × 4.5–6.5 µm. *Conidia* cylindrical, thin-walled, smooth, hyaline, aseptate 8– 9.5(–10.5) × (2–)2.5–3(–3.5) µm, with (5–)6–8(–10) minute apolar guttules. *In vivo* eguttulate and somewhat broader, measuring (8–)9–12(–12.5) × (4.5–)5–5.5(–6.5) µm. Conidial exudates not observed.

Culture characteristics: Colonies on OA 45–50 mm diam after 7 d, margin regular. Immersed mycelium olivaceous black, in some sectors covered by a low mat of floccose white to grey aerial mycelium, towards colony margin the aerial mycelium is gradually becoming more felted and white; reverse olivaceous buff to dark mouse-grey. Colonies on MEA 50–55 mm diam after 7 d, margin regular. Immersed mycelium

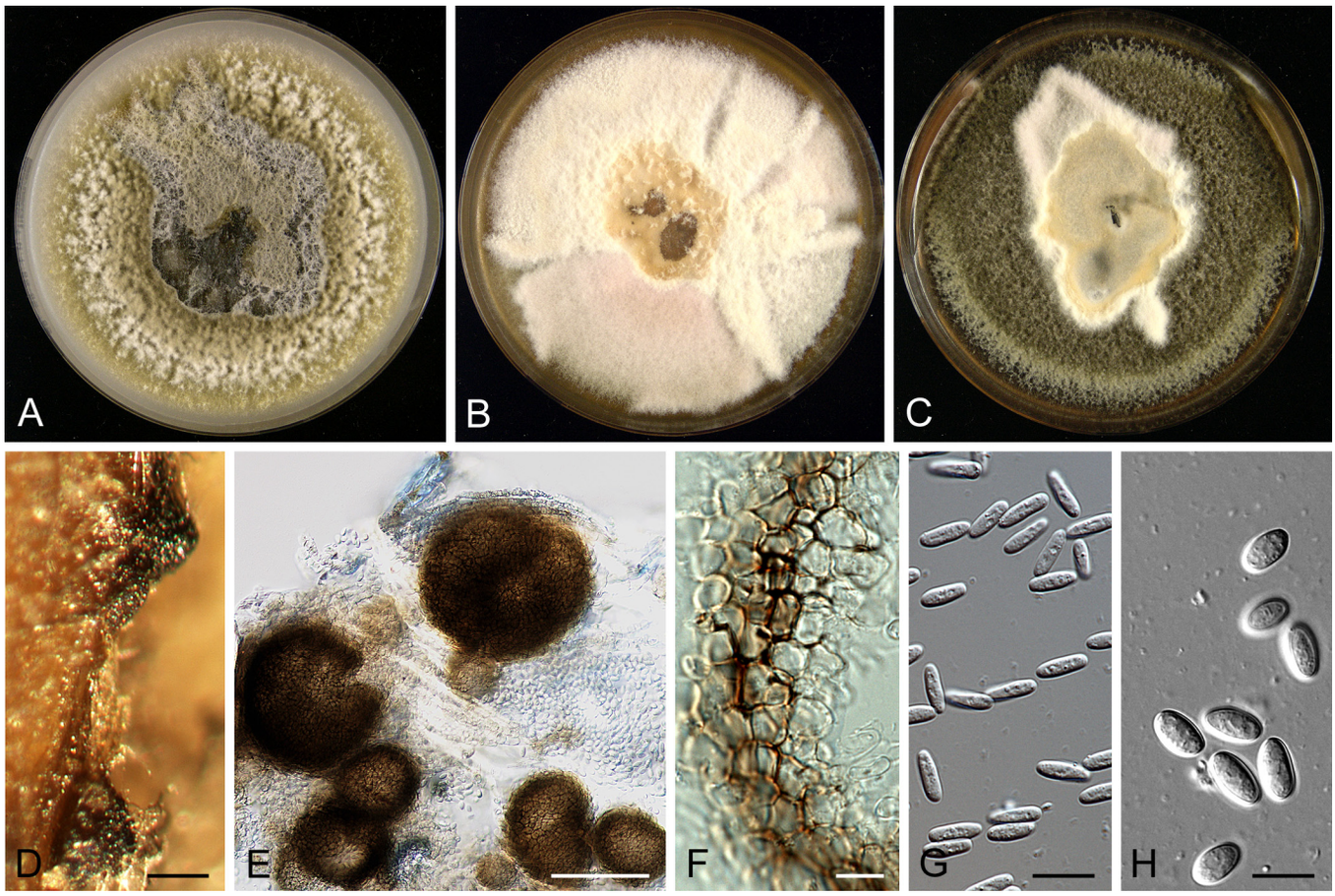


Figure 5.5. *Phoma dimorpha* (CBS 346.82). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D. Pycnidia on stem of *Urtica dioica*. E. Pycnidia. F. Pycnidial wall. G–H. Conidia *in vitro* (G) and *in vivo* (H). Scale bars: D–E = 100 µm, F = 20 µm, G–H = 10 µm.

hyaline, amber or iron-grey. Only sparsely small white tufts of whitish aerial mycelium are produced in older cultures; reverse concolourous. Colonies on CHA 55–60 mm diam after 7 d, margin regular. Immersed mycelium hyaline, honey to isabelline or dark mouse-grey. Aerial mycelium more proliferent near colony margin initially white, later developing to iron-grey with olivaceous grey tinges; reverse black, but hyaline near colony centre. Application of NaOH did not have any effect.

In older cultures white dendritic crystals are formed both in the aerial mycelium and immersed in the agar.

Specimen examined: **Spain**, Canary Isles, Gran Canaria, from phyllocladium of *Opuntiae* sp., June 1982, H.A. van der Aa, **holotype designated here** CBS H-20234, culture ex-holotype CBS 346.82.

Notes: Although sufficient pycnidial primordia are formed on OA, maturation of pycnidia is only incidentally observed *in vitro*. Therefore the characters of the pycnidia and the pycnidial wall described here are based on only three samples. Formation of mature pycnidia can be induced by addition of a sterilised stem piece of stinging nettle (*Urtica dioica*). The conidia that were described from *in vivo* material were obtained using this technique.

Several other *Phoma* species are known from *Opuntiae*, including *Ph. opuntiae* (*Phoma sensu lato*) and *Ph. longicolla* sp. nov. (see below). The conidia of *Ph. opuntiae* are, however, considerably smaller, measuring 2.5–3.5 × 1–1.5 µm (de Gruyter & Noordeloos 1992), whereas the main difference with *Ph. longicolla* are the pycnidia, which are uniostiolate and significantly larger in the latter species.

Group K ***Peyronellaea***:

This group (BPP = 1.00, RBS = 58 %) comprises many of the chlamydospore forming species, including the majority of the species that were accommodated in *Phoma* section *Peyronellaea* (Boerema *et al.*

1965a, 1968, 1971, 1973, 1977). Also *Ph. glomerata*, type species of this section is accommodated here (Boerema 1997). However, as section, *Peyronellaea* has a polyphyletic nature (Aveskamp *et al.* 2009a). *Phoma chrysanthemicola*, *Ph. violicola* and the recently established species *Ph. schachtii* (Aveskamp *et al.* 2009a) have been found to be basal to the *Didymellaceae* (Figure 5.1), whilst several species producing botryoid chlamydospores, representing the genus *Epicoccum* as emended below, are clustered in group M. Also *Ph. infossa* and *Ph. omnivirens*, which have proven to produce dictyochlamydospores in culture (Aveskamp *et al.* 2009a), are not situated in this part of the phylogenetic tree. *Peyronellaea calidophila* and *Ph. microchlamydospora* reside in the basal lineages of this clade.

Also several *Phoma* species that were not included in *Peyronellaea* in the Boeremaean taxonomical system, but that do produce either uni- or multicellular chlamydospores, are included in this clade. In *Ph. gardeniae*, *Ph. narcissi*, and *Ph. zeae-maydis* multicellular chlamydospores have been observed, whereas *Ph. pinodella*, *Ph. arachidicola*, and *Ph. heteroderae* are species that form unicellular chlamydospores. Several species in this clade, however, have never been recorded to produce any unicellular or multicellular chlamydospores. These species are *Ph. alectorolophi*, *Ph. obtusa* and *Ph. protuberans*, which will be treated in a subsequent section of this paragraph, and *Ph. anserina*, *Ph. aurea*, *Ph. nigricans* and *Ph. eucalyptica*. However, two of these species, *Ph. anserina* and *Ph. eucalyptica* are well-known for the formation of swollen cells and anastomosis in culture (de Gruyter & Noordeloos 1992), which may be regarded as a precursor to chlamydospore formation. The ancestral location of *Ph. anserina* in this clade may also be an indication that chlamydospore production has not completely been developed yet in this group. The high posterior probabillity for this group justifies the recognition of a separate genus in the *Didymellaceae*. Therefore the genus name *Peyronellaea* Goid. is re-established, and the associated species are recombined into this genus below.

The plurivorous species *Ph. calorpreferens* and *Ph. heteroderae* share identical LSU, ITS and TUB genes. Also morphologically the representative strains of these species are highly similar. A synonymisation of these species is therefore proposed in this paper.

Another notable subgroup within this clade is a cluster formed by *Didymella pinodes*, *D. lethalis*, *D. arachidicola* and *Ph. pinodella*. Recently, Irinyi *et al.* (2009) synonymised *Ph. sojicola* with *Ph. pinodella*, based on morphological observations and sequence data of ITS, β-tubulin and translation elongation factor 1-α. This indicates that the notorious pathogen of green pea (*Pisum sativum*) is also capable of infecting soybean (*Glycine max*). These observations are supported by the results obtained in the present study. As reported in previous studies (Faris-Mokaiesh *et al.* 1996, Onfroy *et al.* 1999, Fatehi *et al.* 2003, Peever *et al.* 2007), *Ph. pinodella* appears to be very closely related to *D. pinodes* (anam. *Ascochyta pinodes*) and because these species share the same host range they are often confused. Both species can however easily be differentiated on basis of the amount of septate conidia formed *in vitro*, abundantly in *D. pinodes*, and in very small numbers in *Ph. pinodella*.

Because *Ph. pinodella* is morphologically so similar to *Ph. medicaginis*, it was once regarded as a variety of this species by Boerema *et al.* (1965b). The variety was elevated to species rank after careful observation (White & Morgan-Jones 1987a), but the varietal name is however currently still in common use (e.g. Onfroy *et al*. 1999, Fatehi *et al*. 2003, Taylor & Ford 2007). The results obtained in this study however, illustrate a substantial phylogenetical distance to *Ph. medicaginis*, and warrant recognition at species level, in the in the re-instated genus *Peyronellaea*.

The close association of *Ph. arachidicola* with *Ph. pinodella* and *D. pinodes* is reflected by the morphology of these species, which all produce, next to septate and aseptate conidia, also globose to ellipsoidal unicellular chlamydospores, which may be formed in chains. These chlamydospores measure 5–20 µm diam, which is somewhat larger than the species in group N. The close relationship of these three species has been hypothesised before, and was based on chemical analysis of the crystals produced by these taxa (Noordeloos *et al.* 1993).

Didymella arachidicola is a specific pathogen of groundnut (*Arachis hypogaea*), another host plant of the family *Fabaceae* with which the other species in this subclade are also associated.

In the *Ph. pinodella* / *D. pinodes* subcluster (BPP = 1.00, RBS = 94 %), four teleomorph species are found with a coelomycete anamorph state. Next to *D. pinodes*, these are *D. alectorolophi*, *D. arachidicola,* and *D. lethalis*. A fifth teleomorph is the sexual state of *Ph. pinodella* (as *Ph. medicaginis* var. *pinodella*) that is reported and described by Bowen *et al.* (1997), but that has not been named thus far. From a phylogenetic point of view, this record is very plausible as all species in the subclade in which *Ph. pinodella* is embedded,

do form a *Didymella*-like teleomorph. However, as we did not include mating type tests in our studies, and as the species is probably heterothallic (Bowen *et al.* 1997), pseudothecia were not observed in the present study. A formal name for the teleomorph of *Ph. pinodella* could therefore not be proposed here either.

A fifth species in group K that has a known teleomorph is *Ph. zeae-maydis*. This species is however only distantly related to the four species mentioned above. Nevertheless, it can be concluded that the sole teleomorph genus that is associated with group K is *Didymella*-like. This would further support the suggestion (Peever *et al.* 2007) that the teleomorph name for *A. pinodes* that is often referred to by plant pathologists, *Mycosphaerella pinodes,* should be omitted.

Remarkably, three species are found in this clade that are identical based on sequence analyses, but that are morphologically rather distinct. Also sequence comparisons of parts of the actin and calmodulin genes did not reveal any differences between those four strains (Aveskamp & Woudenberg, unpubl. data). *Phoma alectorolophi* and *Ph. protuberans* are associated with *Phoma* section *Sclerophomella* (Boerema *et al.* 1997, de Gruyter *et al.* 2002), because of the thick-walled pycnidia formed in culture and *in vivo*. However, because of the production of relatively large secondary conidia, a link with sections *Heterospora* or *Phyllostictoides* can also be advocated. Colony characters, microscopic features and ecology indicate that the two species should actually be rather distinct. A third taxon found in this group is *Ph. obtusa*, a saprobic species that has a thin pycnidial wall and lacks septate conidia. Nevertheless, these three species are recovered in a clade in which solely chlamydospore-forming species reside, a character that never has been recorded in any of these taxa. The explanation of the contrast between the level of genetic and morphological similarity will be one of the main challenges in *Phoma* taxonomy.

Peyronellaea Goid. ex Togliani, *Ann. Sperim. Agrar*. II **6**: 93. 1952, **emend.** Aveskamp, Gruyter & Verkley.

Conidiomata pycnidial, globose to subglobose, measuring 50–380 µm diam, on agar surface or immersed, solitary or confluent, ostiolate or poroid. *Pycnidial wall* pseudoparenchymatous, counting 2–8 cell layers of which the outer 1–3 are brown or olivaceous pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, ampulliform or doliiform, *ca*. 3.5–7 × 3.5–6 µm. *Conidia* generally aseptate, ellipsoidal to subglobose, thin-walled, smooth, hyaline, but in older cultures conidia may become pigmented, generally measuring 4–15 × 2–4 µm, but larger or septated conidia may occur in at least one species. *Unicellular chlamydospores* often abundantly formed in and on the agar and in the aerial mycelium, globose, intercalary, brown or olivaceous pigmented, measuring 5–22 µm diam. *Multicellular chlamydospores* mainly alternarioid, terminal or intercalary, often in chains, brown or olivaceous pigmented, 10–50 × 7–25 µm. *Pseudothecia* only present in a few species, (sub-)globose, up to 200 µm diam, but in one species also flattened pseudothecia occur. *Asci* cylindrical to clavate, measuring 35–65 × 11–17 µm, always 8-spored, biseriate. *Ascospores* ellipsoid, measuring 12–24 × 4–8 µm, uniseptate, upper cell usually larger than the lower cells.

Type species: *Peyronellaea glomerata* (Corda) Goid. ex Togliani.

Peyronellaea americana (Morgan-Jones & J.F. White) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515596.
Basionym: *Phoma americana* Morgan-Jones & J.F. White, *Mycotaxon* **16** (2): 406. 1983.

Specimens examined: **Argentina**, Buenos Aires Province, Olavarria, from leaves of *Triticum aestivum* cv. Buck Diamante, Aug. 2002, A. Perelló, CBS 112525. **Denmark**, Copenhagen, from seed of *Phaseolus vulgaris*, May 1965, S.B. Mathur, CBS 256.65. **Nigeria**, from *Sorghum vulgare*, 1979, PD 79/58. **South Africa**, from *Zea mays*, 1978, PD 78/1059. **USA**, Arkansas, from pod lesions of *Glycine max*, 1981, H.J. Walters, CBS 568.9797 = ATCC 44494 = PD 94/1544; Georgia, from *Zea mays*, 1985, G.H. Boerema, CBS H-16144, culture CBS 185.85 = PD 80/1191; from *Zea mays*, 1980, PD 80/1143. Unknown origin, from a nematode cyst, 1982, G.H. Boerema, PD 82/1059.

Peyronellaea alectorolophi (Rehm.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515597.
Basionym: *Didymella alectorolophi* Rehm, apud Ade, *Hedwigia* **64**: 294. 1923.
≡ *Phoma alecotorolophi* Boerema, Gruyter & Noordel., *Persoonia* **16** (3): 366. 1997.

Specimen examined: **The Netherlands**, from seed of *Rhinanthus major*, 1993, L 992.167.515, culture CBS 132.96 = PD 93/853.

Peyronellaea anserina **(**Marchal) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515598.
Basionym: *Phoma anserina* Marchal, *Champignon Copr.* **11**: 1891.

Specimens examined: **The Netherlands**, from *Pisum sativum*, 1979, CBS 363.91 = PD 79/712; Ter Apel, from potato flour, 1983, CBS 360.84.

Peyronellaea arachidicola (Khokhr.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515599.
Basionym: *Mycosphaerella arachidicola* Khokhr., *Bolezni i vrediteli maslichnykh kul'tur* **1**(2): 29. 1934.
≡ *Didymella arachidicola* (Khokhr.) Tomilin, *Opredelitel' gribov roda Mycosphaerella Johans*: 285. 1979.
Anamorph: *Phoma arachidicola* Marasas, Pauer & Boerema, *Phytophylactica* **6** (3): 200. 1974.

Specimens examined: **South Africa**, Cape Province, Jan Kempdorp, Vaalharts Research Station, from a leaf of *Arachis hypogaea*, Mar. 1972, G.D. Pauer, **isotype** of *Ph. arachidicola* CBS H-7601, ex-isotype culture CBS 333.75 = ATCC 28333 = IMI 386092 = PREM 44889; **Zimbabwe**, from *Arachis hypogaea*, 1980, CBS 315.90 = PD 80/1190.

Peyronellaea aurea (Gruyter, Noordel. & Boerema) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515600.
Basionym: *Phoma aurea* Gruyter, Noordel. & Boerema, *Persoonia* **15**(3): 394. 1993.

Specimen examined: **New Zealand**, Auckland, from a stem of *Medicago polymorpha*, 1978, **holotype** L 992.177.422, ex-holotype culture CBS 269.93 = PD 78/1087.

Peyronellaea australis Aveskamp, Gruyter & Verkley, **nom. nov.** pro *Phoma nigricans* Ph.R. Johnst. & Boerema. MycoBank MB515601.
≡ *Phoma nigricans* Ph.R. Johnst. & Boerema, *New Zealand J. Bot.* **19** (4): 394. 1982.

Etymology: Epithet refers to the Southern Hemisphere, where this fungus is mainly found.

Specimens examined: **New Zealand**, from *Actinidea chinensis*, 1977, Ph.R. Johnston, PD 77/919; Auckland, Mt. Albert, from a leaf of *Actinidia chinensis*, Apr. 1979, Ph.R. Johnston, **isotype** CBS H-7619, ex-isotype culture CBS 444.81 = PDDCC 6546.

Note: A new name was sought for this species, as the epithet "*nigricans*" already was occupied in *Peyronellaea*, referring to a species which is now synonymised with *Pey. pomorum* var. *circinata* (see below).

Peyronellaea calorpreferens (Boerema, Gruyter & Noordel.) Aveskamp, Gruyter & Verkley, ***comb. nov.*** MycoBank MB515602.
Basionym: *Phoma pomorum* var. *calorpreferens* Boerema, Gruyter & Noordel. apud Boerema, *Persoonia* **15**: 207. 1993.

≡ *Phoma calorpreferens* (Boerema, Gruyter & Noordel.) Aveskamp, Gruyter & Verkley, *Mycologia* **101**: 370. 2009.
= *Phoma heteroderae* Sen Y. Chen, D.W. Dicks. & Kimbr., *Mycologia* **88**: 885.1996.

Conidiomata pycnidial, solitary or confluent, partially or completely immersed in the agar, (sub-)globose or irregular due to the presence of 1(–4) slightly papillate ostioles, measuring (70–)100–200(–250) µm diam. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 2–5 layers thick, with many hyphal outgrows, some setae-like. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, *ca*. 3–5.5 × 3–6.5 µm. *Conidia* broadly ellipsoidal to ovoid to cylindrical, thin-walled, smooth, hyaline, (3.5–)4–8.5(–12) × 2–3.5(–4.5) µm, aseptate, with (1–)2–5(–8) polar guttules. Conidial matrix pale pink. *Chlamydospores* highly variable in shape and size, mostly unicellular but also multicellular. Where unicellular, pale brown to brown, guttulate, intercalary, solitary or in chains, globose, 7.5–19(–26) µm, thick-walled and often with a distinct 'envelope'. Where multicellular dictyosporous alternarioid or botryoid, brown to black, terminal or occasionally intercalary in chains of unicellular chlamydospores, measuring *ca.* (16–)21–55 × (7–)12–30(–33) µm.

Specimens examined: **The Netherlands**, from undefined food material, 1973, G.H. Boerema, **holotype** L 990.290.418, ex-holotype culture CBS 109.92 = PD 73/1405. **USA**, Florida, Gainesville, from eggs of *Heterodera glycines* from greenhouse soil, CBS 630.97 = ATCC 96683 = IMI 361196 = PD 96/2022; from indoor environment, 1993, CBS 875.97 = PD 93/1503.

Notes: *Peyronellaea calorpreferens* is a taxon that was recently elevated from variety level to species rank, as *Phoma calorpreferens* (Aveskamp *et al.* 2009a). Due to its morphological and genetical similarity with *Ph. heteroderae*, it is concluded that both taxa are actually one and the same species. According to the International code of Botanical Nomenclature (McNeal *et al.* 2006) the epithet *calorpreferens* has priority, as its basionym *Ph. pomorum* var. *calorpreferens* was published earlier.

The type of *Peyronellaea calorpreferens* has been recovered from food materials, but Boerema (1993) hypothesizes about the plurivorous nature of this taxon, and mainly records it as a world-wide occurring soil- and seedborne opportunist, whereas Chen *et al.* (1996) record this species (as *Ph. heteroderae*) from eggs of a cyst nematode, *Heterodera glycines*.

Peyronellaea coffeae-arabicae (Aveskamp, Verkley & Gruyter) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515603.
Basionym: *Phoma coffeae-arabicae* Aveskamp, Verkley & Gruyter, *Mycologia* **101** (3): 371. 2009.

Specimens examined: **Ethiopia**, from *Coffea arabica*, 1984, M.M.J. Dorenbosch, **holotype** CBS H-20143, ex-holotype culture CBS 123380 = PD 84/1013; from *Coffea arabica*, 1984, M.M.J. Dorenbosch, CBS 123398 = PD 84/1014.

Peyronellaea curtisii (Berk.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515604.
Basionym: *Hendersonia curtisii* Berk., in Cooke, *Nuovo Giorn. Bot. Ital.* **10**: 19. 1878.
≡ *Stagonosporopsis curtisii* (Berk.) Boerema, in Boerema & Dorenbosch, *Verslagen Meded. Plziektenk. Dienst Wageningen* **157**: 20. 1981.
= *Phyllosticta narcissi* Aderh., *Centralbl. Bakteriol., 2 Abth*. **6**: 632. 1900.
≡ *Phoma narcissi* (Aderh.) Boerema, Gruyter & Noordel., *Persoonia* **15** (2): 215. 1993.

Specimens examined: **The Netherlands**, from *Nerine* sp., May 1992, J. de Gruyter, culture 251.92 = PD 86/1145; from *Sprekelia* sp., PD 92/1460. Unknown origin, from *Ismene* sp., 1971, PD 71/6. Unknown origin, from *Hippeastrum* sp., 1976, PD 76/61.

Peyronellaea eucalyptica (Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515605.

Basionym: *Phoma eucalyptica* Sacc., *Syll. Fung.* **3**: 78. 1884.

Specimens examined: **Australia**, Western Australia, from a leaf of *Eucalyptus* sp., 1979, CBS 377.91 = PD 79/210. **Croatia**, Adriatic Sea, from seawater, 1973, CBS 508.91 = PD 73/1413. **Indonesia**, Sumatra, Sulavesi, from *Eugenia aromatica,* 1982, CBS 378.91 = PD 82/107.

Peyronellaea gardeniae (S. Chandra & Tandon) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515606.
Basionym: *Pyrenochaeta gardeniae* S. Chandra & Tandon, *Mycopathol. Mycol. Appl.* **29**: 274. 1966.
≡ *Phoma gardeniae* (S. Chandra & Tandon) Boerema, in Boerema & Dorenbosch, *Verslagen Meded. Plziektenk. Dienst Wageningen* **156**: 27. 1980.

Specimens examined: **India**, Allahabad, from the leaf of *Gardenia jasminoides*, 1966, S. Chandra and R.N. Tandon, **isotype** CBS H-7605, ex-isotype culture CBS 626.68 = IMI 108771. **Netherlands Antilles**, Curacao, from air sample, 1978, A. Kikstra, CBS 302.79 = PD 79/1156.

Peyronellaea glomerata (Corda) Goid. ex Togliani, *Ann. Sperim. Agrar.* III **6**: 93. 1952.
Basionym: *Coniothyrium glomeratum* Corda, *Icon. Fung.* (Prague) **4**: 39. 1840.
≡ *Phoma glomerata* (Corda) Wollenw. & Hochapfel, *Z. Parasitenk.* **3** (5): 592. 1936.

Specimens examined: **Germany**, Berlin-Zehlendorf, Domäne Düppel, from a tuber of *Solanum tuberosum*, 1936, H.W. Wollenweber, CBS 293.36 = MUCL 9882; Monheim, from *Hordeum sativum*, 1984, M. Hossfeld, CBS 834.84; from indoor environment, 2003, C. Rudolph, CBS 112448. **Romania**, Bukarest, from a church wall-fresco, Nov. 1971, I. Ionita, CBS 133.96 = PD 79/127. **Russia**, Novosibirsk, Hortus Botanicus, from a leaf of *Populus nigra*, 1963, T.T. Kuznetsova, CBS 284.76 = ATCC 26238 = IMI 176748 = VKM F-1842; Novosibirsk, Hortus Botanicus, from a leaf of *Rubus idaeus*, 1963, T.T. Kuznetsova, CBS 287.76 = ATCC 26240 = IMI 176746 = VKM F-1847; Novosibirsk, Hortus Botanicus, from a leaf of *Populus alba*, 1963, T.T. Kuznetsova, CBS 288.76 = ATCC 26243 = VKM F-1845; Novosibirsk, Hortus Botanicus, from a leaf of *Allium nutans*, 1963, T.T. Kuznetsova, CBS 289. 76 = ATCC 26239 = IMI 176745 = VKM F-1846; Novosibirsk, Hortus Botanicus, from a leaf of *Ribes nigrum*, 1963, T.T. Kuznetsova, CBS 290.76 = ATCC 26244 = IMI 176747 = VKM F-1848; from *Heracleum* sp., 1973, PD 73/1415. **The Netherlands**, from a root of *Lycopersicon esculentum*, 1949, D. Verleur, CBS 304.49 = MUCL 9884; from *Chrysanthemum* sp., 1963, CBS 528.66 = PD 63/590; from indoor bathroom environment, 1997, M. Komen, CBS 464.97; from *Medicago sativa*, PD 77/47. **UK**, from air, PD 74/1023. **USA**, Virginia, from *Juniperus* sp., Jan. 2002, A.Y, Rossman, CBS 120109. Unknown origin, from *Cucumis sativus*, PD 81/767; from *Capsicum* sp., PD 83/782.

Peyronellaea lethalis (Ellis & Bartholomew) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515607.
Basionym: *Ascochyta lethalis* Ellis & Bartholomew, *Fungi Columb.* **1808**. 1903.
= *Mycosphaerella lethalis* R. Stone, *Ann. Mycol.* **10**: 587. 1912.
≡ *Didymella lethalis* (R. Stone) Sivan., *Bitunicate Ascomycetes and their Anamorphs*: 424. 1984.
Specimen examined: Unknown origin and substrate, 1925, A.W. Archer, CBS 103.25.

Peyronellaea musae Ph. Joly, *Revue Mycol*. **26**: 97. 1961.
≡ *Phoma jolyana* Piroz. & Morgan-Jones, *Trans. Brit. Mycol. Soc.* **51**: 200. 1968.

Specimens examined: **India**, from fruit of *Mangifera indica*, May 1969, CBS 463.69; from *Malus sylvestris*, PD 83/326.

Notes: *Phoma jolyana* was originally described in the genus *Peyronellaea*, as *Pey. musae*. The epithet "*jolyana*" was later proposed for this species, as the epithet *musae* was already occupied in *Phoma* (Pirozynski & Morgan-Jones 1968). Here, we reinstate this fungus under its original name.

Peyronellaea obtusa (Fuckel) Aveskamp, Gruyter & Verkley, ***comb. nov.*** MycoBank MB515608.
Basionym: *Phoma obtusa* Fuckel, *Jahrb. Nassauischen Vereins Naturk*. **23-24**: 378. 1870.

Specimens examined: **The Netherlands**, from a root of *Daucus carota*, July 1993, J. de Gruyter, CBS 377.93 = PD 80/976; from *Spinacia oleracea*, July 1993, J. de Gruyter, CBS 391.93 = PD 80/87.

Peyronellaea pinodella **(**L.K. Jones) Aveskamp, Gruyter & Verkley, ***comb. nov.*** MycoBank MB515609.
Basionym: *Ascochyta pinodella* L.K. Jones, *Bull. New York State Agric. Exp. Sta.* 547: 10. 1927.
≡ *Phoma medicaginis* var. *pinodella* (L. K. Jones) Boerema apud Boerema, Dorenbosch & Leffring, *Netherlands J. Pl. Pathol.* **71:** 88. 1965.
≡ *Phoma pinodella* **(**L.K. Jones) Morgan-Jones & K.B. Burch, *Mycotaxon* **29**: 485. 1987.

Specimens examined: **Hungary**, from *Glycine max,* 1996, G. Kövics, CBS 567.97 = PD 97/2160; from seed of *Glycine max*, 1997, G. Kövics, CBS 100580 = PD 98/1135. **The Netherlands**, from *Pisum sativum*, 1981, CBS 318.90 = PD 81/729. **USA**, Minnesota, from *Trifolium pretense*, 1966, CBS 531.66.

Notes: *Phoma sojicola*, which was erected in 1999 (Kövics *et al.* 1999), has recently been synonymised with the present species, based on morphological and genetical similarities (Irinyi *et al.* 2009). The present study supports these findings.

Peyronellaea pinodes (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515610.
Basionym: *Sphaeria pinodes* Berk. & A. Bloxam, *Ann. Mag. Nat. Hist.*, *Ser*. III **7**: 454. 1861.
≡ *Didymella pinodes* (Berk. & A. Bloxam) Petr., *Ann. Mycol.* **22** (1/2): 16. 1924.
≡ *Mycosphaerella pinodes* (Berk. & A. Bloxam) Vestergr*., Ann. Mycol.* **10** (5): 581. 1912.
= *Ascochyta pinodes* L.K. Jones, *Bull. New York State Agric. Exp. Sta.* **547**: 4. 1927.

Specimens examined: **Belgium**, Gembloux, from *Pisum sativum*, 1977, G. Sommereyns, CBS 525.77. **Iraq**, Basrah province, from *Pisum sativum*, 1977, CBS 159.78. **Switzerland**, Glarus Kanton, Filzbach, from a leaf of *Primula auricula*, June 1949, E. Müller, CBS 285.49. **The Netherlands**, from an unknown substrate, 1955, M.H. van Raalte, CBS 235.55.

Peyronellaea pomorum **var. *Pomorum*** (Thüm.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515611.
Basionym: *Phoma pomorum* var. *pomorum* Thüm., *Fungi Pomicoli*: 105. 1879.

Specimen examined: **The Netherlands**, Wageningen, from *Polygonum tataricum*, 1964, M.M.J. Dorenbosch, CBS H-16540, culture CBS 539.66 = ATCC 16791 = IMI 122266 = PD 64/914.

Peyronellaea pomorum var. ***circinata*** (Kusnezowa) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515612.
Basionym: *Peyronellaea circinata* Kusnezowa, *Novoste Sist. Nizsh. Rast.* **8**: 189. 1971.
≡ *Phoma jolyana* var. *circinata* (Kusnezowa) Boerema, Dorenb. & Kesteren, *Kew Bull.* **31**: 535. 1977 [1976].
≡ *Phoma pomorum* var. *circinata* (Kusnezowa) Aveskamp, Gruyter & Verkley, *Mycologia* **101** (3): 377. 2009.

= *Peyronellaea nigricans* Kusnezowa, *Novoste Sist. Nizsh. Rast.* **8**: 191. 1971.

Specimens examined: **Russia**, Siberia, Novosibirsk, from *Heracleum dissectum*, 1963, T.T. Kusnezowa, **isotype** CBS H-3747, ex-isotype culture CBS 285.76 = ATCC 26241 = IMI 176742 = VKM F-1843; Siberia, Novosibirsk, from a leaf of *Allium nutans*, 1963, T.T. Kusnezowa, CBS 286.76 = ATCC 26242 = IMI 176743 = VKM F-1844.

Peyronellaea pomorum var. ***cyanea*** (Jooste & Papendorf) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515614.
Basionym: *Phoma cyanea* Jooste & Papendorf, *Mycotaxon* **12**: 444. 1981.
≡ *Phoma pomorum* var. *cyanea* (Jooste & Papendorf) Aveskamp, Gruyter & Verkley, *Mycologia* **101** (3): 377. 2009.

Specimen examined: **South Africa**, Heilbron, from straw of *Triticum* sp., 1972, W.J. Jooste, **holotype** PREM 45736, ex-holotype culture CBS 388.80.

Peyronellaea protuberans (Lév.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515613.
Basionym: *Phoma protuberans* Lév., *Ann. Sci. Nat. Bot*. III **5**: 281. 1846.

Specimen examined: **The Netherlands**, from a leaf of *Lycium halifolium*, 1971, CBS 381.96 = PD 71/706.

Peyronellaea sancta **(**Aveskamp, Gruyter & Verkley) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515615.
Basionym: *Phoma sancta* Aveskamp, Gruyter & Verkley, *Mycologia* **101** (3): 377. 2009.

Specimens examined: **Argentina**, from *Opuntia ficus-indica*, 1997, CBS 644.97. **South Africa**, from dead branches of *Ailanthus altissima*, Oct. 1982, C. Jansen CBS H-16332, ex-holotype culture CBS 281.83. Unknown origin, from *Gleditsia triancantha* culture LEV 15292.

Peyronellaea subglomerata (Boerema, Gruyter & Noordel.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515616.
Basionym: *Phoma subglomerata* Boerema, Gruyter & Noordel., *Persoonia* **15** (2): 204. 1993.

Specimens examined: **USA**, North Dakota, from *Triticum* sp., 1976, CBS 110.92 = PD 76/1010. Unknown origin, from *Zea mays*, 1978, PD 78/1090.

Peyronellaea zeae-maydis (Mukunya & Boothr.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515617.
Basionym: *Mycosphaerella zeae-maydis* Mukunya & Boothr., *Phytopathology* **63**: 530. 1973.
≡ *Didymella zeae-maydis* (Mukunya & Boothr.) Arx, *Beih. Nova Hedwigia* **87**: 288. 1987.
Anamorph: *Phyllosticta maydis* Arny & R.R. Nelson, *Phytopathology* **61**: 1171. 1971.
≡ *Phoma zeae-maydis* Punith., *Mycopathologia* **112** (1): 50. 1990.

Specimen examined: **USA**, Wisconsin, Hancock, from *Zea mays*, June 1969, D.C. Arny, ex-holotype culture CBS 588.69.

Group L:
Phoma draconis, *Ph. henningsii*, *Ph. plurivora* and the novel species *Ph. brasiliensis* cluster basally to the *Epicoccum* species in group M. The species clustered here, however, all lack chlamydospores.

These species do, like the chlamydospore-forming species mentioned above, solely produce unicellular conidia, and have glabrous, thin-walled, pseudoparenchymatous pycnidial walls composed of isodiametric cells.

Phoma brasiliensis Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515618. Figure 5.6.

Conidia cylindrica, glabra, hyalina, continua, 6–9(–10) × 2–3(–3.5) µm, (3–)4–6(–8) guttulis parvis praedita. Matrix conidiorum alba.

Etymology: Epithet refers to the country of origin, Brazil.

Conidiomata pycnidial, mainly solitary but also confluent, globose to irregularly shaped, glabrous, on the agar surface and immersed, (220–)250–370(–550) × (150–)190–290(–320) µm. Usually with a single inconspicuous non-papillate ostiole. *Pycnidial wall* pseudoparenchymatous, composed of 5–9 layers of oblong to isodiametric cells, 18–27 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, globose to flask-shaped, *ca*. 4–5 × 3.5–4 µm. *Conidia* variable in size, cylindrical, thin-walled, smooth, hyaline, aseptate 6–9(–10) × 2–3(–3.5) µm, with (3–)4–6(–8) small polar guttules. Conidial matrix white.

Culture characteristics: Colonies on OA 50–53 mm diam after 7 d, margin regular. Aerial mycelium sparse, tufted near the centre of the colony, white. Immersed mycelium hyaline. Abundant pycnidia produced semi-immersed in concentric rings. Pycnidia in the outer rings pale luteous, darkening towards the centre of the colony via buff, honey, hazel to brown-vinaceous; reverse concolourous. Colonies on MEA 59–63 mm diam after 7 d, margin regular. Immersed mycelium completely covered by a mycelial mat, which is densely floccose, greenish olivaceous to greenish grey, with elements of citrine, olivaceous black and white; reverse concolourous. Hyphae locally containing red amorphous chrystaline material. Colonies on CHA 62–67 mm diam after 7 d, margin regular. Aerial mycelium floccose, white. Abundant dark pycnidia are formed on the agar surface. Application of NaOH results in a luteous discolouration of the agar, later changing to reddish, best to be observed on OA medium.

Specimen examined: **Brazil**, from *Amaranthus* sp., Nov. 2007, E. Rosskopf, **holotype designated here** CBS H-20235, ex-holotype culture CBS 120105.

Notes: This species is thus far only known from a single isolate from a wild *Amaranthus* sp. in Brazil. According to Boerema *et al.* (2004), no other *Phoma* species have been recorded from the same host.

Phoma draconis (Berk. ex Cooke) Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* **159** (*Jaarboek 1982*): 24. 1983.
Basionym: *Phyllosticta draconis* Berk. ex Cooke, *Grevillea* **19**: 8. 1891.

Specimen examined: **Rwanda**, from a leaf of *Dracaena* sp., Jan. 1982, G.H. Boerema, CBS H-16207, culture CBS 186.83 = PD 82/47.

Phoma henningsii Sacc., *Syll. Fung.* **10**: 139. 1892.

Specimen examined: **Kenya**, Maguga, from the bark of *Acacia mearnsii*, June 1992, T.W. Olembo, CBS H-16354, culture CBS 104.80 = PD 74/1017.

Phoma plurivora Ph.R. Johnst., *New Zealand J. Bot.* **19** (2): 181. 1981.

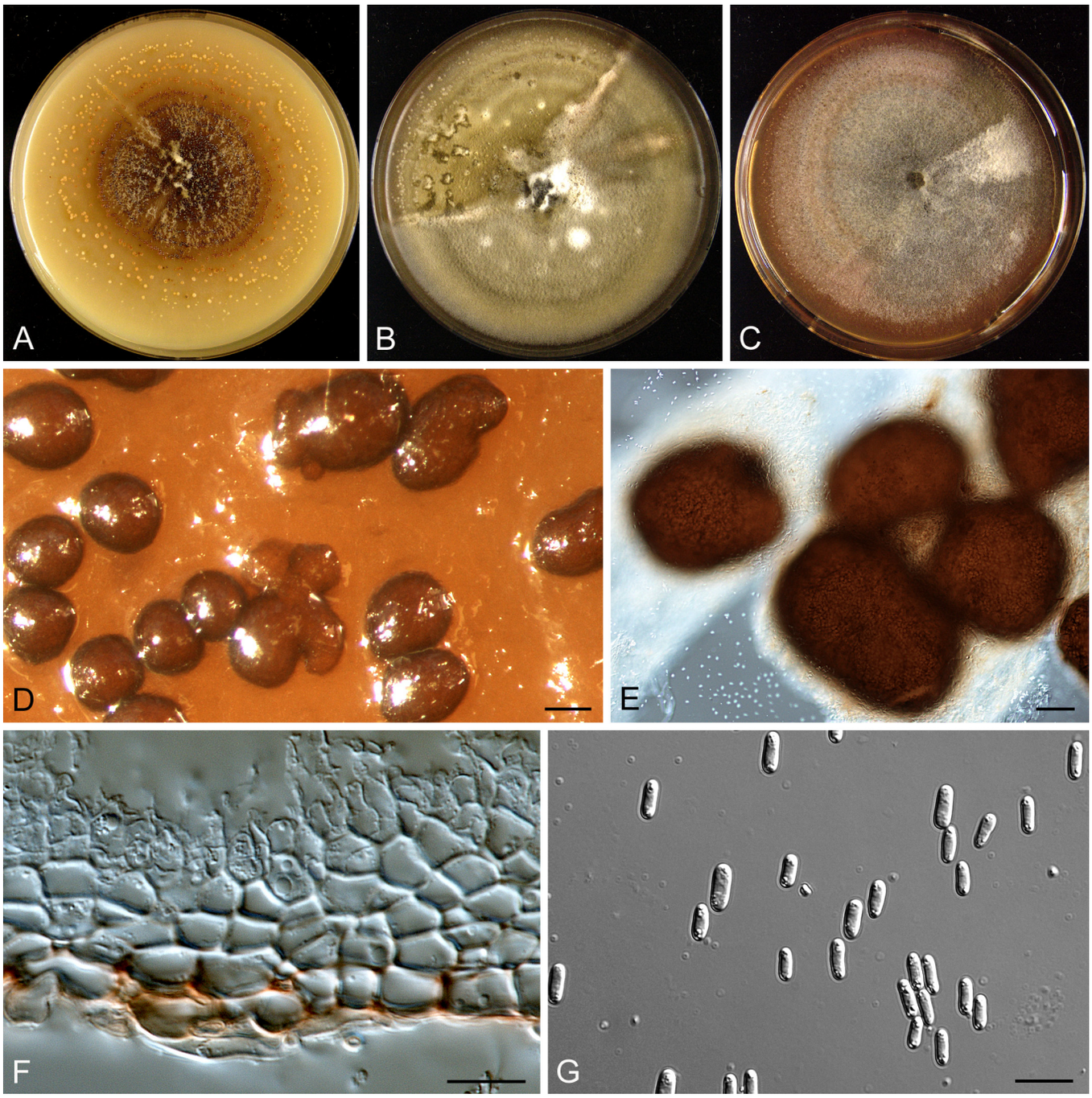


Figure 5.6. *Phoma brasiliensis* (CBS 120105a). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D–E. Pycnidia. F. Section of the pycnidial wall. G. Conidia. Scale bars: D = 200 µm, E = 100 µm, F–G = 10 µm.

Specimens examined: **Australia**, from *Medicago sativa*, 1975, CBS 248.93 = PD 95/907. **New Zealand**, Auckland, Mt Albert, from a leaf of *Setaria sp*., Feb. 1979, Ph.R. Johnston, CBS H-7624, ex-isotype culture CBS 558.81 = PDDCC 6873.

Group M ***Epicoccum***:

This group (BPP = 1.00, RBS = 66 %) comprises three species that are accommodated in the section *Peyronellaea*. The *Peyronellaea* species in this group, *Ph. sorghina*, *Ph. pimprina* and *Epicoccum nigrum* (chlamydospore-based synanamorph of *Ph. epicoccina*; Arenal *et al.* 2000, 2004) are characterised by the production of botryoid or epicoccoid chlamydospores, in contrast to the species in group K, which produce alternarioid dictyochlamydospores. The distinct morphology and phylogenetic position justify the recombination into a separate genus. As the oldest generic name in this clade is *Epicoccum*, new combinations for *Ph. pimprina* and *Ph. sorghina* are proposed below.

Epicoccum Link, *Mag. Gesell. Naturf. Freunde Berlin* **7**: 32. 1815, **emend.** Aveskamp, Gruyter & Verkley. Figure 5.7.

Conidiomata pycnidial, globose to subglobose, measuring 50–250 µm diam, on agar surface or immersed, mostly solitary but incidentally confluent. *Ostioles* papillate or on pronounced necks. *Pycnidial wall* pseudoparenchymatous, counting 2–8 cell layers of which the outer 1–3 are brown olivaceous pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, ampulliform, *ca*. 3–7 × 3–7 µm. *Conidia* variable in shape, initially hyaline but in later stages a slight brownish pigmentation may be found, thin-walled, smooth, always aseptate 3–8.5(–10) × 1.5–4(–4.5) µm. *Chlamydospores* unicellular or multicellular, intercalary or terminal, smooth, verrucose or incidentally tuberculate, subhyaline to dark brown, where unicellular globose, measuring 5–15 µm diam, where multicellular globose or irregular shaped, smooth, verrucose or incidentally tuberculate, measuring 8–35 µm.

Type species: *Epicoccum nigrum* Link.

Epicoccum nigrum Link, *Mag. Gesell. Naturf. Freunde Berlin* **7**: 32. 1815.
≡ *Phoma epicoccina* Punith., M.C. Tulloch & C.M. Leach, *Trans. Brit. Mycol. Soc.* **59**(2): 341 (1972).

Specimens examined: **Germany**, Berlin, from soil, 1985, H.J. Halfmann, CBS 505.85. **The Netherlands**, Geleen, from human toe nail, Dec. 1981, CBS 125.82 = IMI 331914 = CECT 20044; Randwijk, from *Malus* sp., J. Köhl, 2003, CBS 115825. **USA**, Oregon, from seeds of *Dactylis glomerata*, 1967, CBS 173.73 = ATCC 24428 = IMI 164070.

Epicoccum pimprinum (Ph.N. Mathur, S.K. Menon & Thirum.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515619.
Basionym: *Phoma pimprina* Ph.N. Mathur, S.K. Menon & Thirum., *Sydowia* **13**: 146. 1959.

Specimens examined: **India**, Poona, Pimpri, from soil, Mar. 1959, S.K. Menon, ex-isotype culture CBS 246.60 = ATCC 22237 = ATCC 16652 = IMI 81601; from soil, 1977, PD 77/1028.

Epicoccum sorghi (Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515620.
Basionym: *Phyllosticta sorghina* Sacc., *Michelia* **1** (2): 140. 1878.
≡ *Phoma sorghina* (Sacc.) Boerema, Dorenb. & Kesteren, *Persoonia* **7** (2): 139. 1972.
For a complete synonymy see Boerema *et al.* (1977).

Specimens examined: **France**, Antibes, from a twig of *Citrus* sp., 1966, CBS 627.68 = PD 66/926. **Guinea-Bissau**, Gacheu Région, from *Oryza sativa*, Oct. 1978, CBS 181.81. **India**, from a fruit of *Coffea* sp., July 1968, C.V. Subramanian, CBS 846.68; Jabalpur, from *Panicum miliare*, Jan. 1972, D. Sharma, CBS 293.72. **Martinique**, from a leaf of *Lycopersicon esculentum*, June 1989, B. Hostachy, CBS 301.89. **Papua New Guinea**, from *Stellaria* sp., A. Aptroot, Oct. 1995, CBS 886.95; Central Province, Varirata National Park near Port Moresby, from soil, A. Aptroot, Oct. 1995, CBS 986.95. **Puerto Rico**, Mayaguez, from *Sorghum vulgare*, Apr. 1976, R. Alconera, CBS 179.80= PD 76/1018. **South Africa**, Potchefstroom, from a leaf of *Zea mays*, Nov. 1978, W.J. Jooste, CBS 180.80 = PD 78/1100.

Notes: The strains that were previously accommodated in *Ph. sorghina* are morphologically and phylogenetically highly diverse (Aveskamp *et al.* 2009a, Pažoutová 2009), and probably represent multiple species. These species were, however, not treated in the present study.

Group N ***Boeremia*** **gen. nov.**:

This group represents species that are morphologically similar to what is currently known as *Ph. exigua*. Group N is a well-defined clade (BPP = 1.00, RBS = 100 %) and comprises all taxa that were previously

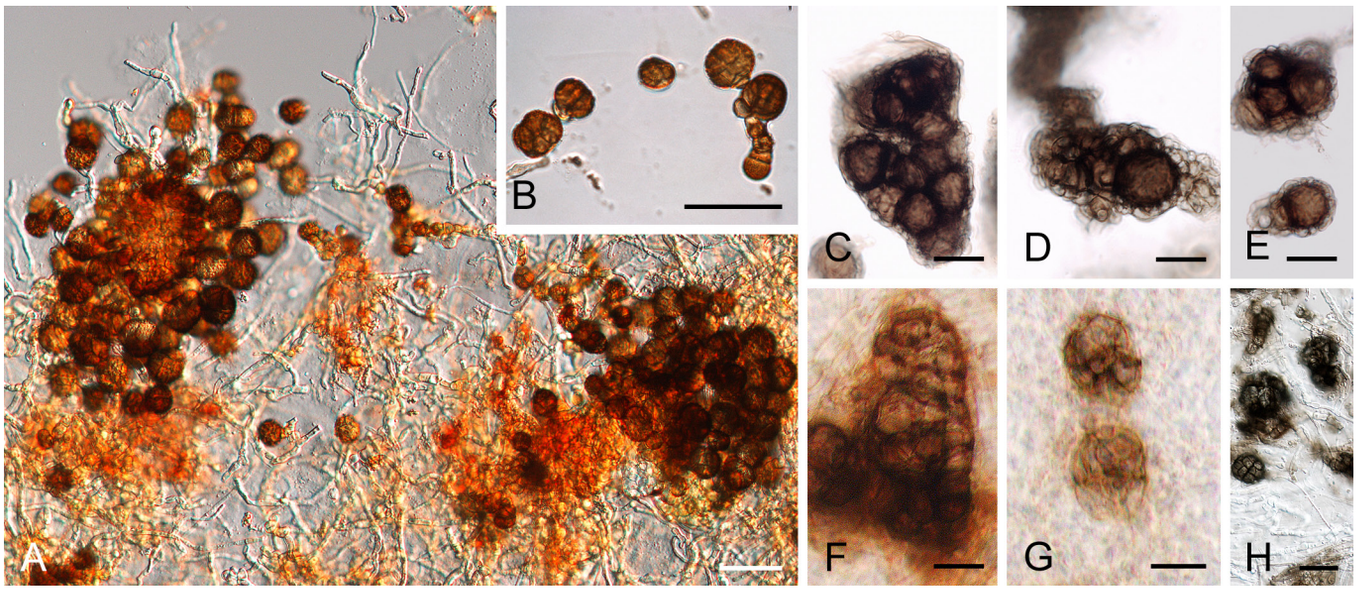


Figure 5.7. Globose chlamydospores of *Epicoccum* spp. A–B. *E. nigrum* (CBS 173.73). C–E. *E. sorghi* (CBS 246.60). F–H. *E. pimprinum* (CBS 179.80). Scale bars: A–B = 50 µm, C–H = 20 µm.

recognised as separate *Ph. exigua* varieties by Abeln *et al.* (2002). *Phoma foveata* and *Ph. sambuci-nigrae* are embedded here as well, two species that previously were known as varieties of *Ph. exigua*, but were elevated to species rank due to their phytopathological relevance (*Ph. foveata*, Boerema *et al.* 1987) or distinct physiological characters (*Ph. sambuci-nigrae*, Monte *et al.* 1991). As already noted by Aveskamp *et al.* (2009b) also *Ph. telephii*, *Ph. strasseri* and *Ph. lycopersici* are closely related. This study also reveals the close relationship with *Ph. tarda*, a pathogen of coffee. *Phoma hedericola*, a frequently occurring causal agent of leaf spots on ivy (*Hedera helix*) and *Ph. crinicola*, a pathogen of *Amaryllidaceae* are embedded in this clade. In contrast to the other species in this clade, which are linked to *Phoma* section *Phyllostictoides*, *Ph. hedericola* and *Ph. crinicola* are associated with *Phoma* section *Phoma*, due to the absence of septate conidia (de Gruyter & Noordeloos 1992, de Gruyter *et al.* 1993). The sequence data of CBS 172.34, a strain recorded as *Dothiorella ulmi*, appeared to be genetically identical to *Ph. exigua*, as was already noted by de Gruyter *et al.* (2009). Based on morphological studies of other strains, *Dothiorella ulmi* was suggested to be recombined into *Plectophomella* (Redfern & Sutton 1981), a genus that is linked to the Pezizomycotina. Morphological features of the present strain appeared to be similar to *Ph. exigua*, suggesting that this strain was probably preserved under an incorrect name, and actually belongs to *Ph. exigua* var. *populi*.

Of the species within this clade, a teleomorph is only named in *Ph. lycopersici* (*Didymella lycopersici*), although Stewart (1957) has reported the existence of pseudothecia of *Ph. tarda* in nature, a finding that also has been reported by Salgado *et al.* (2007). This contradicts with the fact that none of the varieties embedded in the *Ph. exigua* has been found in association with a teleomorph thus far.

For further delineation of this clade, a comparison of actin gene sequences is proposed (Aveskamp *et al*. 2009b), although not all species and varieties in this complex can be recognised using this gene only. Thus far the varieties of *Ph. exigua* could only be delineated using two fingerprint techniques: Amplified Fragment Length Polymorphism (AFLP, Abeln *et al.* 2002) and DAF (DNA Amplification Fingerprinting) using mini-hairpin primers (Aveskamp *et al*. 2009b). Based on this latter technique Aveskamp *et al*. (2009b) recognised two varieties within *Ph. exigua* that had not been described before. These two infraspecific taxa, *Ph. exigua* var. *gilvescens* and var. *pseudolilacis* are treated and described below.

Based on the phylogenetic reconstruction obtained here, the taxa previously known as *Ph. exigua* var. *noackiana* and *Ph. exigua* var. *diversispora* cluster in a distinct clade from the other varieties in this complex, and are elevated to species level here. Also actin sequence data and DAF analysis (Aveskamp *et al*. 2009b), AFLP data (Abeln *et al.* 2002) reveal a basal topology of these species compared to *Ph. exigua*. Morphological data obtained by van der Aa (2000) also suggest that these species are not completely fitting in the *Ph. exigua* concept.

The species and varieties in this clade differ from other *Phoma* taxa based on their ostiole morphology. In contrast to other species, which have a smoothly lined ostiole, the taxa present in this clade have distinct hyaline cells lining their ostiolar openings (Figure 5.8A). In addition, these species, with the exception

of *Ph. hedericola*, produce septate conidia in addition to the regular aseptate ones, although in general the septate conidia are produced in smaller numbers in culture than on the host. These conidia are mostly 1-septate, as only in *Ph. exigua* incidentally multiseptate conidia occur, and are often only slightly larger than the regular aseptate ones (Figure 5.8C). Due to the morphological and genetic distinctiveness, we propose a new generic name for the taxa in this clade.

Boeremia Aveskamp, Gruyter & Verkley, **gen. nov.** MycoBank MB515621. Figure 5.8.

Conidiomata pycnidialia, plerumque globosa vel subglobosa, glabra vel eminentiis sparsis hypharum vestita, superficialia vel in agaro immersa, solitaria vel confluentia, 75–370 µm diam. Ostiola papillata vel epapillata, tempore maturitatis interne cellulis hyalinis papillatis. Paries pycnidii pseudoparenchymatus, e 2–8 stratis cellularum compositus, extima 1–3 strata brunnea. Cellulae conidiogenae phialidicae, hyalinae, glabrae, ampulliformes vel dolliiformes, ca. 3–7.5 × 3–6.5 µm. Conidia hyalina, tenuitunicata, glabra, plerumque continua, 2.5–12 × 2–4 µm, et interdum uni- vel biseptata, usque 15 × 5 µm.

Conidiomata pycnidial conidiomata variable in shape and size, mostly globose to subglobose, glabrous or with few mycelial outgrowths, on agar surface or immersed, solitary or confluent, measuring 75–370 µm diam. *Ostioles* 1–2(–3), non-pappillate or pappillate, lined internally with a pappillate hyaline cells when mature. *Pycnidial wall* pseudoparenchymatous, counting 2–8 cell layers of which the outer 1–3 are brown pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, ampulliform to doliiform, *ca*. 3–7.5 × 3–6.5 µm. *Conidia* variable in shape, hyaline, thin-walled, smooth, mainly aseptate, 2.5–12 × 2–4 µm, but regularly 1(–2)-septate conidia may be found which measure up to 15 × 5 µm. *Pseudothecia*, only rarely recorded in one species *in vivo*, subglobose, up to 300 µm diam. *Asci* cylindrical or subclavate, measuring 50–95 × 6–10 µm, always 8-spored, biseriate. *Ascospores* ellipsoid, measuring 12–18 × 5–6 µm, uniseptate.

Type species: *Boeremia exigua* (Desm.) Aveskamp, Gruyter & Verkley

Etymology: Named after Gerhard H. Boerema, who made great contributions to our understanding of the taxonomy of phomoid fungi.

Boeremia crinicola (Siemasko) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515622.
Basionym: *Phyllosticta crinicola* Siemasko, *Acta Soc. Bot. Poloniae* **1**: 22. 1923.
≡ *Phoma crinicola* (Siemasko) Boerema apud Boerema & Dorenbosch, *Verslagen Meded. Plziektenk. Dienst Wageningen* **153**: 18.1979.

Specimens examined: **The Netherlands**, Haarlem, from a bulb of *Crinum powellii*, Mar. 1976, G.H. Boerema, CBS H-16198, CBS 109.79 = PD 77/747; Alkmaar, from a bulb of *Crinum* sp., 1970, G.H. Boerema, CBS 118.93 = PD 70/195.

Boeremia diversispora (Bubák) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515623.
Basionym: *Phoma diversispora* Bubák, Oest. Bot. Z. **55**: 78. 1905
≡ *Phoma exigua* var. *diversispora* (Bubák) Boerema apud Boerema & van Kesteren, *Gewasbescherming* **11**: 122. 1980

For a complete description see Boerema *et al.* (1981a, 2004), and van der Aa *et al.* (2000).

Specimens examined: **Brazil**, leaf of *Phaseolus*, F. Noack, **holotype** B. **Kenya**, from a pod of *Phaseolus vulgaris*, 1979, G.H. Boerema, **epitype designated here** CBS H-16308, ex-epitype culture CBS 102.80 = CECT 20049 = IMI 331907 = PD 79/61. **The Netherlands**, near Tilburg, from *Phaseolus vulgaris*, 1979, J. de Gruyter, CBS 101194 = PD 79/687 = IMI 373349.

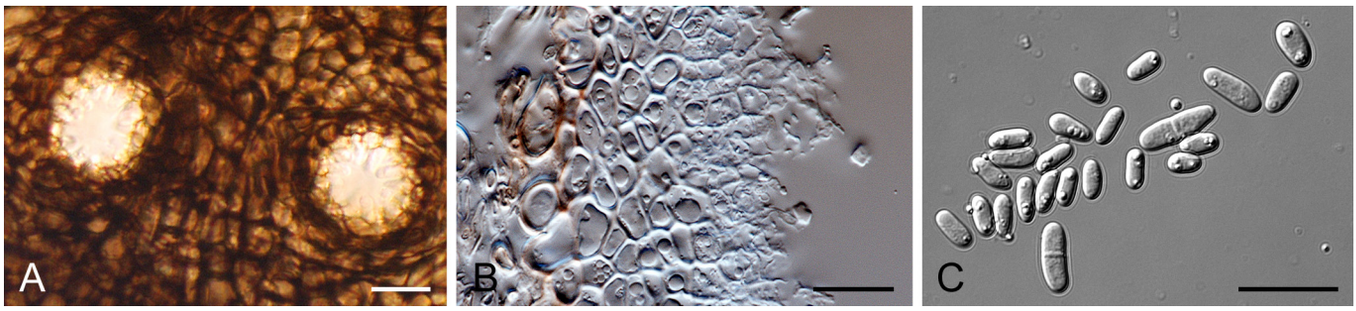


Figure 5.8. *Boeremia* gen nov. A. Ostiole configuration of *B. exigua* var. *exigua* (CBS 431.74). B. Pycnidial wall and conidiogenous cells of *B. telephii* (CBS 760.73). C. Aseptate and septate conidia *B. lycopersici* (CBS 378.67). Scale bars: A = 20 µm, B–C = 10 µm.

Notes: *Phoma diversispora* was originally described by Bubák as a pathogen of cowpea (*Vigna unguiculata*) causing Black Node Disease (van der Aa *et al.* 2000), but was later classified as a variety of *Ph. exigua* by Boerema & Van Kesteren (1980) and Boerema *et al*. (1981a), on basis of its morphology. The present study, however, revealed the *B. exigua* varieties to be phylogenetically distinct from the present species, which justifies re-establishment of the taxon as separate species in the genus *Boeremia*. The present species is closely related to *B. noackiana*, formerly known as *Ph. exigua* var. *noackiana* (see below).

Boeremia exigua var. ***coffeae*** (Henn.) Aveskamp, Gruyter & Verkley, **stat. et comb. nov.** MB515632.
Basionym: *Ascochyta coffeae* Henn., *Hedwigia* 41: 307. 1902; not *Phoma coffeae* Delacr., *Bull. Soc. Mycol. France* 13: 122. 1897.
= *Ascochyta tarda* R.B. Stewart, *Mycologia* **49**: 430. 1957.
≡ *Phoma tarda* (R.B. Stewart) H. Verm., *Coffee Berry Dis. Kenya*: 14. 1979.

For a complete description see de Gruyter *et al.* (2002).

Specimens examined: **Brazil**, Patrocínio, from leaf of *Coffea arabica*, L.H. Pfenning, CBS 119730. **Cameroon**, Bemenda, from *Coffea arabica*, CBS 109183 = PD 2000/10506 = IMI 300060.

Notes: *Boeremia exigua* var. *coffeae* was originally described from leaves of coffee plants (*Coffea arabica*, Stewart 1957) as *Ascochyta coffeae* and *A. tarda*. The observed late euseptation in this species proved to be a character common for *Phoma* species accommodated in section *Phyllostictoides*, leading to a recombination into *Phoma*, as *Ph. tarda*. Phylogenetic results obtained in the present study reveal genetic similarity between the present species and the *B. exigua* species complex. The cultures of *B. exigua* varieties are somewhat slower growing than those of the present species, which completely covers the agar surface (90 mm diam) within 7 d. The pycnidia of *B. exigua* var. *tarda* may grown to up to 255 µm (de Gruyter *et al.* 2002), but other micromorphological characters fit within the scope of *B. exigua* as described for Ph. exigua by van der Aa *et al.* (2000) and de Gruyter *et al.* (2002). It is concluded, therefore, that *Ph. tarda* should be reduced to a variety of the *B. exigua*. Multiple *Phoma* species have been found in association with *Coffea arabica*, such as *Ph. coffeae-arabicae*, *Ph. coffeicola*, *Ph. coffeiphila*, *Ph. costarricensis*, *Ph. excelsa*, and *Ph. pereupyrena* (Saccas 1981, Aveskamp *et al*. 2009a). None of these species however matches the description that is applied to taxa in the *B. exigua* complex.

Boeremia exigua var. ***exigua*** (Desm.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515624.
Basionym: *Phoma exigua* Desm., *Ann. Sci. Nat. Bot*. III **11**: 282. 1849.

Specimens examined: **Germany**, Artern, from *Foeniculum vulgare*, Apr. 1984, S. Petzoldt, CBS 391.84. **The Netherlands**, from a tuber of *Solanum tuberosum*, 1928, CBS 236.28; Emmeloord, from a tuber of *Solanum tuberosum*, 1974, G.H. Boerema, CBS 431.74 = PD 74/2447; Emmeloord, from *Cichorium*

intybus, 1979, G.H. Boerema, CBS 101150 = PD 79/118; Ommen, from *Digitalis* sp., 1990, J. de Gruyter, CBS 101152 = PD 90/835-3.

Boeremia exigua var. ***forsythiae*** (Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515625.
Basionym: *Phyllosticta forsythiae* Sacc., *Michelia* **1**(1): 93. 1997.
≡ *Phoma exigua* var. *forsythiae* (Sacc.) Aa, Boerema & Gruyter, *Persoonia* **17**: 452. 2000.

Specimens examined: **The Netherlands**, from *Forsythia* sp., 1992, J. de Gruyter, CBS 101213 = PD 92/959; from *Forsythia* sp., 1995, J. de Gruyter, CBS 101197= PD 95/721.

Boeremia exigua var. ***gilvescens*** Aveskamp, Gruyter & Verkley, **var. nov.** MycoBank MB515626. Figure 5.9.

Varietas Phomae exiguae similis, sed matrix conidiorum flavida vel luteola. In agaro et in mycelio aereo catenis cellularum inflatarum (11.5–)12.5–27.5(–31) × (5.5–)7.5–14.5(–18) µm.

Etymology: Varietal name refers to the yellow conidial matrix, which distinguishes this variety.

Culture characteristics: Colonies on OA 75–80 mm diam after 7 d, margin regular or irregular. Immersed mycelium sparsely visible due to coverage by the aerial mycelium, hyaline or black to greenish olivaceous, with many pycnidia; reverse mouse-grey to olivaceous. Colonies on MEA 70–75 mm diam after 7 d, margin regular or irregular. Immersed mycelium completely covered by a compact aerial mat, which is smoke-grey with some mouse-grey zones; reverse black. Colonies on CHA at least (75–)80 mm diam after 7 d, but often the agar surface is completely covered, margin regular or somewhat crenate. Immersed mycelium completely covered by a compact smoke-grey mat of aerial mycelium, or, in some zones floccose, olivaceous with white tufts; reverse shows a dendritic leaden-black zone around the colony centre, with black zones near the colony border. Application of NaOH did not have any effect.

Pycnidial and conidial shapes and sizes fit within the *Ph. exigua* species concept. Conidial matrix yellowish or pale luteous. Brown pigmented swollen cells occur in chains in the agar and in the aerial mycelium, measuring (11.5–)12.5–27.5(–31) × (5.5–)7.5–14.5(–18) µm.

Specimens examined: **Philippines**, from *Solanum tuberosum*,1990, L.J. Turkensteen, CBS 101156 = PD 90/731; **The Netherlands**; from a graft of *Ulmus*, 1961, H.M. Heybroek, CBS 373.61; Baarn, from leaves of *Dactylis purpurea*, 1970, H.A. van der Aa, **holotype designated here** CBS H-16281, culture ex-holotype CBS 761.70; Lisse, from *Dahlia*, 1982, J. de Gruyter, CBS 101151 = PD 82/1022.

Notes: This novel variety of *B. exigua*, distinguished from other *B. exigua* varieties on basis of DAF analysis (Aveskamp *et al.* 2009b), is closely related to *B. exigua* var. *exigua*, but different in the colour of its conidial matrix (yellowish) and absence of a positive reaction to NaOH. This variety may be identical to *Ph. exigua* var. *inoxydabilis* Boerema & Vegh, but as the type culture has been lost (van der Aa *et al.* 2000) a proper comparison of the varieties cannot be made. Additionally, *Ph. exigua* var. *inoxydabilis* was originally only known from periwinkle (*Vinca minor*, Vegh *et al.* 1974), whereas the strains associated to the present taxon are isolated from a wide range of host plants.

Boeremia exigua var. ***heteromorpha*** (Schulzer & Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515627.
Basionym: *Phoma heteromorpha* Schulzer & Sacc., *Hedwigia* **23**: 107. 1884.
≡ *Phoma exigua* var. *heteromorpha* (Schulzer & Sacc.) Noordel. & Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* **166**: 109.1989.

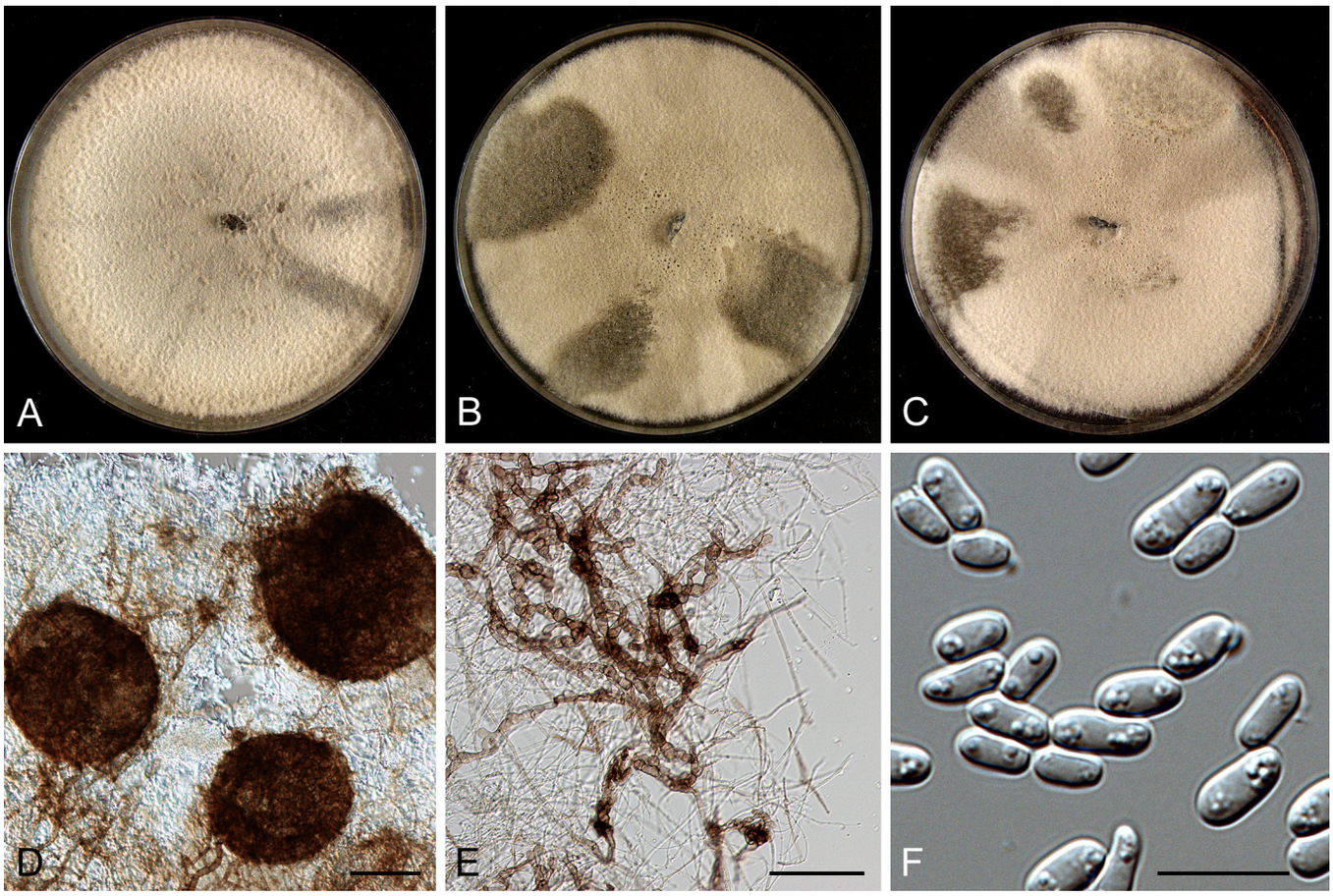


Figure 5.9. *Boeremia exigua* var. *gilvescens* (CBS 101150). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D. Pycnidia. E. Chains of wollen cells. F. Conidia. Scale bars: D = 100 µm, E = 100 µm, F = 10 µm.

Specimens examined: **France**, Antibes, from *Nerium oleander*, 1979, CBS 101196 = PD 79/176. **Italy**, Perugia, from *Nerium oleander*, 1994, A. Zazzerini, CBS 443.94.

Boeremia exigua var. ***lilacis*** (Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515628.
Basionym: *Phoma herbarum* f. *lilacis* Sacc., *Michelia* **2** (1): 93. 1880.

Specimen examined: **The Netherlands**, Wageningen, from a twig of *Syringa vulgaris*, June 1976, G.H. Boerema, CBS H-163131, culture CBS 569.79 = PD 72/741.

Notes: Although in the present study this variety clusters outside the *B. exigua* cluster, it is phylogenetic affiliation is ambiguous. In previous studies in which fingerprint markers and actin sequences were applied to delineate this species complex (Abeln *et al.* 2002, Aveskamp *et al.* 2009b) the present taxon clusters within *Ph. exigua*, and is therefore recombined as *B. exigua* var. *lilacis*. Further analysis of this complex is, however, advocated.

Boeremia exigua var. ***linicola*** (Naumov & Vassiljevsky) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515629.
Basionym: *Ascochyta linicola* Naumov & Vassiljevsky, *Mater. Micol. Fitopatol.* **5**: 3. 1926.

Specimens examined: **The Netherlands**, Zierikzee, from *Linum usitatissimum*, 1928, H.A. Diddens, CBS 114.28; Flevoland, from a stem of *Linum usitatissimum*, 1976, G.H. Boerema, CBS 116.76 = ATCC 32332 = CECT 20022 = CECT 20023 = IMI 197074 = PD 75/544.

Boeremia exigua var. ***populi*** (Gruyter & Ph. Scheer) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515630.
Basionym: *Phoma exigua* var. *populi* Gruyter & Ph. Scheer, *J. Phytopathol.* **146** (8-9): 413. 1998.

Specimens examined: **The Netherlands**, Deil, from a twig of *Populus* X *euramericana* cv. Robusta, Feb. 1993, A.J.Ph. Oort, **holotype** L 995.263.325, ex-holotype culture CBS 100167 = PD 93/217; Rotterdam, from *Salix* sp., 1982, J. de Gruyter, CBS 101202 = PD 82/942.

Boeremia exigua var. ***pseudolilacis*** Aveskamp, Gruyter & Verkley, **var. nov.** MycoBank MB515631. Figure 5.10.

Varietas haec in cultura habitu Phomae exiguae var. exiguae et var. gilvescentis similis, sed matrix conidiorum roseo-bubalina et citius crescens.

Etymology: Refers to the former placement in and close resemblance to *Ph. exigua* var. *lilacis*.

Colonies on OA 70–75 mm diam after 7 d, margin regular. Immersed mycelium black to greenish olivaceous, sparsely visible due to coverage by a mat of mouse-grey woolly to compact aerial mycelium; reverse mouse-grey to olivaceous. Colonies on MEA 70–75 mm diam. after 7 d, margin regular. Immersed mycelium completely covered by a compact aerial mat, which is smoke-grey with some mouse-grey to white zones; reverse black. Colonies on CHA slower growing, 70–80 mm diam after 7 d, margin regular, appearance similar as on MEA.

Application of NaOH did not have any effect. Pycnidial and conidial shapes and sizes fit within the *B. exigua* species concept. Conidial matrix rosy-buff.

Specimen examined: **The Netherlands**, near Boskoop, from *Syringa vulgaris*, 1994, J. de Gruyter, **holotype** CBS H-20371, culture ex-holotype CBS 101207 = PD 94/614.

Notes: This novel variety of *B. exigua*, distinguished from other *B. exigua* varieties on basis of DAF analysis (Aveskamp *et al.* 2009b) and AFLP (Abeln *et al.* 2002), is closely related to *B. exigua* var. *exigua* and *B. exigua* var. *gilvescens*. Upon collection, the strain representing *B. exigua* var. *pseudolilacis* has probably erroneously been identified as var. *lilacis* due to its host association.

Boeremia exigua var. ***viburni*** (Roum. ex. Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515633.
Basionym: *Ascochyta viburni* Roum. ex Sacc., *Syll. Fung.* **3**: 387. 1884.
≡ *Phoma exigua* var. *viburni* (Roum. ex. Sacc.) Boerema apud Gruyter & Ph. Scheer, *J. Phytopathol.* **146**: 414. 1998.

Specimens examined: **The Netherlands**, Boskoop, from *Viburnum opulus*, 1984, G.H. Boerema, CBS 100354 = PD 83/448; from *Lonicera* sp., 1993, J. de Gruyter, CBS 101211 = PD 93/838.

Boeremia foveata (Foister) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515653.
Basionym: *Phoma foveata* Foister, *Trans. & Proc. Bot. Soc. Edinburgh* **33**: 66. 1940.

Specimens examined: **Bulgaria**, from a tuber of *Solanum tuberosum*, 1994, J. de Gruyter, CBS 109176 = CECT 2828 = PD 94/1394. **UK**, from a tuber of *Solanum tuberosum*, Mar. 1937, C.E. Foister, ex-isotype culture CBS 200.37; Northern Ireland, Belfast, from a tuber of *Solanum tuberosum*, 1966, C. Logan, CBS 341.67 = CECT 20055 = IMI 331912.

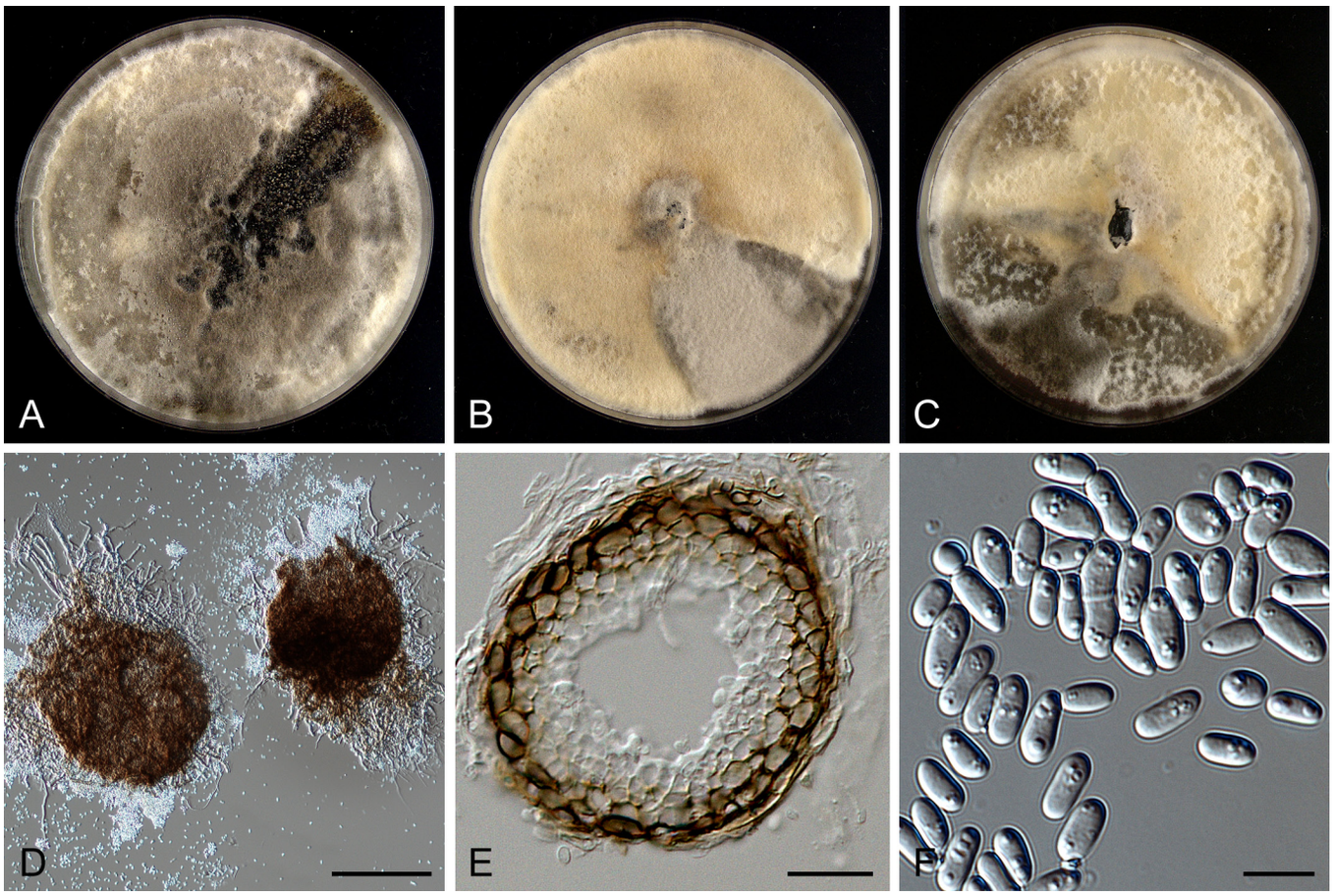


Figure 5.10. *Boeremia exigua* var. *pseudolilacis* (CBS 101207). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D. Pycnidia. E. section of young pycnidium. F. Conidia. Scale bars: D = 100 µm, E = 20 µm, F = 5 µm.

Boeremia hedericola (Durieu & Mont.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515634.
Basionym: *Phyllosticta hedericola* Durieu & Mont., *Flore d'Algérie Cryptog*. **1**: 611. 1849.
≡ *Phoma hedericola* (Durieu & Mont.) Boerema, *Trans. Brit. Mycol. Soc.* **67**: 295. 1976.

Specimens examined: **The Netherlands**, Meppel, from a leaf of *Hedera helix*, 1970, CBS 366.91 = PD 70/811; from *Hedera helix*, 1987, J. de Gruyter, CBS 367.91 = PD 87/229.

Note: Strain CBS 367.91 is sterile.

Boeremia lycopersici (Cooke) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515635.
Basionym: *Phoma lycopersici* Cooke, *Grevelia* **13**: 94. 1885.
Teleomorph: *Didymella lycopersici* Kleb., *Z. Pflanzenkrankh.* **31**: 9. 1921.

Specimens examined: **The Netherlands**, Heerde, from fruit of *Lycopersicon esculentum*, Aug. 1967, G.H. Boerema, CBS 378.67 = PD 76/276; from *Lycopersicon esculentum*, 1984, J. de Gruyter, CBS 109172 = PD 84/143.

Boeremia noackiana (Allesch.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515636.
Basionym: *Phyllosticta noackiana* Allesch., *Bol. Inst. Agron. Campinas* **9**: 85. 1898.
≡ *Phoma exigua* var. *noackiana* (Allesch.) Aa, Boerema & Gruyter, *Persoonia* **17**: 450. 2000.

For a complete description see van der Aa *et al.* (2000).

Specimens examined: **Colombia**, from *Phaseolus vulgaris*, 1979, J. de Gruyter, CBS 101203 = PD 79/1114. **Guatemala**, from *Phaseolus vulgaris*, 1987, IPO Wageningen, CBS 100353 = PD 87/718.

Notes: *Boeremia noackiana* is genetically a sister species to *B. diversispora*, and was also noted by Boerema *et al*. (2004) as "the American cousin". Just like *B. diversispora*, the present species is known from beans, although the main host appears to be *Phaseolus vulgaris.* The two species have many characters in common with *B. exigua* (van der Aa *et al.* 2000, Boerema *et al.* 2004) and with each other, but are distinguished based on enzyme analysis (Obando-Rojas, 1989) and molecular fingerprinting methods such as AFLP (Abeln *et al.* 2002) and DAF (Aveskamp *et al.* 2009b). Additionally, *B. noackiana* is characterised by a relative fast growth rate on MEA: (6–)6.5–7.5 mm diam after 7 d, and is further distinguished from *B. diversispora* by its relatively uniform conidia. Due to the relatively large genetical distance to the *B. exigua* complex, this taxon is elevated to species level.

Boeremia sambuci-nigrae (Sacc.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515637.
Basionym: *Phoma herbarum* f. *sambuci-nigrae* Sacc., *Syll. Fung*. **3**: 133. 1884.
≡ *Phoma exigua* var. *sambuci-nigrae* (Sacc.) Boerema & Höweler, *Persoonia* **5**(1): 26. 1967.
≡ *Phoma sambuci-nigrae* (Sacc.) E. Monte, Bridge & B. Sutton, *Mycopathologia* **115**: 102. 1991.

Specimens examined: **The Netherlands**, Wageningen, from a leaf of *Sambucus nigra*, 1967, **lectotype** CBS H-16314, ex-lectotype culture CBS 629.68 = CECT 20048 = IMI 331913 = PD 67/753; Baarn, Maarschalksbos, from a leaf of *Sambucus nigra*, Nov. 1967, H.A. van der Aa, CBS 104.68= CECT 20010; from *Sambucus nigra*, 1975, G.H. Boerema, CBS 109170 = PD 75/796.

Boeremia strasseri (Moesz) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515638.
Basionym: *Phoma strasseri* Moesz, *Bot. Közlem.* **22**: 45. 1924. nom. nov. pro *Phoma menthae* Strasser, *Verh. zool. Bot. Ges. Wien* **60**: 317. 1910 [non *Phoma menthae* Roum. (date of publication unknown)].

Specimens examined: **The Netherlands**, Arnhem, from a stem of *Mentha* sp., 1973, CBS 126.93 = PD 73/642. **USA**, Oregon, from *Mentha piperita*, 1970, H.A. van der Aa, CBS 261.92 = ATCC 244146 = PD 92/318.

Note: As the older name *Ph. menthae* is illegitimate, the epithet '*strasseri*' prevails.

Boeremia telephii (Vestergr.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515639.
Basionym: *Ascochyta telephii* Vestergr*., Öfvers. Förh. Kongl. Svenska Vetenska.-Akad.* **54**: 41. 1897.
≡ *Phoma telephii* (Vestergr.) Kesteren, *Netherlands J. Pl. Pathol.* **78**: 117.1972.

Specimens examined: **The Netherlands**, Utrecht, from a stem of *Sedum telephium*, 1971, G.H. Boerema, CBS 760.73 = PD 71/1616; from *Sedum spectabile*, 1975, G.H. Boerema, CBS 109175 = PD 79/524.

Group O:

Three species are clustered in group O, which all were accommodated in the Boeremaean section *Phoma*. These species, *Ph. multirostrata*, *Ph. pereupyrena* and *Ph. insulana* are characterised by the production of small (5–15 µm diam), unicellular chlamydospores, comparable to those formed by some species in group K. The absence of septate conidia and a thin pycnidial wall are further characters of the species accommodated in group O.

The strains accommodated in *Ph. multirostrata* reveal a high variation in spore size. Boerema *et al.* (1986) introduced three varieties within this species, but based on morphological observations and DNA sequence analyses, these varieties were not recognised by later researchers and thus the varieties were merged again (Morgan-Jones 1988, Aveskamp *et al.* 2009a).

Phoma insulana (Mont.) Boerema & Malathr., in Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* **158** (Jaarboek 1981): 28. 1982.
Basionym: *Phyllosticta insulana* Mont., *Ann. Sci. Nat. Bot. IV* **5**: 343.1856.

Specimen examined: **Greece**, from the berries of *Olea europaea*, 1980, G.H. Boerema, CBS 252.92 = PD 80/1144.

Phoma multirostrata (Ph.N. Mathur, S.K. Menon & Thirum.) Dorenb. & Boerema, *Mycopathol. Mycol. Appl.* **50** (3): 256. 1973, emend. Aveskamp *et al. Mycologia* **101**: 375. 2009.
Basionym: *Sphaeronaema multirostratum* Ph.N. Mathur, S.K. Menon & Thirum., *Sydowia* **13**: 146. 1959. (as '*Sphaeronema*').

Specimens examined: **India**, Maharashtra, Poona, Talegaon, from poultry farm soil, Mar. 1959, M.J. Thirumalachar, **isotype** CBS H-7616, culture CBS 274.60 = IMI 081598; Maharashtra, Poona, Talegaon, from soil, Mar. 1959, M.J. Thirumalachar, CBS H-16499, culture CBS 368.65 = PD 92/1757. **The Netherlands**, Hoorn, greenhouse, from the stem of *Cucumis sativus*, Aug. 1967, G.H. Boerema, CBS H-16502, culture CBS 110.79 = PD 65/8875. Unknown origin, from *Cucumis sativus*, 1983, PD 83/48.

Phoma pereupyrena Gruyter, Noordel. & Boerema, *Persoonia* **15** (3): 390. 1993.

Specimen examined: **India**, from a leaf of *Coffea arabica,* 1976, CBS 267.92 = PD 76/1014.

Group P:
This well-supported clade (BPP = 1.00, RBS = 97 %) comprises *Ph. dictamnicola* and *Ph. sylvatica*, which are both associated with the section *Sclerophomella* (Boerema *et al.* 1998). In addition, both varieties of *Ph. poolensis* are recovered here. As in the *Sclerophomella* species, an ostiole is commonly absent in *Ph. poolensis* var. *poolensis*, a character which supports the sequence data found in the present study. In contrast, the second variety of this species, *Ph. poolensis* var. *verbascicola*, always produces ostiolate pycnidia (de Gruyter *et al.* 1993). Both *Ph. poolensis* varieties can further be differentiated on the basis of the β-tubulin sequence, and are morphologically distinguishable in the colour of the conidial matrix. The conidia of the type variety are on average somewhat smaller, measuring *ca*. 3.5–5 × 1.5–2 µm, than those of var. *verbascicola*, which measure 3.5–5.5 × 1.5–2.5 µm. Both varieties are known from plant hosts belonging to the *Scophulariaceae*, but whereas var. *poolensis* is recorded as causal agent of leaf spots and basal stem rot in snapdragon (*Antirrhinum majus*), var. *verbascicola* is only known as saprobe of *Verbascum* spp., although inoculation trials indicated that it may also have a role as pathogen (Boerema *et al.* 2004). Given all these differences, it is considered to be justified to erect a separate species for *Ph. poolensis* var. *verbascicola* as *Ph. novae-verbascicola*.

Although none of the species in this group has been confirmed to have a teleomorph (Boerema *et al.* 1998), it has been suggested that *Didymella winteriana* is the teleomorph of *Phoma sylvatica* (Munk 1957). Given the topology of the tree, this association with a *Didymella* species is plausible, although a sexual structure was not observed in the present study, nor in the previous studies of Boerema & de Gruyter (1998).

Phoma dictamnicola Boerema, Gruyter & Noordel., *Persoonia* **15** (1): 90. 1992.

Specimen examined: **The Netherlands**, Arnhem, from a stem of *Dictamnus albus*, 1974, J. de Gruyter, CBS 507.91 = PD 74/148.

Phoma novae-verbascicola Aveskamp, Gruyter & Verkley, **nom. nov.** pro *Phyllosticta verbascicola* Ellis & Kellerm. MycoBank MB515640.

Basionym: *Phyllosticta verbascicola* Ellis & Kellerm., *Bull. Torrey Bot. Club* 11: 115. 1884.
≡ *Phoma poolensis* var. *verbascicola* (Ellis & Kellerm.) Aa & Boerema, in de Gruyter, Noordeloos & Boerema, *Persoonia* **15** (3): 385. 1993. Not *Phoma verbascicola* (Schwein.) Cooke, in Ravenel. 1878.

Etymology: The epithet refers to the host plant, *Verbascum* spp.

For a full description see de Gruyter *et al.* (1993).

Specimens examined: **The Netherlands**, Zeist, Abburg nursery, **holotype** L 9893.00.134; Haarlem, from dead stem material of *Verbascum densiflorum*, 1992, J. de Gruyter, CBS 127.93 = PD 92/347; from stem of *Verbascum* sp., 1974, G.H. Boerema, CBS 114.93 = PD 74/228.

Notes: This species is distinguishable from *Ph. poolensis* by to the presence of 1–2(–5) ostioles, the colourless to whitish matrix and the smaller conidia. On MEA, the aerial mycelium is more compact or woolly than that of *Ph. poolensis*.

The variety epithet could not be elevated to species level, as *Phoma verbascicola* is already occupied. This basionym, however, probably refers to immature pseudothecia of a *Pleospora* species (Boerema *et al.* 1996). Therefore, a new name is proposed here for the present species.

Phoma poolensis Taubenh., *Dis. Greenhouse Crops* **203**. 1919.

Specimens examined: **Denmark**, from a stem of *Antirrhinum majus*, July 1938, Ph. Neergaard, CBS 253.38. **The Netherlands**, Wageningen, from a stem of *Scrophularia nodosa*, 1974, G.H. Boerema, CBS 115.93 = PD 74/206; Bennekom, from a stem of *Antirrhinum majus*, 1973, G.H. Boerema, CBS 116.93= PD 71/884. Unknown origin and substrate, 1920, E.M. Smiley, CBS 113.20 = PD 92/774.

Phoma sylvatica Sacc. *Michelia* **2** (2): 337. 1881.

Specimens examined: **The Netherlands**, Wageningen, from *Melampyrum pratense*, 1983, J. de Gruyter, CBS 135.93 = PD 83/87; Wageningen, from a stem of *Melampyrum pratense*, 1993, J. de Gruyter, CBS 874.97 = PD 93/764.

Group Q:

The *Phoma* species embedded in this group, *Ph. commelinicicola* and *Ph. eupatorii* are morphologically distinct, hence their accommodation in the sections *Phoma* and *Macrospora* respectively. The accommodation of *Chaetasbolisia erysiphoides* in this clade, the type of its genus, is unexpected. Attempted morphological studies revealed that this strain was sterile, and therefore recombination of the species could not be supported by morphological data. The descriptions provided in literature (Sutton 1980, Patel *et al.* 1997, Reynolds 1999) suggest, however, that this genus could very well represent a group of setose *Phoma* species, although this cannot be resolved due to a lack of isolates. The presence of setae is not recorded in other species in group Q, and moreover, is within the *Didymellaceae* only known from *Peyronellaea gardeniae* (Group K), and from pycnidia in some older cultures from *Epicoccum sorghi* (Group M), *Peyronellaea glomerata* (Group K) and *Phoma herbarum* (Boerema *et al*. 2004). The topology and the clustering of these species cannot be further explained by the morphology or ecology, nor by their geographical distribution.

Phoma commelinicola (E. Young) Gruyter, *Persoonia* **18** (1): 93. 2002.
Basionym: *Phyllosticta commelinicola* E. Young, *Mycologia* **7**: 144. 1915.

Specimen examined: **New Zealand**, South Auckland, Alfriston, from *Tradescantia* sp., 1997, K. Ramsay, CBS 100409.

Phoma eupatorii Died., *Ann. Mycol.* **10** (5): 447. 1912.

Specimen examined: **The Netherlands**, Arnhem, from *Eupatorium cannabinum*, 1977, G.H. Boerema, CBS 123.93 = PD 77/1148.

Group R:

This group comprises five species that previously were accommodated in the sections *Phoma*, *Peyronellaea* and *Phyllostictoides*. As the name of *Ph. tropica* already suggests, it concerns a thermotolerant species, which is mainly found in European greenhouses on a wide range of hosts, but which probably has a tropical origin (Schneider & Boerema 1975), as do most other species found in the present clade. The sole host of *Ph. costarricensis* is coffee bean (*Coffea arabica*), while *Ph. piperis* is associated with Indian Long Pepper (*Piper longus*), and the novel species *Ph. minor* has been isolated twice from clove (*Syzygium aromaticum*) in Indonesia. In addition, *Ph. labilis* is a warmth-preferring plurivorous species that has been isolated in European greenhouses and from nature in the Middle East, Turkey and Indonesia (Boerema *et al.* 2004). *Phoma zantedeschiae* is widespread throughout the Western Hemisphere, but always in association with arum or calla (*Zantedeschia* sp.), a genus that is indigenous in southern Africa (Boerema & Hamers 1990). Thus far, however, no data of temperature-growth studies are available for these species except for *Ph. tropica*. Several other thermotolerant species, such as *Ph. calidophila*, *Ph. calorpreferens* and *Ph. multirostrata*, are, however, not accommodated in this group. These three species are soil-borne, in contrast to *Ph. tropica* and *Ph. costarricensis*, which are associated with leaf-spots.

Phoma tropica and *Ph. costarricensis* are both closely related, and colony characters are highly similar. However, the strains available revealed a significant difference in conidial and pycnidial sizes, consistent with the data obtained in previous studies (Schneider & Boerema 1975, de Gruyter & Noordeloos 1992).

Phoma costarricencis Echandi, *Rev. Biol. Trop.* **5**: 83. 1957.

Specimens examined: **Nicaragua**, from a twig of *Coffea* sp., 1991, CBS 506.91 = PD 91/876 = IMI 215229. Unknown origin, from *Coffea arabica*, 1979, CBS 497.91 = PD 79/209.

Notes: Strain CBS 497.91 was initially identified as *Ph. tropica*. The close phylogenetic association between this species and *Ph. costarricensis* concurs with their overlapping morphological characters (see Schneider & Boerema 1975, de Gruyter & Noordeloos 1992).

Phoma labilis Sacc., *Michelia* **2** (7): 341. 1881.

Specimens examined: **Israel**, from a stem of *Rosa* sp., 1970, G.H. Boerema, CBS 479.93 = PD 70/93. **The Netherlands**, Barendrecht, from a stem of *Lycopersicon esculentum*, 1987, J. de Gruyter, CBS 124.93 = PD 87/269.

Phoma minor Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515641. Figure 5.11.

Conidia ellipsoidea, ovoidea vel leniter allantoidea, glabra, hyalina, continua, (3–)3.5–4.5(–5) × 1.8–2.5(–3) µm, (0–)1–3(–4) guttulis minutis praedita. Matrix conidiorum alba.

Etymology: Epithet derived from the small-sized conidia.

Conidiomata pycnidial, solitary, (sub-)globose to broadly ellipsoidal, glabrous or with some hyphal outgrows, on the agar surface and immersed, (125–)150–280(–330) × (125–)150–220(–245) µm. *Ostioles* (1–5), slightly papillate or non-papillate. *Pycnidial wall* pseudoparenchymatous, composed of oblong to isodiametric cells, outer cell layer pigmented, 2–4 layers, 8–15 µm thick. *Conidiogenous cells* phialidic,

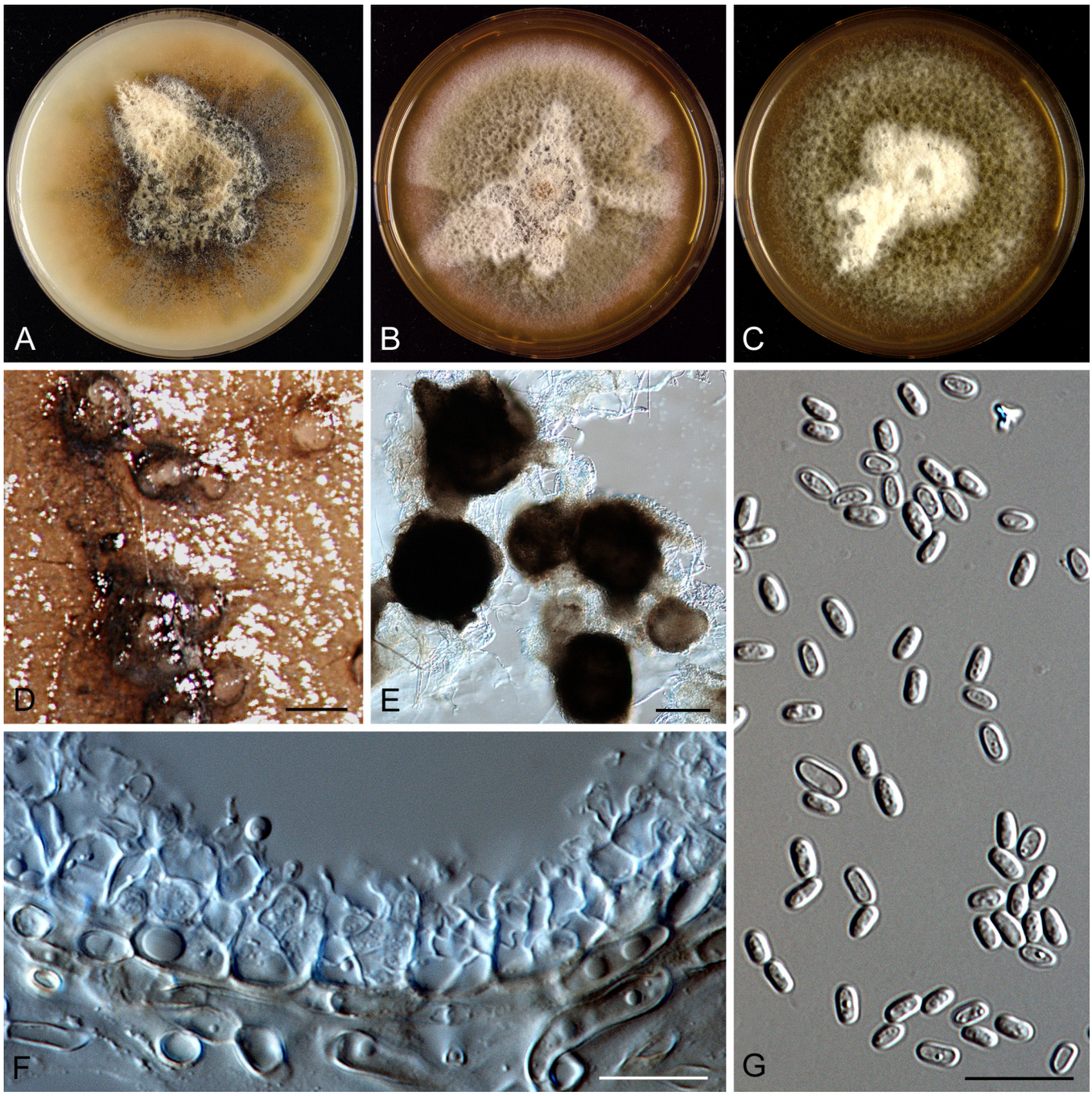


Figure 5.11. *Phoma minor* (CBS 325.82). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D–E. Pycnidia. F. Section of the pycnidial wall. G. Conidia. Scale bars: D = 200 µm, E = 100 µm, F–G = 10 µm.

hyaline, simple, smooth, flask-shaped or somewhat isodiametric, *ca*. 4–5.5(–6.2) × 3–4.5(–4.7) µm. *Conidia* ellipsoidal to ovoid or slightly allantoid, thin-walled, smooth, hyaline, aseptate (3–)3.5–4.5(–5) × 1.8–2.5 (–3) µm, with (0–)1–3(–4) minute guttules. Conidial matrix white.

Culture characteristics: Colonies on OA (44–)45–50(–54) mm diam after 7 d, margin regular. Aerial mycelium flat, grey, but locally well-developed in densely floccose white tufts. Immersed mycelium olivaceous with rosy-buff tinges near the colony margin; reverse concolourous. Colonies on MEA 46–48 mm diam after 7 d, margin regular. Immersed mycelium hyaline, with abundant semi-immersed pycnidia, but almost completely covered by an aerial mycelial mat. Aerial mycelium pluriform, with a compact white mat and some felty glaucous grey or dull green zones, near colony margin white; reverse black to grey olivaceous. Colonies on CHA 50–54 mm diam. after 7 d, margin regular. Aerial mycelium similar as on MEA, although the felty white and glaucous grey zones are less abundant; reverse slate blue to leaden-black. Application of NaOH results in a greenish yellow discolouration of the agar, best to be observed on OA medium.

Specimens examined: **Indonesia**, Sumatra, from *Syzygium aromaticum*, Apr. 1982, R. Kasim, **holotype designated here** CBS H-20236, ex-holotype culture CBS 325.82; Lampung, from *Syzygium aromaticum*, Dec. 1982, H. Vermeulen, CBS 315.83.

Notes: As for *Ph. eucalyptica*, this species has been recorded in association with clove trees (*Syzygium aromaticum*, Boerema *et al.* 2004). Both species, although genetically distinct, have many characters in common, notably the colony characters on OA, the high variation in ostiole number and a similar reaction to application of NaOH. Although *Phoma minor* produces relatively small conidia, the conidia of *Ph. eucalyptica* are even smaller, measuring only 2–4 × 1–2 µm (de Gruyter & Noordeloos 1992).

Phoma piperis (Tassi) Aa & Boerema, *Persoonia* **15** (3): 398. 1993.
Basionym: *Phyllosticta piperis* Tassi, *Boll. Reale Orto Bot. Siena* **3**(2): 28. 1900.

Specimens examined: **The Netherlands**, Tiel, from a leaf of *Peperomia pereskiifolia*, 1988, J. de Gruyter, CBS 268.93 = CBS 108.93 = PD 88/720; Tiel, from *Peperomia* sp., 1990, J. de Gruyter, PD 90/2011.

Phoma tropica R. Schneid. & Boerema, *Phytopathol. Z.* **83** (4): 361. 1975.

Specimen examined: **Germany**, Horrheim, from *Saintpaulia ionantha*, 1973, R. Schneider, **isotype** CBS H-7629, ex-isotype culture CBS 436.75.

Phoma zantedeschiae Dippen., *S. African J. Sci.* **28**: 284. 1931.

Specimen examined: **The Netherlands**, from a bulb of *Zantedeschiae* sp., 1969, G.H. Boerema, CBS 131.93 = PD 69/140.

*Group S **Stagonosporopsis***:

This large group (BPP = 1.00, RBS = 55 %) comprises mainly species with *Stagonosporopsis* synanamorphs. In the Boeremaean classification system, these species were embedded in *Phoma* section *Heterospora* (Boerema *et al.* 1997). As with the other sections, this group also appeared to be artificial. Based on LSU and SSU sequences, the type species of the section *Heterospora*, *Ph. heteromorphospora*, clusters outside the *Didymellaceae* (de Gruyter *et al*. 2009), as do *Ph. samarorum* and *Ph. dimorphospora*. Three species, *Ph. clematidina*, *Ph. glaucii* and *Ph. aquilegiicola* form a separate clade (Group C) within the *Didymellaceae*, and are treated above. Also *Ph. nigripycnidia* and *Ph. narcissi* are not accommodated here.

In contrast to the *Heterospora* species that are absent in this clade, several current *Phoma* taxa recovered here have been associated with the section *Phyllostictoides*, such as *Ph. artemisiicola*, *Ph. caricae-papayae*, *Ph. cucurbitacearum*, *Ph. heliopsidis*, *Ph. rudbeckiae*, and the quarantine-organisms *Ph. ligulicola* var. *ligulicola* and var. *inoxydabilis* (de Gruyter 2002). These are all included in subclade S1 (BPP = 0.93, RBS = 73 %). These species do produce a percentage of multicellular conidia in culture that are often considerably larger than the regular aseptate ones. However, Boerema *et al.* (1997) decided to exclude the *Ph. ligulicola* varieties and *Ph. cucurbitacearum* from section *Heterospora*, as the sizes of the *Stagonosporopsis*-like conidia do not always exceed that of the aseptate conidia in these species. A sister clade to subclade S1 is S2, which hosts the potato pathogens *Ph. andigena* and *Ph. crystalliniformis* – formerly known as *Ph. andina* var. *crystalliniformis*. Both species originate from the Andes region, and are regarded as serious quarantine pathogens in large parts of the world (Smith *et al*. 1992).

In addition, three other subclades can be recognised in this clade. One (S3) comprises the species *Ph. schneiderae* and *Ph. subboltshauseri* (both of the section *Heterospora*) as well as *Ph. astragali*. This species is known as a pathogen of *Astragalus* spp., and is characterised by a high percentage of "distorted" conidia, but thus far, no records have been made of a *Stagonosporopsis*-like synanamorph. Whereas records of *Ph. astragali* and *Ph. schneiderae* are mainly limited to the American continent, *Ph. subboltshauseri* appears to occur worldwide on *Fabaceae*. However, Boerema *et al.* (2004) suggested that the original host of this

species may have been *Phaseolus*, which is native to the Americas.

A fourth and fifth (S4, S5) subclade in this group comprise species that are accommodated in section *Phoma*, and therefore lack any further features than a plain, globose pycnidium and aseptate, hyaline conidia. The species found here are *Ph. dorenboschiae*, *Ph. loticola* (both S4), *Ph. ajacis* and *Ph. valerianellae* (both S5).

In group S several taxa have been found with a teleomorph in *Didymella*, such as *Ph. ligulicola* var. *ligulicola* (telecom *D. ligulicola* var. *ligulicola*), *Ph. ligulicola* var. *inoxydabilis* (*D. ligulicola* var. *inoxydabilis*), and *Ph. cucurbitacearum* (*D. bryoniae*). Also the teleomorph of *Ph. caricae-papayae* has been recovered in this study, and found to be a *Didymella*, which is in line with the other teleomorph observations in this clade. The current teleomorph state of this species is accommodated in *Mycosphaerella* as *M. caricae* (Sivanesan 1990).

As the species in the present clade form a well-defined group within the *Didymellaceae*, the taxa are recombined into the genus *Stagonosporopsis*. This further implies that the names of the *Stagonosporopsis* synanamorphs of *Ph. samarorum* and *Ph. narcissi* (*S. fraxini* and *S. curtisii* respectively) should no longer be used.

Stagonosporopsis Died., *Ann. Mycol.* **10** (2): 142. 1912. **emend.** Aveskamp, Gruyter & Verkley. Figure 5.12.

Conidiomata pycnidial, globose to subglobose, measuring 70–300 µm diam, on agar surface or immersed, solitary or confluent, ostiolate or poroid. *Pycnidial wall* pseudoparenchymatous, counting 2–6 cell layers of which the outer 1–3 are brown/olivaceous pigmented. *Conidiogenous cells* phialidic, hyaline, simple, smooth, ampulliform or doliiform, *ca*. 4–7.5 × 3–6 µm. *Conidia* often in two types: majority aseptate, hyaline, ellipsoidal to subglobose, thin-walled, smooth, measuring (3–)3.5–10 × 1.5–3(–3.5) µm. Conidia of the second type can be produced both *in vivo* and *in vitro* in the same pycnidia as the smaller spores, unicellular or with up to 3 septa, measuring up to 30 × 8 µm. *Pseudothecia*, if present, occurring only *in vivo*, globose to subglobose, sometimes with a somewhat conical neck, measuring 90–230 µm diam. *Asci* cylindrical or subclavate, measuring 50–90 × 9–13 µm, always 8-spored, biseriate. *Ascospores* ellipsoid, fusiform or obovoid, measuring 12–18 × 4–7 µm, uniseptate, guttulate.

Stagonosporopsis actaeae (Allesch.) Died., *Ann. Mycol.* **10**: 141. 1912.
Basionym: *Actinonema actaeae* Allesch., *Ber. bayer. bot. Ges*. **5**: 7. 1897.
= *Phoma actaeae* Boerema, Gruyter & Noordeloos, *Persoonia* **16** (3): 347. 1997.

Specimens examined: **The Netherlands**, Zeist, from a stem of *Cimicifuga simplex*, 1974, G.H. Boerema, CBS 105.96 = PD 74/230; Limburg, Schaersbergerbos, from a leaf of *Actaea spicata*, 1994, J. de Gruyter, L 992.167.501, culture CBS 106.96 = PD 94/1318.

Notes: In contrast to the earlier description of the *Phoma* anamorph of this species (Boerema *et al.* 1997), the larger conidia regularly produces up to 3-septate conidia (see **Fig 5.12A**). In the study mentioned above and in the present one the same strains were examined morphologically.

Stagonosporopsis ajacis (Thüm.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515653.
Basionym: *Phyllosticta ajacis* Thüm., apud Bolle & von Thümen, *Boll. Soc. Adriat. Sci. Nat. Trieste* **6**: 329. 1880.
= *Phoma ajacis* Aa & Boerema, apud Gruyter, Noordeloos & Boerema, *Persoonia* **15** (3): 383. 1993.

Specimens examined: **Kenya**, from *Delphinium* sp., 1990, Hopman, L 993.034.225, culture CBS 177.93 = PD 90/115. **The Netherlands**, Ter Aar, from *Delphinium* sp., 1986, CBS 176.93 = PD 86/547.

Stagonosporopsis andigena (Turkenst.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515655.
Basionym: *Phoma andigena* Turkenst., apud Boerema, Gruyter & Noordeloos, *Persoonia* **16** (1): 131. 1995.

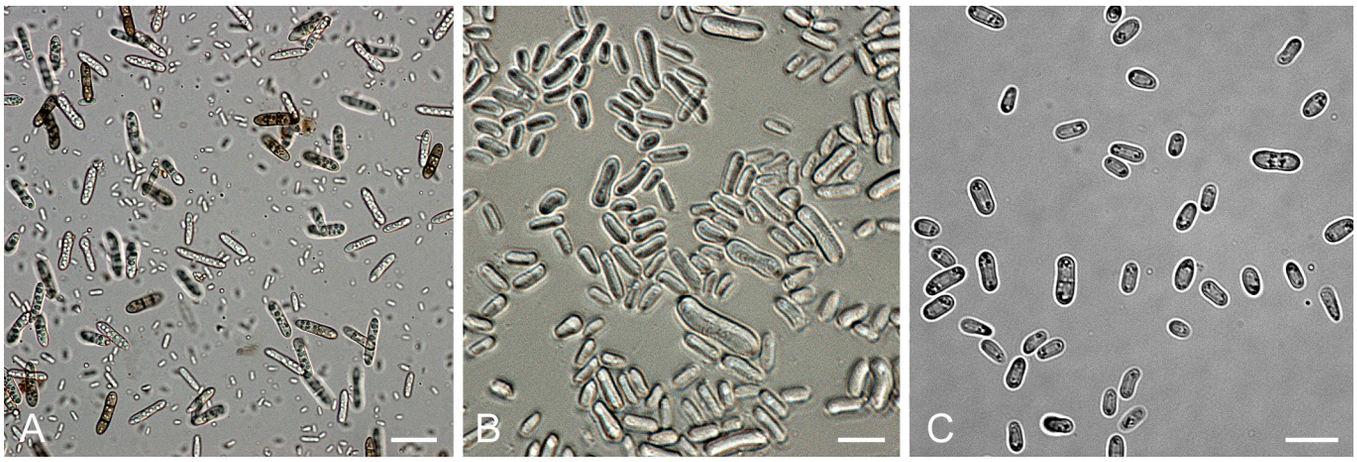


Figure 5.12. Conidial dimorphism in three species of *Stagonosporopsis*. A. *S. actaeae* (CBS 106.96). B. *S. lupini* (CBS 101494). C. *S. cucurbitacearum* (CBS 109171). Scale bars: A = 20 µm, B–C = 10 µm.

Specimens examined: **Peru**, Dep. Junin, Huancayo, near Vallis Mantaro, from a leaf of *Solanum* sp., 1975, L.J. Turkensteen, CBS 101.80 = PD 75/909 = IMI 386090; Dep. Junin, Huancayo, near Vallis Mantaro, from a leaf of *Solanum* sp., 1975, L.J. Turkensteen, CBS 269.80 = PD 75/914.

Stagonosporopsis artemisiicola (Hollós) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515656.
Basionym: *Phoma artemisiicola* Hollós, *Mat. Természettud. Közlem.* **35**: 40. 1926. (as '*artemisaecola*')

Specimen examined: **France**, from a stem base of *Artemisia dracunculus*, 1973, CBS 102636 = PD 73/1409.

Stagonosporopsis astragali (Cooke & Harkn.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515657.
Basionym: *Phoma astragali* Cooke & Harkn., *Grevillea* **13**: 111. 1885.

Specimen examined: Unknown origin, from *Astragalus* sp., 1925, A.W. Archer, CBS 178.25 = MUCL 9915.

Stagonosporopsis caricae (Sydow & Ph. Sydow) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515658.
Basionym: *Mycosphaerella caricae* Sydow & Ph. Sydow, *Ann.. Mycol.* **11**: 403. 1913.
≡ *Phoma caricae-papayae* (Tarr.) Punith., *Trans Brit. Mycol. Soc.* **75**: 340. 1980.
≡ *Ascochyta caricae-papayae* Tarr., *The fungi and plant diseases of Sudan*: 53. 1955.
= *Ascochyta caricae* Pat., *Bull. Soc. Mycol. France* **7**: 178. 1891.
≡ *Phoma caricae* Punith., *CMI Descriptions of Pathogenic Fungi and Bacteria* **634**: 1. 1979.

For description of the teleomorph see Sivanesan (1990). Punithalingam (1979b) provides an extensive description, *Ph. caricae*, a synonym of the anamorph stage.

Specimens examined: **Brazil**, from *Carica papaya*, 2006, J. de Gruyter, PD 06/03082531. **Chile**, from fruit of *Carica papaya*, Feb. 1990, H.A. van der Aa, CBS 248.90. **Indonesia**, Java, Segunung, from *Brassica* sp., Feb. 1976, H. Vermeulen, CBS 282.76.

Notes: *Phoma caricae-papayae* has been associated with an undescribed teleomorph state in *Mycosphaerella* or *Didymella* (Boerema *et al.* 2004). Sivanesan (1990) synonymised *Ph. caricae* with *M. caricae*, apparently not noting that *Ph. caricae* already was synonymised with *Ph. caricae-papayae* by Punithalingam (1980). As *Mycosphaerella* is phylogenetically unrelated to *Phoma* (de Gruyter *et al.* 2009), this taxonomic association is unlikely, and the observed sexual state observed was probably *Didymella*-like.

This species has solely been associated with pawpaw (*Carica papaya*, *Caricaceae*), but a single strain, deposited at CBS as *D. exigua* and that was isolated from *Brassica* leaves from Java, Indonesia (CBS 282.76), was genetically identical to the reference strain of *Ph. caricae-papayae*. Herbarium material of this strain consisted of an inoculated lupine stem on cornmeal agar (CBS H-11960) and represented a conidial state similar to this of *Ph. caricae-papayae*. This indicated that probably the *Didymella* teleomorph had been observed, but that it was preserved under an incorrect name as it was only distantly related to the ex-type strain of *Didymella exigua* (CBS 183.55). This finding provides evidence that *S. caricae* is not restricted to pawpaw.

Stagonosporopsis crystalliniformis (Loer., R. Navarro, M. Lôbo & Turkenst.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515659.
Basionym: *Phoma andina* var. *crystalliniformis* Loer., R. Navarro, M. Lôbo & Turkenst., *Fitopatología* **21** (2): 100. 1986.
≡ *Phoma crystalliniformis* (Loer., R. Navarro, M. Lôbo & Turkenst.) Noordel. & Gruyter, apud Noordeloos, de Gruyter, van Eijk & Roeijmans, *Mycol. Res.* **97**: 1344. 1993.

Specimens examined: **Colombia**, Antioquia, Rionegro, from a stem base of *Lycopersicon esculentum*, 1983, R. Navarro, **holotype** CBS H-3926, ex-holotype culture CBS 713.85 = ATCC 76027 = PD 83/826; Guachacal, from a leaf of *Solanum tuberosum*, Nov. 1985, W.M. Loerakker, CBS 771.85 = IMI 386091 = PD 85/772.

Stagonosporopsis cucurbitacearum (Fr.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515660.
Basionym: *Sphaeria cucurbitacearum* Fr., *Syst. Mycol.* **2**(2): 502. 1823.
≡ *Phoma cucurbitacearum* (Fr.) Sacc., *Syll. Fung.* **3**: 148. 1884.
= *Sphaeria bryoniae* Fuck., *Jahrb. Nassauischen Vereins Naturk.* **23-24**: 112. 1870.
≡ *Didymella bryoniae* (Fuckel) Rehm, *Ber. Naturhist. Vereins Augsburg* **26**: 27. 1881.

Specimens examined: **New Zealand**, from *Cucumis* sp., 1979, CBS 133.96= PD 79/127. **The Netherlands**, Horst, from *Cucumis* sp., 1991, J. de Gruyter, CBS 109171 = PD 91/310.

Notes: Strain CBS 133.96 could not be identified morphologically, as it proved to be sterile.

Stagonosporopsis dennisii Boerema, Gruyter & Noordel., *Persoonia* **16** (3): 350. 1997.
= *Phoma dennisii* Boerema, *Trans. Brit. Mycol. Soc.* **67** (2): 307. 1976.

Specimens examined: **Canada**, Ontario, from a stem of *Solidago canadensis*, 1995, G.Ph. White, CBS 135.96 = IMI 19337 = PD 94/4756. **The Netherlands**, Arnhem, from a stem of *Solidago floribunda*, 1968, CBS 631.68 = PD 68/147.

Stagonosporopsis dorenboschii (Noordel. & Gruyter) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515661.
Basionym: *Phoma dorenboschii* Noordel. & Gruyter, *Persoonia* **15** (1): 83. 1992.

Specimens examined: **The Netherlands**, Rijnsburg, from *Physostegia virginiana*, 1986, D. Kruger, **holotype** L 988.202.121, **isotype** CBS H-7604, ex-holotype culture CBS 426. 90 = IMI 386093 = PD 86/551; from *Physostegia virginiana*, 1986, CBS 320.90 = PD 86/932.

Stagonosporopsis heliopsidis (H.C. Greene) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515662.

Basionym: *Phyllosticta heliopsidis* H.C. Greene, *Trans. Wisconsin Acad. Sci.* **50**: 158. 1961.
≡ *Phoma heliopsidis* (H.C. Greene), Aa & Boerema apud Gruyter, Boerema & van der Aa, *Persoonia* **18** (1): 40. 2002.

Specimens examined: **Canada**, Island of Montréal, from *Ambrosia artemisiifolia*, PD 95/6189 = DAOM 221138. **The Netherlands**, from *Heliopsis patula*, 1974, CBS 109182 = PD 74/231.

Stagonosporopsis hortensis (Sacc. & Malbr.) Petr., *Ann. Mycol.* **19** (1/2): 21. 1921.
Basionym: *Hendersonia hortensis* Sacc. & Malbr., in Saccardo, *Michelia* **2** (8): 629. 1882.
= *Phoma subboltshauserii* Boerema, Gruyter & Noordel., *Persoonia* **16** (3): 360. 1997.

Specimens examined: **The Netherlands**, from an unknown substrate, Mar. 1942, N. Hubbeling, CBS 104.42; from *Phaseolus vulgaris*, 1979, G.H. Boerema, CBS 572.85 = PD 79/269.

Stagonosporopsis ligulicola var. ***ligulicola*** (K.F. Baker, Dimock & L.H. Davis) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515663.
Basionym: *Mycosphaerella ligulicola* K.F. Baker, Dimock & L.H. Davis, *Phytopathology* **39**: 799. 1949.
Anamorph: *Phoma ligulicola* var. *ligulicola* Boerema, *Stud. Mycol.* **32**: 9. 1990.

Specimens examined: **Germany**, Berlin, from *Chrysanthemum indicum*, 1963, R. Schneider, CBS H-11952, culture CBS 500.63 = MUCL 8090. **The Netherlands**, near Lisse, from a leaf of *Chrysanthemum indicum*, 1984, CBS 137.96 = PD 84/75.

Stagonosporopsis ligulicola var. ***inoxydabilis*** (Boerema) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515664.
Basionym: *Didymella ligulicola* var. *inoxydabilis* Boerema, *Stud. Mycol.* **32**: 10. 1990.
Anamorph: *Phoma ligulicola* var. *inoxydabilis* Boerema, *Stud. Mycol.* **32**: 10. 1990.

Specimens examined: **The Netherlands**, from *Chrysanthemum parthenii*, 1981, G.H. Boerema, **holotype** CBS H-7611, culture ex-holotype CBS 425.90 = PD 81/520; from *Matricaria* sp. 1985, J. de Gruyter, PD 85/259.

Stagonosporopsis loticola (Died.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515665.
Basionym: *Phoma loticola* Died., *Kryptog.-Fl. Mark Brandenburg.* 9, Pilze **7** (1): 152. 1912.

Specimens examined: **New Zealand**, Auckland, Mt. Albert, from *Lotus pedunculatus*, 1981, Ph.R. Johnston, **isotype** CBS H-7612, ex-isotype culture CBS 562.81 = PDDCC 6884; Auckland, from the stem of *Lotus tenuis*, 1979, Ph.R. Johnston, CBS 628.97 = PD 79/72.

Stagonosporopsis lupini (Boerema & R. Schneid.) Boerema, Gruyter & Ph. Graaf, *Persoonia* **17** (2): 283. 1999.
Basionym: *Ascochyta lupini* Boerema & R. Schneid., apud Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* **162**: 28. 1984.
≡ *Phoma schneiderae* (Boerema & R. Schneid.) Boerema, Gruyter & Ph. Graaf, *Persoonia* **17** (2): 282. 1999.

Specimens examined: **Peru**, Puno, from *Lupinus mutabilis*, 1980, CBS H-9061, culture CBS 375.84= PD 80/1250. **UK**, Cambridgeshire, Mepal, from *Lupinus albus*, Apr. 1998, Ph. van de Graaf, **holotype** L 998.099.105, ex-holotype culture CBS 101494 = PD 98/5247.

Stagonosporopsis oculo-hominis (Punith.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515666.
Basionym: *Phoma oculi-hominis* Punith., *Trans Brit. Mycol. Soc.* **67**: 142. 1976.
≡ *Phoma dennisii* var. *oculo-hominis* (Punith.) Boerema, Gruyter & Noordel., *Persoonia* **16**: 351. 1997.

Specimen examined: **USA**, Tennessee, Nashville, from a man's corneal ulcer, 23 Apr. 1975, Y.M. Clayton, ex-holotype culture CBS 634.92 = IMI 193307.

For a complete description see Punithalingam (1976) and Boerema *et al.* (1997).

Notes: *Stagonosporopsis oculo-hominis* is a species that thus far has been reported only once in a clinical case in Tennessee, USA, when it was isolated from a man's corneal ulcer (Punithalingam 1976). Due to morphological similarities it has been recombined into a variety of *Ph. dennisii* by Boerema *et al.* (1997), but the genetical data presented here suggest that this entity should be recognised at species level in *Stagonosporopsis*. It is distinguishable from *S. dennisii* by the absence of a diffusible pigment in the agar, and by the absence of a discolouration after application of NaOH to the culture. Further, the septate conidia are significantly smaller than those of *S. dennisii*: 9–16 × 4.5 µm versus 14.5–24 × 4–7 µm, respectively.

Stagonosporopsis rudbeckiae (Fairm.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515667.
Basionym: *Phoma rudbeckiae* Fairm., *Proc. Rochester Acad. Sci.* **1**: 51. 1890.

Specimen examined: **The Netherlands**, from *Rudbeckia bicolor*, 1979, CBS 109180 = PD 79/175.

Stagonosporopsis trachelii (Allesch.) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515668.
Basionym: *Phoma trachelii* Allesch., *Fungi Bavaria exs.* **4**: 360. 1897.
= *Ascochyta bohemica* Kabát & Bubák apud Bubák & Kabát, *Hedwigia* **44**: 361. 1905.
≡ *Stagonosporopsis bohemica* (Kabát & Bubák) Boerema, Gruyter & Noordel., *Persoonia* **16** (3): 361. 1997.

Specimens examined: **Sweden**, Svalöv, from *Campanula isophylla*, 1968, W. Södergren, CBS H-8972, ex-holotype culture 384.68. **The Netherlands**, from a leaf of *Campanula isophylla*, 1977, CBS 379.91 = PD 77/675.

Note: Although this species has been described in *Stagonosporopsis* before (as *S. bohemica*, Boerema *et al.* 1997), this was based on a later homonym, and thus a recombination based on the oldest epithet is proposed here.

Stagonosporopsis valerianellae (Gindrat, Semecnik & Bolay) Aveskamp, Gruyter & Verkley, **comb. nov.** MycoBank MB515669.
Basionym: *Phoma valerianellae* Gindrat, Semecnik & Bolay, *Revue Hort. Suisse Romande* **40**: 350. 1967.

Specimens examined: **The Netherlands**, Wageningen, from *Valerianella locusta* var. *oleracea*, 1966, G.H. Boerema, **holotype** L 965.300.24, **isotype** CBS H-7631, ex-isotype culture CBS 329.67 = PD 66/302; from *Valerianella locusta*, 1982, CBS 273.92 = PD 76/1019.

Residual species in the Didymellaceae:

The following *Phoma* species are embedded in the *Didymellaceae*, but could not be confidently assigned to one of the groups or new genera in this study due to lack of support for their respective clades. Several of the species listed here belong to this family based on LSU and/or ITS sequence data, but due to missing

sequencing data on one of the loci used, these species could not be assigned. These species are provisionally retained under their current holomorph name until further analyses are conducted to place them in the new phylogenetic system.

Didymella macropodii Petr., *Hedwigia* **68**: 219. 1928.
Anamorph: *Phoma nigrificans* (Ph. Karst.) Boerema, Loer. & Wittern, *J. Phytopathol.* **115**(3): 270. 1986.
Basionym: *Sphaeronaema nigrificans* Ph. Karst., *Meddeland. Soc. Fauna Fl. Fenn.* **16**: 17. 1888. (as '*Sphaeronema*').

Specimens examined: **Germany**, from *Brassica napus*, 1982, G.H. Boerema, CBS 100190 = PD 82/736. **Poland**, near Gryfice, from *Thlaspi arvense*, 1990, J. Marcinkowska, CBS 100191. **The Netherlands**, from an unidentified crucifer, 1984, G.H. Boerema, PD 84/512.

Didymella rabiei (Kovatsch.) Arx, in Müller & Arx, *Beitr. Kryptogamenfl. Schweiz* **11** (2): 364. (1962).
Basionym: *Mycosphaerella rabiei* Kovatsch., The blight of chick pea: 70. 1936.
Anamorph: *Phoma rabiei* (Pass.) Khune ex Gruyter, *Persoonia* **18**(1): 89. 2002.
Basionym: *Zythia rabiei* Pass., *Comment. Soc. Crittog. Ital.* **2**(3): 437. 1867.

Specimens examined: **India**, from the seeds of *Cicer arietinum*, 1965, S. Sinha, CBS 534.65. **Syria**, from *Cicer arietinum*, 1981, W. Barz, CBS 581.83A.

Notes: The placement of this teleomorph in either the *Didymella* or *Mycosphaerella* has been debated regularly in the past (Müller and von Arx 1962, von Arx 1987, Trapero-Casas & Kaiser 1992, Wilson & Kaiser 1995, de Gruyter 2002, Barve *et al.* 2003). The most recent emendment was by de Gruyter (2002) who judged in favour of *Mycosphaerella rabiei* Kovatsch. ex Gruyter. However, as the genus *Mycosphaerella* is phylogenetically not linked with the *Pleosporales* (Schoch *et al.* 2006, 2009a, Crous *et al*. 2009b), the placement in *Didymella* appears to be more correct.

Didymella adianticola Aa & Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* **159** (*Jaarboek* 1982): 25. 1983.
Anamorph: *Phoma adianticola* (E. Young) Boerema, *Verslagen Meded. Plziektenk. Dienst Wageningen* **159** (*Jaarboek* 1982): 25. 1983.
Basionym: *Phyllosticta adianticola* E. Young, *Mycologia* **7**: 144. 1915.

Specimens examined: **Costa Rica**, from a leaf of *Polystichum adiantiforme*, 1989, J. de Gruyter, CBS 258.92 = PD 89/1887. **USA**, Florida, from a leaf of *Polystichum adiantiforme*, 1982, G.H. Boerema, CBS H-16142, culture CBS 187.83 = PD 82/128.

Phoma aliena (Fr.) Aa & Boerema, apud Gruyter, Noordeloos & Boerema, *Persoonia* **16**(4): 486. 1998.
Basionym: *Sphaeria aliena* Fr., *Syst. Mycol*. **2**(2): 502. 1823.

Specimens examined: **The Netherlands**, from a twig of *Berberis* sp., 1982, J. de Gruyter, CBS 379.93 = PD 82/945; near Boskoop, from a twig of *Buxus sempervirens*, 1994, J. de Gruyter, CBS 877.97 = PD 94/1401.

Phoma aubrietiae (Moesz) Boerema, *Gewasbescherming* **1**(4): 66. 1970.
Basionym: *Sclerophomella aubrietiae* Moesz, *Balkán-Kutat Tud. Eredm.* **3**: 144. 1926.

Specimens examined: **The Netherlands**, Bodegraven, from seed of *Aubrietia hybrida* cv. Superbissima,

1965, G.H. Boerema, CBS H-16154, culture CBS 383.67 = PD 65/223; from a stem of *Aubrietia* sp., 1970, G.H. Boerema, CBS 627.97 = PD 70/714.

Phoma bulgarica Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515671. Figure 5.13.

Pycnidia solitaria, subglobosa, elongata vel obpyriformia, glabra, epapillata, brunnea, superficialia vel in agaro immersa, (140–)170–250(–295) µm. Pycnidia fertilia non vidi.

Etymology: Epithet refers to the country of origin, Bulgaria.

Conidiomata pycnidial solitary, subglobose to elongated or obpyriform, glabrous, non-papillate, brown, on the surface and immersed in the agar, measuring (140–)170–250(–295) µm. Pycnidia proved to be sterile. In older cultures pycnidial primordia are formed, which are surrounded by clusters of needle-shaped crystals.

Culture characteristics: Colonies on OA, 45–65 mm diam after 7 d, margin regular. Immersed mycelium hyaline, largely covered by mat of felty to compact whitish grey to lavender grey aerial mycelium; reverse iron-grey, but vinaceous-black where the aerial mycelium is present. Colonies on MEA 40–50 mm diam after 7 d, margin regular. Immersed mycelium mainly hyaline, incidentally black when clustering into thicker hyphal strands. Aerial mycelium sparse, flat, olivaceous green to white near the colonies margin; reverse greenish olivaceous to olivaceous black. Colonies on CHA 70–85 mm diam after 7 d, or even covering the total agar surface, margin regular. Immersed mycelium as on MEA. Aerial mycelium occurring around the colony centre, white, compact to floccose; reverse leaden black. Application of NaOH did not have any effect.

Specimens examined: **Bulgaria**, Silkossia, Strandga Mountain, from leafs of *Trachystemon orientale*, 20 June 1980, S. Vanev, **holotype designated here** CBS H-20242, ex-holotype culture CBS 357.84; from *Trachystemon orientale*, 1982, CBS 124515 = PD 82/1058.

Notes: Strain PD 82/1058 differed from CBS 357.84 (which is described above) by a significantly different colony pattern on MEA. This strain was characterised by a growth of *ca*. 20 mm diam. after 7 d, with a strongly lobate margin. White to buff aerial mycelium was present in a few irregular zones, and had a compact to floccose structure. Pycnidial primordial are only produced in culture on MEA after addition of an autoclaved piece of *Urtica dioica* (stinging nettle).

Phoma calidophila Aveskamp, Gruyter & Verkley, *Mycologia* **101**: 368. 2009.

Specimens examined: **Egypt**, from desert soil, Feb. 1980, M.I.A. Abdel-Kader, **neotype** CBS H-20168, ex-neotype culture CBS 448.83. Unknown European origin, from *Cucumis sativus*, 1984, G.H. Boerema, PD 84/109.

Phoma chenopodiicola Gruyter, Noordel. & Boerema, *Persoonia* **15** (3): 395. 1993.

Specimens examined: **Peru**, from a stem of *Chenopodium quinoa* cv. Sajana, 1979, CBS 128.93= PD 79/140; from a stem of *Chenopodium quinoa* cv. Sajana, 1979, CBS 129.93 = PD 89/803.

Phoma complanata (Tode) Desm., *Michelia* **2** (7): 337. 1881.
Basionym: *Sphaeria complanata* Tode, *Fungi Mecklenburg. Sel. (Lüneburg)* **2**: 22. 1791.

Specimens examined: **The Netherlands**, Tilburg, from a stem of *Heracleum sphondylium*, Nov. 1997, H.A. van der Aa, CBS H-16194, culture CBS 100311; from a stem of *Angelica sylvestris*, 1974, G.H. Boerema, CBS 268.92 = PD 75/3.

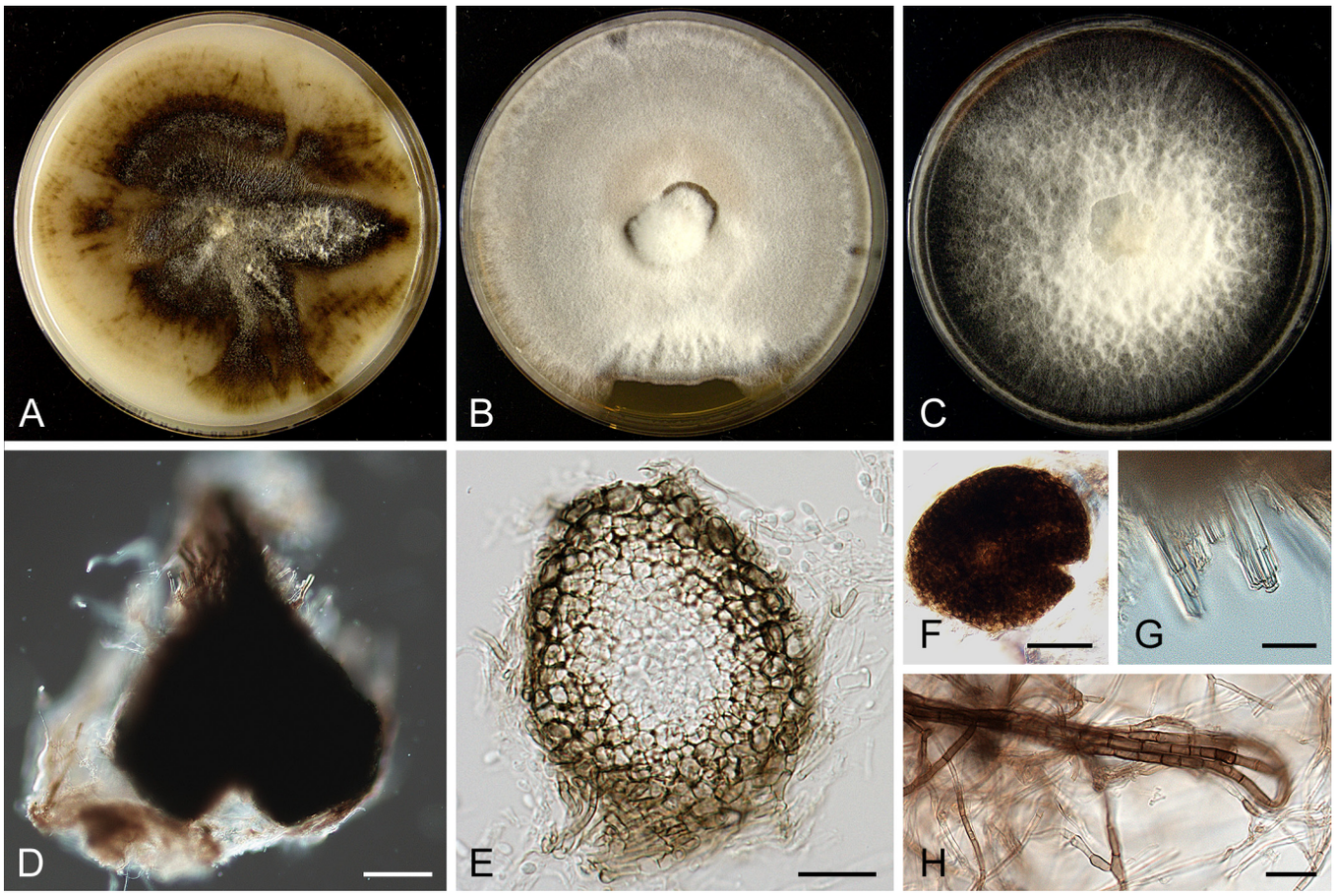


Figure 5.13. *Phoma bulgarica* (CBS 357.84). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D. Pycnidia *in vivo*, isolated from manually infected sterilized stems of *Urtica dioica*. E. Pycnidial section. F. Pycnidium. G. Crystals. H. Hyphal strand. Scale bars: D, F = 100 µm, E, H = 50 µm, G = 10 µm.

Phoma crystallifera Gruyter, Noordel. & Boerema, *Persoonia* **15** (3): 393. 1993.

Specimen examined: **Austria**, Kärnten, Wallenberg near Völkermarkt, from *Chamaespartium sagittale*, Apr. 1982, H.A. van der Aa, **holotype** L 992.177-456, ex-holotype culture CBS 193.82.

Phoma dactylidis Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515671. Figure 5.14.

Conidia dimorpha, intra idem pycnidium formata. Conidia typus 1 ellipsoidea vel ovoidea, interdum leniter allantoidea, glabra, hyalina, continua, 4.5–9(–9) × (2–)2.5–3.5 µm, (2–)3–6(–8) guttulis praedita. Conidia typus 2 cylindrica vel ellipsoidea, glabra, hyalina, saepe uniseptata, (9–)9.5–13.5(–14.5) × (2.5–)3.5–4.5 µm, interdum septata et guttulis (2–)4–8(–15) in quoque cellula. Matrix conidiorum salmonea.
Etymology: Named after the associated plant host genus, *Dactylis* sp.

Conidiomata pycnidial, solitary or confluous, produced on the agar surface, (sub-)globose, with some hyphal outgrows, (115–)135–230(–250) × (75–)95–195(–105) µm. *Ostioles* 1–4(–5), papillate. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 4–8 cell layers, outer 2–4 cell layers pigmented, 10–27 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, *ca*. 4.5–6.5 × 3–5 µm. *Conidia* dimorphic, both originating from the same pycnidia. Conidia of type 1: ellipsoidal to ovoid, sometimes somewhat allantoid, thin-walled, smooth, hyaline, aseptate 4.5–9(–9) × (2–)2.5–3.5 µm, with (2–)3–6(–8) guttules. Regularly also large conidia occur: cylindrical to ellipsoidal, thin-walled, smooth, hyaline, often uniseptate (9–)9.5–13.5(–14.5) × (2.5–)3.5–4.5 µm, but sometimes septate and septate somewhat constricted at the septum, with (2–)4–8(–15) guttules per cell. Conidial matrix salmon.

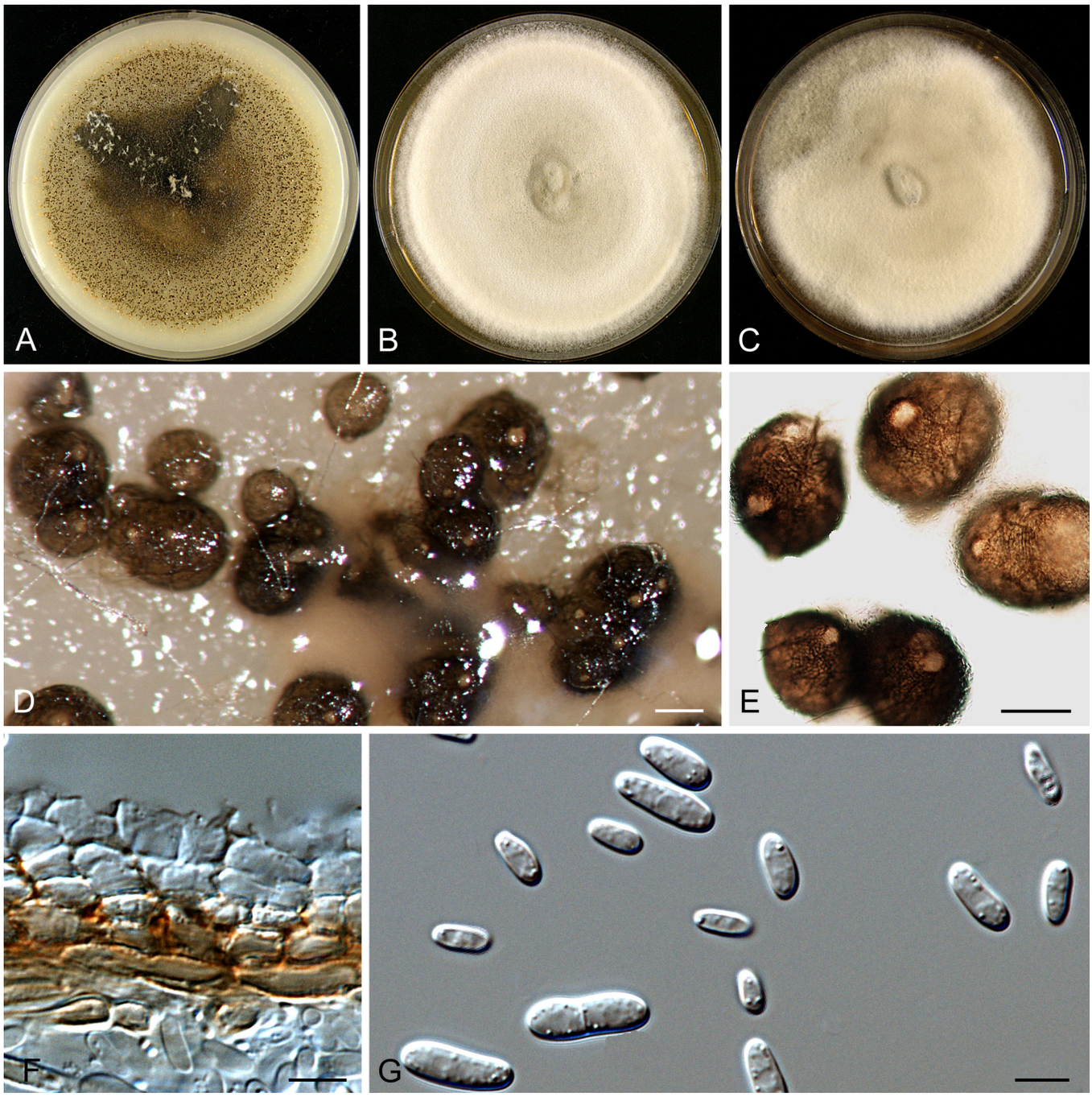


Figure 5.14. *Phoma dactylidis* (CBS 124513). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D–E. Pycnidia. F. Section of the pycnidial wall. G. Conidia. Scale bars: D–E = 100 µm, F–G = 5 µm.

Culture characteristics: Colonies on OA, 40–45 mm diam after 7 d, margin regular. Immersed mycelium hyaline, but some greenish black zones may occur, with tufts of with aerial mycelium. Abundant greenish black pycnidia are scattered over the medium, which are salmon coloured near the colony margin; reverse concolourous. Colonies on MEA 45–50 mm diam after 7 d, margin regular. Immersed mycelium completely covered by a felty greyish white aerial mycelium; reverse grey olivaceous, becoming brown olivaceous near the colony margin. Colonies on CHA similar as on MEA, but somewhat faster growing, 55–60 mm diam. after 7 d; reverse completely black. Application of NaOH results in a slight greenish discolouration of the agar, best to be observed on OA medium.

Specimen examined: **USA**, Oregon, on *Dactylis glomerata*, 1973, **holotype designated here** CBS H-20237, ex-holotype culture CBS 124513 = PD 73/1414.

Notes: *Phoma dactylidis* has thus far only been isolated once from the leaves of *Dactylis glomerata* in Oregon, USA. Other *Phoma* pathogens of *Dactylis* include *Ph. paspali* and *Ph. pratorum,* which both occur in New Zealand, but are relatively distantly related to *Ph. dactylidis*. Additionally, two related taxa have been found on this host, viz. the novel variety *Boeremia exigua* var. *gilvescens* and *Epicoccum nigrum* (Punithalingam *et al.* 1972). The clustering of this species suggests ecological or morphological similarities with *Ph. rhei* (BPP = 1.00; RBS = 100 %).

Phoma destructiva var. ***destructiva*** Plowr., *Gard. Chron. II* **16:** 621. 1881.

Specimens examined: **Guadeloupe**, from fruit of *Lycopersicon esculentum*, 1987, CBS 133.93 = PD 88/961 = IMI 173142. **Tonga**, Friendly Islands, from decaying fruit of *Lycopersicon esculentum*, 1967, G.F. Laundon, CBS H-16200, culture CBS 378.73 = CECT 2877.

Phoma destructiva var. ***diversispora*** Gruyter & Boerema, apud Gruyter, Boerema & van der Aa, *Persoonia* **18** (1): 28. 2002

Specimen examined: **The Netherlands**, Berkel en Rodenrijs, from a leaf of *Lycopersicon esculentum*, Oct. 1977, G.H. Boerema, **holotype** CBS H-16199, ex-holotype culture CBS 162.78 = PD 77/725.

Phoma eupyrena Sacc., *Michelia* **1** (5): 525. 1879.

Specimens examined: **Germany**, Kiel-Kitzeberg, from wheat field soil, 1966, W. Gams, CBS 527.66 = ATCC 22238; **The Netherlands**, from the tuber of *Solanum tuberosum*, 1991, J. de Gruyter, CBS 374.91 = PD 78/391.

Phoma herbarum Westend., *Bull. Acad. Roy. Sci. Belgique*, *Cl. Sci.* **19** (3): 118. 1852.

Specimens examined: **Belgium**, Herb. Crypt. Belge. Fasc. 20, No. 965, **lectotype**, on stems of *Onobrychis viciifolia*, 1854. Sweden, Sofieheim, from wood pulp, Apr. 1937, E. Rennerfelt, CBS 276.37 = MUCL 9920. **The Netherlands**, Emmeloord, from the stem of *Rosa multiflora* cv. Cathayensis, Apr. 1965, G.H. Boerema, CBS 615.75 = PD 73/665 = IMI 199779; Naaldwijk, from a stem base of *Nerium* sp., 1986, J. de Gruyter, CBS 502.91 = PD 82/276; Oirschot, from a twig of *Thuja* sp., 1987, J. de Gruyter, CBS 503. 91 = PD 87/499. **UK**, from paint, Aug. 1936, K.S.G. Cartwright, CBS 109.36. **USA**, Maryland, Washington area, from the fruit of *Malus sylvestris*, July 1963, M.A. Smith, CBS 567.63 = ATCC 15053 = MUCL 9889.

Phoma herbicola Wehm., *Mycologia* **38**: 319. 1946.

Specimen examined: **USA**, Montana, Missoula, head of Seeley Lake, from water, CBS H-16581, culture CBS 629.97 = PD 76/1017.

Phoma huancayensis Turkenst., *Fitopatologia* **13**: 68. 1978.

Specimens examined: **Peru**, Dep. Junin, Huancayo, near Vallis Mantaro, from a stem of *Solanum* sp., Feb. 1974, L.J. Turkensteen, **isotype** CBS H-7609, ex-isotype culture CBS 105.80 = PD 75/908; from *Chenopodium quinoa*, 1977, CBS 390.93 = PD 77/1173.

Phoma longicolla Aveskamp, Gruyter & Verkley, **sp. nov.** MycoBank MB515672. Figure 5.15.

Conidia late ellipsoidea vel ovoidea, glabra, hyalina, continua, 6–8.5(–10) × (3.5–)4–5(–5.5) µm, (2–)3–9(–12) guttulis polaris praedita. Matrix conidiorum cremeo-alba.

Etymology: Refers to the elongated necks of the ostioles.

Conidiomata pycnidial, initially solitary, globose, glabrous, slightly papillate and olivaceous buff, produced on the agar surface, measuring (45–)50–115(–130) µm diam. Later developing to black broadly globose to irregular conidiomata with many white hyphal outgrows and with a clear elongated neck around the ostioles, giving it a irregular shape, measuring (170–)200–270(–285) × (115–)125–205(–220) µm. *Ostioles* 1–3(–4), on a long elongated neck (up to 200 µm long). Often these pycnidia merge to an irregular mass of confluent conidiomata. *Pycnidial wall* pseudoparenchymatous, composed of isodiametric cells, 5–7 layers, 17–22 µm thick. *Conidiogenous cells* phialidic, hyaline, simple, smooth, flask-shaped, *ca*. 4–5 × 3–5 µm. *Conidia* broadly ellipsoidal to ovoid, thin-walled, smooth, hyaline, aseptate 6–8.5(–10) × (3.5–)4–5(–5.5) µm, with (2–)3–9(–12) polar guttules. Conidial matrix crème-white.

Culture characteristics: Colonies on OA 50–55 mm diam. after 7 d, margin regular. Immersed mycelium hyaline with abundant pycnidia, in some sectors covered by a low mat of felty to floccose mouse grey aerial mycelium, with tufts of white mycelium near the colonies margin. In the sectors with aerial mycelium, pycnidia are only sparsely present; reverse hyaline, but leaden black and olivaceous grey where the aerial mycelium is present. Colony on MEA 50–55 mm diam. after 7 d, margin regular. Immersed mycelium completely covered by a floccose crème mat of white aerial mycelium; reverse greenish olivaceous to olivaceous black. Colony on CHA 55–60 mm diam. after 7 d, margin regular. Immersed mycelium brown vinaceous to black. Aerial mycelium is occurring in sectors, felty, pale grey to white; reverse black with incidentally a pale purplish grey zone. Application of NaOH did not have any effect.

Specimens examined: **Spain**, Canary Isles, from *Opuntia* sp., 1980, J. de Gruyter, **holotype designated here** CBS H-20238, ex-holotype culture CBS 124514 = PD 80/1189; Canary Isles, Gran Canaria, from *Opuntia* sp., June 1982, H.A. van der Aa, CBS 347.82.

Notes: This species was isolated twice from *Opuntia* on the Canary Isles. Around the time of the second isolation (CBS 347.82), also *Ph. dimorpha* sp. nov. was isolated from the same location and host substrate. This species is described above. A third species that is found in association with *Opuntia* is *Ph. opuntiae*, which is, however, rather distinct in morphology and phylogeny.

Phoma medicaginis var. ***medicaginis*** Malbr. & Roum. apud Roumeguère, *Fungi Selecti Galliaei Exs.* **37**: 3675. 1886.

Specimens examined: **Czech Republic**, from *Medicago sativa*, CBS 316.90 = CCM F-187. **Italy**, Perugia, from a leaf of *Medicago sativa*, 1963, M. Ribaldi, CBS H- 16483, culture CBS 479.63. **The Netherlands**, from a leaf of *Medicago sativa*, 1966, M.M.J. Dorenbosch, CBS 533.66 = ATCC 16929 = PD 66/370. **Turkey**, Ankara, from *Medicago sativa*, 1942, S. Kuntay, CBS 107.42. **USA**, Minnesota, from *Medicago sativa*, Sep. 1953, M.F. Kernkamp, CBS 110.53; Minnesota, from *Medicago sativa*, Sep. 1953, M.F. Kernkamp, CBS 111.53.

Phoma medicaginis var. ***macrospora*** Boerema, R. Pieters & Hamers, *Netherlands J. Pl. Pathol.* **99** (Suppl. 1): 19. 1993.

Specimens examined: **Canada**, Saskatchewan, Saskatoon, from seed of *Medicago sativa*, 1965, H.W. Mead, CBS 404.65 = IMI 116999. **USA**, Minnesota, from *Medicago sativa*, Sep. 1953, M.F. Kernkamp, **holotype** CBS H-16487, ex-holotype culture CBS 112.53.

Figure 5.15. *Phoma longicolla* (CBS 124514). A–C. Fourteen-day-old colonies on OA (A), MEA (B) and CHA (C). D–F. Pycnidia. G. Section of the pycnidial wall. H. Conidia. Scale bars: E–G = 100 µm, H = 50 µm, I–J = 10 µm.

Phoma microchlamydospora Aveskamp & Verkley, *Mycologia* 101: 374. 2009.

Specimens examined: **UK**, from an unknown vegetable plant, 1990, D. Hyall, CBS 491.90; from leaves of *Eucalyptus* sp., 1994, A.M. Ainsworth, **holotype** CBS H-20147, ex-holotype culture CBS 105.95.

Phoma nebulosa (Pers.) Berk., *Outl. Brit. Fung. (London)*: 314. 1860.
Basionym: *Sphaeria nebulosa* Pers., *Observ. Mycol.* **2**: 69. 1799.

Specimens examined: **Austria**, Kaprun, from a stem of *Urtica dioica*, Jan. 1975, G.H. Boerema, CBS H-16510, culture CBS 503.75= ATCC 32163 = DSM 63391 = IMI 194766 = PD 75/4. **The Netherlands**, from a stem of *Mercurialis perennis*, 1983, CBS 117.93 = PD 83/90.

Phoma negriana Thüm., *Die Pilze des Weinstockes*, *Vienna*: 185. 1878. Originally described as '*Ph. negrianum*'.

Specimens examined: **Germany**, Oberdollendorf am Rhein, from *Vitis vinifera*, July 1969, L. Kiewnik, CBS H-16511, culture CBS 358.71. **The Netherlands**, from *Vitis vinifera*, 1979, PD 79/74; from *Vitis vinifera*, 1979, PD 79/75; from *Vitis vinifera*, 1979, PD 79/76.

Phoma nigripycnidia Boerema, Gruyter & Noordel., *Persoonia* **16** (3): 356. 1997.

Specimen examined: **Russia**, from a leaf of *Vicia cracca*, 1969, M. Ondrej, **holotype** L 992.163.150, ex-holotype culture CBS 116.96 = CCMF 243 = PD 95/7930.

Phoma omnivirens Aveskamp, Verkley & Gruyter, *Mycologia* 101: 375. 2009.

Specimens examined: **Belgium**, Gembloux, from *Phaseolus vulgaris*, 1968, L. Obando, **holotype** CBS H-20151, ex-holotype culture CBS 341.86. **India**, Japalbur, from an unknown substrate, 1977, D.Ph. Tiwari, CBS 654.77. **Papua New Guinea**, Varirata National Park, from soil, Aug. 1995, A. Aptroot, CBS 991.95. Varirata National Park. From soil, Aug. 1995, A. Aptroot, CBS 992.95. **Tanzania**, from *Statice* sp., 1990, J. de Gruyter, CBS 123397 = PD 90/1555. **The Netherlands**, from *Chrysanthemum indicum*, 1981, J. de Gruyter, CBS 123396 = PD 81/122.

Phoma putaminum Speg., *Atti Soc. Crittog. Ital.* **3**: 66. 1881.

Specimens examined: **The Netherlands**, from a branch of *Ulmus* sp., 1975, G.H. Boerema, CBS 372.91 = PD 75/960. **Denmark**, from the rhizosphere of *Malus sylvestris*, Mar. 1968, E. Sønderhousen, CBS 130.69 = CECT 20054 = IMI 331916.

Phoma rhei (Ellis & Everh.) Aa & Boerema apud Gruyter, Boerema & van der Aa, *Persoonia* **18** (1): 42. 2002.
Basionym: *Ascochyta rhei* Ellis & Everh., *Proc. Acad. Nat. Sci. Philadelphia* **1893**: 160. 1893.

Specimen examined: **New Zealand**, from a leaf of *Rheum rhaponticum*, CBS 109177 = LEV 15165 = PD 2000/9941.

Phoma selaginellicola Gruyter, Noordel., Aa & Boerema, *Persoonia* **15** (3): 399. 1993.

Specimen examined: **The Netherlands**, from a leaf of *Selaginella* sp., 1977, G.H. Boerema, CBS 122.93 = PD 77/1049.

Phoma versabilis Boerema, Loer. & Hamers, *Persoonia* **16** (2): 154. 1996.

Specimens examined: **Germany**, Westfalen, Oberdresselendorf, from stems of *Cardamine impatiens*, Oct. 1925, A. Ludwig, **holotype** L 995.229.369. **The Netherlands**, Wageningen, from a stem of *Silene* sp., 1982, G.H. Boerema, CBS 876. 97 = PD 82/1008; from *Stellaria media*, 2000, J. de Gruyter, PD 2000/1379.

Discussion

What is *Phoma*?

According to the generic concept which is applied today, species of *Phoma* are relatively simple coelomycetes that are characterised by the *in vitro* production of mainly unicellular, hyaline conidia from monophialidic, doliiform to flask-shaped conidiogenous cells in pycnidial conidiomata (Boerema & Bollen 1975).

Many species that currently are accommodated in sections *Paraphoma*, *Pilosa* and *Plenodomus* are phylogenetically basal to the *Didymellaceae*, in which most other *Phoma* taxa, including the type species are accommodated. These results support the work of Reddy *et al.* (1998), who advocated that the genus *Plenodomus* should be reinstalled as a separate genus. Torres *et al.* (2005b) subsequently made a novel description in this genus, *Pl. morganjonesii*. A paper by de Gruyter *et al.* is in preparation, in which all species of *Phoma* section *Plenodomus* recognised by Boerema *et al.* (1994, 1996) and Boerema & de Gruyter (1999), will be taxonomically revised.

However, in the present study, it has become clear that the phylogenetic boundaries between *Phoma* and several closely related genera that are defined on their conidial characters are ambiguous. Species that produce consistently two-celled hyaline conidia classified therefore traditionally in the genus *Ascochyta* appear to have evolved independently multiple times during evolution together with typical *Phoma* taxa, in several lineages of the pleosporalean tree (Figure 5.1). Also other conidial characters, such as the pigmentation of spores, as formed by *Phoma clematidis-rectae* (formerly in *Coniothyrium*) and *Microsphaeropsis olivacea*, appear not to be reliable for the delimitation of the genus *Phoma*. Thus, based on the trees presented in this study, it can be concluded that *Phoma*, as defined by Saccardo (1880, 1884) and emended by Boerema & Bollen (1975) is highly polyphyletic.

The close relation of *Phoma* with *Ascochyta* has often been observed before, as strains of both genera are often highly similar in morphology (Wollenweber & Hochapfel 1937, Brewer & Boerema 1965, Boerema & Bollen 1975, Boerema 1997), physiology (Noordeloos *et al.* 1993, Faris-Mokaiesh *et al.* 1995), pathogenicity (Mendes-Perreira *et al.* 1999, Davidson *et al.* 2009) and nucleotide sequences (Faris-Mokaiesh *et al.* 1995, Fatehi *et al.* 2003, Schoch *et al.* 2006, Peever *et al.* 2007, Chilvers *et al.* 2009, de Gruyter *et al.* 2009). In the Saccardoan system, both genera were only distinguished by the presence or absence of conidial septa, and by the type of substrate: *Ascochyta* species were considered to be specific leaf-pathogens, whereas *Phoma* was solely associated with stem lesions (Boerema & Bollen 1975).

Brewer & Boerema (1965) contrasted the septation process of the conidia in *Ascochyta pisi* to this process in *Phoma exigua*. These authors suggested that in *Phoma* species euseptation occurs only after secession, whereas in *Ascochyta* the septation of the spores was considered to be an elemental part of conidiogenesis. Later, this was determined to be a genus-specific character (Boerema 1970). Additionally, Boerema and Bollen (1975) stated that both genera are distinct in conidiogenesis. According to these authors, the *Ascochyta* species produce conidia from either an accumulation of annelations, which give the conidigenous cell an annelidic appearance, or from a gradually thickening collar of periclinal annelations. In contrast, *Phoma* species produce true phialides with a collarette. This micromorphological difference of the conidiogenesis can only be observed using electron microscopy, as the appearance of a *Phoma* collarette is highly similar to the periclinal thickening of *Ascochyta* species. This observation is however not consistent with the conidial ontogeny of *Ph. fumosa*, which was observed to be annellidic by Sutton & Sandhu (1969).

The application of these characters for the purpose of generic delimitation was heavily questioned (Punithalingam 1979a), and nowadays these characters are hardly applied in the taxonomy of both genera, simply because the use of electron microscopy is expensive and sectioning of pycnidia is too time consuming. Due to this unclear classification system, and to the fact that not all species produce exclusively septate or aseptate conidia, species had synonyms in both genera (Boerema 1972, Boerema & Dorenbosch 1973, van der Aa *et al.* 2000, Mel'nik 2000). Even nowadays the status of many species is unclear as *Phoma* and *Ascochyta* synonyms are often used simultaneously. Examples are *Ph. rabiei* and its synonym *A. rabiei* (Singh & Reddy 1993, Singh *et al.* 1997, Barve *et al.* 2003, Chongo *et al.* 2004, Pande *et al.* 2005, Hernandez-Bello *et al.* 2006, Peever *et al.* 2007), and *Ph. gossypiicola* and its synonym, *A. gossypii*

(e.g. Shen *et al.* 2005). The concept of *Ph. clematidina* has appeared to comprise several taxa belonging in multiple genera, amongst which a *Didymella* with an unnamed *Ascochyta* anamorph (Woudenberg *et al.* 2009).

The results presented in this study further suggest a close relation between *Microsphaeropsis* and *Phoma*. Morphological studies of members of both genera (Jones 1976) reveal that conidiogenesis is similar, although the conidia of *Microsphaeropsis* differ from those of *Phoma* by the dark pigmentation and the presence of a double-layered cell wall. The pigmentation occurs only after conidial secession. Therefore, young pycnidia with colourless pycnidia may be easily confused with a *Phoma* species (Boerema *et al*. 2004).

In general, it can be concluded that *Phoma* should only be regarded as a general concept, as members sharing this morphology are found throughout the *Pleosporales*, although most members are found in the *Didymellaceae*. The type species of *Phoma* is only distantly related to the other members of this genus, but relatively close to *Ascochyta pisi*, the type species of the older name *Ascochyta*. However, based on the results observed in the present study, this genus is poorly elucidated. Therefore, we opt to retain the taxonomy of *Phoma* as is, with the exception of the groups that can be resolved further, such as *Boeremia*, *Epicoccum*, *Peyronellaea* and *Stagonosporopsis*.

Taxonomic revisions

The observations presented in the present paper suggest that LSU and SSU data, which contain approximately 270 informative sites in the alignment, are sufficient to distinguish various major groups in *Pleosporales*. However, other, more variable loci should also be analysed to determine the phylogenetical basis for the species that are congeneric with the ex-type strain of *Phoma*. These species were found throughout the pleosporalean phylogeny that was reconstructed in the present paper. Molecular studies on the species that are currently accommodated in the section *Plenodomus* and *Pilosa* are in progress (de Gruyter *et al.* in prep.).

The type species of the genus *Phoma*, *Ph. herbarum,* resides in the *Didymellaceae* clade, a result that is in congruence with the observations of de Gruyter *et al.* (2009). However, based on the data generated in the present study, also the type species of *Ascochyta* (*A. pisi*), *Chaetasbolisia* (*C. erysiphoides*), and *Microsphaeropsis* (*M. olivaceae*) are located in the same group (Figure 5.2). Of those species, *Phoma* carries the oldest name, which was deposited by Fries in 1821, but as *Phoma sensu* Saccardo (1880) was conserved against *Phoma* Fries (McNeill *et al.* 2006), the genus *Ascochyta*, which was erected in 1830, would be the preferred name for the species in this genus. Nevertheless, because of the impact that recombination of *Phoma* in *Ascochyta* would have in phytopathology, we suggest to keep both generic names in use for the unresolved species in the *Didymellaceae*, disregarding the fact that both names are polyphyletic. Both genera can be regarded as polyphyletic concepts, until a proper study of the teleomorph genera related to the *Didymellaceae* has been conducted. Also the younger genera *Chaetasbolisia* and *Microsphaeropsis* should be retained as separate taxonomic entities, until at least all taxa are restudied both morphologically and phylogenetically. However, the clades that are resolved, and that are characterised by shared morphological or physiological characters, or have a shared ecological role, are elevated to generic level here. Consequences of this approach are the reinstatement of the genus *Peyronellaea* Goid., expansion of the formerly monotypic genus *Epicoccum* Link, emendment of the concept of *Stagonosporopsis* Died. and the erection of the novel genus *Boeremia*.

Teleomorph relations

In *Phoma* several teleomorphs have been recognised, but for the majority of *Phoma* species the sexual structures have yet to be discovered, as the induction of these structures requires special conditions; or simply because the species has lost its ability to propagate sexually. Boerema *et al.* (2004) only recognised *ca*. 40 species that produce teleomorphs.

The finding of multiple teleomorphs with phenotypically indistinguishable associated anamorphs is not uncommon in mycology, yet unwanted, and should be resolved in due course as more data become available. For example, such a situation also applies to major genera such as *Aspergillus* (Pitt & Samson 2007), *Botryosphaeria* (Crous *et al.* 2006b), *Geotrichum* (De Hoog & Smith 2004), *Mycosphaerella* (Crous *et al.* 2009a) and *Penicillium* (Pitt 1979).

Boerema *et al.* (2004) linked *Phoma* to four teleomorph genera: *Didymella*, *Leptosphaeria*, *Mycosphaerella* and *Pleospora*. In recent studies it was shown that the association of *Phoma* with *Mycosphaerella* was untenable, because the involved teleomorphs were apparently morphologically similar but in fact *Didymella*. The genus *Mycosphaerella* is phylogenetically distinct and not even associated with the *Pleosporales* (Schoch *et al.* 2006, 2009a, Crous *et al*. 2009b), whereas their associated *Phoma* anamorphs proved to be genetically similar to *Didymella* (de Gruyter *et al.* 2009). As a consequence, the pawpaw (*Carica papaya*) pathogen *M. caricae* has been recombined into *D. caricae* in the present study.

Also the *Didymellaceae* clade is not yet completely resolved. Next to *Didymella*, also *Leptosphaerulina* and *Macroventuria* are accommodated in the *Didymellaceae*. *Macroventuria* resembles *Venturia* (van der Aa *et al.* 1971); the ascospore morphology being highly comparable to that of *Didymella*. In contrast, *Leptosphaerulina* is distinct in morphology, producing ascospores with longitudinal and transverse septa, more resembling the ascospores of *Pleospora* and *Cucurbitaria* (von Arx 1981). *Didymella* is a poorly studied genus that is in need of a comprehensive revision, as it plays such a crucial role in the delimitation of phytopathologically important genera. When studied more intensively, this genus may very well be split up into multiple genera that have a proper morphological basis.

Sexual states have thus far only been reported for a limited number of *Phoma* species. It seems unlikely that the ability to produce sexual reproductive structures is lost in so many species, whilst other, closely related species, or even species that emerge from these "asexual" species, do have a teleomorph state. It may be assumed that the sexual state of these species is cryptic, and can only be induced under the right conditions. These teleomorph structures, that probably much resemble the sexual structures formed by the genus *Didymella*, are probably the missing links that are required for further taxonomical delineation of the species in the *Didymellaceae*.

Can the sections be maintained?

The present study was initiated chiefly to clarify the status of *Phoma* and to judge the validity of the sections introduced by Boerema (1997). Aveskamp *et al.* (2008) already illustrated the ambiguity of some sections, as multiple characters that are regarded to be section-specific may be present in a single species. For example, *Ph. zeae-maydis* was regarded as the type species of the section *Macrospora*, due to the presence of its relatively large aseptate spores (de Gruyter 2002). However, this species also produces multicellular chlamydospores, resembling the chlamydospores formed in species that are accommodated in the section *Peyronellaea*. The recombination of this species into *Pey. zeae-maydis* in the present study, which is based on DNA phylogeny, indicates that the spore size is not an informative character at above-species level.

Another example of the ambiguity of the Boeremaean section is *Ph. destructiva*. Infraspecific taxa of this species are accommodated in two sections: *Ph. destructiva* var. *diversispora* was accommodated in section *Phyllostictoides*, wheras the type variety was linked to section *Phoma* due to the absence of septate conidia. Boerema *et al.* (2004) acknowledged this ambiguity problem and were forced to key out several species in multiple sectional dichotomous keys. In the previous study of de Gruyter *et al*. (2009) this ambiguity could not be illustrated as only sectional representatives were included. Here it is illustrated that, although some sections can be partially maintained, most of the sections are not supported from an evolutionary perspective.

Section Heterospora:

The majority of the species that were ascribed to *Phoma* section *Heterospora* is recovered in Group R, from which the species are all recombined into the genus *Stagonosporopsis* in the present paper. The type species of section *Heterospora* however, *Ph. heteromorphospora*, is recovered basal to the *Didymellaceae* together with *Ph. dimorphospora*. Also *Ph. samarorum* is not retrieved in the main *Phoma* clade, but is associated with the *Phaeosphaeriaceae*.

Also within the *Didymellaceae*, the *Heterospora* section appears to be polyphyletic as *Ph. aquilegiicola*, *Ph. glaucii* and *Ph. clematidina* are distantly related to most other *Heterospora* species and form a distinct clade together with another *Clematis* pathogen, *Ph. clematidis-rectae*, a species that has been regularly confused with the *Phoma clematidina* complex (Woudenberg *et al.* 2009). The species in this clade can be distinguished from the main body of the *Heterospora* species as they lack the production of large *Stagonospora*-type conidia in culture, although smaller, septate conidia may occur.

Section Macrospora:
The five large-spored species of the section *Macrospora* included in this study are found scattered throughout the *Didymellaceae*, indicating that spore size is not a good taxonomic criterion for delimiting taxa above species level. *Phoma zeae-maydis* is genetically similar to most *Peyronellaea* species. This association is supported by the finding of dictyochlamydospores in most species in this clade (Aveskamp *et al.* 2009a).

Section Paraphoma:
Also *Phoma* section *Paraphoma* (van der Aa *et al.* 1990) appears to be polyphyletic. The section comprises 12 taxa that produce pycnidial conidiomata with setae (de Gruyter & Boerema 2002). Members of this section are found in clades 5, 6, and 8 of Figure 5.1. *Phoma gardeniae* is the only setae-producing species known in the *Didymellaceae*. Because of its ability to produce dictyochlamydospores, and based on the DNA phylogeny presented in Figure 5.2, it is recombined into the genus *Peyronellaea* here.

The type species for the former section *Paraphoma* is *Ph. radicina*, which is accommodated in the *Phaeosphaeriaceae* group (clade 6). Remarkably, no other species that were ascribed to the section *Paraphoma* are found in the same family. Instead, *Ph. chrysanthemicola* (formerly ascribed to the section *Peyronellaea*) is found in close association with *Ph. radicina*. Both species are recognised as soil fungi and have a wide distribution with records from Europe, North-America and Asia (Boerema *et al.* 2004). The close association between *Ph. samarorum*, *Ph. chrysanthemicola* and *Ph. radicina* has been recorded before in a phylogenetical reconstruction of the section *Peyronellaea* in a study of Aveskamp *et al.* 2009a. The resolution of the clade in that study was, however, higher as the complete ITS regions 1 and 2 were applied in genetic analyses (Aveskamp *et al.* 2009a). Further linkage of the morphological and ecological characters to the phylogeny will be one of the main challenges for taxonomists working on the species in this group.

A third *Paraphoma* species, *Ph. terricola*, is recovered in clade 5 of Figure 5.1, which resembles the *Cucurbitariaceae*. This family also hosts the setae-lacking species *Ph. pratorum*, which was classified in section *Phoma*. Several other coelomycete fungi are accommodated here as well, including *Phialophorophoma litoralis*, *Pleurophoma cava*, a sterile strain that once has been identified as *Coniothyrium* sp. and various *Pyrenochaeta* species. The close morphological relation between the genera *Pyrenochaeta*, *Pleurophoma* and *Phoma* section *Paraphoma* was already noted by Boerema *et al.* (1996) and Grondona *et al.* (1997). Like *Phialophorophoma litoralis* and *Pleurophoma cava*, *Pyrenochaeta* is characterised by the formation of elongated, filiform, multiseptate conidiophores, a character that is however not found in the various *Phoma* species embedded in this clade (de Gruyter *et al.* 2009). A further delineation of the species associated with the genera *Pyrenochaeta* and *Pleurophoma* and the *Phoma* section *Paraphoma* will be provided in a follow-up paper by de Gruyter *et al.* (2010).

Section Peyronellaea:
The chlamydospore-producing species have been treated before by Aveskamp *et al.* (2009a), who revealed that also *Phoma* section *Peyronellaea* is artificial from an evolutionary point of view. Most species, including the type *Ph. glomerata*, cluster in group K of Figure 5.2, along with many other (uni- and multicellular) chlamydospore producing species. To be in accordance with the phylogenetic results, this cluster is elevated to generic level, which is named after the section *Peyronellaea.* A second group of species belonging to this section is recovered in clade L, which groups species that produce botryoid or epicoccoid dictyochlamydospores, including *Epicoccum nigrum*. Two species, *Ph. pimprina* and *Ph. sorghina* are recombined into *Epicoccum* here. Species that produce pseudoscleroid chlamydospores, such as *Ph. violicicola* and *Ph. chrysanthemicola* were found to cluster outside the *Didymellaceae*.

Section Phoma:
Species ascribed to *Phoma* section *Phoma* are retrieved in practically all clades of the trees produced in the present study. This supports the general idea that this section has been used as a "waste-bin" for phomoid taxa that could not be placed in other sections or genera due to the lack or presence of typical sectional characters.

The type species of this section, and also of the genus as a whole, is *Ph. herbarum* (Boerema 1964). The reference strains of this species are accommodated amongst the basal polytomous species of the *Didymellaceae*. This suggests that it has branched off from most other members of this family in an early

phase of the development of the *Didymellaceae* and probably evolved further without recombining with other taxa.

Although the description of *Ph. crinicola* is highly similar to that of other species in the *Ph. exigua* clade presented in Figure 5.2, it has never been recognised as such due to the absence of septate conidia. Nevertheless, the remaining characters do not contradict with the description given for *Ph. exigua* (van der Aa *et al.* 2000). The pycnidia of *Ph. crinicola* usually carry a single ostiole, but pycnidia are regularly observed lacking an apparent ostiole. This may correspond with the ostiolar openings of many species found within the *exigua* clade, which are often lined or filled with papillate, hyaline cells.

Similar findings are *Ph. aurea* and *Ph. nigricans* in clade K, which is mainly filled with chlamydospore-forming species that were previously associated with the section *Peyronellaea*. Both species were originally described from New Zealand (Johnston & Boerema 1981, de Gruyter *et al.* 1993), but may be commonly present on the whole Australasian continent (de Gruyter *et al.* 1993, 1998). Two other species, belonging to section *Phoma*, but found in this clade are *Ph. anserina* and *Ph. eucalyptica*. Both species produce swollen cells in older cultures (de Gruyter & Noordeloos 1992), which may be an initial phase of chlamydospore formation.

Fifteen species are phylogenetically only distantly related to the *Didymellaceae*, and should therefore be excluded from the genus. These species include the current *Ph. apiicola*, *Ph. capitulum*, *Ph. fallens*, *Ph. fimeti*, *Ph. flavescens*, *Ph. flavigena*, *Ph. glaucispora*, *Ph. haematocycla*, *Ph. lini*, *Ph. minutispora*, *Ph. multipora*, *Ph. opuntiae*, *Ph. pratorum*, *Ph. valerianae*, and *Ph. vasinfecta*. The problem in recombining these species is, however, the absence of characters that could link these taxa to a specific genus. No teleomorphs are known in this group.

Section Phyllostictoides:
All taxa belonging to *Phoma* section *Phyllostictoides* are retrieved in the *Didymellaceae* clade of Figure 5.1 (Clade 8). This is remarkable as this large section has been regarded, just like section *Phoma*, to be a repository for all species that could not be accommodated elsewhere. Nevertheless, within the *Didymellaceae* this section falls apart as species occur in many distinct clades.

The major body of the *Phyllostictoides* species is retrieved in group N, in which all *Ph. exigua*-related species and varieties are found (Aveskamp *et al.* 2009b), as well as *Ph. crinicola* and *Ph. hedericola*, which were associated with *Phoma* section *Phoma*. A second group in which many *Phyllostictoides* taxa cluster is clade R. This clade comprises many species of the former section *Heterospora,* and several species that were excluded from this section and transferred to *Phyllostictoides* by Boerema *et al.* (1997), such as *Ph. cucurbitacearum* and *Ph. ligulicola*.

Section Pilosa:
Only one of both members of the section Pilosa was included in the present study. The type of this section, *Ph. betae*, produces a teleomorph in *Pleospora*, a genus that is typified by *Pl. herbarum*. Both species are related and are found in the *Pleosporaceae* and *Leptosphaeriaceae* clade, although the genetic distance between these species is significant. This finding illustrates the difficulties that are experienced when delineating the *Pleosporaceae* (Dong *et al.* 1998).

Section Plenodomus:
Thus far the only section created by Boerema that still may be monophyletic is the section *Plenodomus*, of which all the members are found in the *Leptosphaeriaceae*. However, some species associated with other sections, such as *Ph. apiicola*, *Ph. valerianae*, *Ph. vasinfecta* (section *Phoma)* and *Ph. violicola* (section *Peyronellaea*) are also linked to this clade and are found to be closely related to the *Plenodomus* species. The section *Plenodomus* is associated with a *Leptosphaeria* teleomorph, but for the aberrant *Phoma* states found in this clade, no teleomorphs are known. Boerema *et al.* (2004) mentioned five *Leptosphaeria* species that produce *Phoma* anamorphs, but that do not fit within the *Plenodomus* concept. These species, including *L. sacchari*, *L. haematitis*, *L. libanotis*, *L. purpurea* and *L. weimeri* were however not to our disposal, and were therefore not studied. Apparently the genus *Leptosphaeria* produces multiple anamorphs.

Most taxonomic studies on the *Leptosphaeriaceae* reveal a monophyletic group, although in these studies, only a limited number of species, belonging to either *Leptosphaeria* or *Phoma* section *Plenodomus*, have been included (Morales *et al.* 1995, Reddy *et al.* 1998, Torres *et al.* 2005b). Other studies indicate that this genus is

paraphyletic (Dong *et al.* 1998, Câmara *et al.* 2002). Due to the inclusion of only two *Leptosphaeria* species in the present study, it cannot be unambiguously stated whether this section is mono- or paraphyletic.

Both species included, *L. maculans* and *L. biglobosa*, are assumed to represent a heterogeneous assemblage of cryptic taxa (Howlett *et al*. 2001, Mendes-Pereira *et al*. 2003, Barrins *et al*. 2004, Voigt *et al*. 2005). Although many recombinations have been made in the past, this has obscured a proper understanding of *Phoma* section *Plenodomus* and *Leptosphaeria* (Boerema *et al.* 1996). Due to the complexity of this group, we will attempt to resolve its phylogeny in a separate paper (de Gruyter *et al.* prep.).

Section Sclerophomella:
The thickened, sclerotisised pycnidial wall, and the formation of poroid pycnidial openings instead of an ostiole, are the main characters of *Phoma* section *Sclerophomella*. These characters appear not to reflect the evolutionary history of the genus. Only in group O, a cluster of species is retrieved that is known for their ostiole absence, although not in all species the thickened pycnidial wall is observed. Most other species belonging to section *Sclerophomella* appear to be unrelated as they have emerged from non-*Sclerophomella* multiple times during evolution. Therefore these species are found scattered throughout the phylogeny of *Pleosporales*. The type species of this section is *Ph. complanata*, which is found in the basal polytomy of the *Didymellaceae*.

Many of the morphological characters that were used by Boerema *et al.* (1997) to create an infrageneric subdivision of *Phoma*, appear not to be evolutionary informative when compared to sequence data. The main characters that were applied to distinguish sections, like the thickness of the pycnidial walls, chlamydospore structure and presence of *Stagonosporopsis* synanamorphs are only of limited value. Several characters, such as percentage of septated spores may be genetically driven, but are certainly also highly influenced by the growth media and culturing conditions (Rai 2000). This has led to much confusion surrounding the taxonomic placement of many species in either *Ascochyta* or *Phoma*, such as *A. rabiei* (e.g. Barve *et al.* 2003, Pande *et al.* 2005, Peever *et al.* 2007) vs. *Ph. rabiei* (e.g. Singh & Reddy 1993, Singh *et al.* 1997, de Gruyter 2002).

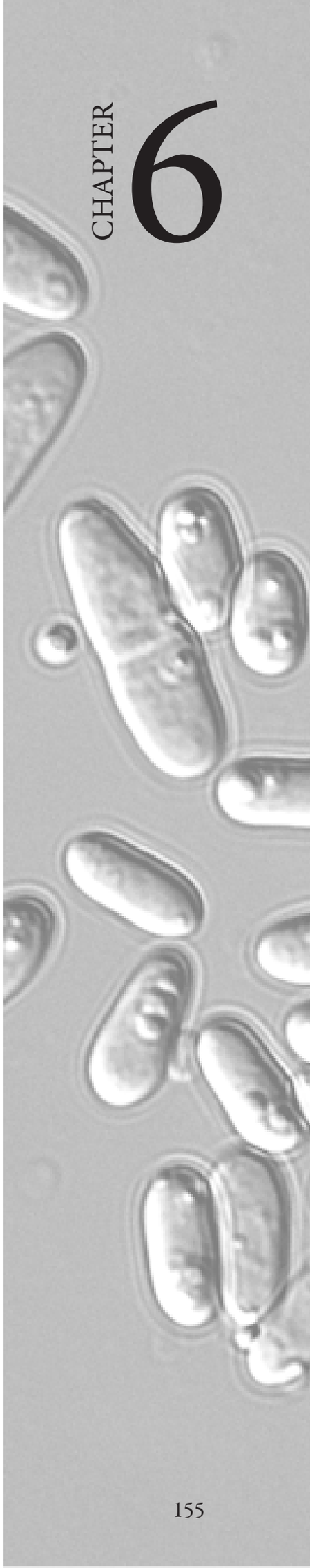
In short, the Boeremaean sectional subdivision is hardly of any evolutionary relevance, suggesting that future classification of taxonomic novelties into these sections should be avoided. Nevertheless, the morphological identification system that was developed based on this subdivision (Boerema *et al.* 2004) is still applicable, as this system can be still aid in morphological species recognition.

DNA Barcoding
A further aim of this study was the development of species-specific DNA barcodes for species of *Phoma*. The preferred DNA barcode region for *Fungi* is ITS (Druzhininia *et al.* 2005, Summerbell *et al*. 2005, Seifert 2008, 2009). Cytochrome Oxidase I (COI) was for a long time considered to be a good candidate gene for barcoding fungi (Seifert *et al.* 2007, Nguyen & Seifert 2008), although some recent studies indicate the variation between copies within a single strain (Geiser *et al.* 2007, Gilmore *et al.* 2009). Also Aveskamp *et al.* (2009b) found that the COI locus was not robust, and thus far, COI barcodes have only been applied in an oligonucleotide array identification system for *Penicillium* spp. (Chen *et al.* 2009). The value of ITS as primary barcode region is, however, not sufficient to delineate all taxa. Especially amongst the species clustered in clade N, which represents the species that are associated to the *Ph. exigua* species complex, ITS is not sufficient to distinguish the various species. This finding is in congruence with results obtained in previous studies, in which the ITS region has been applied in an attempt to distinguish the species within the *Ph. exigua* complex but without success (Abeln *et al.* 2002, MacDonald *et al.* 2000, Cullen *et al.* 2006). Nevertheless, the other taxa included in this study have been found on long-branched clades, which are mainly due to the variation in TUB and ITS sequences. Another locus that is considered to be helpful for developing DNA barcodes, and which can distinguish many more taxa in the *Ph. exigua* complex is the Actin gene (Aveskamp *et al.* 2009b), which is sequenced with a primer combination developed by Carbone & Kohn (1999). This locus has, however, not been included in the present study, as infraspecific genetic variation, even within the *Didymellaceae*, was too high to align the obtained sequences. Also Calmodulin and Translation Elongation Factor 1-α loci have been tested, but none of the primers combinations used (Carbone & Kohn 1999) could guarantee successful amplification of all strains.

Observations and results presented here represent only a preliminary step towards resolving questions related to the taxonomy of the genus *Phoma*. With the numerous species awaiting to be discovered, the taxonomic system of this complex will probably be changed again as more clades are added. Nevertheless, it is hoped that the present study on *Phoma* systematics, together with the "*Phoma Identification Manual*", will provide a solid foundation on which *Didymellaceae* in general, and the *Phoma* species in particular, can be further delineated.

CHAPTER 6

General discussion



Species of *Phoma* occur worldwide on countless substrates, and are known to represent endophytes, saprobes and plant pathogens. Because several of these taxa are pathogenic, causing serious damage and yield loss to economically important crops, several *Phoma* species are regarded as quarantine organisms, which pose problems to organisations involved in plant health inspections.

Phoma biology

The genus *Phoma* was originally introduced for coelomycetes with single-celled, hyaline conidia that mainly occurred on plant stems (Saccardo 1880). Although one-celled, hyaline conidia are still used as common character, the genus presently comprises organisms with a range of different lifestyles occurring on many plants organs, but also on a series of other organic and inorganic materials. Some species even cause infections in vertebrates, including humans (Zaitz *et al*. 1997, de Hoog *et al*. 2000, Balis *et al*. 2006). Species of *Phoma* can be mycophilic (Hutchinson *et al*. 1994, Sullivan & White 2000), or even lichenicolous (Hawksworth & Cole 2004). More than 100 species have been documented, however, to be important plant pathogens. Many of these are economically important being linked to either important diseases such as Blackleg in *Brassicaceae* (Fitt *et al*. 2006), or are of quarantine importance. Identifying isolates of *Phoma* up to species level requires special skills and expertise. Therefore, the need to develop fast and reliable molecular tools for their detection and identification is welcomed by the plant health organisations (Miric *et al*. 1999, Macdonald *et al*. 2000, Somai *et al*. 2002b, Koch & Utkhede, 2004, Balmas *et al*. 2005, Licciardella *et al*. 2006, Cullen *et al*. 2007).

The life cycle

Although a few hundred species are currently recognised, the life cycles of these fungi are generally similar. Infection of plants occurs through wounds that are caused by cultivation practices, or via direct penetration of the epidermis (Williams 1992, Agrios 1997, Roustaee *et al*. 2000, West *et al*. 2001, van de Graaf *et al*. 2002). After a latent period where no symptoms are visible, globose or flask-shaped pycnidia appear. These pycnidia are present either on, or in the epidermis of the roots, stems or leaves and produces a white to pink conidial mass. Conidia are dispersed by water or wind, or incidentally by small animals (Perrotta & Graniti 1988). When a host is absent, most species survive on dead organic material in the soil, either as chlamydospores, or by forming ascomata that give rise to ascospores for long-range dispersal and infection.

Taxonomy of *Phoma* and *Phoma*-like taxa

Boerema *et al*. (2004) published a taxonomic handbook on *Phoma*, in which the genus *Phoma* was divided into nine sections (Chapter 2). This classification system was criticised, however, for the unclear discrimination between *Phoma* and several related or even convergent genera, such as *Ascochyta*, *Stagonosporopsis* and *Phyllosticta*. Furthermore, the relation between *Ascochyta* and *Phoma* species that produce septate conidia remained unresolved, with both genera having teleomorphs in the genus *Didymella* (Barve *et al*. 2003, Fatehi *et al*. 2003, Peever *et al*. 2007).

Species linked to *Phoma* section *Peyronellaea* are typified by the production of dictyochlamydospores, and thus have additional characters to be used in taxon delineation. However, the production of such chlamydospores is also known in some other sections of *Phoma*. Based on the phylogenetic data obtained in this thesis (Chapter 3), most dictyochlamydospore-producing taxa were shown to cluster together with *Ph. herbarum*, the type species of the genus *Phoma* (section *Phoma*), but also with taxa of two other sections, namely *Phyllostictoides* and *Macrospora*. Based on these results the subdivision of the genus *Phoma* (Boerema 1997) into these sections can therefore be questioned. Furthermore, results from Chapter 3 also demonstrate that single morphological characters should not be used to discriminate between taxa, and definitely not between sections of the genus *Phoma*.

In Chapter 3 it was also shown that the taxa that clustered in section *Peyronellaea* comprise a genetically heterogenous group. The ability to produce dictyochlamydospores has probably been lost and gained multiple times during the evolution of the *Pleosporales*. This character is also easily lost in culture (Boerema *et al*.

1965a, Dorenbosch 1970). Chlamydospores are generally considered to be a survival propagules in fungi to survive harsh conditions, though they may also represent some form of ecological adaptation. In Chapter 5 an attempt was made to delineate generic boundaries by means of integrating morphological and phylogenetic data, and to come to a generic circumscription that is more correct from an evolutionary point of view. A total of 324 strains were included in the analyses of which most belonged to *Phoma* taxa, whilst 54 were related to pleosporalean fungi. In total, 206 taxa were investigated, of which 159 are known to have affinities to *Phoma*. The phylogenetic analyses revealed that the current Boeremaean subdivision was highly polyphyletic. In fact, *Phoma* species clustered in six distinct clades within the *Pleosporales*, and even represented different families. The majority of the species, however, including the generic type, clustered in a recently established family, *Didymellaceae*, which segregated into at least 18 distinct subclusters.

In Chapter 1, a schematic overview of the relations between the several genera and sections associated with the *Phoma* complex was presented (Figure 1.1). During the course of this research, the understanding of the complex changed significantly. A schematic representation of the classification of the taxa associated with *Phoma sensu lato* is presented in figure 6.1.

Asexual-sexual connections

In *Phoma* several teleomorphs have been recognised, but for the majority of *Phoma* species the sexual structures have yet to be discovered. Boerema *et al*. (2004) linked *Phoma* to four teleomorph genera: *Didymella*, *Leptosphaeria*, *Mycosphaerella* and *Pleospora*.

In this study the phylogenetic affinity of the sexual genera *Pleospora* and *Leptosphaeria* was briefly investigated. Based on morphological studies, a one-on-one relation was suggested to exist between these teleomorph genera and the *Phoma* sections *Pilosa* and *Plenodomus* respectively (Boerema *et al*. 1994, Boerema 2003). Molecular data presented in this thesis and in an affiliated study (de Gruyter *et al*. 2009) suggest that for those taxa this approach can be maintained, although the various sections will be elevated to a separate, generic level, as the taxa involved are not incorporated in the *Didymellaceae*.

The hypothesis that *Phoma* section *Macrospora* is linked with *Mycosphaerella* teleomorphs, as suggested by Punithalingam (1990), should be abandoned on the basis of the phylogenetic analyses presented in Chapter 5. The teleomorphs in fact resemble *Didymella*. This genus should be considered as the main, or probably even the sole teleomorph of true *Phoma* species. However, in a recent study on a novel species with an unnamed *Phoma* anamorph, the new genus name *Atrodidymella* has been introduced to accommodate this teleomorph (Davey & Currah 2009), which is also phylogenetically linked to the *Didymellaceae*. Furthermore, *Leptosphaerulina* and *Macroventuria* are also accommodated in the *Didymellaceae*. *Macroventuria* resembles *Venturia* (van der Aa 1971) but ascospore morphology is highly comparable to that of *Didymella*. In contrast, *Leptosphaerulina* is distinct in morphology, producing ascospores with longitudinal and transverse septa (von Arx 1981).

Most *Didymella* species have an asexual state in either *Phoma* or *Ascochyta*. The complexity of this group of species is further illustrated by Woudenberg *et al*. (2011), who discuss the co-occurrence of closely related species with different anamorphs on the same hosts.

DNA barcoding of *Phoma*

During the course of the present study, a series of genome regions were tested for their value in discriminating the various *Phoma* taxa. Many of the tested loci are already successfully applied in several species recognition assays for fungi, such as COI (Seifert *et al*. 2007), ITS (Druzhinina *et al*. 2005, James *et al*. 2006, Rossman 2007, Schoch *et al*. 2009a, b, 2012, Seifert 2009), actin (Voigt & Wöstemeyer 2000), translation elongation factor 1-α (James *et al*. 2006, Schoch *et al*. 2009a, b, Balajee *et al*. 2009), β-tubulin (Geiser *et al*. 2007, Balajee *et al*. 2009) and calmodulin (Geiser *et al*. 2007, Balajee *et al*. 2009). These loci could, however, not be used in a universal *Phoma* identification tool based on DNA barcoding, as the discriminatory power of these loci was either too low, or was only applicable to a certain subset of the taxa included in this study. The main problem was the large genetic diversity in the genus *Phoma*. Although Boerema *et al*. (2004) reduced the number of *Phoma* species by recombining and transferring species to other genera, it appeared that the form-genus represented taxa of at least three different families (de Gruyter *et al*. 2009).

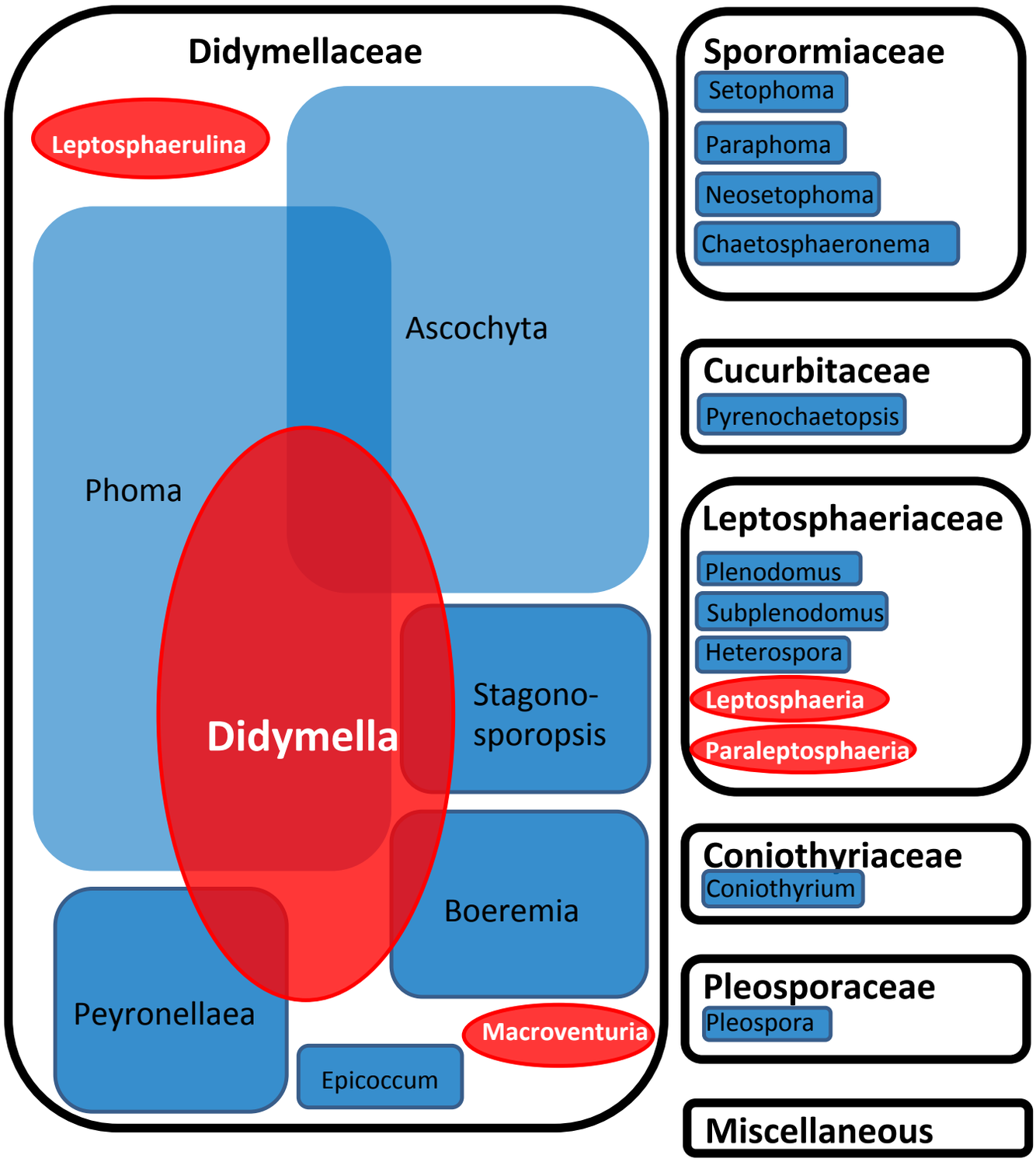


Fig. 6.1. New diagrammatic representation of the distribution of species associated with the former *Phoma* generic complex, with an emphasis on the *Didymellaceae*. The species are redistributed over several families (boxed in white) and genera (blue). Associated teleomorph genera are indicated in red.

However, even when limiting the quest for a universal DNA barcoding region to the *Phoma* species residing in the recently erected family *Didymellaceae*, which houses the species of *Phoma* that are linked to the teleomorph genus *Didymella* (de Gruyter *et al*. 2009), the most common loci in fungal evolutional research still appear to be too variable at infra-specific level. In that light, the quest for a "golden gene", a single locus that is applicable to many genera, as suggested by Hebert *et al*. (2003), Stoeckle (2003) and Seifert (2009), appears to be unrealistic based on the results obtained with the currently tested loci. As the

main question of this study –what exactly is *Phoma*?– is only partially resolved, a DNA barcoding system that is based on multiple gene loci is the best solution to develop a molecular identification system.

Molecular species identification and detection

One of the goals of this research project was to develop a DNA-based method for distinguishing and identifying *Phoma*-like species. However, although development of specific DNA barcodes was advocated, no single locus could be identified with sufficient inter-specific variation and low intra-specific variation to establish a sequence database for all the species treated by Boerema *et al*. (2004). Therefore, multiple loci were used to reconstruct the phylogeny of *Phoma*-like taxa, including β-tubulin, the internal transcribed spacer regions 1 and 2 and the intervening 5.8 S nrRNA gene (ITS), the Large Subunit (28S nrRNA gene) and the Small Subunit (18S nrRNA gene) of the nrRNA gene operon. In Chapter 4, molecular markers were developed to discriminate varieties within what is currently known as the *Boeremia exigua* (previously *Ph. exigua*) complex. At present, the complex includes seven varieties and six related species. Only for *Ph. tarda*, which has been reduced to the varietal status of *B. exigua* var. *coffeae* (described in Chapter 5), no taxon-specific primer combination was generated. The developed molecular markers were based on actin gene sequence data and SCAR-based markers. This technique is considered to be a promising tool for assessing intraspecific genetic variation. Using the obtained DAF-profiles, we were able to design oligonucleotide primer combinations that can aid in identifying taxa at sub-species level. One of the pitfalls of DAF-based identification of *Ph. exigua* varieties is that it is based on the absence or presence of two extremely short nucleotide sequences. Mutations or sexual recombination may affect the target DNA of the DAF primers, causing false negative identification results. However, it should be noted that thus far no evidence has been found for sexual reproduction of *Ph. exigua*. Converting such amplified products to SCAR-based markers circumvent some of the problems of false negatives due to point mutations in the primer binding sites of the DAF primers.

Identification tools

Initial diagnostics of plant diseases and associated pathogens mainly relies on illustrations of disease symptoms and fungal structures, whilst in-depth fungal identifications mainly rely on dichotomous keys based on macro- and micro-morphological characters. For many fungal genera, such keys and illustrations are sometimes hardly available to plant quarantine inspectors or the keys do not cover all of the species in a genus. One of the main goals of the present study that led to this dissertation was to develop an online, publicly available identification tool that combined illustrations with morphological characters and DNA data of *Phoma* spp.

For this identification tool, the same material as used by Boerema *et al*. (2004) was restudied and most macro-morphological and phytopathogenic characters were confirmed. In addition, partial DNA sequences of actin, β-tubulin, calmodulin, the internal transcribed spacer regions 1 and 2 and the intervening 5.8 S nrRNA gene (ITS), 28S nrRNA gene and 18S nrRNA gene were included as additional tools for identification.

A polyphasic identification key, which combines both morphological and molecular characters of the *Phoma* species that are covered in the present study, has been placed online in the Q-bank database (www.q-bank.eu, Fig. 6.2). This website serves as a reference for identification and detection of phytosanitary relevant organisms. Next to the *Phoma* database, there are online identification tools and descriptions available for the genera *Ceratocystis*, *Colletotrichum*, *Monilinia*, *Mycosphaerella* spp. of European quarantine importance, *Phytophthora* and *Stenocarpella*, as well as for many groups of pathogenic bacteria, insects, nematodes, phytoplasmas, viruses, viroids and invasive plant species. The descriptions of the items in the databases, and the link between data and vouchered specimens or cultures, are an indispensable tool to identify and detect harmful quarantine organisms.

The software behind this website, BioloMICS (Robert *et al*. 2011), makes it possible to search for both phenotypic and sequence data, and is therefore a more reliable tool than, for example, a BLAST-search on GenBank, in which many accessions have been incorrectly named (Bridge *et al*. 2003, 2004, Nilsson *et al*. 2006, 2008). The BioloMICS software makes simultaneously searching multiple loci in a single action possible, contrary to GenBank where each locus needs to be blasted individually.

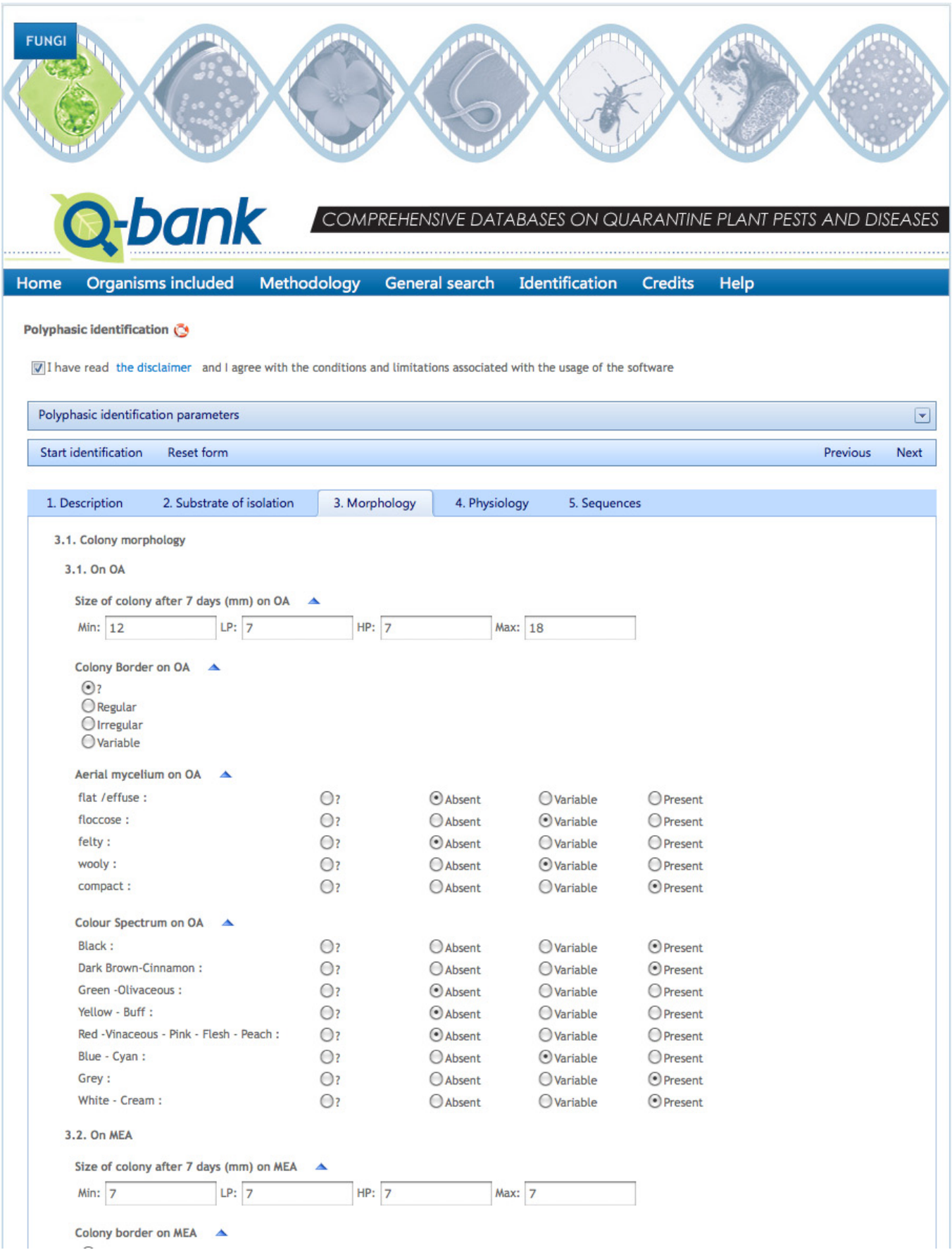


Fig. 6.2. Screen shot illustrating the *Phoma* query page on www.q-bank.eu.

Species identifications

Although DNA barcoding is generally considered as a technique that is very useful in species identification, the biggest limitation remains the content of the DNA library, which makes DNA barcoding less suited for species discovery. Nevertheless, when screening an existing collection carefully, using a set of informative loci, the rate of species discovery can be increased significantly (Puillandre *et al*. 2012). Such an approach led to the discovery of 13 new species and two new taxa at varietal level that were recognised in the collections of the CBS-KNAW Fungal Biodiversity Centre and the Dutch Plant Protection Service. Those novelties have been described in Chapters 3 and 5 of this thesis.

The basic dataset that was developed during this study comprised physiological, micro- and macro-morphological information of more than 700 strains. The dataset itself is considered to be highly informative and was therefore itself published in various open access on-line databases, including the above-mentioned Q-bank and MycoBank. Furthermore, the vast majority of DNA sequences were submitted to NCBI's GenBank nucleotide database. The availability of this dataset has increased the speed in taxon discovery in this species complex and in related genera significantly. For example, Tsuneda *et al*. (2011) described a new genus, *Endophoma*, which phylogenetically clusters with *Ph. eupyrena*. The redefined genus *Epicoccum* has been split up further to accommodate the genetically diverse species *E. nigrum* (Fávaro *et al*. 2011). De Gruyter *et al*. (2010) used this dataset to obtain a better delimitation of *Phoma* and the related genera *Paraphoma*, *Pyrenochaeta* and *Pleurophoma*. Furthermore this dataset has aided in further delimitation of the allied genera *Phomopsis* (Udayanga *et al*. 2010) and *Phyllosticta* (Wikee *et al*. 2011).

Concluding remarks

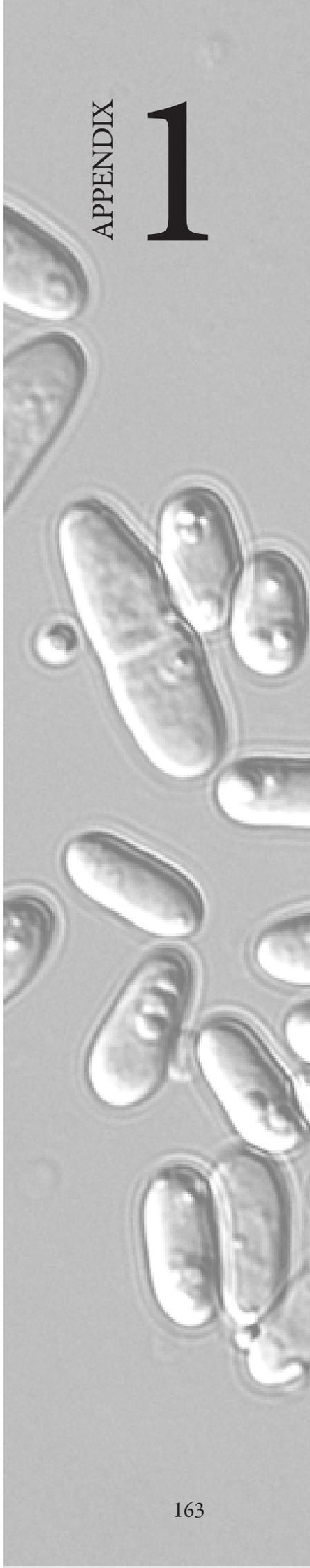
The research presented in this thesis increases our understanding of the diversity of the species in the genus *Phoma* and covers various taxonomical aspects of this broadly defined group of plant pathogens.

The different strains obtained were studied and compared on morphological, physiological and cultural characters, as well as DNA phylogeny. Thirteen new species and two new varieties were recognised and described in this study. One of the reasons to initiate the present study, was to clarify the status of *Phoma,* and to judge the validity of the sections introduced by Boerema (1997). Results obtained in this study demonstrated that although some sections can be partially maintained, most of the sections appeared not supported from an evolutionary perspective (e.g. *Phoma* section *Peyronellaea*). Based on the results obtained here, it seems prudent that future classification of taxonomic novelties into these sections should be avoided.

These data also led to a significant renaming of the species in the *Phoma* complex, which is more consistent with teleomorph names, and in correlation with chlamydospore structure, spore shape and other relevant characters. Examination of all strains available to us, as well as herbarium material used in the original *Phoma* studies of Boerema and co-workers (Boerema *et al*. 2004), allowed the development of an online identification tool. The results obtained in this study have greatly facilitated the accurate identification of *Phoma* species and new isolates. This approach has laid the foundation for the identification of present and future collections of *Phoma* and *Phoma*-like taxa. It is essential that the database thus be expanded to incorporate new accessions that get deposited in the culture collections at the CBS and the National Reference Laboratory of the Dutch Plant Protection service in future.

APPENDIX 1

References



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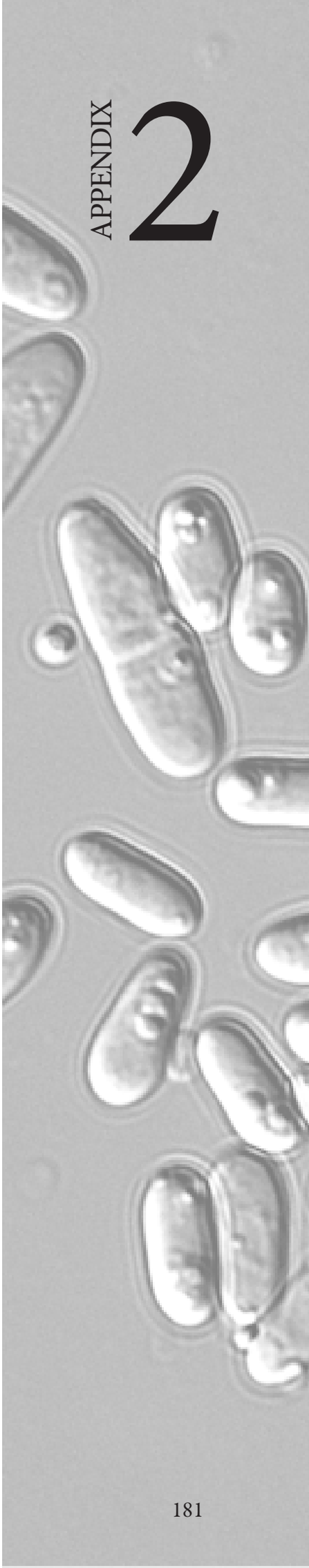
APPENDIX 2

Summary



This thesis treats the taxonomy of a generic complex presently known as *Phoma* Sacc. emend Boerema & Bollen. This group of fungi comprises more than 200 taxa at species or variety level that are characterised by the production of hyaline, non-septate conidial spores in pycnidial conidiomata. The genus is omnipresent in the environment, and exponents can be found on a wide range of host substrates.

For many years the genus *Phoma* was the main research topic of a group of mycologists at the Dutch National Plant Protection Service. The studies conducted in the last decennia of the previous century culminated in a handbook that monographed the majority of the species in the above-mentioned generic complex. This handbook marked the end of the era in which the taxonomy of this genus mainly relied on morphological observations and cultural descriptions. However, it can also be regarded as the starting point of the present study. The aim of the present project was to integrate DNA-based identification methods into the taxonomic system established by the previously mentioned group of researchers. The major part of this study therefore deals with the validation of current generic and species concepts.

An extensive literature review of the biology, taxonomy and identification methods to the species in this genus is provided in **Chapter 2**, with specific reference to the progress that has been made in *Phoma* taxonomy after the publication of the abovementioned handbook. The advantages and disadvantages of the current taxonomical system are discussed. Furthermore, this chapter describes the general biology of the species in this fungal group, including their life cycles, distribution and host substrates. The importance of the genus for plant health and quarantine issues is illustrated, and the development of a rapid and robust identification technique based on DNA barcodes is advocated.

Chapter 3 treats species in *Phoma* section *Peyronellaea*. Species in this section are typified by the production of dictyochlamydospores, and thus have additional morphological characters to use in taxon delineation in comparison with species in the other *Phoma* sections. All species in this group were subjected to a morphological re-examination and phylogenetic analyses employing ITS, actin, and β-tubulin nucleotide sequences. Based on multi-gene analyses, *Phoma* section *Peyronellaea* could not be maintained as a taxonomic entity, due to the polyphasic nature of taxa in this section. The morphological study revealed that for five species a taxonomic revision was required. A further five species appeared to be new to science, including *Ph. microchlamydospora*, *Ph. omnivirens*, and *Ph. schachtii.* Also the taxonomic novelties *Ph. coffeae-arabicae* and *Ph. sancta* are described here, and are allocated to the genus *Peyronellaea*, re-erected in Chapter 5.

In **Chapter 4** the diversity among species and varieties belonging to the *Ph. exigua* species complex is investigated. The *Ph. exigua* species complex includes nine taxa at varietal level and four species that have a high morphological similarity both *in vivo* and *in vitro*, whilst historical relations with plant hosts cannot be maintained. Among this group, both omnipresent saprobes as well as host-specific plant pathogens are present – including the potato pathogen *Ph. foveata*.

The diversity in this complex is studied by means of Internal Transcribed Spacer regions 1 & 2 and intervening 5.8S nrDNA (ITS) and actin nucleotide sequence analyses and a DNA fingerprinting technique rarely used to study fungal diversity. This technique, DNA Amplification Fingerprinting (DAF) employs short, arbitrary primers that form a loop, or a mini-hairpin, under specific temperature conditions and is frequently used in molecular plant breeding. The amplified DNA fragments were isolated and sequenced in order to develop taxon-specific markers and primer combinations based on the SCARs (Sequence Characterised Amplified Regions) and actin sequence data generated. These tools can aid rapid identification of this morphologically highly similar set of taxa.

Two separate taxa were recognised within the type variety *Ph. exigua* var. *exigua*. In the following chapter these taxa are described and all species and varieties in this complex are recombined into the new genus *Boeremia*.

Chapter 5 provides further details about the taxonomy and phylogeny of the species of interest, with a special focus on the taxa that are phylogenetically placed with *Didymellaceae*. In total 206 taxa were treated, of which 159 have affinities with *Phoma*.

The genus is circumscribed in the first section of this chapter. The phylogeny was reconstructed using 28S nrDNA (Large Subunit) and 18S nrDNA (Small Subunit) sequence data. It was shown that the currently used Boeremaean subdivision of the phomoid taxa and the phylogeny were inconsistent, as the genus was highly polyphyletic. Species belonging to the form-genus *Phoma* were retrieved in as much as six distinct clades within *Pleosporales*. These clades even represent different families. The majority of the phomoid taxa, including the type species *Ph. herbarum* and most exponents of the sections *Macrospora, Peyronellaea, Heterospora*, and *Phyllostictioides*, were found in a single clade that represented the

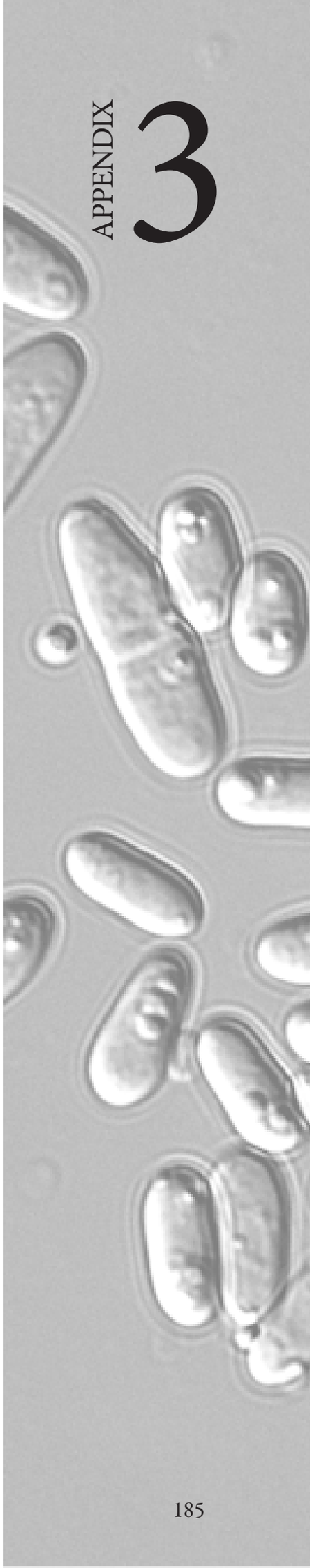
Didymellaceae. Most species that are associated with the *Phoma* sections *Plenodomus* and *Pilosa* cluster with the *Leptosphaeriaceae* and *Pleosporaceae* clades. Furthermore, some species were also found to cluster in the *Sporormiaceae* and *Cucurbitariaceae* clades.

In the second part of this chapter, the phylogenetic variation of the species and varieties in *Didymellaceae* is further assessed, using a phylogenetic reconstruction that is based on DNA sequences of the Large Subunit, ITS, and part of the β-tubulin (TUB) gene region. Besides the teleomorph genus *Didymella*, members of the teleomorph genera *Leptosphaerulina* and *Macroventuria* were also found to cluster in this clade. Based on the reconstructed phylogeny, *Didymellaceae* segregate into at least 18 distinct clusters, of which many can be associated with specific morphological characters. Furthermore, a number of taxa did not match any of these clusters, suggesting that an evolutionary correct subdivision of the *Phoma* species in *Didymellaceae* is even more complex. Taxa in four of these phylogenetic clusters were also defined well enough by means of morphology to elevate these groups to new or reinstalled genera, namely *Stagonosporopsis*, *Epicoccum*, *Boeremia* and *Peyronellaea*. A total of 61 taxa were recombined and several new species of *Phoma* were introduced, namely *Ph. brasiliensis*, *Ph. bulgarica*, *Ph. dactylidis*, *Ph. dimorpha*, *Ph. longicolla*, *Ph. minor*, *Ph. pedeiae* and *Ph. saxea*. Furthermore, two new varieties were described, *Boeremia exigua* var. *gilvescens* and *B. exigua* var. *pseudolilacis*.

Finally, the results presented in this dissertation are highlighted and discussed in **Chapter 6**. In total 13 species of *Phoma* and two taxa at varietal level were newly described during the course of this study. Moreover the taxonomic status of species in the form-genus *Phoma* were further clarified, and insight provided into the phylogenetic status of *Didymellaceae*, a fungal family that was recently established, comprising most species of *Phoma*, *Ascochyta* and *Didymella*. All macro- and micromorphological data obtained in this study, as well as the DNA sequences, were placed online in a publicly available polyphasic identification database (www.q-bank.eu). This will enable scientists and institutes involved in plant health to correctly identify phomoid species. Rapid identification of these species based on the tools and data generated in this study, can facilitate swift clearing of plant material and arable products during export and import, and prevent the spread of quarantine organisms.

APPENDIX 3

Samenvatting



Dit proefschrift beschrijft de taxonomie van het complexe schimmelgeslacht dat bekend staat als *Phoma* Sacc. emend Boerema & Bollen. Deze groep schimmels bevat meer dan 200 taxa op soorts- of variëteitsniveau, die gekarakteriseerd worden door de vorming van ongesepteerde, pigmentloze sporen in pycnidia. Het geslacht is wijdverspreid, en vertegenwoordigers ervan worden op vele verschillende substraten gevonden.

Voor een groep mycologen destijds verbonden aan de Nederlandse Plantenziektekundige Dienst was de taxonomie van het geslacht *Phoma* een belangrijk onderzoeksthema. Het daar uitgevoerde onderzoek in de laatste twee decennia van de afgelopen eeuw leidde tot de publicatie van een handboek waarin nagenoeg alle soorten in dit complexe geslacht staan beschreven. Dit handboek markeerde het einde van het tijdperk waarin de taxonomie van dit geslacht hoofdzakelijk gebaseerd was op morfologische observaties en beschrijvingen van cultures. Daarentegen kan het ook beschouwd worden als het startpunt van het onderzoek waar dit proefschrift deel van uitmaakt. Dit promotieonderzoek had als doel om op DNA merkers gebaseerde identificatiemethoden te integreren in het taxonomische systeem dat opgezet was door voornoemde onderzoekers. Het grootste deel van dit promotieonderzoek behelst de validatie van de huidige geslachts- en soortsconcepten.

En uitgebreid literatuuronderzoek over de biologie, taxonomie en identificatiemethoden van de soorten in dit geslacht is beschreven in **Hoofdstuk 2**. Speciale aandacht is gegeven aan de voortgang die is gemaakt in *Phoma* taxonomie ná het verschijnen van bovengenoemd handboek. Tevens worden de voor- en nadelen van het huidige taxonomische systeem bediscussieerd. Daarnaast wordt er in dit hoofdstuk de algemene biologische kenmerken van de soorten binnen dit schimmelgeslacht beschreven, inclusief de levenscyclus, geografische verspreiding en substraten. Het belang van deze groep schimmels in plantgezondheid en quarantainezaken is beschreven. De ontwikkeling van een snelle en robuuste identificatiemethode gebaseerd op de DNA barcoding, wordt aanbevolen.

Hoofdstuk 3 beschrijft een verkennende studie binnen *Phoma* sectie *Peyronellaea*. Soorten binnen deze sectie worden gekarakteriseerd door de vorming van dictyochlamydosporen, en beschikken dus ten opzichte van soorten in andere secties additionele morfologische eigenschappen. Alle soorten in deze groep zijn morfologisch opnieuw bestudeerd en fylogenetisch geanalyseerd. Daarbij is gebruik gemaakt van DNA sequenties welke codeerden voor Internal Transcribed Spacer regio's 1 & 2 en 5.8S nrDNA (ITS), actine en β-tubuline. Op basis van deze multi-gen analyses was het niet mogelijk om *Phoma* sectie *Peyronellaea* als taxonomische entiteit in stand te houden, omdat de groep zeer polyfyletisch is. Het morfologische deel van deze studie toonde aan dat voor vijf soorten een taxonomische samenvoeging nodig was. Verder werden er vijf nieuwe soorten ontdekt, waaronder *Ph. microchlamydospora*, *Ph. omnivirens* en *Ph. schachtii.* Ook de nieuwe soorten *Ph. coffeae-arabicae* en *Ph. sancta* zijn in dit hoofdstuk beschreven. Echter, in hoofdstuk 5 zijn deze soorten verder samengevoegd in het nieuwe geslacht *Peyronellaea*.

In **Hoofdstuk 4** is de diversiteit van de soorten en variëteiten in het *Ph. exigua* soortscomplex onderzocht. Deze taxonomisch complexe groep bevat vier taxa op soortsniveau en negen variëteiten. Deze zijn zowel *in vivo* als *in vitro* morfologisch zeer gelijkend, terwijl er ook geen sprake is van een duidelijke een-op-een interactie met een vaste waardplant. Binnen deze groep bevinden zich zowel wijdverspreide saproben als specifieke plant pathogenen, zoals het aardappelpathogeen *Ph. foveata*.

De diversiteit in dit complex is bestudeerd met behulp van DNA sequentie analyse van ITS en actine en een nieuwe vingerprinttechniek die nog niet eerder gebruikt is voor schimmels. Deze techniek, DNA Amplification Fingerprinting (DAF) maakt gebruik van korte, specifieke primers die een kleine lus vormen onder de juiste temperatuurcondities en vaak gebruikt worden in moleculaire plantentaxonomie en plantenveredeling . De hiermee geamplificeerde DNA fragmenten werden geïsoleerd en gesequenced, met als doel om taxon-specifieke markers te ontwikkelen.. Deze markers zijn gebaseerd op de SCARs (Sequence Characterised Amplified Regions) en actine sequenties. Met deze methode is het mogelijk een snelle identificatie uit te voeren van deze morfologisch sterk gelijke groep schimmels.

Binnen de typevariëteit *Ph. exigua* var. *exigua* zijn twee aparte taxa te onderscheiden. In het volgende hoofdstuk zijn deze taxa verder beschreven en taxonomisch op naam gebracht. Verder zijn alle taxa in dit complex ondergebracht in het nieuwe geslacht *Boeremia*.

Hoofdstuk 5 betreft een gedetailleerde studie over de taxonomie en fylogenie van de soorten binnen *Phoma*, en is voornamelijk toegespitst op de taxa die fylogenetisch gegroepeerd zijn binnen de *Didymellaceae*. In totaal zijn 206 taxa behandeld; hiervan hebben er 159 een *Phoma* anamorf.

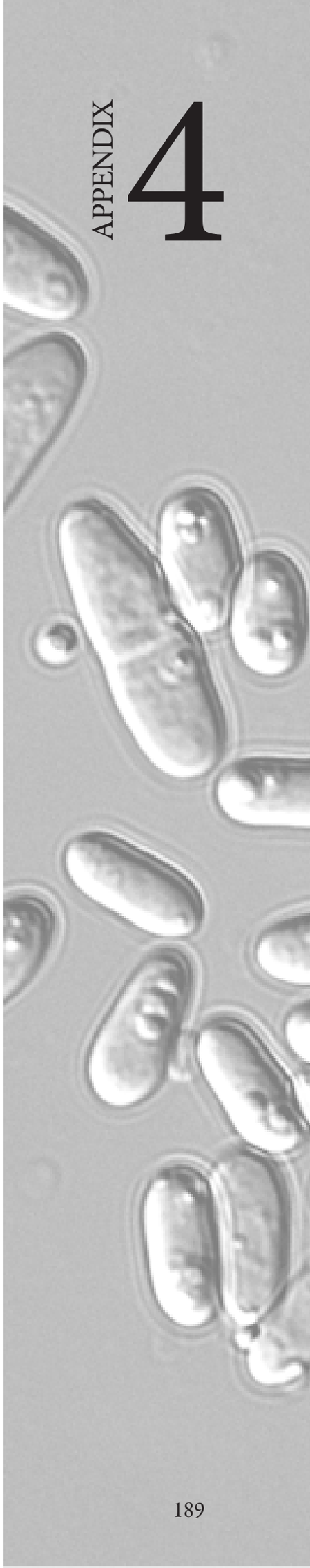
In het eerste gedeelte van dit hoofdstuk is de algemene fylogenie weergegeven voor dit geslacht. De fylogenie is gereconstrueerd met DNA sequenties van 28S nrDNA (Large Subunit) en 18S nrDNA (Small Subunit). Hieruit blijkt dat de huidige, door Boerema voorgestelde onderverdeling van de *Phoma*-achtige taxa inconsistent is, omdat het geslacht zeer polyfyletisch blijkt te zijn. Soorten van het vorm-geslacht *Phoma* zijn gevonden in zes verschillende clusters binnen de *Pleosporales*. Deze clusters vertegenwoordigen verschillende families. De meerderheid van deze *Phoma*-achtigen, waaronder de typesoort *Ph. herbarum* en de meeste exponenten uit de secties *Macrospora, Peyronellaea, Heterospora* en *Phyllostictioides*, groeperen in een enkele cluster die geassocieerd is met de *Didymellaceae*. De meeste soorten die geassocieerd worden met de *Phoma* secties *Plenodomus* en *Pilosa* groeperen in de *Leptosphaeriaceae* en *Pleosporaceae* cluster. Daarnaast zijn er soorten gevonden die clusteren in de *Sporormiaceae* en *Cucurbitariaceae* families.

Het tweede gedeelte van dit hoofdstuk is de fylogenetische variatie van de soorten en variëteiten binnen de *Didymellaceae* familie bestudeerd. Hierbij is gebruik gemaakt van een fylogenie gebaseerd op DNA sequenties van de Large Subunit, de ITS en een gedeelte van het β-tubuline (TUB) gen. Naast het teleomorf geslacht *Didymella* zijn er ook vertegenwoordigers gevonden van de teleomorfe geslachten *Leptosphaerulina* en *Macroventuria* in dit cluster. Op basis van de gereconstrueerde fylogenie, wordt de *Didymellaceae* familie in ten minste 18 clusters opgesplitst. Veel clusters kunnen gekoppeld worden aan specifieke morfologische eigenschappen. Verder is er een aantal taxa gevonden die buiten de clusters vallen, wat suggereert dat het reconstrueren van de evolutie van de *Didymellaceae* waarschijnlijk nog complexer is dan eerder vermoed. Taxa in vier van deze fylogenetische clusters konden ook op basis van morfologie met voldoende zekerheid gekarakteriseerd worden om ze in aparte geslachten onder te verdelen. Deze nieuw voorgestelde geslachten betreffen *Stagonosporopsis*, *Epicoccum*, *Boeremia* en *Peyronellaea*. In totaal zijn er in dit hoofdstuk 61 taxa samengevoegd. Daarnaast zijn er verschillende nieuwe soorten beschreven, te weten *Ph. brasiliensis*, *Ph. bulgarica*, *Ph. dactylidis*, *Ph. dimorpha*, *Ph. longicolla*, *Ph. minor*, *Ph. pedeiae* en *Ph. saxea*. Bovendien zijn er twee nieuwe variëteiten beschreven: *Boeremia exigua* var. *gilvescens* en *B. exigua* var. *pseudolilacis*.

Tot slot worden in **Hoofdstuk 6** de resultaten uit deze studie bediscussieerd en samengevat. In totaal zijn 13 nieuwe soorten en twee variëteiten beschreven. Daarnaast is de taxonomische status van de soorten in het vorm-geslacht *Phoma* verduidelijkt en zijn verdere inzichten verkregen over de fylogenetische status van de *Didymellaceae*, een familie binnen de schimmels die de meeste *Phoma*, *Ascochyta* en *Didymella* soorten bevat. Alle macro- en micromorfologische gegevens die in deze studie zijn verzameld, alsmede de DNA sequenties die zijn gegenereerd, zijn gepubliceerd in een publiek toegankelijke polyfasische identificatie databank (www.q-bank.eu). Deze databank kan wetenschappers en medewerkers van instituten die betrokken zijn bij plantenziektekundig onderzoek, in staat stellen om *Phoma*-achtige schimmels snel te identificeren. Snelle identificatie van deze soorten, op basis van de gegevens die in dit onderzoek zijn gegenereerd, is van belang bij het vlot inklaren van planten en plantaardige producten bij export en import waardoor verspreiding van quarantaineziekten sterk kan worden terug gedrongen.

APPENDIX 4

Acknowledgements



This study started unexpectedly at the diagnostic mycological section of the former Dutch Plant Protection Service in Wageningen (PD). Published research from this group on *Phoma* taxonomy indirectly led to the establishment of a new PhD position at CBS in 2005. Coincidentally I was working at the PD in Wageningen, which may have helped to gain the position in Utrecht. For the work conducted there, I am much indebted to many persons who helped me in one way or another.

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The project really started when Joyce Woudenberg joined the *Phoma*-team as technician. Joyce, I am very much indebted to you for all your hard work in the laboratory and your enormous precision in data handling. Now you have embarked on your own PhD project and I am convinced that your talents in the lab and in the analyses will result in a wonderful thesis.

Two guest researchers were of great help to me. Elena Turco helped me so enthusiastically with the DAF research and Jens Laurids Sørensen is much credited for his work with the *Peyronellaea* species.

Of course I am also in indebted to the other members of the FES project at CBS. Lute-Harm Zwiers, many thanks for your technical guidance and the loads of work you did on managing the FES project. Vincent Robert, thank you for helping to set up the database now available in Q-bank. Henk Brouwer and Arthur de Cock, I should offer you my sincere apologies for my jokes about the "fake-fungal status" of the wonderful organisms you were dealing with. Moreover, I want to thank you for the great cooperation in setting up a workable Q-bank database. Collin Gerritzen, you are one of the most talented lab-rats that I have ever known. I am glad I have worked with you at the CBS, and even more so that we now again work together in Horst.

Mahdi Arzanlou, we have shared an office for three years – although it appears to have been a much shorter period (as we would always tend to say: "Time flies like an arrow, fruit flies like a banana"). Marizeth Groenewald, you provided me with loads of practical advice during my PhD. My thanks go to both of you for our nice discussions and collaboration.

We had many people from over the world in our research group, with whom I could share a laugh, or have an interesting conversation about fungi, science, culture, and life in general. Mieke Starink, Lizel Mostert, Lam-Duong Minh, Uwe Simon, Gavin Hunter, Ratchadawan (Joy) Cheewangkoon, Lorenzo Lombard, Ulrike Damm, Konstanze Bensch, Chirlei Glienke, Annemiek Schilder, Salwa Essakhi, William Quaedvlieg, and all other persons with whom I shared an office for a shorter or longer period: Many thanks for our pleasant conversations and for being quiet at times when it was needed.

The CBS is further equipped with many skilled people who are essential for a good research organization. Manon Verweij, many thanks for your great help with the design of this thesis and for your perfect organizational skills. Arien van Iperen, thank you for your help with the deposit of strains and herbarium

material and for the maintenance of the working collections. To Marjan Vermaas I am highly indebted for her assistance in preparing the photo-plates and “guarding” the computers in the library on which the phylogenetic analyses ran. Many thanks to Cecilé Gueidan for her assistance in conducting the phylogenetic analyses and the useful discussions we had on the taxonomical status of many species. Jeroen Korving of the Hubrecht Laboratory is thanked for his help in preparing the microtome sections.

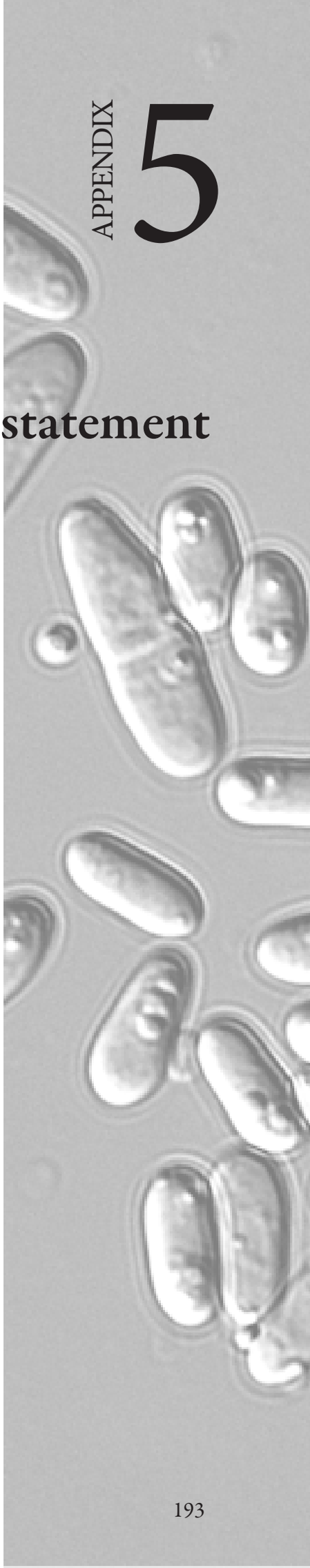
I would also like to express my appreciation to the persons who have provided us with the new fungal strains, or who were kindly willing to release strains in the CBS collection from restrictions. These include Amy Rossman (Systematic Botany and Mycology Laboratory, USA), Wolfgang Krumbein and Anna Gorbushina (University of Oldenburg, Germany), Mónica Murace and Analía Perelló (Universidad Nacional de La Plata, Argentina), Keith Seifert, (Agriculture and Agri-Food Canada) and Ludwig Pfenning (Federal University of Lavras, Brazil). In this respect I should not forget to express my great gratitude to Karin Rosendahl from the Plant Protection Service in Wageningen, who provided us with hundreds of strains from the PD-collection.

However, most strains used in this study were preserved in an excellent state by “the hidden force of the CBS”, namely the Collection department. Trix Merkx, Jannie Holtman, Gerrit Stegehuis, Francis Claus, Yda Vlug, and all others from your team: you are doing a great job!

I know I have been postponing the finishing of the PhD for several years, and without the encouragement of many of you, I would not have made it. I experienced the greatest encouragement from my family - my parents, my siblings, my in-laws-, but above all, my lovely children and my wonderful wife. Marleen –I know it has been a difficult time for you when I spent those evenings and weekends to “work on my PhD”, but without your support this booklet would probably never have been written.

APPENDIX 5

Graduate school education statement form



Research School *Bio*diversity

BioDIVERSITEIT

PHD PROFICIENCY CERTIFICATE

Maikel Aveskamp

PhD Thesis Title: *Taxonomy and Identification in the form-genus Phoma*

Graduated at: Wageningen University

Has successfully fulfilled the education program according to the criteria of the Research School.

Period: 1 June 2005 - 31 May 2010

For list: see appendix

Date: 26 January 2012

Scientific Director Research School Biodiversity:

Prof. dr. M.S.M. Sosef

Signature:

*Bio*DIVERSITEIT

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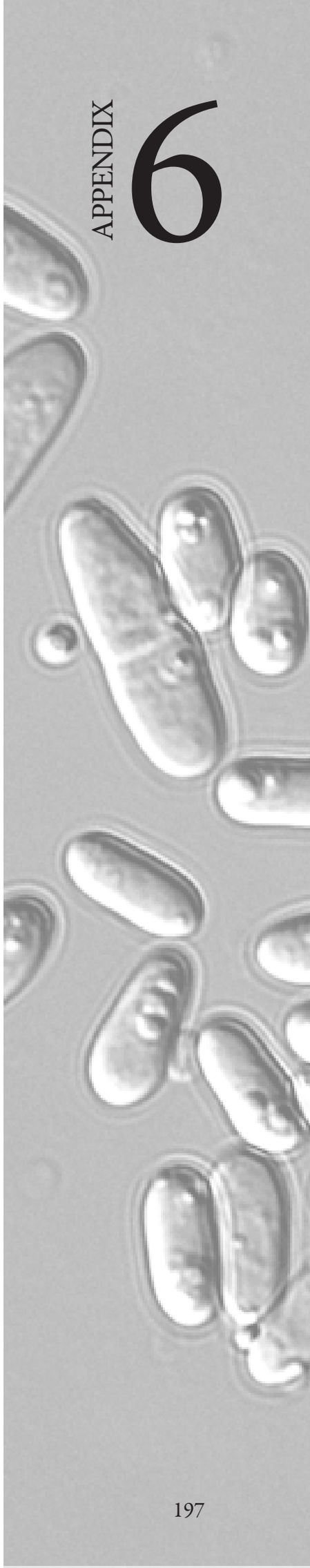
Appendix to PhD Proficiency certificate of Maikel Aveskamp

1. Start-up phase	*date*	*CE*
First presentation of your project (mandatory)		
Phylogeny and DNA barcoding of the genus Phoma	*November 2005*	1.0
Writing or rewriting a project proposal		
Phylogeny and DNA barcoding of the genus Phoma	*September 2005*	4.5
Subtotal Start-up phase		5.5
2. Scientific Exposure		
PhD day (mandatory)		
PhD day Research School Biodiversity, Wageningen University	*1 December 2005*	0.3
PhD day Research School Biodiversity, CBS. Oral Presentation	*8 December 2006*	0.3
PhD day Research School Biodiversity, University of Amsterdam. Oral & PosterPresentation	*13 December 2007*	0.3
PhD day Research School Biodiversity, Naturalis/ Leiden University	*4 December 2008*	0.3
CBS/Naturalis/NHN/IBED/EPS colloquia, study days, etc. (mandatory)		
CBS Seminar series	*2005-2009*	4.0
Colloquia Laboratory of Phytopathology	*2005-2009*	0.6
International symposia and congresses (mandatory)		
International Mycological Congres IMC 8, Cairns, Australia. Poster presentation	*21–25 August 2006*	1.5
DNA Barcoding in Europe, Leiden, the Netherlands. Poster presentation	*3–5 October 2006*	0.9
CBS Symposium Fungi and Health, Amsterdam, The Netherlands. Poster Presentation.	*13–14 November 2007*	0.6
9th International Congress on Plant Pathology ICPP9, Torino, Italy. Oral & Poster Presentataion	*24–29 August 2008*	1.8
46th Congress of the Southern African Society for Plant Pathology. 2 Oral presentations	*25–28 January 2009*	1.2
Subtotal Scientific Exposure		11.8
3. In-Depth Training		
PhD courses (mandatory)		
Advanced topics in phylogeny reconstruction, NHN, Leiden		3.6
Molecular Phylogenies: Reconstruction and interpretation, Wageningen Univ.	*16–20 October 2006*	1.5
CBS Course on Mycology, CBS, Utrecht	*5–17 February 2007*	2.8
Writing Scientific English, KNAW, Amsterdam	*October–December 2007*	3.7
Journal club		
Individual research training		
Subtotal In-Depth Studies		11.6

4) Personal development	*date*	*CE*
Skill training courses (mandatory)		
Gelcompar II and BioNumerics, Sint-Martens-Latem, Belgium	*12–13 December 2005*	0.6
Introduction to phylogenetic analysis, NHN, Leiden	*February 2006*	2.3
Goal Oriented Working and Planning, KNAW/Boertien Training, Utrecht	*March–April 2006*	0.6
Elemental training Photoshop for Publications, CBS, Utrecht	*October 2008*	0.5
Organisation of PhD students day, course or conference		
Organisation committee PhD day Research School Biodiversity 2006	*8 December 2006*	2.0
Membership of Board, Committee or PhD council		
Subtotal Personal Development		6.0
TOTAL NUMBER OF CREDIT POINTS		**34.9**

APPENDIX 6

List of publications



Refereed scientific papers

Aveskamp MM, Gruyter de J, 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, Verkley GJM, Gruyter de J, 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 de J, 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.

Aveskamp MM, Gruyter de J, 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.

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, 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 (2013). Redisposition of *Phoma*-like anamorphs in *Pleosporales*. *Studies in Mycology* **75**: 1–36.

Luong TM, Huynh LMT, Tran U, Dau VT, Burgess LW, Phan HT, **Aveskamp MM**, Vo AD. (2008). First report of *Phoma terrestris* causing pink root rot of Chinese onion in Vietnam. *Australasian Plant Disease Notes* **3**: 147–149.

Sørensen JL, **Aveskamp MM**, Thrane U, Andersen B (2010). Chemical characterization of *Phoma pomorum* isolated from Danish maize. *International Journal of Food Microbiology* **136**: 310–317.

Paz Z, Komon-Zelazowska M, Druzhinina IS, **Aveskamp MM**, Shnaiderman A, Aluma Y, Carmeli A, Ilan M, Yarden O (2010). Diversity and potential antifungal properties of fungi associated with a Mediterranean sponge. *Fungal Diversity* **42**: 17–26.

Woudenberg JHC, **Aveskamp MM**, Gruyter de J, Spiers AG, Crous PW (2009). Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **22**: 56–62.

Abstracts and Presentations

Aveskamp MM, Wingelaar GJ (2005). Elimination of *Phytophthora ramorum* in the tunnel-composting process. *In*: *Sudden Oak Death Second Science Symposium The State of Our Knowledge,* 18–21 January 2005, Monterey, California, United States. Poster presentation.

Aveskamp MM, Baal van PJM, Gruyter de J (2005). Effect of sanitary measures on the survival of *P. ramorum* in soil. *Sudden Oak Death Second Science Symposium The State of Our Knowledge,* 18–21 January 2005, Monterey, California, United States. Poster presentation.

Aveskamp MM, Gruyter de J, Crous PW (2006). A phylogenetic re-evaluation of the sections within the genus *Phoma*. *International Mycological Congres IMC 8*, 21–25 August 2006, Cairns, Australia. Poster presentation.

Aveskamp MM, Gruyter de J, Groenewald JZ, Summerbell RC, Verkley GJM, Crous PW (2006). A phylogenetic study on the sections in the genus *Phoma*. *Joint Meeting of the American Phytopathological Society, the Canadian Phytopathological Society and the Mycological Society of America*, 29 July –2 August 2006, Québec City, Canada. Poster presentation.

Aveskamp MM, Gruyter de J, Woudenberg JHC, Crous PW (2007). DNA Barcoding the fungal genus Phoma: sequence typing in a generic complex. *DNA Barcoding in Europe*, 3–5 October, Leiden, the Netherlands. Poster presentation.

Aveskamp MM, Gruyter de J (2008). Taxonomy of plant pathogenic fungi: *Phoma*. *9th International*

Congress of Plant Pathology, 24–29 August, Torino, Italy. Oral presentation.

Aveskamp MM, Turco E, Groenewald JZ, Gruyter de J, Crous PW (2008). Distinguishing varieties within the *Phoma exigua* complex using DAF-based DNA barcodes. *9th International Congress of Plant Pathology*, 24–29 August, Torino, Italy. Poster presentation.

Aveskamp MM, Turco E, Woudenberg JHC, Groenewald JZ, Gruyter de J, Crous PW (2009). DNA Amplified fingerprinting using mini-hairpin primers as a tool to identify taxon-specific SCARs in *Phoma exigua*. *46th Congress of the Southern African Society for Plant Pathology*, 25–28 January 2009, Gordon's Bay, South Africa. Oral presentation.

Aveskamp MM, Gruyter de J, Woudenberg JHC, Crous PW (2009). Defining genus and specie concepts in the genus *Phoma*: a labyrinth of species complexes of major phytopathological importance. *46th Congress of the Southern African Society for Plant Pathology*, 25–28 January 2009, Gordon's Bay, South Africa. Oral presentation.

Gruyter J de, **Aveskamp MM**, Crous PW (2006). Barcoding the genus *Phoma*: The next phase for *Phoma* taxonomic research in The Netherlands. *International Mycological Congres IMC 8*, 21–25 August 2006, Cairns, Australia. Poster presentation.

Gruyter J de, **Aveskamp MM**, Woudenberg JHC, Verkley GJM, Groenewald JZ, Crous PW (2010). Redisposition of *Phoma* anamorphs in the *Leptosphaeriaceae* and *Pleosporaceae*. *International Mycological Congres IMC 9*, 1–6 August 2010, Edinburgh, United Kingdom. Poster presentation.

Hoog de GS, Borman A, Ahmed AOA, **Aveskamp MM** (2006). Phylogeny of *Madurella*-like agents of black-grain mycetoma. *16th Congress of the International Society for Human and Animal Mycology*, 25–29 June, Paris, France. Poster presentation.

Sørensen JL, **Aveskamp MM**, Hansen MAE, Thrane U, Crous PW, Andersen B (2008). Polyphasic analysis of *Phoma* section *Peyronellaea*. *CBS Symposium Fungi and Health*, 13–14 November, Amsterdam, the Netherlands. Poster presentation.

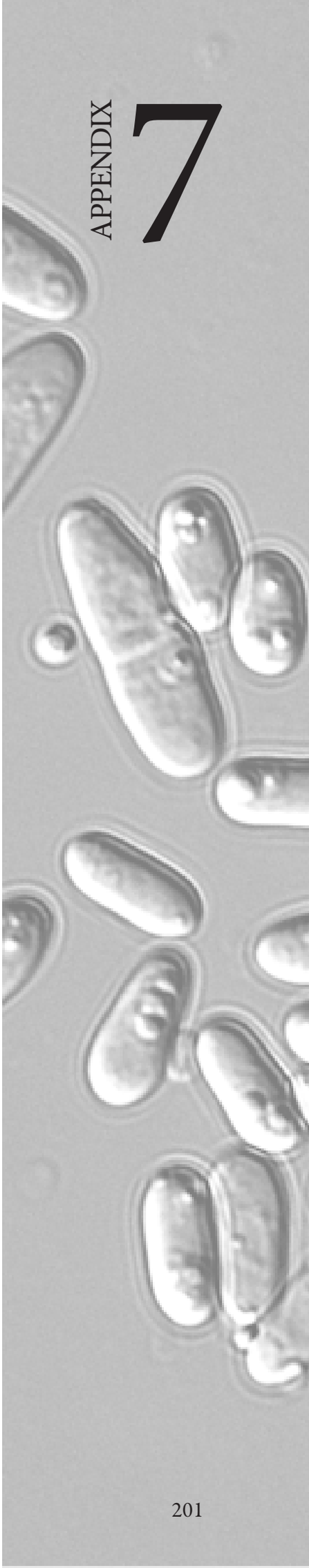
Other publications

Aveskamp MM, Verkley GJM, Crous PW (2006). *Phoma* barcoding: De volgende fase in taxonomisch onderzoek in een complex schimmelgeslacht. *Gewasbescherming* **37**: 266–269.

Zwiers L-H, **Aveskamp MM**, Bonants P, Brouwer H, Cock de A, Damm U, Gruyter de J, Meekes E, Verstappen E, Woudenberg J (2011). Taxonomie van plant-pathogene schimmels als basis voor identificatie en detectie. *Gewasbescherming* **43**: 57–62.

APPENDIX 7

Glossary

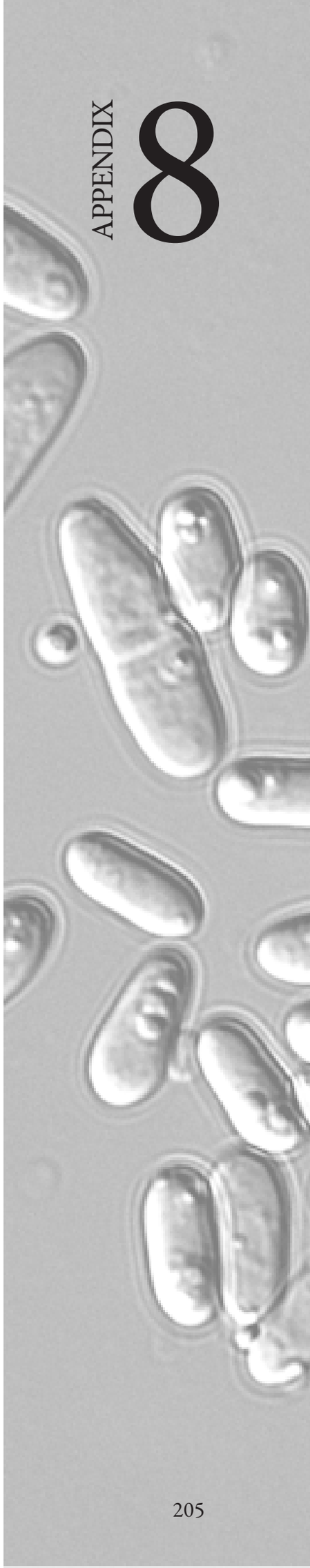


aggregated near together, thereby forming a cluster or mass of individual units
allantoid curved, cylindrical (sausage-shaped)
ampulliform flask-like in form
anamorph asexual or mitosporic form of propagation (= imperfect state)
annellation elongation of a conidiogenous cell with progressive conidiogenesis
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
compact solid, firm in texture
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)
dictyo(chlamydo)spores with both transversely and longitudinally septate 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
felty consisting of matted, compressed hyphae
filiform thread-like
floccose loosely cottony or woolly, or more densely agglomerated into small bundles
genus novum new genus
glabrous smooth, not hairy
guttule lipid storage organelle
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
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
pyriform pear-like in form
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 (≡), 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
var. nov. varietas novus, new variety
vouchered specimen is a preserved sample deposited for future reference gen. nov.
wooly resembling wool in appearance

APPENDIX 8

Curriculum vitae



Maikel Martijn Aveskamp was born on the 4th of December in Losser, located in the Twente region in the eastern parts of the Netherlands. In 1998, he graduated from pre-university secondary education at the Twents Carmel Lyceum in Oldenzaal.

In the same year, he initiated his study in 'Plant Breeding and Crop Protection' at what is currently known as Wageningen University. The subjects of his MSc-thesis were Entomology (Laboratory of Entomology, Wageningen University) and Integrated Crop Protection (Plant Research International and the Laboratory of Nematology, Wageningen University), which led to a graduation in January 2003.

From April 2003 until May 2005, he worked at the Plant Protection Service of the former Dutch Ministry of Agriculture, Nature Conservation and Food Safety. Here, in the mycological laboratory of the diagnostics department, he worked on several projects on the diagnostics and ecology of Horse Chesnut Bleeding Canker and the causal agent of Sudden Oak Death, *Phytophtora ramorum*.

In June 2005 he pursued a PhD through a project at the Fungal Biodiversity Center (CBS-KNAW) in Utrecht under the supervision of Prof. dr Pedro Crous. The research on the taxonomy, identification and diagnostics of the form-genus *Phoma* carried out during this study is described in this thesis.

Since June 2009, he holds a position as head of mushroom R&D at Limgroup BV.

The research for 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).

The Training and Supervision Plan was completed at the Graduate School Biodiversity.

Layout and design:
Manon van den Hoeven-Verweij

Front cover insets from left to right:
Pycnidia and section of a pynidial wall of *Phoma longicolla*, conidia of *Stagonosporopsis cucurbitacearum*

Back cover insets:
Fourteen-day-old colonies of *Phoma brasiliensis* on oatmeal agar

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